Studies of how to improve translocation outcomes of *Apteryx mantelli* focusing on breeding, hybrids, diversity, and telomeres

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Overall Abstract

Genetic diversity contributes to the resilience of populations and, thus, to their potential to adapt to change and rebound after episodes of population decline. At the same time, many threatened species are confined to small populations with severely reduced access to gene flow. Since lost connectivity can result in inbreeding, translocations have become an increasingly important tool used by conservationists. However, the relative risks associated with inbreeding are difficult to weigh against potential negative fitness effects of outcrossing and hybridization. North Island brown kiwi, *Apteryx mantelli*, have a long, documented history of management and many severely isolated populations. The goal of this thesis was to determine current gaps in knowledge for successful genetic management of *A. mantelli*, explore closing those gaps using established hybrid populations and make recommendations for future translocations. First, information from past studies of *Apteryx* genetics was synthesised, which drew attention to the fact that available genetic data are insufficient for informing genetic management, predicting translocation outcomes, and linking genetic diversity to population fitness and local adaptation. Genome science combined with a strategic sampling regime was identified as crucial for acquiring the missing data. Second, an in-depth theoretical and empirical analysis of *A. mantelli* breeding behaviour was conducted. The results of this analysis showed that *A. mantelli* have the potential for polygamy, shows no signs of assortative mating, and breed in groups in certain conditions. These features of *A. mantelli* behaviour increase the likelihood of successful genetic rescue after reinforcement translocations. Next, the genetic diversity of the mixed-origin *A. mantelli* population on Ponui Island was investigated. Genotyping-by-sequencing analyses showed that this population constitutes a hybrid swarm in which

founding parental genomes remain represented and levels of diversity are high compared to reference mainland populations. In addition to these studies, I conducted the first investigation of *Apteryx* telomeres. My theoretical analyses and empirical findings showed that telomere analyses of as long-lived species as *A. mantelli* are challenging and that telomere length is unlikely to be a suitable marker for determining *Apteryx* age and viability. Taken together, I suggest that the success of the hybrid population on Ponui Island indicates that mixed origin translocations should be considered as part of *Apteryx* management. However, I stress the need to (1) determine the role of local adaptation in *Apteryx* diversification, (2) study the impact of inbreeding, and (3) undertake investigations into informative markers of age and fitness on the individual and population-level. Investigation of epigenetic regulation of gene expression will be highly interesting for both these quests.

Key Words

Admixture; Age; Aotearoa; Apteryx; Attrition; Aves; Birds; Breeding Strategy; Breeding System; Conservation; Conservation Genetics; DNA Quality; Fragmentation; Genetic Diversity; Genetic Rescue; Genomics; Genotype by Sequencing; Group Breeding; Heterozygous; Hybrid; Hybrid; Vigour; Inbreeding; Incubation; Introduction; Kiwi; Kiwi Recovery Plan; Management Unit; Mate Choice; Monogamy; New Zealand; Outbreeding Depression; Polygamy; Ponui Island; qPCR; Reinforcement; Telomeres; Translocation

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Preface

My first encounter with kiwi was as a volunteer for the Department of Conservation (DOC) in 2013. I was accompanying an experienced kiwi practitioner. We were supposed to go out in the evening to a known nest, and wait until it got dark and the incubating dad left. I was going to watch her grab the chick, assist her while fitting a radio transmitter to its leg, maybe take a photo, and then watch her put the bird back. That plan was exciting enough, but, suddenly, this night became much more memorable when the practitioner's arm turned out to be too short and I was asked to have a go at extracting the chick(s) from their deep burrow. I will never forget the two feisty fluff-balls in the light of my headtorch.

Unfortunately, almost equally vivid is my memory from just a few weeks later when the radio transmitters gave us mortality signals for both siblings. In unmanaged areas, less than one in 20 kiwi chicks survive, and, even in managed areas, an incursion of one single stoat, ferret, or dog can have devastating consequences. However, while hundreds of people and millions of dollars are devoted to controlling these invasive pests, I hoped to contribute to long-term diverse and thriving kiwi populations in a different way. In fact, my work centred around one single sentence in the current Kiwi Recovery Plan, '*there is confusion about how to manage hybrid and mixed provenance individuals, as well as individuals and populations that are suspected to contain deleterious alleles/genes*', and consequently around the practice that unites the history – and probably future – of these confusing populations: translocations. While I am convinced that this work is important for kiwi, it is also part of my vision to increase the scientific as well as public understanding for processes that increase, diminish, and alter biodiversity; only through information and understanding can we generate fascination,

and only fascination can lead to the passion and motivation necessary for preserving earth's diversity for generations to come.

I dedicate this thesis to:

…all the hard-working kiwi practitioners and conservation rangers out there. Especially Greg Moorcroft and the Whakatane Kiwi Team; it is thanks to you this journey was possible.

…the birds we disturbed to collect the data analysed herein, in particular those on Ponui Island and in the Pūkaha National Wildlife Centre which had been tracked for years but who passed away in 2020 due to drought and ferret predation, respectively.

… the amazing spiral of coincidences that brought me and my fantastic wife together. If it was not for a friend of your supervisor, for ONE, for a timely click on a bookmarked webpage, for reorganisation within DOC, for a shared love for birds, and a number of other things, we would never have ended up in New Zealand, at the same time, both studying kiwi. In fact, we could have spent years in Palmy and never met, if it was not for that we both are part of the small, inclusive cult known as Roller Derby. If all this does not mean we were meant to be, I don't know what does! Jag älskar dig!

…my family and my home. There is no better way to express my feelings for my home than this song from and about the area where I grew up written by Augusta Amalia Wilén in 1897. Originally performed by Lisa Cecilia Thomasson-Bosiö, but regularly sang by my great-grandmother Clarah Undin:

Lappflickan

"Bland Ovikens skyhöga snökrönta fjäll
bland grönskande furor där ligger mitt tjäll
där växte jag upp likt en lilja ur knopp
där lärde jag mig blicka mot himmelen opp

Det tjället var ringa dess dörr var så låg
men aldrig en kärare boning jag såg
jag var arm uppå guld men gud var mig huld
jag kände ej världen dess synd och dess skuld

Då fyllde en brinnande längtan min håg
att skåda den värld som bak fjällena låg
se dess bländande prakt
Och jag flydde den trakt
där barndomens änglar kring vaggan stå vakt

Och sen har jag farit så vida omkring
och skådat i världen mångt undersamt ting
Men jag längtar ibland hem till fjällbäckens strand
till min hembygd där norrskenet flammar ibland

Nog vänder jag åter till hemmet en gång
och sjunger bland Ovikens fjällar min sång
Sjunger glad och förnöjd
Och i jublande fröjd
går min sång emot blånande himmelens höjd"

Very direct translation into English by Malin Undin 2021

"Among the sky-high, snow-covered mountains of Oviken; among verdant pines lies my home; there I grew up like a budding lily; there I learnt to gaze to the skies;

The home was modest, the door was so small; but never a dearer home I saw; I was poor in terms of money, but the powers stood me by; I was unaware of the terrors and sins of the world;

So, a burning desire filled my soul; to set my eye upon the world beyond the mountains; see its dazzling beauty; so, I left the region; there the angels of childhood had guarded my sleep;

And since I have travelled near and far; and seen in the world many fascinating things; but sometimes I miss my home by the mountain stream shore; miss my home where the northern light sometimes glow

Sure, one day I shall turn back; to sing my song among the mountains of Oviken; singing happy and at peace; and in bubbling joy; my song turns to the bluish heights of the sky"

Svensk Populärvetenskaplig Sammanfattning

Genetisk mångfald är en helt avgörande resurs för hotade arter och deras förmåga att återhämta och anpassa sig vid miljöförändringar. Således är det djupt oroande att många hotade arter idag utgörs av små, avgränsade populationer med drastiskt minskad tillgång till genetiskt utbyte med artfränder från andra områden. På grund av detta har translokeringar – det vill säga avsiktlig förflyttning av individer – blivit ett allt viktigare verktyg inom naturvård som används för att återskapa mångfald inom, och kontakt mellan, populationer. Tyvärr så återstår osäkerhet kring hur translokeringar ska genomföras för att minimera risken att generera populationer med låg eller ojämnt fördelad genetisk mångfald till följd av små startpopulationer eller hybridisering med oönskade följder.

Forskningen som presenteras i den här avhandlingen har utförts på brun kivi, *Apteryx mantelli*, och utnyttjat att det inom denna art finns ett spann av populationer som representerar olika grader av isolering och olika bakgrund vad gäller tidigare naturvårdsåtgärder. Genom att studera utvalda populationer utefter detta spann flyttar mina resultat fram vår kunskap om, och förståelse för, faktorer som avgör och indikerar utgången av translokeringar. För det första konstaterar jag att trots ett mycket stort intresse för kivigenetik under de senaste decennierna, så har de data som producerats litet värde för att kunna förbättra strategier för bevarande av genetisk mångfald. I nästa kapitel diskuterar jag mer generellt att våra bristande möjligheter att formulera en förutsägbar och välfungerande praxis för translokeringar beror också på otillräcklig förståelse för sambandet mellan ärftlig mångfald, negativa konsekvenser av inavel och positiva effekter av hybridisering.

En avgörande faktor för utgången av den typ av translokeringar som för samman individer från flera olika populationer är med vem och hur många som parning sker. Min djupgående genomgång av insamlade data från kivi, kombinerat med teoretiska och empiriska data rörande häckningsstrategier för fåglar i allmänhet, konstaterade tre viktiga fakta: (1) *A. mantelli* har teoretisk potential för polygami, (2) *A. mantelli* i en blandad population parar sig inte företrädesvis med individer av samma bakgrund, och (3) *A. mantelli* kan under vissa omständigheter häcka i grupp. Dessa tre faktorer tyder på att supplementerande-translokeringar av *A. mantelli* – det vill säga förflyttande av individer från en population till en annan – har goda förutsättningar för att resultera i positiva effekter för små, inavlade populationer. Detta brukar kallas för ’genetic-rescue’-effekt.

I nästa del av avhandlingen, demonstrerar jag – med hjälp av tiotusentals genetiska markörer spridda över hela genomet – att *A. mantelli* populationen på ön Ponui utgörs av en så kallad hybridsvärm där båda originalgenomen fortfarande finns representerade i hög grad. Denna hybridpopulation har upplevt snabb tillväxt och har nu (över 50 år efter de två translokeringar som grundade populationen) höga nivåer av genetisk mångfald till och med jämfört med stora fastlandspopulationer som kan antas vara det närmaste vi har ett mått på ’naturlig’ nivå av mångfald. Mina undersökningar tydde på att timing för translokeringen, släktskap mellan de introducerade fåglarna, samt hur lång tid som passerat är faktorer som alla starkt påverkar mångfalden i en hybrid population. Trots detta anser jag att bevisen för positiva effekter av translokeringar som blandar fåglar från flera områden var så pass övertygande att denna typ av åtgärd bör spela en större roll i framtida åtgärdsprogram för kivi.

I den fjärde delen av avhandlingen presenterar jag resultaten från mina studier av kivi telomerer. Telomerer finns i ändarna på alla kromosomer men krymper vid varje celldelning. Detta gör att dess längd kan indikera individers ålder och populationers demografiska sammansättning. Baserat på mina egna laboratorieresultat samt en sammanställning av tidigare resultat från andra fågelarter, så visade det sig tyvärr att telomerlängd troligen inte är den hett eftertraktade markören för kiviålder och hälsa som man letat så länge efter. Dessutom fann jag att efterhandskonstruerade studier av telomerer för arter som är så pass långlivade som kivi är svåra att genomföra och tolka på grund av de strikta kraven på DNA av högsta kvalitet.

Sammantaget tyder mina resultat på att vi borde omvärdera de nuvarande nationella och regionala åtgärdsprogrammen för kivi vilket uppmanar till att i största möjliga mån minimera genetiskt och geografiskt avstånd mellan populationer man translokerar till och från. Denna inställning riskerar nämligen att minska snarare än bevara genetisk mångfald. Jag föreslår att de två viktigaste kvarstående frågorna för att förbättra hur kivitranslokeringar genomförs är: (1) att utreda hur stor del av de genetiska skillnaderna mellan kivier från olika områden som är ett resultat av lokalanpassning och hur stor del som är slump, samt (2) att undersöka i detalj på vilket sätt inavel påverkar individers och populationers hälsa och reproduktion. Studier av så kallade epigenetiska modifikationer av DNA – det vill säga DNA-förändringar som inte påverkar själva sekvensen utan endast i vilken utsträckning olika delar översätts till RNA och proteiner – skulle kunna bidra till svaren på båda dessa frågor. Dessutom har epigenetiska förändringar visat sig utgöra lovande åldersmarkörer i andra långlivade arter, och ett sätt att åldersbestämma vuxna individer är en av pusselbitarna som fattas för att kunna utvärdera kivipopulationer både innan och efter translokeringar.

Thesis structure, outline, and content

This thesis is divided into five parts, each of which are further split into chapters. Each chapter has a title page featuring a word cloud of the 100 most frequently used words followed by an abstract and an introduction. Beyond that, the format varies, but the structure for each chapter is overall consistent with either a published review or an original research paper. The parts and chapters are ordered to tell a story from cover to cover but are also written to be read independently. The exception to the latter is that repetition has been reduced through referencing parts of Materials and Methods to previous chapters. All research has been preceded by consultation with Mana Tangata and conducted with permission from the Massey University Animal Ethics Committee and Department of Conservation (see Appendix A5). Two chapters have been published prior to submission and slightly modified versions of two further chapters have been submitted for review (see Appendix 6). Multiple aspects of my work have also been presented at scientific conferences (see Appendix 7).

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equal success of all founders, 101 out of 120 sample birds were found to be of mixed ancestry i.e., hybrids.

This data chapter examines two other *A. mantelli* populations with mixed origin: those at the Pūkaha National Wildlife Centre and the Remutaka Forest Park, and is phrased and formatted as a report for the management team at these sites. This chapter examines in greater detail than was previously possible the composition of the of the founder populations for these sites, concluding that when taking relatedness into account, the starting populations were as small as 32 and 24 birds respectively, far fewer than the recommended 40 birds. Moreover, genomic analyses suggested that while the overall diversity in the Pūkaha and Remutaka populations combined was high, there was large variation at the individual-level with birds ranging from being hybrids with heritage from three separate management units to being highly inbred.

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PART I – BACKGROUND

Genetic management is becoming increasingly unavoidable for successful conservation, and translocations remain one of the leading tools available for generating augmented gene flow. In this part, I describe *Apteryx* conservation, focusing on how it will be affected by ongoing, worldwide changes to the environment, management attitudes, and technology that sets the scene for the protection of biological diversity. I then go on to discuss our current understanding of *Apteryx* genetics and genetic rescue, focusing on why neither is sufficient for developing successful genetic management plans, and describing how *A. mantelli* constitutes a unique study system for filling the knowledge gaps.

Chapter 1.1

General introduction: Kiwi conservation in a changing world

All supervisors provided feedback on one or more drafts of this chapter.

Abstract

Forest clearing and predation by invasive mammals have severely reduced *Apteryx* distribution and density throughout Aotearoa New Zealand and continue to do so in unmanaged areas. Hence, kiwi conservation receives a lot of attention, and, besides pest control, translocating individuals between sites is a key part of ongoing management. However, questions remain regarding the best practice for such translocations. For instance, historic translocations have resulted in some populations being regarded as unwanted hybrids. The rapid, ongoing environmental change, the increasing recognition of the importance of preserving adaptive potential, the introduction of genomic tools, and an awakening regarding the role of indigenous guardians in sustainable conservation constitute five key phenomena; in light of which, it is time to re-evaluate the current *Apteryx* translocation policies. However, to develop new policies for predictable and successful conservation outcomes, several outstanding questions remain. Intriguingly, the unwanted *A. mantelli* hybrid populations are among the most promising study systems for answering questions about (1) when translocations are likely to be beneficial, (2) what constitutes appropriate source and target populations, (3) how can the currently dominating population genetics focused models of translocation success be improved, (4) what does the breeding system and mate choice look like in *A. mantelli* and how will this impact the potential for genetic rescue, and (5) what are the most informative markers for assessing *Apteryx* population status, viability, and demographics?

1.1.1 General introduction

In their strategic plan 2017-2020, the International Union for Conservation of Nature (IUCN) states that '*With sufficient funding, effective law enforcement, removal of the major threats, solid research, appropriate technology, and perseverance, no threatened species need to ever become Extinct, and many could return to play their historical role as part of the complex natural ecosystems that have enriched planet Earth.*' Consequently, researchers, practitioners, and governmental bodies all have their role to play in saving our biodiversity. Currently, biodiversity keeps declining on a global level, and the main threats remain habitat destruction for the purpose of agriculture, invasive alien species, and overexploitation (Hoffmann *et al.* 2010, Hoffmann & Sgro 2011, IUCN 2020). However, while the IUCN Red List status has been deteriorating for many bird species over recent decades, conservation efforts have led to noticeable increases in population size and/or range for several species, even allowing a few to move to a less threatened category (Figure 1.1.1; Hoffmann *et al.* 2010). When the IUCN Red List data are broken down by the main threat identified, alien invasive species is the category with the most success stories relative to the number of deteriorating species. Specifically, 12 species moved to a less threatened category thanks to conservation efforts against invasive alien species compared to 32 species moving to a more threatened category due to such invaders (Hoffmann *et al.* 2010). This finding is particularly encouraging from a New Zealand conservation perspective, not the least in the context of 'Predator free 2050' (Russell *et al.* 2015). However, besides the removal of invasive alien species, the IUCN recognises translocations as an important and growing field for conservation management, not the least since any type of habitat degradation tends to cause fragmentation and reduce connectivity (IUCN/SSC 2013, Moehrenschlager 2018).

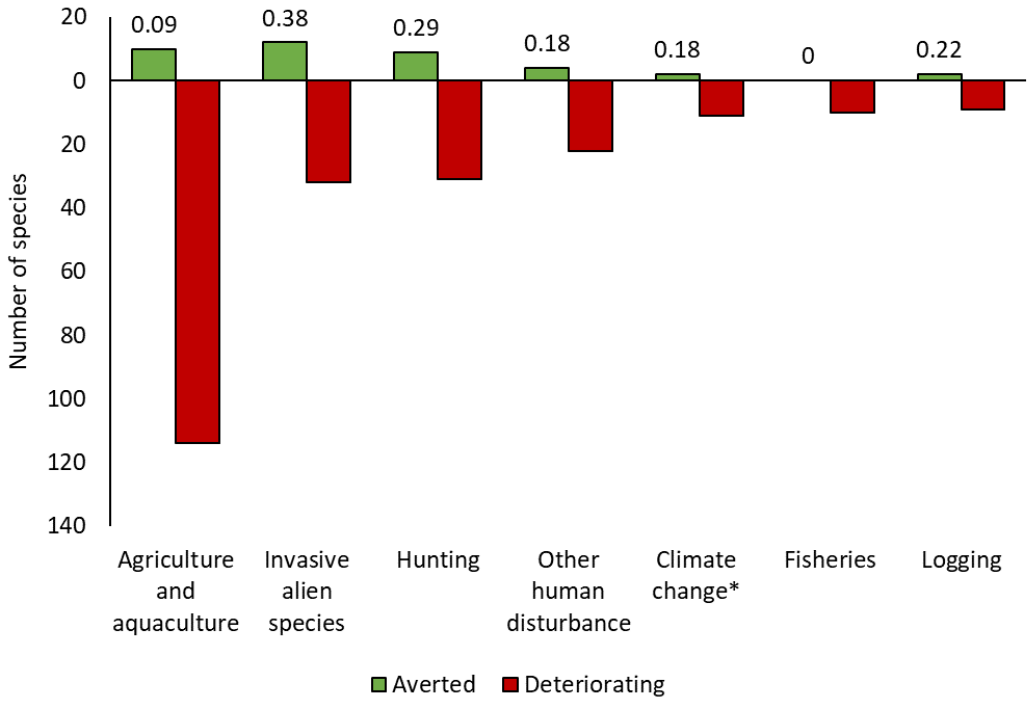


Figure 1.1.1. Bar graph illustrating main reasons for bird species moving to another category on the 2010 IUCN Red List. Species moving to a more threatened category are indicated in red, and green indicates a move to a less threatened category compared to previous editions of the list. Threats have been split into seven types along the x-axis. For species becoming more threatened, red bars indicate what type of threat caused the decline. For species deemed less threatened, green bars indicate the type of threat that was removed or controlled to allow the improvement. Only the threat listed as having the most impact is shown even though in reality a combination of threats is often involved. The opposite direction of the bars is emphasising the opposite direction of the threat status change of the species they represent. Numbers above green bars indicate the proportional size of the green bar to the red. Data from Hoffmann *et al.* (2010). *The threat type 'climate change' includes changes to wildfire regimes and extreme weather events.

Translocations can be done to introduce, reintroduce, or reinforce a taxon in a geographic area (IUCN/SSC 2013, Armstrong *et al.* 2019; 'taxon', in this case, refers to a lineage at any taxonomic level such as a genus, species, subspecies, population, management unit, or evolutionary unit). There is a considerable amount of variation both in the number of available case studies from different avian groups (Sutherland *et*

al. 2019) and in the methods and strategies used (Batson *et al.* 2015). Yet, overall, support is strong for both introduction and reinforcement being beneficial interventions in bird conservation with high success rates (Miskelly & Powlesland 2013, Jamieson 2015, Sutherland *et al.* 2019, Skikne *et al.* 2020). Reinforcement (also referred to as 'supplementation', see, for instance, Germano *et al.* 2018) involves adding additional individuals into existing populations, for instance, to increase population size (also referred to as achieving a 'rescue effect'). However, reinforcement is increasingly recognised as a tool to maintain, restore, add, or change genetic diversity and counteract inbreeding (Weeks *et al.* 2011, Hoffmann *et al.* 2015, Frankham *et al.* 2017). Such missions and/or their outcome, when successful, are commonly referred to as genetic restoration or genetic rescue (Ingvarsson 2001, Frankham 2015, Whiteley *et al.* 2015, Bell *et al.* 2019, Ralls *et al.* 2020).

For the *Apteryx* spp. (kiwi birds), conservation motivated translocations have taken place for at least 100 years (Colbourne 2005). Originally, the reason for these was often to momentarily save birds threatened by forest clearing by moving them to a safer habitat. However, increasing understanding of the consequences of low genetic diversity, fragmentation, small founder sizes, bottlenecks, poor recruitment, and inbreeding have spiked interest in this type of intervention over the last 25 years (Colbourne *et al.* 2005, Gasson 2005, Cromarty & Alderson 2013, Miskelly & Powlesland 2013, Innes *et al.* 2015, Innes *et al.* 2016, Robertson *et al.* 2019).

1.1.2 *Apteryx* and their conservation

Over 16 million years ago, the ancestors of *Apteryx* flew to what is today known as Aotearoa New Zealand (Worthy *et al.* 2013, Mitchell *et al.* 2014, Weir *et al.* 2016). It is intriguing to think of the flying *Apteryx* ancestor self-introducing to Aotearoa New

Zealand in a similar way that the silvereye (*Zosterops lateralis*), welcome swallow (*Hirundo neoxena*), western cattle egret (*Bubulcus ibis*), masked lapwing (*Vanellus miles*), Eurasian coot (*Fulica atra*), white-faced heron (*Egretta novaehollandiae*), swamp harrier (*Circus approximans*), black-fronted dotterel (*Elseyornis melanops*), and western barn owl (*Tyto alba*) have done much more recently, suggesting that the avian fauna of Aotearoa New Zealand has always been dynamic (Trewick & Gibb 2010).

The *Apteryx* ancestors evolved into one of the most peculiar bird genera, owing at least partly to an environment lacking mammalian predators and competitors that allowed them to lose flight and populate a nocturnal niche where scent, hearing, and touch – rather than sight – became their main senses (Sales 2005, Cunningham *et al.* 2009, Castro *et al.* 2010, Castro 2011, Zelenitsky *et al.* 2011, Le Duc *et al.* 2015). *Apteryx* dispersed and radiated, and, during their peak distribution, they are believed to have inhabited all areas of Aotearoa New Zealand's current three main islands (North Island, South Island, and Stewart Island/Rakiura), except for the alpine zone of the South Island (Shepherd & Lambert 2008, Shepherd *et al.* 2012, Weir *et al.* 2016, Germano *et al.* 2018).

Distribution of, and connectivity between, *Apteryx* species and populations have changed historically along with the climate, changing rivers, and erupting volcanoes (Weir *et al.* 2016, White *et al.* 2018). However, the most catastrophic change has been caused by forest clearing and the introduction of invasive species (Taborsky 1988, McLennan *et al.* 1996, McLennan *et al.* 2004, Innes *et al.* 2010, Robertson *et al.* 2011). This is partly because the same adaptations that made *Apteryx* well suited for the Aotearoa New Zealand's environment made these birds vulnerable to mammalian predation. Consequently, after human arrival to Aotearoa New Zealand, all five extant *Apteryx* species have been drastically reduced in number and are now largely confined

to more-or-less geographically isolated populations in dire need of active conservation management (McLennan & Potter 1992, Miller & Pierce 1995, Taylor 2014, Innes *et al.* 2015, Kiwis for Kiwi 2016, Robertson *et al.* 2016, Germano *et al.* 2018; Figure 1.1.2). Some populations are strictly isolated since one of the oldest *Apteryx* management strategies has been translocating threatened birds to predator-free islands (Colbourne 2005; Figure 1.1.3). While such islands generally feature suitable kiwi habitat, they are naturally of limited size, completely cut off from gene flow, and the founders were often few (Colbourne 2005).

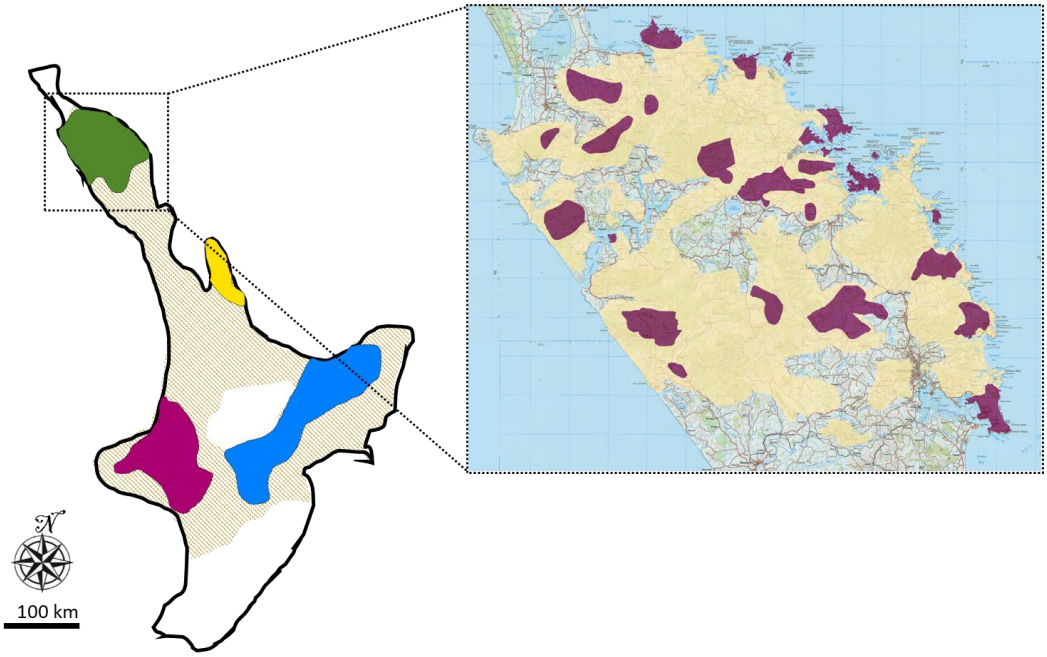


Figure 1.1.2. Map of the historic and current distribution of North Island brown kiwi. The inferred continuous historic distribution is illustrated with light patterned shading. Current, fragmented distribution is split by colour into the four management units recognised within this species: Northland (green), Coromandel (yellow), Western (pink) and Eastern (blue; Germano *et al.* 2018; see Table 1.1.1). Enlarged map to the right illustrates the current distribution of *A. mantelli* Northland in more detail highlighting the fragmented distribution even on a regional scale. Light yellow represents areas where *A. mantelli* Northland are present in very low density, and purple indicates areas of relatively high kiwi density (≥ five calls per hour during call count surveys; DOC GIS Team/R. Fuchs, unpubl. 2019).

The drastic decline, combined with the iconic status of kiwi in Aotearoa New Zealand, has created widespread, and needed, interest in long-term sustainable and successful conservation of this group (Peters *et al.* 2015, Kiwis for Kiwi 2016, Germano *et al.* 2018). The main management strategies in place are (1) trapping and/or poisoning of invasive mammalian predators (e.g., stoats, rats, possums, and ferrets) and (2) translocating either eggs or newly hatched chicks to captive facilities or predator-free crèches – generally islands or areas surrounded with a predator-proof fence. The latter strategy is referred to as Operation Nest Egg or ONE (McLennan *et al.* 1996, Colbourne *et al.* 2005, Robertson *et al.* 2011, Germano *et al.* 2018). At ONE facilities, the chicks are kept until they are large enough to not be eaten by stoats (i.e., at a weight of ≥1000 g; McLennan *et al.* 2004) after which they are released. The exception is *A. owenii*. Due to the smaller size of this species, birds are never deemed to reach a stoat proof weight and, consequently, the entire population of about 1800 individuals is kept confined to off-shore islands and a few predator-free on-shore sanctuaries (Taylor 2014, Germano *et al.* 2018, Robertson *et al.* 2019). Both predator eradication, ONE, and translocations are implemented by the Department of Conservation (DOC) but also commonly by local community groups and iwi/hapū (iwi is a tribe and hapū a subtribe of the indigenous Māori people of Aotearoa New Zealand; see further below; Cromarty 2013). In addition, the so-called kōhanga (creche) kiwi program is a third strategy tightly intertwined with ONE. The kōhanga kiwi program is lead and implemented by the national charity Kiwis for Kiwi (www.kiwisforkiwi.org). In this program, *Apteryx* pairs are held in predator-free areas, acting as a source population from which a proportion of the juveniles are 'harvested' and translocated to boost existing or found new populations (Innes *et al.* 2015, Innes *et al.* 2016, Kiwis for Kiwi 2016).

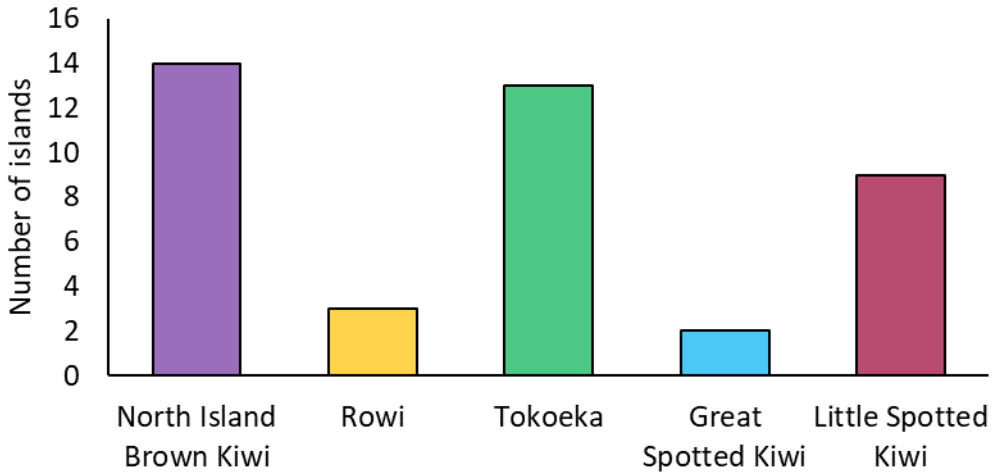


Figure 1.1.3. Bar graph illustrating the minimum number of Aotearoa New Zealand offshore islands with *Apteryx* spp. The North Island and South Island are not included. These populations are the result of translocations. The exception is five to seven of the islands, including Rakiura, on which Tokoeka (*A. australis*; including Rakiura) is believed to occur naturally. Some of these five to seven islands have also received birds through translocations. Due to poor records, more islands might have received birds that never established a population. Data from Colbourne (2005).

One hot topic of discussion since the beginning of *Apteryx* conservation is how to adequately split the genus into species and further into management units (Butler & McLennan 1990, Robertson 1996, Holzapfel *et al.* 2008, Germano *et al.* 2018). Studies utilising traditional genetic markers such as allozymes, mitochondrial DNA, and microsatellites have identified genetic differences within *Apteryx* since the 1980s. Based on these studies, five extant species have been recognised. In addition, identification of differences within these species (generally defined as non-shared mitochondrial haplotypes) means that a total of at least 15 separate *Apteryx* taxa have been identified (Table 1.1.1; Shepherd *et al.* 2012, Weir *et al.* 2016). A minimum of four of these taxa are believed extinct: the North Island lineages of *A. rowi* and *A. owenii* as well as, two separate lineages of *A. australis* on the eastern side of the South Island (Shepherd *et al.*

2012, Weir *et al.* 2016). However, due to the limited information in the markers used, it remains unclear to what extent the differences between these taxa are linked to local adaptation or merely a consequence of geographic distance followed by isolation (Shepherd & Lambert 2008, Shepherd *et al.* 2012, Reitzel *et al.* 2013, Manthey & Moyle 2015, Weir *et al.* 2016, Flanagan *et al.* 2018, Junaid *et al.* 2018, Galla *et al.* 2020, Rey *et al.* 2020). See Chapter 1.2 for further details.

Despite this uncertainty, the current Kiwi Recovery Plan (KRP) recognises 14 extant conservation management units (MUs, also referred to as taxa) based on identified or inferred barriers to gene flow (McLennan & McCann 2002, Burbidge *et al.* 2003, Weir *et al.* 2016, Germano *et al.* 2018, White *et al.* 2018), and a national target has been set to reaching and maintaining at least 2% annual growth of each of these units independently (Innes *et al.* 2015, Kiwis for Kiwi 2016, Germano *et al.* 2018). Currently, the conservation status is improving for several *Apteryx* MUs (Figure 1.1.4). However, such positive population trends are the result of rapid growth in managed populations, while unmanaged populations keep declining within all taxa (Robertson & de Monchy 2012, Innes *et al.* 2015, Germano *et al.* 2018).

Table 1.1.1. The scientific and common names of the five extant *Apteryx* species and their current splitting into 14 management units.

		Latin	Common names*	Management units
Apteryx	Brown kiwi	*A. mantelli***	North Island brown kiwi (NIBK); northern brown kiwi; brown kiwi; parauri; kiwi nui	Northland
				Western (or Taranaki)
				Eastern
				Coromandel
		A. rowi	rowi; Okarito brown kiwi	Rowi
		A. australis	tokoeka; South Island brown kiwi (SIBK); southern brown kiwi	Haast
				Northern Fiordland
				Southern Fiordland
				Rakiura***
	Spotted kiwi	*A. owneii*	little spotted kiwi (LSK); kiwi pukupuku; little grey kiwi	Little Spotted Kiwi
		A. haastii	great spotted kiwi (GSK); roroa; great grey kiwi	Northwest Nelson
				Westport
				Paparoa Range
				Arthur's Pass

*Other names are also sometimes used locally.

**Old scientific names such as *A. australis mantelli* or *A. bulleri* still occasionally appear.

***Occasionally referred to as its own subspecies named *A. australis lawryi* or Stewart Island tokoeka.

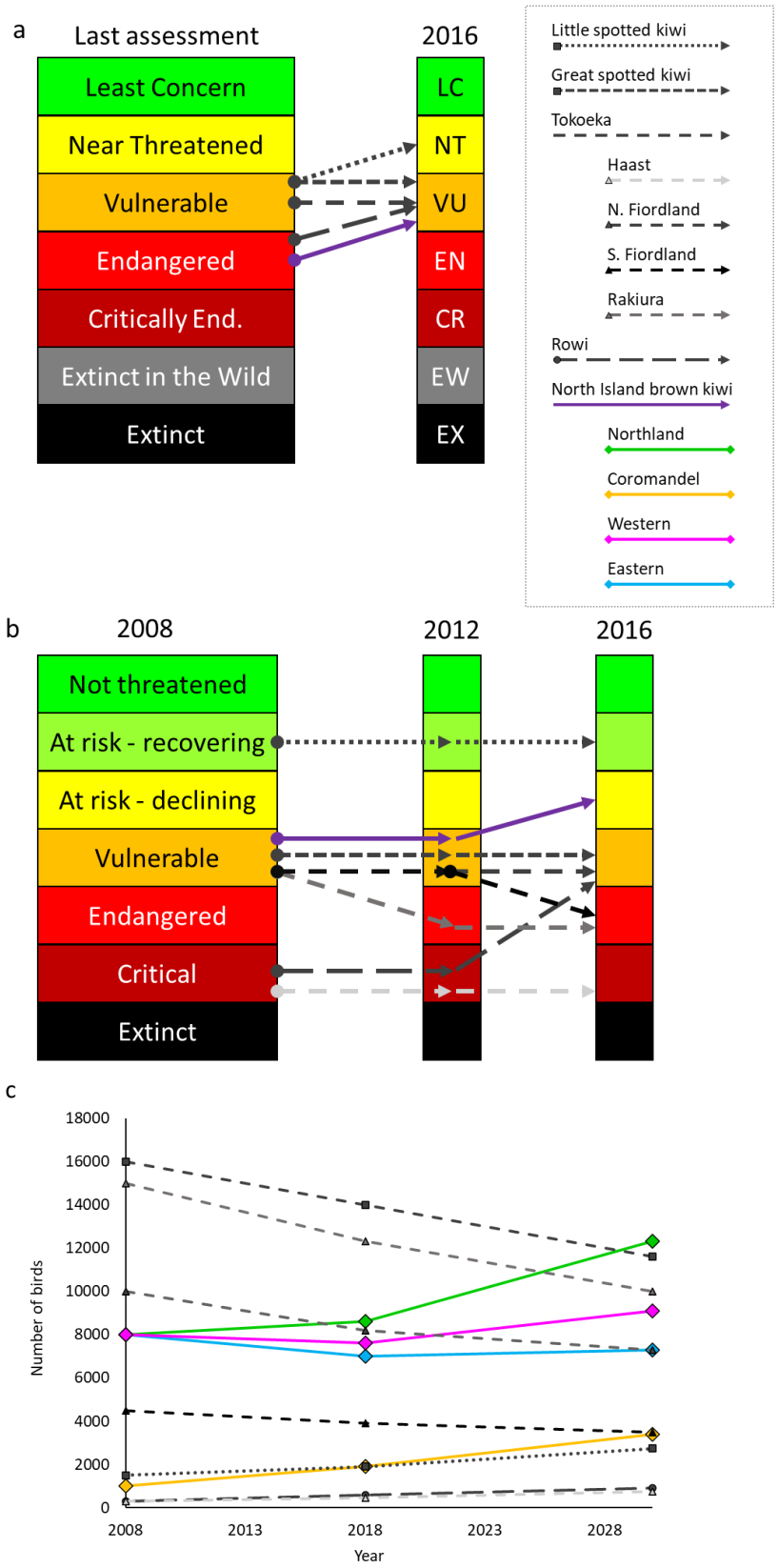


Figure 1.1.4. Illustration of current, past, and predicted threat statuses and population trends for *Apteryx* spp. In (a), each of the five *Apteryx* species are indicated by an arrow starting at the IUCN Red List category they were deemed to fall into in previous assessments and pointing towards their categorisation in the 2016 edition of the list (right column). (b) illustrates the

Aotearoa New Zealand national equivalent of the Red List and the categorisations of each *Apteryx* species in 2008, 2012, and 2016, respectively (Miskelly *et al.* 2008, Robertson *et al.* 2013, Robertson *et al.* 2016). In (b), *A. australis* is split into the four management units; the remaining four species are reported at the species level only (Germano *et al.* 2018). (c) depicts the estimated number of birds for each taxon/management unit in 2008 and 2018, and the predicted number in 2030 if current management remains the same (in the case of *A. haastii* for which only species-level information is available; Germano *et al.* 2018). *Apteryx mantelli* and the four management units within this species are coloured to signify them being the focus of this thesis.

The national *Apteryx* management policy promotes maintaining the range and genetic diversity of all 14 management units independently (Germano *et al.* 2018). While this has been interpreted as preserving the genetic differences between units, the KRP also states that that the genetic diversity shall be 'enhanced as much as is feasible' (Germano *et al.* 2018). This lack of clarity has resulted in several *Apteryx* populations today being regarded as hybrids unfit for further conservation interventions since they originate from a mixture of two or more management units (Herbert & Daugherty 2002, Colbourne 2005, Keast *et al.* 2010, Shepherd *et al.* 2012, Germano *et al.* 2018). For example, at least five *Apteryx* populations established through translocations to offshore islands have received birds from multiple management units (Colbourne 2005). On four of these islands, admixture between the units is confirmed or assumed, and these birds are now regarded as hybrids (on the fifth site breeding was never recorded and there are no longer *Apteryx* on this island; Colbourne 2005). One of these hybrid populations is situated on Ponui Island in the Hauraki Gulf, and this population plays a central role in this thesis.

The results of genetic studies (Shepherd *et al.* 2012, Grueber & Jamieson 2013, Ramstad *et al.* 2013, Taylor 2014, Weir *et al.* 2016), combined with the rapid and locally ongoing decline, and current and historic management regimes suggest that

consideration of genetic diversity, and loss thereof, warrant increased focus of future *Apteryx* conservation. From a genetic perspective, ecosystem-level management such as trapping and poisoning can have clear benefits by providing protection for a larger number of birds and, thereby, creating a greater potential for retaining genetic diversity and evolutionary potential (if effective enough; Innes *et al.* 2015, Innes *et al.* 2016, Germano *et al.* 2018). In addition, this type of management can render spill-over effects benefiting other species, allowing *Apteryx* spp. to act as what is often called umbrella species (Robertson *et al.* 2011, Gilby *et al.* 2017, Johnson *et al.* 2017). A concern with the other two strategies described above is that both the ONE and kōhanga programs inevitably reduce effective population sizes by restricting successful reproduction to a small subset of birds, leading to increased risk of losing genetic diversity (Lacy 1987, Frankham 1995, Luikart *et al.* 1998, Innes *et al.* 2016). Furthermore, the limited number of adults founding a kōhanga site will reduce the opportunity for mate choice and may even alter breeding behaviour. Lastly, both the kōhanga and the ONE strategies risk shifting the birds' (and their gene pools') exposure to natural selection, possibly affecting their potential for adapting to their future environment (Willoughby *et al.* 2017). The protocol currently in place for managing the loss of genetic diversity when implementing the kōhanga program is to limit the harvesting per founder male to a set number of chicks. However, concerns have been raised over the scientific justification and limited effectiveness of this cap (Innes *et al.* 2015, Innes *et al.* 2016).

With or without trapping, poisoning, ONE, and/or kōhanga, translocations are likely to remain a significant part of *Apteryx* conservation. Currently, the policy and practice for *Apteryx* translocations are restricting any movement of birds to within management units, and sometimes even stricter recommendations of minimising distance between source and target are in place (Powlesland 1988, Pierce *et al.* 2006, Craig *et al.* 2011,

Scrimgeour & Pickett 2011, Germano *et al.* 2018). A contributing factor to this strict policy is a recommendation that was provided to the Department of Conservation in 2016 stating that hybrid populations are of no conservation value beyond captive display for advocacy and not fit to use as translocation sources (Letter to Kiwi Recovery Group shared with Ponui Island landowners and Ponui Island Kiwi Research Team, 2016). However, others are questioning this reasoning, highlighting, for instance, that hybrid populations have been observed to experience rapid and persistent population growth indicative of high fitness (Cunningham & Castro 2011). Among other things, such hybrid success suggests that we do not fully understand the drivers behind *Apteryx* diversity and, thus, not what to expect once populations or management units are mixed (see further Chapter 1.3). This lack of knowledge is currently leaving conservationists with unclear, and possibly even incorrect, data on which to base their policies. Indeed, the KRP states that one of the actions needed is to determine 'the risk and benefits of hybrid man-mixed provenance populations and the role they can play in kiwi management'. While studying the hybrid populations will be crucial for evaluating such risks and benefits, they are also likely to provide further understanding that would influence the management of this entire genus. In addition, below I present five key changes to the global premises for conservation management that all support that a reconsideration of the current *Apteryx* genetic management and translocation policies is timely.

1.1.3 Conservation times are changing

The biological, political, and scientific environments have always been changing. However, while the speed has never been constant, the rate of change is currently at a historic peak; and this speed risks having particularly severe impacts for long-lived species with low dispersal ability such as *Apteryx* spp. (Koprowski & Krausman 2019,

Young & Duchicela 2020). There are (at least) five such rapid, global changes that directly relate to *Apteryx* conservation in general and translocation policy specifically: the rapid transformation of the environment, the revitalised attitude towards incorporating genetics in conservation, the advancement of new methods for studying genomics, the expanding focus of conservation of processes, and the awakening regarding the role of indigenous communities in appropriate and successful management of threatened fauna and flora (Figure 1.1.5). I describe these one by one in more detail below.

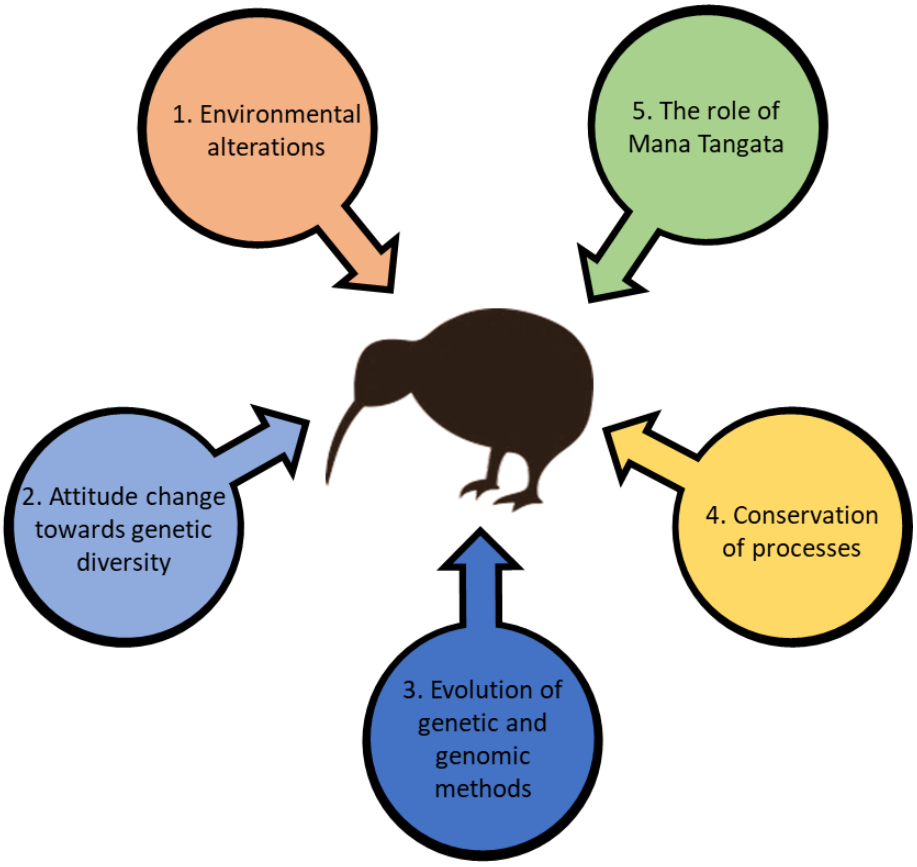


Figure 1.1.5. Schematic illustration of five changes affecting conservation management for *Apteryx* and other threatened taxa. In particular, these changes will impact translocation strategy and policy.

The first and, arguably, most noticeable change is that of the biological environment. Humans have altered, degraded, and/or destroyed the habitat of most species on earth

(Hooke *et al.* 2012, Kuemmerle *et al.* 2016, Kennedy *et al.* 2019). Examples are alterations to species composition by introduction of alien species (Pyšek *et al.* 2020), degradation through pollution (Gouveia *et al.* 2019), and destruction through deforestation (Giam 2017, Aleman *et al.* 2018). These changes have been extremely rapid from a historical and evolutionary perspective, and are causing fitness reduction for many taxa (Dayananda *et al.* 2016, Zimova *et al.* 2016, Mayor *et al.* 2017, Amélineau *et al.* 2019). Fitness reduction can, for instance, be linked to the fact that habitat degradation has led to fragmentation. Fragmentation, in turn, has (1) left many taxa confined to small and/or suboptimal habitat patches that contain smaller, less dense or sometimes denser populations than would have been the norm over evolutionary time and (2) cut off populations from immigration and emigration (Cattarino *et al.* 2016, Winiarski *et al.* 2017). This isolation results in genetic drift and forces mating between close relatives, resulting in inbreeding and a reduced or suboptimal gene pool (Lacy 1987, Lynch 1991, Crnokrak & Roff 1999, Roff 2002, Wright *et al.* 2008, Höglund 2009). Consequently, many taxa are confined to a situation where they need adaptive genetic variation more than ever to cope with change, while their access to such variation is likely to be at its historical lowest due to isolation (Whiteley *et al.* 2015, Zimova *et al.* 2016, Frankham *et al.* 2017, Ralls *et al.* 2018). Another related way in which changes to habitat quality and population density risks lowering fitness is when this alters opportunities for animals to express their entire repertoire of behaviours (Walker *et al.* 2008, Parker *et al.* 2012). For example, a plausible scenario is that small population sizes and isolation leads to reduced opportunity for mate choice and sociability, which can lower reproductive success (Bayne & Hobson 2001, Banks *et al.* 2007, Winiarski *et al.* 2017).

The second important change is the recent acceleration towards a consensus around the importance of considering genetics in conservation management (Frankham 2005, Jamieson 2007, Pierson *et al.* 2016). More specifically, this change involves increased priority and recognition of the value of (1) conserving genetic diversity, (2) populations' access to variation for retaining evolutionary potential, and (3) using genetic and genomic tools to evaluate and monitor population status and management success (Frankham 2010, Jamieson 2015, Whiteley *et al.* 2015, Dresser *et al.* 2017, Carroll *et al.* 2018, Hunter *et al.* 2018, Hoban *et al.* 2020, Ralls *et al.* 2020). There has been long-lasting scepticism towards genetic management for several reasons; first, conservation genetics (and now genomics) has frequently been alleged of mainly being an academic field with little application to practical conservation (Caughley 1994, Hedrick *et al.* 1996, Sarre & Georges 2009, Shafer *et al.* 2015, Garner *et al.* 2016). Among other things, this opinion has been linked to a lack of clear examples where reduced genetic diversity has caused the extinction of a taxon (Frankham 2005). In addition, even when recognising that low genetic diversity could pose a threat, more immediate threats, such as habitat loss, predation, and poaching, have been argued to drive population decreases at a rate that drown any genetic effects (Jamieson 2007, Jamieson 2015, Shafer *et al.* 2015). Lastly, genetic studies have been considered not worth the expense, in particular since studies using traditional genetic markers (such as allozymes, mitochondrial DNA and microsatellites) provide a comparatively low level of detail and resolution, limiting the outcomes of such studies to vague management recommendations (Caughley 1994, Bonin *et al.* 2007, Love Stowell *et al.* 2017, Puckett 2017, Wennerström *et al.* 2017).

In contrast to this sceptical view, the number of cases where genetic degradation has been directly linked to increased extinction risk is aggregating (Crnokrak & Roff 1999, Spielman *et al.* 2004, Frankham 2005). For instance, for the Tasmanian devil

(*Sarcophilus harrisii*) the direct cause of decline is the Devil Facial Tumour Disease (DFTD; Miller *et al.* 2012, Hogg *et al.* 2017), but increasing evidence suggests that the rapid spread of, and lack of sufficient immune response against, this disease is linked to the low genetic diversity within this species (Miller *et al.* 2012, Hendricks *et al.* 2017, Hogg *et al.* 2017). Another example is the substantially lower breeding success among second- compared to first-generation birds after translocation of *A. owenii*, resulting in the expectation that this population will decline rapidly once the F1 generation has ceased contributing to the population (Taylor 2014, Taylor *et al.* 2017c). This reduction in fitness has been suggested to be caused by recessive deleterious alleles which become expressed due to inbreeding, but direct tests of this have not been conducted. Recent research even suggests that inbreeding led to detrimental skeletal alterations in Neanderthals and that this might have contributed to their extinction (Ríos *et al.* 2019).

Even though it remains hard to prove or quantify the specific contribution of genetics in most extinctions or near-extinction events, the overall insight of the significant short- and long-term benefits of genetic diversity and the potential of genetic rescue is spreading (Hamilton & Miller 2016, Love Stowell *et al.* 2017, Ralls *et al.* 2018, vonHoldt *et al.* 2018, Bell *et al.* 2019, Ralls *et al.* 2020). More specifically, a diverse gene pool has the potential to increase population growth rate and, thus, either slow down decline while other threats are addressed or increase recovery rate once other threats have been reduced or eliminated (Bouzat *et al.* 2008, Jamieson 2015). Focusing conservation on the management of genetic diversity has been used to widen the perspective of conservation beyond the species level (UN 1992, Kahilainen *et al.* 2014). However, in practice, focusing solely on preserving genetic diversity regardless of its nature and cause can increase extinction risk (Weeks *et al.* 2016). *Apteryx* serves as an example of when the urge to conserve genetic diversity has resulted in restrictive

translocation policy (see above). Over the last decade, concerns have been raised regarding plausible inbreeding and/or lack of genetic diversity in small, isolated kiwi populations. Particularly, there has been debate about the appropriate management of island population descending from a small number of founders (Taylor 2014, Germano *et al.* 2018, Robertson *et al.* 2019). In this scenario, there is potentially a trade-off between keeping translocation policy restrictive and increasing the opportunity for interventions to result in genetic rescue (Weeks *et al.* 2016, Frankham *et al.* 2017, Ralls *et al.* 2018, Ralls *et al.* 2020). However, no studies of genetic rescue in *Apteryx* have been conducted so far.

The third change, intricately linked to change number two, is the rapid development of methods for studying genetics. Even though genetic diversity and genotype identity has been studied for decades, the advancements of sequencing methodology, computational techniques, and bioinformatics have enabled a move from genetic to genomic studies (Crandall *et al.* 2000, Allendorf *et al.* 2010, Frankham 2010, Narum *et al.* 2013). Key differentiating features of genomic methods are that they (1) allow for analyses of a wide variety of species rather than a few model organisms and (2) enable analyses of the sequence, function, and relationships of genes throughout the entire genome (Elshire *et al.* 2011, Narum *et al.* 2013, Reitzel *et al.* 2013, Hess *et al.* 2015, Ottenburghs *et al.* 2017, Hunter *et al.* 2018, Leroy *et al.* 2018, Picq *et al.* 2018, Dorant *et al.* 2019). The vast increase in the number of markers analysed, and their spread across the coding as well as the 'neutral' regions of the DNA, means that genomic studies have, for instance, the power to discriminate between divergence due to random genetic drift, and differences linked to selection and local adaptation. In other words, rather than simply stating *that* taxa differ, genomic studies can take us closer to understanding *how* and *why* (Funk *et al.* 2012, Reitzel *et al.* 2013, Campagna *et al.* 2015, Johnson *et al.* 2015,

Schmickl *et al.* 2017, Flanagan *et al.* 2018, vonHoldt *et al.* 2018, Pereira-Dias *et al.* 2019).

Consequently, an important role for genomics in conservation will likely be in enabling more accurate and meaningful division of management units (Funk *et al.* 2012, Galla *et al.* 2020). Thanks to their potential for diagnosing and quantifying differences and relationships (Dorant *et al.* 2019), genomic studies also have great potential for improving our identification of source and target populations for translocations (Ransler *et al.* 2011, Malone *et al.* 2018). Fortunately, birds are well placed for genomic studies thanks to (1) a rapidly increasing number of annotated genomes for this group that simplifies the interpretation of genomic data (for the sequenced species but also for related taxa; Campagna *et al.* 2015, Winger 2017, Galla *et al.* 2019, Rexer-Huber *et al.* 2019) and (2) the fact that birds have nucleated red blood cells, which sets researches up for relatively easy access to the high-quality DNA required for successful genomic analyses (Arctander 1988, Oyler-McCance *et al.* 2016).

The fourth change is the recently intensified discussion regarding conservation focused on preserving processes, rather than species or taxa *per se*, and a widening of what desirable processes to preserve can be (Ricklesfs *et al.* 1984, Harvey *et al.* 2017, Matzek *et al.* 2019). Most attention has been given to the preservation of ecosystem services (Luck *et al.* 2003, Bennett *et al.* 2009, Lunt *et al.* 2013, Matzek *et al.* 2019), and, even though this has received extensive research attention for over 40 years, it is only recently that such services have become central to the debate about conservation prioritisation, focus, and funding (Polasky *et al.* 2012, Matzek *et al.* 2019). Some authors point out that the only way to ensure functioning ecosystems is to preserve the evolutionary potential allowing species and populations to adapt to their changing environment (Hoffmann *et al.* 2015, Zimova *et al.* 2016, Hochholdinger & Baldauf

2018, vonHoldt *et al.* 2018, Taylor & Larson 2019). Hence, a key feature of future conservation will be actively facilitating access to genetic diversity and adaptive variation through, for instance, preserving or creating potential for taxon admixture (Crandall *et al.* 2000, Abbott *et al.* 2013, Carlson *et al.* 2014, Hoffmann *et al.* 2015). However, increasingly, it has been highlighted that allelic diversity is likely necessary but not sufficient for long term success; thus, focus is also needed on improving our understanding of the interactions between genotype, phenotype, environment, and fitness (Steeves *et al.* 2017, Junaid *et al.* 2018, Rey *et al.* 2020).

The conservation of processes relates to ecological replacement, i.e., using taxonomically or functionally similar species to maintain ecosystem function after native species have gone (functionally) extinct (Steeves *et al.* 2017, Armstrong *et al.* 2019). In Aotearoa New Zealand, this discussion has so far mainly concerned species such as the Snares Island snipe (*Coenocorypha huegeli*), South Island kokako (*Callaeas cinerea*), and Takahe (*Porphyrio hochstetteri*) who could potentially replace extinct sister species on either of Aotearoa New Zealand's two main islands (Bunin & Jamieson 1995, Trewick 1996, Miskelly & Powlesland 2013). Fossil records suggest that *Apteryx* distribution was essentially continuous before human arrival and that some areas now lacking kiwi used to be populated with taxa that are now extinct (Shepherd *et al.* 2012, Weir *et al.* 2016, Germano *et al.* 2018). This suggests that replacement translocations could play a role in future *Apteryx* conservation, but thorough consideration would be warranted regarding which birds to use for reintroduction into which sites (Malone *et al.* 2018).

E Kotahitanga o te Kaupapa

- Togetherness to Achieve our Objective

A fifth major change currently underway in the management and research communities is a steady but slow awakening regarding the role of indigenous people in successful conservation. In the case of Aotearoa New Zealand, Māori are the people with mana whenua (traditional authority) over the land. Conservation sciences in general, and conservation genetics in particular, have repeatedly been accused of distancing themselves from hands-on conservation (Caughley 1994, Hedrick *et al.* 1996, Sarre & Georges 2009, Garner *et al.* 2016). This has rendered a gap between the scientific findings and their application by those executing the management (Shafer *et al.* 2015, Love Stowell *et al.* 2017, Taylor *et al.* 2017d). Arguably this gap has been widest between classic 'western' scientists and indigenous people across the globe (Bohensky & Maru 2011, Collier-Robinson *et al.* 2019, Stevens *et al.* 2020). Simultaneously, conservation science results are of little use if they do not affect management practices, and, therefore, bridging this gap is crucial for achieving long-term sustainable management programs and healthy, resilient populations. Frankly, this gap needs to be sealed with a two-way bridge that also enables traditional views, practices, and knowledge to affect how, where, when, and why research is conducted. Such bridges are needed worldwide and will likely differ in the detail. However, much of the overall pattern will arguably remain the same.

For hundreds of years, Māori hapū (Māori sub-tribes) have been exercising kaitiakitanga (guardianship) in their respective rohe (tribal area of authority), which includes taking on the responsibility to manage the awa and moana (rivers and seas), the whenua (land) as well as the species and the ecosystems found therein. In the modern context, there are legislative drivers to consult with local Māori regarding many

activities carried out within their rohe thanks to Te Tiriti o Waitangi (the Treaty of Waitangi, 1840) which gives Māori co-management of all natural resources with the Crown. Unfortunately, the iwi consultation in the context of research and sample collection has often been of low standard and limited to a project stage when permits are applied for, but research questions, experimental design, and sampling regime have already been defined. Consequently, mana tangata (traditional and authoritative people of the land) are often excluded from having input into project planning and the opportunity to incorporate research results in their kaitiakitanga.

Massey University Te Kunenga Ki Purehuroa states: '*As a Tiriti-led University we are committed to demonstrating authentic leadership in a contemporary Aotearoa New Zealand as we uphold Te Tiriti o Waitangi, the founding document of our nation, and its principles through our practice. Massey embraces this not just as an obligation but also as a real opportunity for the nation and its peoples. The University will champion new strategies for advancement and integration of te reo Māori* [the language of Māori] *and Māori knowledge. It will demonstrate informed practices consistent with tikanga Māori* [Māori costumes and practices] *and will embrace kaupapa Māori* [Māori principles and values] *across our activities. Massey's teaching and research will contribute to advanced outcomes for whānau* [extended family], *hapū and iwi* [tribe]' (Massey University 'Strategy 2018-2022'). In other words, work done in association with a Tiriti-lead institution, such as Massey University Te Kunenga Ki Purehuroa, shall aspire to achieve significantly better consultation with mana tangata than the commonly seen approach described above. E kotahitanga o te kaupapa – togetherness to achieve our objective – is, in short, what this is all about.

Extensive consultation is particularly important for work in the wildlife, ecology, and conservation fields since such work generally include (1) sampling and storage of plants

or animals that are considered taonga (treasures) in Māori culture, (2) collection of material from within the rohe of particular hapū, and (3) generation of results that have the potential (and often even the purpose) of affecting recommended kaitiakitanga and conservation strategy of species and ecosystems. In te ao Māori (the Māori world) the hapū is the paramount organisational unit of a community and the main unit of action for questions concerning the rohe. For any given activity, such as a research project, the hapū requests to be informed; in particular about what the exact plan is, when, and how it will be executed and, perhaps most importantly, who is doing it, and where. To follow tikanga Māori, this information should be communicated kanohi ki te kanohi (face to face) during the regularly occurring hapū hui (hapū meetings). The most notable feature of a hapū hui is that anyone that has something to say can stand up, say it, and will be listened to. During this project, I have had the privilege to take part in a number of such hui and had my powhiri (official welcome) in several different marae (Māori community hall). These meetings take time, especially when considering travel, but they have been crucial for the building of sustainable trust that has been critical for successful sample collection for our research program.

The hapū hui can also be a vital source of Mātauranga Māori (traditional information). One part of Mātauranga Māori is the knowledge of tapu (sacred sites), commonly old burial sites. In addition, kaumatua (elders) and kaitiaki (guardians) possess invaluable knowledge of local terrain, geographical, and weather-related circumstances. Hence, involving mana tangata at the planning stage of a project is a way of showing appropriate respect that can also greatly assist in making fieldwork more efficient. Another highly valued part of Mātauranga Māori is whakapapa. A strict interpretation of whakapapa is the knowledge of your personal genealogy and the ability to name all ancestors (which can be up to about 58 generations) back to the waka (boat/vessel) by

which your ancestor arrived in Aotearoa New Zealand. In wider terms, however, whakapapa refers to relationships and connections among all things through common ancestry, provenance, affiliation, and/or affection. This tradition of discussion around whakapapa has generated an inherent understanding of concepts such as heritability, genetics, genetic diversity, inbreeding, and hybridisation in the Māori community. In other words, topics such as translocations and conservation genetics tend to awaken interesting, insightful discussion around a project's aim and purpose. In addition, the strong and culturally grounded feeling of right and liability to exert appropriate kaitiakitanga over taonga species paves the way for discussion regarding the implications and incorporations of future results. Thus, it is concerning that the reporting back of outcomes and data to the hapū has often been lacking even in projects being conducted after the most common type of iwi consultation as described above. To be frank, a key component in a sustainable future for mana whenua themselves is employment opportunities that allow people to stay in their rohe. This is another major reason why communicating any results that can contribute to opportunities for paid work is important.

The perhaps trickiest, yet especially important, topic of discussion during the consultation with mana tangata is the long-term and short-term fate of any collected material. This is a complicated discussion since it involves adjusting the storage of samples and handling of data in ways that must remain in place even after your personal involvement in the project is done. Internationally and within Aotearoa New Zealand, interesting work is going on in this space to create a system for cultural labels. In short, the idea with such labels is that they form metadata that will link objects, samples, and/or information back to its geographical and/or cultural origin; these labels would

specify what they might be used for and when and how consultation would be required to allow further use (see for instance https://localcontexts.org/about/).

This thesis is part of the Whakapapa of North Island Brown Kiwi research program, which began in 2012 when mana tangata, and especially Te Patukeha and Ngati Kuta Hapū from Te Rawhiti in Ipipiri, Tai Tokerau (Bay of Islands, Northland), decided to exert their kaitiaki role over kiwi in their rohe. One important objective of the research program is to empower the people closest to day-to-day conservation work by providing tools for making informed management decisions. In other words, our work will never be about coming and telling anyone what or how to do it. Mana tangata, and especially Te Patukeha and Ngati Kuta Hapū from Rawhiti, have been involved in every step of setting up and executing this research. In 2012, these hapū started working together with researchers from Massey to plan this project, getting approval from other hapū whose rohe we were interested in sampling from, and finding suitable students. In parallel with multiple and reoccurring hui, several members of the Rawhiti community were also trained in kiwi handling, and many of them have been directly involved in collecting the data presented in this thesis. In February 2020, we had our first hui with the purpose of bringing the results back to the community. The Whakapapa of North Island Brown Kiwi research program was initiated and is still led by the Te Patukeha and Ngati Kuta Hapū. However, projects initiated by researchers, students, or the industry would arguably have no reason to develop and proceed any differently.

I hope that the work of the Whakapapa of North Island Brown Kiwi program can serve as an inspiration for others to work in partnership with Māori. I also hope that all Massey University research from now on will include thorough and inclusive hapū consultation before, during, and after any work taking place in their rohe and/or on samples originating from there (recognising that all of Aotearoa New Zealand falls

within the rohe of some hapū). Ideally, such consultations should involve participation in face-to-face hui to involve local kaimahi (manpower) and Mātauranga Māori in the planning, fieldwork, and discussion around the implications of the results – before as well as after they are gathered. This is showing appropriate respect for people and their culture, but it is also a way of learning and getting the opportunity to utilise a resource not available in any other way; in other words, it is a way to improve research questions as well as outcomes. To our surprise, the university as well as permitting procedures are lagging in enabling full involvement of hapū in research. For instance, there is no practice in place for Māori consultation for work on captive individuals of native species, and, more notably, there is no opportunity for a hapū to stand as the applicant for a DOC wildlife permit.

A prevailing argument used for justifying inadequate hapū consultation is lacking knowledge of who to contact. This should not need to be a limiting factor. Locating the closest marae to the area of research interest is likely the best and easiest starting point. Most marae have their contact details on www.MāoriMaps.com. In addition, the marae are rarely empty; thus, knocking on the door while being in the area is another straightforward point of entry. During my doctoral research, my work has included contact with close to 25 hapū spread across the North Island. It is a time-consuming process, and I was lucky enough to have great help from my team. I strongly believe it was worth the effort, not the least since I think it serves to increase the chances that the work I have done will contribute to improved kiwi conservation.

1.1.4 Aim and scope

At the intersection of the five changes described above lies decision making about future translocations of *Apteryx*. While translocations can be efficient tools, these interventions are also risky and require extensive resources in terms of money and labour; thus, rigorous planning based on well-supported theories is paramount for assuring their success (Bouzat *et al.* 2008, IUCN/SSC 2013, Batson *et al.* 2015, Flanagan *et al.* 2018, Armstrong *et al.* 2019). This PhD thesis is the first in a series of two (the second by Angelia Hura) conducted as part of the Whakapapa of North Island Brown Kiwi research program. The overarching goal of this work is to increase our understanding of the ecology, genetics, and history of *Apteryx,* and, in particular of *A. mantelli,* to facilitate improving the guidelines for translocation by integrating a sounder scientific foundation.

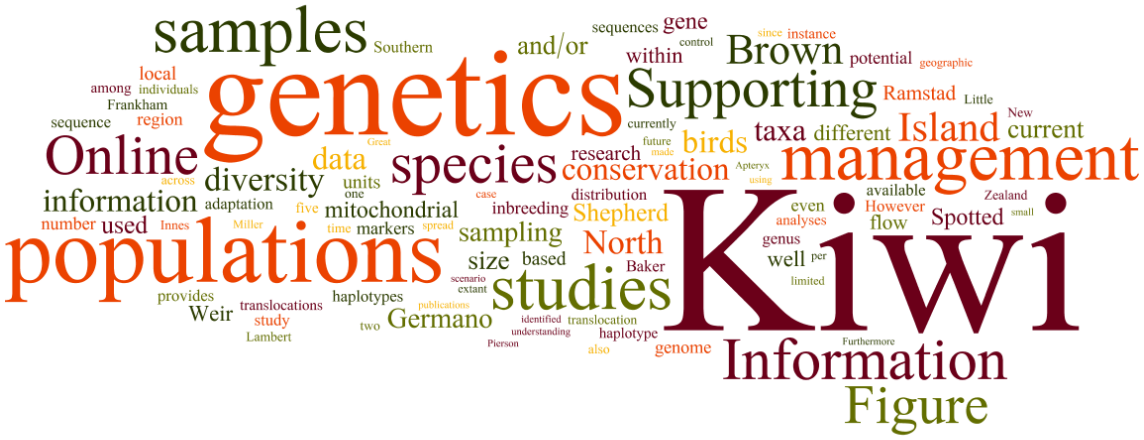
To contribute towards this goal, my thesis has four separate but aligned focal areas. First (Chapters 1.2 and 1.3), I critically review what we do and do not know, about *Apteryx* genetics and, in general, about genetic rescue, inbreeding, and outbreeding depression, to discuss how the lack of knowledge affects current conservation management and translocation policy. Second (Chapters 2.1, 2.2, & 2.3), I examine breeding strategies and mate choice in *A. mantelli* since this suite of behaviours has great implications for translocation outcomes, the risk for outbreeding depression, and the potential for genetic rescue. Third (Chapter 3.1), I attempt to elucidate what a kiwi hybrid is by taking the closest look so far at a number of populations with varying translocation histories. More specifically, my study revolves around the unintentional 'common garden' set up that was created when eight *A. mantelli* Northland from Waipoua and six *A. mantelli* Western from Little Barrier Island were translocated to Ponui Island in 1964. I compare genomic diversity and composition of this hybrid

population to its parental ancestors, as well as to one island population with a single origin and two other mainland populations – one with, and one without, a known historic bottleneck. This is done to examine whether the benefits of introducing additional genetic variation out-weighs the risk of causing outbreeding depression or homogenisation. In addition, Chapter 3.2 looks at two other hybrid populations for which admixture has happened more recently, i.e., the *A. mantelli* populations at the Pūkaha National Wildlife Centre and the Remutaka Forest Park. Before ending with a general discussion (Chapter 5.1), Part 4 deals with an important obstacle for conservation: how to prioritise among and evaluate populations. The Kiwi Recovery Group has recognised the lack of an accurate method for ageing adult *Apteryx* as a hurdle for achieving long-term sustainable management of this genus. Telomeres are the repetitive ends of all chromosomes, and telomere length has been found to correlate with age, mortality risk, life expectancy, and stress exposure in multiple bird species, which make telomeres a good candidate marker for viability on an individual as well as population level (Monaghan 2014, Smith *et al.* 2016, Monaghan *et al.* 2018, Wilbourn *et al.* 2018). To investigate if this is the case for *Apteryx*, I take the first-ever look at their telomeres.

While the topics of this thesis may appear disparate, the research conducted (lab-, field- as well as literature-based) all contributes directly towards increasing our understanding of how to best utilise translocations as a tool for *Apteryx* conservation and maximise chances of success. Thus, ultimately, my aim with this research is to contribute towards diverse and thriving *Apteryx* populations throughout Aotearoa New Zealand for many, many generations to come.

Chapter 1.2

Gaps in genetic knowledge affect conservation management of Kiwi *Apteryx* species



This Chapter has been published as: Undin, M., Hills, S. F. K., Lockhart, P. J., & Castro, I. (2021). Gaps in genetic knowledge affect conservation management of kiwi (*Apteryx*) species. Ibis doi: 10.1111/ibi.12951. All supervisors provided feedback on one or more drafts of this chapter. See further Appendix 6.

Abstract

Worldwide, there is growing appreciation of the importance of integrating genetic information into conservation management. However, there are commonly occurring problems which impact on doing this successfully. This issue is well illustrated by kiwi *Apteryx* spp. Like many endangered taxa, extant kiwi populations are small, fragmented, and isolated, raising concerns of potential inbreeding depression. Accordingly, kiwi conservation includes discussion of genetic management and translocations. To date, kiwi has been the subject of 41 genetic studies. Here we provide the first synthetic review of these studies. We conclude that despite the impressive study effort, current understanding of kiwi genetics is nevertheless insufficient to guide genetic management, predict translocation outcomes, and manage biological diversity to achieve future sustainability of the species. We explain why and outline research questions that can be addressed with new genome science technologies. Using new technologies to build on the foundation of work already undertaken, we see great opportunity to address key issues for North Island Brown Kiwi that are central to the conservation concerns for many threatened species. Specifically, we suggest that historical, disjunct, mainland populations combined with translocations of North Island Brown Kiwi, *A. mantelli*, to offshore islands have generated unique case studies to investigate the effects of fragmentation, cessation of gene flow, inbreeding, outbreeding, and hybridisation on biological diversity.

1.2.1 Introduction

With climate change, habitat destruction, and increasing isolation of small populations, the future success of many threatened species will rely on active genetic management such as augmented gene flow through reinforcement translocations (Whiteley *et al.*

2015, Pierson *et al.* 2016, Frankham *et al.* 2017). However, despite an often-stated desire to incorporate genetic information and genetic goals into conservation, only a small portion of management plans include analyses of genetic data - 50% in the USA, 11% in Europe and 12% in Australia (Pierson *et al.* 2016). When plans do include genetic information or intentions to collect such data, present and historical gene flow among populations, and its consequence for the fitness of populations, is rarely considered (Pierson *et al.* 2016).

In cases where genetic data has been available for developing management plans, this information had often been insufficient because i) the data were collected with a different research question in mind, ii) the sampling was insufficient in terms of resolution through time and space, often due to a necessarily opportunistic approach, iii) the findings lacked significance because of the small number of genetic markers available at the time, and/or iv) the planning relied on meta-analyses of different studies that used different methods and were conducted with different research aims. For instance, one review of genetic studies from the Baltic Sea identified 214 studies covering 61 species; however, the review authors considered that only seven of those studies provided sufficient genetic information for facilitating informed conservation management planning (Wennerström *et al.* 2017). Generally, genetic data are more readily available for big or otherwise charismatic species because the study of such species receives more funding which encourages more research effort (Witzenberger & Hochkirch 2011, Wennerström *et al.* 2017).

Despite this widespread complete or partial lack of essential genetic information, approximately 50% of management plans conclude that translocations are among the best options for population management (Pierson *et al.* 2016). Furthermore, policy and guidelines around genetic management and translocations of wild populations are often

based on population models whose parameters are difficult to measure and interpret (Frankham *et al.* 2014, Frankham *et al.* 2017). This situation has the potential to lead to incorrect or imprecise estimates of key parameters for models on which predictions and management actions are based. This can, in turn, affect decisions concerning the optimal number of founding individuals used in translocations, the length of time between interventions, the maximum distance between the source and target populations, and what constitutes a sustainable effective population size. Consequently, poor estimates have the potential to lead to policies, rules, and practises that do not result in successful conservation outcomes (Frankham *et al.* 2014, Innes *et al.* 2016, Dussex *et al.* 2018, Ralls *et al.* 2018).

An increasing number of authors suggest that the most promising way ahead is to obtain genome and epigenome level data (Funk *et al.* 2012, Garner *et al.* 2016, Flanagan *et al.* 2018, vonHoldt *et al.* 2018, Ramstad & Dunning 2020, Rey *et al.* 2020). Technology for genome-wide analysis has rapidly advanced (Reitzel *et al.* 2013, Leaché *et al.* 2014, Hess *et al.* 2015, Hunter *et al.* 2018, Leroy *et al.* 2018). The costs of genome analyses are also rapidly decreasing with bioinformatic pipelines becoming more readily accessible for turning genome sequences into useful information for conservation management and population monitoring (Hoffmann *et al.* 2015, Dresser *et al.* 2017, Puckett 2017, Carroll *et al.* 2018, Galla *et al.* 2020). Methods such as reduced-representation-sequencing (RRS) enable genome-wide marker-scans that have the potential to generate information beyond taxonomic relationships. This technology has provided insight into local adaptation, ecological interactions, and associations between genotype and phenotype (Elshire *et al.* 2011, Narum *et al.* 2013, Escudero *et al.* 2014, Picq *et al.* 2018, Taylor & Larson 2019). The technology can also be used to identify and develop informative, single-locus genetic markers useful for screening species and

populations (Norman *et al.* 2013, Hess *et al.* 2015, Garner *et al.* 2016, Ramstad & Dunning 2020). Genetic variants can also be associated with specific phenotypes of interest, and provide a low-cost method of monitoring these phenotypes (Voelckel *et al.* 2017, Callaway 2019).

New Zealand was without terrestrial mammals for more than 80 million years, and their arrival accompanying human settlement has severely impacted native flora and fauna. Whilst many species flourished in the past, population numbers for most native birds have been decreasing at alarming rates since the introduction of invasive, predatory mammals (Holdaway 1989, Innes *et al.* 2010, Robertson *et al.* 2016). Several native species have already gone extinct, but through intensive conservation efforts, some have been rescued from the brink of extinction. For example, the Black Robin or Kakaruia *Petroica traversi,* was reduced to five individuals including one breeding female (Merton 1992), the Little Spotted Kiwi or Kiwi Pukupuku *Apteryx owenii*, was reduced to seven individuals of which five bred (Ramstad *et al.* 2013), and the Kakapo was reduced to approximately 50 individuals (Lloyd & Powlesland 1994). The population sizes of these species have now substantially improved, although their genetic diversity appears to have been compromised (Robertson & Fraser 2009, Ramstad *et al.* 2013, Kennedy *et al.* 2014, Taylor 2014). A key to the successful rescue of these and several other species in New Zealand has been translocating individuals to locations – particularly islands – that have been freed from mammalian pests (Armstrong & Craig 1995, Miskelly & Powlesland 2013, Jamieson 2015). Translocations continue to be an important part of New Zealand conservation as a tool to increase population numbers and restore parts of the former range of species. Recently, the discussions around translocations are also increasingly focused on counteracting the loss of their genetic diversity (Germano *et al.* 2018, Robertson *et al.* 2019).

The genus *Apteryx* is endemic to New Zealand, and throughout the country, kiwi are considered taonga (treasure), one of the most respected tamariki a Tāne (children of the god Tane), and a symbol of status by the Māori people. This is evident from the high value put on kākakahu, kahu huruhuru, or kahu kiwi. The wearing of these cloaks woven from kiwi feathers remains restricted to chiefs, and some of the cloaks were considered so important that they were given individual names (Pendergrast 1984, 1987, 1997, Tamarapa 2011). Because of this high value, trade and gifting of feathers, cloaks, and even live kiwi has been common in different parts of New Zealand (see for example Hartnup *et al.* 2011). Examples include occasions of arranged marriage made to forge allegiances between iwi (tribes). The uncommon features of kiwi have more recently made them a symbol of the uniqueness of New Zealand and hence a national icon (teara.govt.nz/en/kiwi). However, despite being unusual birds, kiwi face some of the most common conservation challenges worldwide: habitat degradation, fragmentation, disrupted gene flow, small population sizes, and threats from invasive alien species (McLennan *et al.* 1996, McLennan & McCann 2002, Sales 2005, Taylor 2014). Kiwi were once a common and widespread genus across much of New Zealand (Miller & Pierce 1995, Shepherd *et al.* 2012, Germano *et al.* 2018). Currently, these birds are confined to more or less isolated habitat fragments of varying size, and population density (McLennan & Potter 1992, Miller & Pierce 1995, Pierce *et al.* 2006, Blue & Blunden 2010) and only kiwi at sites under intensive management experience population growth (Miller & Pierce 1995, Innes *et al.* 2015, Germano *et al.* 2018). Fortunately, the taonga and icon status of kiwi has brought substantial interest in their conservation.

However, there exist two competing narratives around the background of the remaining kiwi populations. One theory, hereafter referred to as the ‘pied’ scenario, suggests that

even when widely distributed, local adaptation led to differences among kiwi from distinct populations, and these divisions are tens of thousands of years old (Weir *et al.* 2016; Supplementary Figure S1.2.1). The competing theory, hereafter referred to as the 'rainbow' scenario, suggests that there was once relatively continuous distribution but that the reduced migratory capacity of kiwi compared to flying birds generated isolation-by-distance that has been exaggerated by population fragmentation, closely related to the process referred to as 'speciation-by-extinction' (Shepherd & Lambert 2008; Supplementary Figure S1.2.1). Since these scenarios have different implications for conservation, this uncertainty generates debate around the appropriate approach to kiwi management, specifically regarding whether it is desirable or potentially harmful to restore connectivity between populations.

To test the pied *versus* the rainbow hypothesis, as well as to elucidate other outstanding kiwi questions, hope has been pinned on better understanding of genetic variation. Recently, Ramstad and Dunning (2020) highlighted the potential benefits of more extensive genome-level studies of kiwi. However, the position of ratites and kiwi in the family tree of birds has been subject to lengthy debate (Prager *et al.* 1976, de Boer 1980). In addition, kiwi possess many challenging features, such as very subtle (if any) morphological differentiation between species and sexes, and all species being nocturnal, long-lived, and relatively rare (Sales 2005, Cunningham & Castro 2011, Shepherd *et al.* 2013). Consequently, kiwi genetics have already received much research attention. Thus, perhaps understandably, some voices in the kiwi conservation community argue that we should prioritise making more of the available genetic results for conservation policy development, rather than conducting additional resource- and time-consuming research.

To highlight the current limitations of our knowledge we provide here a synthesis of all genetic studies on kiwi. While this review is explicitly focused on kiwi, the insights provided are relevant for conservation of numerous other fragmented species in need of sustainable plans for management of genetic diversity. We comment on questions such as: Why have samples been collected? What questions have been asked and what markers have been used? Where and when have samples been collected? How has research effort been spread across time, space, and taxonomic units? Can results be combined to further inform conservation decision making? And if not, why? To address the latter, we analyse the biggest combinable dataset of kiwi genetic possible. Finally, we discuss why we believe that North Island Brown Kiwi could provide one of the most informative study systems for developing sustainable management strategies of fragmented biodiversity. As we explain, this is in part due to a long and well documented history of translocations combined with the existence of relatively pristine populations for comparison.

1.2.2 Materials and Methods

An exhaustive literature search was based on a keyword search for ‘kiwi’ OR ‘*Apteryx*’ AND ‘genetics’ in Google Scholar, and a manual search through reference lists and citation lists of the identified articles. Published kiwi sequences were found through searching the National Center for Biotechnology Information (NCBI) database.

1.2.2.1 Markers used, questions asked, and spread of sampling effort

The identified publications were categorised based on the aims and research question(s) addressed. In addition, information on sample size, sample localities, time of publication, and the type of molecular markers used was recorded (Supplementary Tables 1.2.1 & 1.2.2). This information was often obtained through supplied electronic resource material. The collation of these data allowed us to examine sample resolution

and how research effort had been spread geographically. In some instances, this information and clarifications were obtained from the corresponding author of research publications.

Samples were split into taxonomic groups based on the current Kiwi Management Plan (2018-2028; Germano *et al.* 2018). NZ Topo Map (www.topomap.co.nz) in combination with Google maps (www.google.se/maps/) were used to identify sampling locations and to split samples into locality groups. The number of samples from each locality group and taxon were then summed across publications as a record of the spread of invested research effort. The feathers from kahu kiwi (Māori cloaks) analysed by Hartnup *et al.* (2011) were excluded from our compilation due to uncertainty concerning the number of individuals represented by the feathers in these cloaks. Even after excluding this study, it was sometimes impossible to discount the possibility that there was resampling of the same individual birds and reuse of samples in different studies. The exception was the 16 papers reporting analyses of mitochondrial sequences, for which sample identity, species and taxon identity, and sampling locality was successfully tracked allowing to account for reuse of sampling (Supplementary Table 1.2.3).

1.2.2.2 Combining datasets and analyses of Mitochondrial DNA sequence

Although there was large variation of markers and methods used across publications, 511 individuals have been sequenced for a portion of the mitochondrial genome control region, and comparative analyses of these data provided an opportunity to obtain an overview of the relationships among kiwi from different geographic regions. Mitochondrial control region sequences from these birds were aligned and compared using the ‘map to reference’ function in Geneious 11.1.5 (Biomatters ltd), with the

complete mitochondrial North Island Brown Kiwi genome used as a reference (KU695537; Liu *et al.* 2017) for individual accession numbers for all sequences see Supplementary Table 1.2.3). The alignment was then trimmed to the longest common region (175bp of the D-loop control region, out of which 35 sites were polymorphic) with the highest sample coverage (488 samples). Distinct haplotypes were recognised if the sequences differed by at least one base pair for at least one sample and were named based on increasing dissimilarity compared to the reference sequence.

The 53 haplotypes identified, were used to generate a nation-wide mitochondrial haplotype map accounting for sample size, and a haplotype network was generated using the Median Joining Network algorithm of Bandelt *et al.* (1999) implemented in PopArt v1.7 (GitHub). Site pattern compatibility within these data were also visualized using NeighborNet (Bryant & Moulton 2004) implemented in SplitsTree4.0 (Huson & Bryant 2005). Lastly, the Kiwi Recovery Plan 2018-2028's information on the current distribution (Germano *et al.* 2018) and the authors' information about sample origin was used for comparing haplotype occurrence in present-day samples with haplotypes in historic samples and samples obtained outside the extant distribution range. Cytochrome b sequences were analysed in the same way as the D-loop control region. However, due to the smaller sample size (113 birds) and the limited additional resolution added, we focus our discussion below on the control region results. Analysed datasets are available via Dryad doi.org/10.5061/dryad.1vhhmgqrq.

1.2.3 Results

We identified 41 publications published up until June 2019 on kiwi genetics. Of these, 35 were peer reviewed articles, two were New Zealand Department of Conservation (DOC) reports, and two each were MSc and PhD theses (Supplementary Table 1.2.1).

The sample size of the studies ranged from one to several hundred birds, or thousands of feathers (Supplementary Tables 1.2.1 & 1.2.2; Hartnup *et al.* 2011).

1.2.3.1 Markers used and questions asked

Molecular markers used to study kiwi have included allozymes (Powlesland 1988, Baker *et al.* 1995, Herbert & Daugherty 2002), mitochondrial DNA sequences (e.g., Cooper *et al.* 1992, Baker *et al.* 1995, Phillips *et al.* 2009, White *et al.* 2018; see further Supplementary Table 1.2.1), nuclear microsatellites (Shepherd & Lambert 2006, Jensen *et al.* 2008, Ramstad *et al.* 2010, Ziesemann 2011, Ramstad *et al.* 2013, Taylor 2014, Ramón-Laca *et al.* 2018; Figure 1.2.1), and whole genomes (Le Duc *et al.* 2015, Sackton *et al.* 2019). A small number of studies have also investigated genetic variation in particular, targeted nuclear genes (Binney 2007, Hackett *et al.* 2008, Harshman *et al.* 2008, Miller *et al.* 2011a, Haddrath & Baker 2012, Grueber & Jamieson 2013, Grueber *et al.* 2015), and one published study has obtained genotype-by-sequencing (GBS) data (Weir *et al.* 2016; Figure 1.2.1). The first characterisation of the kiwi genome was made in 1980 when de Boer (1980) mapped the karyogram of Southern Brown Kiwi or Tokoeka *A. australis*. Since then, three species have had their mitochondrial genome sequenced, Great Spotted Kiwi or Roroa *A. haastii* (Haddrath & Baker 2001), Little Spotted Kiwi (Crimp 2010) and North Island Brown Kiwi (Liu *et al.* 2017); and four species have had their entire genome sequenced, North Island Brown Kiwi (Le Duc *et al.* 2015), Great Spotted Kiwi, Little Spotted Kiwi and Okarito Kiwi or Rowi *A. rowi* (Sackton *et al.* 2019).

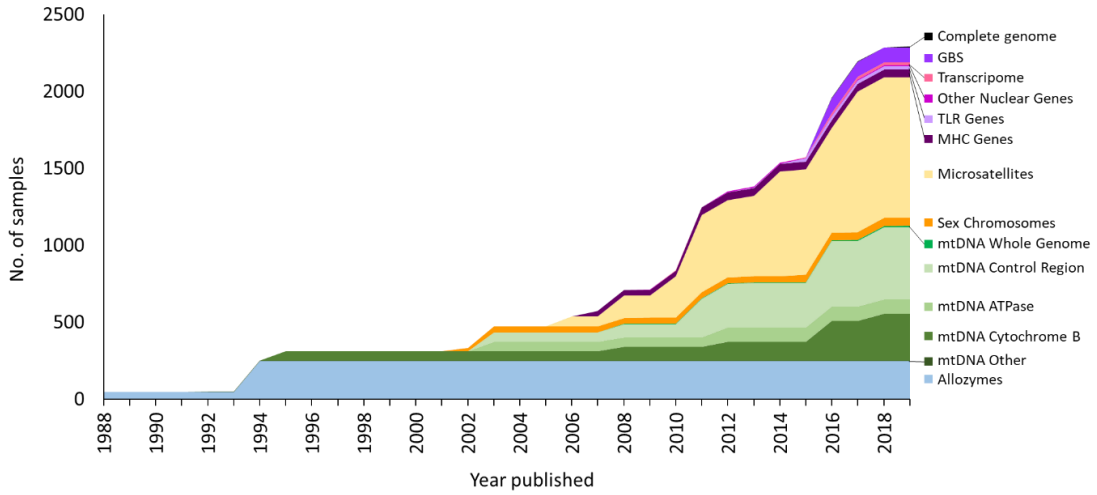


Figure 1.2.1. Stacked area graph showing cumulative sample size and molecular marker types used across all 41 publications on kiwi genetics to date.

The research questions addressed by the studies can be summarized under three categories: applied studies addressing specific research questions of kiwi biology, phylogenetic studies exploring origins and evolutionary relationships, and studies assessing the genetic diversity of kiwi (Supplementary Table 1.2.1). Among the applied studies, several were devoted to the development of a reliable method to sex juvenile kiwi (Huynen *et al.* 2002, Huynen *et al.* 2003, Dawson *et al.* 2015, Ramón-Laca *et al.* 2018). Other applied studies have suggested that North Island Brown Kiwi are probably colour blind (Le Duc *et al.* 2015), and not as monogamous as previously thought (Ziesemann 2011, Vieco-Gálvez 2019).

Phylogenetic studies of kiwi have focused on resolving the relationships among the ratites or Palaeognathae, and the position of this early diverging, monophyletic group within Aves. For a long time, the prevailing theory was that phylogenetic divergence of the ratite genera should be strongly correlated with geographic distribution because the loss of flight was thought to have happened only once within the group (Wiley 1988). The first genetic phylogeny immediately questioned this theory, and a key finding was

that the two ratite groups in New Zealand – kiwi, and moa, Dinornithiformes – are not sister clades (Cooper *et al.* 1992). The independent origin of kiwi and moa was later confirmed by studies including the entire mitochondrial (mt) genome from two moa species alongside the mt genomes of extant ratites (Cooper *et al.* 2001, Haddrath & Baker 2001), as well as by phylogenies based on nuclear markers (Hackett *et al.* 2008, Harshman *et al.* 2008, Haddrath & Baker 2012). Mitchell *et al.* (2014) were finally able to include a sufficiently long sequence of mtDNA from elephant bird, Aepyornithidae, in their analyses to suggest that this extinct taxon from Madagascar is a sister group to kiwi, and this has seemingly put an end to the vicariance hypothesis of ratite diversification (Figure 1.2.2).

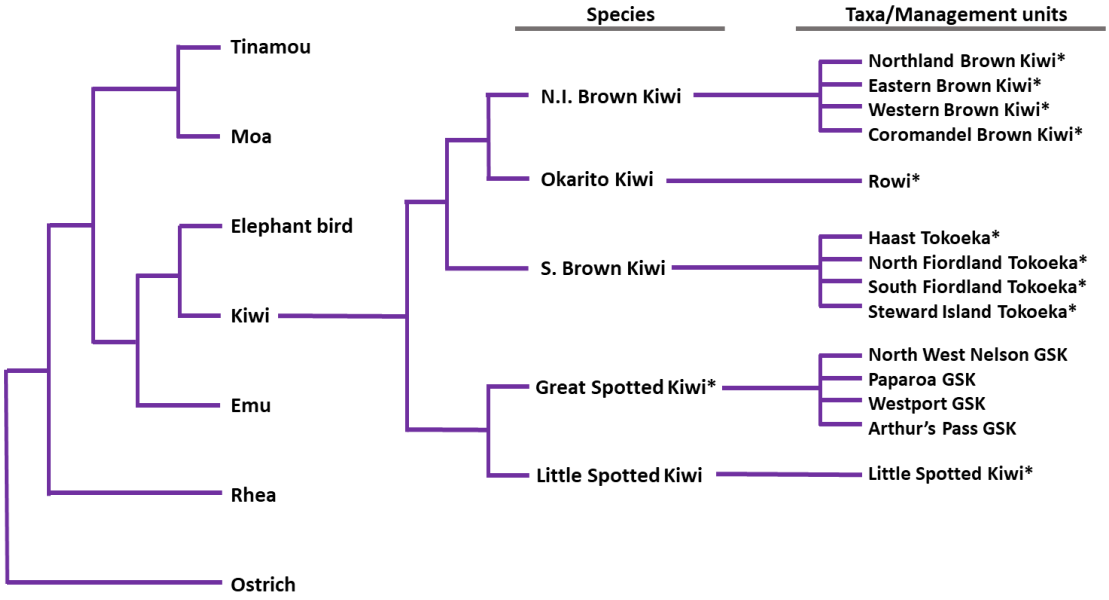


Figure 1.2.2. Schematic illustration of phylogenetic relationships between the five extant and two extinct groups of ratites (Mitchell *et al.* 2014) as well as the five extant species of kiwi (Burbidge *et al.* 2003). The 14 currently recognised management units – also referred to as taxa – within kiwi are shown (Germano *et al.* 2018). Branch lengths do not indicate genetic distance or divergence time in this illustration. * indicate the 11 taxa described by Weir *et al.* (2016).

Concurrently with the phylogenetic examinations at higher taxonomic levels within Aves and the ratites, genus-level studies have also changed the view of historical relationships within *Apteryx*. Thanks to these studies, the kiwi is currently recognized as consisting of five extant species: three species of brown kiwi known as Southern Brown Kiwi, North Island Brown Kiwi, and Okarito Kiwi, and two species of spotted kiwi known as Great Spotted Kiwi, and Little Spotted Kiwi. In addition, five to six extinct lineages within the genus have been identified but the taxonomic level of these lineages has not been determined (Shepherd *et al.* 2012, Weir *et al.* 2016). Since the entire extant Little Spotted Kiwi population of about 1800 birds stem from as few as five breeding founders (Ramstad *et al.* 2013), most studies concerning kiwi genetic diversity and inbreeding have focused on this species (Supplementary Table 1.2.1; Ramstad *et al.* 2010, Miller *et al.* 2011a, Shepherd *et al.* 2012, Taylor 2014). Such studies have reported strong evidence of inbreeding depression, traces of several historic bottlenecks, and exceptionally low diversity in, for instance, the major histocompatibility complex (MHC) genes (Ramstad *et al.* 2010, Miller *et al.* 2011a, Taylor 2014).

The latest species added to the *Apteryx* genus was Okarito Kiwi which was not genetically verified until 2003 (Burbidge *et al.* 2003, Tennyson *et al.* 2003). Interestingly, despite currently being restricted to the South Island, Okarito Kiwi once inhabited the southern part of the North Island and seem more closely related to North Island than Southern Brown Kiwi suggesting that the brown kiwi clade originated on the South Island and later expanded its range north (Figure 1.2.2; Baker *et al.* 1995, Burbidge *et al.* 2003, Shepherd & Lambert 2008, Weir *et al.* 2016). Furthermore, ever since the first pilot studies of kiwi genetics in the 1980s (Powlesland 1988), allozymes (Baker *et al.* 1995, Herbert & Daugherty 2002), mtDNA (Baker *et al.* 1995, Burbidge *et al.* 2003, Shepherd & Lambert 2008, Weir *et al.* 2016), and GBS data (Weir *et al.* 2016)

have all indicated striking diversity between geographic areas where kiwi are found. These studies suggest that even seemingly small natural barriers, such as rivers, have left noticeable genetic traces, confirming the relatively low dispersal tendency or capability of kiwi, making these flightless birds more like mammals than other genera in Aves (Burbidge *et al.* 2003, White *et al.* 2018, Brunke *et al.* 2019).

Partly thanks to these observations of diversity and apparent gene flow barriers, a discussion has been ongoing for decades about the most appropriate way to separate kiwi into species, subspecies, and taxa, as well as conservation management units (Butler & McLennan 1990, Robertson 1996, Holzapfel *et al.* 2008, Germano *et al.* 2018). The most recent and extensive phylogenetic study supports a split into five species and a further split of North Island Brown Kiwi into *A. m.* Northland, *A. m.* Western, *A. m.* Coromandel and *A. m.* Eastern, and of Southern Brown Kiwi into *A. a.* South Fiordland, *A. a.* North Fiordland, *A. a.* Haast and *A. a.* Stewart Island respectively resulting in a total of eleven lineages (Figure 1.2.2; Weir *et al.* 2016). This splitting had already received acceptance by the New Zealand Department of Conservation (DOC) in 2008 (Holzapfel *et al.* 2008). Subsequently, more detailed study of the Southern Brown Kiwi identified landscape-level barriers to gene flow (Weir *et al.* 2016, White *et al.* 2018). This resulted in a splitting of Great Spotted Kiwi into four lineages based solely on the identification of geographic features deemed likely to pose comparable barriers to gene flow as seen in Southern Brown Kiwi (McLennan & McCann 2002). However, to date, no genetic studies have been conducted to further test this inferred population structure within Great Spotted Kiwi.

In total there are thus 14 identified lineages within the *Apteryx* genus (Figure 1.2.2). In management publications, each lineage is referred to as a ‘taxon’, and each of these 14 taxa is currently recognised as a separate management unit (Robertson & Colbourne

2017, Germano *et al.* 2018). The current management guidelines strictly advise against any admixture between the management units in the wild as well as in captivity (Germano *et al.* 2018). This strict policy has, for instance, resulted in some kiwi populations being considered unwanted hybrids excluded from further conservation interventions. In particular, these birds are hybrid offspring resulting from historic translocations of birds from different management units (Innes *et al.* 2015, Germano *et al.* 2018). Furthermore, some North Island Brown Kiwi populations as close as 50 km apart have been distinguished genetically based on allozymes (Powlesland 1988). Rightly or wrongly, this has been interpreted as evidence for local adaptation and used to argue that translocations should be restricted to occurring between populations separated by less than 50 km even within management units, especially in Northland.

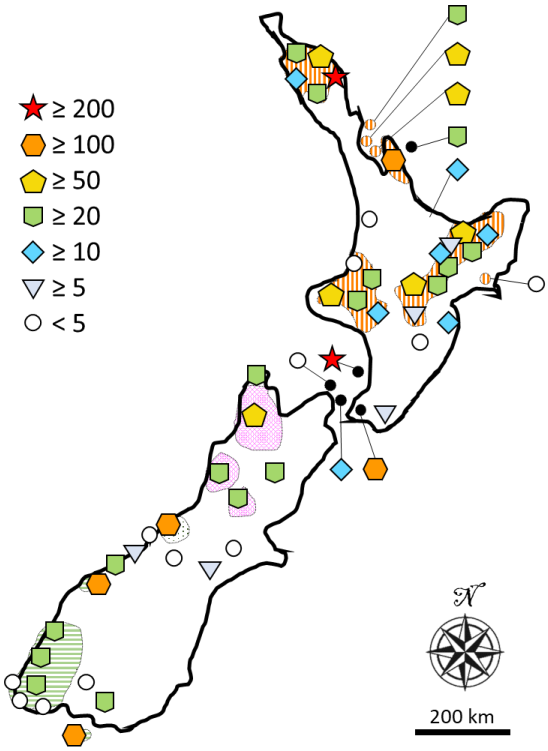
Weir *et al.* (2016) investigated the role of glaciation in the diversification of New Zealand fauna using kiwi as an example. They did not hold that lineages they identified should be considered distinct management units. On the contrary, they stated that 'it is unlikely that all of these kiwi lineages represent distinct biological species' and they highlighted the need to investigate phenotypic differences among lineages such as vocalisation and olfaction, and whether any differences could be linked to fitness. More recently, Dussex *et al.* (2018) raised concern about potential misinterpretation by others of the results presented by Weir *et al.* (2016). These authors highlighted the need to investigate how observed genetic differences between populations relate to phenotype, local adaptation, reproductive isolation, and incompatibility between taxa.

Our examination of genetic studies of kiwi confirms that distinct management units have consistently been associated with distinct taxa based on the argument that differences between them represent local adaptation. However, there is yet little evidence for this suggestion. As we outline in the next section, limitations in the extent

and geographic distribution of past sampling efforts have resulted in missing information that is crucial for improving the delimitation of management units, as well as our understanding of local adaptation.

1.2.3.2 Spread of sampling effort

Resampling of birds and reuse of samples between publications combined with incomplete data on sample origin made our quantification of the research effort across the 41 identified studies more challenging. Total sample size across all studies indicates that a maximum of 2559 birds have been the subject of study (Figures 1.2.1 & 1.2.3; Supplementary Table 1.2.2). A more conservative estimate accounting for resampling is a total sample size of *ca.* 2000 individual birds. Although this number is impressive, the spread of the sampling has been very uneven both geographically and taxonomically (Figure 1.2.3; Supplementary Figure S1.2.2). Even in the most extensively sampled taxon, North Island Brown Kiwi Eastern, we found that only four populations were represented with a sample size of eight or more birds, and for other taxa, sample size and resolution have been even less optimal (Supplementary Figure S1.2.2b). We also found that there has been a strong bias towards sampling from facilities used for egg and chick rearing undertaken as part of the national Operation Nest Egg (ONE), and Kohanga Kiwi programs, respectively (such as Rainbow Springs in Rotorua; Colbourne *et al.* 2005, Innes *et al.* 2016; Supplementary Table 1.2.3). Since this form of intensive management is unevenly spread thought New Zealand, this bias means that some populations under other or no management are underrepresented in genetic studies.



	Population est. in 2018
Little Spotted Kiwi	1 900
Great Spotted Kiwi	14 000
North Island Brown Kiwi	25 100
Northland	8 600
Coromandel	1 900
Eastern	7 000
Western	7 600
Rowi	600
Tokoeka	24 850
Haast	450
N. Fiordland	8 200
S. Fiordland	3 900
Rakiura	12 300

Figure 1.2.3. Geographic and taxonomic spread of the sampling and research effort invested across the 41 reviewed studies of *Apteryx* genetics. Sampling has covered most of the current (patterned shading; Germano *et al.* 2018) and some of the historic distribution of kiwi but the spread has been uneven. Polygons indicate general sampling locations, and the shape of the polygon represents the sum of the number of samples per location across publications, from less than five (circle) to over 200 (star; which is the case only for Kapiti Island and the Whangarei area). Polygons are also shaded in a heat map fashion. Note that these numbers are not equal to the number of birds sampled since samples and birds have in some cases been reused between publications. The sampling of some smaller populations has been indicated by lines to allow discernment of sampling from currently extant and locally extinct populations. This map is based on 2 280 samples, the remaining 279 samples utilised for kiwi genetic studies (spread across multiple publications) were of unspecified origin and are thus not included (154 North Island Brown Kiwi, 33 Southern Brown Kiwi, 6 Okarito Kiwi, 44 Little Spotted Kiwi, and 42 Great Spotted Kiwi; see Supplementary Table 1.2.2).

1.2.3.3 Combining and interpreting datasets

None of the studies conducted provided a dataset that could be utilised to test the 'pied' and the 'rainbow' hypothesis for kiwi population history. Thus, we explored the potential for compiling studies to produce a dataset that would be suitable. However, the large differences in methods and markers used between studies mean that the datasets and published results cannot easily be combined into a meta-analysis. The largest number of birds have been analysed using microsatellites (Figure 1.2.1). However, the same satellites have not been used in all these studies. The second most used marker has been the mitochondrial control region (Figure 1.2.1). We collated sequence information from all 511 kiwi sequenced for any mitochondrial DNA region into a multiple sequence alignment combined with a database of sample meta-data (Supplementary Table 1.2.3). Due to slight differences in which part of the control sequence was analysed the largest comparable kiwi genetics dataset was found to consist of 488 birds, representing all five species and thirteen of the fourteen management units. For this subset, we analysed the longest shared region which was found to be a 175 bp section of the mitochondrial D-loop control region containing 35 variable sites (Figure 1.2.4; Supplementary Figures S1.2.3 & S1.2.4).

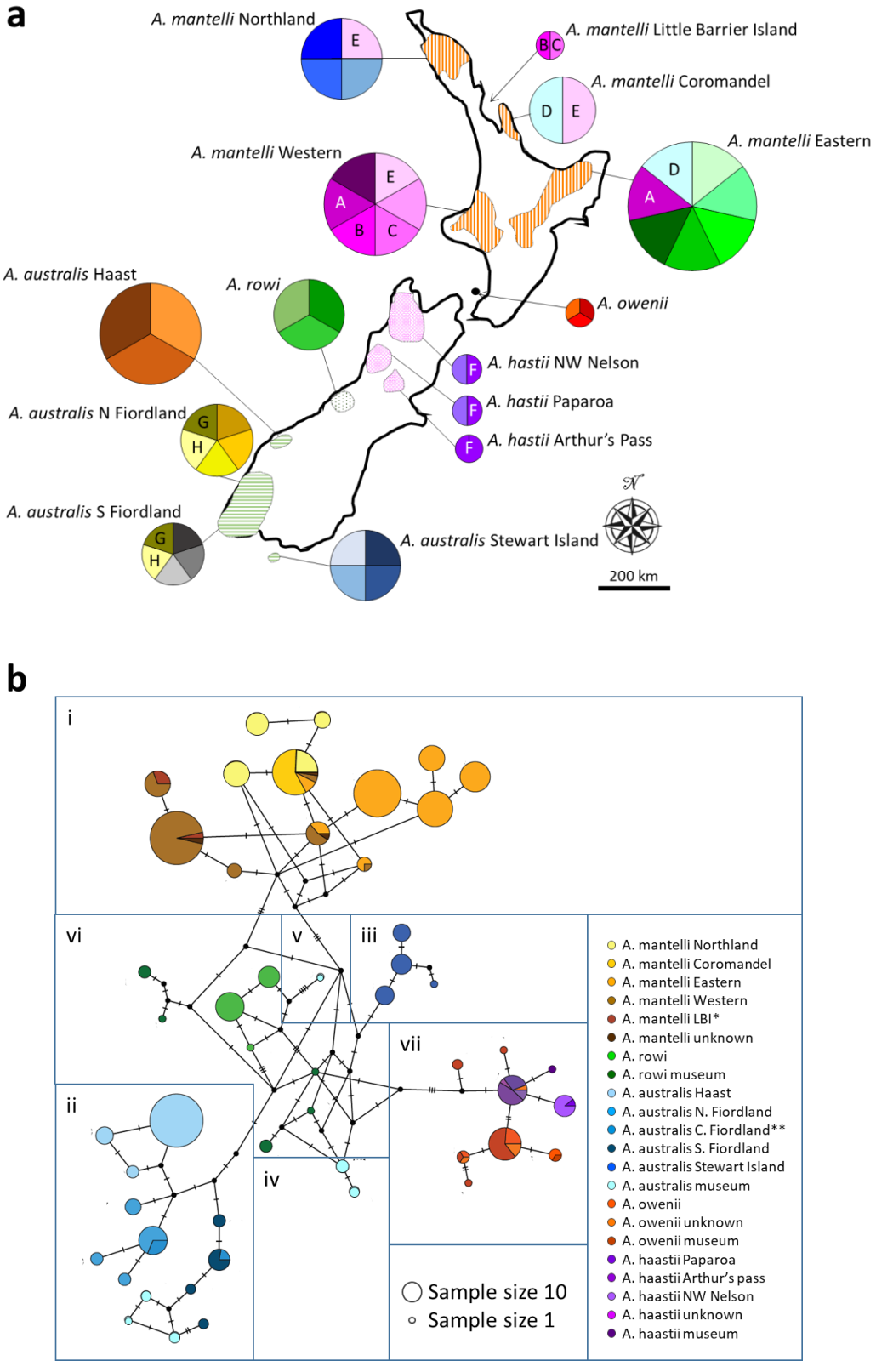


Figure 1.2.4. Map of New Zealand showing the distribution of, and relationships between, mitochondrial haplotypes (sequence versions) based on a 175 bp sequence of the kiwi mitochondrial control region. (a) Illustrates presence (but not relative frequency) and

geographical distributions of the haplotypes. Only samples from extant taxa were included (compare Supplementary Table 1.2.3) and all 14 taxa except Great Spotted Kiwi Westport are represented. Little Barrier Island (LBI) is kept separate since the taxonomic status of these birds remains unclear (Colbourne 2005). Size of pie charts represents sample size. Each pie piece represents a taxon-unique haplotype except when letters indicate shared haplotypes. (b) Haplotype network of the 53 haplotypes identified across all 488 samples. This analysis is for the largest dataset combined from kiwi genetic studies to date. Each pie represents one haplotype, pie size represents relative number of samples, and colours represent management unit in which they have been found i.e., multi-coloured pies represent shared haplotypes equivalent to the shared letter is panel a. The network has been divided into boxes for clarity and illustrates that this short mitochondrial fragment does not provide enough information to resolve important relationships within this genus despite the large sample size, not on the species or the management unit level. North Island Brown Kiwi resolves as a separate cluster (i), but the four management units within this species share haplotypes. Most of the Southern Brown Kiwi samples form a resolved cluster (ii), except for the Stewart Island samples (iii) and some of the museum samples (iv and v) which form a partly unresolved grouping together with the Okarito Brown Kiwi samples (vi). Great Spotted Kiwi and Little Spotted Kiwi cluster together but do not resolve into two separate species (vii). 'Museum' refers to haplotypes only identified in museum samples, some of which represent areas where kiwi is now locally extinct. * = Little Barrier Island, ** = Central Fiordland, neither of these are established management units, but the identity of these birds in remains under debate. Nodes and intersecting short lines represent mutational steps (or theoretical haplotypes) that are not represented in the sample set. Branch lengths are arbitrary and do not indicate genetic distance.

A haplotype network constructed with these data could not resolve the taxonomy within kiwi and failed to separate the two species of spotted kiwi (Figure 1.2.4b). Furthermore, NeighborNet analysis showed that there was significant incompatibility of site patterns for this haplotype data (Supplementary Figure S1.2.3). This observation cautions against overinterpretation since the incompatibility suggests multiple alternative ways to interpret the relationships among the sequences and taxa. Eight haplotypes are shared among different management units (Figure 1.2.4). However, it is not clear whether theses shared haplotypes are remaining variants ancestral to the formation of distinct

populations (Joly *et al.* 2009), explained by more recent gene flow, or for that matter represent errors in annotation of the location details. There are also limitations to analysing sequences of such short length (Hudson & Turelli 2003, Zink & Barrowclough 2008, Jeffries *et al.* 2016, Hodel *et al.* 2017), and concerns about overinterpretation of phylogenetic estimates when only a small proportion of genomes (mitochondrial and nuclear) are analysed. For instance, nuclear microsatellites, mtDNA sequence polymorphisms, allozymes, and restriction endonuclease digestion patterns, can all experience noticeable differences in coalescent time, meaning that dating the divergence times of populations can be affected by what sections of the genome is used for the analyses (Allendorf *et al.* 2010, Putman & Carbone 2014).

An informative but concerning finding from the collated mitochondrial dataset was that of the 53 haplotypes found, 16 haplotypes have only been identified in museum specimens or in samples from areas where kiwi no longer exist (Supplementary Figure S1.2.4; Shepherd & Lambert 2008, Shepherd *et al.* 2012). Many of these historic samples are of unknown age, but they originate from a span from 40 to at least 4000 years ago (Shepherd & Lambert 2008, Shepherd *et al.* 2012). This temporal pattern of haplotypes is a likely indication of that substantial kiwi diversity that has already been lost. Another interesting observation was that our compilation found support for one of the most surprising results reported in the Weir *et al.* (2016) study, namely a split between Northern and a Southern Fiordland Southern Brown Kiwi. Weir *et al.* (2016) analysed a longer (1 710 bp) mtDNA sequence combining mitochondrial D-loop control region and Cytochrome b region, but their sample size was limited to 28 birds. Our analyses of all 43 Fiordland kiwi with available published sequence for mitochondrial D-loop control region supports this split and confirms that individuals with haplotypes from both taxa live in sympatry in the central parts of Fiordland (Figure 1.2.5).

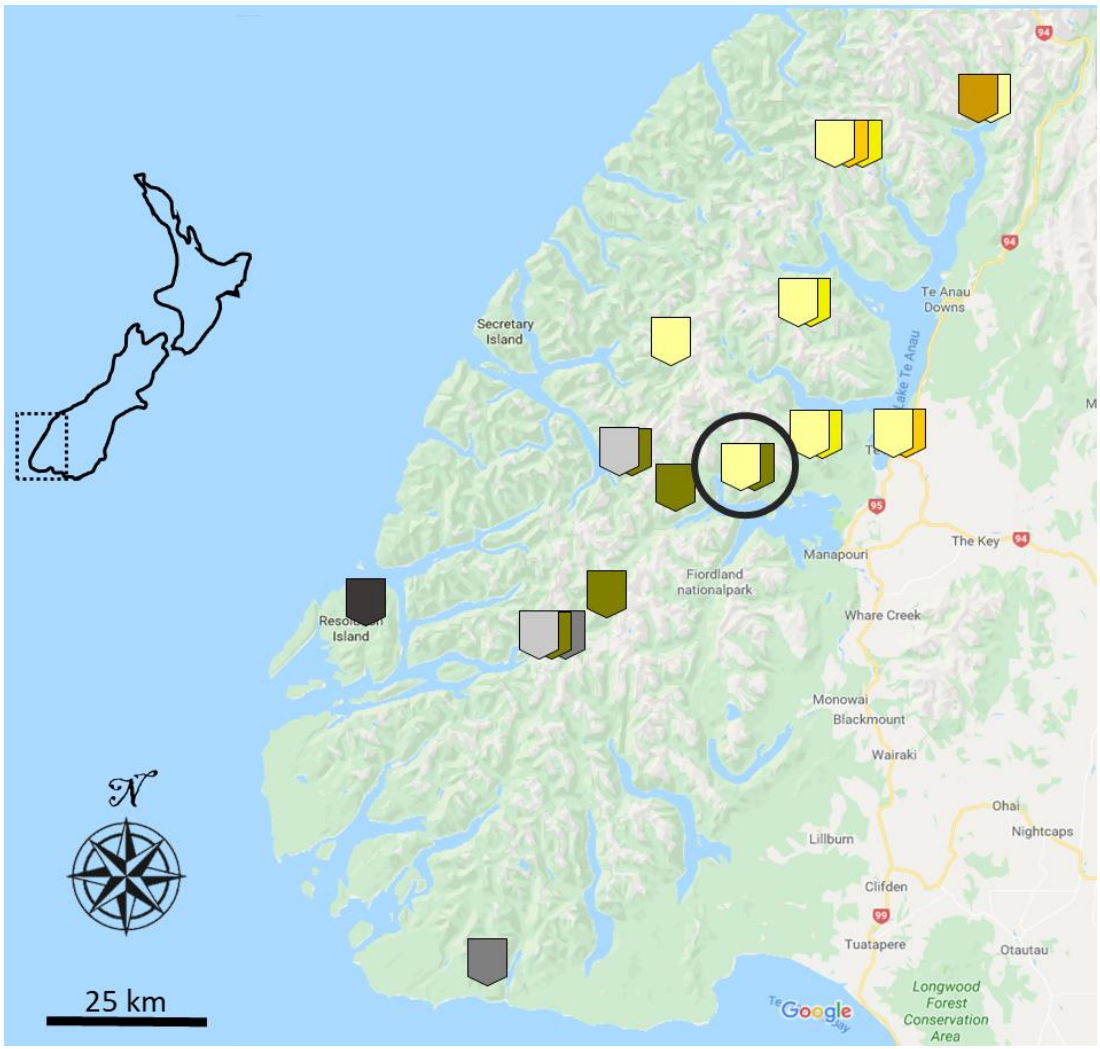


Figure 1.2.5. Map of Fiordland showing the distribution of Southern brown kiwi mitochondrial haplotypes in this area (*n* = 43). Each colour represents a different haplotype (mitochondrial sequence variant). Despite the larger sample size, these results are consistent with those presented by Weir *et al.* (2016; n = 28) and hence with a separation of a Northern and a Southern lineage in this area. The exception to this separation is apparent intermixture at one location highlighted with a circle. Inset indicates the location of Fiordland within New Zealand. Background map from google.com/maps.

1.2.4 Discussion

Collectively, genetic research over the last 35 years has brought substantial insight into the ecology, phylogeny, and physiology of kiwi. However, despite more than 40 studies conducted with over 2000 kiwi sampled, the knowledge gained is still insufficient to enable reliable predictions of conservation action outcomes and population success. Our review highlights that, while published findings do indicate that kiwi differ genetically between areas, there is little understanding of the extent of local adaptation, or of inbreeding and outbreeding depression in populations. A test of the 'pied' versus the 'rainbow' scenario cannot be conducted and further conclusions about the nature of the differences cannot currently be drawn based on available data. Similar to the findings of Wennerström *et al.* (2017), who reviewed genetic studies conducted in the Baltic Sea, these limitations are a consequence of 1) data having been collected with different research questions in mind, 2) sampling resolution in time and space having been suboptimal - often due to necessarily opportunistic sampling - and 3) studies having used genetic markers available and affordable at the time. Whilst this should not be seen as a criticism of past research efforts – opportunistic sampling regimes are commonly employed for work on threatened species since this is often the only feasible way of gathering samples (Callaghan *et al.* 2020, Turvey *et al.* 2020) – a concern for the conservation of kiwi in New Zealand is that available genetic data has been used to justify some management actions despite being insufficient for this purpose (Innes *et al.* 2015, Innes *et al.* 2016, Kiwis for Kiwi 2016, Germano *et al.* 2018).

Regardless of which narrative best describes the population history of kiwi, a large proportion of kiwi individuals are confined to more or less isolated populations with limited potential for external gene flow (Potter 1990, Miller & Pierce 1995, Germano *et al.* 2018). This situation will expose populations to the effects of genetic drift and

inbreeding, which will lead to loss of genetic diversity and can generate genetic differences among kiwi populations not linked to local adaptation (Lacy 1987, Michaelides *et al.* 2014, Frankham *et al.* 2017). In such a situation, managing disjunct populations separately might be counterproductive since this could further reduce the adaptive potential of these populations and their ability to cope with ongoing environmental change and habitat loss (Mace & Purvis 2008, Shepherd & Lambert 2008, Frankham *et al.* 2011, Li *et al.* 2015, Weeks *et al.* 2016). Improving our knowledge of the adaptive differences of populations is clearly important, as is further discussion concerning augmentation of gene flow through translocations. We argue that testing the pied vs the rainbow scenario should be a priority in future research since this will help determine the importance of adaptation in the differentiation of kiwi populations. This knowledge is key for future sustainable management and will, for instance, be crucial for deciding what constitutes appropriate source and target populations for translocations.

A common concern among conservation practitioners is that translocation risks disturbing local adaptation (Allendorf *et al.* 2001, Love Stowell *et al.* 2017, Ralls *et al.* 2020). The observation that there are several examples of successful kiwi populations that have resulted from multi-origin and long-distance translocations could suggest that local adaptation plays a relatively small role in the genetic differentiation of kiwi taxa. Regardless of this, even in cases where genetic differences between populations are adaptive, restoring connectivity between them might still be beneficial if translocations and admixture lead to overall increased diversity, or to beneficial introgression (i.e., incorporation) of adaptive gene variants (Grant & Grant 2014, Schmickl *et al.* 2017, Burgarella *et al.* 2019). In addition, some authors argue that selection forces that generate local adaptation should also be sufficient to prevent unwanted genetic

homogenization in cases where habitats remain relatively intact (Hendry *et al.* 2007, Todesco *et al.* 2016). Evaluation of this hypothesis is needed and important. Measures of isolation-by-distance, which indicate increasing genetic differences with geographic distance, have received much attention in the kiwi conservation community (Germano *et al.* 2018, White *et al.* 2018). This phenomenon is explained by limited dispersal and gene flow between populations, but it does not provide evidence for local adaptation (Slatkin 1993). Nevertheless, observations of isolation-by-distance for continuous as well as discrete populations have commonly been used to argue that populations should be managed as separate conservation units (Wright *et al.* 2015). For ground-dwelling birds such as kiwi that have limited capacity for dispersal, we suggest that it is inappropriate to interpret relationships between genetic and geographic distances in this way.

Evidence for local adaptation of kiwi populations might be obtained in several ways, and application of newer genetic technologies may prove insightful. Useful data are likely to be obtained with whole-genome sequencing or reduced-representation-sequencing (RRS; such as GBS or RADseq; Elshire *et al.* 2011, Etter *et al.* 2012), and variant protocol that provides information on methylated sites (van Gurp *et al.* 2016). With RRS technology, tens of thousands of loci across the genome can be examined, enabling analyses of gene regions subject to both purifying and diversifying selection directly linked to local adaptation and gene-environment associations (Narum *et al.* 2013, Gaughran *et al.* 2017, Richmond *et al.* 2017, Picq *et al.* 2018). Thanks to this resolution, RRS studies hold promise to not only finally test the 'pied' and the 'rainbow' hypotheses, but they could also be used to verify the current split into management units, identify suitable source populations for translocations (especially for successful genetic rescue), and shed light on the evolutionary and ecological

significance of the growing number of reported kiwi hybrids (Herbert & Daugherty 2002, Burbidge *et al.* 2003, Shepherd *et al.* 2012, Narum *et al.* 2013, Wennerström *et al.* 2017, Picq *et al.* 2018). Some initial work with RRS (specifically GBS) has been undertaken on kiwi (Weir *et al.* 2016). However, this study relied on somewhat opportunistic sampling and sample size per area was low (n = 1-7 per population except for 10 Southern Brown Kiwi Stewart Island and 14 Okarito Kiwi) resulting in relatively high confidence for higher order taxonomic splits but leaving questions about diversity within taxa (and thus current management units) unanswered.

Direct use of monitoring based on genomic methods is still largely confined to species of high commercial value, but cases from conservation management are emerging (Garner *et al.* 2016). Four examples are conservation of the Tiger *Panthera tigris tigris* (Natesh *et al.* 2017), the Southern Emu-wren *Stipiturus malachurus* (Bradford *et al.* 2018), the Pacific Lamprey *Entosphenus tridentatus* (Hess *et al.* 2013, Hess *et al.* 2015) and the Tasmanian Devil *Sarcophilus harrisii* (Miller *et al.* 2011b, Hogg *et al.* 2017). In New Zealand, the recovery groups for the critically endangered Kakapo, or Kākāpō *Strigops habroptilus* (through the Kākāpō125+ sequencing project) and Black Stilt or Kakī *Himantopus novaezelandiae* (Galla *et al.* 2019, Galla *et al.* 2020) are leading in this field. A further case study with great potential for providing conservation insights for fragmented species in need of sustainable conservation genetic management would be North Island Brown Kiwi.

In contrast to many fragmented threatened species, North Island Brown Kiwi offers the opportunity to compare the genetic diversity of restricted island populations with known founder population size, time, and provenance to that of mainland populations of various sizes, degrees of isolation, management regimes, and geographic origin. Such comparisons would enable the development of a landscape genomic model of current

and historic gene flow as well as facilitate analyses of segregating genetic loci indicative of selection and local adaptation (Storfer *et al.* 2018). Also of great interest would be to examine and compare the epigenetic diversity of these populations in order to further link genotype and phenotype to environmental factors and thus to local adaptation (Hochholdinger & Baldauf 2018, Miryeganeh & Saze 2019, Rey *et al.* 2020). Furthermore, the targeted sampling of known hybrid kiwi populations or individuals and comparison of these to their parental populations has great potential for investigations into the relative contributions of allelic variation and epigenetic regulation to inbreeding depression, loss of local adaptation (or outbreeding depression), and genetic rescue (or heterosis; Garner *et al.* 2016, Flanagan *et al.* 2018, vonHoldt *et al.* 2018, Ramstad & Dunning 2020, Rey *et al.* 2020).

We emphasize that the success of such future study requires an experimental design and sampling regime that focus on a more representative geographical spread of sampling. This must consider genetic variation both within and between populations and capture present and historical gene flow (Funk *et al.* 2012, Wright *et al.* 2015, Garner *et al.* 2016, Wennerström *et al.* 2017, Flanagan *et al.* 2018). Collecting samples from a maximum number of populations within each taxon, rather than maximising sample size *per se*, would likely prove more important for these studies (Landguth & Schwartz 2014). However, studying evidence for local adaptation could be approached in different ways and determining what constitutes appropriate sample size and spread will vary depending on the methods used and the exact hypotheses tested. For example, identifying local adaptations as indicated by outlier allele frequencies suggestive of genes under diversifying selection, or by correlations between allele frequency and environmental factors, will require larger sample sizes (at least eight to 12 samples per site; Landguth & Schwartz 2014, Pastenes *et al.* 2017, Zhen *et al.* 2017, Homola *et al.*

2019). On the other hand, local adaptation indicated by differences in patterns of gene expression or genomic methylation profiles might require fewer samples (three or fewer depending on the method of comparative analysis; Zhang *et al.* 2018, Xin *et al.* 2019).

1.2.5 Conclusion

We have reviewed the current state of understanding of kiwi genetics. We appreciate and acknowledge that the studies conducted to date provide a foundation to advance understanding of this iconic genus in New Zealand. However, our main conclusion is that the current knowledge of kiwi genetics is still insufficient to inform conservation decisions and develop robust management plans for long-term sustainable and diverse kiwi populations. A serious deficiency is that we currently do not understand the nature and extent of the genetic differences observed among taxa and populations. This includes not only a lack of understanding about genetic variation but also lack of knowledge about the plastic expression of this variation. Improving understanding of both these factors is important as together they contribute to the biological diversity upon which selection acts.

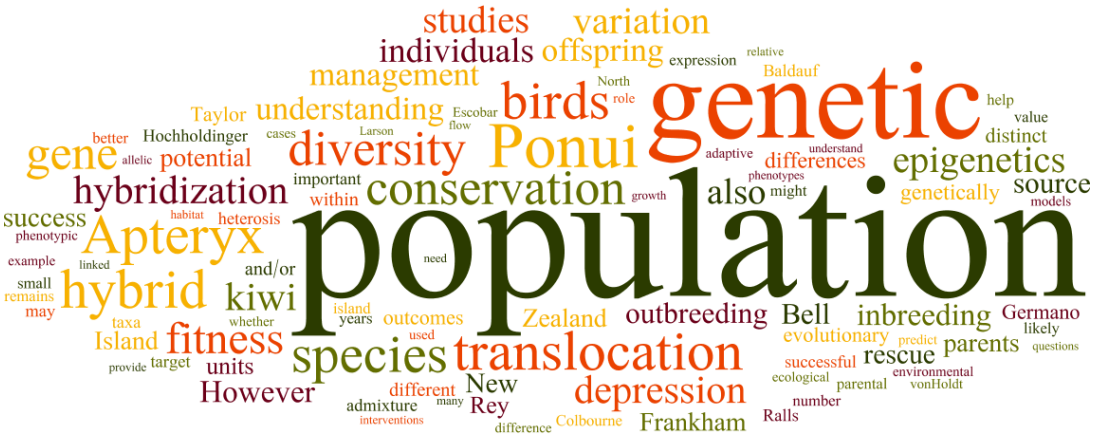
Currently, the main kiwi management strategy involves trapping or poisoning of invasive mammalian predators (Robertson *et al.* 2011, Robertson & de Monchy 2012, Innes *et al.* 2015). However, translocating adults, juveniles, or eggs to predator-free environments has also been a key strategy for all five kiwi species and translocations are currently increasing in importance (Colbourne 2005, Colbourne *et al.* 2005, Innes *et al.* 2016, Kiwis for Kiwi 2016, Germano *et al.* 2018). Predicting successful translocation outcomes that involve genetic rescue requires detailed information about the genetic and epigenetic variation of the target as well as the source populations (Groszmann *et al.* 2013, Biémont & Vieira 2014, vonHoldt *et al.* 2018, Rey *et al.* 2020). Decision making

in the absence of this information risks leading to management strategies that are insufficient or even harmful for future population sustainability. This is a situation facing conservationists worldwide. The dilemma is that there is a desire to rapidly improve population numbers of threatened species while maintaining, increasing, or restoring genetic diversity, but that sufficient genetic data to support successful management is not available.

With the North Island Brown Kiwi, there is the opportunity to advance studies on a 'scientific umbrella' species that will aid not only conservation of kiwi but also other less well funded and researched species in need of successful genetic management. Lastly, we concur with Ramstad and Dunning (2020) in emphasizing the added value of building on earlier genetic studies of kiwi with newer methods of genome science. Specifically, we suggest that studies of the North Island Brown Kiwi system would benefit from using RRS methodology, combined with sampling from as many populations as possible including those with a documented translocation history. Added benefits could be gained if the studies conducted become part of a coordinated, nationwide genetic survey of kiwi utilising a pre-defined protocol and a marker type that would facilitate future meta-analyses.

Chapter 1.3

Genetic rescue and the plight of Ponui hybrids



Contribution: This Chapter has been published as: Undin, M., Lockhart, P. J., Hills, S. F. K., & Castro, I. (2021). Genetic rescue and the plight of Ponui hybrids. Frontiers in Conservation Science 1, 10.3389/fcosc.2020.622191. All supervisors provided feedback on one or more drafts of this chapter. The expertise of Peter Lockhart was crucial for this chapter. See further Appendix 6.

Abstract

Long-term sustainable and resilient populations is a key goal of conservation. How to best achieve this is controversial. There are, for instance, polarized views concerning the fitness and conservation value of hybrid populations founded through multi-origin translocations. A classic example concerns *Apteryx* (kiwi) in New Zealand. The *A. mantelli* of Ponui Island constitute a hybrid population where the birds are highly successful in their island habitat. A key dilemma for managers is understanding the reason for this success. Are the hybrid birds of Ponui Island of 'no future conservation value' as recently asserted, or do they represent an outstanding example of genetic rescue and an important resource for future translocations? There has been a paradigm shift in scientific thinking concerning hybrids, but the ecological significance of admixed genomes remains difficult to assess. This limits what we can currently predict in conservation science. New understanding from genome science challenges the sufficiency of population genetic models to inform decision making and suggests instead that the contrasting outcomes of hybridization, 'outbreeding depression', and 'heterosis', require understanding additional factors that modulate gene and protein expression and how these factors are influenced by the environment. We discuss these findings and the investigations that might help us to better understand the birds of Ponui Island, inform conservation management of kiwi and provide insight relevant for the future survival of *Apteryx*.

1.3.1 Introduction

The arrival of humans to New Zealand and the accompanying deforestation and predation by introduced mammals have decimated many native and endemic species (Holdaway 1989, Robertson *et al.* 2016). In responding to this catastrophic

development, New Zealand conservation efforts have attracted international recognition for their bold and pioneering methods aimed at rescuing species from the brink of extinction. Most notably, strategies for translocation have influenced practices worldwide (Armstrong & McLean 1995, Armstrong & Seddon 2008). Despite this, controversy remains in New Zealand and elsewhere for how to best achieve long-term sustainable and resilient populations (Love Stowell *et al.* 2017, Ralls *et al.* 2018, vonHoldt *et al.* 2018, Ralls *et al.* 2020). Specifically, the outcome of translocations involving the mixing of individuals from genetically distinct populations remains difficult to predict and questions have been raised about the future fate and conservation value of admixed populations originating from such historic translocations.

An iconic example concerns the flightless, nocturnal genus *Apteryx*, or kiwi birds, which once were common and widespread throughout New Zealand but now are confined mainly to isolated and/or small populations relying on active management to achieve net growth (Innes *et al.* 2015, Germano *et al.* 2018). *Apteryx* face many threats such as habitat degradation, fragmentation, disrupted gene flow, and small population sizes (McLennan & Potter 1992, McLennan & McCann 2002, Germano *et al.* 2018). However, depredation by invasive mammals, in particular stoats (*Mustela erminea*), ferrets (*M. furo*), and dogs (*Canis lupus familiaris*), have been identified as the main cause of their decline (Taborsky 1988, McLennan *et al.* 1996, Innes *et al.* 2010, Germano *et al.* 2018). For example, in unmanaged populations, about 95% of *Apteryx* chicks are predated by stoats (McLennan *et al.* 1996, Innes *et al.* 2010). Consequently, *Apteryx* management focuses on trapping and poisoning mammalian predators and on artificial rearing of eggs and chicks collected from the wild under a program referred to as Operation Nest Egg (ONE; Colbourne *et al.* 2005). In addition, a program called ‘kōhanga kiwi’ involves sites where *Apteryx* pairs are held and allowed to breed in

predator-free areas and a proportion of the chicks are ‘harvested’ and used to supplement existing or establish new populations (Innes *et al.* 2016, Kiwis for Kiwi 2016). A principle of kōhanga kiwi sites is that they are founded by 40 unrelated birds. However, relatedness is inferred based on geographic origin rather than genetic testing (Innes *et al.* 2016, Kiwis for Kiwi 2016).

Apteryx genetics have received extensive research attention (Ramstad & Dunning 2020, see further Chapter 2.2). Based on findings from these studies, *Apteryx* is currently split into five extant species (Tennyson *et al.* 2003) and further into 14 management units based on observed or inferred barriers to gene flow (Powlesland 1988, Burbidge *et al.* 2003, Weir *et al.* 2016, Germano *et al.* 2018). The exploration of *Apteryx* diversity has so far focused on describing observations from a taxonomic perspective. Hence, parameters based on population genetic models have not been estimated and there is little understanding of migration between populations, inbreeding within populations, and local adaptation (Shepherd & Lambert 2008, Germano *et al.* 2018). Consequently, lack of studies of genetic variation within management units, the nature of the genetic difference between taxa, and the prevalence of in- and outbreeding, means that far-reaching *Apteryx* conservation decisions and policy are currently being implemented despite a lack of crucial information (McLennan & McCann 2002, Dussex *et al.* 2018).

One policy linked to the demarcation of 14 management units is that *Apteryx* translocations are strictly limited to movement within units (Pierce *et al.* 2006, Innes *et al.* 2016, Germano *et al.* 2018). Another is that several *Apteryx* populations, regarded as hybrid populations because they originated from translocations involving two or more units, are considered unsuitable sources as well as targets for future translocations (Herbert & Daugherty 2002, Colbourne 2005, Shepherd *et al.* 2012, Germano *et al.* 2018). One such hybrid population is present on Ponui Island. Concerned for the rapid

decline of kiwi on the North Island, in 1964 the landowner Peter Chamberlin on Ponui (Chamberlin's) Island in the Hauraki Gulf had 14 North Island brown kiwi (*Apteryx mantelli*) translocated to the island (Colbourne 2005). Six of these birds came from Hauturu-o-Toi (also known as Little Barrier Island; Colbourne 2005; Figure 1.3.1). The remaining eight birds came from the Waipoua Forest in the northernmost part of the North Island mainland (Colbourne 2005). In turn, the origin of the population on Hauturu-o-Toi was a translocation from Taranaki in the early 1900s, but anecdotal evidence suggests that additional birds of unknown sources may have been present on Hauturu-o-Toi prior to this translocation (Baker *et al.* 1995, Burbidge *et al.* 2003, Colbourne 2005; Figure 1.3.1). Because the parental populations belong to different management units, the Ponui Island birds are classified as hybrids. The initial success of the translocated birds and their Ponui Island hatched offspring is unknown, but the current high density is evidence for rapid and extensive population growth within a small number of generations. *Apteryx* typically reach sexual maturity around four years of age and live to be over 40 years old (Sales 2005, Robertson & de Monchy 2012). Consequently, in the 56 years that have passed since their introduction, the current population on Ponui Island is likely best described as a hybrid swarm where some individuals birds will be of pure Waipoua or Hauturu-o-Toi origin, others will be F1 hybrids or offspring from crossing and backcrossing between parental types and/or F1 individuals (Hwang *et al.* 2011, Hamilton & Miller 2016). While Ponui Island lacks stoats, about one third of the *A. mantelli* chicks on the island are preyed upon by feral cats (*Felis catus*) every year and ship rats (*Rattus rattus*) likely compete with chicks for food (Shapiro 2005, Dixon 2015, Strang 2018). Despite this presence of invasive mammals, the population density on Ponui Island is suggestive of a population growth rate otherwise only seen in populations in predator-free sanctuaries and/or sites where

juvenile mortality is reduced through Operation Nest Egg (Colbourne *et al.* 2005, Robertson & de Monchy 2012). The Ponui Island *Apteryx* population has been extensively studied over the past 17 years, providing ground-breaking data on habitat utilization, diet, parasite impact, disease, anatomy, social interactions, and causes of chick and egg mortality (e.g. Cunningham & Castro 2011, Ziesemann *et al.* 2011, Hiscox 2014, Wilson 2014, Dixon 2015, Reynolds *et al.* 2017, Abourachid *et al.* 2019, Bansal 2020, Vieco-Galvez *et al.* 2020). No studies have so far investigated the impact of their mixed heritage on the success of the birds on Ponui Island.

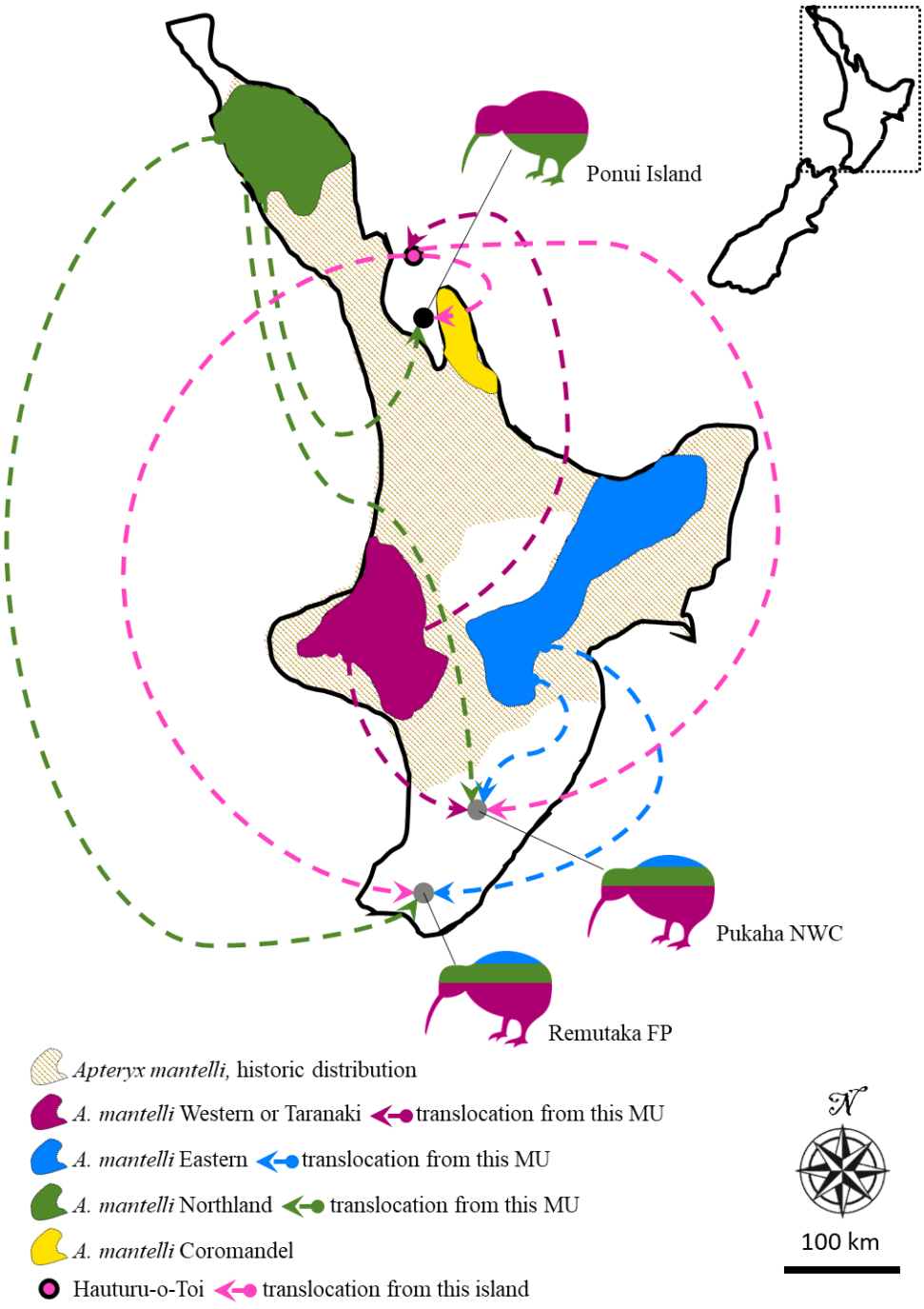


Figure 1.3.1. Map of the North Island of New Zealand illustrating the historic, as well as, current distribution of *Apteryx mantelli*, North Island brown kiwi, and how this species is further split into four geographically disjunct and genetically distinct management units (MU, or taxa; Burbidge *et al.* 2003, Weir *et al.* 2016, Germano *et al.* 2018). Arrows and kiwi silhouettes represent three separate cases where multi-origin translocations over the last 100 years have resulted in hybrid populations. Silhouette coloring roughly illustrates the proportional representation of different taxa in the founding population at each site. Translocations from Hauturu-o-Toi (also known as Little Barrier Island) are colored in a different shade of pink for

clarity and to highlight the somewhat disputed MU identity of this island. FP = Forest park. NWC = National Wildlife Centre. Inset show the location of the zoomed in map in New Zealand.

Despite the lack of studies, the mixed provenance and the untested assumption that the birds introduced to Ponui Island had limited genetic diversity, it was concluded that the Ponui Island birds 'have no genetic value whatsoever for use in restoration' (Letter to Department of Conservation and the Kiwi Recovery Group shared with Ponui Island landowners and Ponui Island Kiwi Research Team, 2016). The suggestion is that the Ponui Island birds suffer from (1) inbreeding depression resulting from the small number of founding birds and (2) outbreeding depression resulting from anthropogenically-mediated hybridization of birds deemed likely to be adapted to different local conditions (Allendorf *et al.* 2001). These potential problems have not impacted policymaking around translocations and admixture of geographically adjacent populations. On the contrary, translocations of birds within management units to boost population numbers and attempt to maintain genetic diversity remains a paramount part of ongoing *Apteryx* conservation (Kiwis for Kiwi 2016, Germano *et al.* 2018).

The plight of the Ponui Island kiwi illustrates the importance of improving our understanding of inbreeding, outbreeding depression, and the sometimes contrasting outcomes of hybridization. This understanding is also needed to realize the full potential of genetic rescue and other forms of translocation involving endangered species. In our contribution, we highlight why study of the Ponui Island birds will be informative for (1) illuminating the ecological and evolutionary significance of hybrids, (2) establishing evidence-based principles for identifying and matching source and target populations for translocations involving admixture, (3) proposing the most appropriate source(s) of individuals for repopulating areas after local extinctions, and (4) evaluating the biological relevance of *Apteryx* management units in New Zealand. We emphasize that

addressing these four questions is key for the long-term successful conservation of *Apteryx* whose populations are highly fragmented and are arguably in need of augmented gene flow given their insular New Zealand habitat. We think that if these questions could be answered for *Apteryx* in New Zealand, they may be informative for conservation practices elsewhere. We see an important role for integrating genome science in ecological studies of *Apteryx* that will help us to better understand the evolutionary significance of hybrids and their conservation value.

One reason discussion of hybridization in conservation can be problematic, and often fruitless, stems from a lack of agreement concerning the many criteria for delimiting species (Mallet 2013). The prevalence of hybridization in plant evolution and the challenges of delimiting plant species has recently led to emphasis being placed on what occurs when taxa from distinct evolutionary lines are brought together, rather than on whether or not these taxa are named as distinct species (Winkworth *et al.* 2005, Abbott *et al.* 2013, Hoffmann *et al.* 2015, Canestrelli *et al.* 2016, Chan *et al.* 2019). We adopt this approach, and this review accepts the definition of hybridization used by Canestrelli *et al.* (2016) as 'mating between individuals from genetically distinct populations that produces offspring'. We also adopt the perspective that it may not be helpful to distinguish hybridization that results from human intervention or otherwise since, for philosophical as well as practical reasons, the causes of some hybridization events are almost impossible to disentangle (Allendorf *et al.* 2001).

1.3.2 Population genetic models and predicting hybrid success

Hybridization will impact genetic diversity and plasticity, however, predicting the success of managed gene flow requires understanding not only the genetic diversity and plasticity of species, but also of the extent and directionality of the gene flow and how

admixture between genetically diverged populations and lineages affect reproductive success (Abbott *et al.* 2013). Models for the accumulative effects of particular alleles (gene variants) and overall allelic diversity in the genomes of organisms have been used since the early 1900s to help explain the relative fitness (reproductive success) of offspring when the parents are genetically similar (inbreeding) as well as genetically dissimilar (outbreeding or hybridization; Roff 2002, Wright *et al.* 2008, Hochholdinger & Baldauf 2018). The Partial Dominance hypothesis (Davenport 1908) predicts that mating between close relatives increases the number of genes in offspring where the same sub-optimal (deleterious) variant of a gene is inherited from both parents. This is said to decrease the fitness of the offspring. The Overdominance hypothesis (East 1908) proposes that it is not deleterious genes *per se*, but that an increased number of genes with the same variant inherited from both parents reduces the fitness of the offspring. These alternative explanations for inbreeding depression – reduced fitness of offspring relative to parents – are still largely unresolved (e.g. Roff 2002, Wright *et al.* 2008). While there is good reason that population genetic models based on inheritance of gene variants remain a leading explanation for heterosis and genetic rescue, as well as inbreeding and outbreeding depression, after a hundred years of empirical testing, there is now a chorus of voices questioning the sufficiency of these models (Groszmann *et al.* 2013, O'Dea *et al.* 2016, Hochholdinger & Baldauf 2018, Bell *et al.* 2019, Rey *et al.* 2020).

Heterosis or hybrid vigor is expressed as a release from inbreeding depression inferred from relatively higher fitness of offspring compared to its genetically distinct parents (Whitlock *et al.* 2000, Hochholdinger & Baldauf 2018, Bell *et al.* 2019). The superior performance of first-generation (F_1) hybrids is a well-documented phenomenon of ecological and agricultural advantage. It has evolutionary significance for adaptation of

populations and generation of novel species (Johansen-Morris & Latta 2006, Janes & Hamilton 2017, Hochholdinger & Baldauf 2018, Junaid *et al.* 2018, Koide *et al.* 2019, Miryeganeh & Saze 2019, Taylor & Larson 2019). When differences in phenotype and underlying gene expression are much greater or much less between progeny and parents than the additive difference between the parents, it is sometimes referred to as transgressive segregation (Stelkens *et al.* 2014, Koide *et al.* 2019). However, whether the mechanisms of heterosis and transgressive segregation are different, the same, or whether one is a subset of the other remains unclear. According to some definitions, the difference lies in that while transgressive segregation results in individuals that 'express trait values that fall outside the range of both parent species' in either direction (Stelkens *et al.* 2014), heterosis only refers to an 'increased fitness relative to more 'pure-bred' individuals' (Whitlock *et al.* 2000). Others suggest that the difference lies in having a population *versus* an individual perspective, for instance, heterosis is 'usually ascribed to the average fitness of the hybrid offspring' while transgressive segregation refers to 'the presence of extreme phenotypes (in either a negative or a positive direction) relative to the parental phenotypes' that make particular individuals 'more fit […] than either of the parents' (Johansen-Morris & Latta 2006). Another perspective is that the difference concerns at what point in time the positive fitness following hybridization is observed. Heterosis is said to be evident in the 'observation that cross-pollinated hybrids are more vigorous than their parents [….] calculated as the difference in the phenotypic performance of a trait between a hybrid and the average of its two distinct parents' (Hochholdinger & Baldauf 2018) or the 'elevated fitness of F1 hybrids relative to their parents' (Bell *et al.* 2019). In contrast transgressive segregation produces 'hybrid progeny phenotypes that exceed the parental phenotypes […which are] heritably stable' (Koide *et al.* 2019).

Outbreeding depression, on the other hand, is the reduced fitness of admixed offspring compared to their genetically distinct parents (Marr *et al.* 2002, Goldberg *et al.* 2005, Frankham *et al.* 2011, Barmentlo *et al.* 2018). Population genetic models have also been used to explain this phenomenon (Lynch 1991, Marr *et al.* 2002, Goldberg *et al.* 2005, Frankham *et al.* 2011, Whitlock *et al.* 2013, Kardos *et al.* 2016). Hybrid offspring will be heterozygous for many genes, and it is suggested this could cause an averaging or intermediate effect that makes the progeny adapted to neither parental habitat (Edmands & Timmerman 2003). In subsequent generations of interbreeding it has been suggested that the depression could worsen, because particular combinations of gene variants which have been of adaptive value to parental lineages in their environments may no longer co-occur in the genomes of the hybrid offspring (Lynch 1991, Allendorf *et al.* 2001, Edmands 2007). In the worst cases, where parental species have evolved differences in their chromosome karyotypes (the packaged form of their DNA sequences) it is possible that the chromosomes will not pair properly during meiosis and this will affect the fertility of the hybrid offspring (e.g. the famous case of donkeys and horses producing sterile mules; Rieseberg 2001).

A growing number of studies support the view that outbreeding depression is more likely to occur when genetic differences are linked with local adaptation to specific environments. In contrast, isolation *per se* even for many generations, under similar selection pressure rarely leads to symptoms of outbreeding depression upon admixture (Barton 1996, Orr & Smith 1998, Hendry *et al.* 2000, Rundle *et al.* 2000, Nosil *et al.* 2002, Rundle 2003, Frankham *et al.* 2011). However, disruption of adapted phenotypes cannot explain some reported instances of outbreeding depression. For example, adaptive differences between parental linages fail to explain why several crosses between the same lineages can generate very different outcomes for offspring fitness

and phenotypes (Johansen-Morris & Latta 2006, Escobar *et al.* 2008, Barmentlo *et al.* 2018). The importance of local adaptation in *Apteryx* evolution remains untested. However, there are examples of both within and between species hybrids in kiwi that are vital, reproducing, and even very successful (Herbert & Daugherty 2002, Cunningham & Castro 2011, Shepherd *et al.* 2012). There are also cases of long-distance translocations of *Apteryx* that are reported to be successful (Colbourne 2005, Robertson *et al.* 2019). While more rigorous investigations need to be conducted, these observations may suggest a limited role of local adaptation in the disjunct management units.

Despite considerable effort, finding the so-called 'sweet spot' of genetic and/or phenotypic distance between taxa that will produce heterosis and not outbreeding depression has not been possible, raising the question of whether the concept of a sweet spot is useful at all (Tallmon *et al.* 2004, Edmands 2007, Escobar *et al.* 2008, Stelkens *et al.* 2014, Kardos *et al.* 2016, Barmentlo *et al.* 2018, Bell *et al.* 2019, Koide *et al.* 2019). Further complicating the picture, several studies have found that crossing of different lineages within the same species has sometimes resulted in outbreeding depression and sometimes in heterosis (Edmands 1999, Rundle *et al.* 2000, Marr *et al.* 2002, Escobar *et al.* 2008, Whitlock *et al.* 2013). Occasionally, these outcomes have differed depending on which lineage was maternal and which was paternal (Escobar *et al.* 2008, Barmentlo *et al.* 2018). While at other times, hybridization has produced some traits with both negative and positive consequences for fitness (Johansen-Morris & Latta 2006, Escobar *et al.* 2008). Similarly, Whitlock *et al.* (2013), have also reported that the frequency and magnitude of outbreeding depression have differed depending on what trait was the subject of study. A further anomaly not explained by population genetic models is the resilience towards inbreeding in some species (Jamieson 2015).

This is perhaps most striking in recovery success following population bottlenecks (Heber *et al.* 2013, Ramstad *et al.* 2013, Frankham 2015, Jamieson 2015). This success has been attributed to the purging of deleterious gene variants, but empirical evidence for this speculation of losing the worst alleles due to homozygous expression has not yet been forthcoming and an alternative explanation might be needed (Crnokrak & Barrett 2002, Kennedy *et al.* 2014, López-Cortegano *et al.* 2018). Also anomalous is the observation that inbreeding depression can manifest differently between environments, suggesting that understanding genetic variation alone is insufficient to predict fitness outcomes (Keller *et al.* 2002, Cheptou & Donohue 2013). These examples suggest that critical information is missing, and an improved conceptual framework is needed to understand the fitness of populations that we wish to manage (Escobar *et al.* 2008, Hochholdinger & Baldauf 2018, Rey *et al.* 2020). With this increasing realization, attention has recently turned to the phenomenon of epigenetics and the extent to which chemical modification of the DNA in response to environmental signals also contributes to an organism's fitness.

Gene variants (alleles), specifically for transcription factors and in regulatory regions, are thought to play a major role in altering the dynamics of an organism's transcriptome, with consequences for the dynamics of its proteome, morphology, physiology, and behavior (Johnston *et al.* 2019, Lai *et al.* 2019). However, the expression of genes is also modulated through chemical modification of DNA, RNA, and proteins in processes linked to environmental signals - a phenomenon known as epigenetics (Donohue 2014, Junaid *et al.* 2018). Mechanisms of epigenetic regulation are thought to have a role in the plastic (varied) expression of genes and phenotypes (Bonduriansky *et al.* 2012, Groszmann *et al.* 2013, Li *et al.* 2018b, Thiebaut *et al.* 2019). Such regulation has been linked to the phenotypic divergence of populations and

can affect the width of a niche and the capacity to fulfil roles in an ecosystem (Miryeganeh & Saze 2019, Thiebaut *et al.* 2019, Rey *et al.* 2020). Epigenetic change has also been linked to altered patterns of gene expression associated with transgressive segregation and heterosis in hybrid offspring (Groszmann *et al.* 2013, Junaid *et al.* 2018, Botet & Keurentjes 2020). Recent work in plants suggests that epigenetic regulation plays a crucial role in hybrid vigor and that non-additive and yet not random differences in the patterns of chemical DNA modification (methylation of Cytosine bases) between parents and hybrid offspring contributes to phenotypic differences (Kawanabe *et al.* 2016, Junaid *et al.* 2018, Lauss *et al.* 2018, Miryeganeh & Saze 2019, Sinha *et al.* 2020). Other studies have also found that inbred and outbred lines exhibit different epigenetic profiles and that manipulating these profiles can revert symptoms of inbreeding depression (Vergeer *et al.* 2012). Taken together, these findings strongly suggest that accounting for both allelic and epigenetic variation is likely to be necessary to predict fitness outcomes for hybrid populations (Rey *et al.* 2020). While most studies to date involve plants, common mechanisms of eukaryotic gene expression suggest similar studies in animals will reveal similar results.

1.3.3 Genetic rescue

Prolonged periods of low (effective) population size and/or restricted gene flow are a growing concern among conservationists and there is increasing interest to utilize genetically motivated management to address the loss of genetic diversity and its negative effects (IUCN/SSC 2013, Frankham 2015, Hoffmann *et al.* 2015, Whiteley *et al.* 2015, Frankham *et al.* 2017, Ralls *et al.* 2018, Bell *et al.* 2019, Chan *et al.* 2019, Taylor & Larson 2019). In practice, management interventions to increase genetic diversity usually entail translocation of individuals from a source to a genetically distinct target population with the aim of intentionally generating intermixed (hybrid)

populations of the same species (Armstrong & McLean 1995, Weeks *et al.* 2011, IUCN/SSC 2013, Pierson *et al.* 2016, Wennerström *et al.* 2017, Flanagan *et al.* 2018). Efforts to increase genetic diversity have also entailed management of connectivity between distinct populations or different forms of guided mate choice (Soulé 1985, Pierson *et al.* 2016, Wennerström *et al.* 2017, Flanagan *et al.* 2018). These translocations involving genetically distinct populations differ from more classic supplementary translocations (also referred to as reinforcement translocations) which have the goal to increase population size directly by adding more individuals, and differ from reintroduction translocations where the purpose and focus are to re-establish populations with genetic stock obtained from within its historical range (IUCN/SSC 2013, Armstrong *et al.* 2019). All the above-mentioned types of interventions have been suggested for *Apteryx*. However, these proposals have been made without studies having been conducted to quantify inbreeding and/or inbreeding depression in neither source nor target populations (Innes *et al.* 2015, Kiwis for Kiwi 2016, Germano *et al.* 2018).

The interbreeding of individuals from populations with the aim of increasing fitness is now commonly referred to as genetic rescue (Johnson *et al.* 2010, Hedrick *et al.* 2011, Frankham 2015, Ochoa *et al.* 2017, Bell *et al.* 2019). 'Rescue', in this case, refers to decreasing the extinction risk in the target population and is commonly evaluated by an observed population growth rate increase after genetic admixture (Ingvarsson 2001, Hedrick *et al.* 2011, Frankham 2015, Whiteley *et al.* 2015, Bell *et al.* 2019). A growing number of authors argue that this prospect of alleviating extinction risk by augmented gene flow is underutilized (Frankham *et al.* 2011, Frankham 2015, Whiteley *et al.* 2015, Love Stowell *et al.* 2017, Ralls *et al.* 2018, Bell *et al.* 2019, Ralls *et al.* 2020). Since 1964, the 14 birds released on Ponui Island have produced an estimated population of

over 1700 birds, or one bird per hectare, which makes the Ponui Island brown kiwi population one of the densest in the world having experienced an equivalent of on average 9% annual population growth (Potter 1990, McLennan & Potter 1992). Thus, even though this was not an intention of the original translocations, the Ponui Island population might be one of New Zealand's best examples of genetic rescue.

Conceptually, genetic rescue and hybrid vigor likely describe the same biological phenomenon resulting from hybridization. However, in conservation, genetic rescue is rarely or never promoted as hybridization due to the negative connotations of this word (Allendorf *et al.* 2001, Wayne & Shaffer 2016, Love Stowell *et al.* 2017, Bell *et al.* 2019, Chan *et al.* 2019, Taylor & Larson 2019). Hitherto, most attempts of genetic rescue have focused on utilizing source populations with smallest possible genetic difference to the target population (Edmands 2007, Frankham *et al.* 2017, Ralls *et al.* 2018, Ralls *et al.* 2020). However, discussion about the trade-off between increasing genetic diversity and maintaining genetic integrity is becoming increasingly relevant. Recently, several authors have discussed the ambiguity and sometimes mismatch in genetically motivated conservation interventions. For instance, vonHoldt *et al.* (2018) highlighted that there is a need for debate around our understanding of the evolutionary significance of hybridization and its implications for conservation management, and Ralls *et al.* (2018) called for a 'paradigm shift in the genetic management of fragmented populations'.

As for hybrid vigor, the allele centered modelling of genetic rescue (Davenport 1908) is still the commonly suggested explanation for successful population growth, leading to the prediction that the magnitude of genetic rescue is a function of the severity of the genetic load in the target population (Bell *et al.* 2019). A variation on this model, while still having an allele focus, acknowledges the role of the size of the source population

(the bigger the better) and the amount of adaptive genetic difference between target and source population (the less the better, Whiteley *et al.* 2015, Bell *et al.* 2019). In reviewing 156 published cases, Frankham (2015) identified that in practice the two most important conditions for successful genetic rescue were (1) that the source population was not inbred, and (2) that the target population experienced some level of environmental stress. Importantly, the review of Frankham (2015) found that severe inbreeding in both target and source population did not have to exclude observed genetic rescue, such as in the case of Mexican wolves (*Canis lupus baileyi*; Fredrickson *et al.* 2007), however, the magnitude of rescue was larger when outbred sources were used. The noticeable discrepancy between theory and empirical finding highlights the need to identify what is missing from our understanding of inbreeding depression, outbreeding depression and heterosis. Quantification of these phenomena requires a baseline for comparison, and in cases where species are confined to small isolated populations and have been so for a long time, what can be learnt from comparing these populations may be limited (Hedrick & Fredrickson 2010, Heber & Briskie 2013, Ramstad *et al.* 2013, Taylor 2014). An exemplary case concern *A. owenii*. This is the only *Apteryx* species in which inbreeding has been thoroughly studied. Results show evidence of several bottlenecks and extremely low variation in the MHC genes (Ramstad *et al.* 2010, Miller *et al.* 2011a, Shepherd *et al.* 2012, Taylor 2014, Taylor *et al.* 2017c). However, the fact that extant members of this species originate from as few as five founders, means that findings might not be applicable to other *Apteryx* species (Ramstad *et al.* 2013).

While debate remains surrounding the degree to which genetic load contributes to extinction risk, there will remain debate as to the magnitude of threat aversion or loss of extinction risk that can be expected from genetic rescue (Bell *et al.* 2019). One

challenge for evaluating the success of translocations involving birds from multiple sources is that long term outcomes might not be immediately apparent. While some studies have suggested that the effect of genetic rescue is greatest in the first- and/or second-generation (Johansen-Morris & Latta 2006, Bell *et al.* 2019), other studies have shown that initial generations can suffer reduced fitness as a consequence of outbreeding depression. In such cases, crossing, backcrossing, and genomic recombination can later generate a hybrid swarm with higher average fitness than the parental populations (Hwang *et al.* 2011). While these findings are somewhat contradictory, they caution against drawing conclusions too soon after translocations involving interpopulation crosses. The Ponui Island birds, resulting from a mixed translocation that occurred more than 50 years ago, offer the opportunity to investigate the longer-term fitness consequences of admixture.

The successful introduction of diversity resulting in phenotypic diversity on which selection can act and adaptations evolve in response to environmental conditions is said to diffusely depend on the genomic makeup of the introduced individuals (Bell *et al.* 2019). It will also depend on how the genome is packaged and expressed in individuals, and how this affects the fitness of individuals (Hochholdinger & Baldauf 2018, Li *et al.* 2018a, Botet & Keurentjes 2020). This is an area of research where there are many outstanding questions, but where knowledge is increasing rapidly (Hochholdinger & Baldauf 2018, Rey *et al.* 2020). Genome Science that links genetic variation, genome expression and local adaptation will be key, and this may require rethinking our measures of biodiversity.

1.3.4 Assessing biodiversity

Since the ratification of the Convention on Biological Diversity (CBD) in 1992, the world has agreed to acknowledge and preserve three levels of biodiversity: ecosystem diversity, species diversity and genetic diversity (UN 1992). The latter is mainly justified because standing genetic diversity – allelic variation – is thought to contribute most to evolutionary potential of species and thus to their capacity to adapt to a rapidly changing environment (e.g. Haenel *et al.* 2019, Lai *et al.* 2019). In practice, this has led to genetic differences being used as an objective criterion to measure diversity, delimit and identify species, subspecies, taxa, lineages, evolutionary significant units (ESUs), or management units, often driven by improving opportunity for legal protection, funding and/or threat acknowledgement (Rojas 1992, Godfray *et al.* 2004, Palsbøll *et al.* 2007, Lohman *et al.* 2010, Wayne & Shaffer 2016, Cobley 2017, Groves *et al.* 2017, Taylor *et al.* 2017a, Taylor *et al.* 2017b). The prevailing standpoint in such cases has been that preserving genetic diversity means maintaining the observed differences (see for example Palsbøll *et al.* 2007, Weir *et al.* 2016, Richmond *et al.* 2017, Taylor *et al.* 2017d, Germano *et al.* 2018). As mentioned above, the *Apteryx* genus serves as a typical example of both these tendencies (Tennyson *et al.* 2003, Weir *et al.* 2016, Germano *et al.* 2018).

Numerous genetic studies that have identified populations or other groups within species and genera, such as *Apteryx*, as genetically distinct have been based on markers such as microsatellites, allozymes, mitochondrial DNA sequence, or sequence fragments of a small number of selected genes (Baker *et al.* 1995, Cooper *et al.* 2001, Haddrath & Baker 2001, Burbidge *et al.* 2003, Haddrath & Baker 2012, Shepherd *et al.* 2013). These traditional markers have successfully been used to provide measures of

genetic diversity and infer evolutionary relationships (Mitchell *et al.* 2014, Weir *et al.* 2016). However, this approach can be limiting when only a small number of gene loci are investigated and/or when loci evolve at different rates of evolutionary change within and between taxa (Allendorf *et al.* 2010, Funk *et al.* 2012, Steiner *et al.* 2013, Putman & Carbone 2014). Low resolution can lead to underestimation and/or miss interpretation of the complex history of species and populations (Goldstein *et al.* 1996, Hudson & Turelli 2003, Zink & Barrowclough 2008, Funk *et al.* 2012, Steiner *et al.* 2013, Jeffries *et al.* 2016, Kardos *et al.* 2016, Hodel *et al.* 2017, Richmond *et al.* 2017, Galla *et al.* 2020). Another limitation of the most commonly used genetic markers is that they are not linked to traits of functional adaptive significance and have restricted ability for determining the cause and nature of ecological distinctiveness of taxa (Allendorf *et al.* 2010, Funk *et al.* 2012, Wennerström *et al.* 2017, Leroy *et al.* 2018). Hence, there are limitations to the ability of such markers to inform and evaluate the consequences of hybridization and translocation success based on possible outbreeding and inbreeding effects (Funk *et al.* 2012, Hess *et al.* 2013, Frankham 2015, Whiteley *et al.* 2015, Flanagan *et al.* 2018, Bell *et al.* 2019, Taylor & Larson 2019).

Newer molecular tools allow researchers to reduce these limitations. For example, genomic methods, such as reduced-representation-sequencing (for instance through protocols like genotyping-by-sequencing) (1) provide information from thousands of loci spread throughout the entire genome, and (2) cover both neutral, non-coding sequences and genes under selection (Elshire *et al.* 2011, Funk *et al.* 2012, Hess *et al.* 2013, Narum *et al.* 2013, Reitzel *et al.* 2013, Hunter *et al.* 2018, Leroy *et al.* 2018, Picq *et al.* 2018). This resolution allows for much more detailed genetic characterization of closely related taxa, and thus also of historic as well as recent hybridization and introgression between them and the population growth effects over time such admixture

has had (Elshire *et al.* 2011, Weeks *et al.* 2011, Narum *et al.* 2013, Escudero *et al.* 2014, Leaché *et al.* 2014, Gaughran *et al.* 2017, Schmickl *et al.* 2017, Zhen *et al.* 2017, Picq *et al.* 2018, Taylor & Larson 2019). In addition, genome-wide association studies (GWAS), provide insight into the nature of the physiological and behavioral differences of populations (Hess *et al.* 2013, Flanagan *et al.* 2018, Hunter *et al.* 2018). Methodological advances mean that genomics is likely to take on a larger role in conservation biology, in delimiting management units, in identifying suitable source populations and in evaluating translocation outcomes (Funk *et al.* 2012, Flanagan *et al.* 2018, vonHoldt *et al.* 2018, Galla *et al.* 2019, Ramstad & Dunning 2020, Russello *et al.* 2020). Even so, the potential for genomics to contribute to conservation science may not be fully realized while focus remains on genetic variation alone which in many cases will be insufficient to make reliable predictions for managing biodiversity.

While our understanding of diversity, resilience and the contrasting outcomes of hybridization is informed by genetic variation, the importance of epigenetic variation is increasingly being recognized (Cheptou & Donohue 2013, Groszmann *et al.* 2013, Biémont & Vieira 2014). Epigenetic changes accompanying environmental change play a key role in plastic responses that occur at a faster rate than mutational change and the sorting of allelic variation (Hochholdinger & Baldauf 2018, Miryeganeh & Saze 2019, Rey *et al.* 2020). Epigenetic factors could explain why some natural populations are more fit than others, and also why some species that have crashed to very low levels of genetic diversity and adaptive potential can still rebound successfully (Heber *et al.* 2013, Ramstad *et al.* 2013, Frankham 2015, Jamieson 2015). The contribution of epigenetic processes to evolutionary and ecological success requires more study and understanding, but the potential of ‘conservation epigenetics’ is both exciting and promising (Rey *et al.* 2020). Differences in epigenetic markers between populations

could help us to identify and categorize evolutionary significant units (ESUs) and whether species with low genetic variation might nevertheless be resilient to environmental change. This in turn will help us to better predict the viability and differences between populations – information that could be crucial for identifying suitable source- and target populations for translocations. Epigenetic monitoring, as a complement to genetic investigations, could provide more ecologically significant information than studies of genetic variation alone and improve prediction of what interventions are likely to be most successful (Hochholdinger & Baldauf 2018, Miryeganeh & Saze 2019, Rey *et al.* 2020).

Investigation of epigenetic processes will involve studies of DNA, RNA, and proteins. However, a place to start at the population level is with DNA methylation profiles (Sepers *et al.* 2019, Rey *et al.* 2020). Of interest for conservation planning would be understanding variation among individuals and between populations, and whether ESUs are categorized appropriately, determining whether source populations show differences from translocated and admixed populations and whether there is evidence of population fragmentation sooner than is appreciated from studies of genetic variation (Rey *et al.* 2020). Variant protocols of reduced-representation-sequencing, such as DREAM, EpiGBS and bsRADseq offer the potential to characterize partial methylation profiles (Jelinek & Madzo 2016, Trucchi *et al.* 2016, van Gurp *et al.* 2016). However, these protocols have limitations since they capture only a small subset of the entire epigenome. A more complete methylome profile is possible using whole-genome bisulfite sequencing (Lister *et al.* 2009, Hansen *et al.* 2012). Locus specific bisulfite sequencing is also possible (Hernández *et al.* 2013, Lam *et al.* 2020). Another alternative is the so-called assay for transposase-accessible chromatin using sequencing

(ATAC-seq) which maps genome-wide chromatin accessibility, which is tightly linked to gene expression (Miskimen *et al.* 2017). However, both the latter methods introduce the cost of whole-genome sequencing. Whatever might be the methodological developments to come in the fast-developing field of high throughput sequencing technologies, a focus on both genomics and epigenetics is likely to provide the insight that is needed to understand hybridization, and its contribution to biological diversity and successful conservation strategies (Goulet *et al.* 2017, vonHoldt *et al.* 2018, Chan *et al.* 2019, Taylor & Larson 2019, Rey *et al.* 2020).

1.3.5 The hybrid birds of Ponui

In 2016, the hybrid birds of Ponui Island were said to be of no value for kiwi conservation other than as specimens for public display. However, this conclusion needs to be reconsidered in light of recent findings and modern tools of genome science. There is no doubt that allelic diversity and the genetic background of individuals is important for individual fitness, population sustainability and genetic rescue. However, knowledge of genetic variation alone appears insufficient to fully understand the link between genotype, transcriptome, phenotype, and fitness. Focusing solely on allelic variation makes for attractive and accessible models. However, the poor performance of these models to predict outcomes has the potential to damage the relationship between researchers and practitioners. This motivates the need for research investigating the interactions and relative contributions of genotype, epigenetics, and the environment for understanding phenotypic diversity, reproductive success, and adaptive potential.

The Ponui Island birds, which are highly successful in their island habitat, provide an exciting model system to investigate ecological success and potentially the evolutionary and ecological significance of hybridization. In addition, this system could help to

inform how conservation translocations can effectively utilize genome-level data to achieve their goals. Ecological factors might help explain the success of this population relative to the mainland and other island populations. For instance, compared to unmanaged populations the juvenile kiwi on Ponui Island experience much lower mortality from predation (Shapiro 2005, Dixon 2015, Strang 2018). However, this mortality is still higher than observed in the most extensively managed populations (Colbourne *et al.* 2005, Robertson & de Monchy 2012). Thus, even if juvenile mortality contributes to population success it may still be important to determine other significant factors. Is the secret to their success the loss of recessive deleterious effects? Is it phenotypic diversity – and if so, how did that come about? Mainly through allelic admixture? Or via epigenetics and thus transcriptome variation? To gain understanding, the next step will be to compare the genomic diversity of Ponui Island birds with their parent populations and with other mainland populations whose history has not involved extreme genetic bottlenecks associated with the founding of island populations by a small number of translocated birds. Epigenetic studies might initially focus on methylation profiles and their density (Rey *et al.* 2020). A study could seek to answer questions such as: Is there epigenetic variation within and between populations of *A. mantelli*? How do the epigenetic profiles of Ponui Island birds compare with other populations? How do the methylation profiles change over generations on Ponui Island? How do they compare between parents and offspring? Does the genetic distance between parents influence the non-additivity of the epigenetic profile of offspring? What are the methylation patterns for genes of potential adaptive value? Following this investigation, important studies would compare genomic and epigenetic diversity in relation to transcriptome variation, and to how the resulting phenotypic variation relates to fitness, and measures of inbreeding-, outbreeding depression and/or hybrid vigor. A

key for meaningful interpretation of transcriptome analyses will be detailed knowledge of individual birds (including information on generation, age, health, and sex) and here again, Ponui Island could prove suitable after 17 years of extensive studies on this population.

The questions that could be addressed by investigations of the Ponui Island birds are not only relevant to *Apteryx*, improved understanding of genomics and epigenetics and thus of the nature of population differences is key for conservation of all fragmented populations in need of augmented genetic influx (Tallmon *et al.* 2004, Edmands 2007, Escobar *et al.* 2008, Stelkens *et al.* 2014, Barmentlo *et al.* 2018, vonHoldt *et al.* 2018, Bell *et al.* 2019, Koide *et al.* 2019, Rey *et al.* 2020). Worldwide, habitat and population fragmentation has rendered a situation where focusing solely on species-level conservation may lead to either inbreeding or homogenization, both of which results in loss of genetic diversity. Retaining evolutionary potential is arguably one of the main challenges for conservationists across the globe, not only because of the intrinsic value of diversity but also for providing populations with the ability to adapt to our changing environment. Because of this challenge, we support the call for a paradigm shift in conservation that includes redefining admixture and hybridization (Canestrelli *et al.* 2016, Ralls *et al.* 2018, vonHoldt *et al.* 2018, Taylor & Larson 2019). We believe that it is wrong to dismiss a prolific population of a threatened species as unimportant in an unsubstantiated way when there remains uncertainty as to the best way of managing the species. This is even more so when these populations have the potential to serve as sources for ongoing, translocation focused interventions – a literal source for individuals and a source of increased knowledge. Lastly, we recognize the importance of multidisciplinary studies that are needed to help better understand and predict hybridization outcomes.

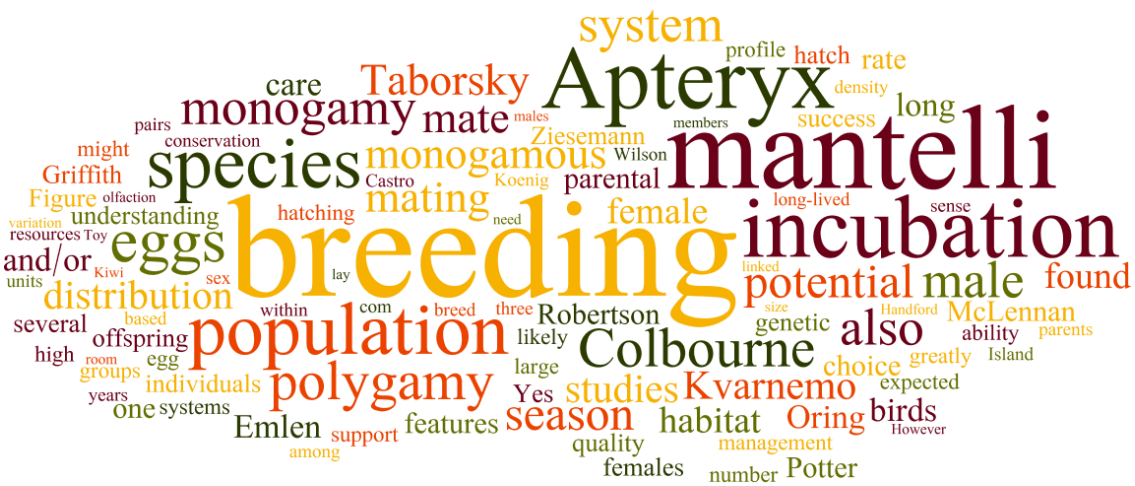
The questions that could be addressed with genetic and epigenetic investigations of the Ponui Island birds are not only relevant for *Apteryx*. Making meaningful measurements of population differences is important for determining what interventions are appropriate to ensure the sustainability of fragmented populations (Tallmon *et al.* 2004, Edmands 2007, Escobar *et al.* 2008, Stelkens *et al.* 2014, Barmentlo *et al.* 2018, vonHoldt *et al.* 2018, Bell *et al.* 2019, Koide *et al.* 2019, Rey *et al.* 2020). These measurements require embracing the complexity of epigenetic phenomena and understanding how it interacts with genetic variation in affecting the fitness of individuals in different environments. Improved understanding will in turn help us to better understand the adaptive potential and resilience of species to environmental stresses and change. We support the call being made by others for a paradigm shift in conservation to rethink the negative connotations of admixture and hybridization (Canestrelli *et al.* 2016, Ralls *et al.* 2018, vonHoldt *et al.* 2018, Taylor & Larson 2019). Measures of fitness of individuals and populations need to consider both temporal and environmental factors. Furthermore, until interactions between the environment of Ponui Island and the epigenomes of its kiwi are better understood, we believe that it would be wrong to dismiss a prolific population of a threatened species. This is even more important when this population has the potential to serve as a source for ongoing, translocation focused interventions – a literal source of individuals and a source of increased knowledge to be drawn upon in decision making. Lastly, we acknowledge the importance of integrating contributions from other disciplines when using novel tools from genome science to improve understanding and better predict intervention outcomes. An important goal is to inform and develop practices that meet conservation aspirations.

PART II – BREEDING

Elucidating with whom *Apteryx* individuals pair, and if, when, and why, it might be with more than one mate, is crucial for reliably predict outcomes of management interventions, such as translocations. Hence, in this part, I first present a review of empirical and theoretical data to make predictions about the expected breeding system in *A. mantelli*. Second, I utilise the mixed *A. mantelli* population on Ponui Island to study mate choice and discuss its implications for future genetic rescue attempts. Last, I present the most thorough description of the *A. mantelli* breeding system so far and discuss potential drivers for why some *A. mantelli* in a high-density population breed in groups rather than socially monogamous pairs.

Chapter 2.1

The expected breeding system in *Apteryx mantelli,* a long-lived bird with huge eggs and male-only incubation.



All supervisors provided feedback on one or more drafts of this chapter. The expertise of Isabel Castro and Emilie Vallee, and the hard work of previous members of the Ponui Island team were crucial for this chapter.

Abstract

Understanding the breeding system and mating strategy of a species is important for making accurate population predictions and evaluating conservation interventions. Theoretical and empirical studies have provided substantial knowledge of what generates polygamy potential as well as the typical character profile of a monogamous bird species. Here I discuss the expected breeding system of North Island brown kiwi, *Apteryx mantelli*, in light of this knowledge and the phylogenetic context of this species. *Apteryx mantelli* has so far only been described as monogamous. However, although *A. mantelli* have several features matching the typical monogamy profile (big investment in offspring by both parents, long life expectancy, and a well-developed sense of smell), they arguably have as many features consistent with potential for polygamy (uneven quality and distribution of resources, long and asynchronous breeding season, and super-precocial chicks). This, combined with recent studies discovering mixed parentages among *A. mantelli* eggs incubated by the same male, suggests that categorising this species as strictly monogamous might be incorrect. I argue that further study of *A. mantelli* mating behaviour could benefit future conservation planning for this species and should focus on determining the number of associated mates and their genetic relatedness as well as, the drivers of identified breeding strategies. Importantly, such studies would also further our overall understanding of the breeding biology of birds.

2.1.1 Introduction

An increasing number of animal populations are in need of active management to persist. One type of intervention growing in importance is translocations (IUCN/SSC 2013, IUCN 2020). Management generally aims to generate population growth, and one

way this can be achieved through translocations is when introduction of new genetic material alleviates symptoms of inbreeding depression. This phenomenon is known as genetic rescue (Ingvarsson 2001, Frankham 2015, Ralls *et al.* 2018, Bell *et al.* 2019). Key factors governing if and how quickly such rescue can be observed following a translocation event are how breeding units are formed and how many individuals they contain. Both these factors have the potential to greatly impact individual reproductive success, the rate of genetic admixture, population growth rate, and ultimately viability (Adams *et al.* 2011, Bradley *et al.* 2014, Hoover *et al.* 2018, Koenig *et al.* 2019, Thavornkanlapachai *et al.* 2019). Consequently, understanding the breeding system of a focus species, and how it relates to properties such as habitat quality and population density, will provide crucial input for predicting management outcomes in general and specifically translocation outcomes.

Monogamy was formerly considered the dominating breeding system in birds with few exceptions (Lack 1968, Griffith *et al.* 2002). However, long-term studies and genetic analyses of birds have more recently been finding (1) wide-spread polygamy, and (2) that, even for species residing in pairs, extra-pair mating and mixed parentage is relatively common, leading to the coining of the terms ‘social monogamy’ contra ‘genetic monogamy’ (Hughes 1998, Griffith *et al.* 2002). Emlen and Oring (1977) introduced the concept of ‘potential for polygamy’ and the idea that monogamy is an exception occurring only when a potential for polygamy is non-existent or cannot be utilised. The main component of this potential is the ability of one sex to monopolize either individuals of the opposite sex or enough resources to support successful breeding with more than one mate (Emlen & Oring 1977). Thus, the distribution of resources and mates in space and time are predicted to be key factors determining the size of the potential for polygamy and which sex should preferentially be able to utilise

it (Emlen & Oring 1977). Furthermore, Emlen and Oring (1977) suggested that other factors which can increase the potential for polygamy are having a long and/or asynchronous breeding season, as well as offspring that do not require biparental care.

Since the publication of their influential paper, a rather narrow 'monogamy profile' for birds has indeed been emerging, but empirical support is stronger for some factors suggested as important by Emlen and Oring (1977) than others (Griffith *et al.* 2002, Kvarnemo 2018). As predicted, habitat limitation and, more specifically, low variability in habitat quality seem to be the conditions strongest associated with monogamy in birds (Griffith *et al.* 2002, Kvarnemo 2018). Substantial evidence has also been gathered for a link between monogamy and need for parental care, though for multiple species it remains to be tested whether extensive parental care is a cause or a consequence of this mating system (Marks *et al.* 1999, Kvarnemo 2018). The role of mate availability remains unclear, with most support for a link between mate availability and divorce rate (Taborsky & Taborsky 1999, Culina *et al.* 2015, Kvarnemo 2018), and arguably there is a point where monogamy with high divorce rate is better described as sequential polygamy or promiscuity. Support is also mixed for an effect of breeding synchrony on the prevalence of polygamy (Spottiswoode & Møller 2004, Kvarnemo 2018). Importantly, phylogeny and genetic predisposition have been found to be important explanations of mating system distribution among and within taxa (Björklund 1990, Mabry *et al.* 2013, Koenig & Dickinson 2016). Lastly, prevalence of monogamy seemingly increases with longevity in birds (Griffith *et al.* 2002, Sánchez-Macouzet *et al.* 2014, Kvarnemo 2018, Leach *et al.* 2020), and such long-term stable monogamy can be expected to render strong selection for the ability to pair with the 'correct' mate. Consequently, monogamy and a long lifespan have also been associated with mechanisms for active and accurate mate choice such as well-developed olfaction

(McNamara & Forslund 1996, Brown 1998, Zelano & Edwards 2002, Bried *et al.* 2003, Bonneaud *et al.* 2006, Bonadonna & Sanz-Aguilar 2012, Strandh *et al.* 2012, Kamiya *et al.* 2014, Caro *et al.* 2015, Culina *et al.* 2015, Hoover *et al.* 2018, Kvarnemo 2018, O'Connor *et al.* 2019).

The extensive empirical and theoretical support for resource distribution being a key factor determining breeding strategy strongly suggests that changes to habitat quality and/or continuity can affect mating systems and behaviour. Hence concern is warranted for the potential of an ongoing, worldwide shift in breeding behaviour of wild animals caused by anthropogenic habitat alteration, destruction, and fragmentation (Banks *et al.* 2007, Caro & Sherman 2011, Winiarski *et al.* 2017, Buchholz *et al.* 2019). Consequently, increasing our understanding of the exact drivers for and conditions under which different breeding strategies evolve, take effect, and change holds promise to greatly assist future improvements of conservation management and policy (Koenig & Dickinson 2016, Griesser *et al.* 2017, Koenig 2017, Klug 2018). It has been highlighted that, groups and species that display several mating systems and breeding strategies in parallel will be crucial study systems for furthering this field (Klug 2018). Consequently, thanks to their interesting mixture of mating systems, several study systems of interest can be found within the ratites (Palaeognathae).

2.1.2 The variable ratites

The Palaeognathae or ratites, consisting of Struthioidae or ostriches, Rheidae or rheas, *Dromarius* or emus, Tinamidae or tinamous, and Apterygidae or kiwi display a wide variety of mating systems and strategies (Handford & Mares 1985, Coddington & Cockburn 1995, Mitchell *et al.* 2014). Ostriches have been observed to breed in monogamous pairs, sequential polyandry, and, commonly, with three females

cooperatively sharing one male and one nest (Kimwele & Graves 2003). Rheas frequently breed cooperatively with multiple females mating with one or several males and laying eggs in one or several nests, thereby creating a spectrum from polyandry to polygynandry to polygyny (Handford & Mares 1985, Codenotti & Alvarez 2001). Emus seem to breed either as monogamous pairs or in a polyandrous fashion (Handford & Mares 1985, Coddington & Cockburn 1995). Tinamous show large variation among species ranging from monogamy to polyandry to cooperative polygyny (Handford & Mares 1985, Brennan 2012, Giraldo-Deck *et al.* 2017, Solano-Ugalde *et al.* 2018). Both extinct Palaeognathae groups also show interesting features linked to breeding behaviour with the moa having extreme reverse sexual dimorphism, and the eggs of elephant birds famously being found together in large quantities suggestive of shared nesting (Bunce *et al.* 2003, Clarke *et al.* 2006, Olson & Turvey 2013).

For the kiwi (*Apteryx* spp.), which are nocturnal, the partaking adult(s) leave the nest only once to several times per night during incubation, spending only a few hours total away from the egg(s). This predictable activity decline during incubation followed by a further decline during hatching and the rapid activity increase observed if eggs are deserted have allowed for the development of leg-fitted radio transmitters for *Apteryx* that can link a males' level of activity to his incubation status and indicate one of the following stages: non-incubating, incubating, hatching, or deserting (wildtech.co.nz/Kiwi.aspx; Colbourne 2002, Taylor *et al.* 2014, Robertson & Colbourne 2017). Thanks to these transmitters, much has been learnt about *Apteryx* incubation behaviour and success rate. In addition, the transmitters currently play a direct role in *Apteryx* conservation by allowing practitioners to collect eggs from areas deemed unsafe for chicks to substantially increase juvenile survival and population recruitment success (Colbourne *et al.* 2005, Robertson & de Monchy 2012, Kiwis for Kiwi 2016,

Robertson & Colbourne 2017, Germano *et al.* 2018). *Apteryx* are extraordinarily long-lived with individuals known to have reached over 40 years and lifespans suggested to be up to 80 or 100 years (Robertson & Colbourne 2004, Barlow 2011, Weiser *et al.* 2013). *Apteryx* also have a very well-developed sense of smell (Corfield *et al.* 2014) and a huge uropygial gland (Reynolds *et al.* 2017), suggesting chemical identification and evaluation might occur in this genus (Cunningham *et al.* 2009, Castro *et al.* 2010). While longevity and well-developed olfaction are features associated with monogamy and thorough mate choice, the mating system in this genus shows large variation (Colbourne 2002, Sales 2005, Kvarnemo 2018).

None of the five extant *Apteryx* species practice female-only incubation. In three species, *A. australis*, *A. rowi*, and *A. haastii*, incubation duties are shared between the male and female, and sometimes additional breeding unit members (Eason 1988, Sturmer & Grant 1988, McLennan & McCann 1991, Colbourne 2002, Jahn *et al.* 2013). On Stewart Island (also known as Rakiura), the vast majority of *A. a. lawryi* breed in groups of mixed sexes and ages of up to seven or more individuals (Colbourne 2002, Feenstra pers. comm 2019). Some of these group members have been identified as offspring from previous seasons, and multiple group members partake in the incubation of the eggs (Colbourne 2002, Feenstra unpubl. 2020). Based on observations of 'extra' individuals present in nest burrows and higher parental activity during incubation than expected, it has been suggested that *Apteryx australis* on the New Zealand mainland also breed in groups but at lower prevalence compared to on Rakiura (Tansell, J. pers. comm. 2020). In *A. rowi*, three types of breeding units seem to occur: monogamous pairs, long-term stable trios consisting of one adult male and two adult females, and units where juveniles stay with their parents for up to two breeding seasons after hatching (Colbourne 2002, Dearlove, T. pers. comm. 2020). The activity of some such

staying *A. rowi* juveniles has been found to be consistent with partaking in incubation (Dearlove, T. pers. comm. 2020). For *A. haastii*, the number of individuals involved in incubation seems to vary between populations and could possibly be density-dependent (Halley, J., & Young, S. pers. comm. 2020). In this species, offspring tend to stay around their parents' nesting area until they are sexually mature (at around 4 years of age), and while the juveniles do not seem to partake in incubation (with some possible exceptions; Halley, J. pers. com. 2020), they are frequently found together with their parents and/or the newly hatched chicks (Jahn *et al.* 2013, Halley, J., & Young, S. pers. comm. 2020). *Apteryx owenii* has, to my knowledge, only been described as breeding in monogamous pairs with male-only incubation (Jolly 1989). Common to all *Apteryx* species is that members of breeding units (pairs or groups) can be found together all year round in shared burrows or roosts. It is worth noting that most of this knowledge comes from *Apteryx* populations under intensive management, with low densities and/or small population sizes. Since these conditions are drastically different compared to pre-human New Zealand (Holdaway 1989, Miller & Pierce 1995, Robertson *et al.* 2016), and because parentage tends to be inferred rather than genetically tested, there is still a lot to learn about historic as well as current *Apteryx* breeding biology.

2.1.3 The perhaps not so monogamous *Apteryx mantelli*

The most common (albeit still threatened) and most researched *Apteryx* species is the North Island brown kiwi or *A. mantelli* (Robertson *et al.* 2016, Germano *et al.* 2018, IUCN 2020). Like all *Apteryx* species, *A. mantelli* lay exceptionally large eggs and have a long incubation period (about 65-90 days; McLennan 1988, Jolly 1989, Potter 1989, Colbourne 2002, Sales 2005). The onset of the *A. mantelli* breeding season is noticeably asynchronous with the laying of the first clutch stretching, on average, across three months every year (Figure 2.1.1; Ziesemann *et al.* 2011, Wilson 2014, Dixon 2015,

Bansal 2020; Castro unpubl. 2020), and the season proceeds for about five to eight months per year (Potter 1989, Colbourne 2002, Sales 2005, Wilson 2014, Bansal 2020). Several previous studies have concluded that *A. mantelli* are socially monogamous and that the father singlehandedly incubates the egg(s) (Colbourne & Kleinpaste 1983, McLennan 1988, Potter 1989, Taborsky & Taborsky 1992, 1999), but, anecdotally, incubation by the female or a second male has been reported (McLennan 1988, Colbourne 2002, Ziesemann 2011). The large egg and long, male-dominated incubation suggests that the female and male *A. mantelli* both invest heavily in their offspring even though parental care is supposed to end at egg-laying for the female and at hatching for the male since no food provisioning of the super-precocial offspring occurs (Jolly 1989, Colbourne *et al.* 2005).

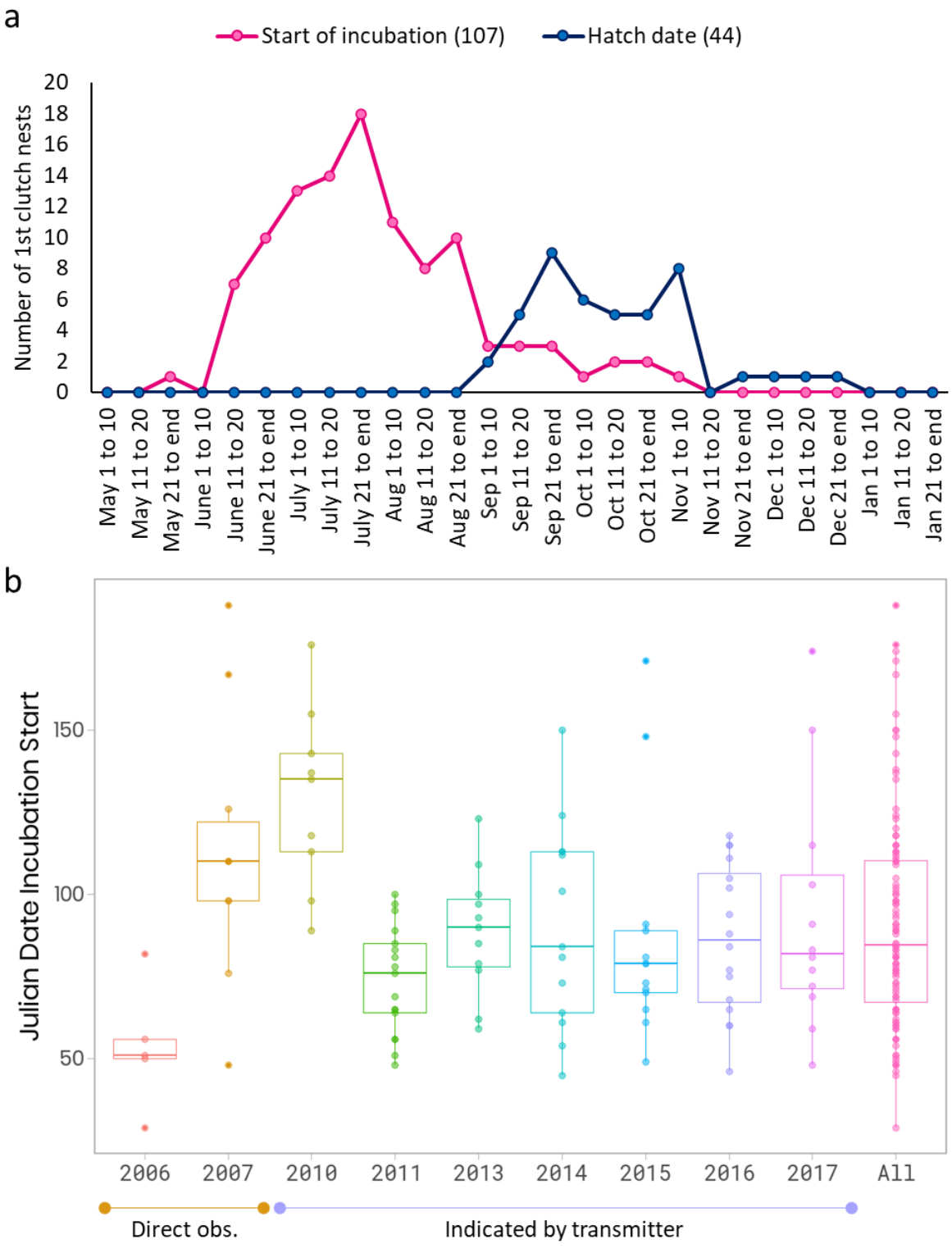


Figure 2.1.1. Illustration of the timing of the *A. mantelli* breeding season on Ponui Island. Timing is defined as the date incubation starts (pink line in panel a; all of panel b) and the date of hatching (a; blue line) of the first clutch. In (a) the number of clutches laid (pink) and the number of clutches hatched (blue) are summarized across years in ten-day slots. In (b) the

variation between and within years in the onset of incubation is illustrated with boxplots highlighting the median date and the quartiles with each datapoint (nest) represented by one dot. The overall median incubation start-date was July 24 but varied between June 21in 2006 to September 13 in 2010. The unit of the y-axis in panel b is the Julian date with 1 set to May 1, making day 100 August 9. Years listed indicate the respective breeding season; for instance, an egg hatched in January 2007 fall under the 2006 season. Data combined from Ziesemann (2011), Wilson (2014), Dixon (2015), Bansal (2020) and Castro unpubl. (2020) with partitions under the graph indicating that two different methods were used to obtain the data.

Despite the size, female *A. mantelli* can lay eggs as close as 20 days apart and up to eight times per season (McLennan 1988, Colbourne 2002). This high productivity is likely made possible through a combination of their skeletal composition (Currey & Alexander 1985, Dennison & Kooyman 1991), a calcium- and energy-rich diet (Dixon 2015), and a comparatively thin eggshell (Vieco-Galvez *et al.* 2020), which together makes *A. mantelli* less calcium limited than expected based on egg size alone (Vieco-Gálvez 2019). In addition, female *Apteryx* have two functioning ovaries, which is a rare feature within Aves, and it has been theorised that these allow a second egg to start forming before the first one is laid (Kinsky 1971). The ultimate reason for the short period between eggs might be the relatively high hatching failure rate (Figure 2.1.2; McLennan 1988, Potter 1989, Robertson & de Monchy 2012, Black, J. pers. comm. 2020, Toy pers. comm. 2020), which would have selected for an ability to lay replacements clutches (Emlen & Oring 1977). Frequent need for relaying has in turn been suggested as a key driver of male-only incubation (Emlen & Oring 1977). Interestingly, male-only incubating *A. mantelli* seemingly tend to have lower hatching success than other *Apteryx* species in which the male and female share incubation (Figure 2.1.2). Further studies would be needed to elucidate if this higher failure rate is consistent, and whether lack of female incubation in *A. mantelli* is a cause or a consequence of this. Potentially, a higher failure rate could have contributed to females

reducing their input in incubation to increase their ability to produce replacement clutches (Emlen & Oring 1977). Alternatively, it is plausible that male-only incubation could have evolved for other reasons, and, as a side-effect, a higher success rate linked to shared incubation has been lost. For the latter feature to become fixed, the fitness loss must somehow have been compensated for, possibly by the females being available for further mating when not involved in incubation. A third important consideration is that increased likelihood of hatching failure is a common symptom of inbreeding depression following tight bottlenecks in birds (Briskie 2004); thus individual, population, and species level genetic diversity could potentially be important cofactors governing observed differences in hatching success.

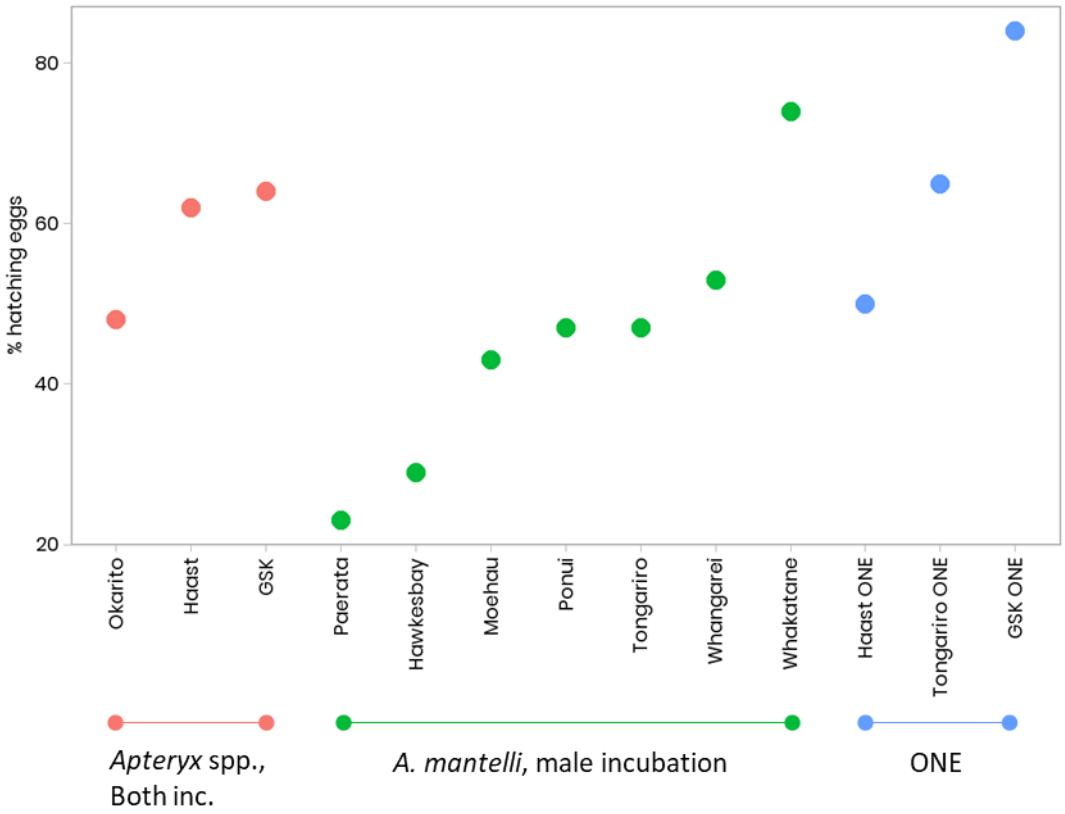


Figure 2.1.2. Percentage of *Apteryx* eggs successfully hatching in 13 populations. Values are split by incubation mode and ordered based on success rate. Examples come from three populations of *Apteryx* species where male and female share incubation (left), seven populations of *A. mantelli* in which the male does all the incubation, and three populations in which eggs are

collected and artificially incubated in captivity – a protocol known as Operation Nest Egg or ONE (Colbourne *et al.* 2005); data from McLennan (1988), Potter (1989), Robertson and de Monchy (2012), Wilson (2014), Toy pers. comm. 2020, and Black pers. comm. 2020.

Territoriality varies notably between *A. mantelli* populations and has been found to be exceptionally low or even absent in some areas where the birds instead roam vastly overlapping home ranges while birds in other areas aggressively defend territory borders (Potter 1990, McLennan & Potter 1992, Taborsky & Taborsky 1999, Ziesemann 2011). Potentially this variation in territoriality could be linked to variability in territory quality. Support for this hypothesis is that hatching success has been found to vary with burrow type (Figure 2.1.3; Castro unpublished) and so has foraging success with habitat type (Cunningham & Castro 2011, Dixon 2015). If this is the case territoriality can be expected to depend on habitat and be influenced by population density (Nicola *et al.* 2015, Ippi *et al.* 2018). However, another suggestion is that territoriality in *A. mantelli* could be linked to mate guarding by males, and, thus, that it is expected to be absent in female-biased and potentially in overall high-density populations (Taborsky & Taborsky 1999).

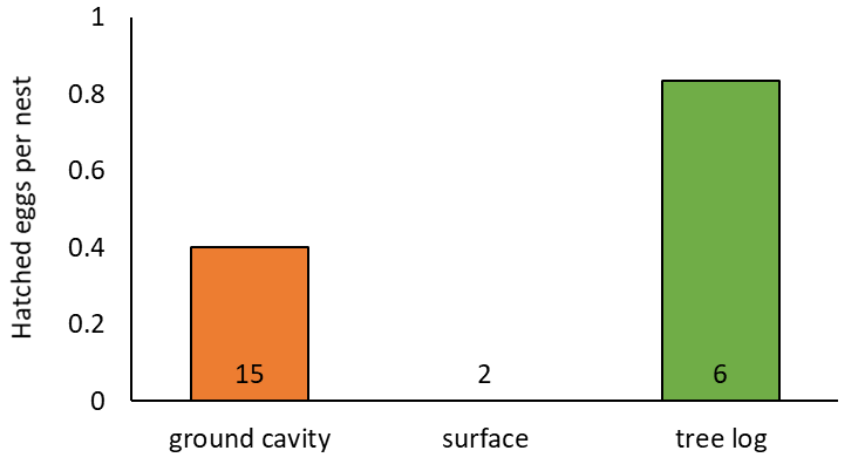


Figure 2.1.3. Variation in nest-success with burrow type for *A. mantelli* on Ponui Island. Data from Ziesemann (2011) and Wilson (2014). Numbers indicate sample sizes.

In summary, the uneven distribution of high-quality resources, the lack of parental care after hatching, the long and asynchronous breeding season, and the phylogenetic background all suggest that *A. mantelli* have potential for polygamy (Table 2.1.1; Emlen & Oring 1977). The large eggs, the long incubation, and the relatively rapid relaying likely limits the number of eggs one male can incubate in a season to fewer than one female can produce, suggesting that if polygamy potential exists, females are the more likely sex to be free to utilise it, resulting in polyandry (Lislevand & Thomas 2006, Abourachid *et al.* 2019). The long incubation also likely reduces the males' opportunity to mate-guard or monopolise on females in other ways, also supporting potential for polygamy and, specifically, polyandry. At the same time, the large investment from both parents, the high pair fidelity in most populations, the long lifespan, and the highly developed olfaction sense are all features matching the profile of monogamous bird species (Table 2.1.1; Taborsky & Taborsky 1999, Kvarnemo 2018). Thus, I suggest that the theoretically expected breeding system of *A. mantelli* is a combination of monogamy and polygamy, specifically polyandry.

Table 2.1.1. List of features of *A. mantelli* breeding and life-history and whether they are more consistent with potential for polygamy or the profile of monogamous bird species (based on Emlen & Oring 1977, Kvarnemo 2018).

Polygamy potential	**Support in *Apteryx mantelli***
Uneven distribution of resources	Yes - both distribution and quality uneven
Uneven distribution of mates	No - grouping of males or females does not occur
Long/Asynchronous breeding season	Yes
No need for bi parental care	Yes - female care ends at laying and male at hatching*
One sex is limiting and determine the type of polygamy	Yes - number of eggs male can incubate < number of eggs female can lay

Monogamy profile	**Support in *Apteryx mantelli***
Low habitat variability	No - both distribution and quality uneven
Need for parental care	Yes - big investment in offspring by both male and female
Longevity	Yes - over 40 years
Mate choice mechanisms for instance through olfaction	Yes - well-developed sense of smell

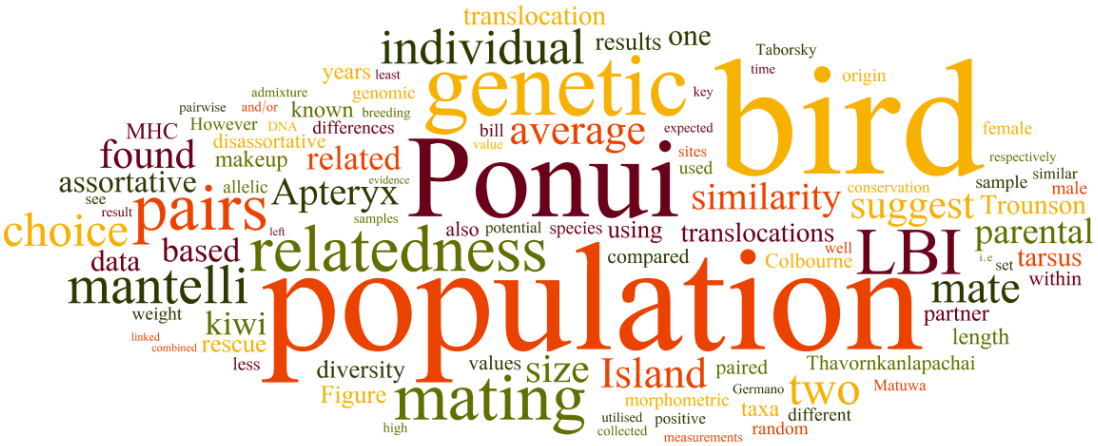
*chicks frequently stay around the nest and/or dad for days to weeks after hatching. No active parental care, such as food provisioning, occurs during this time; still, it is possible that chicks experience benefits by staying such as reduced predation risk or energy expenditure.

As stated above, the only described mating system in *A. mantelli* so far is monogamy. However, recent studies have found intriguing mixed parentage among eggs incubated by the same male in several *A. mantelli* populations, suggesting that the mating system might have more variability than previously appreciated (Ziesemann 2011, Vieco-Gálvez 2019). Importantly, the studies that have described *A. mantelli* as strictly monogamous have taken place under population densities that are now the norm, but these are severely reduced compared to the inferred historical densities (Potter 1990, Robertson *et al.* 2016, Germano *et al.* 2018). There are indeed several examples of species where the occurrence of different breeding systems changes with population density, habitat alterations, and/or lack of available territories (Pribil & Searcy 2001, Walker *et al.* 2008, Griffith *et al.* 2010). In line with this, I suggest that close

investigation of the *A. mantelli* breeding system, and how it changes with habitat features and population density, holds promise of furthering our understanding of how to best manage this species. In addition, such studies have the chance to further the general understanding of the drivers of breeding systems. Lastly, the breeding system is closely intertwined with mate choice. Studies of mate choice in these long-lived birds with few morphological distinguishing features, but with a highly developed sense of smell, would be important to improve our ability to predict outcomes of management interventions, such as translocations with the aim of achieving genetic rescue. Methods targeting genomic markers will likely play a crucial role in such investigations thanks to their high resolution which overcomes some of the limitations of the more traditional genetic markers such as microsatellites (Putman & Carbone 2014, Kardos *et al.* 2016, Oyler-McCance *et al.* 2016). For example, the resolution provided by genomic studies have great potential to prove beneficial when parental candidates are close relatives, or when multiple genetic loci or phenotypic characteristic are associated with kin-recognition and mate choice (Hughes 1998, Hatchwell *et al.* 1999, Baglione *et al.* 2002c, Griffith *et al.* 2002, Lettink *et al.* 2002, Richardson *et al.* 2002, Cockburn 2006, Ziesemann 2011, Preston *et al.* 2013, Koenig & Dickinson 2016, Leedale *et al.* 2018, Galla *et al.* 2020).

Chapter 2.2

Mixed mating in a multi-origin population suggests high potential for genetic rescue in North Island brown kiwi, *Apteryx mantelli*



This Chapter has been invited for submission to a special issue of Frontiers in Conservation Science called ‘Animal Behavior After Translocation into Novel Environments’. All supervisors provided feedback on one or more drafts of this chapter. The expertise of the Elshire Group, Tea Break Bioinformatics and Isabel Castro, the help from Brianna Nelson and Trish McLenachan, the hard work of Natasha, Stephen, Mo, Yi, other field helpers, and previous members of the Ponui Island team, and, not the least, the hard work and expertise of Richard Witehira, and the support from Mana Tangata and landowners were crucial for this chapter. See further Appendix 6.

Abstract

Reinforcement translocations are increasingly utilised in conservation with the goal of achieving genetic rescue. However, concerns regarding undesirable results such as genetic homogenisation or replacement are widespread. One factor influencing translocation outcomes is the rate at which the resident and the introduced individuals interbreed. Consequently, post-release mate choice is a key behaviour to consider. Here I studied mating and its consequences for genomic admixture in the North Island brown kiwi *Apteryx mantelli* population on Ponui Island which was founded by two translocation events over 50 years ago. The two source populations used are now recognised as belonging to two separate management units where birds differ in size and are genetically differentiated. I examined the correlation between male and female morphometrics for 17 known pairs and quantified the relatedness of 20 pairs from this admixed population. In addition, I compared the genetic similarity and makeup of 106 Ponui Island birds, including 23 known pairs, to birds representing the source populations for the original translocations. I found no evidence for size-assortative mating. On the contrary, genomic SNP data suggested that kiwi of one feather did not flock together, meaning that mate choice resulted in pairing between individuals that were less related than expected by random chance. Furthermore, the birds in the current Ponui Island population were found to fall along a gradient of genomic composition consistent with non-clustered representation of the two parental genomes. These findings indicate potential for successful genetic rescue in future *Apteryx* reinforcement translocations, a potential that is currently underutilised due to restrictive translocation policies. Thus, I suggest that reconsideration of these policies could render great benefits for the future diversity of this iconic genus in New Zealand.

2.2.1 Introduction

Translocations are increasingly utilised in conservation management to reintroduce species within their former ranges, introduce them to new sites predicted to be suitable for them, or reinforce already existing populations (IUCN/SSC 2013). One type of reinforcement translocation involves moving genetically distinct individuals into a target population. When such interventions result in a fitness increase, it is commonly referred to as genetic rescue (Ingvarsson 2001, Hedrick *et al.* 2011, Whiteley *et al.* 2015, Bell *et al.* 2019). Support is growing for genetic rescue being an effective tool, especially for target populations showing symptoms of inbreeding depression (Frankham 2015, Whiteley *et al.* 2015, Bell *et al.* 2019, Ralls *et al.* 2020). Yet, several recent publications argue that genetic rescue has not been attempted as often as it should (Frankham 2015, Ralls *et al.* 2018, Ralls *et al.* 2020). One reason for hesitation towards this deliberate admixture of individuals is the perceived high risk of causing loss of unique genetic diversity through homogenisation or genetic replacement (Love Stowell *et al.* 2017, vonHoldt *et al.* 2018, Ralls *et al.* 2020).

A key factor determining whether a translocation results in rescue, homogenisation, or replacement is whether, and at what rate, introduced and original individuals interbreed (Hedrick & Fredrickson 2010, Adams *et al.* 2011, Weiser *et al.* 2013, Mussmann *et al.* 2017, Thavornkanlapachai *et al.* 2019). Consequently, mate choice, and specifically assortative mating, has the potential to greatly affect the outcome of translocations (Bradley *et al.* 2014, Engler *et al.* 2019). Assortative mating is defined as a positive or negative correlation between phenotypic or genetic traits of paired males and females (Jiang *et al.* 2013). Some level of positive assortative preference (‘like mates alike’) is fundamental in biology since this is a key mechanism for generating new as well as

preserving existing species, subspecies, and ecotypes (Verzijden *et al.* 2005, Kopp *et al.* 2017, Schumer *et al.* 2017, Janicke *et al.* 2019). However, the results of positive assortative mating are context- and situation-dependent (Schumer *et al.* 2017). For instance, in birds as well as other groups, this behaviour has been shown to maintain sharp morphological separation in hybrid zones (Semenov *et al.* 2017) but has also been suggested to explain cases of instant speciation through hybridisation (Melo *et al.* 2009, Hermansen *et al.* 2011). This mating strategy has been linked to preference for matching plumage colouration or pattern in several bird species (Andersson *et al.* 1998, Masello & Quillfeldt 2003, Semenov *et al.* 2017, Sun *et al.* 2019). Yet, the most discussed and studied type of positive assortative mating is size matching between males and females, generally resulting in big individuals mating with other big individuals and small with small (Jiang *et al.* 2013, Janicke *et al.* 2019).

The opposite of positive assortative mating is negative assortative mating or 'opposites attract', which is also known as disassortative mating (Jiang *et al.* 2013). Several studies of birds have found strong evidence of disassortative mating being a key mechanism for ensuring high fitness of offspring, avoiding incest, and reducing inbreeding (Walters *et al.* 1988, Nelson-Flower 2009, Nelson-Flower *et al.* 2012, Riehl & Stern 2015, Riehl 2017). Most research on disassortative mating has focused on the major histocompatibility complex (MHC; Brown 1998, Bonneaud *et al.* 2006, Kamiya *et al.* 2014, Santos *et al.* 2016, O'Connor *et al.* 2019). Growing evidence suggests a strong heterozygotic advantage for these immune system genes, resulting in fitness benefits for pairs with differing or otherwise compatible MHC profiles (Strandh *et al.* 2012, Kamiya *et al.* 2014, Brambilla *et al.* 2018, Hoover *et al.* 2018). However, disassortative mating has also been linked to other traits. A famous example is the white-throated sparrow

(*Zonotrichia albicollis*). Within this species, mating almost exclusively occurs between individuals of the opposite of the two prevalent colour morphs (Hedrick *et al.* 2018).

Whether positive or negative, assortative mating is by necessity linked to mechanisms for detecting compatibility, phenotypic similarity, and/or genetic kinship (Kopp *et al.* 2017). The mechanisms behind such kin-recognition vary greatly between species, but can be based on visual (Walters & Garcia 2016, Sun *et al.* 2019), audible (Humphries 2013), and/or scent cues (Zelano & Edwards 2002, Bonadonna & Sanz-Aguilar 2012, Strandh *et al.* 2012, Hoover *et al.* 2018), and the ability to detect (dis)similarity can be learnt or innate (Riehl & Stern 2015). Zelano and Edwards (2002) speculated that the most likely birds to evolve the ability to make active mate choice based on MHC profiles and compatibility would (1) lack the opportunity for kin-recognition based on social recognition, for instance, due to having precocial young leaving the parents at an early stage, (2) have at least one parent whose only or main contribution to the offspring is genetic material, (3) be long-lived, (4) be monogamous, and (5) have a highly developed olfactory sense. Part of the reasoning behind this is that a long life-span and high partner fidelity generates selection for mechanisms identifying optimal partners (Kvarnemo 2018). Intriguingly, when comparing across Aves, Zelenitsky *et al.* (2011) concluded that two extant groups stand out by being associated with large size of the olfaction bulb and positive selection for this part of the brain compared to their sister clades: the genus *Apteryx* (the kiwi birds) and the order Procellariiformes (the petrels and allies, including albatrosses and shearwaters). Supporting Zelano and Edwards (2002), studies of Procellariiformes have found olfaction-based kin-recognition in European storm petrels, *Hydrobates pelagicus,* and blue petrels, *Halobaena caerulea* (Bonadonna *et al.* 2004, Bonadonna & Sanz-Aguilar 2012) as well as MHC-based

disassortative mating in Leach's storm-petrel (*Oceanodroma leucorhoa*) and *H. caerulea* (Strandh *et al.* 2012, Hoover *et al.* 2018).

Strikingly, the second group identified by Zelenitsky *et al.* (2011) as being selected for olfactory capacity – *Apteryx* – meets all five criteria proposed by Zelano and Edwards (2002). In particular, the North Island brown kiwi, *Apteryx mantelli* (1) has super-precocial chicks, allowing conservationists to collect eggs and hatch them in captivity without the presence of a parent (Colbourne *et al.* 2005), (2) has male-only incubation (Colbourne 2002), (3) can live for at least 40 years (Barlow 2011, Robertson & de Monchy 2012), (4) shows high levels of pair fidelity and monogamy (Taborsky & Taborsky 1999), and (5) uses scent as a primary sense (Cunningham *et al.* 2009, Castro *et al.* 2010).

Here I exploit research opportunities provided by the unique high-density *A. mantelli* population on Ponui Island that has been thoroughly researched for the last 17 years. This population is the result of two translocation events over 50 years ago. The origin of the translocated birds was Little Barrier Island and the Waipoua Forest, and these populations have more recently been deemed to belong to different genetic taxa and management units within *A. mantelli* (Burbidge *et al.* 2003, Colbourne 2005, Germano *et al.* 2018). The unique features of *A. mantelli*, combined with the research effort put in and the multi-origin background of the study population, make the *A. mantelli* on Ponui Island ideal for mate choice research. I examine size matching and genetic (dis)similarity of 17 to 23 pairs by utilising morphometric data collected over 17 years combined with high-density SNP data obtained through genotype-by-sequencing (GBS; a method for reduced representation sequencing; Elshire *et al.* 2011). I then discuss what the results suggest about *A. mantelli* mate choice and the plausible implications of

such choice for future *A. mantelli* conservation efforts focusing on reinforcement translocations aiming to achieve genetic rescue.

2.2.2 Materials and Methods

2.2.2.1 Study sites and sampling

Ponui Island is 1770 ha, and its kiwi density is today estimated to one bird per ha. Over the last 17 years, at any given time between 30 and 50 adult *A. mantelli* on Ponui Island have been tagged with radio transmitters. Tracking these transmitterised birds, combined with detailed studies of their habitat use, foraging, and breeding (partly using camera traps) has provided extensive data on individual identities and associations between these individuals. The Ponui Island population was founded through two translocations in 1964 of eight birds from the Waipoua Forest (*A. mantelli* Northland) and six birds from Hauturu-o-Toi (also known as Little Barrier Island (LBI); Colbourne 2005). The LBI birds are usually considered *A. mantelli* Western, but some claim their origin as hybrid or unclear (Burbidge *et al.* 2003, Colbourne 2005, Germano *et al.* 2018). Unfortunately, data could not be collected directly from these parental populations due to low population density (Waipoua) and restricted access (LBI). Fortunately, we were able to source samples from sites considered to be sufficiently similar to the true parental populations to act as representative of them for our analyses. Waipoua was represented by birds from Trounson Kauri Park (hereafter Trounson) since these sites are geographically close and movement of birds between them has been recorded several times (Meduna & Donovan pers. comm. 2020). Little Barrier Island samples were collected from the Remutaka Forest and the Pūkaha National Wildlife Centre (also known as Mt Bruce) from birds that were translocated to these sites from

LBI in 2010 and 2008, respectively. Throughout this text, these birds will be referred to as birds from LBI to reduce confusion.

The full dataset utilised herein contained 11 birds from LBI, 20 birds from Trounson, and 106 birds from Ponui Island, including 23 known pairs which are the main focus of the study presented herein. However, for specific analyses, datasets consisted of 17, 20, or 23 pairs and 74 or 106 Ponui Island birds, see further below. For details on how pairs were defined, see Chapter 2.3.

2.2.2.2 Blood sampling and morphometric measurements

Blood sampling and measurements of the birds were conducted in accordance with the Kiwi Best Practice Manual (Robertson & Colbourne 2017), the Massey University Animal Ethics Committee (MUAEC) permits 06/05, 07/144, 16/92, and 18/83, and the Department of Conservation Wildlife permits AK-14969-RES, AK-21519-FAU, 50249-FAU and 70875-RES. Blood was sampled on Ponui Island in four separate cohorts: in 2004, 2006-2008, 2010, and 2017-2018 (see further Appendix A1). Most of the samples utilised were collected in 2017, but older samples were used to increase sample size and spatial spread across Ponui Island. Samples from the parental populations were collected in 2020. For birds fitted with transmitters (most of the Ponui Island birds analysed, all the studied pairs, and most of the LBI birds), blood sampling and measuring occurred together with the annual transmitter replacement. A licenced kiwi dog assisted with catching the remaining birds, which were caught specifically for this study.

The morphometric measurements taken were weight, tarsus length (TL), tarsus width (TW), bill length, weight to TL ratio, and bill length to TL ratio. Body condition was

calculated based on weight and TW following Taborsky and Taborsky (1999). All morphometric parameters were available for 17 out of the 23 Ponui Island pairs, and Pearson correlations between female and male values were investigated using R (R core team version 3.6.2). To make the morphometric comparison meaningful, only measurements of adult birds from LBI and Trounson were used in these analyses (n = 5 from LBI and 15 from Trounson). Birds were considered adults if they were known to have bred or, when the breeding status was unknown, based on their sex, size, and weight combined; females were considered adult if weighing > 2000 g, or > 1700 g if having a TW > 11 mm or a bill > 113 mm; males were considered adults if weighting > 1700 g, or > 1400 g if having a TL > 90 mm or a bill > 90 mm.

2.2.2.3 DNA purification and sequencing

DNA was extracted from 10-50 µl thawed *A. mantelli* blood using a High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The manufacturer's instructions were followed with the exception that the DNA was eluted twice using 50 µl of elution buffer for each centrifugation round. The DNA extraction success and quality were validated using agarose gel electrophoresis (1% (w/v) agarose in 1x TAE buffer) and the concentration of DNA was measured with the Qubit 2.0 fluorometer using the dsDNA High Sensitivity assay (Life Technologies, CA, USA). The GBS library was generated following the Elshire *et al.* (2011) method and included the following changes: 100 ng of genomic DNA were used, 1.44 ng of total adapters were used, the genomic DNA was restricted with the enzyme EcoT22i, and the library was amplified with 18 PCR cycles. For further details GBS library preparation, sequencing, SNP calling, and bioinformatic pipelines see Chapter 3.1.

2.2.2.4 Relatedness and genetic makeup

Single nucleotide polymorphism (SNP) calling was carried out in the STACKS 2.5 pipeline. After linkage disequilibrium (LD) pruning and reduction to one variable site per loci, 51 691 SNPs were utilised for analyses. A re-assembly of the published *A. mantelli* genome (Le Duc *et al.* 2015) was used as reference. Unscaled pairwise relatedness (allelic similarity) values were derived using KGD (Dodds *et al.* 2015). These values were used to quantify the relatedness between the male and female in each pair in three ways. To evaluate whether paired individuals were more or less related than expected under a scenario or random mating, test one, compared pairwise relatedness between members of known pairs (n = 20) to the average relatedness of each paired individual to 74 Ponui Island birds using a t-test. Test two, compared ranked relatedness of the female to the male and vice versa for each pair to a ranked relatedness under a scenario of random mating. A ranking of 1 suggested that the partner represented the least related bird within the dataset and a ranking of 73 that the partner was the most related bird in the dataset. Random mating was simulated using 10*40 randomly drawn ranks between 1 and 73. Lastly, to relate the genetic makeup of the Ponui Island birds to the mixed heritage of the population, genetic similarity between partners was plotted by using the average pairwise relatedness of each paired individual (n = 23 Ponui Island pairs) to the 11 birds from LBI. The average relatedness to LBI for all 106 Ponui Island birds in the dataset was used as a threshold value to split the relatedness space into four quadrants after which the average relatedness of each paired individual to birds from LBI was used to classify pairs as being of ‘similar’ or ‘different’ genetic makeup.

To explore the effect of mate choice over time on the summarised genome across the Ponui Island population, the average relatedness to the LBI and the Trounson birds, was calculated for each of the Ponui Island birds in the sample (*n* = 106 Ponui Island birds). These two factors of relatedness were then linearly normalised to span from 1 to 0; i.e., the Ponui Island birds most related to Trounson had a value of 1 and the bird least related a value of 0, and same was done for the relatedness of Ponui Island birds to LBI birds. The relatedness of each Ponui Island bird to LBI was then plotted against the same bird's relatedness to Trounson to explore potential skews towards either parental population and/or patterns of discontinuous spread (or clustering) along the relatedness gradient suggestive of positive assortative mating over time.

2.2.3 Results

2.2.3.1 Morphology

The birds of the two parental populations to Ponui Island were found to differ in size, with birds of LBI origin being smaller on average (Figure 2.2.1). This was true for bill length (t = -3.78, df = 6.60, p-value = 0.008), tarsus length (t = -3.22, df = 8.29, p-value = 0.012), as well as bill to tarsus ratio (t = -6.93, df = 12.24, p-value < 0.001). The span of individual morphometric values of the Ponui Island birds covered the range of both parental populations (Figure 2.2.1), and the distribution within the Ponui Island population was continuous when accounting for the differences in male and female size (Robertson & Colbourne 2017). None of the analysed morphometric characteristics (bill length, weight, tarsus length, tarsus width, bill to tarsus and weight to tarsus ratio, or body condition) showed any support for size assortative mating among the Ponui Island pairs (*n* = 17 pairs; Figure 2.2.2).

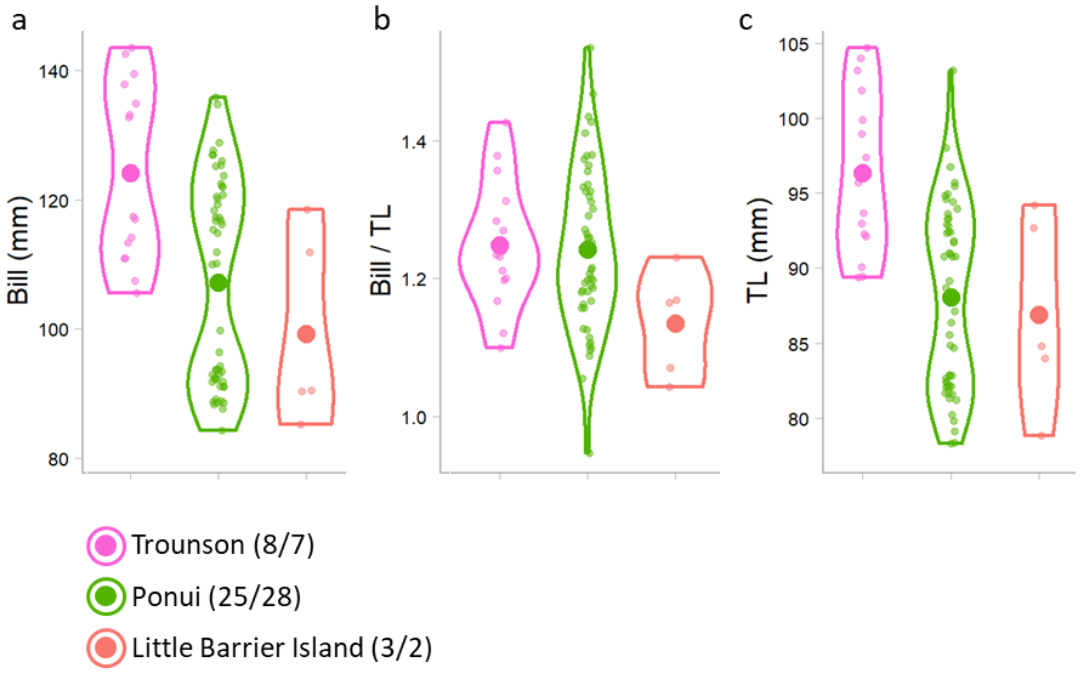


Figure 2.2.1. Violin plots comparing morphometrics of adult *A. mantelli* from Little Barrier Island, Ponui Island, and Trounson Kauri Park, specifically bill length (a), tarsus length (c), and the ratio between the two (b). Small dots represent each data point. Large dots represent the average for each population. The shape of the violin outline indicates the distribution of the data points. Sample size is provided in the legend after each population as males/females.

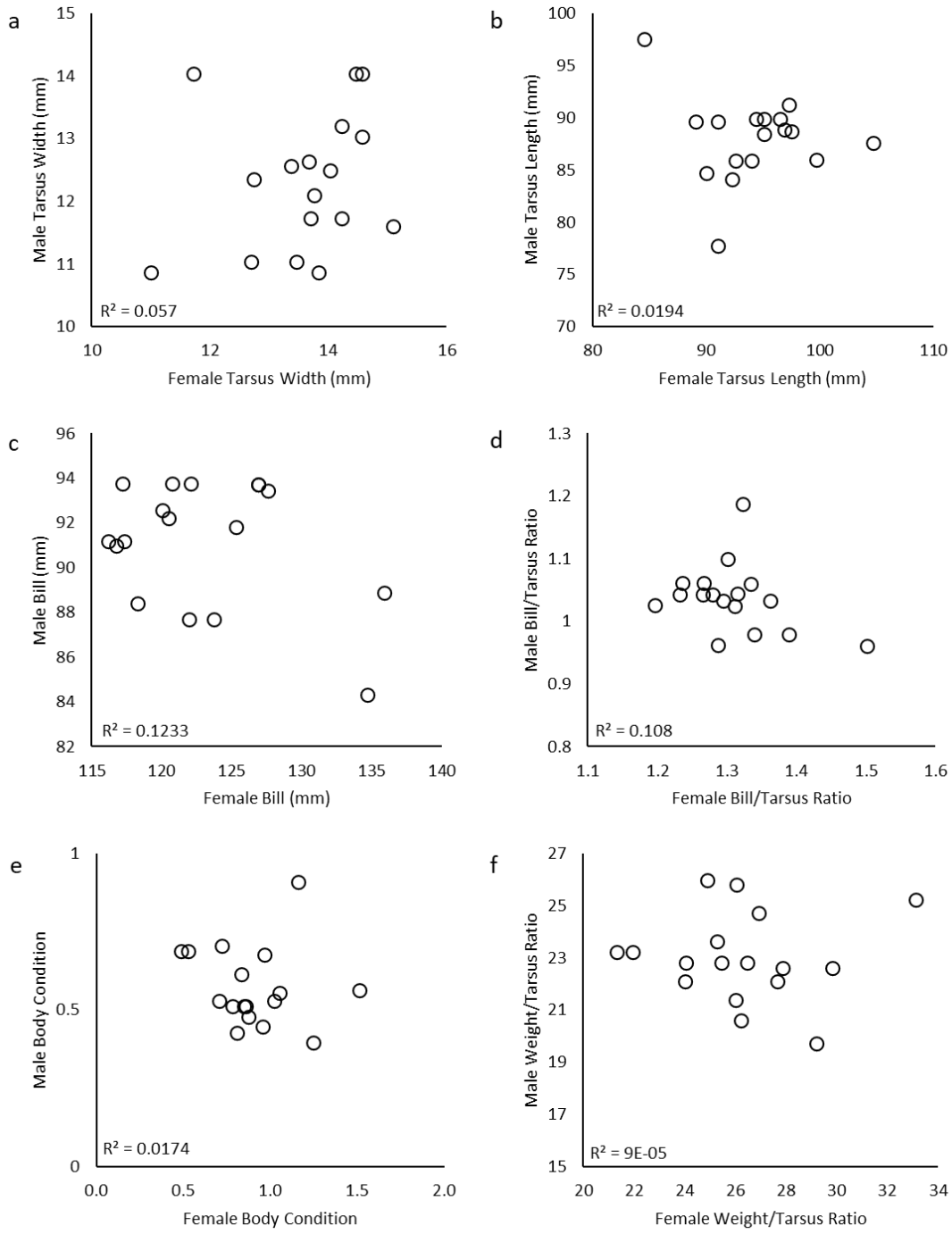


Figure 2.2.2. Correlations between morphometric measurements of paired male and female *A. mantelli* from Ponui Island. The unit for weight is grams. Bill, tarsus length, and tarsus width are given in millimetres. Body condition is based on a combination of weight and tarsus width (Taborsky 1999). None of the correlations were found to be significant. R^2 values refer to linear regression.

2.2.3.2 Genetic similarity between partners

Overall, the allelic similarity within pairs was found to be significantly lower compared to the paired individuals average relatedness to all other Ponui Island birds in the dataset (0.057 vs 0.011, respectively; t = -4.06, df = 39, p-value < 0.001; *n* = 20 pairs and 74 birds overall; Figure 2.2.3). Looking at individuals, 75% of the paired birds had a lower value of allelic similarity to their partner compared to their average similarity to the full dataset. The results based on ranked relatedness further supported that pairs were less related than expected under random mating (t = 2.43, df = 73.25, p-value = 0.018). On average, 69% of the birds in the full dataset had a higher ranked relatedness than the partner of each individual. In five cases, the partner was in fact one of the top three least related individuals in the dataset. However, there was large variation, and one pair (Genesis and Susie) was found to have an allelic similarity close to that of first-degree relatives.

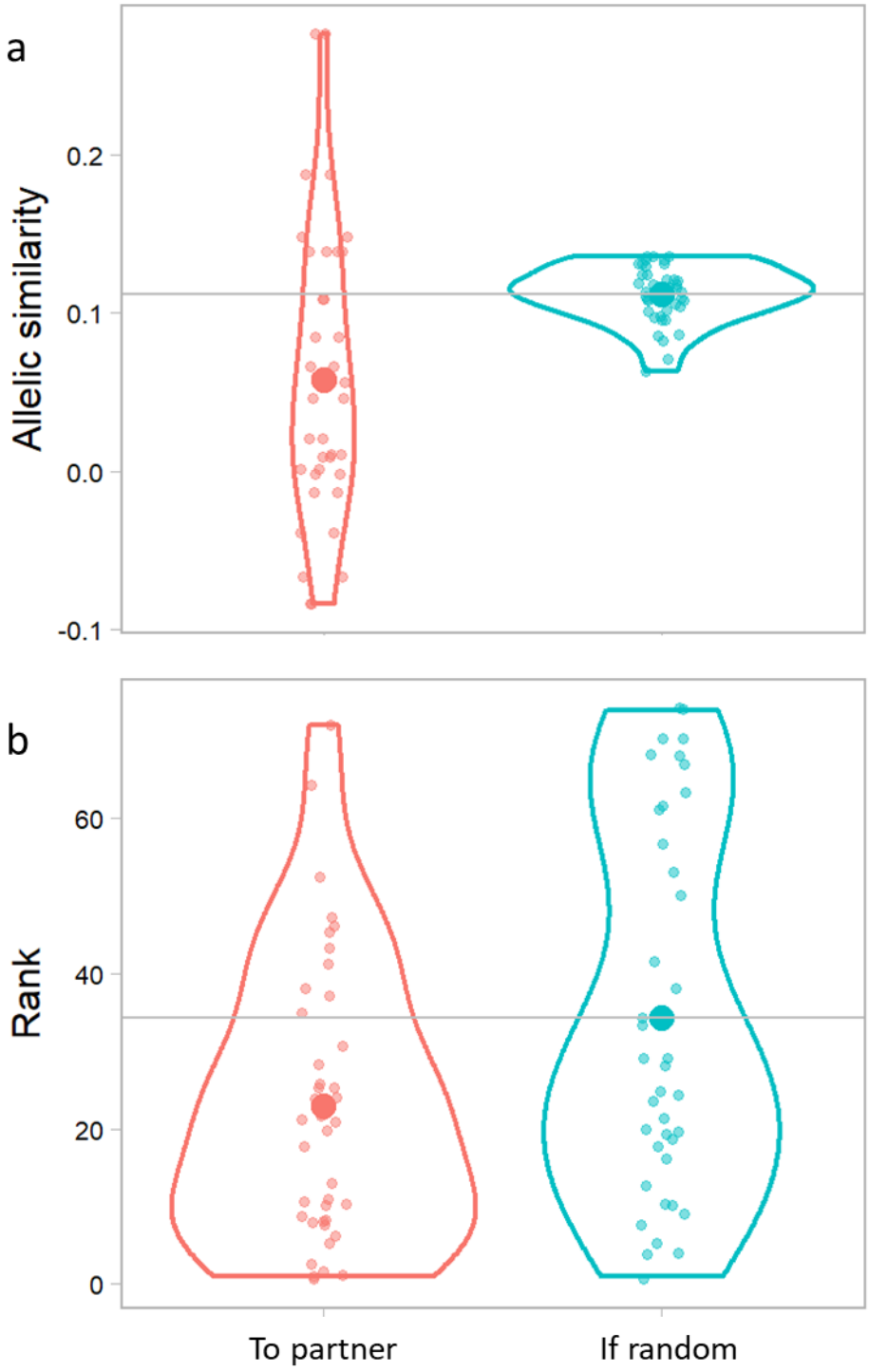


Figure 2.2.3. Violin plots illustrating relatedness between paired individuals (*n* = 20 pairs) compared to the expectation based on random mating. (a) illustrates pairwise relatedness based on allelic similarity (lower values suggest less similarity) between each paired bird and its partner (left) compared to each paired bird's average relatedness to the entire sample set (right; *n* = 74). (b) ranked (or relative) relatedness of each paired bird and its partner (left) compared to

one example iteration of 40 rank values drawn at random. The least related individual in the sample set was assigned rank 1 and the most related rank 73. Small dots represent each data point, and the shape of the outline represents their distribution. Large dots represent the average. Horizontal grey lines illustrate the significantly lower average for the observed pairs compared to the null hypothesis of random mating.

2.2.3.3 Population genetic consequences of mate choice over time

An equal number of pairs could be classified as similar (both male and female over or under average relatedness to LBI) and different (one bird less and one more related to LBI than average; 10 pairs *versus* 10 pairs; Figure 2.2.4a). The remaining three pairs were placed in neither category due to either or both partners falling on the threshold value (0.07; Figure 2.2.4a).

Looking at the effect of mate choice over time on the genetic makeup of the Ponui Island population suggested that there was strong negative correlation between relatedness to LBI and Trounson. This correlation formed an undisrupted gradient and indicated no clustering (Figure 2.2.4b).

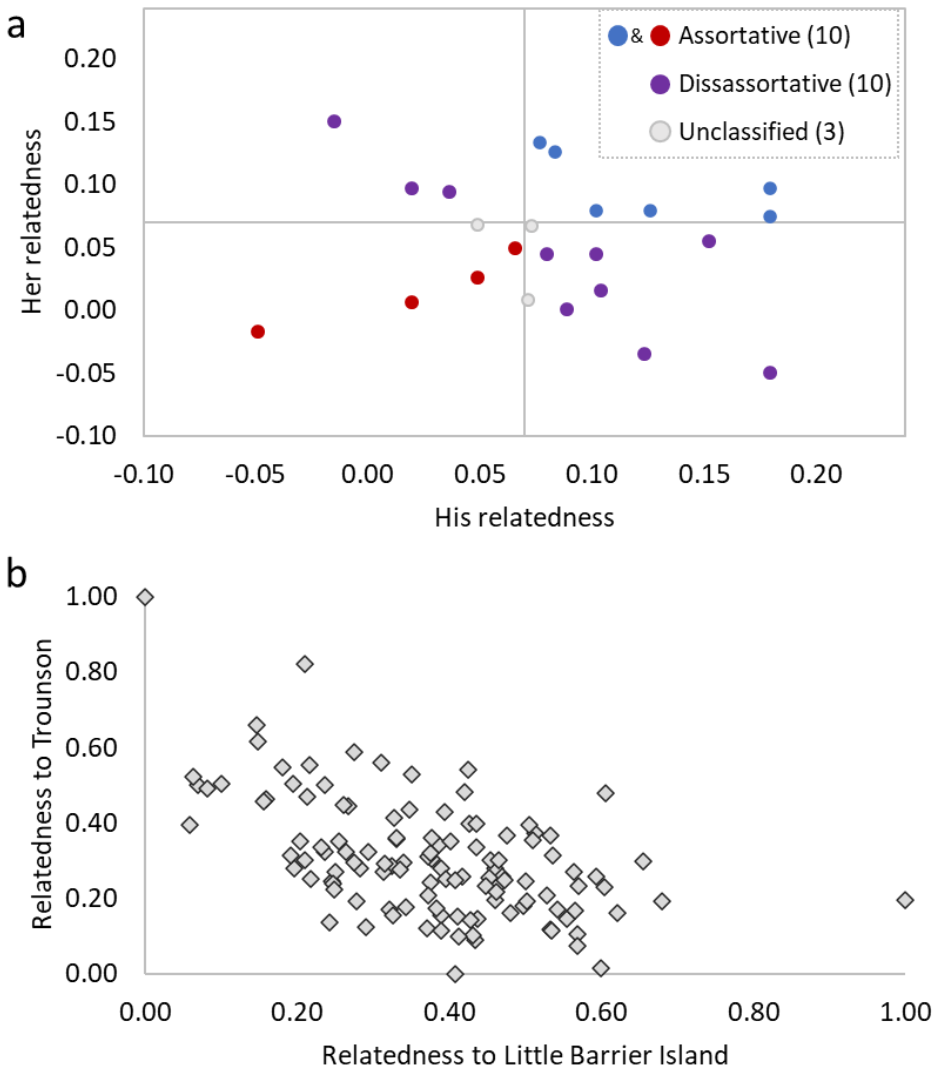


Figure 2.2.4. Genetic makeup of Ponui Island pairs (a) and the Ponui Island population overall (b). In panel (a) 23 Ponui Island pairs are represented with one dot each and grey lines divide the graph into quadrants using the average relatedness of 106 Ponui Island birds to the 11 LBI birds as threshold value. In pairs falling in the lower left quadrant (red dots), both the male and female are less related to LBI than the average Ponui Island bird and, thus, classified as 'assortative' or 'similar'. Pairs in the upper right quadrant (blue) are also 'assortative' or 'similar' (both less related to LBI that average), while the top left and bottom right quadrant contains pairs where the male and the female fall on opposite sides of the threshold and are, hence, referred to as 'disassortative' or 'different' pairs (purple). Three pairs fall on the threshold value and remain unclassified (grey). In panel (b) each of the 106 Ponui Island birds is represented by a diamond indicating their normalised relatedness to LBI and Trounson birds. Relatedness values were spread uniformly across the gradient, i.e., the scatter plot indicates no genetic clustering suggestive of positive assortative mating. Compare Figure 3.1.7a.

2.2.4 Discussion

Taken together I found no evidence for positive assortative mating in *A. mantelli*. This was supported both by morphometric data, which indicated no size assortative mating, and genetic data, which suggested an equal likelihood of pairing with an individual of similar or dissimilar genetic makeup. In fact, pairwise allelic similarity and ranked relatedness suggested that mates were less related to each other than expected by chance.

Our limited morphometric sample set from LBI and Trounson suggested that these populations differ morphologically with LBI individuals being smaller. Hence, size assortative mating following the translocation of birds from these populations to Ponui Island could have resulted in limited admixture. On the contrary, the morphological distributions of Ponui Island birds spanned that of both parental populations. This result is striking since size matching has frequently been observed across multiple animal taxa including birds (Delestrade 2001, Helfenstein *et al.* 2004, Ippi *et al.* 2018). The apparent grouping in the two size clusters suggested in Figure 2.2.1 can be explained by sex differences in size rather than the presence of two morphotypes on Ponui Island. In addition, the genetic analyses indicated a continuum of relatedness to the two founder genomes without clustering. Both these lines of evidence suggest that the Ponui Island population in 2017 – close to six decades after the translocation – has retained genetic material from both parental populations and that the population today constitutes a successful hybrid swarm with large individual variation in genomic makeup and no clear separation into a LBI and a Waipoua cluster within the population (see further Chapter 3.1).

Based on allelic similarity, mate choice on Ponui Island tended to be disassortative. The Ponui Island population originating from two distinct taxa (Colbourne 2005, Germano *et al.* 2018) might have artificially increased the opportunity for disassortative mating in this population. On the other hand, Ponui Island represents one of very few *Apteryx* populations that exhibit what is thought to resemble kiwi densities found before introduction of exotic predators (Potter 1990, McLennan & Potter 1992, Miles *et al.* 1997, Craig *et al.* 2011, Germano *et al.* 2018) and, consequently, a comparatively 'natural' frequency of mate choice opportunity. Thus, these results warrant further investigation. Disassortative mating based on phenotype has been found to be rare (Jiang *et al.* 2013), and *A. mantelli* fit the profile of a bird expected to exhibit mate choice linked to MHC compatibility, for instance, because of its excellent sense of smell (Zelano & Edwards 2002, Cunningham *et al.* 2009, Castro *et al.* 2010, Corfield *et al.* 2014, Le Duc *et al.* 2015). Thus, I suggest that scent-guided disassortative mating to maximise MHC diversity/compatibility and/or minimize inbreeding is a likely mechanism at play for mate choice in *Apteryx* that requires further investigation. Studies of MHC diversity and structure in *Apteryx* have so far focused on the two species with the most drastic bottleneck history: *A. rowi* and *A. owenii* (Binney 2007, Miller *et al.* 2011a). The MHC diversity in *A. owenii* was found to be extremely low (Binney 2007, Miller *et al.* 2011a). If MHC profiles are important for *Apteryx* mate choice as well as fitness, this low diversity could add an additional hurdle to *A. owenii* recovery. On the contrary, MHC diversity in *A. rowi* was found to be higher than expected based on their small current population size and recent tight bottleneck (Binney 2007). This finding could suggest that a population reduction to about 150 birds (as in *A. rowi*) still allowed for mate choice to keep MHC diversity high, while a population minimum as low as five breeding birds (as in *A. owenii*) left little or no variation for mate choice to act

upon. Further studies are needed to verify the existence of a mechanism for kin-recognition in *Apteryx* and to identify the cues involved. A first step would be to further examine the genetic dissimilarity between partners and investigate if it is predominantly linked to and/or exaggerated in certain regions of the genome (Beaumont & Balding 2004, Fitzpatrick *et al.* 2010, Keller *et al.* 2013). Such studies would not only increase our understanding of *Apteryx* breeding but also take us closer to understanding the significance and nature of the genetic differences observed between *Apteryx* taxa today (see further Chapter 1.2).

An interesting parallel to the *A. mantelli* population on Ponui Island is the population of burrowing bettongs (or boodie, *Bettongia lesuer*) in Matuwa (Western Australia). This population was established in 2010 through the translocations of individuals from Barrow Island and Dryandra (Thavornkanlapachai *et al.* 2019). Similar to the case of Ponui Island, the Barrow Island and Dryandra populations represent two distinct taxa within *B. lesuer* (sometimes referred to as different subspecies; Thavornkanlapachai *et al.* 2019). A second similarity is that the Dryandra population, similar to LBI, was the result of a previous translocation from Dorre Island. Thirdly, these taxa show marked differences in phenotype, with the Barrow Island bettongs being substantially smaller (Short & Turner 1999, Donaldson *et al.* 2017). This size difference remained after translocation, suggesting a genetic origin (Thavornkanlapachai *et al.* 2019). When exploring the results of this multi-origin introduction, Thavornkanlapachai *et al.* (2019) and Rick *et al.* (2019) found that due to the timing of the two translocation events, the first breeding at Matuwa happened almost exclusively within the two taxa. However, after three years, over half of the offspring in the Matuwa population were found to be of mixed origin (Thavornkanlapachai *et al.* 2019). Moreover, even though not all haplotypes in the two parental populations were found to be present in Matuwa, genetic

variation was overall higher in the mixed population compared to the parental populations (Rick *et al.* 2019, Thavornkanlapachai *et al.* 2019). Interestingly, for some measurements, the F1 intermixed offspring in Matuwa were larger than the larger of the two parental populations, a phenomenon referred to as transgressive segregation (Koide *et al.* 2019, Thavornkanlapachai *et al.* 2019).

There are some key differences between the bettongs at Matuwa and the kiwi on Ponui Island that will affect how the intermixture contributes to the genomic composition of the population. First, while the Ponui Island population was founded with only 14 *A. mantelli*, Matuwa received over 150 founders, suggesting the founding population might have harboured more diversity. Second, *B. lesuer* breeds in a harem-like or polygynous system (whereas *A. mantelli* predominantly breeds in long-term stable monogamous pairs), potentially skewing breeding success to a smaller number of *Bettongia* males while also allowing for more variation in parentage between years (Sander *et al.* 1997, Taborsky & Taborsky 1999, Colbourne 2002; however, see further Chapters 2.1 and 2.3). Thirdly, the *Bettongia* taxa are believed to have been separated for about 8000 years while some authors suggest the separation between Western and Northland kiwi to be substantially longer (Weir *et al.* 2016). Lastly, Thavornkanlapachai *et al.* (2019) found clear patterns of size matching that resulted in interbreeding happening predominantly between females of Barrow Island phenotype (i.e., the smaller taxa) and males of Dryandra phenotype (i.e., the larger taxa). One result of this 'directional' admixture was that genetic examination based on mitochondrial *versus* nuclear genes painted very different pictures. The contrasting distribution of mitochondrial and nuclear haplotypes would be an interesting parameter to explore in *A. mantelli* to further our understanding of mate choice in this species. Despite their differences, the *A. mantelli* on Ponui Island and the *B. lesuer* in Matuwa

can both be seen as examples where neither genetic nor phenotypic differences need to be hurdles for generating successful and genetically diverse populations through active augmented geneflow, translocations, and admixture (Frankham 2015, Frankham *et al.* 2017, vonHoldt *et al.* 2018, Rick *et al.* 2019, Hoffmann *et al.* 2020).

2.2.5 Conclusion

Our results suggest that *A. mantelli* do not mate assortatively, but rather seem to have the ability to distinguish kin from non-kin – an ability they seem to utilise to breed with dissimilar mates. This behaviour has the potential to work as a counterforce that reduces inbreeding (Walters *et al.* 1988, Nelson-Flower 2009, Nelson-Flower *et al.* 2012, Riehl & Stern 2015, Riehl 2017). Importantly, this suggests that interbreeding between resident and introduced kiwi is likely to happen after reinforcement translocations, and, thus, that genetic rescue is a probable outcome of such interventions. However, it also raises concern that, for the vast majority of *Apteryx*, the opportunity for mate choice is substantially restricted either due to low population densities, small population sizes, or artificial settings such as translocations to so-called kōhanga kiwi sites (sites where predators have been excluded, and the goal is to breed kiwi and harvest juveniles for introduction, reintroduction, and/or reinforcement translocations; Innes *et al.* 2016).

Currently, a discussion is ongoing about the potential impact of inbreeding on *Apteryx* fitness and viability (Weiser *et al.* 2013, Innes *et al.* 2015, Innes *et al.* 2016, Germano *et al.* 2018). At the same time, policy around translocations is restrictive, strongly recommending minimisation of the geographic as well as the genetic distance between target and source population (Craig *et al.* 2011, Scrimgeour & Pickett 2011, Innes *et al.* 2016, Kiwis for Kiwi 2016, Germano *et al.* 2018). Arguments for this policy is that mixing populations that are too different risks resulting in outbreeding depression, loss

of local adaptation, and overall loss of diversity. However, our results suggest that genomic admixture is a slow process that retains diversity from both parental populations. This steady but slow mixture of individuals could prove ideal for balancing the two key conservation goals of introducing genetic material without eradicating local diversity or homogenising population differences. Further support that there is currently underutilised potential for genetic rescue in *Apteryx* is the astonishing success of the Ponui Island population. This hybrid population has, on average, exhibited 9% annual population growth for over 50 years and is now one of the densest in the country despite the presence of some species of introduced predators and competitors (Cunningham & Castro 2011, Strang 2018). Consequently, I suggest that the results presented herein should contribute to conservation decision-making for *A. mantelli* as well as other species by (1) encouraging discussion about genetic rescue, (2) widening the view of what could constitute suitable source populations for translocations, and (3) emphasising how conservation management plans could benefit from incorporating opportunity to express the full range of natural behaviours – such as mate choice – as an explicit goal.

Chapter 2.3

Access to resources, rather than kin-selection, is the likely driver of group breeding and shared incubation in *Apteryx mantelli*

All supervisors provided feedback on one or more drafts of this chapter. The expertise of the Elshire Group, Tea Break Bioinformatics and Isabel Castro, the help from Brianna Nelson and Trish McLenachan, and the hard work of Natasha, Stephen, Mo, Yi, other field helpers, and previous members of the Ponui Island were crucial for this chapter.

Abstract

Prevalence of breeding strategies, for instance cooperative breeding, has been linked to changing environmental conditions, survival rate, population density, and territory quality. Consequently, breeding strategies and changes to breeding behaviours warrant more attention from a threat status, population viability, and conservation outcome perspective. *Apteryx mantelli* has so far only been reported to breed in monogamous pairs. However, studies have taken place in populations decimated by habitat destruction and invasive predators. For the first time, I describe the mating system in an *A. mantelli* population whose density is similar to that inferred during pre-human conditions. Seventeen years of study of this population suggests that the *A. mantelli* mating system is much more flexible than previously acknowledged. Specifically, I describe five different male breeding strategies, including regular occurrence of shared incubation and group breeding with varying sex compositions. High resolution SNP data suggested that kinship could have been a driver of group breeding behaviour in one case, but overall relatedness between breeding unit members was low. Rather than kin selection, the key drivers of group breeding seemed to be acquiring access to resources such as time and beneficial habitat. This variability makes *A. mantelli* a promising candidate system for further ground-breaking study of mating strategies. In addition, I suggest that investigations of the occurrence of group breeding in other *A. mantelli* populations could positively affect conservation policy concerning estimates of population density and success.

2.3.1 Introduction

Climate change, deforestation, and invasive species have repeatedly been causally linked to population decline and increased extinction risk. However, predicting which species will suffer, and in what way, remains challenging (Hoffmann *et al.* 2010,

Hoffmann *et al.* 2011, Frankham *et al.* 2017, IUCN 2020). Behavioural adaptation is believed to be an important factor governing how individual fitness, population viability, and threat status is affected by habitat alterations and environmental change (Charmantier *et al.* 2008, Massaro *et al.* 2008, Caro & Sherman 2011, Kalbitzer & Chapman 2018, Buchholz *et al.* 2019, Ducatez *et al.* 2020). Examples of such adaptations are changes to migration, time budgeting, diet, and phenology (Tuomainen & Candolin 2011, Merilä & Hendry 2014, Howard *et al.* 2018, Amélineau *et al.* 2019, Ducatez *et al.* 2020). Other behavioural adaptations that can greatly impact population viability are changes to breeding behaviours (Tuomainen & Candolin 2011, Sibly *et al.* 2012). There is convincing support for breeding strategy being driven by predation risk, territory quality or availability, and population density (Emlen & Oring 1977, Bayne & Hobson 2001, Banks *et al.* 2007, Griffith *et al.* 2010, Sibly *et al.* 2012, Winiarski *et al.* 2017, Kvarnemo 2018). Since all these factors are affected by anthropogenic environmental modifications, concern is warranted for an ongoing global shift in animal breeding behaviour. One example is that the presence and prevalence of cooperative breeding have been linked to environmental change (Griesser *et al.* 2017). Consequently, this breeding strategy warrants more attention from a conservation perspective.

Cooperative breeding is defined herein as a mating system in which more than a pair of conspecific individuals work together to raise young at a single nest or from a single brood. This breeding strategy is a relatively common among birds and has been recorded in several hundred species from more than 70 families (Koenig & Dickinson 2004, Cockburn 2006, Riehl 2013). The phylogenetic signal associated with cooperative breeding is relatively strong; the taxonomic distribution is uneven, ranging from single species within some groups (e.g., grosbeaks, wagtails, terns, and owls) to all or most

species in others (for instance anis, babblers, mousebirds, and fairy-wrens; Cockburn 2003, Koenig & Dickinson 2004, 2016). Perhaps most famously, as many as 45 species within Corvidae (crows and allies) exhibit cooperative breeding (Iwaniuk & Arnold 2004, Koenig & Dickinson 2004, Ekman & Ericson 2006). Examples of cooperatively breeding species in New Zealand are pukeko (*Porphyrio melanotus*; Craig 1980), rifleman (*Acanthisitta chloris*; Preston *et al.* 2016), and whitehead (*Mohoua albicilla*; McLean & Gill 1988).

While being considered one breeding strategy, there is large variation in how cooperative breeding is expressed among birds (Table 2.3.1). For many cooperatively breeding bird species, a breeding unit is a group consisting of a 'main pair' and several other individuals sometimes referred to as 'joiners' or 'helpers' (Strahl 1988, Haig *et al.* 1994, Canestrari *et al.* 2008, Riehl 2013, Preston *et al.* 2016, Koenig *et al.* 2019); but on other occasions, the groups are better described as multiple 'equal' individuals breeding together (Yuan *et al.* 2004, Riehl 2013). Breeding unit size is often variable both within and between species, ranging from two or three up to over 15 cooperating individuals (Heinsohn & Cockburn 1994, Hatchwell *et al.* 2004). In most species, only a proportion of the breeding units are groups while the rest are pairs (Woolfenden & Fitzpatrick 1978, Strahl 1988, Heinsohn & Cockburn 1994, Dickinson & Akre 1998, Miller & Jones 1999), but a few obligate cooperative breeding species are known (Courchamp *et al.* 1999, Riehl 2013). In the majority of cooperative breeders, group members are close relatives such as siblings or offspring from earlier clutches of the main pair; groups are thus often referred to as families (Woolfenden & Fitzpatrick 1978, Hunter 1985, Strahl 1988, Canestrari *et al.* 2008, Barve *et al.* 2019), but other species have non-related group members or a mixture of both (Reyer 1980, Yuan *et al.* 2004, Cox & Slater 2007, Riehl 2013). Commonly, there is a male bias among group members (Walters *et al.*

1988, Dickinson & Akre 1998, Baglione *et al.* 2002b, Hatchwell *et al.* 2004, Cox & Slater 2007, Barve *et al.* 2019), but female-biased groups do exist (Caffrey 1992, Richardson *et al.* 2002). In a few species, there is a clear division of labour between group members and in some of these, the extent of specialisation depends on group size (Reyer 1984, Ridley & Raihani 2007, Canestrari *et al.* 2008, Nelson-Flower *et al.* 2012). Lastly, a plethora of (sometimes contradictory) environmental conditions have been suggested to be strongly linked to cooperative breeding (Emlen 1982b, 1982a, Cockburn & Russell 2011, Riehl 2013, Griesser *et al.* 2017, Koenig 2017, Lukas & Clutton-Brock 2017), and many cooperative breeding species are territorial (Woolfenden & Fitzpatrick 1978, Walters *et al.* 1988, Baglione *et al.* 2002b, Cox & Slater 2007), but not all (Caffrey 1992, Hatchwell *et al.* 2004).

The most common way of assisting as a group member is food provisioning of the communally raised chicks (Reyer 1980, Hunter 1985, Sydeman 1989, McGowan & Woolfenden 1990, Hatchwell *et al.* 2004, Canestrari *et al.* 2008, Koenig & Walters 2016, Liebl *et al.* 2016, Preston *et al.* 2016). In addition, territory defence (Woolfenden & Fitzpatrick 1978, Hunter 1985), reducing risk of depredation (Raihani & Ridley 2007, Ridley 2016, Koenig *et al.* 2019), reducing exposure to parasitism (Ridley & Raihani 2007, Canestrari *et al.* 2009), nest building (Baglione *et al.* 2002a), and incubation (Jamieson *et al.* 1994, Fessl *et al.* 1996, Yuan *et al.* 2004) are other ways of assistance that have been recorded.

In some species, multiple group members have a chance of siring offspring (Baglione *et al.* 2002c, Richardson *et al.* 2002, Riehl 2013, 2017). However, in other species, some group members appear to be strictly non-breeding helpers (Canestrari *et al.* 2008, Preston *et al.* 2013, Koenig & Dickinson 2016, Koenig *et al.* 2019). There are several, not mutually exclusive, ways through which such members can gain fitness despite not

being the parent of the raised chicks. Closely related members can gain indirect fitness (also known as kin selection) by increasing the group's reproductive output through increasing the survival and/or success of the breeding pair and/or their chicks (Stallcup & Woolfenden 1978, Reyer 1984, Hunter 1985, Sydeman *et al.* 1988, Gregory 1990, Hatchwell *et al.* 2004, Canestrari *et al.* 2008, Brouwer *et al.* 2012, Preston *et al.* 2013, Liebl *et al.* 2016, Preston *et al.* 2016, Hammers *et al.* 2019, Koenig *et al.* 2019). For instance, sharing provisioning can be a way of increasing chick growth rate, survival, and/or fledging success (Shen *et al.* 2010, Koenig & Dickinson 2016, Liebl *et al.* 2016) or a way to reduce parental workload, which can increase future chances of successful reproduction (Sydeman 1989, Gregory 1990, Heinsohn 1992). Cooperative breeding can also enable synchronised provisioning, which has been found to reduce nest-predation and sibling competition (Shen *et al.* 2010). Furthermore, being a group member can increase future reproductive success if group living increases survival and/or lifespan (Ekman *et al.* 1999, Ekman *et al.* 2000), enables the acquirement of parenting skills (Komdeur 1996, Hatchwell *et al.* 1999), or promotes getting help in the future (Kingma *et al.* 2011).

Table 2.3.1. List of characteristics associated with group breeding in birds, the references in which they are discussed, and the species featured in those publications. M = male, F = female, Y = yes, N = no. Empty squares do not indicate that this trait is not a feature of that particular species, but simply that it has not been referred to in the references cited.

	Shared…			Helpers…							Drivers				Features								
	…incubation	…nest building	…territory defence	…get help in the future	…acquire parental skills	…increase lifespan	…improve pair fitness	…reduce parasitism	…improve chick fitness	…improve pair and chick fitness	Habitat quality	Population density	Territory access	Safer together	Territorial?	Labour division	Gender bias in group composition	Most group members close relatives	Multiple equal pairs breeding together	Group breeding obligate?	Synchronized chick provisioning	Evidence of kin recognition	
Acorn woodpecker, *Melanerpes formicivorus*			**x**							**x**		**x**	**x**				**M**	**Y**					Barve *et al.* 2019, Koenig *et al.* 2019
Brown headed nuthatch, *Sitta pusilla*															**Y**		**M**	**N**		**N**			Miller & Jones 1999, Cox & Slater 2007
Carrion crow, *Corvus corone*		**x**						**x**		**x**				**x**	**Y**	**x**	**M**	**Y**				**x**	Canestrari *et al.* 2008, 2009, Baglione *et al.* 2002a, 2002b, 2003, 2005
Chestnut-crowned babbler, *Pomatostomus ruficeps*									**x**					**x**									Chappell *et al.* 2016, Liebl *et al.* 2016
Florida scrub jay,			**x**				**x**						**x**		**Y**			**Y**		**N**			Woolfenden & Fitzpatrick 1978

Aphelocoma coerulescens																							
Hoatzin, *Opisthocomus hoazin*																		**Y**		**N**			Strahl 1988
Long-tailed tit, *Aegithalos caudatus*					**x**										**N**		**M**			**N**		**x**	Hatchwell *et al.* 1999, 2004, Nam et al. 2010, Leedale *et al.* 2020
Moustache warbler, *Acrocephalus melanopogon*	**x**																						Fessl *et al.* 1996
Pied kingfishers, *Ceryle rudis*										**x**	**x**					**x**		**N**					Reyer 1980, 1984
Pukeko, *Porphyrio melanotus*	**x**																						Jamieson *et al.* 1994
Purple gallinule, *Porphyrio martinicus*			**x**						**x**									**Y**					Hunter 1985
Purple-crowned fairy wren, *Malurus coronatus*				**x**																			Kingma *et al.* 2011
Pygmy nuthatch, *Sitta pygmaea*									**x**														Sydeman *et al.* 1988
Red-cockaded woodpecker, *Leuconotopicus borealis*											**x**		**x**		**Y**		**M**						Walters *et al.* 1988

Rifleman, *Acanthisitta chloris*									**x**												**x**	Gregory 1990, Preston *et al.* 2013, 2016
Seychelles warblers, *Acrocephalus sechellensis*					**x**				**x**								**F**					Richardson *et al.* 2002, Brouwer *et al.* 2012, Komdeur 1996
Siberian jay*, *Perisoreus infaustus*						**x**						**x**		**x**								Ekman *et al.* 1999, 2000
Southern pied babbler, *Turdoides bicolor*			**x**					**x**					**x**	**x**		**x**						Ridley & Raihani 2007, Nelson-Flower *et al.* 2012, Ridley 2016
Taiwan yuhina, *Yuhina brunneiceps*	**x**																	**N**	**x**		**x**	Yuan *et al.* 2004, Shen *et al.* 2010
Western American crow, *Corvus brachyrhynchos hesperis*															**N**		**F**					Caffrey 1992
Western bluebird, *Sialia mexicana*																	**M**			**N**		Dickinson & Akre 1998
White-winged choughs, *Corcorax melanorhamphos*																				**Y**		Heinsohn & Cockburn 1994

*Siberian jays are typically considered family living, but not cooperative breeders.

Many attempts have been made to find an overarching explanation as to what drives the evolution of cooperative breeding despite this large variation in how cooperative breeding is executed (Riehl 2013, Koenig & Dickinson 2016, Griesser *et al.* 2017, Riehl 2017). One recent theory based on a meta-analysis suggests that the only way the evolution of cooperative breeding can be understood is if it is regarded as a two-step process; step one is transitioning from non-family living to family living, and step two involves a change in behaviour where family members transition from being passive to actively assist in breeding (Griesser *et al.* 2017, Koenig 2017). The most common way to take the first step seems to be delayed dispersal of juveniles; the usually occurs when living alone is costly or dangerous while, at the same time, the cost for the parents of accepting the stayers is relatively low thanks to high and stable resource access (Ekman *et al.* 1999, Baglione *et al.* 2005, Chappell *et al.* 2016, Ridley 2016, Griesser *et al.* 2017). In addition to increased survival, direct benefits of delaying dispersal can be increased chances to inherit or otherwise acquire a good territory, particularly if such territories are rare (Woolfenden & Fitzpatrick 1978, Walters *et al.* 1988, Ridley 2016, Barve *et al.* 2019). Consequently, delayed dispersal resulting in family living should be expected to occur more often in higher density populations (Ekman *et al.* 1999, Barve *et al.* 2019), or areas with uneven resource distribution (Reyer 1980, Lacey *et al.* 2016). Once family living is established, cooperative breeding seems to typically evolve when environmental change suddenly makes offering as well as accepting assistance beneficial for both the staying juvenile(s) and their parents (Griesser *et al.* 2017, Koenig 2017). However, this hypothesis does not explain why some other species, seemingly with the same features, do not breed cooperatively, nor what the drivers are in groups where members are not close relatives (Griesser *et al.* 2017, Koenig 2017).

Our understanding of cooperative breeding would greatly benefit from focusing on species and groups where several breeding strategies exist in parallel, such as Palaeognathae (Handford & Mares 1985, Coddington & Cockburn 1995, Codenotti & Alvarez 2001, Kimwele & Graves 2003, Brennan 2012, Giraldo-Deck *et al.* 2017, Klug 2018, Solano-Ugalde *et al.* 2018). In the Palaeognathae genus *Apteryx*, *A. australis lawryi* and *A. rowi* are facultative cooperative breeders while *A. oweni* has only been reported to breed monogamously (Jolly 1989, Colbourne 2002, Feenstra unpubl. 2020, see further 2.3). Hence, *Apteryx* is an interesting study system for learning more about drivers of cooperative breeding on an evolutionary scale as well as within- and between populations. Another beneficial feature of *Apteryx* is that their super-precocial offspring enables studies that naturally rule out chick provisioning as a driver. Here I combine data on roost sharing, breeding, activity, morphometrics, and genomics collected over 17 years in a high-density *A. mantelli* population to (1) present the most thorough description of the mating strategy of this species so far and (2) investigate the proximate benefits of the identified breeding strategies. Specifically, I test if alternative strategies to monogamy could allow *A. mantelli* to (i) reduce incubation effort, and/or (ii) get access to preferable resources, and (iii) whether either or both these things could result in increased resilience to stressful conditions for non-monogamous individuals. Lastly, I discuss the potential consequences of breeding system flexibility on *A. mantelli* conservation.

2.3.2 Materials and Methods

2.3.2.1 Study site and species

The study population was situated on Ponui Island in the Hauraki Gulf [36 55’ S, 175 11’ E]. This *A. mantelli* population originated in 1964 through two translocations of

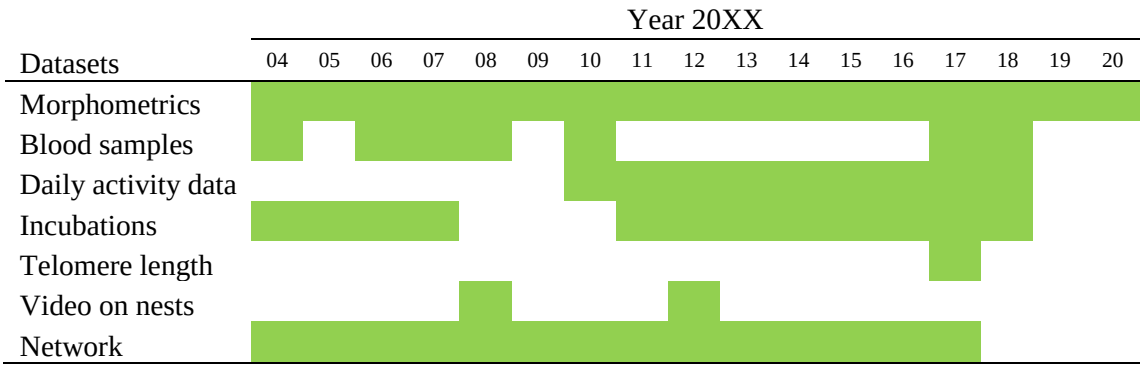
eight and six birds from Waipoua Forest and Hauturu-o-Toi (Little Barrier Island), respectively (Colbourne 2005). This population is unusual in that between 30 and 50 individuals (approximately equal numbers of males and females) have been fitted with radio transmitters and closely monitored for the last 17 years. In addition, the Ponui Island population represents (1) a population density that is rarely seen today but presumed common historically and (2) a population exposed to comparatively low levels of introduced mammalian predators (the feral cat, Felis catus, is the only introduced predator of kiwi on Ponui Island; Strang 2018). Other features of this population are a low prevalence of second and thirds clutches as well as comparatively low territoriality (high site fidelity but lack of defence against other individuals; Ziesemann *et al.* 2011, Wilson 2014).

2.3.2.2 Defining breeding units

During each season, radio-tagged birds were regularly tracked and located to verify if they shared roosts. The frequency of tracking varied over time both within and between years. A breeding unit was classified as two (pair) or more birds (group) that were found together repeatedly, consistently, and more frequently compared to other individuals. In addition, at least one breeding attempt had to be observed or inferred from activity. The data on which birds were found together and how often were compiled using the network tool in the R package igraph (Csardi 2006) to assist in identifying breeding units. Comprehensive video monitoring of nests and roosts in 2008 and 2012 further increased the confidence in the identification and delimitation of breeding units. *Apteryx mantelli* in this population have defined home ranges but these overlap extensively (Ziesemann 2011); hence, overlapping home range was not enough to define a breeding unit. Birds with overlapping home ranges who had been

encountered utilising the same roost but that were not deemed to be part of the same breeding unit were referred to as ‘sharers’.

Table 2.3.2. List of the major datasets analysed herein, indicating which year the data were collected.



2.3.2.3 Incubation and breeding strategies

From 2010 onwards, some or all the leg-fitted VHF transmitters used in the Ponui Island population were Chick timer transmitters™ (wildtech.co.nz/Kiwi.aspx). Chick timer transmitters use the daily activity of males to infer the start and end of incubation, the day of hatching of the first chick, and nest desertions. The transmitters output several types of information including the number of days since the start or end of incubation, the minutes of activity per 24 hours for the previous night, the night before previous, and the average for the last four days (in 10 minute units). These data were accessed manually using a hand-held receiver and aerial once per month to several times per week. In addition, the inbuilt data loggers record activity data that was downloaded annually once the transmitters were retrieved from the bird. The capacity of the dataloggers was limited to the last nine months so since transmitters were changed annually in March, downloaded values for daily activity was available from approximately early July to late March.

Downloaded data only provided the amount of activity and not the status of the male (incubating, not incubating, egg hatched, deserted, or dead). Thus, the downloaded and the manual data combined were used to determine the average start date of incubation of the first clutch, the number of full-long incubations in a season, the number of initiated incubations in a season, and the number of days incubating during a full-long incubation bout (Table 2.3.2). A full-long incubation bout was deemed as any activity dip consistent with incubation longer than 65 days (McLennan 1988, Colbourne 2002). The daily activity for males from the same breeding unit was overlaid on each other to identify synchronised activity-dips consistent with shared incubation. These data were also overlaid with the manually collected data on when transmitter indicated incubation and hatching to investigate if shared incubation reduced the reliability and accuracy of the Chick timers™. Males were also occasionally observed together in the nest burrows (see further Ziesemann 2011).

2.3.2.4 Activity

Using the data downloaded from the transmitters, I calculated the average daily activity for each consecutive 30-day period (day 1 to day 30, day 2 to day 31 etc). From this, the sex-specific timing of the 30 consecutive days with highest (Max30) and lowest (Min30) average daily activity was extracted. Since incubation is done by males and involves a substantial reduction in daily activity, only seasons with at least one activity dip consistent with incubation were included for males to render comparable Min30 values. For females, all years were included. The manually collected data were used as a complement to detect the possibility of activity peaks outside the time frame of the downloaded data (late March to start of July); since the manual data were not continuous, the reported average activity for the last 4 days was used. The daily activity during Max30 and Min30 were then used to compare activity levels with respect to

breeding strategy. For females, one additional activity parameter was extracted, since female kiwi lay huge eggs. To study any activity dips around laying, the daily activity during the overall (i.e., not necessarily consecutive) days with the lowest activity was extracted and compared. The options of analysing the 10, 9, 8, 7, 6, 5, 4, or 3 days of lowest activity were explored, and four days were chosen for further analyses since it provided the widest variation. See Table 2.3.3 for detailed definitions and abbreviations.

2.3.2.5 Morphometrics and stress

During each annual health-check and transmitter replacement, the following measurements were taken from each bird (following the Kiwi best practice manual; Robertson & Colbourne 2017): weight, bill length, tarsus length (TL), tarsus depth, and tarsus width (TW). All except weight were measured as three repeats. Body condition (BC) was calculated based on the method developed by Taborsky and Taborsky (1999) using the latest value from weight and TW. Two principal component analyses (one for male and one for females) were run to examine the overall morphometric similarity between group and pair breeding birds (R package tidyverse; Wickham *et al.* 2019). These analyses included BC, TL, Bill, Weight, TW, the ratio between TL and Bill, and the relatedness to Little Barrier Island (see below). To investigate stress resilience as a potential driver of breeding strategy, percentual weight loss between 2012 and 2013 was calculated and compared. This was because 2013 was a severe drought year with less than 100 mm rain during the summer. See Table 2.3.3 for detailed definitions and abbreviations.

2.3.2.6 Blood samples

Blood samples were collected in 2004, 2006-2008, 2010, and 2017 in accordance with the Kiwi Best Practice Manual (Robertson & Colbourne 2017), the Massey University

Animal Ethics Committee (MUAEC) permits 06/05, 07/144, and 16/92, and the Department of Conservation Wildlife permits AK-14969-RES, AK-21519-FAU, and 50249-FAU. DNA was extracted from 10-50 µl thawed whole blood using a High Pure PCR template preparation kit (Roche). Manufacturer's instructions were followed with the exception that elution was done in two rounds with 50 µl of elution buffer each centrifugation round. The DNA extraction success and quality were validated using agarose gel electrophoresis (1.5-2% agarose in 1x TAE buffer) and the concentration of DNA was measured using the Qubit dsDNA High Sensitivity assay (Life Technologies, CA, USA). The DNA was then diluted to a concentration of 20-100 ng per microliter, plated with 30 µl per sample, and submitted for genotype-by-sequencing (GBS) library prep by the Elshire group (Elshire *et al.* 2011). In addition, relative telomere length (RTL) was quantified using qPCR (Criscuolo *et al.* 2009, Eastwood *et al.* 2018). See Chapters 2.2, 3.1, and 4.2 for further details.

2.3.2.7 Relatedness

The GBS library was generated following the method of Elshire *et al.* (2011) including the following changes: 100 ng of genomic DNA were used, 1.44 ng of total adapters were used, the genomic DNAs were restricted with EcoT22i enzyme, and the library was amplified with 18 PCR cycles. Based on the GBS data, the allelic similarity was generated based on a re-assembly of the published *A. mantelli* genome (Le Duc *et al.* 2015). SNP calling was carried out in the STACKS 2.5 pipeline. After LD pruning and reduction to one variable site per loci, 51 691 SNPs were utilised for analyses. Unscaled pairwise relatedness (allelic similarity) values were derived using KGD (Dodds *et al.* 2015).

In total 120 Ponui Island birds were included in the analyses. Fourteen of these were left out of the analysis of overall relatedness due to being known offspring of other birds in

the dataset. The remaining 106 birds included all members of 23 pairs and seven groups. Pairwise relatedness of all members within the groups was compared to relatedness (1) between known first degree relatives (n = 42 pairwise comparisons from 5 families), (2) within pairs, (3) between shares, and (4) of each sampled individual to all other sampled birds (106*106). In addition, the average relatedness of each pair and group member to 13 birds from Little Barrier Island was quantified. This comparison was to elucidate an effect of heritage on breeding strategy and to complement the morphometric analyses since *A. mantelli* from Little Barrier Island tend to be smaller than birds from Northland (see Section 2.2 for further information).

2.3.2.8 Statistical analyses and relating factors to breeding strategy

All statistical analyses were performed in R version 3.6.2, and sequential (also known as Holm's) Bonferroni correction was used to account for multiple testing (R-Development core team). All data were deemed to conform to normal distributions based on graphical evaluation of histograms and qqplots (R-Development core team). The sample sizes for all categories were defined as the number of bird-years, i.e., the sum of the number of birds times the number of years they were represented in the data; all individuals did not contribute equally since data were missing for some individuals in some years.

For males, data were split and compared in three ways: (1) pairs *versus* groups, (2) multi-male groups with shared incubation ('Helped' males) *versus* all other breeding unit formation with a single incubator, and (3) helped males *versus* the single incubators further split into male from: multi-male groups with one incubator (No help), male-female-female groups (MFF), and pairs (Pair) respectively. Females were split into the same categories as males based on the strategy of the male(s) in their breeding unit.

Females were also split based on the number of initiated incubations by their associated male(s) as a proxy for the number of eggs laid per season.

Data split into pairs *versus* groups were analysed using t-tests. Relatedness data were examined using linear models (lm; R-Development core team). In order to introduce a random effect (year and bird id), I used linear mixed-effects models (lmer; R package lme4; Bates *et al.* 2015), for the analyses with Max30, Min30, Lowest4, start date, and incubation days as response variables. A generalized linear mixed-effects model (glmer; R package lme4; Bates *et al.* 2015), was used to analyse the likelihood of birds being found together to allow a logit link function. For all models, Anova (R package car; Fox & Weisberg 2019) was used to evaluate statistical significance, combined with Tukey tests (R packages multcomp; Hothorn *et al.* 2008, and agricolae; de Mendiburu 2020) for post-hoc analyses.

Table 2.3.3. List of categories and factors analysed with respect to breeding strategy as they are referred to in the text and their definitions.

Factor	Definition
Full-long incubation	Incubation > 65 days
Initiated incubation	Incubation < 65 days
Days incubating	Number of days with activity consistent with incubation and/or when incubation was observed
Max30	Average number of hours active per night during the 30 consecutive days with the highest average activity*
Min30	Average number of hours active during the 30 consecutive days with the lowest average activity. Only including years with full-long incubation for males*
Lowest4	Average number of hours active during the four overall days with lowest activity (females only)*
Weight	Weight in grams at transmitter change in late March
Bill	Bill length in mm at transmitter change in late March
TW	Tarsus width in mm at transmitter change in late March
BC	Body condition calculated based on Weight and TW at transmitter change in late March
BillTL	Ratio between Bill and Tarsus length at transmitter change in late March
Weight loss	Weight loss in grams between 2012 and the drought year 2013

RTL	Relative telomere length
To LBI	Average relatedness to 13 birds from Little Barrier Island (one of two parental populations to the *A. mantelli* population on Ponui Island)
Pair	Breeding unit with only one female and only one male member
Group	Any breeding unit with additional members compared to a Pair. Birds were considered group breeding if they were ever observed breeding in a group even if they at some other point in time were breeding as a pair
Sharers	Birds with overlapping home range and shared burrow not deemed part of the same breeding unit
All	The full dataset of *A. mantelli* from Ponui Island, excluding known offspring to avoid bias
1st degree relative	The pairwise relationship between a parent to a sibling or between (presumed) full siblings

*From the daily activity data, i.e., between early July and late March.

2.3.3 Results

In total, 42 breeding units were identified and in line with previous publications, social monogamy was the most common mating strategy. However, 21% (9 out of 42) of the monitored units were found to be groups of more than two individuals (Figure 2.3.1 & 2.3.2).

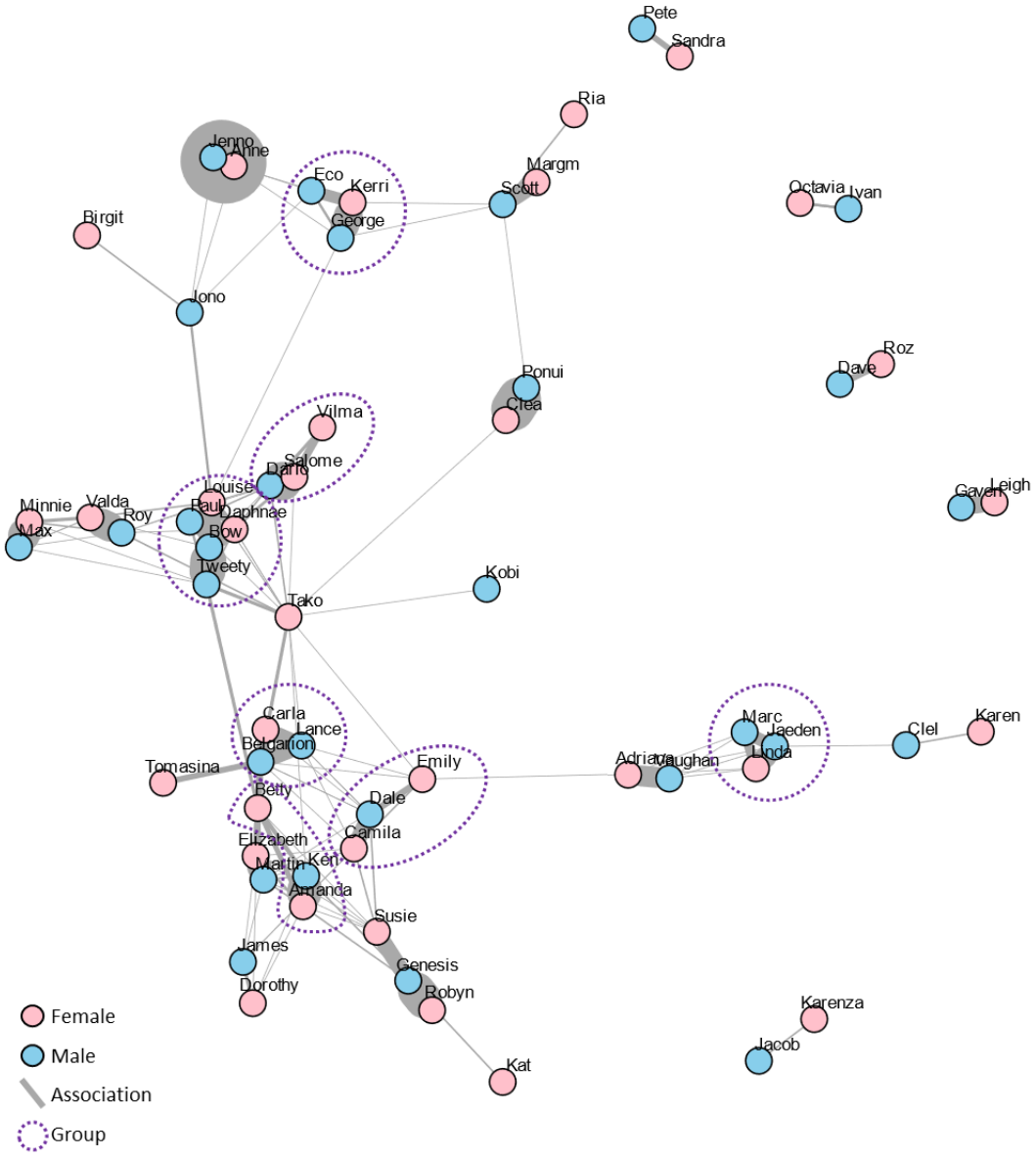


Figure 2.3.1. Network illustrating relationships between the monitored birds in the Ponui Island population. Weight of grey edges scale to the number of times two nodes (birds) were found roosting together. The seven breeding units with more than two individuals are indicated.

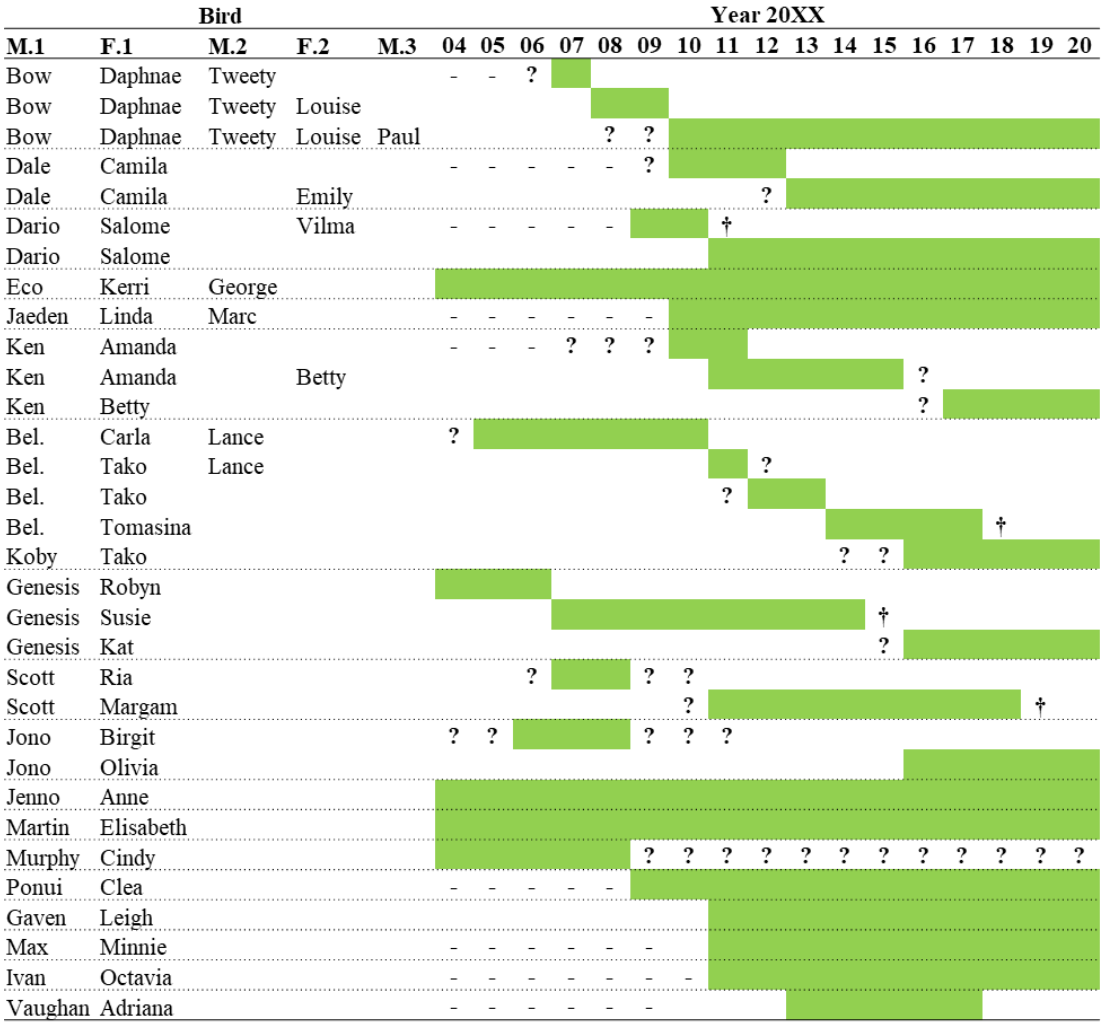


Figure 2.3.2. Examples illustrating the dynamics and duration for 31 (of the total of 42) *A. mantelli* breeding units monitored on Ponui Island. Dotted lines separating units involving partly the same individuals for clarity; M = male. F = female. Green = was a confirmed unit during the breeding season this year; White = was not a unit at this year; - = not yet part of study; ? = status unknown; † = relation ended due to death.

2.3.3.1 Breeding strategies

Four different breeding unit compositions were found: male-female pairs, female-female-male (MFF) groups, male-male-female (MMF) groups, and one case of a two-female-three-male quintet (5tet; Figure 2.3.1 & 2.3.2). Members of groups and pairs were commonly found roosting together, while roost-sharing with other kiwi was rare (Figure 2.3.1). The likelihood of finding a bird together with any other bird in its unit

was equally high for pairs and groups (47% and 45%, respectively; Supplementary Figure S2.3.1a). Hence, the likelihood of finding a group breeding bird together with a specific member of its unit was lower compared to pairs and was particularly low for female-female duos within groups.

Synchronised activity-dips, consistent with cooperative incubation, occurred consistently for two of the four groups with multiple males (Figure 2.3.3). In the remaining multi-male units, the activity of only one male per group indicated incubation. Consequently, combining group compositions with the presence or absence of shared incubation, I identified a total of five mating strategies among male *A. mantelli* in this population: (1) breeding in a multi-male group and share incubation; (2) breeding in a multi-male group and (a) being the sole incubator or (b) not participating in incubation; (3) breeding in a male-female-female group; (4) breeding in a socially monogamous pair (Figure 2.3.4). Strategies 2, 3, and 4 are jointly referred to as single incubator strategies (Figure 2.3.4). Relationships were found to be overall stable and there tended to be a 'main pair' within each group (Figure 2.3.2; Supplementary Figure 2.3.1a). However, dynamics such as divorces and joining of new members were also observed. Despite the high population density, divorce could be followed by several years as unmated (see for instance Jono in Figure 2.3.2). Dynamics could also be more subtle; for instance, within the quintet, the most likely duo to encounter together changed over time from Bow and Daphnae, to Paul and Daphnae, to Bow and Tweety (Supplementary Figure 2.3.1b).

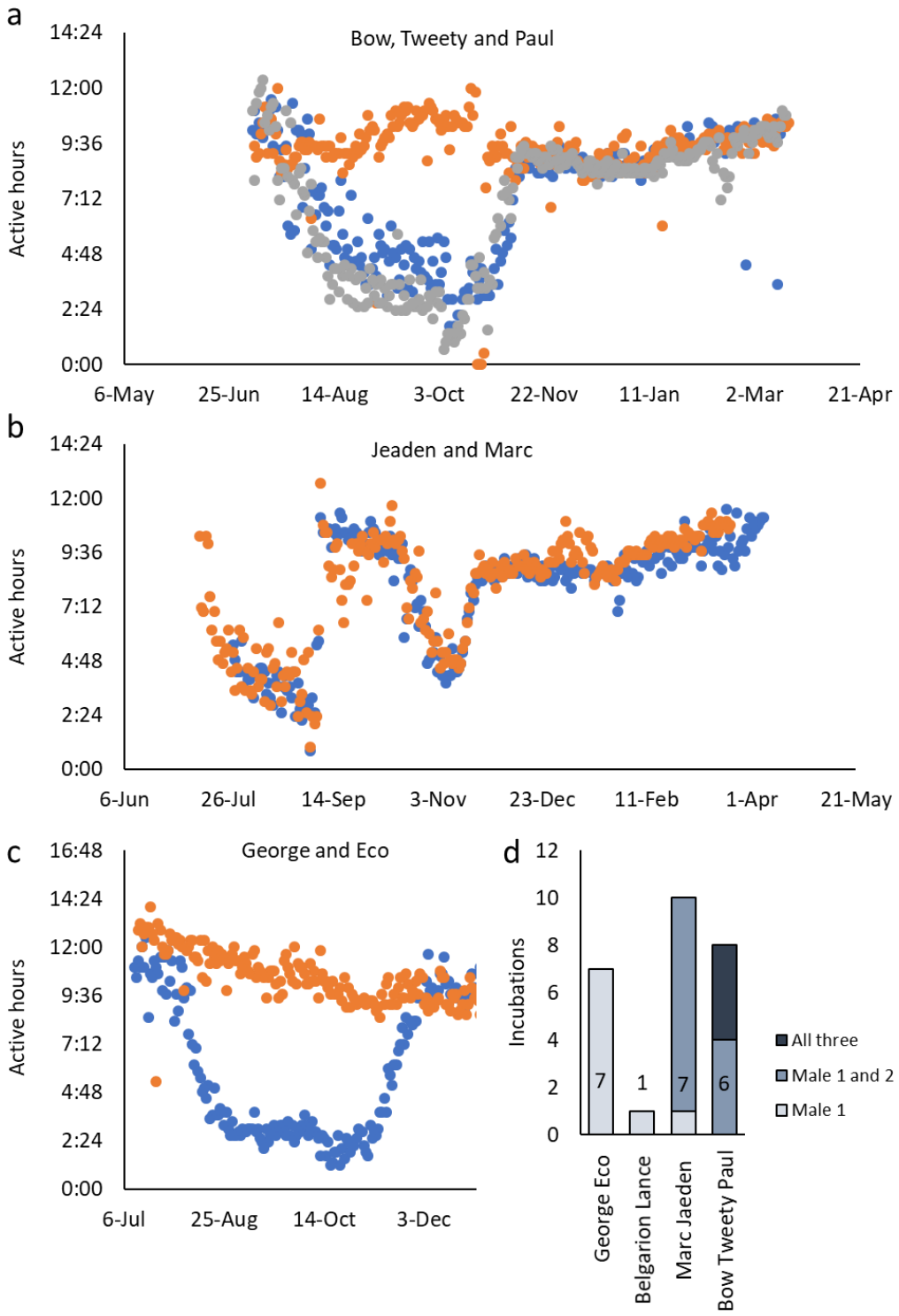


Figure 2.3.3. Three examples of over laid activity patterns of *A. mantelli* males within the same breeding units. In example (a), two males display synchronised activity consistent with shared incubation (Tweety in blue; Bow in grey). The activity of the third male in this group (Paul in orange) was not consistent with incubation during the year displayed with only three days of low activity, all occurring seemingly after the hatching of the egg. In example (b), Jaeden and Marc both display two synchronised initiated (but not full-long) incubations. Example (c)

illustrates a group with two males out of which one did all observed incubations by himself (George). Panel (d) denotes how many incubations were observed in the activity data and how many males were involved in each incubation for each of the multimale groups. Numbers denote years of data; only years with activity data for all males in the group were included.

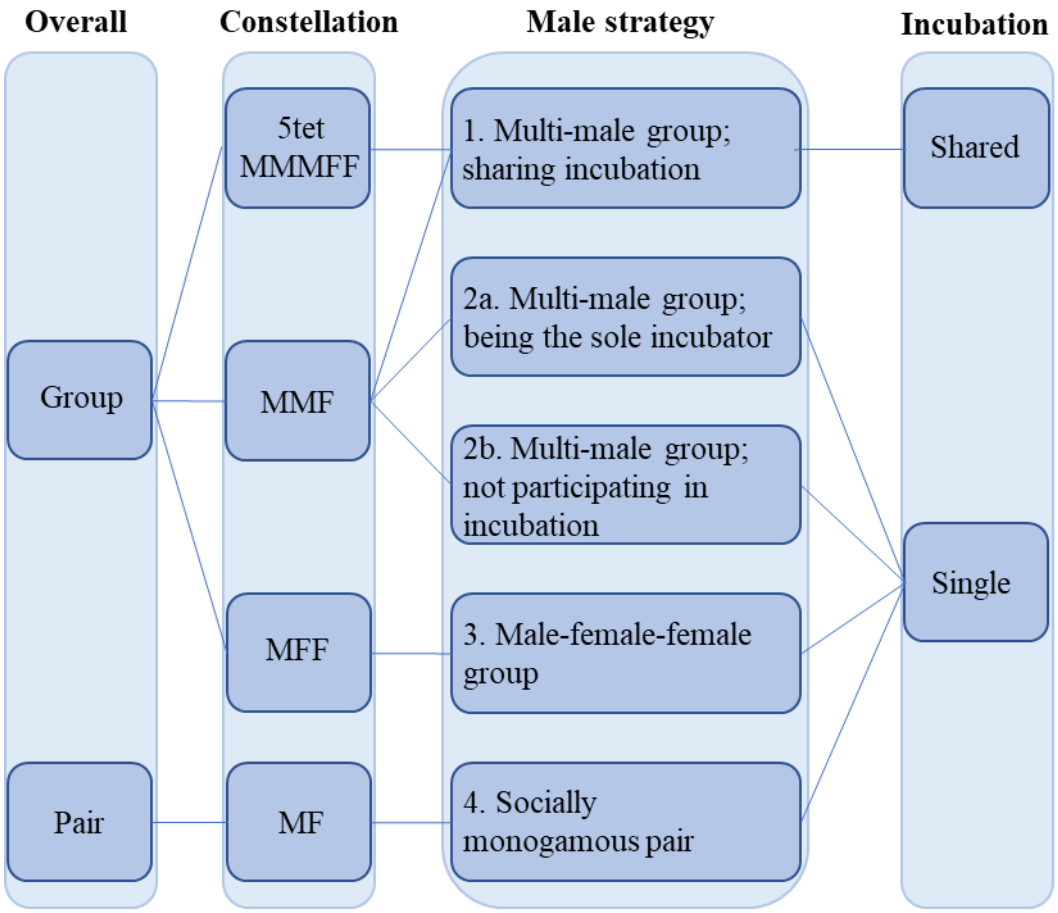


Figure 2.3.4. Flow diagram illustrating how studied birds fell into categories based on different features of their breeding. Males fell into categories based on their own breeding strategy, and females based on the strategy of their associated male(s). Strategy numbers refer to those used in the main text. 5tet = quintet; M = male and F = female.

Table 2.3.4. Statistical results from analyses of relationships between behaviour, morphometric, relatedness, and breeding strategy. **Bold** numbers highlight statistical significance (Holms-Bonferroni adjusted $p < 0.05$), *italics* indicate trends that approached significance (Holms-Bonferroni adjusted $0.05 < p > 0.1$).

Model	Random effect	Category	Sample set	Resp. variable	Fixed effect	Chisq, f, or t / df / p-value	*n*
lmer							
Model 1	Year	Activity	Male*	Max30	Help / No Help / MFF / Pair	**421.87 / 3 / < 0.001**	1980
Model 2	Year	Activity	Male*	Min30	Help / No Help / MFF / Pair	**92.08 / 3 / < 0.001**	1980
Model 3	Year	Activity	Female	Max30	Group vs Pair	**35.49 / 1 / <0.001**	3510
					0/1/2 incubations	**117.35 / 2 / <0.001**	3510
Model 4	Year	Activity	Female	Min30	Group vs Pair	**29.90 / 1 / <0.001**	3510
					0/1/2 incubations	**8.53 / 2 / 0.01**	3510
Model 5	Year	Activity	Female	Lowest4	Group vs Pair	*2.70 / 1 / 0.10*	468
					0/1/2 incubations	0.56 / 1 / 0.45	468
Model 6	Bird ID	Start	Male*		Group vs Pair	1.60 / 3 / 0.66	65
Model 7	Bird ID	Incubation days	Male*		Help vs Single**	1.51 / 1 / 0.22	36
glmer							
Model 8	-	Found together	All		Group vs Pair	1.67 / 1 / 0.20	32
lm							
Model 9	-	Relatedness	All		All / 1st degree / Group / Pairs	**407.01 / 3 / <0.001**	199
Model 10		Relatedness	Sharers		Same sex / MF /Random***	**30.16 / 2 / <0.001**	204
t-test							
T1	-	Morphometrics	Male	Bill	Group vs Pair	1.14 / 21 / 0.27	24
T2	-	Morphometrics	Male	Bill/TL	Group vs Pair	0.78 / 21 / 0.19	24
T3	-	Morphometrics	Male	TW	Group vs Pair	1.35 / 21 / 0.84	24
T4	-	Morphometrics	Male	Weight	Group vs Pair	0.20 / 22 /0.84	24
T5	-	Morphometrics	Male	To LBI	Group vs Pair	-0.49 / 21 / 0.63	24
T6	-	Morphometrics	Female	Bill	Group vs Pair	-2.89 / 25 / 0.14	27
T7	-	Morphometrics	Female	Bill/TL	Group vs Pair	**-2.89 / 22 / 0.01**	27
T8	-	Morphometrics	Female	TW	Group vs Pair	-1.14 / 22 / 0.27	27
T9	-	Morphometrics	Female	Weight	Group vs Pair	0.60 / 22 / 0.56	27

T10	-	Morphometrics	Female	To LBI	Group vs Pair	0.57 / 25 / 0.57	27
T11	-	Stress	All	RTL	Group vs Pair	0.35 / 29 / 0.73	32
T12	-	Stress	All	Body condition	Group vs Pair	0.59 / 40 / 0.56	44
T13	-	Stress	All	Weigh loss 2013	Group vs Pair	-1.60 / 27 / 0.12	30

*Male in these cases refers only to males incubating during that season; ** mode of incubation

2.3.3.2 Activity

Females' lowest activity (Min30) was found to be concentrated in late spring and summer (November to January) while male's Min30 was slightly earlier (September and October), coinciding with the end of incubation of the first clutch. Females Max30 occurred in winter (July through September), while the timing of male Max30 showed two peaks, one in March and one between mid-July and mid-October (possibly depending on the timing of incubation; Supplementary Figure 2.3.2). The data collected manually indicated a third, and possibly even higher, peak in male activity in May to June.

Looking only at years with full-long incubation bouts, male Max30 and Min30 (based on the daily data downloaded from the transmitter) was found to differ based on breeding strategy (Table 2.3.4; Figure 2.3.5). Males sharing incubation were found to spend on average about one hour less active per night during their Max30 compared to males in pairs and males in multimale groups not sharing incubation (Figure 2.3.5a; Table 2.3.4). Males in MFF groups were found to fall in between (Figure 2.3.5a). During incubations, sharing males were found to be the most active, followed by paired males and non-sharing males in multimale groups, while MFF males were the least active (Figure 2.3.5b; Table 2.3.4). The generally opposite directions of these differences in activity meant that, on average, single incubating males reduced their activity by 70% while males sharing males only reduced it by 62% during incubation. The activity patterns for males of all strategies were found to be similar enough to the programmed algorithm to trigger the incubation mode on their Chick timer transmitters™. However, there was a tendency of shared incubation to reduce the accuracy of the hatching trigger (Supplementary Figure S2.3.3-S2.3.5).

Due to low sample size, female activity could only be analysed by splitting into groups *versus* pairs, and on the number of initiated incubations by associated male(s) (used as a proxy for number of eggs laid). I found no significant difference between categories for either splitting for Max30 or Lowest4 (Supplementary Figure 2.3.6; Table 2.3.4). However, for Min30 there was significant difference found with group females being on average 13 minutes more active per night compared to pair females, and zero-egg females being on average nine minutes more active per night compared to one-egg females (Supplementary Figure S2.3.6; Table 2.3.4).

2.3.3.3 Incubations

While shared incubation appeared to reduce the number of days in a full-long incubation bout and increase the number of full-long and initiated incubations per year compared to other breeding strategies, these differences were not statistically significant (Table 2.3.3 and 2.3.4; Supplementary Figures S2.3.7 & S2.3.8). In addition, males that shared incubation were not found to have an earlier onset of the breeding season (Table 2.3.4).

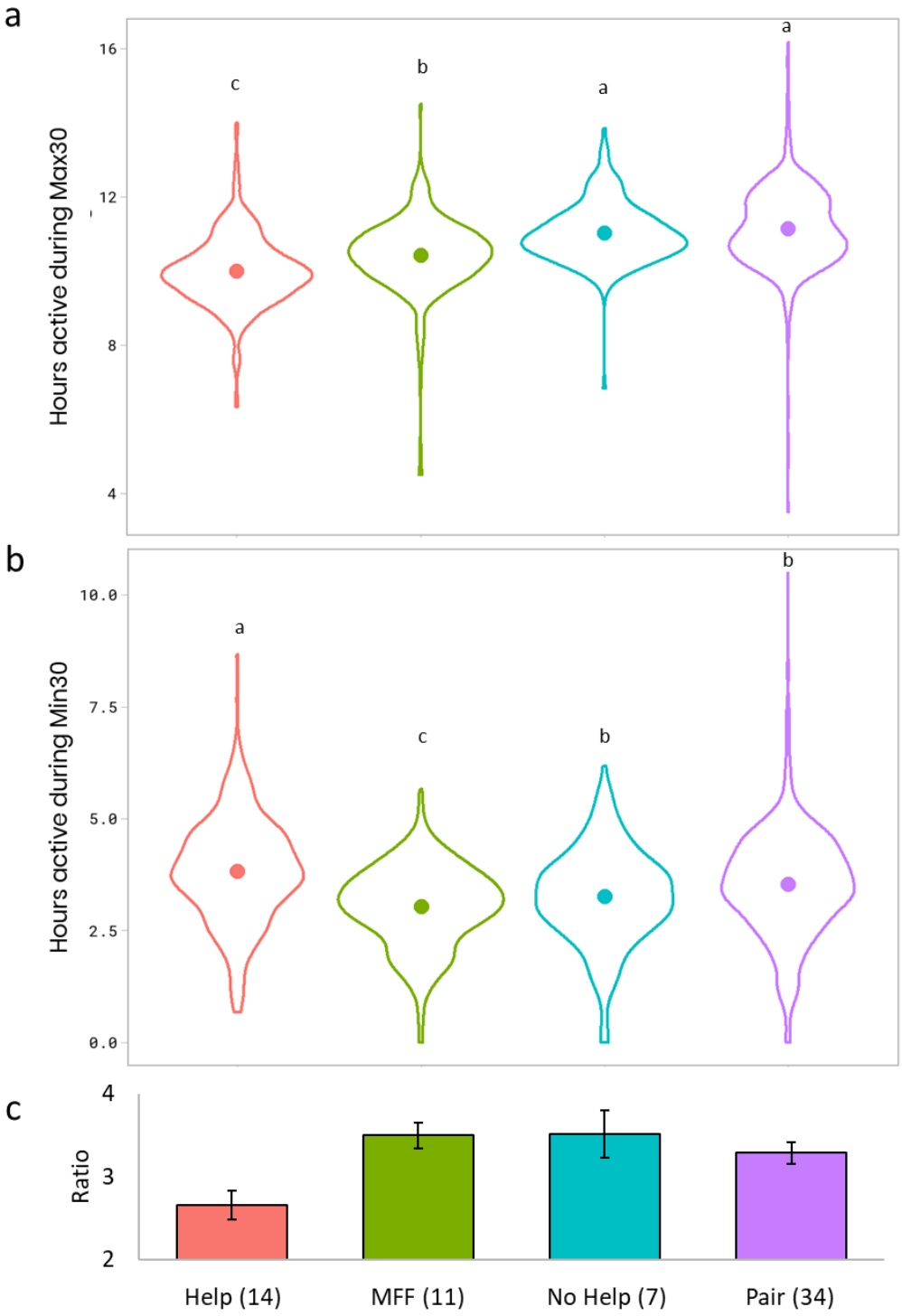


Figure 2.3.5. Comparison of the 30 consecutive days of highest (Max30; a) and lowest (Min30; b) male activity per night in relation to breeding strategy. The categories are multi-male groups observed to share incubation (Help), male-female-female (MFF) groups, male-male-female groups never observed to share incubation (No help), and socially monogamous pairs (Pair). Only years with a full-long incubation bout were included in the analyses. Different, lowercase letters denote statistically significant differences. Numbers in brackets denote sample size in

number of bird-years. (c) displays the ratio between Max30 and Min30 to highlight the fact that the significant differences in (a) and (b) go in opposite directions.

2.3.3.4 Relatedness

Pairwise allelic similarity (relatedness) among members of breeding groups (*n* = all 23 members of 7 groups) was found to be significantly lower than for known first-degree relatives (*n* = 34 offspring-parent and sibling comparisons) and the same as the average relatedness among all sampled individuals (*n* = 106*106; 14 known offspring excluded; Figure 2.3.6; Table 2.3.4). One pairwise relationship suggested possible first-degree relatedness between two group members (Kerri and George; Figure 2.3.6b group C). Two chicks incubated by George were included in the dataset, and the genetic analyses of these suggested that George and Kerri were the biological parents of one and Eco and Kerri of the other (confirming the results based on microsatellites by Ziesemann 2011).

Furthermore, relatedness among birds with overlapping home ranges not part of the same breeding unit was found to be as high as the relatedness among all sampled individuals (Table 2.3.4; Supplementary Figure S2.3.9). However, when broken down further, relatedness was found to be higher than the overall average between same sex, but lower among opposite sex pairwise comparisons (Table 2.3.4; Supplementary Figure S2.3.9).

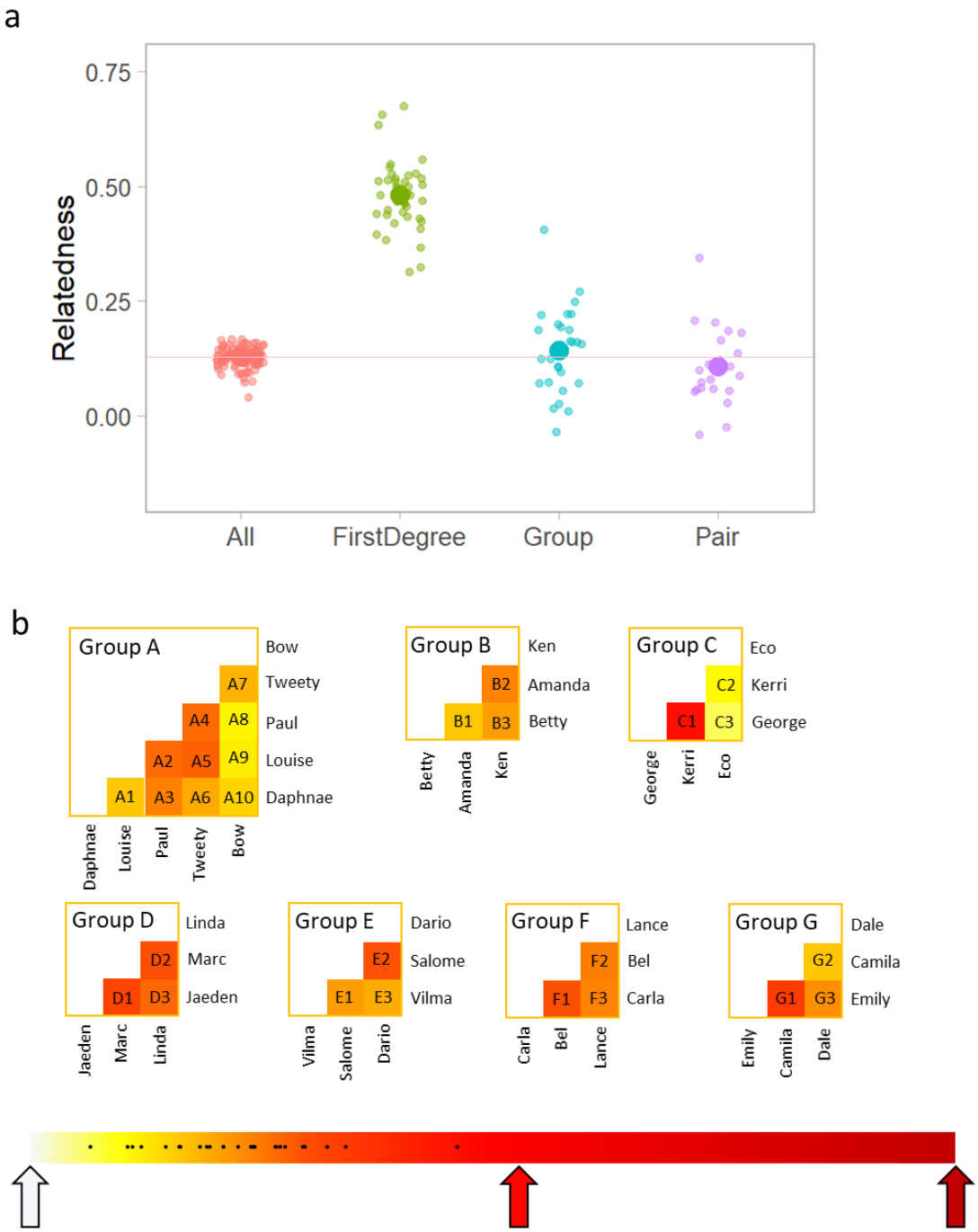


Figure 2.3.6. Comparison of pairwise allelic similarity (inferred relatedness) within and between groups, pairs, first degree relatives, and all sampled individuals from Ponui Island. 'All' refers to the average pairwise relatedness of each individual to all other sampled individuals (n = 106 after exclusion of 14 known offspring). Small dots indicate each pairwise comparison, and large dots show averages for each category. The red line highlights the average among All for clarity. The relatedness within groups did not differ statistically from this overall average. Panel (b) illustrates the individual pairwise relatedness within the seven analysed groups (Group A-G) in a heat map fashion. The three reference points along the colour scale indicate the lowest pairwise relatedness within the dataset (light arrow to the far left), the

average relatedness of known first degree relatives (centre arrow; equivalent of the large green dot in panel (a)), and relatedness of one (dark arrow to the right). Dots along the scale represent each pairwise comparison.

2.3.3.5 Morphometrics and stress

For males, I found no statistically significant morphometric differences associated with breeding in groups or pairs, including no difference in the level of relatedness to the Little Barrer Island birds (Table 2.3.4). Neither did I find any clear separation when all morphometric parameters were combined into a principal component analysis (Figure 2.3.7a). However, group-breeding females were found to be smaller than pair breeding females (Table 2.3.4). This was mainly driven by a lower bill to tarsus ratio but not by their relatedness to birds form Little Barrier Island (Figure 2.3.7b). There appeared to be a tendency for group-breeding individuals to better persist drought and stress, as evident through a smaller weight loss and longer telomeres, but neither of these differences between group- and pair breeding individuals were found to be significant (Table 2.3.4).

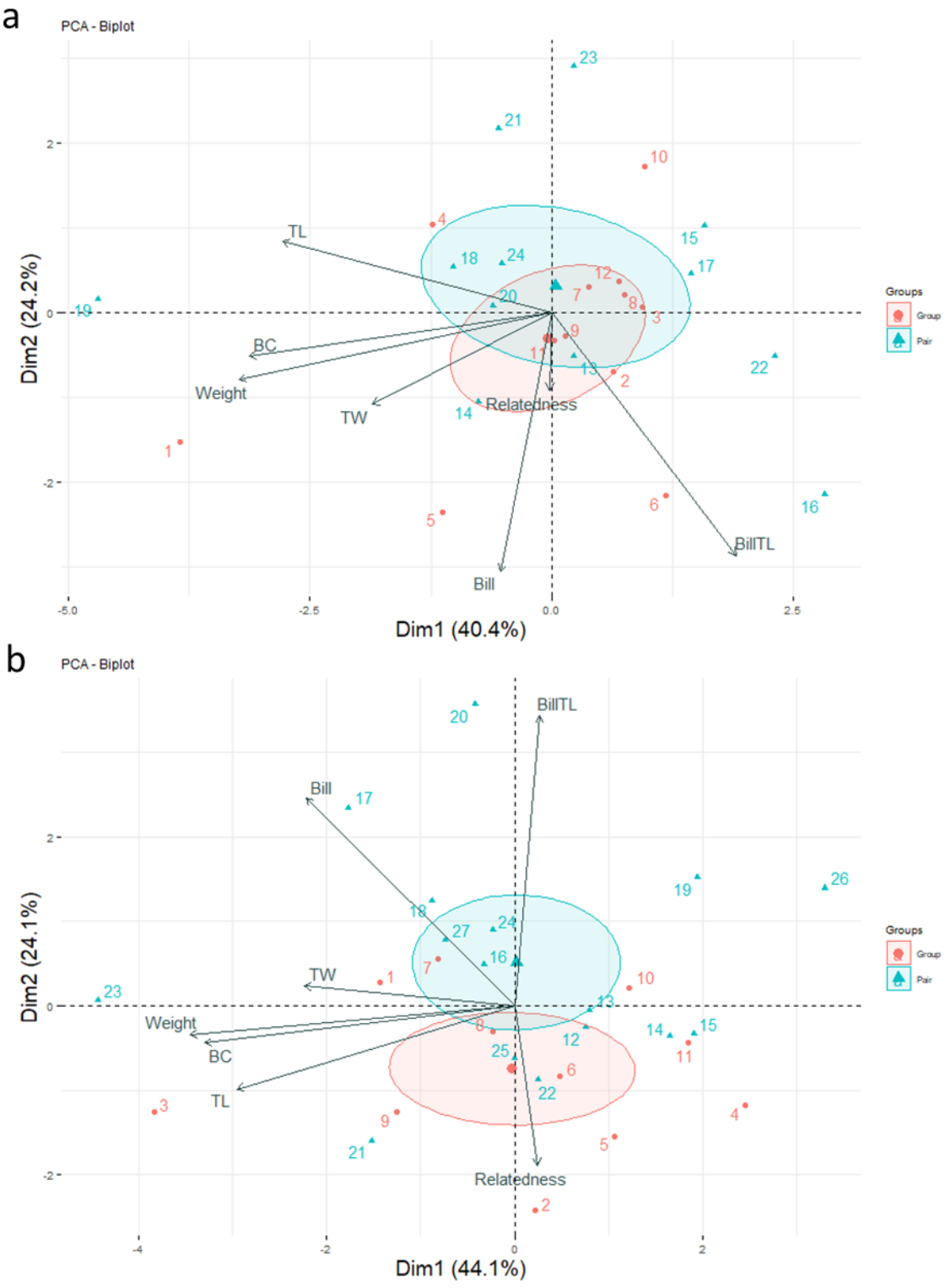


Figure 2.3.7. Principal component analyses (PCA) comparing the morphometric measurements (see Table 2.3.3) of birds breeding in groups to birds breeding in pairs. (a) shows males, (b) shows females. Group breeding females were found to be smaller compared to pair breeding females.

2.3.4 Discussion

To my knowledge, this is the first description of any alternative mating strategy to social monogamy in *A. mantelli*. This study presents five main findings. (1) Four types of breeding unit compositions occurred in the study population: socially monogamous pairs (79% of breeding units), trios with two males (10%), trios with two females (9%), and, in one case, a group of five birds (2%). (2) In two out of four multi-male groups, shared incubation was the norm and associated with lower daily activity outside but higher activity during incubation. (3) In at least one group, one male (Eco) remained part of the group and fathered offspring without ever partaking in incubation during the study period. (4) Group members were generally not closely related enough to be siblings or juveniles from previous seasons. *Apteryx mantelli* shares several features with the typical monogamous bird (Kvarnemo 2018). However, looking closer, *A. mantelli* biology and behaviour also have several characteristics that match the theory of potential for polygamy (Emlen & Oring 1977). In addition, their phylogeny suggests that *A. mantelli* should be genetically predisposed for flexibility in their mating system (Handford & Mares 1985, Coddington & Cockburn 1995, Colbourne 2002; see further Chapter 2.1). Consequently, maybe the biggest surprise is not that I found group breeding *A. mantelli*, but that no one else has done so previously. I suggest that this could be a consequence of previous studies being conducted in populations where density and juvenile survival has been decimated by habitat destruction and depredation from invasive pests (Taborsky 1988, McLennan *et al.* 1996, Caro & Sherman 2011).

Breeding in a group implies investing in someone else's young, increasing competition by sharing home range, and reducing flexibility by increasing resource requirements per breeding unit (Reyer 1984, Heinsohn & Cockburn 1994, Cockburn 2003, Canestrari *et al.* 2007, Sorato *et al.* 2016). All of these could reduce the fitness of the individual bird;

thus, in each case cooperative breeding has evolved, a combination of factors are expected to (1) enable individuals to gain fitness by being part of a group, and (2) constrain individuals from successfully breeding in independent pairs (Cockburn 2003, Baglione *et al.* 2005, Ekman & Ericson 2006, Cockburn & Russell 2011, Griesser *et al.* 2017). Some of the factors driving group breeding in other species can be ruled out for *A. mantelli*. First, *Apteryx* lack chick provisioning, hence, providing more food or reducing parental feeding load cannot be driving breeding strategy in this genus. In addition, reducing the risk of parasitism from other bird species seems a very unlikely factor. Lastly, with one exception (see below), the levels of relatedness among members analysed herein was too low for kin-selection to become an effective driver of group breeding.

Instead, other factors must be generating benefits with group breeding in *A. mantelli*. The long incubation is costly for male *A. mantelli*, both in terms of personal body condition (McLennan 1988) and time sacrificed that cannot be spent on further copulation, mate guarding, or other forms of socialising. I found that males in groups with shared incubation spent about one hour less active per night during their peak activity, likely consistent with less time spent foraging (and to some extent socialising). In addition, the males sharing incubation spent more time active during that time, and even if the difference was small per night, it added up to about 24 hours of extra activity during a full-long incubation bout. As the differences in activity during incubation and non-incubation between shared and single incubation went in opposite directions, the activity ratio might be the most telling figure; sharing males only reduce their activity by about 60% during incubation while males with all other strategies reduce their activity by 70%. Thus, I suggest that for males, the benefits of group breeding appear to be that (1) the father of the egg can reduce investment in incubation, and (2) the non-

fathering males get access to a preferable home range, likely in terms of food availability and/or quality. Notably, I found no differences between group and pair breeding birds in terms of drought resistance or telomere length. Hence, the suggested increased access to preferable habitat for group breeders did not seem to increase stress resilience.

While saying that, access to preferable territory seems insufficient to explain why some males regularly accept doing all the incubation despite (sometimes) not being the father of the egg. Instead, I suggest that the group consisting of George, Eco, and Kerri, should be seen as a special case of conspecific brood parasitism (Zink & Lyon 2015). This hypothesis implies that George accepts to incubate Eco's (the biological father's) eggs due to his high relatedness to Kerri (the mother) and thus to the offspring. This relatedness reduces George's cost of this investment (Griffin *et al.* 2013). At the same time, Kerri's motivation to effectively engage in extra-pair copulation with Eco can also be the close genetic similarity between George and herself; high relatedness has been found to correlate with the prevalence of extra-pair mating in Seychelles warbler (*Acrocephalus sechellensis*; Richardson *et al.* 2005), splendid fairy-wren (Malurus splendens; Tarvin *et al.* 2005), and red junglefowl (*Gallus gallus*; Løvlie *et al.* 2013). It has been suggested that this is a way to increase the chances of high fitness in the offspring. However, this hypothesis implies that *A. mantelli* has a mechanism for kin-recognition (Komdeur & Hatchwell 1999). Such mechanisms have been observed in a number of cooperatively breeding species, and studies have found that the ability to distinguish between kin and non-kin can be used to reduce incest/inbreeding (Walters *et al.* 1988, Nelson-Flower 2009, Nelson-Flower *et al.* 2012, Riehl & Stern 2015, Riehl 2017), preferentially help related birds and thus increase inclusive fitness (Baglione *et al.* 2003, Hatchwell *et al.* 2004, Nam *et al.* 2010, Preston *et al.* 2013, Green *et al.* 2016,

Preston *et al.* 2016, Leedale *et al.* 2020), and reduce territory defence when neighbours are close kin (Ridley 2016). The mechanism for kin-recognition remains elusive for many species (Nam *et al.* 2010, Griesser *et al.* 2015). However, the well-developed sense of smell, long lifespan, and high pair fidelity of *A. mantelli* indicates that a likely mechanism for kin-recognition is olfaction-based recognition linked to major histocompatibility complex (MHC) genotype (Zelano & Edwards 2002, Lundström *et al.* 2009, Castro *et al.* 2010, Zelenitsky *et al.* 2011, Hoover *et al.* 2018; see further Chapter 2.2).

Multiple questions around the drivers of shared incubation in this *A. mantelli* population are remaining. In pre-human New Zealand, native species such as weka (*Gallirallus australis*), kea (*Nestor notabilis*), and tuatara (*Sphenodon punctatus*) would have posed threats to *Apteryx* eggs (and potentially chicks; Jolly 1989). In addition, the relatively thin shell combined with the microbe promoting environment within the nest burrow are other proposed causes of *Apteryx* egg failure (Hiscox 2014, Vieco-Gálvez 2019). This suggests that the long egg stage of *Apteryx* is a rather vulnerable period. Interestingly, there seems to be some flexibility in the *Apteryx* embryonic development; the reported incubation time for male *A. mantelli* varies from about 65 to over 90 days, and males commonly start the incubation with a 'semi-incubation' period with relatively high activity resulting in eggs from two-egg clutches hatching closer together than they are laid (McLennan 1988, Jolly 1989, Colbourne 2002). Considering this, I found it surprising that shared incubation did not reduce the number of days between laying and hatching. Instead, rather than utilising this developmental flexibility to shorten the incubation, the behaviour of studied males suggested that there must be stronger drivers for them to prioritise reducing their personal incubation effort. One potential such driver could be increased chances of future successful breeding. Further study is needed to

elucidate whether the number of days between laying and hatching has any consequences for the chick; for instance, if a longer incubation results in more rapid post-hatching growth this could indicate a potential trade-off between time spent at the two vulnerable life stages egg and chick and increase motivation for fathers to spend a long time incubating.

It remains even more challenging to explain the male-female-female groups. Female *Apteryx* could pay a high cost by sharing males since males can only incubate a limited number of eggs (Winkler & Walters 1983, Abourachid *et al.* 2019). Thus, based on the long incubation occupying the males and that the female egg-laying capacity exceeds the males' incubation capacity, males could be expected to be the limiting sex, resulting in polyandry (Emlen & Oring 1977), but interestingly I found a multitude of group compositions: male-female; male-female-female, male-male-female, and male-male-male-female-female. I did not find much support for a connection between female activity and male breeding strategy. Together with the timing of the highest and lowest activity of females, this suggests that female activity is probably driven by food availability to a greater extent than breeding. Furthermore, it seems unlikely that the cause of sharing one male on Ponui Island is lack of potential partners since, in that scenario, the sex composition of all groups should be biased in the same direction. On the other hand, divorce was sometimes followed by several years of being unpaired, potentially suggesting an overall lack of available (suitable) partners. Perhaps the answer relates to that group breeding females tended to be smaller; the impact of size in competition over partners is another area of interest for future studies of *Apteryx* breeding behaviour.

Two key differences between the *A. mantelli* group breeding on Ponui Island and that reported for *A. rowi*, *A. haastii*, and *A. australis* is that many helpers in these other

species are (1) young, and (2) close relatives (Colbourne 2002, Jahn *et al.* 2013, Feenstra unpubl. 2020, Dearlove, T. pers. comm. 2020, Halley, J., Toy, R., and Young, S. pers. comm. 2020). These observations are consistent with kin-selection being an important driver for helpers in these species. The young age of the helpers and the long lifespan of *Apteryx* also suggests that learning crucial parenting skills is a plausible driver. If the latter was the case on Ponui Island, groups would be expected to dissolve after a certain time, which was not observed. On the contrary, most of the group breeding birds studied herein were at least ten years old. I agree with previous authors suggesting that the milder climate in the area of distribution of *A. mantelli* (and to certain extent *A. owenii*) is key to understanding the differences in incubation behaviour between the five *Apteryx* species (Colbourne 2002, Vieco-Galvez *et al.* 2020). A colder and harsher climate likely makes shared incubation obligate and might also generate additional benefits of family living (for the juveniles as well as the parents) in *A. rowi*, *A. haastii,* and *A. australis* (Colbourne 2002). Such family living could then have evolved into group breeding in line with the two-step process suggested by Griesser *et al.* (2017). Genetic data suggest that *A. mantelli* originated on the South Island and spread north (Burbidge *et al.* 2003, Tennyson *et al.* 2003, Shepherd & Lambert 2008, Weir *et al.* 2016), hence, shared incubation might have been the basal state in the brown kiwi clade. The milder climate, possibly combined with a difference in habitat, could then have rendered shared incubation non-obligate on the North Island, resulting in evolution of male-only incubation. The ability for male *A. mantelli* to single-handedly take care of incubation means that female *A. mantelli* are freed up to relay failed clutches, but also to socialise and potentially mate with other males. Notably, an individual's breeding strategy on Ponui Island was found to be independent of relatedness to Little Barrier Island birds, which is an important observation since some

sources suggest that the Little Barrier Island population might not be pure *A. mantelli* but rather a mix of *A. mantelli* and one of the other *Apteryx* species for which group breeding is common (Colbourne 2002, 2005).

The differences within the *Apteryx* genus suggest that comparative studies of their breeding effort, parentage distribution, and nest success would be interesting and highly informative for better understanding drivers of breeding behaviour within as well as outside this group. A particularly important focus for understanding *Apteryx* group breeding would be determining the distribution of parentage both among breeding unit members and non-members. Ideally, such a study would compare lifetime reproductive success between all the categories of *A. mantelli* identified herein: (1) fathers transferring some of their incubation burden to others; (2) males partaking in the incubation of eggs that are not theirs; (3) males associated with a group who does not incubate; (4) males in socially monogamous pairs; (5) females associated with more than one male; (6) females sharing male(s) with other females; and lastly, (7) females in socially monogamous pairs. However, such study would be challenging, firstly due to the long lifespan of *A. mantelli* (up to at least 40 years). Further, crucial investigations would focus on whether *Apteryx* has a mechanism for kin recognition and/or knowledge of the parentage of individual eggs. If a mechanism for the latter is identified, egg-adult relatedness could then be compared to individual incubation effort. Perhaps differences in relatedness to the individual egg could be the explanation for the noticeable difference in incubation effort invested between clutches for some members of multi-male groups. Other key studies would verify the direct benefits for males sharing incubation. A starting point for such work would be measuring bodyweight just before and just after the onset of incubation to investigate if sharing incubation allows for a smaller weight loss.

Should we expect group breeding in *A. mantelli* to be a Ponui Island specific phenomenon? There are several examples of species where the prevalence and format of cooperation changes with population density, habitat, and/or limited availability of territories (Pribil & Searcy 2001, Walker *et al.* 2008, Griffith *et al.* 2010) and of species where the prevalence and occurrence of group breeding differ among populations and/or areas (Baglione *et al.* 2002a, Lacey *et al.* 2016). This suggests that group breeding could occur only in some *A. mantelli* populations. Anecdotal stories from *A. mantelli* practitioners suggests that males in other populations are sometimes observed continuously nesting two years straight, incubating new eggs very shortly after a known partner has been found dead (Black, J. pers. com. 2020), or attempting to incubate three egg-clutches (Rewha, R. pers. comm. 2020). Vieco-Gálvez (2019) found a high degree of mixed parentage both within and between years in the *A. mantelli* population at Maungataniwha. This population is managed using Operation Nest Egg, which means that eggs are removed from nests and incubated in captivity to improve juvenile survival (Colbourne *et al.* 2005). One possible explanation for the mixed parentage observed in this population could thus be that removing eggs resembles repetitive nest failures, which have been shown to increase the divorce rate in other monogamous birds (Culina *et al.* 2015). However, the results of Vieco-Gálvez (2019) are more in line with either extra pair mating or group breeding than divorces since chick genotypes were mixed rather than replaced consecutively. Based on these observations, I suggest that group breeding is likely occurring in other *A. mantelli* populations.

Our results combined with those of Vieco-Gálvez (2019) highlight the issue that for many species data collection on behaviour is limited to unnatural, already diminishing, fragmented, human affected, and/or managed populations (Pribil & Searcy 2001, Griffith *et al.* 2010, Caro & Sherman 2011). A benefit of studying behaviour in the *A.*

mantelli population on Ponui Island is that this is one of few areas experiencing what is thought to be historical population densities. However, this population has also been vastly affected by humans. For instance (1) Ponui Island is an off-shore island, limiting any longer distance dispersal, (2) the mixed origin of this population likely allows for pairings among genetically more dissimilar birds than would be expected under natural conditions, and (3) just like all *Apteryx* populations, the Ponui Island population live in a habitat with a substantially altered species composition. Along the same lines, while Maungataniwha represents a population without a mixed translocation history or ocean boundaries, the practice to remove eggs hardly represents natural breeding conditions. In addition, humans have likely changed some of the key selection pressures around breeding strategies of New Zealand birds (Massaro *et al.* 2008). For instance, the introduction of mammalian predators might have rendered a direct negative effect of group breeding for *Apteryx* if increased bird presence at the nest was linked to increased predation risk. In several other cases where cooperative breeding has been lost there seems to be a relationship between abandoning group breeding and exposure to a harsher, less predictable habitat making the more flexible and mobile strategy of pair breeding beneficial (Carmen 2004, Ekman & Ericson 2006, Koenig & Dickinson 2016). From an *Apteryx* perspective, the environment after human arrival to New Zealand indeed represents harsher and less predictable habitat. For all these reasons, the current prevalence of group breeding in *A. mantelli* could possibly be quite different from the situation a few hundred years ago.

2.3.5 Conclusion and possible implications

Herein I provide strong evidence for previously unappreciated flexibility in the mating system of *A. mantelli*. I found strongest support for the drivers of group breeding being access to beneficial habitat and opportunity for a smaller behavioural change between

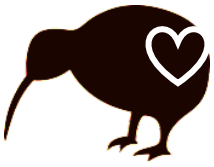
breeding and non-breeding season. However, this was only the case for males. This study is raising interesting questions regarding the scale at which drivers of breeding strategy, such as potential for polygamy, act and whether, even within one population of one species, the circumstances in each individual home range is the determining factor. In his review on the topic, Klug (2018) highlights that studying drivers of breeding strategy choice within species exhibiting multiple strategies is crucial for bringing this research field forwards. Thus, further studies of *Apteryx* breeding are not only needed to delimit the conditions under which breeding flexibility in *A. mantelli* takes effect but could provide long-sought knowledge influential far beyond this genus. One key question is the role of population density.

Does it matter if some *A. mantelli* breed in groups? Many *A. mantelli* conservation projects monitor birds in areas where chick survival is minute due to stoat predation, thus, some of these projects rely on catching the chicks at a few days of age and translocating it to an area of lower predator density. Such interventions rely on an accurately triggered hatching mode on their transmitter fitted males. Our results suggest that occurrence of group breeding could lower the accuracy of the hatch trigger and thus have a direct effect on the outcomes for these management projects. More generally, group breeding frequency is likely to affect the long-sought-after relationship between kiwi call counts, the number of breeding units, and population growth rate. Lastly, group breeding frequency could warrant reconsideration of previous calculations of carrying capacities, recommended founder population sizes, and criteria for determining conservation success since the breeding system can have a large effect on the outcome of management interventions (Vila *et al.* 2003, Adams *et al.* 2011, Bateson *et al.* 2014, Kardos *et al.* 2018, Thavornkanlapachai *et al.* 2019). The latter is relevant to

translocations aiming to achieve genetic rescue, especially if it turns out that parentage within groups is generally mixed or changing over time.

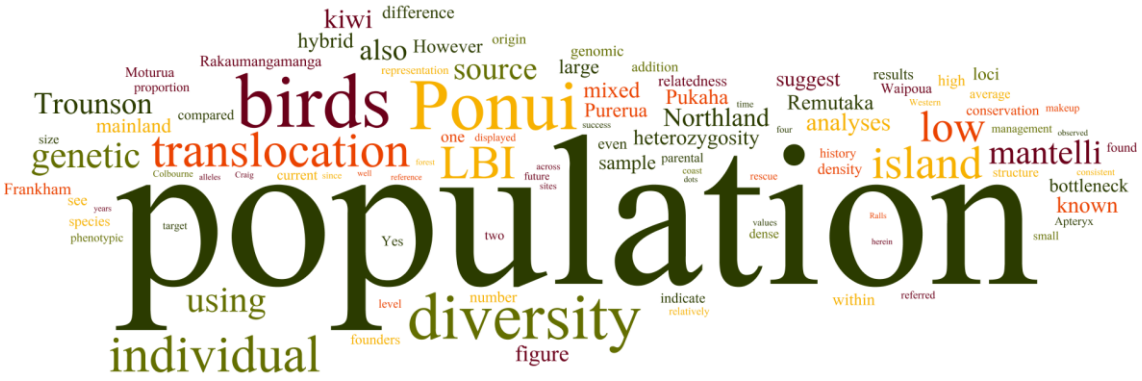
PART III – HYBRIDS

To achieve genetic rescue, some level of genetic difference is required between the source and target populations. However, differences also risk causing outbreeding depression, replacement, or genetic homogenisation. To increase our understanding of whether there is an appropriate level of difference, I take advantage of three populations with mixed-taxon origin and compare them genetically and phenotypically to unmixed populations. I discuss the implications of my results for the management of kiwi from Ponui Island, the Remutaka Forest, and the Pūkaha National Wildlife Centre, future *Apteryx* translocations, and whether it is justified to deem *A. mantelli* hybrids as being 'of no conservation value'.



Chapter 3.1

What is a kiwi hybrid?



All supervisors provided feedback on one or more drafts of this chapter. The expertise of the Elshire Group, Tea Break Bioinformatics and Simon Hills, the help from Brianna Nelson and Trish McLenachan, the hard work of Ange Hura, Natasha, Stephen, Mo, Yi, other field helpers and previous members of the Ponui Island team, and not the least, the hard work and expertise of Richard Witehira, and the support form Mana Tangata and landowners were crucial for this chapter.

Abstract

Human impact over the last several hundred years has resulted in most *Apteryx mantelli* being confined to isolated populations. Many of these populations have a recorded history of translocations, bottlenecks, and/or management. For instance, some populations are known to have been founded with few or mixed founders, and there is currently confusion around where these fit into the current and future management of *A. mantelli*. Herein, I focus on the mixed, or hybrid, *A. mantelli* population on Ponui Island. I compared the diversity for the Ponui Island population to other island populations with a single translocation origin from one area, growing but bottlenecked mainland populations, as well as comparatively pristine, large, and dense mainland populations. I also compared the genetic identity of the Ponui Island birds to reference birds representing the supposed source populations used to found this population in 1964, i.e., Little Barrier Island and the Waipoua Forest. The results show that Ponui Island had the highest diversity and lowest relatedness between individuals of the compared populations. At least eighty percent of the Ponui Island birds had a mixed genomic makeup, but no birds were found to cluster to 100% with the reference birds from Little Barrier Island. I suggest that the large, dense, diverse, and relatively easily accessible *A. mantelli* population on Ponui Island is a successful hybrid swarm that likely represents the positive effects of genetic rescue. Consequently, I suggest that Ponui Island can be a promising source for future kiwi translocations, depending on the goal of such interventions.

3.1.1 Introduction

Natural selection is the force by which the world's biodiversity is shaped to adapt to changing circumstances (Darwin 1859). However, to achieve this, selection relies on access to heritable, phenotypic variation (Hoffmann & Sgro 2011, Lai *et al.* 2019). In today's world, which is characterised by habitat degradation and fragmentation of populations, an increasing number of taxa rely on human-assisted gene flow to maintain such diversity (Cromarty & Alderson 2013, IUCN/SSC 2013, Frankham *et al.* 2017, Sutherland *et al.* 2019). Consequently, one of the most prominent conservation challenges of the twenty-first century is to accurately identify, evaluate, and prioritise among populations potentially (1) in need of assisted gene flow or (2) appropriate to use as sources for successful reinforcement translocations (Zeisset & Beebee 2013, Batson *et al.* 2015, Frankham 2015).

Reinforcement translocations have been a conservation strategy for a long time (Frankham *et al.* 2011, Miskelly & Powlesland 2013) and were originally used to boost population numbers by the sheer act of adding more individuals to achieve so-called rescue effect. However, apart from benefits linked to a larger population, introduction of individuals can result in, for instance, reversal of inbreeding depression, a wider range of phenotypes, increased heterozygosity, increased allelic variation, or increased plasticity. When such changes result in increased fitness – often defined as increased population growth rate – it is referred to as genetic rescue (Ingvarsson 2001, Whiteley *et al.* 2015). Recent progress in our appreciation of the importance of genetic diversity and adaptive potential has thus generated growing interest in using reinforcement to maintain, save, restore, and even add to or change the genetic makeup in a target population (Allendorf *et al.* 2010, Whiteley *et al.* 2015, Garner *et al.* 2016).

However, there are also ways in which translocations can result in reduced diversity such as (1) genetic homogenisation (also referred to as genetic sweeping or swamping), (2) genetic replacement, or (3) when outbreeding depression results in lower fitness of mixed progeny. Since these are all caused by genetic, phenotypic, or fitness differences between source and target populations, the way to reduce the risk of such unwanted outcomes has commonly been minimising the genetic difference between source and target populations (Edmands 2007, IUCN/SSC 2013, Ralls *et al.* 2018). Since genetic identity is rarely known, geographic proximity is often assumed to equal genetic similarity. When no sufficiently similar source population has been identifiable, inaction has often been regarded as the safer option from a conservation perspective (Frankham 2015, Ralls *et al.* 2018). This way of prioritising minimal difference often excludes a large proportion of populations from being candidate sources or target for translations (Craig *et al.* 2011, IUCN/SSC 2013, Frankham *et al.* 2017, Martin *et al.* 2017, DOC 2018). One example is that mixed or hybrid populations are often excluded from acting as translocation sources and targets, even when admixture has been within recognised species (Allendorf *et al.* 2001, Craig *et al.* 2011, Scrimgeour & Pickett 2011, Germano *et al.* 2018), particularly if hybrids are the results of human interventions, such as historic multi-origin translocations or when one of the parental taxa are substantially more common than the other (Love Stowell *et al.* 2017, van Wyk *et al.* 2017).

These restrictive attitudes towards what constitutes suitable translocation targets and sources and the prevailing reluctance of generating hybrids have likely had detrimental consequences for the future perseverance of several populations and species (Frankham 2015, Hamilton & Miller 2016, Love Stowell *et al.* 2017, Ralls *et al.* 2018, Bell *et al.* 2019). In fact, meta-analyses of over 150 published case studies have suggested that the

three most important conditions for translocations successfully generating genetic rescue was not similarity between source and target, but rather that (1) the source population was not inbred, (2) the target population experienced some level of environmental stress, and (3) genetic diversity was maximised in the target population after translocation (Zeisset & Beebee 2013, Frankham 2015, Frankham *et al.* 2017, Ralls *et al.* 2018).

At the same time, our ability to accurately predict the outcomes of mixed origin translocations remains limited (Chapter 1.3; Undin *et al.* 2021b), and, since conservation translocations by default involve threatened species, the opportunity for experimentally addressing what constitutes the best translocation strategy are next to non-existent (Hilton & Richardson 2004, Janes & Hamilton 2017). Luckily, a promising alternative to such studies is revisiting sites of well-documented, historic translocations. Increasing evidence also supports that such studies would benefit from utilising genomic analyses (Funk *et al.* 2012, Garner *et al.* 2016, Galla *et al.* 2020). This is because the resolution provided by genomic analyses enables identification of individuals, populations, and species of hybrid origin; quantification of introgression; and investigation of how and why lineages differ (Hohenlohe *et al.* 2013, Ottenburghs *et al.* 2017), which is crucial information for predicting the likely consequences of admixture (Frankham *et al.* 2011, Flanagan *et al.* 2018). High-density single nucleotide polymorphism (SNP) libraries obtained by reduced representation sequencing (RRS) methods such as Genotype-by-sequencing (GBS; Elshire *et al.* 2011) is one type of dataset that provides high enough resolution for complex analyses while also avoiding the high costs and data complexity associated with whole-genome sequencing (Puckett 2017). In contrast to traditional genetic markers such as allozymes, microsatellites, and mitochondrial sequence, RRS identifies thousands of polymorphic loci from genomic

regions that are ‘neutral’ as well as those under selection (Narum *et al.* 2013, Picq *et al.* 2018). In addition, RRS enables genotyping of individuals without the need for a separate marker identification step, which is highly valuable for the analyses of non-model organisms (Elshire *et al.* 2011, Etter *et al.* 2012).

The *Apteryx* genus is currently recognised as consisting of five species that have been further split into 14 management units (MU; McLennan & McCann 2002, Burbidge *et al.* 2003, Tennyson *et al.* 2003, Weir *et al.* 2016, Germano *et al.* 2018). These units are managed separately and shall remain independent according to national policy (Craig *et al.* 2011, Scrimgeour & Pickett 2011, Germano *et al.* 2018). However, translocations to areas with lower (or no) pressure from invasive predators have been part of *Apteryx* conservation for over a century, and some such interventions have brought together birds from what are now considered different MUs (Colbourne 2005, Cromarty & Alderson 2013, Miskelly & Powlesland 2013). The birds from such populations are referred to as hybrids and have been deemed unfit for further translocations due the ‘confusion about how to manage hybrid and mixed provenance individuals’ (Innes *et al.* 2015, Barlow 2018, Germano *et al.* 2018). Specifically, it has been suggested that birds in hybrid populations will suffer from (a) inbreeding depression due to their small number of founders, and (b) outbreeding depression based on the assumption that differences between *Apteryx* MUs are linked to local adaptation. However, no comparative studies of the fitness and diversity of hybrids and ‘pure’ *Apteryx* have been conducted, the interbreeding between birds from different taxa has only been assumed, and, most importantly, available genetic data do not allow us to determine the role of local adaptation for *Apteryx* diversification (Undin *et al.* 2021a, Undin *et al.* 2021b; i.e., Chapter 1.2 & 1.3).

Fortunately, the translocation, population, and management histories are well documented for many *A. mantelli* (North Island brown kiwi) populations. For instance, the timing of the translocation(s), as well as the number of founders and their origins have often been recorded (Colbourne 2005). This array of populations constitutes an ideal study system for increasing our understanding of, for example, the consequences of hybridisation, the premises for genetic rescue, and how to best identify source populations in the future. Herein I utilise this study system and specifically focus on the dense hybrid *A. mantelli* population on Ponui Island. This population resulted from two translocations in 1964 that combined birds from the Northland and the Western *A. mantelli* MUs, and is thus one of the populations described above as causing 'confusion'. I compare the Ponui Island population to (1) its ancestral (parental) populations, (2) mainland populations with no recorded history of translocations (albeit in one case with known bottleneck history), (3) another island population with the same number of founders but where all came from the same MU, and (4) two other admixed sites partly sharing ancestry with the Ponui Island birds to which translocations have happened more recently. In addition, I look at the genetic structure of 120 Ponui Island birds to determine the level of admixture and the current representation of the two parental genomes. By doing this I test the hypothesis that this population constitutes a hybrid swarm where repeated interbreeding has resulted in high diversity, against the previous outlaid hypothesis that this population suffers from high levels of inbreeding and outbreeding depression. Specifically, I address (1) whether the patterns of phenotypic diversity are consistent with founder effects, demographic swamping, additive mixing, or transgressive segregation; (2) if the Ponui Island population has low genetic diversity and heterozygosity consistent with inbreeding and/or few mixed individuals suggestive of reproductive incompatibility or outbreeding depression; or,

alternatively (3) if the mixed parentage of Ponui Island has generated higher diversity and lower levels of inbreeding compared to the reference populations. Finally, I discuss how my results relate to *Apteryx* conservation in general, to the current recovery plan, and to the future management of the Ponui Island population. Increased understanding of successful translocations, hybridisation, and how to achieve genetic rescue is instrumental for conservation management of fragmented populations of all species but will be particularly beneficial for *Apteryx* spp. and other long-lived species with reduced dispersal ability (Young & Duchicela 2020).

3.1.2 Materials and Methods

3.1.2.1 Study species

The nocturnal, flightless, ratite genus *Apteryx* is endemic to Aotearoa New Zealand. Genetic analyses recognise five extant species which are further split into 14 MUs or taxa; four of these are within *A. mantelli* and are referred to as Northland, Coromandel, Western, and Eastern, respectively. Despite lacking understanding around the nature of and history behind the genetic differences (see further Chapters 1.2 and 1.3), current conservation focuses on managing and maintain the taxa separately. Historic management has resulted in current *A. mantelli* populations falling along a gradient from non-managed to highly managed and from low to extensive influence of translocations and/or bottlenecking (Colbourne 2005, Robertson & de Monchy 2012, Innes *et al.* 2015).

3.1.2.2 Study populations, translocation history, and blood sampling

The nine focus *A. mantelli* populations referred to and/or analysed in this study are described in detail below (summary in Table 3.1.1 and Figure 3.1.1). In addition,

reference samples from populations were included in the analyses of relatedness to represent the Eastern and Coromandel MUs as well as additional areas within the Northland MU.

Ponui Island is a 1770 ha island in the Hauraki Gulf. The *A. mantelli* population on this island was founded through two translocations in 1964, the first of which consisted of six birds from Little Barrier Island (usually considered *A. mantelli* Western but by others considered hybrids; Colbourne 2005, Holzapfel *et al.* 2008, Scrimgeour & Pickett 2011) and later an additional eight birds from the Waipoua Forest were brought (*A. mantelli* Northland; Colbourne 2005). In 1999, the population was estimated to be 350 adults, and today the estimated density is one bird per hectare, making it one of the densest in the world (McLennan 1988, Miller & Pierce 1995, Colbourne 2005, Craig 2019). Over the last 17 years, at any given time 30 to 50 birds in this population have been closely monitored using radio transmitters.

The **Waipoua** Forest is located in the western part of Northland, and the kiwi population here used to be referred to as 'probably the largest population now extant in the North Island' with an estimated population of 3000 to 5000 birds (Butler & McLennan 1990). However, in recent years the density has suffered a rapid decline (Craig *et al.* 2011). In addition, the Waipoua forest features exceedingly challenging terrain for monitoring, as well as sampling, of birds. Unfortunately, the terrain in combination with the low density meant that I was unable to collect samples in the Waipoua forest for this study.

The **Trounson** Kauri Park is a 445 ha forest about 10 km in a straight line from the Waipoua Forest. This is within the known dispersal distance of *A. mantelli* (Forbes 2009) and movement of tagged birds have been recorded several times between these

areas (Tom Donovan pers. comm. 2020). Consequently, the *A. mantelli* in these forests are likely to have a shared gene pool. The Trounson Kauri Park is managed as one of five 'mainland islands' in Aotearoa New Zealand. These are areas of special interest where the Department of Conservation (DOC) executes extensive management of invasive species aiming to restore the habitat within the area while also focusing on research into improving management strategies (Gillies *et al.* 2003). The name 'mainland islands' refers to that the goal is reaching pest-levels otherwise only observed on off-shore islands. When this extensive trapping regime was deployed in 1996, the *A. mantelli* population density had remained comparatively unaffected and the density has stayed high since with the number of pairs estimated to 66 in 2007 (Craig *et al.* 2011, Craig 2019). Thus, I refer to the Trounson Kauri Park population as a mainland population without bottleneck history.

Little Barrier Island (**LBI**) also known as Hauturu-o-Toi is a 3083 ha island. This island is protected and is regarded as one of the most pristine and diverse areas left in New Zealand. Consequently, access is limited and biosecurity measures rigorous. The island did have feral cats until a successful eradication mission enabled declaring it completely pest free in 1980. The combination of the state of the forest, the size, and the offshore distance meant that LBI was discussed as a potential place to utilise as a safe haven and a source site for species translocations for over 100 years. The island has a thriving kiwi population. In 2002 it was estimated to 200-300 pairs, and since about 2008 the population is assumed to be at its inferred carrying capacity of *ca*. 1000 birds (Holzapfel *et al.* 2008). The most widespread suggestion is that the current kiwi population on LBI stem from a translocation of 16 individuals from Taranaki (*A. mantelli* Western) in 1919 (Colbourne 2005). In addition, several other releases of kiwi onto the island have been suggested, for instance, one of albino *A. mantelli* from Taranaki in 1913 and one of *A.*

haastii from the South Island in 1915 (Oliver 1922, Oliver 1930). In addition, it has been suggested that *A. mantelli* were native to the island prior to human interventions. If any of the earlier translocations happened, it is unknown if the birds established on LBI or if birds from different sources interbred. There is some support for mixed heritage such as unique mitochondrial alleles (Herbert & Daugherty 2002, Burbidge *et al.* 2003) and a seemingly unique species of lice (Palma 1991). However, reanalyses of the mitochondrial sequences do not suggest mixture with anything as taxonomically distant as *A. haastii* (see further Chapter1.2). This insecurity around the origin and composition of the founding population has resulted in the taxon identity of the LBI birds regularly being referred to as mixed or unknown, even if the island currently fall under the *A. mantelli* Western Taxon Plan (Holzapfel *et al.* 2008, Scrimgeour & Pickett 2011). Unfortunately, I was granted permission for sampling on LBI just in time for the April 2020 Covid-19 level-4 lockdown, so no samples were acquired directly from LBI for this thesis.

The **Remutaka** Forest and the **Pūkaha** National Wildlife Centre are two sites with *A. mantelli* populations outside the known historic range of this species. Both populations were established by the release of a mixture of previously captive birds and birds translocated from LBI (20 and 30 LBI birds in 2008 and 2010 respectively; see further Chapter 3.2). These populations have been followed closely, thus birds with known 'pure' LBI background (a combination of the actual translocated individuals and their offspring) are still present. Such birds will be referred to as LBI birds throughout the remainder of the text.

The **Purerua** Peninsula is situated on the east coast of Northland. Like the Trounson Kauri Park, Purerua features a high-density *A. mantelli* Northland population (Craig

2019). The population has no known translocation or reinforcement history (neither in nor out of the population), but before management intensified in the last decades, the population did go through a bottleneck of unknown size.

Moturua is a 163 ha island in the Bay of Islands. The *A. mantelli* population here was founded in 1983 and 1985 by the release of 14 relatively local *A. mantelli* Northland (nine females and five males) rescued from forestry missions (Colbourne 2005). In 2006, the population was estimated to be 40 pairs. A large part of the island is today a nature reserve managed by the Department of Conservation (DOC); the remaining is privately owned.

Rakaumangamanga, or Cape Brett, is the tribal land of Te Patukeha and Ngati Kuta Hapū. This peninsula has strong cultural significance by being referred to as the first point at which Kupe (the first human that arrived in Aotearoa New Zealand) landed, as well as one of three corners of the Polynesian triangle. The *A. mantelli* on Rakaumangamanga was down to very low densities and restricted to a small area at the far end of the peninsula. However, since a pest control regime was established in 2003, the numbers and distribution of *A. mantelli* have steadily increased, recently reaching the human settlements at the base of the peninsula. No translocations are known to have occurred in or out of the Rakaumangamanga population.

Table 3.1.1. Summary of background and current status of the focus *A. mantelli* populations discussed.

Population	Role	Sampled?	Location	Site type	Translocation	Bottleneck - timing	Bottleneck - Min size	MU Composition	Populations status
Ponui Island	Successful hybrid population	Yes	Northland - East coast	Island	Yes – 2*	Founded 1964	14	Hybrid	Large and dense. Limited by size of island
Waipoua Forest	One parent to the Ponui Island population	No	Northland - West coast	Mainland	No known	Currently in decline		Pure Northland	Comparatively low density
Trounson Kauri Park	Dense population acting as proxy for Waipoua	Yes	Northland - West coast	Mainland	No known	No known bottleneck		Pure Northland	Large and dense
Little Barrier Island (LBI)	One parent to the Ponui Island population	No	Northland - East coast	Island	Yes - at least 1	Founded 1919[‡]	16	Pure Western OR Hybrid[‡]	Large and dense. Limited by size of island
Pūkaha National Wildlife Centre	Source of birds originally from LBI	Yes	Southern North Island[§]	Mainland - Isolated	Yes - multiple over 18 years	Founded 2000	57 (32)[¤]	Hybrid - Founders from LBI (in 2010) but also from the Western, Northland, and Eastern MUs	Comparatively low density

Remutaka Forest Park	Source of birds originally from LBI	Yes	Southern North Island[§]	Mainland - Isolated	Yes - multiple over 6 years	Founded 2006	31 (24)[¤]	Hybrid - Founders from LBI (in 2008) but also from the Western, Northland, and Eastern MUs	Comparatively low density
Purerua Peninsula	Large mainland population	Yes	Northland - East coast	Mainland	No known	Yes	Unknown	Pure Northland	Large and dense
Moturua	Single source island population	Yes	Northland - East coast	Island	Yes - over 3 years	Founded 1983-85	14	Pure Northland	Medium density[¥]
Rakaumangamanga – Cape Brett	Mainland population with bottleneck history	Yes	Northland - East coast	Mainland	No known	Until 2003†	Unknown	Pure Northland	Comparatively low density

[*]Both in the same year.

[ǂ]Some evidence exists for kiwi being on the island before this translocation (Palma 1991), but even if there were, their contribution to the current population is unknown (Colbourne et al. 2005).

[§]Outside the historic range of *A. mantelli.*

[¤]High relatedness among some individuals account for the lower number in brackets.

[¥]'Medium' density refers to a density substantially lower than Ponui Island, Purerua Peninsula, etc. but higher than Rakaumangamanga.

†Assumed to have reached a minimum right before pest control started in 2003.

For all populations except Ponui Island, blood sampling was conducted in 2019 and 2020 under the Massey University Animal Ethics Committee (MUAEC) permits 06/05, 07/144, 16/92, 18/82, and 18/83 and the Department of Conservation Wildlife permits AK-14969-RES, AK-21519-FAU, 50249-FAU, 70826-CAP, and 70875-RES. Following the Kiwi Best Practice Manual, blood was sampled by licensed kiwi practitioners from the metatarsal vein. With the exception of two birds in the Trounson Kauri forest, these were all birds without transmitters; detection and catching involved a combination of daytime tracking with a certified kiwi conservation dog and handler, and night-time catching relying mainly on encounter capturing but also, to a lesser extent, attracting birds using whistle or playback. The method used was found to not influence the bird composition sampled or the blood values collected (see further Appendix A2).

On Ponui Island, blood was sampled in four separate cohorts: in 2004, 2006-2008, 2010, and 2017-2018 (see further Appendix A1). Most of the sample utilised here was sampled in 2017, but older samples were used to increase sample size and spread across the island.

3.1.2.3 Phenotypic diversity

To investigate patterns of phenotypic diversity consistent with founder effects, additive phenotypic diversity, and transgressive segregation, the phenotypic spread of four blood and four morphometric parameters was compared between populations. Specifically, the spreads of total protein levels, glucose concentration, packed cell volume (PCV) and haemoglobin concentration (HB), bill length, tarsus width (TW), tarsus length (TL), and bill length to TL ratio were graphically compared using the commands geom_violin and geom_jitter within the function ggplot from the R package tidyverse (Wickham *et al.* 2019). For the morphometric parameters, only adult birds were included. However,

since the focus was on the spread of phenotypes, males and females were analysed together despite size differences between the sexes; this allowed for a larger sample size per population.

3.1.2.4 Genetic diversity

3.1.2.4.1 DNA isolation

See Chapter 2.2.

3.1.2.4.2 GBS library preparation and sequencing

Pair-ended Genotype-by-sequencing (GBS) library and sequencing preparation as well as associated quality checks were done by The Elshire Group Limited. GBS libraries were constructed using 100 ng of genomic DNA, 1.44 ng of adapters, the restriction enzyme EcoT22i, and 18 PCR cycles (Elshire *et al.* 2011). In total, 282 samples from 260 unique birds were analysed across three 96-well plates (see further Appendix A1). Sample location within plates was randomised and each plate contained one positive and one negative control. Sequencing was performed on an Illumina HiSeq XTen with 2 x 150 bp paired-end reads.

3.1.2.4.3 SNP calling, filtering, and trimming

Processing of raw read data, including filtering, trimming, alignment, and SNP calling, was done by Tea Break Bioinformatics. Due to the questionable quality of the previously published *A. mantelli* genome (Le Duc *et al.* 2015), fragments from this study were aligned to a re-assembled genome using Meraculous 2.2.5.1 (Chapman *et al.* 2011). The 1 538 639 658 raw GBS reads were demultiplexed using Axe (axe-demux; Murray & Borevitz 2018), adapters and barcodes were trimmed using the batch_trim.pl

script (www.github.com/Lanilen/GBS-PreProcess), and pair matching was carried out using Bowtie 2 (Langmead & Salzberg 2012).

SNP-calling was conducted in STACKS 2.5 (Catchen *et al.* 2013) using the populations program set for the EcoT22i enzyme, bootstrapping, and site merging. The output was provided as text files formatted for genepop and STRUCTURE (see below), fasta files for loci and samples, and as a single snp file. This was all done using the following command line: --vcf -r 0.1 --min-maf 0.1 -e ecoT22I --ordered-export --bootstrap --merge-sites --genepop --structure --fasta-loci --fasta-samples --fasta-samples-raw --write-single-snp. Each step was done for each plate separately, after which the graphical output from Kinship-using-GBS-with-Depth-adjustment program (KGD; Dodds *et al.* 2015) and Tensorflow Projector (www.projector.tensorflow.org/) were used to verify the absence of bias or batch effects. Stacks 2.5 was then rerun with the same parameter-setting for a combined dataset of all three plates. The output from this run was trimmed to only include the first variable site per locus. The number of individuals and populations utilised differed between populations (see below), thus all loci might not be variable within each analysed datasets.

3.1.2.4.4 Relatedness

The KGD output (see above) was used to acquire a matrix of pairwise relatedness (also referred to as allelic similarity) values accounting for differences in SNP read depth. Such relatedness values have previously been shown to be highly correlative with known pedigree-based values of relatedness (see for instance Galla *et al.* 2020). The unscaled relatedness value of each bird to all other sample birds from the same population were used to compare average relatedness within populations as a proxy for inbreeding. Linear model (lm; R core team) was used to test population differences

statistically. The HSD-test was used for post-hoc analyses (de Mendiburu 2020), and, to ensure no significant impact was due to the substantially larger sample size from Ponui Island, the average pairwise relatedness within this population was calculated based on 13 randomly drawn subsets of between five and 100 individuals for comparison. For the analysis of average relatedness, the birds from LBI sampled at Pūkaha and Remutaka were analysed separately as well as included together with all other Pūkaha and Remutaka birds.

In addition, the KDG relatedness output also features a dendrogram that clusters individuals based on relatedness. This clustering included the focus as well as the reference populations and was used as additional verification of the structure results (see below), as well as the assumption of the parental populations of Ponui Island.

3.1.2.4.5 Diversity

The function genedivFis in the R packages genepop (Rousset 2008) was used to analyse genetic diversity within and divergence between populations. For quantification of the average, proportional heterozygosity within individuals ('1-Qintra') and proportion of not shared alleles between individuals within the same population ('1-Qinter'), all diploid loci (>35 000) with at least two individuals sequenced were included. For complementary quantification of the proportion of individuals being heterozygous in each population, I used 500 loci for which heterozygosity was observed for the major allele and the most common minor allele. For these analyses, birds from Pūkaha and Remutaka were considered one population, since the sample size was too small for separate analyses.

3.1.2.4.6 Structure

To investigate population structure with the hybrid Ponui Island population, 153 individuals (120 from Ponui Island, 13 from LBI, and 20 from Trounson) were analysed using STRUCTURE 2.3.4 (Pritchard *et al.* 2000, Hubisz *et al.* 2009). Each K between 1 and 8 was explored using the admixture model, a 10000 run burn-in, 90000 generations, and 10 iterations. Structure_threader (www.github.com/StuntsPT/Structure_threader) was used to automate and parallelize the runs. Best K was evaluated using the Evanno method (Evanno *et al.* 2005) and Structure harvester version v0.6.94 (Earl & vonHoldt 2012). In order to validate the interpretation of the clustering with the highest statistical support as well as the expected number of clusters (three), the results of the structure analyses were cross-compared to the relatedness data by (a) comparing the cluster identity of each bird and (b) correlating proportional likelihood of assignment to cluster one (at K = 3) to average relatedness to the LBI birds for each Ponui Island bird.

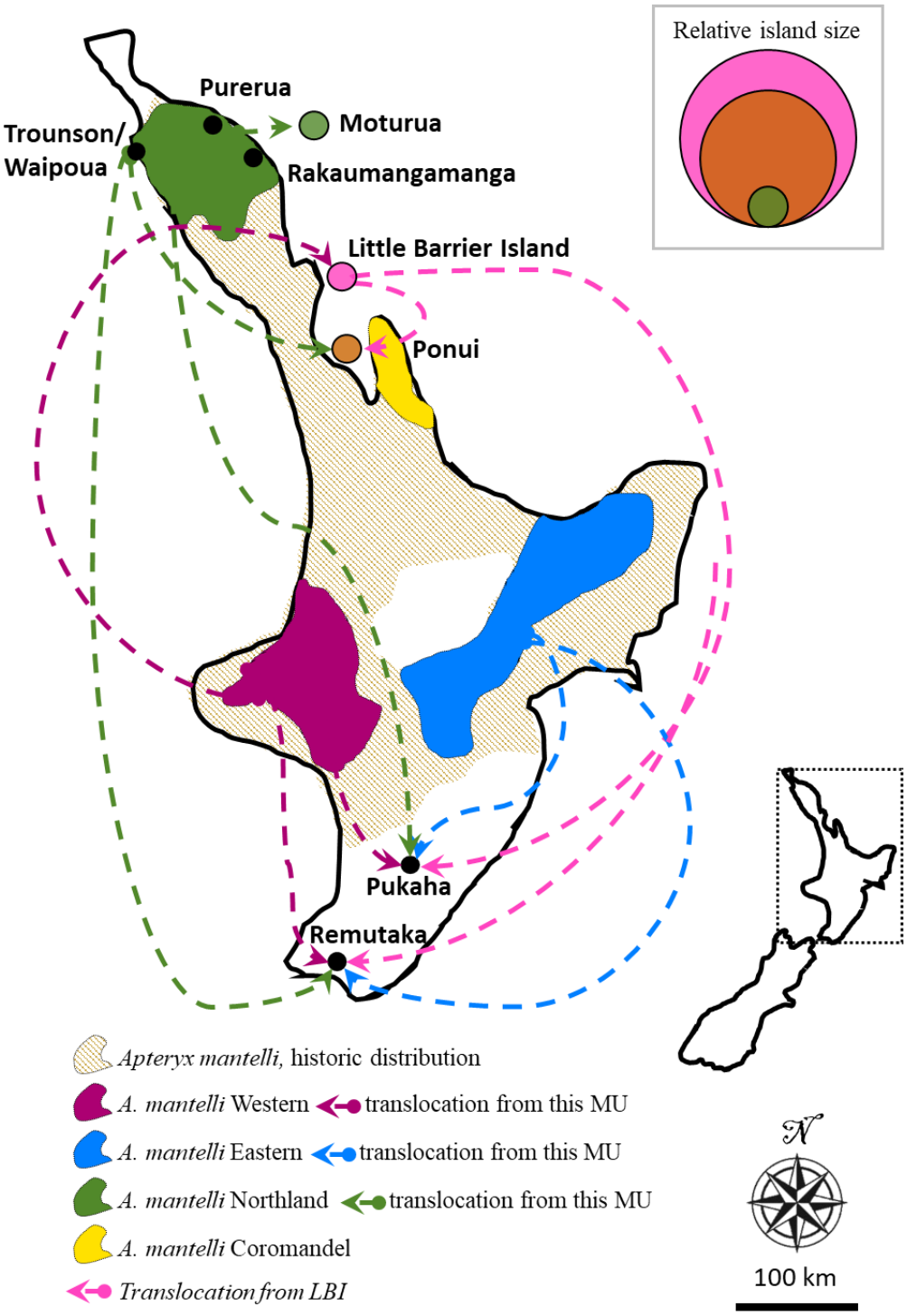


Figure 3.1.1. Map indicating the location of *A. mantelli* populations sampled and discussed in relation to genetic diversity and translocation history (complementary data in Table 3.1.1). Coloured dots indicate islands, and the top right inset their relative size. Patterned area indicates the historic distribution of *A. mantelli* (Shepherd *et al.* 2012, Germano *et al.* 2018). Filled areas indicate the current distribution, with colours delimiting management units. Arrows indicate the origin of translocated founders.

3.1.3 Results

To quantify, evaluate, and compare the diversity of the hybrid *A. mantelli* on Ponui Island, samples from 209 birds from seven focus populations (Table 3.1.1) were utilised plus an additional 51 reference samples. The genomic analyses included 51691 loci with an average call rate of 0.60 (STD 0.07) and an average sample depth of 24 (STD 12).

3.1.3.1 Phenotypic diversity

Overall, the analyses of phenotypic diversity indicate that the Ponui Island population is morphologically diverse, consistent with a genomic contribution from both parental populations. The distribution of bill lengths and bill length to tarsus length ratios found in LBI and Trounson had limited overlap, with Trounson birds being bigger. The equivalent distribution for the Ponui Island population spanned values that encompassed both LBI and Trounson distributions combined, a pattern consistent with hybridisation with additive phenotypic diversity (Figure 3.1.2a & c). The distribution of tarsus widths and tarsus lengths in the Ponui Island population included values both larger and smaller than that of Trounson and LBI, a pattern consistent with hybridisation followed by transgressive segregation resulting in extreme phenotypes (Figure 3.1.2b & d). Furthermore, the relatively narrow and offset variation observed in the Moturua and Rakaumangamanga, is consistent with so-called founder effect, and can be expected due to the tight bottlenecks of these populations (Figure 3.1.2). There were few clear trends in the haematology data. However, the wide span of PCV and glucose concentration values observed in the Ponui Island population compared to LBI and Trounson is consistent with increased phenotypic plasticity associated with transgressive segregation (Figure 3.1.3b & c). Notably, the small sample sizes of adult individuals from some populations rendered it impossible to quantify the contribution of differences between male and female kiwi as well as environmental factors to the phenotypic patterns

observed. For instance, the noticeable difference between Trounson and Purerua in terms of glucose as well as the discrepancy between the Pūkaha plus Remutaka birds and the remaining populations could both be consistent with either genetic differences (possibly local adaptation) or phenotypic plasticity combined with environmental differences between sites (Figure 3.1.3a, d, & c). The latter might be more likely in the case of Remutaka and Pūkaha considering the climatic difference between Northland and the location of these populations.

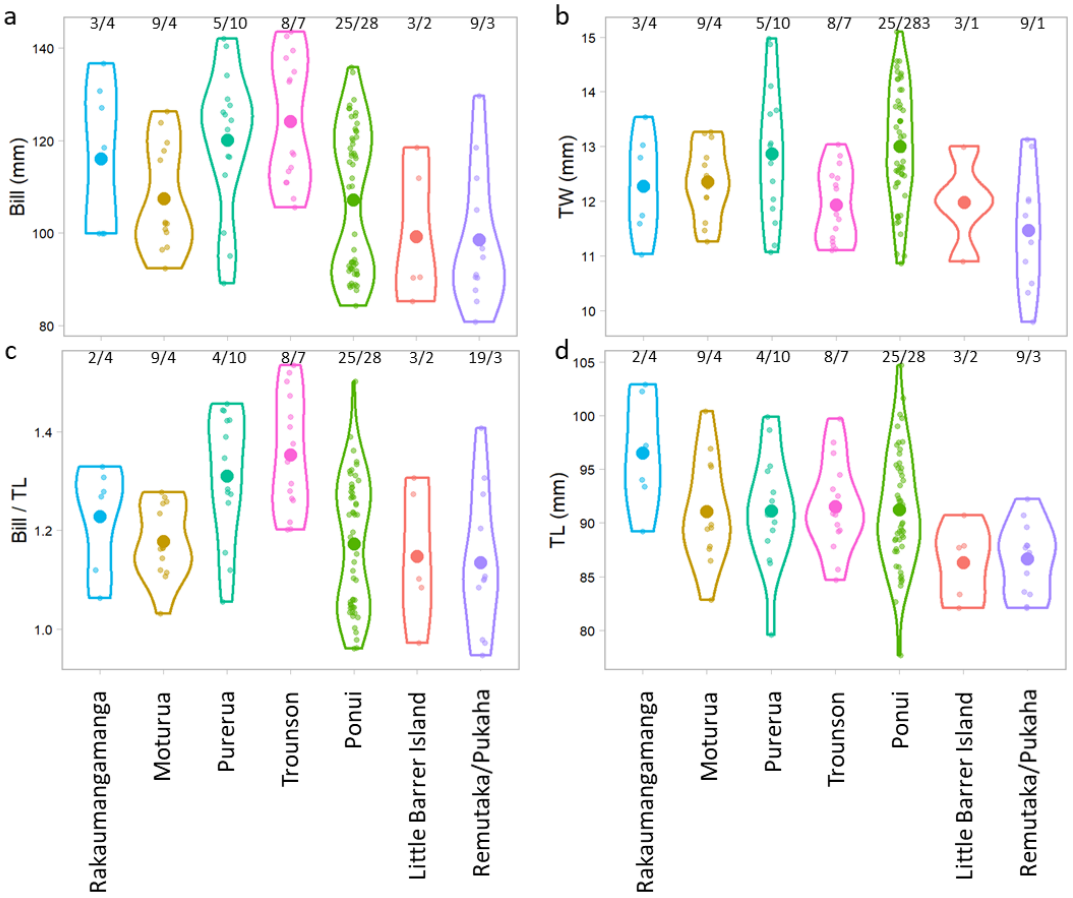


Figure 3.1.2. Violin plots illustrating phenotypic diversity with respect to morphometric measurements of adult individuals from seven *A. mantelli* populations. Specifically, the average values and spread are displayed for bill length (a), tarsus width (b), ratio between bill length and tarsus length (c), and tarsus length (d). Large dots indicate mean; small dots indicate each data point and the shape of the outline their distribution. Sample size is specified above each violin as males/females (male *A. mantelli* are on average about 20% smaller than females; Miles *et al.* 1997, Sales 2005, Robertson & Colbourne 2017). The Remutaka and Pūkaha populations are

displayed together to the far right in each panel, but LBI origin individuals from both sites are, in addition, displayed separately.

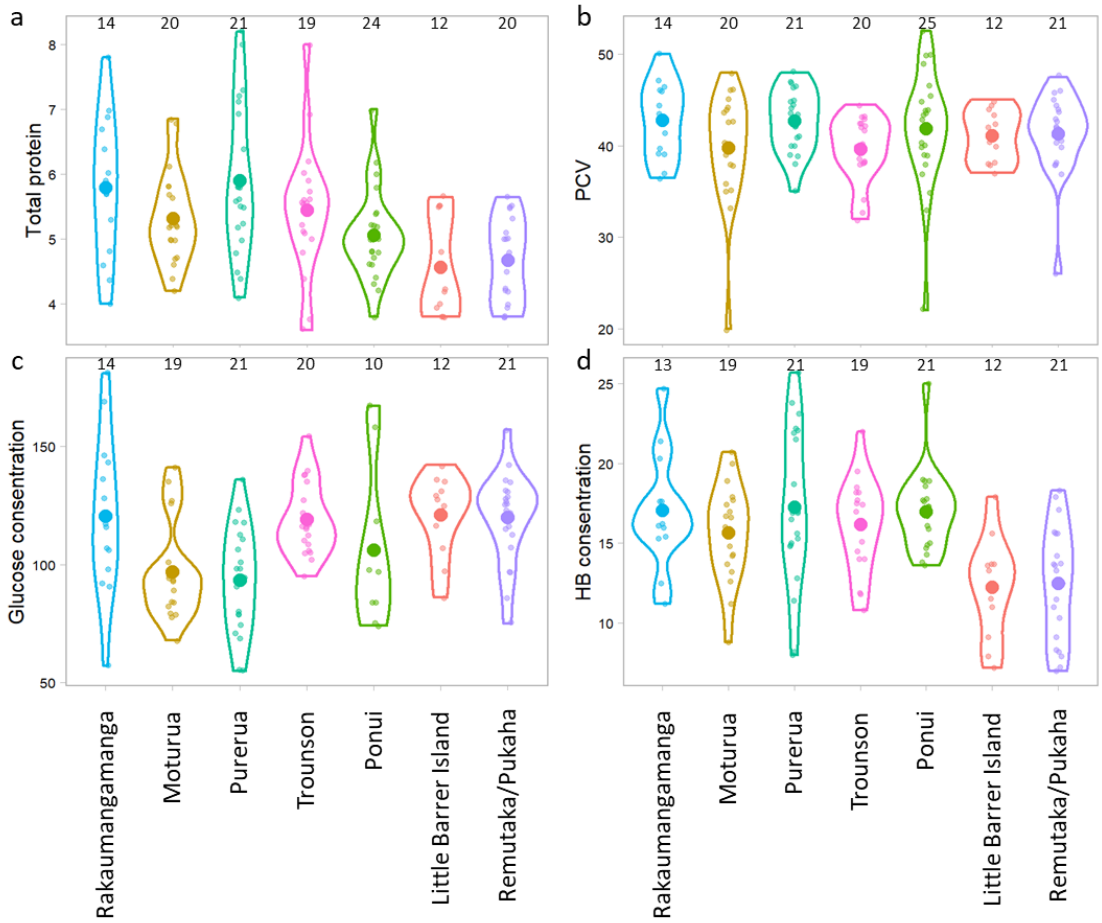


Figure 3.1.3. Violin plots illustrating phenotypic diversity with respect to four haematologic characters of seven *A. mantelli* populations. Specifically, the average values and spreads are displayed for total plasma protein (a); packed cell volume (b); glucose concentration (c); haemoglobin concentration (d). Large dots indicate mean; small dots indicate each data point and the shape of the outline their distribution. Sample size is specified above each violin. The Remutaka and Pūkaha populations are displayed together to the far right in each panel, but LBI origin individuals from both sites are, in addition, displayed separately.

3.1.3.2 Genetic diversity

Birds from Ponui Island were found to have the lowest average pairwise relatedness out of all populations analysed (Figure 3.1.4). Purerua, Moturua, and Rakaumangamanga had the highest average relatedness; Trounson birds were less related than these three but more than Ponui Island birds. Pūkaha and Remutaka birds were more related than Ponui Island birds but statistically inseparable from all other populations due to high variability among individuals.

In terms of individual level of heterozygosity, the average for all populations fell within 4.5 percentage points (16.7-21.2%). Ordered from highest to lowest individual heterozygosity, the population were: Ponui Island, Purerua, Trounson, Remutaka and Pūkaha, Rakaumangamanga, and Moturua (Figure 3.1.5a). For the proportion of individuals being heterozygous, Ponui Island was again the most diverse population followed by Rakaumangamanga, Trounson, Remutaka and Pūkaha, Moturua, and Purerua in descending order (Figure 3.1.5b). Lastly, for the proportion of non-shared alleles among individuals, Ponui Island had the highest followed by Trounson, Remutaka and Pūkaha, Rakaumangamanga, Purerua, and Moturua in descending order (Figure 3.1.5c).

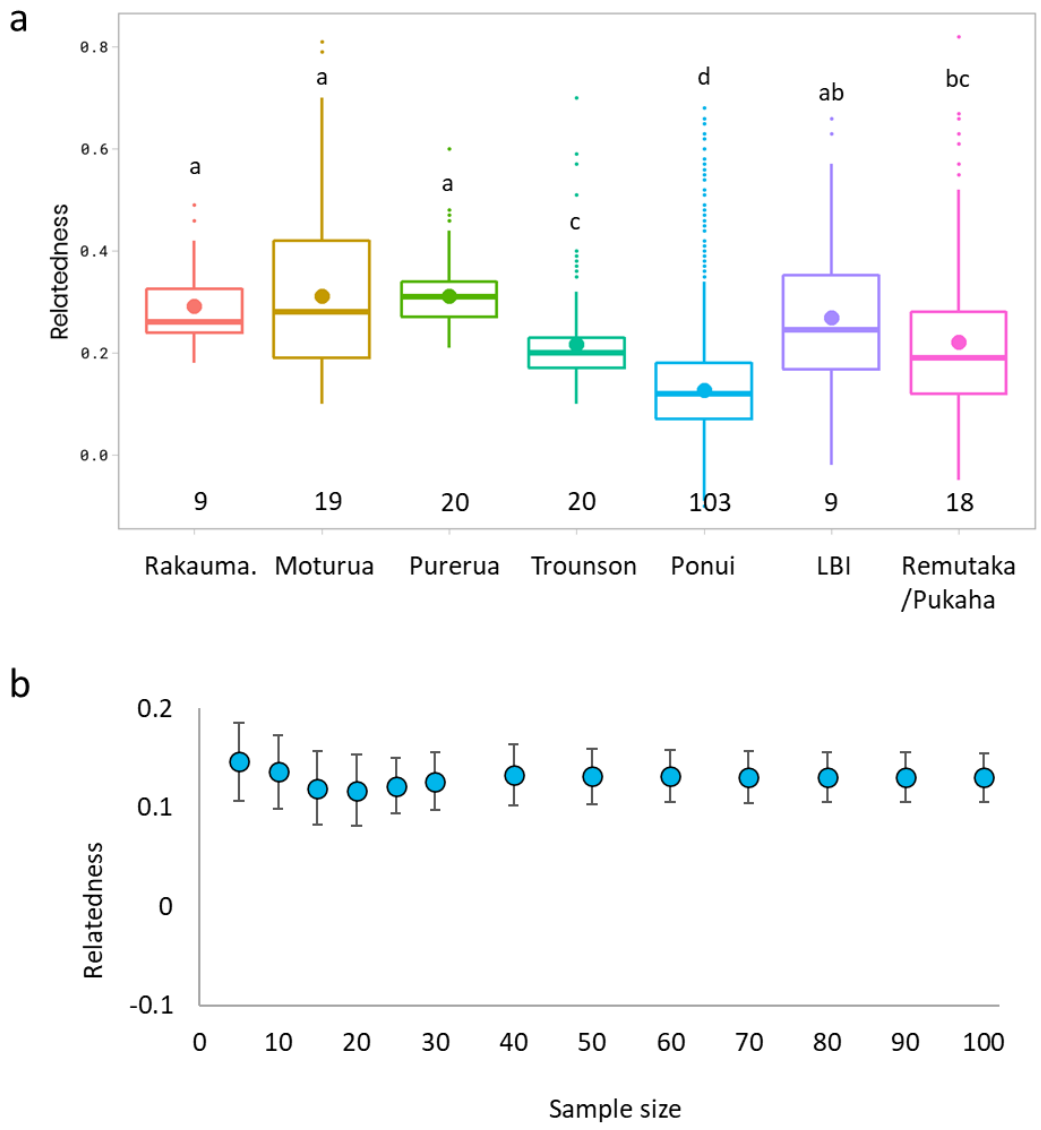


Figure 3.1.4. Pairwise relatedness (or allelic similarity) between individuals within seven *A. mantelli* populations. Lower values indicate lower relatedness and are consistent with lower inbreeding. In (a) boxplots indicate median values and interquartile distance. Large dots indicate the mean for each population. Different lower-case letters indicate statistically significant differences. For population details see Table 3.1.1. The Remutaka and Pūkaha populations are displayed together to the far right in each panel, but LBI origin individuals from both sites are in addition displayed separately. In (b), the average pairwise relatedness within the Ponui Island population has been recalculated based on an increasing number of random individuals from the population to ensure that differences indicated in (a) are not an artefact due to differences in sample size between populations. Error bars indicate standard deviation. Numbers along x-axis in both panels denote sample sizes (n); the number of pairwise comparisons was thus ($\frac{n(n-1)}{2}$).

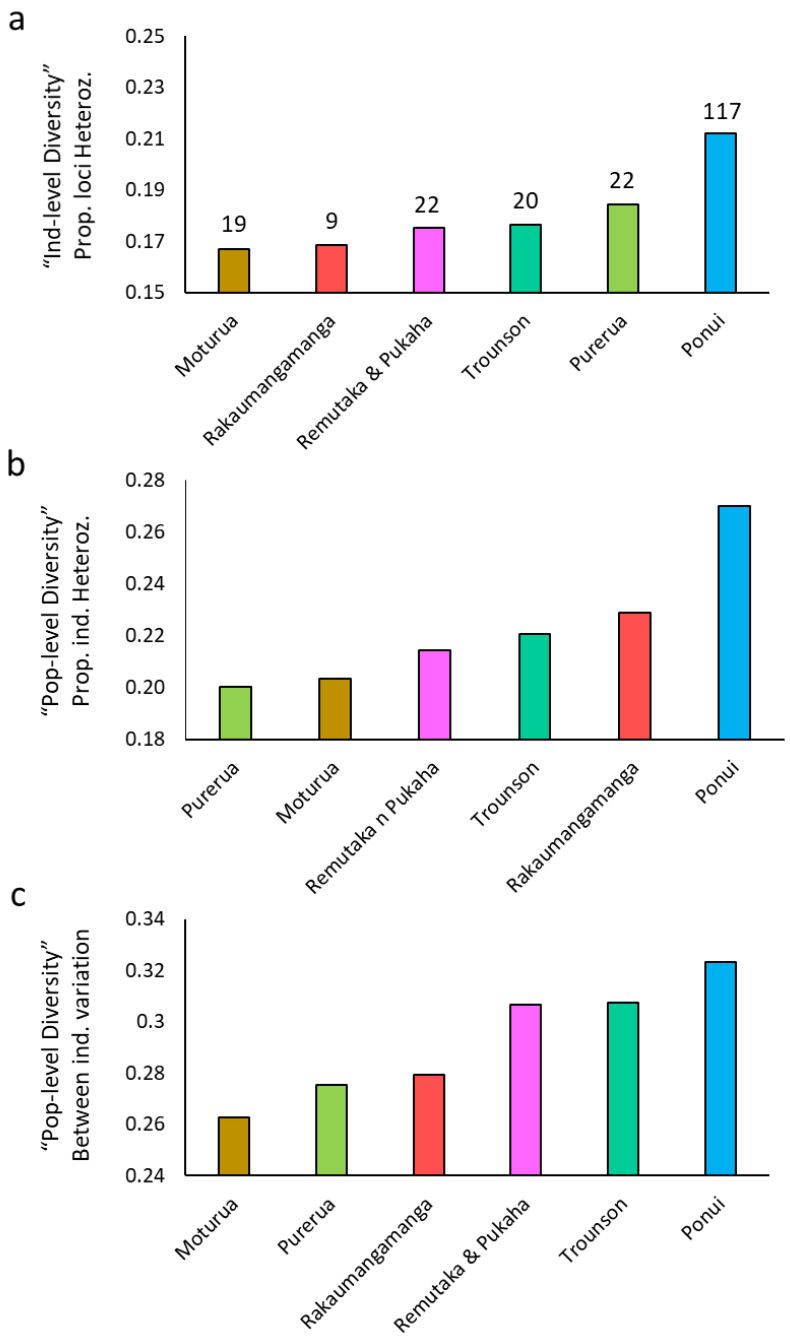


Figure 3.1.5. Comparison of genetic diversity for *A. mantelli* from seven sites. Populations ordered from lowest (less diversity; left) to highest (largest diversity; right). The Remutaka and Pūkaha populations are displayed together. Panel (a) illustrates the average proportion of heterozygous loci in an individual (n > 35000 loci with at least two individuals typed). Panel (b) illustrates the average proportion of heterozygous individuals per loci in a population (n = 500 heterozygous loci). Panel (c) illustrates the average proportion of non-shared alleles between each pairwise combination of individuals within the population (n > 35000 loci with at least two individuals typed). Numbers above bars in (a) indicate the sample sizes used in all three analyses.

3.1.3.3 Genetic clustering and structure

The dendrogram based on allelic similarity (pairwise relatedness) identified a total of nine clusters or clades (Figure 3.1.6a). One of the clades consisted only of birds from LBI and grouped together with three clades containing only Ponui Island birds. The birds from Trounson were split across three clades, one of which contained the remaining Ponui birds, and two containing a mixture of Trounson birds and the other *A. mantelli* Northland birds. Together all Ponui, all LBI and most of the Trounson birds formed a monophyletic group, while the reference birds from the Eastern and the Coromandel taxa and the birds from other Northland populations formed a separate group together.

The structure analyses found the strongest support for a division in five clusters (K = 5). At this K-value, almost no individuals had a 100% probability of assignment to a particular cluster (Figure 3.1.6b). However, when overlaying the dendrogram based on allelic similarity and the cluster-assignment probabilities at K = 5, the results indicated coherence; each dendrogram clade was represented by a distinct assignment probability profile (Figure 3.1.6c). This coherence between method support that the identified clusters represent true genetic lineages.

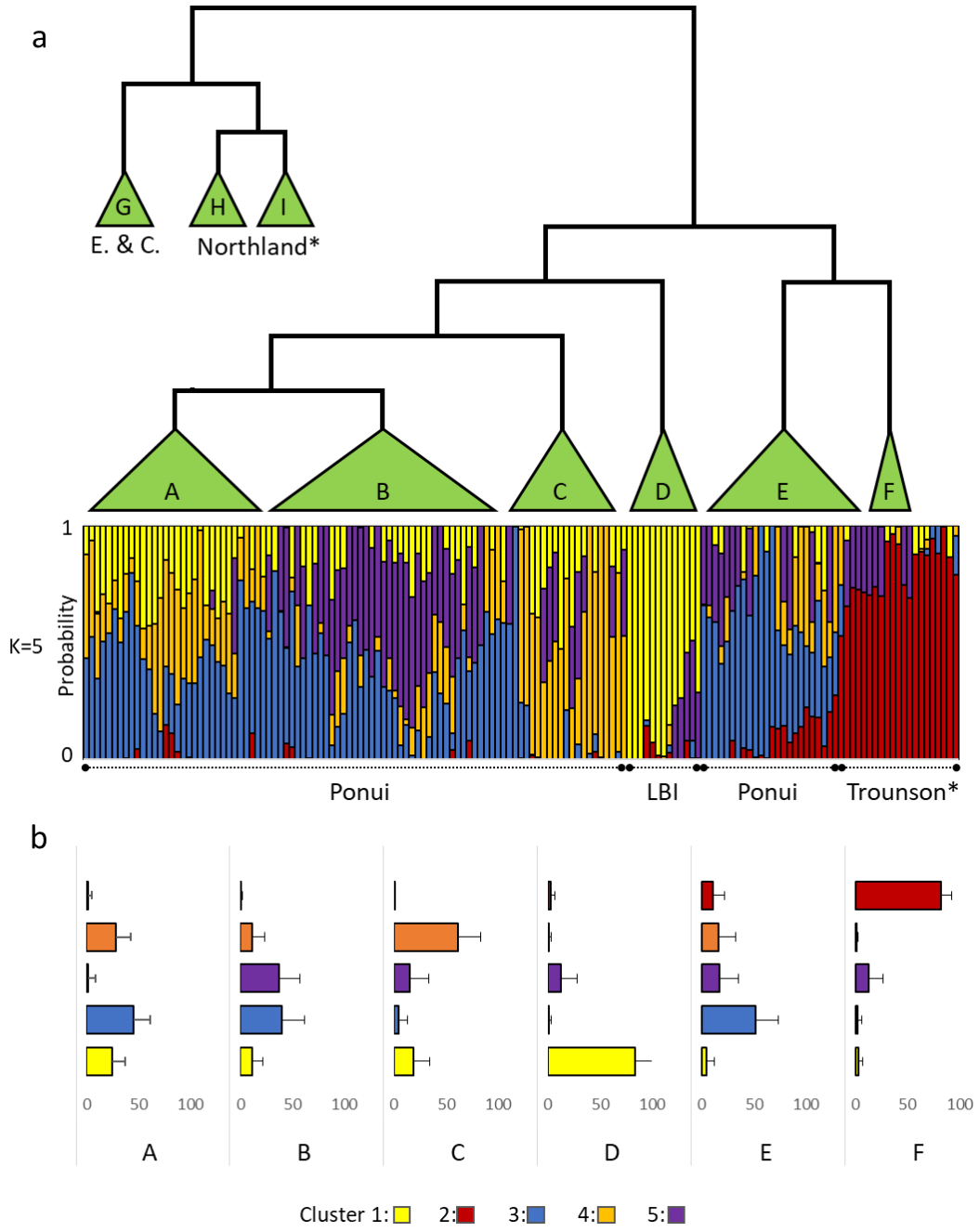


Figure 3.1.6. Clustering of *A. mantelli* individuals based on allelic similarity. (a) Schematic dendrogram of relatedness based on allelic similarity illustrating the nine clades that the analysed *A. mantelli* individuals clustered into. Birds from Ponui Island comprise clades A, B, C, and E. Birds representing the parental populations cluster into clades D and F. Clade G include birds from Eastern and Coromandel taxa ('E. & C.'), and clades H and I from other Northland populations. As expected, the birds from Ponui Island group more closely with birds from LBI and Trounson compared to birds from any of the other reference populations; a monophyletic division of the dendrogram that includes all Ponui Island birds also includes all LBI and most of the Trounson birds. (b) Structure plot showing the assignment probability of

analysed individuals to each cluster at K = 5 since clustering had the highest statistical support. Individuals are ordered in the structure plot to align with the clade that they were assigned to in the dendrogram above (the dendrogram includes more individuals than the structure analyses). Some individuals from the Trounson population clustered into more distantly related clades, as is denoted with an asterisk (*). Each column represents one individual and its colouration the probability of assignment to each of the five clusters. The mixed colouration of most columns suggests that the birds have a complex genetic background that does not resolve into easily defined groups. However, birds aligned under the same dendrogram clade show higher colour similarity compared to birds from different crown groups. (c) Average probability assignment to each cluster for individuals comprising clades A to F (error bars indicate standard deviation). The distinctness of these 'assignment profiles' suggests overall agreement between the two methods of clustering.

Since the main question addressed by the clustering was how the representation of the two parental populations are currently distributed in the Ponui Island population, K = 3 was utilised for further analyses (Figure 3.1.7a). At this level of clustering, the assignment probability for the dominating cluster of each parental population was 90%, and the corresponding cluster assignments of the Ponui Island birds suggested that birds translocated from LBI to Remutaka and Pūkaha were a good proxy for the birds once translocated to Ponui Island. However, the Trounson population was found to not be a good proxy for the second parental population of Ponui Island. This could indicate low levels of gene flow between Trounson and Waipoua, or that the historic records of the translocation origin are misleading. Thus, for further investigation of the makeup of the Ponui Island birds, I assumed assignment to cluster 1 (yellow in Figure 3.1.7 K = 3) to be a proxy for minimum proportional representation of LBI derived genomic material and inferred that the rest represented the second parental population. This assumption was further supported by a high correlation between individuals' probability of assignment to cluster one and their average relatedness to the LBI birds (Figure 3.1.7b). Based on this assumption, out of 120 Ponui Island birds, zero had a 100% LBI makeup,

19 had 100% ‘other’ makeup, and the remaining 101 had a mixed (hybrid) genetic makeup. Summed across individuals, cluster one represented 27% of the allelic diversity among the Ponui Island birds, compared to the expected 43% LBI make up based on the equal success of all 14 founders.

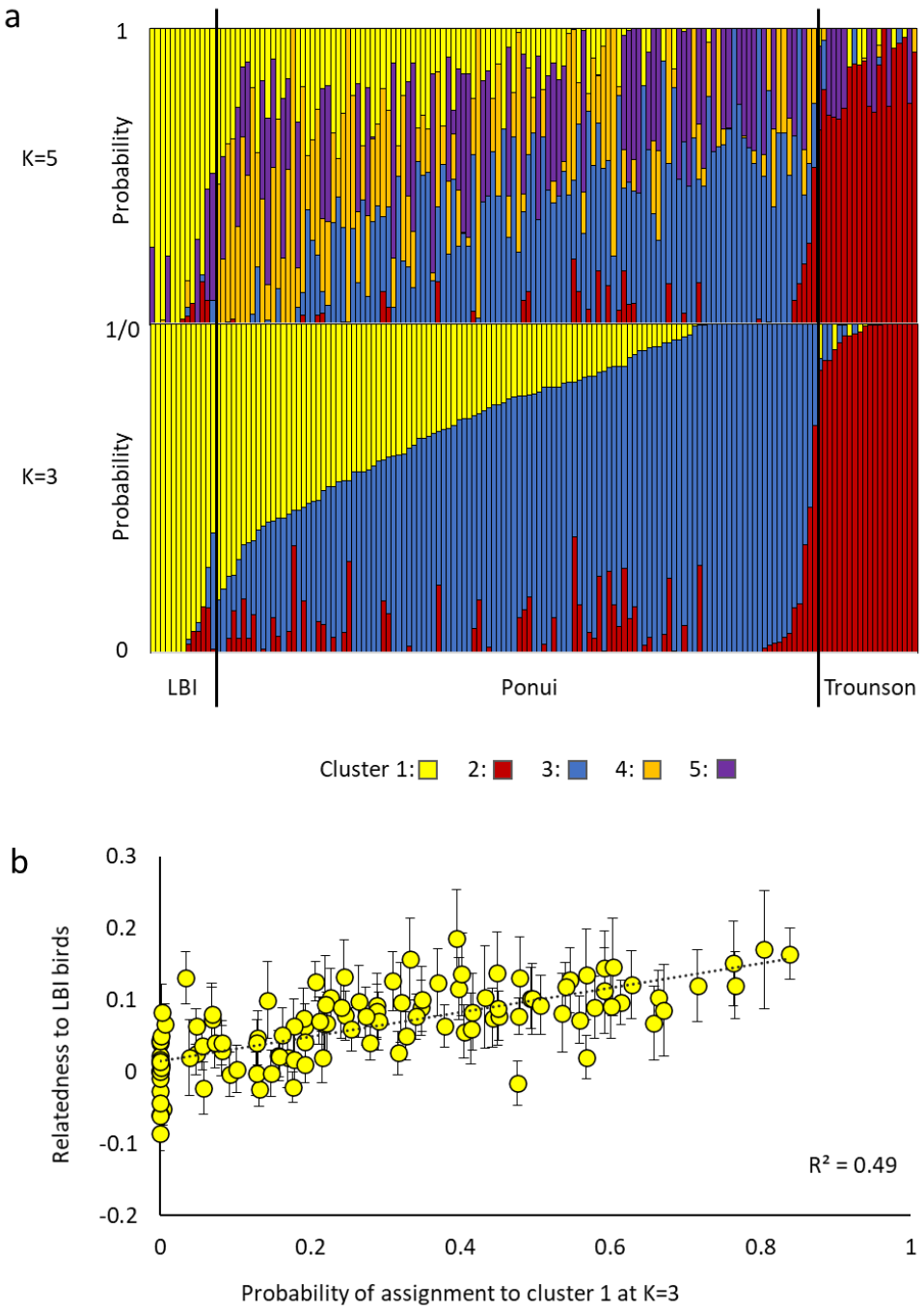


Figure 3.1.7. Genetic structure of the hybrid *A. mantelli* population on Ponui Island. Two results from the structure analyses are shown in (a). The analyses included 13 birds from LBI,

120 birds from Ponui Island, and 20 from Trounson. Each vertical column represents one individual, and the colours represent the proportion of the genome or the probability of the individual clustering with the 5 (top row) or 3 (lower row) identified clusters (K). K = 5 had the strongest statistical support. K = 3 illustrates the gradual representation of genomic identity matching LBI heritage in Ponui Island individuals (yellow part of each column). Individuals appear in the same order in both graphs. Black lines indicate the limits between populations. Compare Figure 2.2.4b. In (b) the individual probability of assignment to cluster one (the dominating LBI cluster) has been correlated to the average relatedness to the LBI birds for each Ponui Island bird defined by allelic similarity. Error bars indicate STD for relatedness.

3.1.4 Discussion

The main result presented herein is that the within-species hybrid population on Ponui Island was found to be more diverse than all reference populations analysed. Specifically, the mixed *A. mantelli* population on Ponui Island was not only more diverse than other bottlenecked populations with single founder origins, but also significantly more diverse than large, dense mainland populations with no known bottleneck history. This pattern was true for indicators of inbreeding (relatedness and heterozygosity; see also Ziesemann 2011) and overall diversity (non-shared alleles). In addition, this pattern was supported by the spread of phenotypic diversity. The high diversity, impressive density, and rapid population growth observed in the Ponui Island population are consistent with the definition of genetic rescue (Frankham 2015, Whiteley *et al.* 2015, Bell *et al.* 2019), even if this term is generally used to describe the outcome after reinforcement rather than introduction translocations. Combined with the structure analyses and the wide phenotypic distribution, this suggests that the current population on Ponui Island constitutes a successful hybrid swarm where about 80% of the sampled individuals (101 out of 120) had a mixed genetic makeup. In fact, this percentage represents a conservative estimate since LBI heritage was defined as the

probability of assignment to the dominating LBI cluster, but not all the reference birds from LBI were assigned with 100% probability with this cluster meaning that the true representation of LBI genomic in the Ponui Island population may be higher. The other caveat to the results of the structure analyses and the comparisons of phenotypic variation was that the structure analyses revealed Trounson to not be a good proxy for the second parental population of Ponui Island. This mismatch is highly interesting since it either suggests that genetic makeup can be distinct even across the short distance between Trounson and Waipoua, or that the historic records are mistakenly identifying Waipoua as the translocation source. Genetic comparison of the Ponui Island birds to reference birds of other origins – ideally including Waipoua – would be needed to elucidate this.

The Ponui Island population was consistently found to be the most diverse population in all four analyses of the genomic data. While the differences may appear small, the large number of markers analysed, means that even five percentage points lower heterozygosity equals about 2000 extra homozygous loci where heterozygosity benefit could be missed or recessive alleles become expressed, which are the two leading hypotheses of the cause of inbreeding depression (Davenport 1908, East 1908, Lynch 1991). In a recent publication from a leading group on the topic, Ralls *et al.* (2018) convincingly add to the discussion that as little as 10% loss of heterozygosity should be a cause of concern for population managers. In comparison to this suggestion, all reference populations herein had an average heterozygosity at least 10% lower than the Ponui Island population, both at the individual and population-wide level. At the same time, all other populations had >90% of the level of heterozygosity observed in the relatively pristine Trounson population, making it uncertain which is a better proxy for the expected level of diversity in a large outbred kiwi population. Unfortunately, no

comparative studies have been done to link *Apteryx* diversity to fitness. Such studies are urgently needed to guide future management of populations at risk of inbreeding depression.

Considering the remaining populations analysed, a number of interesting observations can be made. The *A. mantelli* population on Moturua was founded with only 14 individuals of local stock, and, as expected, the diversity in the population was low. The Moturua population is less dense and has experienced slower growth than the Ponui Island population. While it is tempting to blame this slow growth rate on low diversity, the low carrying capacity of this small island must also be considered. In addition, the diversity in Moturua was not lower than in the large, dense mainland population on Purerua Peninsula. The bottlenecked population on Rakaumangamanga also had a level of diversity on par with Moturua and Purerua. The unexpected skew in the Rakaumangamanga population with a high number of heterozygous individuals but relatively few heterozygous loci could be a random consequence of a founder effect. It will be interesting to follow the future development of this population, especially with the recent ramping up of the pest control in the area led by Te Patukeha and Ngati Kuta Hapū. The kiwi diversity in the Trounson Kauri Forest was closer to expectation in a mainland population with relatively high and stable population numbers over time. While the level of diversity was lower than on Ponui Island, the diversity in Trounson was arguably high enough to warrant even higher priority than at present to the successful management of this population. The low number of founders but high diversity in the Ponui Island population suggests that founder variation may be more important than quantity. Meanwhile, the results from Remutaka and Pūkaha suggested that, while variation between individuals was high, a relatively large proportion of the individuals were genetically inbred. This suggests that, unsurprisingly, many other

factors than founder composition are important for the outcome of a multi-origin translocation. In terms of Pūkaha and Remutaka, such factors are likely to include the time since establishment, combined with suboptimal initial pairing within the cohort of introduced birds and among close relatives (see further Chapter 3.2). Taken together, these discrepancies between population growth, size, and diversity are not unique to *Apteryx* (Angeloni *et al.* 2011, Malone *et al.* 2018), and they support the discussion of Undin *et al.* (2021a & b; Chapter 1.2 & 1.3) stating that measuring allelic diversity alone is not sufficient to explain population success and that further investigation into other aspects of contributing to the intricate relationships between genotype, phenotype, plasticity, resilience, compatibility, and surrounding environment are urgently needed.

One crucial component of such investigations is determining the role of local adaptation in the separation of the taxa within the *Apteryx* genus. Initially, genetic analysis can identify segments of the genomes that differ between taxa and alleles that are overrepresented in certain taxa. This information can then be cross-referenced with knowledge about the function of genes at these loci (Campagna *et al.* 2015, Ottenburghs *et al.* 2017, Rexer-Huber *et al.* 2019). In a following step, important information would be gained from studying what has happened to the relative allele frequencies for these identified segments after admixture or translocations to areas with different habitats (Beaumont & Balding 2004, Fitzpatrick *et al.* 2010, Keller *et al.* 2013, Rexer-Huber *et al.* 2019). The work by Ramstad and Dunning (2020) has already identified some key genes of interest. In addition, studying genetic sequence *per se* may not be enough for such investigations since key differences in phenotype can be the result of expression level rather than sequence changes. Ramstad and Dunning (2020) highlight the importance of transcriptome analyses and that much could be gained through comparison epigenetic profiles (as suggested by for instance by Rey *et al.* 2020).

Furthermore, these molecular studies would greatly benefit from being combined with field studies of fitness parameters such as reproductive success and survival which can be correlated to translocation and bottleneck histories as well as to habitat parameters of interest. Issues with such studies are, of course, the long lifespan of kiwi and the difficulty in accurately determining their age, thus further research into identifying fitness markers that are accurate and can be gained without decades of field study should also be prioritised (see further Chapters 4.1-4.3).

Even when found to be high, the diversity of a mixed population is rarely equal to the sum of variation in the parental populations (Dresser *et al.* 2017, Rick *et al.* 2019, Thavornkanlapachai *et al.* 2019). This can be because translocations inevitably constitute bottlenecks excluding some diversity from the source, but can also be due to an unequal spread of reproductive success among founders. The structure analyses herein suggested that no sampled Ponui Island bird was of 100% LBI identity and that the representation of LBI derived allelic diversity across the sampleset was about 40% lower than expected, assuming equal success for all founders. Equal success of all founders after a translocation is unlikely to be a fair expectation due to, for instance, the high sensitivity to stochastic demographic events (random casualties) while the population is small. While saying this, the lower than expected representation of LBI derived genomic signal is suggestive of a consistently lower reproductive success for these birds compared to birds from the second source population. A very similar scenario was found in the mixed burrowing bettong (*Bettongia lesueur*) population at the fenced-off mainland area, Matuwa, in Western Australia. One of the sources for this population was an island with previous translocation history. Individuals with a >50% genetic identity from this bottlenecked population were found to experience lower reproductive rates compared to other individuals in the mixed population (Todesco *et al.*

2016, Rick *et al.* 2019). However, this aspect of lower fitness was outweighed by hybrid individuals with 50% or less of the island genomic makeup having higher survival than pure individuals from the second source population. This counterforce was strong enough for island genetics to remain at a relatively high and steady level in the mixed population across all years of study (Rick *et al.* 2019). The low proportional representation of LBI derived genomic signal in the Ponui Island population could thus suggest that the parental birds have experienced local adaptation that does not suit the conditions on Ponui Island, founder effects, low diversity, demographic stochasticity (for example, the random death of two or three out of the six LBI founders would be a substantial proportional change), or some combination of these factors. Investigation into the genetic makeup and diversity in the current LBI population could partly elucidate this. Importantly, the observation that hybridisation between birds as distant as *A. rowi* and *A. owenii* has resulted in birds that later successfully produced offspring together, suggests that levels of reproductive incompatibility are low in the *Apteryx* genus.

3.1.5 Conclusion - Did I reduce the confusion?

As mentioned above, the current Kiwi Recovery Plan (Germano *et al.* 2018) states that there is '…confusion about how to manage hybrid and mixed provenance individuals…'. One 'confusion' is the suitability of the Ponui Island population to act as a source for future translocations. Herein I show that Ponui Island would not be a source of individuals with 'pure' genetics from either parental population – over 80% of the birds had mixed genomic makeup. On the other hand, even a smaller number of birds from Ponui Island are essentially guaranteed to provide a target population with a boost of genetic diversity and alleles originally from both parental areas. An additional key consideration is that the Ponui Island population is dense, making 'harvesting'

individuals unlikely to affect the viability of the population. Lastly, the density and terrain could facilitate catching a desirable number of birds in a short amount of time.

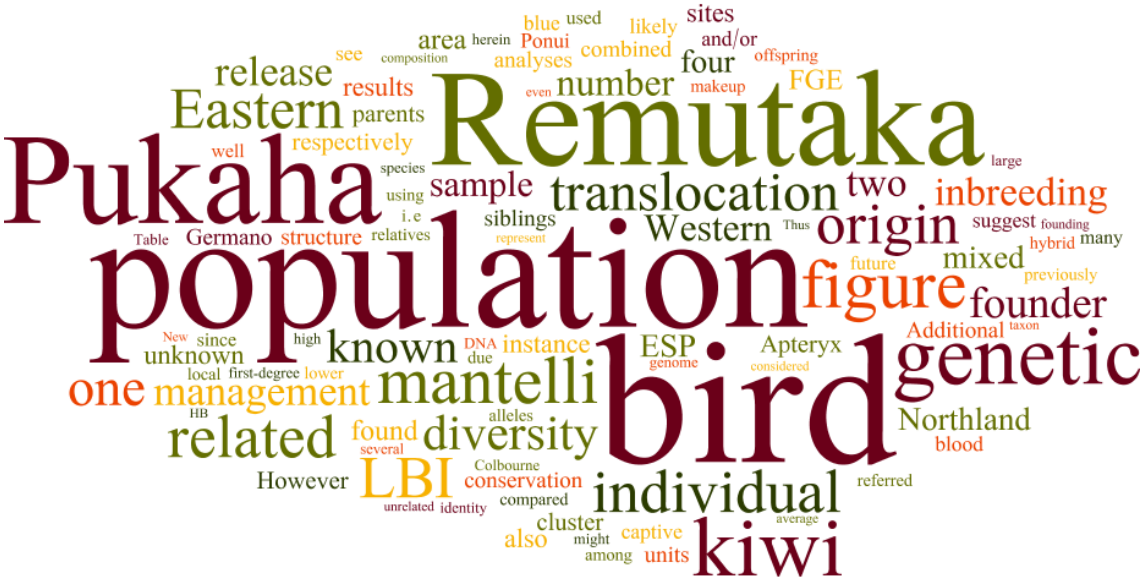
The last two features would also be true from Purerua, but my results show that birds sourced from this population would contribute substantially less diversity compared to Ponui Island birds. On the other hand, the results could be interpreted as that populations with as low diversity as Purerua can seemingly thrive. This, in turn, could suggest that even bottlenecked populations, like Moturua and Rakaumangamanga, could be sources diverse enough to generate successful populations. However, a growing body of work suggests that maximising diversity in the target population – primarily through using outbred, diverse sources – is the main feature correlating with translocation success and genetic rescue (Zeisset & Beebee 2013, Frankham 2015, Whiteley *et al.* 2015, Frankham *et al.* 2017, Ralls *et al.* 2018, Ralls *et al.* 2020) and that this is particularly important under suboptimal habitat conditions (Crnokrak & Roff 1999, Frankham 2015). Thus, if populations with relatively low diversity are the only ones deemed as suitable sources, combining birds from several such bottlenecked populations could compensate for lacking diversity locally and improve future prospect for the target population (Hedrick & Fredrickson 2010, Frankham 2015). This type of mixing would also allow for translocating fewer individuals from each source population, which arguably would mean a smaller risk of having a negative impact on the population at the source site. Is the success of the Ponui Island population evidence for that mixed sourcing mean that 14 founders are enough for future kiwi introductions? Possibly, but fewer founders not only reduce the genetic representation in the target or new population, it also increases the susceptibility to random stochastic events. Thus, I would suggest that aiming for around 40 founders – as suggested in the current guidelines – will have genetic as well as demographic benefits. What the results herein

show, though, is that populations founded with less than 40 should not be deemed failed or of no value based on this criterion alone.

The results herein also highlight that, in addition to our lacking understanding of evolutionary diversification of kiwi, the low diversity in Purerua and the mismatch between Ponui Island and Trounson also suggest that our understanding of recent *Apteryx* population history is incomplete. Further elucidating the history and whakapapa of kiwi is thus pivotal for science to correctly inform future conservation management. However, in saying that, whether Ponui Island and other mixed populations constitute suitable translocation sources or not really comes down to what decision-makers deem to be the goal of *Apteryx* conservation and genetic management. Thus, 'what is a kiwi hybrid' will always partly remain a political rather than a strictly scientific question.

Chapter 3.2

Special cases for special birds: history and future of the kiwi populations in the Remutaka Forest Park and at the Pūkaha National Wildlife Centre



This Chapter has been written as a report for the management teams at the Remutaka Forest Park and at the Pūkaha National Wildlife Centre, the language has been adjusted to reflect this. All supervisors provided feedback on one or more drafts of this chapter. The expertise of the Elshire Group and Tea Break Bioinformatics, the help from Brianna Nelson and Trish McLenachan, the hard work of Ange Hura, Natasha, Stephen, Mo, Yi and other field helpers, and not the least, the hard work and expertise of Richard Witehira, and the support from Mana Tangata and landowners were crucial for this chapter.

Abstract

Apteryx (kiwi) conservation has received a lot of attention over the last 30 years. More recently, the role of genetic diversity in kiwi management has gained notice. Combined with the split into 14 separate management units, this increased genetic focus has generated questions about populations that are the result of historic translocation – especially when birds have been sourced from multiple areas and/or founders were few. Two sites with such hybrid *A. mantelli* populations are the Remutaka Forest Park and the Pūkaha National Wildlife Centre. In this study, I used pedigree data combined with genomics to determine the composition of the founding as well as the current populations at these sites and to determine their individual and population-level diversity. I found that the effective starting population at both sites was smaller than the recommended 40 unrelated founders – 24 and 32, respectively. However, when combined, the Remutaka and Pūkaha populations harbour as much diversity as a large mainland population with no known bottlenecks. Relatedness and individual diversity suggested that many kiwi at these sites are not hybrids but in fact inbred, but also that the populations are seemingly going towards a larger proportion of hybrid individuals. Based on the results, I suggest that a worthwhile first step would be considering Remutaka and Pūkaha as one meta-population where birds are strategically moved between the two sites to minimize relatedness. I agree with the authors of the Western Taxon Plan (Scrimgeour & Pickett 2011) that a promising future prospect would be using the Remutaka and Pūkaha kiwi for supplementations into the area where the distributions of the Eastern and Western management units meet. However, I advise that such translocations should become part of a long-term strategy aiming to create steppingstone populations to restore connectivity and promote genetic exchange between what we currently think of as Eastern and Western *A. mantelli*.

3.2.1 Introduction

The New Zealand native flora and fauna have suffered dramatically over the last centuries from a combination of drastic loss of native forest and the introduction of several invasive mammalian pest-species, acting as previously unprecedented predators and/or competitors (Holdaway 1989, Lovegrove 1992, McLennan *et al.* 1996, Innes *et al.* 2010, Robertson *et al.* 2016, O'Donnell *et al.* 2017). However, over the last decades, concern about preserving and restoring the environment has grown rapidly among the New Zealand public, resulting in an impressive number of conservation groups around the country, commonly running on a more or less volunteer basis with support from local authorities, regional councils, and/or the Department of Conservation (DOC; IUCN/SSC 2013, Peters *et al.* 2015, Kiwis for Kiwi 2016, Germano *et al.* 2018). The goal for most such groups is restoring forest ecosystems, often through trapping and poisoning introduced mammals and/or planting native species, usually in combination with some form of monitoring of the native wildlife (Peters *et al.* 2015). Since many of these projects are geographically isolated from other areas of comparatively pristine or managed forest, they rely heavily on translocations to diversify their fauna even after extensive restoration efforts have been conducted (DOC 2012, Cromarty & Alderson 2013; Table 3.2.1). This is in line with the growing utilisation of conservation translocations and increasing involvement of community groups in such interventions worldwide (IUCN 2013). In fact, translocations play a comparatively large role in New Zealand bird conservation, partly due to the reduced or lost flight ability – and consequently limited dispersal capacity – of many of New Zealand's native species (Armstrong & McLean 1995, Clout & Craig 1995, Roots 2006, Miskelly & Powlesland 2013, Sackton *et al.* 2019).

One avian group highly reliant on human-mediated translocations is the kiwi, or *Apteryx*, genus (Colbourne 2005, Germano *et al.* 2018). The five species in this genus have suffered greatly from degradation and fragmentation of habitat and, in particular, from mustelid predation of chicks resulting in severely reduced recruitment success (McLennan *et al.* 1996, Germano *et al.* 2018). The kiwi is considered a national icon in New Zealand, and *Apteryx* conservation is receiving much attention with a government-supported goal to achieve a nationwide, annual population growth of 2% (Germano *et al.* 2018, teara.govt.nz/en/kiwi, nzbirds.com/birds/kiwipeople.html). To ensure a nationwide spread of the conservation efforts, the *Apteryx* genus has been divided into 14 management units, and the goal has been set to 2% independent annual growth of these units (Germano *et al.* 2018). For instance, North Island brown kiwi, *A. mantelli* is today recognised as consisting of four management units (also referred to as taxa) which are genetically and geographically separate: *A. mantelli* Northland, Coromandel, Western, and Eastern (Weir *et al.* 2016, Germano *et al.* 2018). Admixture among management units has been deemed undesirable (DOC 2004, Germano *et al.* 2018). In fact, birds in two populations generated through multi-origin translocations have been deemed of 'no genetic value whatsoever for use in restoration' by an expert panel approached by DOC. The panel further suggested that 'the best conservation use of these birds would be to move them to a secure captive facility and use them for advocacy'.

Table 3.2.1. Glossary of terms and abbreviations.

Term	Definition used herein
Alleles	Variants of the same gene that differ in their nucleotide sequence.
Heterozygous	Having two different alleles of the same gene; one inherited from either parent. Proportion of heterozygosity is thus a measure of genetic diversity. The opposite is called Homozygous
Fragmentation	The degradation of habitat that results in loss of connectivity, i.e., cessation of migration, dispersal, and mating, between areas within the distribution of a species.
Inbreeding	Mating between close relatives often due to fragmentation and small population size. This increases the risk of inheriting the same allele from both parents which reduces heterozygosity.
First-order relative	The relationship between either an offspring to a parent or between full siblings, i.e., between individuals that theoretically share half of their genetic material.
Translocation	Human-mediated movement of living organisms from one area to another.
Introduction	Translocation to an area outside the indigenous range of a species. Also referred to as assisted colonisation. As a comparison, reintroduction refers to the reestablishment through translocation within the know historic range of a species. When this results in negative fitness consequences, it is referred to as inbreeding depression.
FGE	The number of individuals introduced to an area will be larger than the number of Founder Genome Equivalents (FGE) if some individuals die before they reproduce, or two or more founders are related. The latter means, for instance, that if four introduced birds are full siblings, they only contribute two FGE to the effective starting population. Thus, when estimating, for instance, the risk of inbreeding, the number of FGE and their origin is a more informative value than the number of birds released.
Reinforcement	Translocation of additional individuals into an existing population. Also referred to as Supplementation (see for instance the Kiwi Recovery Plan)
Hybrid	Individual resulting from mating between genetically distinct parents. Often this refers to mixing separate species, but herein I use the word hybrid to refer to *Apteryx mantelli* individuals with heritage from more than one management unit (also referred to as taxa).

Local adaptation	A specific genetic makeup (generally a combination of several alleles) that result in a phenotype that gives individuals higher fitness under the local conditions. While the alleles arise through random mutation, natural selection is required for local adaptation to occur. Generally, populations need to be over a certain size for selection to be an efficient force.
Outbreeding depression	When hybrid offspring experience lower fitness than either or both parents. This can be due to that the parents represent local adaptation to differing local condition or that the genetic differences between the parents are large enough to cause incompatibilities.

In line with the national goals and the icon status of these birds, kiwi management is a focus for many of the above described community conservation initiatives (Cromarty & Alderson 2013, Peters *et al.* 2015). Two examples of extensive and long-term restoration projects to which *A. mantelli* have been introduced through translocation are the Remutaka Forest Park (www.Remutaka.nz) and the Pūkaha National Wildlife Centre (www.Pūkaha.org.nz), hereafter referred to as Remutaka and Pūkaha, respectively. The background of the kiwi populations at these sites is similar. Both started with the release of a small number of previously captive birds (Table 3.2.2). Many of these releases were part of a conscious move, initiated partly by the Zoo and Aquarium Association (ZAA), to focus the captive *A. mantelli* population on successfully breeding 'purebred' *A. mantelli* Eastern individuals (DOC 2004, Barlow 2011, Robertson & Colbourne 2017, Barlow 2018, Germano *et al.* 2018). Several of the released birds were considered hybrids since their parents were from different taxa within *A. mantelli* or their origins were not fully known (Barlow 2011).

In addition to the previously captive birds, a one-off translocation event from Hauturu-o-Toi (Little Barrier Island; hereafter referred to as LBI) was conducted to both Pūkaha and Remutaka (Table 3.2.2). The LBI birds have been treated with caution by DOC, and their suitability for conservation translocations have been debated (Holzapfel *et al.*

2008). This is due to their unclear background; birds from the Taranaki region were released on LBI about 100 years ago, but anecdotal accounts suggest that this might not be the only founding event on the island (Herbert & Daugherty 2002, Colbourne 2005, Scrimgeour & Pickett 2011, Kiwis for Kiwi 2016, Letter to Department of Conservation and the Kiwi Recovery Group shared with Ponui Island landowners and Ponui Island kiwi research team 2016). Lastly, both Remutaka and Pūkaha are situated in the southernmost part of the North Island, placing them outside the historic range of *A. mantelli*, but within that of the extinct North Island taxa of *A. rowi* and *A. owenii* (Figure 3.2.1; Shepherd *et al.* 2012). Due to the already mixed makeup of the Remutaka and Pūkaha populations, the negligible risk of birds from these sites ever mixing with other kiwi, but also the worry of the low number of founders, an exception was made and the translocations from LBI were granted permission.

At both Pūkaha and Remutaka, birds have been monitored closely and Operation Nest Egg (ONE) has been utilised to some degree at both sites to improve chick survival (Colbourne *et al.* 2005, Robertson & Colbourne 2017). Thanks to the extensive effort of the respective management teams, both populations are doing well, and population numbers are increasing. The exceptions are occasional detrimental invasions of ferrets killing adult and juvenile birds at Pūkaha (Blackie 2015, Germano *et al.* 2018) and mast years, during which stoat predation of chicks and juveniles increases at Remutaka following a sharp increase in the rat population (Cameron 2015).

The mixed origin and the location of these sites outside the historic range of *A. mantelli* (Figure 3.2.1) suggest that studying the populations at Pūkaha and Remutaka could help answer several outstanding questions of great importance for future kiwi conservation. Specifically, such studies could assist in improving guidelines around *Apteryx* translocations. For instance, these populations could help (1) reveal to what extent the

differences observed among the four *A. mantelli* management units are caused by local adaptation, (2) determine if combining geographically and/or genetically distant kiwi results in outbreeding depression or genetic rescue, and (3) evaluate if the current guidelines of founding *Apteryx* populations with 40 unrelated individuals is justified. Furthermore, the management teams at both sites recognise that the relatively small founder populations and the geographical isolation of these birds can become problematic in the future (see also Scrimgeour & Pickett 2011). Meanwhile, there is reluctance from the leading national kiwi conservation bodies to allow further translocations in or out of these populations due to the unwillingness to increase and/or spread the number of hybrid kiwi (Herbert & Daugherty 2002, Scrimgeour & Pickett 2011, Germano *et al.* 2018).

Herein I combined the known history of the Remutaka and Pūkaha populations with data on genomics, morphology, and haematology parameters in order to evaluate (1) the size and composition of the effective founding population at these sites, (2) the genetic diversity in the current populations and its relationship with the mixed origin, (3) the genetic identity of particular birds of interest, and (4) whether there are any indications of the mixed origin and/or the geographic location of these populations having resulted in birds of poor condition. Lastly, I discuss the results focusing on the future management of the *A. mantelli* at Pūkaha and Remutaka, as well as *Apteryx* New Zealand-wide, in light of the suggestions made in the Western Taxon Plan as well as the negative connotation and experienced confusion around hybrid population expressed in the national Kiwi Recovery Plan.

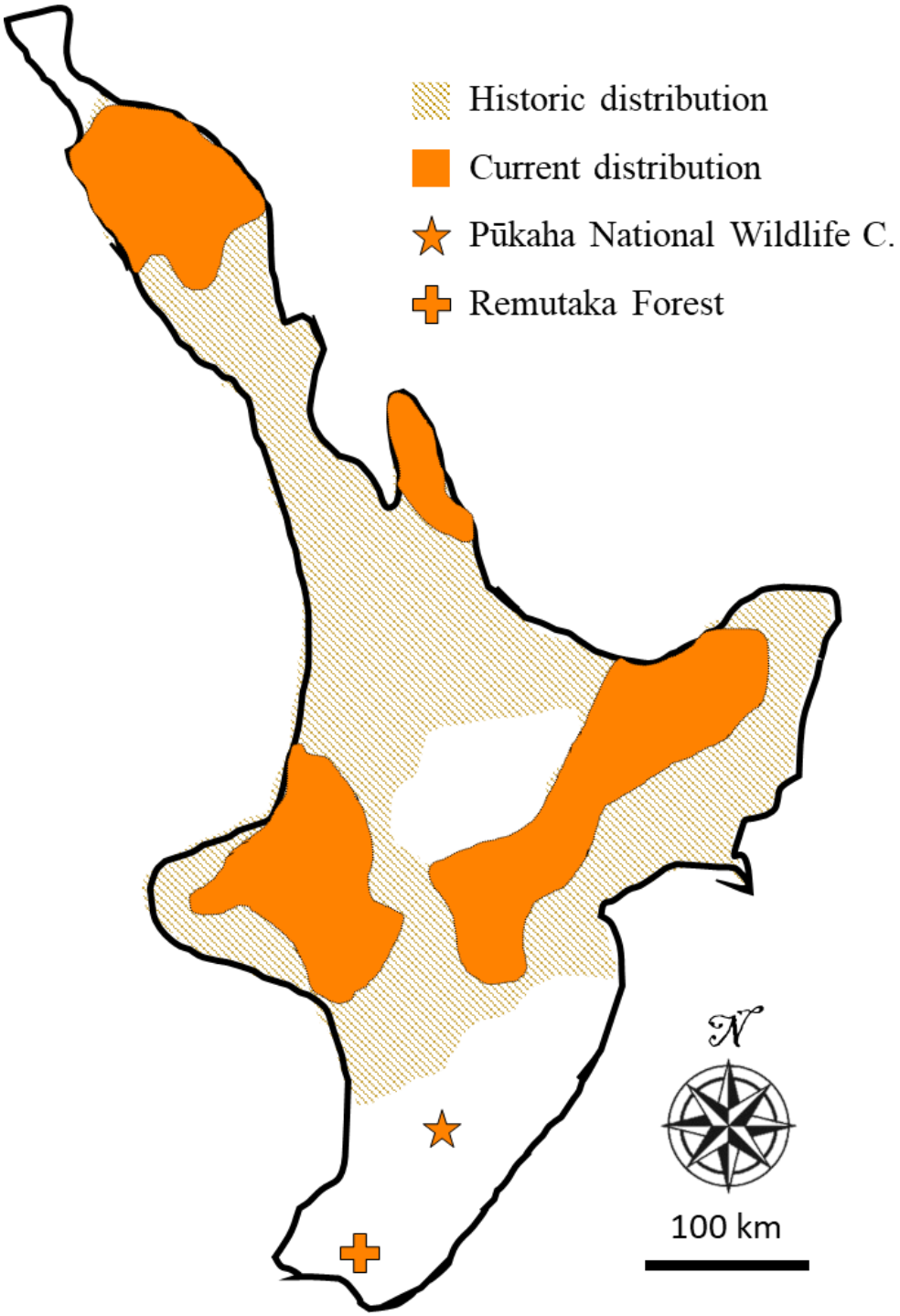


Figure 3.2.1. Map illustrating the location of the Pūkaha National Wildlife Centre and the Remutaka Forest Park in relation to current (Germano *et al.* 2018) and pre-human (Shepherd & Lambert 2008, Shepherd *et al.* 2012, Germano *et al.* 2018) distribution of *Apteryx mantelli*.

3.2.2 Materials and Methods

To address these questions, blood samples for DNA extraction, morphometrics (bill length, tarsus length, tarsus width, and weight), and haematology parameters (haemoglobin concentration, glucose concentration, packed cell volume, and total protein content) were collected from a subset of birds from Pūkaha and Remutaka. Genetic diversity (measured as relatedness or allelic similarity, individual homozygosity, population-level homozygosity, and proportion of shared alleles), haematology values, and morphometrics were then compared to other *A. mantelli* populations.

3.2.2.1 Sample collection

Blood sample collection took place during 2020 in accordance with the Massey University Animal Ethics Committee (permit 18/83), the Department of Conservation (permit 70875), the Kiwi Best Practice Manual (Robertson & Colbourne 2017), and with consent and great assistance from the hapū, employees, trustees, and volunteers at both sites. Birds were either held in an enclosure (Pūkaha), previously fitted with transmitters and, thus, tracked down and sampled (both sites), or found using a certified kiwi dog (Remutaka). Night-time catching was also attempted, but the only successful catch was of an already transmitterised bird (Eddie) in Remutaka. Night catching and tracking with a dog was done following the initiative of the Remutaka management team since they, at the time, aimed to increase their number of transmitterised birds. At the time of sampling, bill and tarsus were measured using callipers and birds were weighed.

3.2.2.2 Size and composition of effective starting populations

The number of founder genome equivalents (FGE; see for instance Jamieson 2015), defined herein as the number of unrelated founders that survived more than one year after release, was used as a measure of effective starting population at each site and at both sites combined. Birds were deemed unrelated if they lacked first-order relationships (parent to offspring or sibling to sibling) with other released birds at that site. Unrelated birds surviving more than one year after release were equivalent to one FGE each. In cases where two or more related birds were released, this was considered to add only two FGEs (regardless of the number of relatives). For example, four siblings (Stud ID 320, 330, 342, and 323) were all released at Pūkaha; these four birds only added two FGE to the starting population. This is equivalent to considering their parents (Stud ID 68 and 141) part of the starting population (see Additional Figure Ad3.2.1 at the end of the chapter). Survival was known since all released individuals were initially fitted with radio transmitters and tracked.

Stud book data from the SPARKS database (pers. comm. T Jenkinson March and April 2020) was used to determine the taxon origin of each bird in the starting population. For example, a bird stemming from Northland contributed one FGE to this taxon, while a bird in the starting population with one parent from Northland and one from the Western management unit contributed 0.5 FGE to each of these taxa.

3.2.2.3 DNA extraction, identification of SNP markers, and analyses of genetic diversity

The blood samples were used to extract high-quality DNA using a High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). Extracted DNA was then sent to The Elshire Group for Genotype-by-sequencing (GBS) library preparation following

their published protocol (Elshire *et al.* 2011) using the restriction enzyme EcoT22i. The resulting sequences were then demultiplexed, trimmed, matched, and aligned to a reference *A. mantelli* genome, in collaboration with Tea Break Bioinformatics. The software STACKS was then used to identify single nucleotide polymorphisms (SNPs, i.e., sites in the DNA where the genetic code differ at a single base between at least two sampled individuals) in the dataset and create output files for further analyses. Further analyses were done using Genepop, Structure, and KGD to quantify genetic diversity, clustering, and relatedness, respectively. For more details see Chapter 3.1.

In brief, the reasons for using SNPs as genetic markers – compared to, for instance, microsatellites or mitochondrial DNA – are that (1) there is no need for a separate process that identifies markers of interest, (2) the number of markers and thus the details in the results are much higher (herein I used 51691 SNPs), and (3) these markers are spread randomly across the entire genome, which means that they cover both coding genes under selection and so-called 'neutral' DNA. However, to determine the nucleotide sequence at each of these markers with high confidence requires high quality DNA; therefore, I relied on blood samples even though blood sampling is more invasive and challenging compared to the collection of feathers or droppings.

3.2.3 Results

3.2.3.1 Number of Founder Genome Equivalents (FGE) and their origin

Based on the amalgamated records, I could conclude that Remutaka has had 31 and Pūkaha has had 57 *A. mantelli* released (Table 3.2.2). However, for Remutaka, four birds died within one year of release, and, among the surviving, previously captive, birds, four were siblings (Additional Figure Ad3.2.1). For Pūkaha, 14 birds (2 previously captive and 12 from LBI) died within one year of release. Among the

surviving previously captive birds, 16 were siblings, one a half-sibling, and one was the father to six of those siblings (Table 3.2.2; Additional Figure Ad3.2.1). Thus, accounting for relatedness and deaths, the number of founder genome equivalents (FGE) making up the starting population was calculated to be 24 birds for Remutaka and 32 for Pūkaha (Table 3.2.2; Figure 3.2.2). Relatedness between birds released at Pūkaha and Remutaka further reduced the number of FGE for the two populations combined from 56 to 52.5 birds (Table 3.2.2; Figure 3.2.2).

Table 3.2.2. Timing of and sources for *A. mantelli* releases at the Pūkaha National Wildlife Centre and the Remutaka Forest Park. The table lists the number of female (F) and male (M) *A. mantelli* released, how many of them survived for more than one year after release (for adults) or long enough to reach adulthood (juveniles; sex not specified), and how many founder genome equivalents (FGE) they contributed after accounting for relatedness (sex not specified).

Site	Source	Year of release	Released F/M	Survived	FGE
Remutaka	Captive	2006-2012	3/8	8	5
Remutaka	LBI	2008	11/9	19	19
Pūkaha	Captive	2000-2018	12/15	25	14
Pūkaha	LBI	2010	16/14	18	18

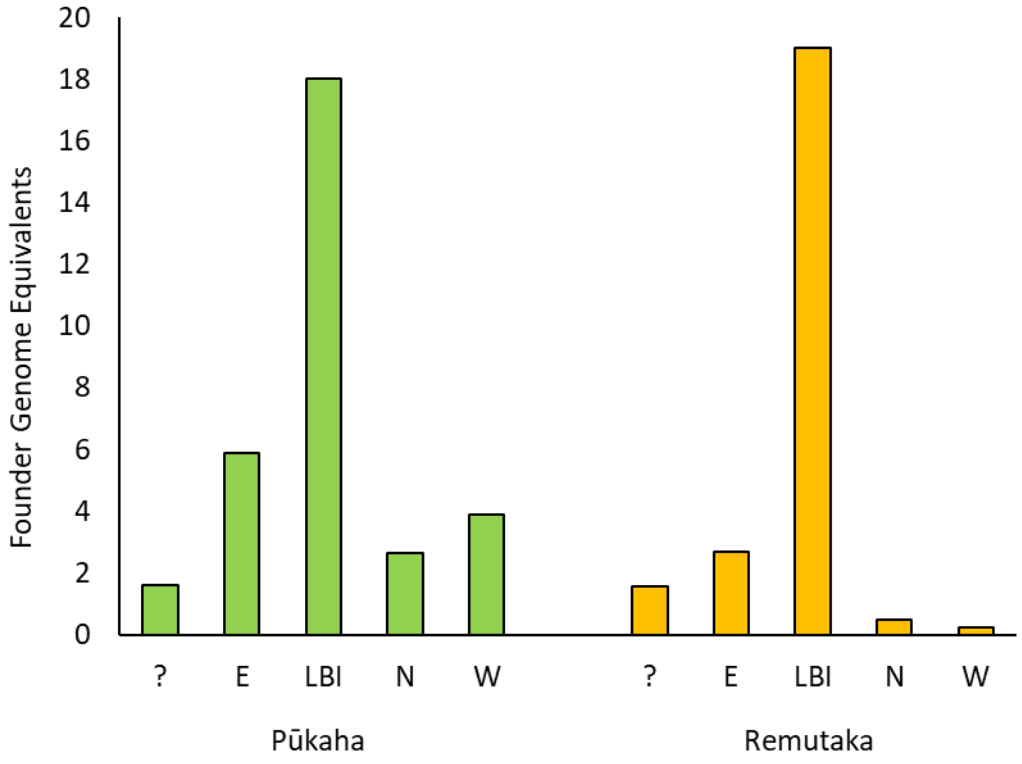


Figure 3.2.2. The number and composition of founder genome equivalents (FGE) starting the *A. mantelli* populations at the Pūkaha National Wildlife Centre and the Remutaka Forest Park. Founder genome equivalents are split into the categories *A. mantelli* Western (W), Northland (N), and Eastern (E) management units, respectively, and *A. mantelli* from Hauturu-o-Toi (LBI), and of unknown origin (?).

The origin of the FGEs at both sites was found to be dominated by Hauturu-o-Toi (LBI) birds (Figures 3.2.2 and 3.2.3). Due to the translocation history of this island, it currently remains debated whether the identity of the LBI birds should be considered Western taxon, hybrid, or unknown (Baker *et al.* 1995, Herbert & Daugherty 2002, Burbidge *et al.* 2003, Colbourne *et al.* 2005, Holzapfel *et al.* 2008). In terms of the remaining founders, both Remutaka and Pūkaha have a noticeable proportion of Eastern taxon origin (13 and 18 % of the FGEs, respectively) and a smaller number of FGEs of Western (non-LBI), Northland, and unknown origin (Figure 3.2.1 and 3.2.2).

For this research, 22 blood samples were collected, 10 from Pūkaha and 12 from Remutaka. These represented 18 of the 52.5 FGE in the starting populations of the two populations combined, four through direct sampling and the remaining through the sampling of offspring and/or grand-offspring (Figure 3; Additional Figure Ad3.2.2 & Ad3.2.3).

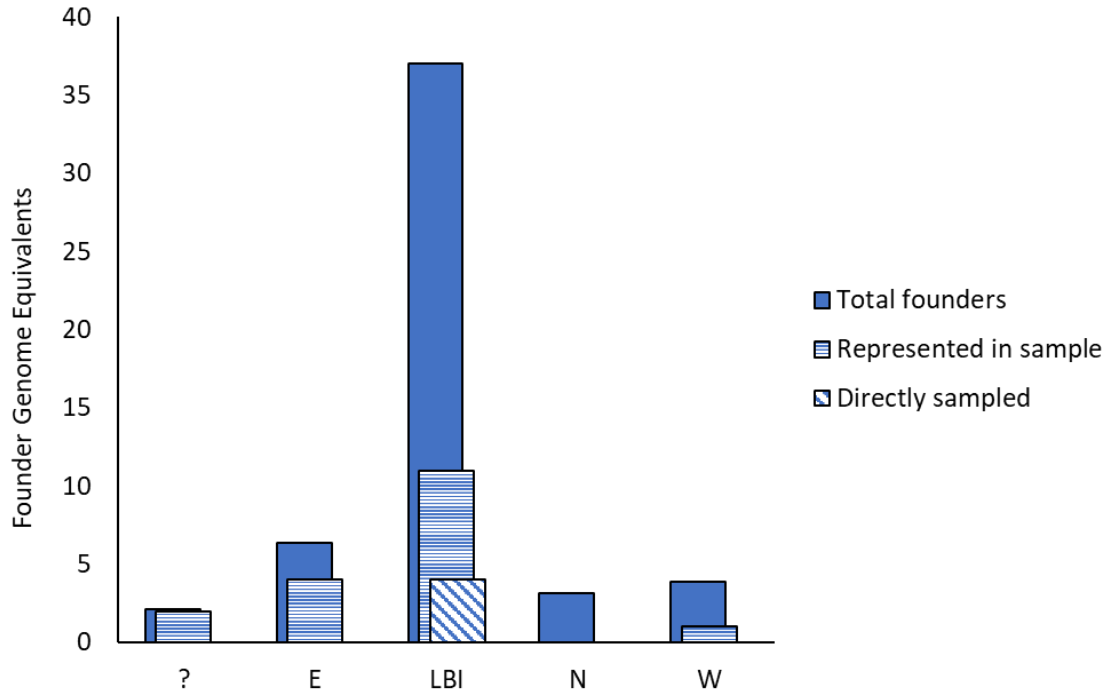


Figure 3.2.3. The combined number of founder genome equivalents for the Pūkaha National Wildlife Centre and the Remutaka Forest Park populations and their representation in my samples. The total number of FGEs are broken down to illustrate representation of the *Apteryx mantelli* Western (W), Northland (N), and Eastern (E) management units, *A. mantelli* from Hauturu-o-Toi (LBI), and *A. mantelli* of unknown origin (?). Striped bars indicate how many of these founders were represented in the blood samples analysed herein. Four birds were represented by direct sampling (diagonal stripes) the remaining through sampling of offspring and/or grand-offspring.

3.2.3.2 Genetic diversity, structure, and identity

For analyses of genetic diversity, structure, and identity, the birds from Pūkaha and Remutaka were combined and treated as one population. For this combined population, the average relatedness, individual heterozygosity, population-level heterozygosity, and proportion of shared alleles (gene variants) among individuals were quantified and compared to five other *A. mantelli* populations with different translocation and bottleneck histories. The results suggested that the combined Pūkaha and Remutaka population harboured (1) more genetic diversity compared to an island population with a single translocation origin (Moturua in the Bay of Islands) and a drastically bottlenecked mainland population (Rakaumangamanga in the Bay of Islands), (2) comparable levels of diversity to a mainland population with no known bottlenecks (the Trounson Kauri Park), and (3) lower levels of diversity that an older hybrid population (Ponui Island in the Hauraki Gulf with mixed *A. mantelli* LBI-Northland origin; see further Chapter 3.2). These levels of diversity relative to other populations followed the same pattern regardless of whether genetic diversity was quantified as the individual heterozygosity, pairwise relatedness (both strongly related to inbreeding), proportion of heterozygous individuals, or proportion of shared alleles (the latter two related to inbreeding but also strongly related to the overall amount of genetic variability).

Noticeably, variation in pairwise relatedness was large, and the level of individual compared to population-level diversity low. This suggests that while the combined Pūkaha and Remutaka population is diverse, there is a notable number of individual birds with high levels of inbreeding. These results are in line with the known pedigrees (Additional Figure Ad3.2.2 & Ad3.2.4).

Another way to explore inbreeding and diversity is to compare relatedness values between known relatives. Based on pedigree data, I compared the pairwise relatedness

of 15 Pūkaha and Remutaka individuals from five families to the relatedness between known first-degree relatives from the longer established hybrid population on Ponui Island. This analysis further supported the hypothesis that some individuals in the Pūkaha and Remutaka populations are highly inbred. In fact, all 15 pairwise comparisons of Pūkaha and Remutaka first-degree relatives approached, equalled, or exceeded the relatedness between a Ponui Island chick and its parents in a case when the parents themselves were closely related enough to be siblings (Figure 3.2.4). The relatedness analyses also revealed a previously unknown first-degree relationship between two of the founders from LBI, Solomon and Marcel. These males likely represent either father and son or brothers.

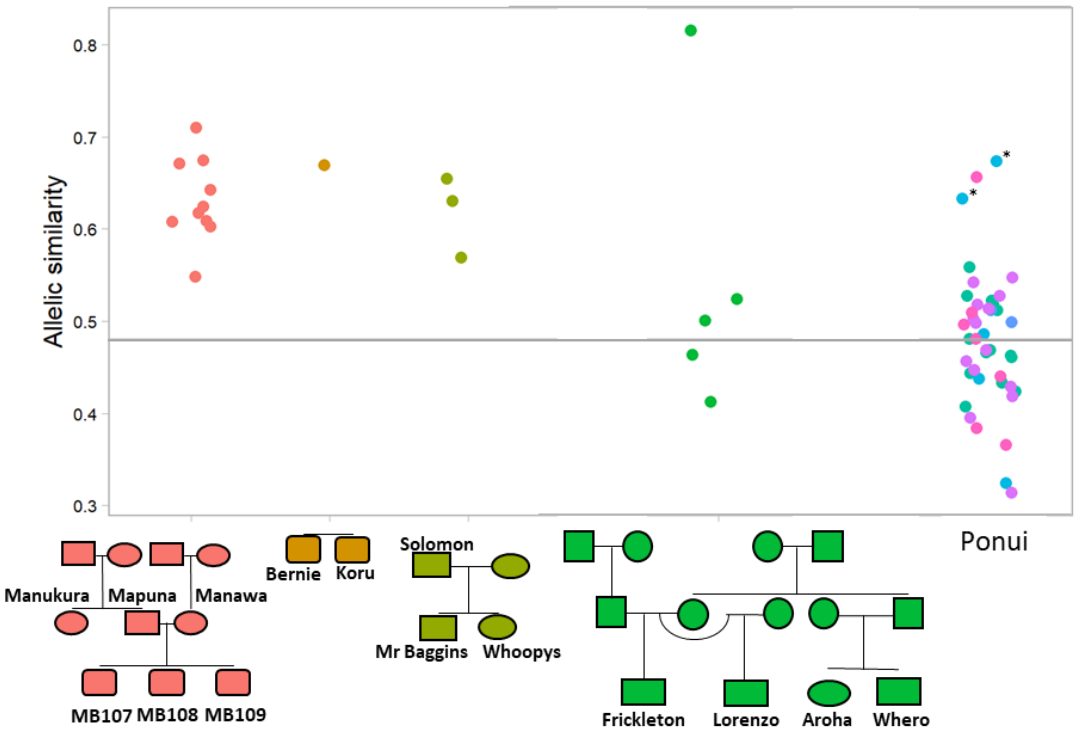


Figure 3.2.4. Pairwise relatedness (also referred to as allelic similarity) between known relatives in the Pūkaha National Wildlife Centre and the Remutaka Forest Park *A. mantelli* populations compared to first-degree relatives (sibling to sibling or parent to offspring) from the hybrid *A. mantelli* population on Ponui Island. Each dot indicates the relatedness between two

birds. In terms of Pūkaha and Remutaka, birds from five families are illustrated (see family trees lining up with dots of matching colour; only named birds were analysed). Specifically, the following relationships are indicated: In red (far left), Mapuna to his sister Manakura and his three offspring MB107-109 as well as MB107-109 to their mother Manawa. The orange dot represents relatedness between the brothers Bernie and Koru. Light green dots represent Solomon to his two offspring Mr. Baggins and Whoopsy, and the siblings to each other. In dark green, the dot at the very top represents the relatedness between Aroha and Whero, whose parents are known to be siblings, and the cluster further down represents relatedness between these siblings and their cousins Frickleton, and Lorenzo, plus the cousins' relatedness to each other. Thus, the four lower dark green dots are the only ones representing second-degree, rather than first-degree, relations. The horizontal grey line indicates the mean relatedness value of the Ponui Island first-degree relatives and highlights that all depicted first-degree relations from Pūkaha and Remutaka fall clearly above this line, indicating closer relatedness (i.e., higher levels of inbreeding). Two Ponui Island dots are indicated with '*', which highlight the relatedness between a mother (Kerri) and father (George) to their daughter (Esther) as a point of reference since Kerri and George themselves were found to be closely related enough to be siblings (see further Chapter 2.3).

The structure analyses found the strongest statistical support for grouping the genomic data in four clusters (usually referred to as K = 4; Figure 3.2.5). By comparing to reference birds from known, pure, wild Eastern and Northland origin as well as to Remutaka and Pūkaha birds with known pedigrees, the three first clusters were determined to represent Eastern, Northland, and LBI or Western ancestry, respectively (Figure 3.2.5a & b). The fact that birds with known Western heritage and LBI birds grouped together supports the hypothesis that the LBI population stems from Taranaki. The fourth cluster is challenging to interpret since no birds fell entirely in this cluster, but it was represented in birds of Eastern, Northland, as well as LBI origin. This cluster could be a signal of shared genetic similarities linked to historic connectivity throughout the North Island, more recent genetic exchange among the current management units, or neither. When the dataset was deliberately divided into three groups (K = 3), the Eastern

and LBI clusters remained very similar, and the unknown cluster merged with Northland, suggesting that they were most similar among the four clusters (Figure 3.2.5). Based on this interpretation, I was able to infer the background of the four sampled birds with unknown pedigrees. Koru and Bernie are both known to be sons of Kakama. However, Kakama was caught unmarked within the Pūkaha reserve so is of unknown origin, and his partner was unknown. The genetic structure analyses suggest that either Kakama or his partner was of completely Eastern origin and the other of mixed Eastern and Northland origin. Serenity is the daughter of Hapu and Jack, both of which were unmarked birds caught in the Pūkaha reserve with unknown origin. The genetic analyses suggest that one of these parents is of LBI origin and the other of Eastern origin. Rua, who was caught in Remutaka using a licenced kiwi dog and handler in 2020, most likely has the same parental combination as Serenity: one parent from LBI and one of Eastern origin (Figure 3.2.5a).

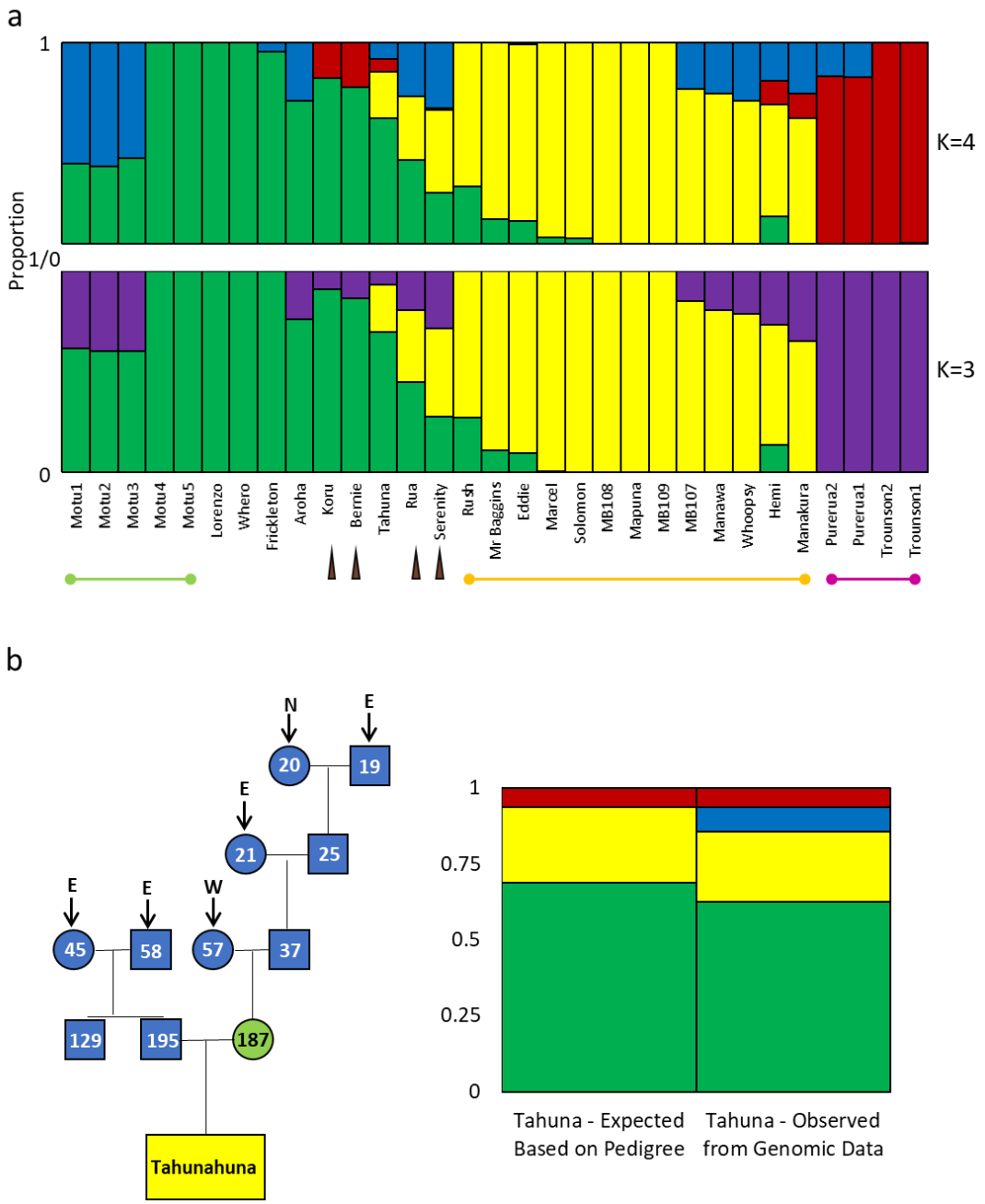


Figure 3.2.5. Genetic structure of the samples from the Pūkaha National Wildlife Centre and the Remutaka Forest Park. Panel (a) illustrates two results from the structure analyses. In these plots, each vertical column illustrates one bird, and the colours represent the proportion of its genome that is grouping with each of the four (top row) or three (lower row) clusters identified by the structure software. Individuals appear in the same order in both graphs. Green and purple lines under graphs highlight the known Eastern and Northland birds included as references, respectively. The yellow line highlights birds with know 100% LBI heritage. Arrows indicate the four birds of fully unknown background prior to this analysis. Focusing on the top row, the simplest possible interpretation is that yellow indicates LBI, green Eastern, and red Northland

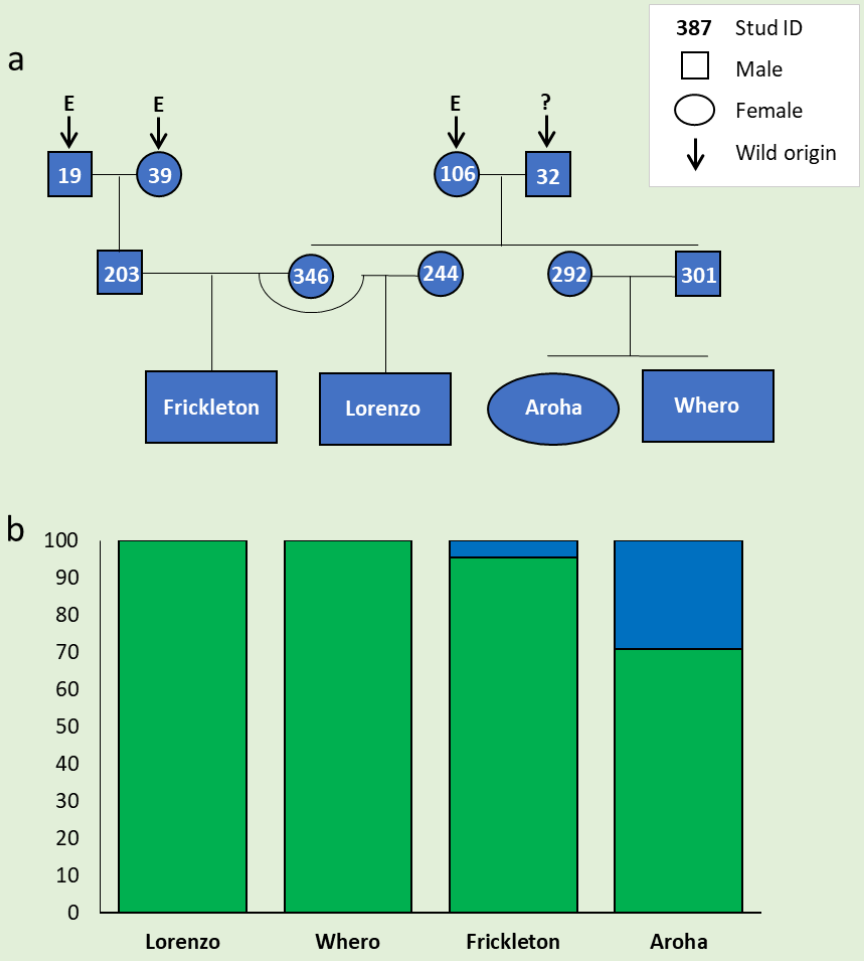
heritage. Multiple colours in a column suggests, but is not proof of, mixed origin (Ottenburghs *et al.* 2017). The blue cluster is the most difficult to interpret since no individuals appear completely blue and blue is represented in birds with known Eastern, LBI, and Northland origin. It is possible that this represents incomplete sorting between the genetic makeup of management units (i.e., that they still share signal of historic connectivity), or it could be a signal of more recent gene flow (i.e., birds migrating or being moved between areas). Comparing the first and second row, the blue merges with the red cluster, suggesting that the makeup of the blue cluster is most similar to the genetic profile typical in Northland. Panel (b) illustrates an example of a bird with a known mixed heritage, Tahunahuna, to illustrate how the result of the structure analyses compare to the known pedigree. The yellow section of Tahunahuna's column supports that the birds on LBI indeed stem from *A. mantelli* Western. Unfortunately, Tahunahuna brings little additional clarity to the mysterious blue cluster. In the pedigree, circles indicating females, squares males, and numbers their stud IDs (compare Additional Figure Ad3.2.1 & Ad3.2.2).

Box 1. The mysterious Stud ID 32

When the decision was made to focus the captive *A. mantelli* population on purebred *A. mantelli* Eastern, several individuals of unknown or mixed origin were released at Pūkaha and Remutaka. Many of these birds were related to one male that was later released to Pūkaha, known as Mr Kiwi or Stud ID 32. This male was allegedly captured in 1975, but unfortunately, his origin was only specified as the North Island. Mr Kiwi was prolific and fathered a substantial proportion of the captive birds hatched at Willowbank Wildlife Reserve, Christchurch, which is one of the main sources for released captive birds at both Pūkaha and Remutaka. Four of the birds represented in my blood samples had Mr Kiwi as their maternal, or both maternal and paternal, grandfather. Their remaining grandparents were all wild-caught *A. mantelli* Eastern.

Most of the genetic makeup of these four grand-chicks grouped with the green cluster that represents Eastern genotype, but two of them, Aroha and Whero, also have genetic material that group with the mysterious blue cluster (see figure in this box and compare Figure 3.2.5). A combination of blue and green genetic identity was observed for three of the five control

birds of known Eastern origin included. However, the blue cluster was also found in birds of known Western and Northland origin. This means that it is likely that Frickleton, Lorenzo, Aroha, and Whero all represent 100% Eastern origin; however, due to the presence of 'blue' genetics, there is a small chance that Mr Kiwi was not Eastern, and his origin might for now need to remain listed as unknown.



3.2.3.3 Health

The sampled birds from Pūkaha and Remutaka fell well inside the interval of other sampled populations in terms of weight, body condition, glucose, packed cell volume, and total blood protein content. However, for haemoglobin concentration (HB), values were low compared to other populations with most of the Pūkaha and all the Remutaka samples falling below the overall average across populations (Figure 3.2.6). The

average HB value was found to be significantly lower in Remutaka than in five of the seven comparison populations, while the Pūkaha average was not found to be statistically different from Remutaka or any of the other populations (Figure 3.2.6).

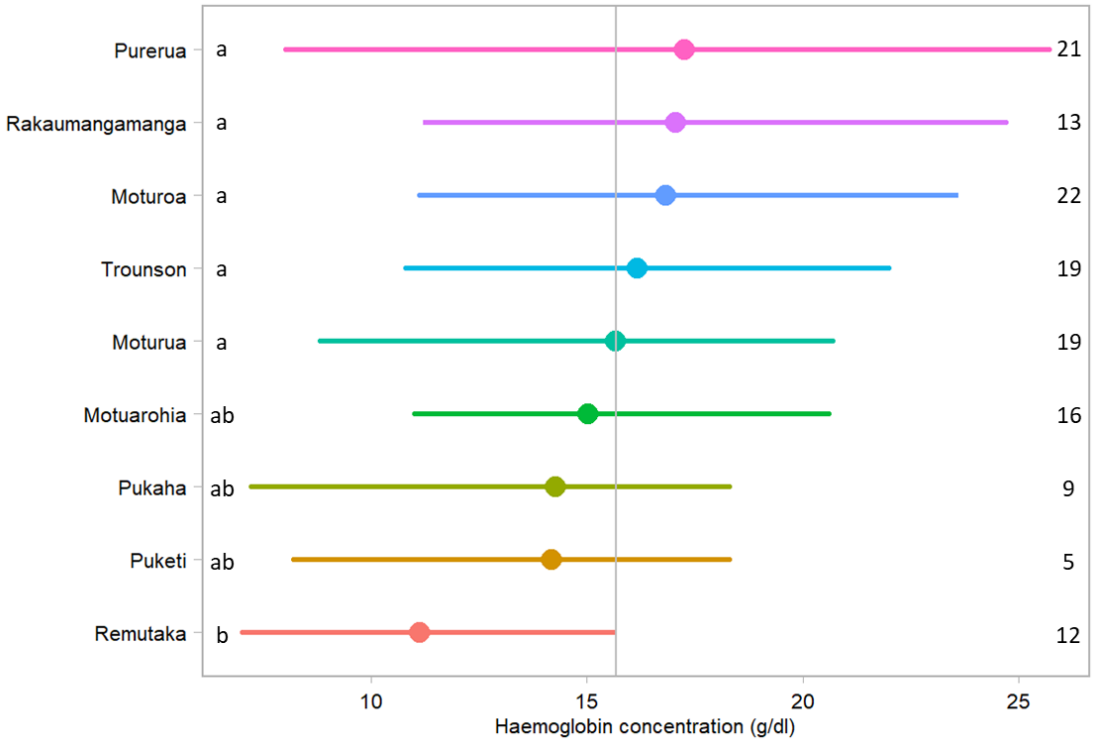


Figure 3.2.6. Comparison of the haemoglobin concentration in whole blood of nine *A. mantelli* populations including the Pūkaha National Wildlife Centre and the Remutaka Forest Park. Coloured lines indicate the span from the lowest to the highest values observed with the dot indicating the average. Populations have been ordered by decreasing average concentration. The vertical grey line indicates the overall average across all samples. Different letters to the far left indicate significant difference, i.e., the 'b' by Remutaka indicates significantly lower values for this population than all populations marked with only 'a', while populations marked with 'ab' are statistically not higher than 'b' nor lower than 'a'. Numbers to the far right indicate sample size.

3.2.4 Discussion, conclusions, and suggestions

Based on the results presented herein, should the mixed-origin introductions of *A. mantelli* to Pūkaha and Remutaka be deemed success stories? The kiwi at both sites are indeed experiencing encouraging, high recruitment rates, and my analyses illustrate that they harbour comparatively high levels of genetic diversity. A caveat to the latter is that, on an individual level, many birds still represent single-origin genetic makeup, and some of these have relatively high inbreeding levels (see further below).

Previously, *Apteryx* introductions have been deemed unsuccessful if fewer than 40 birds have been released (Scrimgeour & Pickett 2011, Kiwis for Kiwi 2016, Robertson & Colbourne 2017); and the number of birds released at Remutaka and Pūkaha has been 31 and 57, respectively, placing Remutaka under but Pūkaha above this threshold. However, rather than the number of individuals released, what matters for retainment of genetic diversity is how many of those contribute to the population and how closely related the contributors are to each other (Hedrick & Fredrickson 2010, Weeks *et al.* 2015, Rick *et al.* 2019). Hence, I calculated the number of founder genome equivalents (FGE) that the birds surviving more than one year after release represented. This reduced the starting populations to 24 and 32 birds, respectively. Even these numbers represent a maximum number of FGE since (1) all birds surviving more than one year might not have bred (in fact, at Remutaka, only 16 of the 19 surviving founders from Little Barrier Island are known to have bred successfully); (2) at least early on, there are examples from both Pūkaha and Remutaka of breeding between very close relatives, which reduces the effective number of genomes (Additional Figure Ad3.2.1 & Ad3.2.4); (3) I only accounted for first-degree relationships when estimating the number of FGEs, but several birds deemed ‘unrelated’ were in fact second-degree relatives, such as aunt/uncle and niece/nephew (Additional Figure Ad3.2.1); (4) my estimation treated

the translocated birds from LBI as unrelated, which is unlikely to be accurate (in fact, the genetic analyses suggested that two of the founding males form LBI were closely enough related to be brothers). Strictly speaking, all *A. mantelli* will, of course, be related, especially all individuals within an isolated site such as Little Barrier Island, but what is meant by unrelated is not defined in the Kiwi Recovery Plan or the Best Practice Manual, leaving this parameter open for interpretation (Robertson & Colbourne 2017, Germano *et al.* 2018).

Consequently, an important question for future *Apteryx* translocations is whether 40 released birds is a useful cut off value. The successful, high-density *A. mantelli* population on Ponui Island has been identified as more genetically diverse than large mainland populations despite only 14 birds having been released on this island (eight *A. mantelli* Northland and six birds from LBI; see Chapter 3.1). At the same time, Weiser *et al.* (2013) found through computer modelling that an *Apteryx* population founded with 40 individuals would need to receive, on average, one immigrant/supplementary bird every two years to retain 90% of the allelic diversity (which is a widely accepted goal for genetic management; Batson *et al.* 2015, Weeks *et al.* 2015, Frankham *et al.* 2017). In general, if comparing across bird introductions worldwide, 30 birds has been the median number of individuals released, and the overall success of bird introductions have been high (Sutherland *et al.* 2019, Skikne *et al.* 2020). Another indicator that the number 40 is rather arbitrary is that, for captive *Apteryx* populations, 20 founders have been deemed sufficient (Barlow 2018). Consequently, I stress that the genetic composition of the founders, their success after release, and the rate of further supplementation are all factors defining for the success and genetic diversity in an introduced population; hence, a set number of founders (40 or otherwise) is unlikely to

be an informative indicator of translocation success (Johnson *et al.* 2010, Benson *et al.* 2011, Ransler *et al.* 2011, Ralls *et al.* 2018, Rick *et al.* 2019).

Combining founders from several different areas is one way of compensating for a small number of founders. However, naturally, such mixing of birds will only generate a diverse population if the birds interbreed (Weiser *et al.* 2013). From this perspective, there are two factors that have delayed the admixture at Pūkaha and Remutaka, which in turn might have delayed any positive effects of increased diversity such as genetic rescue (Ingvarsson 2001, Frankham 2015, Bell *et al.* 2019). First, of the 20 LBI birds released at Remutaka, only one is known to have paired with a local bird straight after release; the rest created pairs within the cohort just released. My research on the hybrid population of Ponui Island suggests that this mating among the LBI birds is unlikely to be caused by a preference for like mates (see Chapter 2.2). Instead, I suggest that a more likely explanation is that few local birds were available for partnering at the time of release. This phenomenon of mating happening preferentially within the translocated cohort due to limited availability has been observed in other species (Thavornkanlapachai *et al.* 2019). However, the long lifespan and high pair fidelity of kiwi mean that the proportion of mixed pairs formed straight after a translocation will have a greater impact on the consequent mixing than it would have in many other species. Thus, active measurements might be warranted for kiwi translocations that increase the likelihood of initial mixed mating. One relatively simple way to approach this, which has proven successful for other species (for instance Florida panthers), is to introduce individuals of only one sex (Hedrick & Fredrickson 2010). The second reason for the comparatively higher levels of diversity in the population compared to the individual level at both these sites is that there have been several cases of skewed reproductive success followed by mating between closely related birds. For instance, the

mysterious Mr Kiwi, or Stud 32, fathered a large number of chicks, many of which were released at Pūkaha and Remutaka since the unknown origin of Mr Kiwi made his offspring unwanted in the captive breeding stock (see Box 1; Barlow 2011, Barlow 2018). Some of these offspring then mated with each other, rapidly increasing average levels of inbreeding. Encouragingly, despite the slow start, admixture is currently ongoing at Pūkaha and Remutaka; for instance, all four unknown birds analysed turned out to have mixed heritage, and three of these birds are known to have more siblings. Based on this, I expect the observed discrepancy between individual and population-level diversity to shrink over time and the genetic structure of the Pūkaha and Remutaka populations to become more similar to that on Ponui Island, where over 80% of sampled individuals were found to be of mixed genetic makeup.

However, while Pūkaha and Remutaka could indeed experience an increase in diversity over the near future, populations isolated from genetic influx will inevitably aggregate inbreeding and lose diversity over time due to genetic drift. Thus, since genetic diversity on individual and population-level is crucial for survival and persistence, especially in the longer term (Frankham *et al.* 2017), and loss of genetic diversity is irreversible, one option for future management of Pūkaha and Remutaka would involve further translocations, which would be in accordance with the current Western Taxon Plan (WTP; which is the regional Kiwi Recovery Plan under which Pūkaha and Remutaka, as well as LBI, fall; Scrimgeour & Pickett 2011). The key questions will be from and to where birds should be translocated. I suggest that the easiest way to increase diversity at both sites would be through strategic movement of birds between Remutaka and Pūkaha in a way that minimises relatedness. This would create a meta-population based on 52.5 FGE, and, while this combined population will still be small enough to inevitably lose

diversity over time, managing them together would reduce the need for external supplementation (Weiser *et al.* 2013).

The WTP suggests that further reinforcement translocations to Remutaka and Pūkaha could come from LBI (Scrimgeour & Pickett 2011). However, further translocation from inaccessible places like LBI will likely necessitate that a large group of birds would be moved at once, which will increase the risk of mating predominantly happening within the translocated cohort. While this could be avoided by introducing individuals of only one sex, determining the sex of *A. mantelli* in the field is challenging why this might not be practical. I suggest that more gradual supplementation from mainland populations might be more beneficial – as well as less costly – especially if the mixed gene pool at Pūkaha and Remutaka can allow managers to have few restrictions of what to consider suitable source populations. The WTP also points out that establishing the true origin of the birds in LBI is desirable. While I only had one available bird with known partial Western origin, the genetic structure of this bird supports the theory that the birds on LBI stem from one or more translocation(s) from the Taranaki area.

Another factor that could influence what is considered adequate sources for further supplementation could be what is deemed a desirable destiny of the birds at Pūkaha and Remutaka. The WTP discusses the potential of Pūkaha and Remutaka to act as sources for future translocations, specifically, to areas where kiwi density is very low and genetics suggest an identity in between *A. mantelli* Eastern and Western; such as the Ruahine ranges and the Kaimanawa area (see Supplementary Figure S1.2.2). I think this could be a good plan, given some thorough consideration, for a number of reasons. First, one concern raised against further translocations of the Remutaka and Pūkaha birds is that the small founder size means that are likely inbred. My results suggest that,

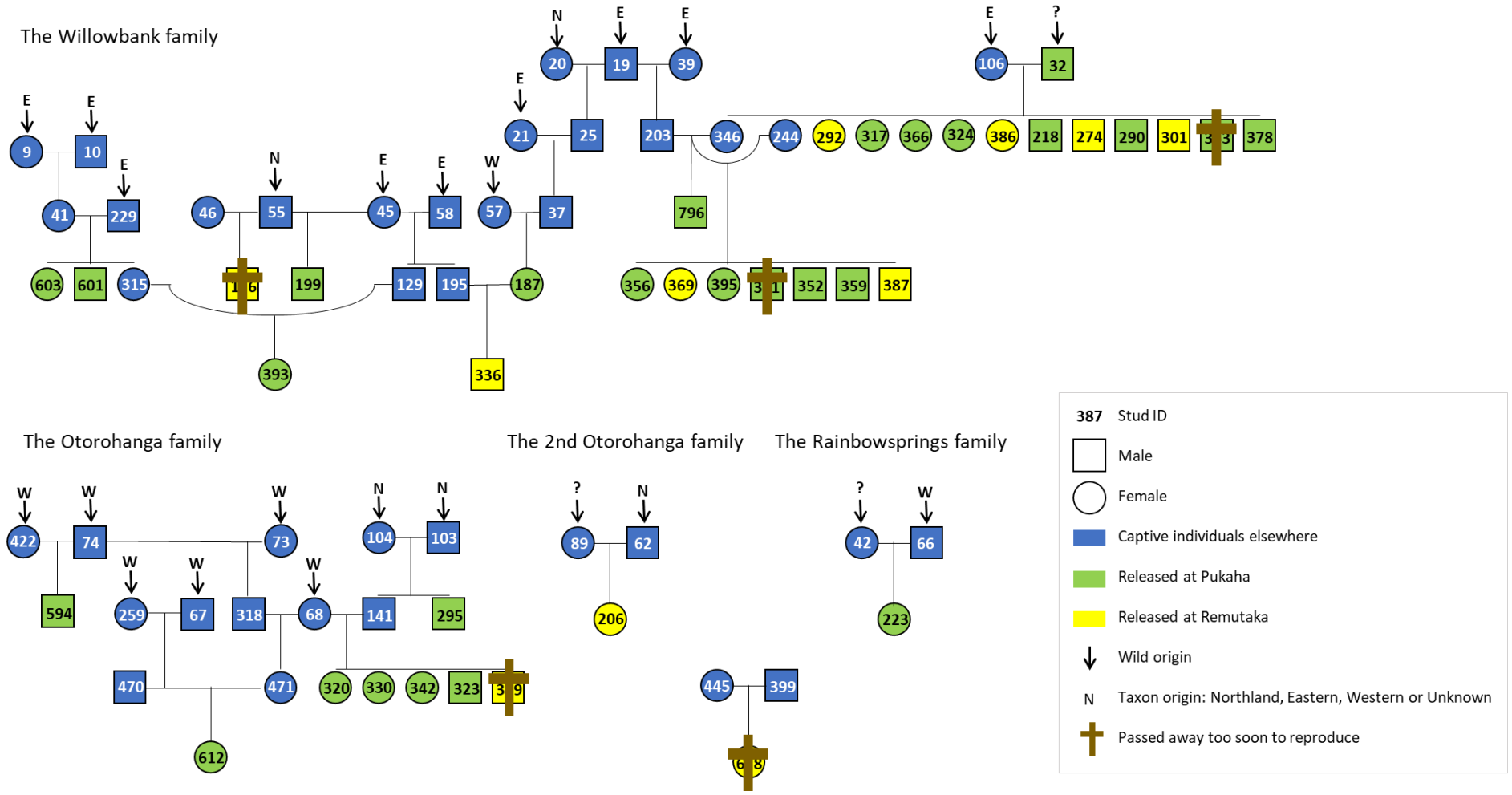
indeed, some kiwi at Pūkaha and Remutaka are inbred, but also that many are diverse and outbred. Second, when discussing the potential of using inbred source populations, the idea frequently comes up that translocating inbred individuals risks spreading 'bad' alleles. However, disproportional occurrence of bad (or deleterious) alleles could only be expected in an inbred population if it has been isolated for a sufficiently long time for deleterious alleles to (a) appear through random mutation, and (b) spread through the population due to genetic drift and lack of selection. For a long-lived organism like kiwi, concerning aggregation of deleterious alleles would take hundreds of years. Thus, risk of spreading deleterious alleles is not a valid argument against utilising the Remutaka and Pūkaha birds for further translocations. Thirdly, while concern is valid that inbred source populations will contribute less diversity per translocated unit, encouraging meta-analyses have found that even inbred supplementary individuals can generate rescue effect, although usually a smaller effect compared to outbred sources (Hedrick & Fredrickson 2010, Frankham 2015). This is especially the case if individuals from several inbred populations of different origin are combined (Fredrickson *et al.* 2007, Ransler *et al.* 2011). Lastly, the 'intermediate' identity, both genetically and geographically, of the kiwi in the potential target areas, suggests that the current strict split into management units is somewhat artificial, which should further encourage the utilisation of mixed translocations and establishment of connectivity among current units.

Consequently, I suggest that translocations of Remutaka and Pūkaha kiwi to the cross-over area between *A. mantelli* Eastern and Western could be beneficial, especially if combined with birds from elsewhere. Specifically, it could be a way to recreate past connectivity, successfully diversify these management units, and make kiwi in both areas more resilient against future environmental change. For this vision to come true,

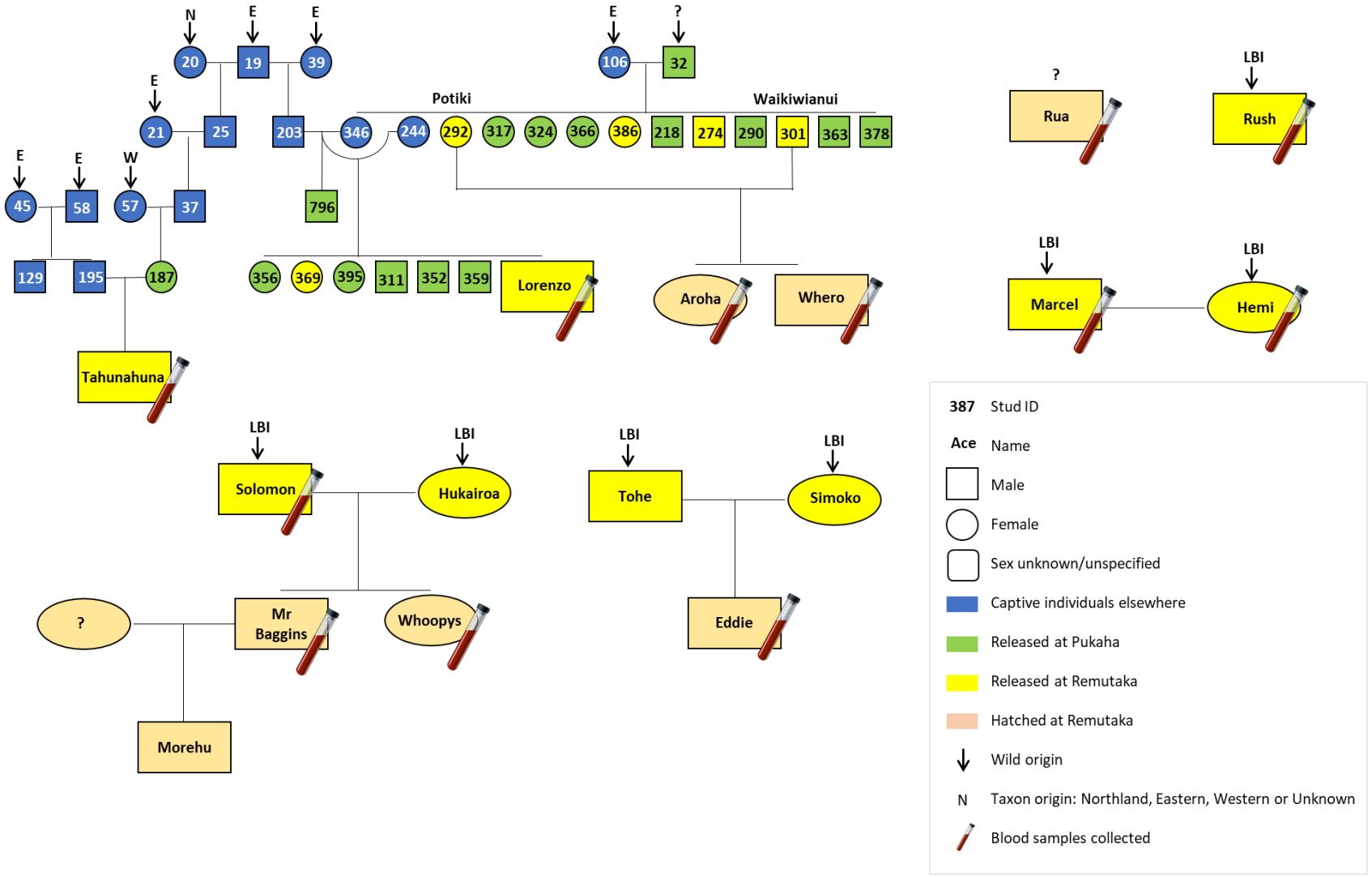
translocations would have to be done strategically to generate a continuum of steppingstone populations close enough together to facilitate kiwi migration between them. Similar approaches have been done successfully in other taxa, for instance, Scandinavian arctic foxes (Hasselgren *et al.* 2018, Hemphill *et al.* 2020). However, while spacing steppingstone populations 250 km apart was sufficient for arctic foxes, the maximum distance between kiwi population would have to be much smaller. I suggest that the current knowledge of kiwi movement is insufficient to determine what the appropriate distance between populations would be, but it likely is in the order of 10 km and habitat-dependent (Potter 1990, Forbes 2009). If utilising *A. mantelli* from Remutaka and Pūkaha is deemed desirable for creating such a continuum of kiwi across the North Island, genetic analyses of the specific candidate birds would be warranted to maximise the amount of contributed diversity.

Lastly, translocations are complex interventions that involve many other aspects in addition to genetic diversity. One example of another potential issue for the Remutaka and Pūkaha populations is their location outside the historic range of *A. mantelli*. I found that the Remutaka and Pūkaha birds fell within the range of other populations for most parameters measured, but one exception of potential concern is the low haemoglobin concentration (HB). High HB is generally regarded as a reliable indicator of health in birds (providing it does not exceed an upper limit; Lill *et al.* 2013b, Kaliński *et al.* 2015, Minias 2015, Glądalski *et al.* 2016) that has been associated with higher weight and/or fat stores (Lill *et al.* 2013a, Minias 2015), reduced parasite load (in particular for chicks; Minias 2015), higher food abundance and/or a more pristine habitat (Bańbura *et al.* 2007, Kaliński *et al.* 2015, Glądalski *et al.* 2016, Minias 2016), and higher chick survival (Kaliński *et al.* 2015, Glądalski *et al.* 2016). In my full dataset across nine *A. mantelli* populations, I found no correlation between HB and weight,

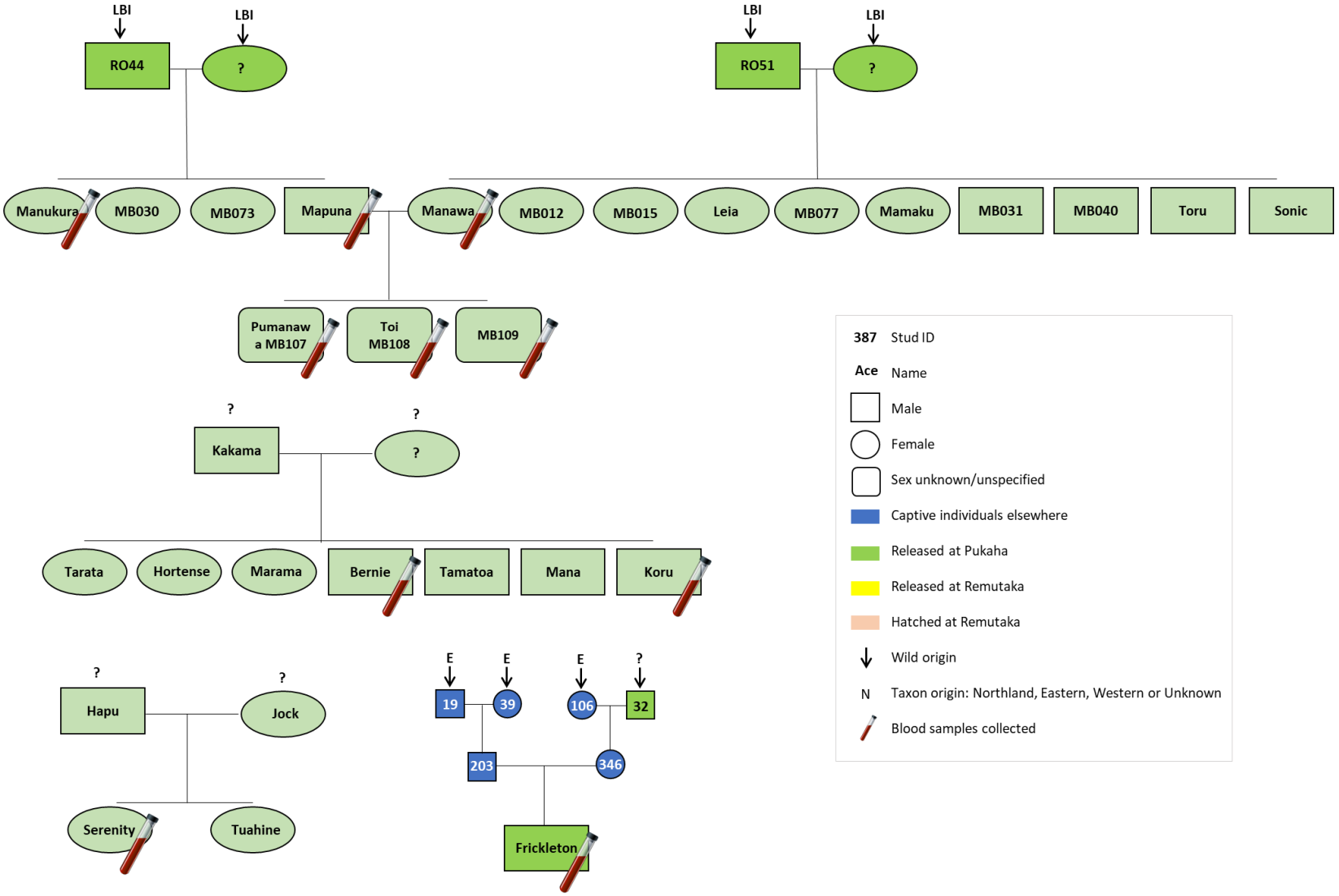
tarsus width (an indicator of size), or body condition (Taborsky & Taborsky 1999; *n* = 140, 134, and 133, respectively; Supplementary Figure S3.2.1). In addition, Bansal (2020) found that HB did not change after the removal of ectoparasites in adult *A. mantelli*. Thus, I suggest that the most likely explanation for the comparatively low HB level at Remutaka might be related to food availability or food quality. While I would not call this an immediate concern for the persistence of this population – the sampled birds all seemed to be in good condition considering the 2020 drought – these results could warrant further study into the habitat at Remutaka and the diet of their kiwi. Such research could, for instance, contribute greatly to making the outcomes of future kiwi translocations more predictable.



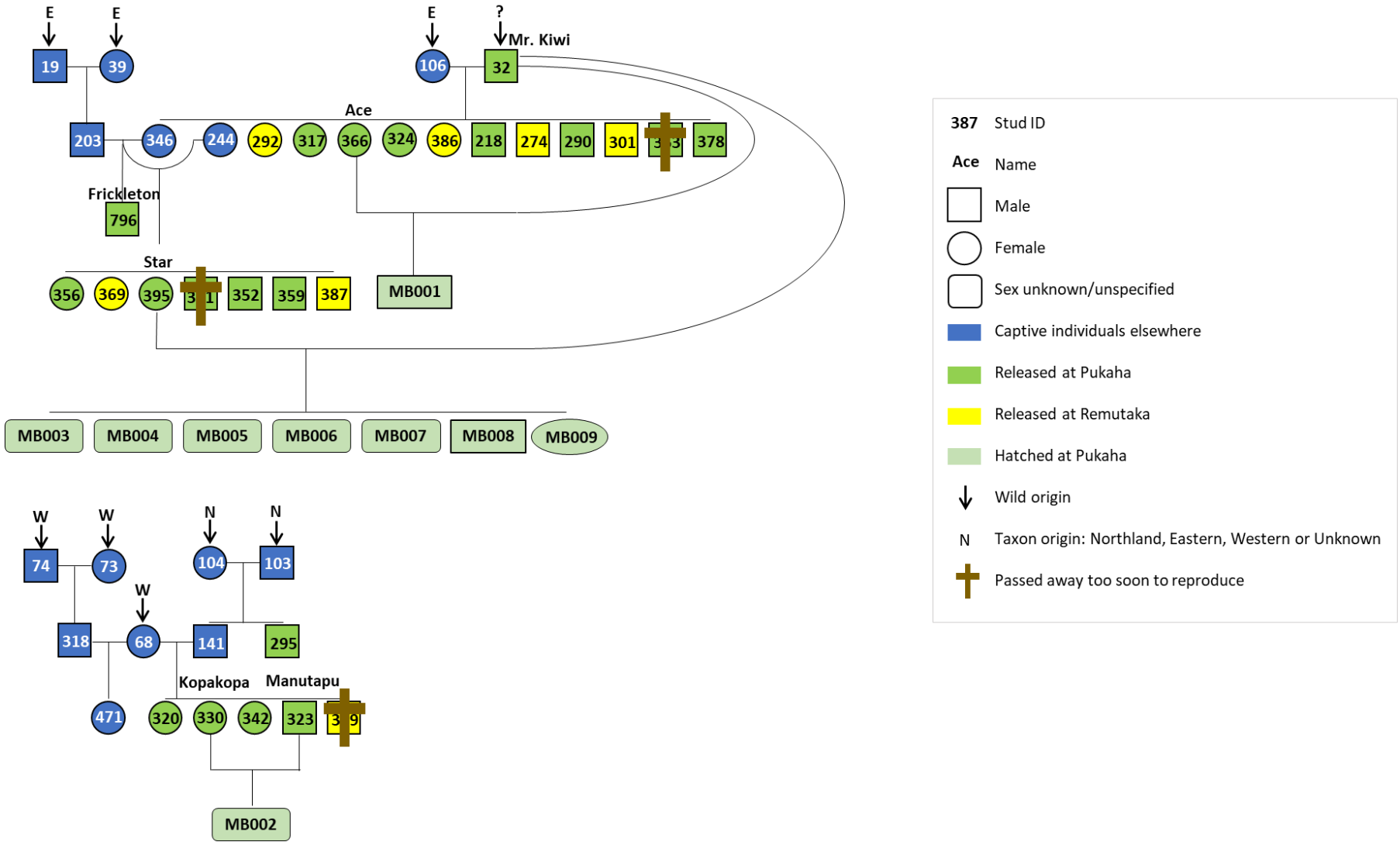
Additional Figure Ad3.1.1. Taxon origin of and relationships between previously captive *A. mantelli* released at the Pūkaha National Wildlife Centre and the Remutaka Forest Park.



Additional Figure Ad3.1.2. Taxon background and relationships between the Remutaka Forest Park birds from which blood samples were collected.



Additional Figure Ad3.1.3. Taxon background and relationships between Pūkaha birds from which blood samples were collected.



Additional Figure Ad3.1.4. Examples of A. mantelli breeding between sister/brother, father/daughter, father/granddaughter at the Pūkaha National Wildlife Centre. Only partial family trees shown (compare Additional Figure Ad3.2.1).

PART IV – TELOMERES

Age distribution is a fundamental indicator of population viability. Consequently, the ability to determine age has the potential to greatly aid identifying target populations in need of influx and suitable source populations for translocations. In addition, such quantification is key for accurate evaluation of translocation outcomes and population viability. *Apteryx* are long-lived and hitherto no method has been found for ageing adult individuals. Hence, in this part, I explore the relationship between *A. mantelli* telomere length and age, growth, and stress with a cross-sectional, longitudinal, as well as a modelling approach. Then I discuss whether telomeres could be the long-sought-after marker to evaluate *Apteryx* age specifically, and individual and population health more generally.

Chapter 4.1

Pilot study: What co-varies with telomere length in wild *Apteryx mantelli*?

All supervisors provided feedback on one or more drafts of this chapter. The expertise of Kristene Gedye, the help from Brianna Nelson and Trish McLenachan, and the hard work of Natasha, Stephen, Mo, Yi, and other field helpers were crucial for this chapter.

Abstract

The growing need for conservation calls for accurate and informative ways to evaluate and compare populations of threatened species. Increasing interest is turned towards molecular markers and one of the candidates receiving the most attention is telomeres. Telomere length and the rate of telomere attrition have been found to correlate with age, stress exposure, and mortality risk in many species, making these promising markers of individual- as well as population-level viability. *Apteryx* is a long-lived genus relying on active conservation management, but for which methods of ageing adult individuals and evaluating parameters such as inbreeding and outbreeding depression is currently lacking. This first-ever pilot study examined what factors co-vary with telomere length in *A. mantelli* and found large variation among individuals, but no correlation between relative telomere length and age, size, growth, or stress. The correlations that were found between haemoglobin concentration, packed cell volume, relative telomere length, and age suggests that environmental impacts on telomere attrition differ too much between populations to infer general patterns. While this does not rule out telomere length as an informative marker of health in *A. mantelli* individuals as well as populations, it suggests that further studies are needed and that these require more controlled settings.

4.1.1 Introduction

Worldwide, conservationists are faced with an ever-increasing number of small, isolated populations at risk of local extinction, combined with a limited pot of conservation dollars (IUCN 2020). This situation renders the need to prioritize among, and hence to somehow evaluate, such populations (Joseph *et al.* 2009, Malone *et al.* 2018, McGowan *et al.* 2020). One reason evaluating the viability and conservation status of long-lived

species can be challenging is that population numbers can stay high for a long time after recruitment has started to decline (Kuussaari *et al.* 2009, Robertson & de Monchy 2012, Taylor 2014, Edwards *et al.* 2019), a phenomenon leading to so called extinction debt (Kuussaari *et al.* 2009). This phenomenon is particularly problematic to detect in species for which age estimation is limited to general categories such as 'juvenile', 'subadult', and 'adult' (Holmes & Martin 2009, Wolfe *et al.* 2010, Töpfer 2018). Under such conditions, conservation managers risk being limited to imprecise evaluation of, for instance, the outcomes of historic translocations or the premises for future ones (Dimond & Armstrong 2007, Margalida *et al.* 2011, Goudarzi *et al.* 2015). To overcome this problem, molecular markers indicative of individual health and population viability are receiving increasing attention (Jarman *et al.* 2015). Telomere length and rate of telomere attrition are among the markers of highest research interest (Smith *et al.* 2016, Monaghan *et al.* 2018, Monaghan & Ozanne 2018).

Telomere attrition has been identified as one of nine hallmarks of ageing since it (1) is a natural part of becoming older, (2) increasing the rate of attrition accelerates ageing, and (3) experimental elongation of telomeres generate phenotypes associated with slowed or reversed ageing (Allsopp *et al.* 1995, Olovnikov 1996, López-Otín *et al.* 2013). Examples of the latter are extended lifespan in mice (Criscuolo *et al.* 2018), and faster feather regrowth in Zebra finches after experimental telomere elongation (Reichert *et al.* 2014). This relationship between ageing and telomere shortening is widely established across taxonomic groups, not the least in birds (Juola *et al.* 2006, Pauliny *et al.* 2006, Bize *et al.* 2009, Salomons *et al.* 2009, Heidinger *et al.* 2012, Angelier *et al.* 2013, Barrett *et al.* 2013, Mizutani *et al.* 2013, Aydinonat *et al.* 2014, Boonekamp *et al.* 2014, Bebbington *et al.* 2016). In addition, many studies have found telomere length to be as good or even a better predictor of mortality risk and/or life expectancy when compared

to chronological (actual) age (Pauliny *et al.* 2006, Bize *et al.* 2009, Salomons *et al.* 2009, Geiger *et al.* 2011, Heidinger *et al.* 2012, Angelier *et al.* 2013, Barrett *et al.* 2013, Watson *et al.* 2015, Bauer *et al.* 2018), rendering expanding interest in examining telomere dynamics in connection to evolution and multiple aspects of ecology (Ringsby *et al.* 2015, Tricola *et al.* 2018).

During every round of DNA replication, a small segment at the end of each chromosome is lost due to the physical need for the DNA polymerase to attach to the DNA strand - the so-called 'end-copy-problem' (Allsopp *et al.* 1995, Olovnikov 1996, Shay & Wright 2000, Blasco 2007). In most stem cells, the lost sequence is replaced by the activity of the enzyme telomerase. However, in most species, this enzyme is downregulated in somatic cells, hence cell division results in telomere attrition (Allsopp *et al.* 1995, Olovnikov 1996, Shay & Wright 2000, Blasco 2007). Because of this inability to regrow telomeres, theory predicts that any type of stress resulting in (body) size change would affect telomere length and rate of attrition through the same mechanism as increasing age (Allsopp *et al.* 1995, Olovnikov 1996, Shay & Wright 2000, Blasco 2007). In line with this, Geiger *et al.* (2011), for example, found that juvenile king penguins (*Hydrobates pelagicus*) with a period of rapid catch-up growth to reach expected weight for their age after a period of low food availability had shorter telomeres than juveniles that were able to grow more slowly and steadily. Additional support for this idea is that larger individuals within a species have been found to age faster (dogs; Kraus *et al.* 2013), and to lose telomeres faster (sparrows; Ringsby *et al.* 2015).

More generally, studies have concluded that the genetic influence on telomere length is largest at birth suggesting that there are significant environmental factors impacting their attrition (Seeker *et al.* 2018). The leading hypothesis regarding the link between

non-growth-related environmental stressors and telomere shortening is that telomeric DNA is especially sensitive to reactive oxygen agents (ROS; Houben *et al.* 2008, Haussmann & Marchetto 2010, Monaghan & Ozanne 2018). Elevated ROS levels are in turn a consequence of stress (Passos *et al.* 2007, Geiger *et al.* 2011). Examples of environmental stressors found to be linked to accelerated telomere attrition are food shortage years in Leach's storm petrel (*Oceanodroma leucorhoa*; Watson *et al.* 2015) as well as Seychelle warblers (*Acrocephalus sechellensis;* Spurgin *et al.* 2018), and bad weather years in black-tailed gulls (*Larus crassirostris*; Mizutani *et al.* 2013). Furthermore, Salmon *et al.* (2016) undertook a reciprocal translocation study of great tit (*Parus major*) chicks and found that regardless of where the eggs were laid, telomeres degraded faster in an urban and more stressful environment compared to a rural rearing environment. It has also been found that solo-living captive individuals of the social African grey parrot (*Psittacus erithacus*) have shorter telomeres than other captive conspecifics living in pairs (Aydinonat *et al.* 2014). Intriguingly, Bebbington *et al.* (2016) found that in Seychelle warblers, homozygosity (quantified using microsatellites), was correlated with shorter telomeres for adults in general and with shorter telomeres for juveniles during years of food shortage, suggesting that increased telomere attrition could potentially be a factor contributing to inbreeding depression. Lastly, a growing number of studies in humans have connected telomere attrition rate to pollutants such as cadmium, lead, arsenic, and various organic compounds, but such studies in birds have so far given unclear and somewhat ambiguous results (see review by Louzon *et al.* 2019).

There remains some debate regarding to what extent telomere attrition is a cause or rather 'simply' a marker of ageing, stress, and/or sickness (Bateson *et al.* 2015, Bateson & Nettle 2018, Bauer *et al.* 2018, Criscuolo *et al.* 2018, Young 2018). However, taking

all the above together, measuring the telomere length of individuals and length distributions in populations could be informative for comparison and evaluation regardless of the causality (Haussmann & Marchetto 2010, Monaghan *et al.* 2018, Spurgin *et al.* 2018). Consequently, telomere length is a marker with great potential be informative for evaluating historic translocation success, current translocation needs, and the suitability of different populations to act as sources for such translocations (Haussmann & Marchetto 2010, Monaghan *et al.* 2018, Spurgin *et al.* 2018).

The kiwi genus *Apteryx* is an exemplar of an extraordinarily long-lived and threatened group for which the lack of methods for (1) evaluating and predicting translocation outcomes, and (2) ageing adults beyond the age of four (while the oldest known individual is currently 42 years old) are currently limiting further improvements of conservation policy (Barlow 2011, Robertson & de Monchy 2012, Innes *et al.* 2015, Innes *et al.* 2016, Robertson & Colbourne 2017, Germano *et al.* 2018). Currently, *Apteryx* individuals can at best be split into four, broad age classes based on bill length, behaviour, weight, and growth (Table 4.1.1; Sales 2005, Robertson & Colbourne 2017). Even these classes are somewhat ambiguous, and a major challenge is that subadult females can be mistaken for adult males (Robertson & Colbourne 2017). To explore if this can be overcome with molecular methods, I herein present the first ever investigation of telomere dynamics in *Apteryx*. In addition, beyond a potential indicator of age and health, *Apteryx* telomeres warrant attention since a recent genomic analysis identified positive selection for a gene named telomere maintenance 2 (TELO2 or Tel2P) as a key locus differentiating the *Apteryx* clade (Ramstad & Dunning 2020).

This study was conducted in three parts. The first part explored the relationship between telomere length and age of *A. mantelli* individuals from several populations of known age ranging from four months to 21 years old. The second part utilised samples from

Ponui Island, which is one of the most extensively studied *A. mantelli* populations. For this part, data has been collected for the last 17 years, enabling investigation of the relationships between telomere length and size, growth, stress, haematology, activity, and habitat utilisation in adult *A. mantelli* (of unknown age). The third and last step was to explore whether any relationships found in the Ponui Island population during step two could be verified in adult birds from other populations. Finally, the results are discussed from a perspective of the potential for telomere length to act as an informative marker of age and/or a worthwhile measurement for population comparisons in *Apteryx*. Specifically, telomere measurements are discussed in the light of improving how appropriate target and source populations are identified and how *Apteryx* translocation success is evaluated.

Table 4.1.1. Definitions of age categories used for *Apteryx* spp. and their definitions (weights do not apply to the smaller *A. owenii*; Robertson & Colbourne 2017). Thresholds for bill length are frequently used as a complement to the parameters in this table, but this measure needs to be somewhat adjusted for each population and thus is not included here (McLennan *et al.* 2004, Cunningham & Castro 2011, Robertson & Colbourne 2017).

	Chick	Juvenile	Subadult	Adult
Behaviour	Still returning to father/nest	Independent of nest**	Not yet breeding**	Breeding
	and	and	and	or
Age	0 to 10-50 days*	< 6 months	> 6 months, < 4 years	> 4 years
	and	and	and	or
Growth	Still growing	Still growing	Still growing	Bill growth < 1.5mm / 6 month
	and	and		and
Weight	< 600g	< 1200g		> 1200g

*Depending on when it is no longer returning to the dad and/or nest

**For *A. rowi* and *A. australis* juveniles and subadults often remain and/or regularly return to the nest/parental territory throughout juvenile- and subadult-hood.

4.1.2 Materials and Methods

4.1.2.1 Samples

This study was based on blood samples collected between 2017 and 2020 from North Island brown kiwi (*Apteryx mantelli*). Samples came from seven populations. Forty-four samples came from Ponui Island in the Hauraki Gulf along the eastern coast of New Zealand's North Island. The actual (chronological) age of the Ponui Island birds was not known, but they were adults. Of the remaining samples, 17 were of known age having been followed since hatching. An additional eight sampled birds were deemed to be

between three and nine months based on size and weight. When utilised in the analyses of age and telomere length, these juveniles were all given the approximate age of 0.5 years. The non-Ponui samples could be split into two distinct groups based on geographical origin: A Southern group and a Northern group. Eleven of the known age birds were adults from Southern *A. mantelli* populations. An additional 20 adults from Northern populations were also sampled and analysed. Together, the Southern and Northern adults were used for investigating if any relationships found on Ponui Island seemed generic across areas. See Table 4.1.2 for further details.

Table 4.1.2. Samples utilised for different aspects of telomere analyses and their origin. 'RTL' refers to relative telomere length, 'Uk' to unknown, and 'Southern' and 'Northern' to how the non-Ponui samples were grouped for analyses.

				Used for analyses of RTL's relation to		
ID	**Population**	**Year of sampling**	**Age (yo)**	**Actual age**	**Other factors on Ponui I.**	**Other factors in other pop.**
MB108	Pūkaha	2020	0.3	X		
MB107	Pūkaha	2020	0.4	X		
MB109	Pūkaha	2020	0.4	X		
RAKPET2	Rakauma.*	2019	0.5**	X		
ROAANG1	Moturoa	2020	0.5**	X		
ROAJAC4	Moturoa	2020	0.5**	X		
ROAMAL1	Moturoa	2020	0.5**	X		
RUAMAL1	Moturua	2019	0.5**	X		
RUANAT2	Moturua	2019	0.5**	X		
TROMAL12	Trounson	2020	0.5**	X		
TROMAL9	Trounson	2020	0.5**	X		
Koru	Pūkaha	2020	2	X		
Serenity	Pūkaha	2020	2	X		
TROMAL7	Trounson	2020	4	X		
Bernie	Pūkaha	2020	5	X		X - Southern
Manawa	Pūkaha	2020	5	X		X - Southern
Frickleton	Remutaka	2020	7	X		X - Southern
Mr Baggins	Remutaka	2020	7	X		X - Southern
Mapuna	Pūkaha	2020	8	X		X - Southern
Manakura	Pūkaha	2020	9	X		X - Southern
Eddie	Remutaka	2020	10	X		X - Southern
Aroha	Remutaka	2020	11	X		X - Southern
Whero	Remutaka	2020	11	X		X - Southern
Whoopsy	Remutaka	2020	11	X		X - Southern

Lorenzo	Remutaka	2020	18	X		X - Southern
Tahuna	Remutaka	2020	21	X		X - Southern
Anne	Ponui I.	2017	Uk. Adult		X	
Belgarion	Ponui I.	2017	Uk. Adult		X	
Betty	Ponui I.	2017	Uk. Adult		X	
Blandy	Ponui I.	2017	Uk. Adult		X	
Bow	Ponui I.	2017	Uk. Adult		X	
Camila	Ponui I.	2017	Uk. Adult		X	
Charlie	Ponui I.	2017	Uk. Adult		X	
Clea	Ponui I.	2017	Uk. Adult		X	
Clel	Ponui I.	2017	Uk. Adult		X	
Daphnae	Ponui I.	2017	Uk. Adult		X	
Dario	Ponui I.	2017	Uk. Adult		X	
Denis	Ponui I.	2017	Uk. Adult		X	
Elisabeth	Ponui I.	2017	Uk. Adult		X	
Emily	Ponui I.	2017	Uk. Adult		X	
Gaven	Ponui I.	2017	Uk. Adult		X	
Genesis	Ponui I.	2017	Uk. Adult		X	
George	Ponui I.	2017	Uk. Adult		X	
Ivan	Ponui I.	2017	Uk. Adult		X	
Jacob	Ponui I.	2017	Uk. Adult		X	
Jaeden	Ponui I.	2017	Uk. Adult		X	
Jenno	Ponui I.	2017	Uk. Adult		X	
Jono	Ponui I.	2017	Uk. Adult		X	
Kat	Ponui I.	2017	Uk. Adult		X	
Ken	Ponui I.	2017	Uk. Adult		X	
Kerri	Ponui I.	2017	Uk. Adult		X	
Kobi	Ponui I.	2017	Adult		X	

			Uk.		
Leigh	Ponui I.	2017	Adult	X	
			Uk.		
Linda	Ponui I.	2017	Adult	X	
			Uk.		
Louise	Ponui I.	2017	Adult	X	
			Uk.		
Margm	Ponui I.	2017	Adult	X	
			Uk.		
Mark	Ponui I.	2017	Adult	X	
			Uk.		
Mauro	Ponui I.	2017	Adult	X	
			Uk.		
Max	Ponui I.	2017	Adult	X	
			Uk.		
Minnie	Ponui I.	2017	Adult	X	
			Uk.		
Octavia	Ponui I.	2017	Adult	X	
			Uk.		
Olivia	Ponui I.	2017	Adult	X	
			Uk.		
Paul	Ponui I.	2017	Adult	X	
			Uk.		
Ponui	Ponui I.	2017	Adult	X	
			Uk.		
Salome	Ponui I.	2017	Adult	X	
			Uk.		
Scott	Ponui I.	2017	Adult	X	
			Uk.		
Tako	Ponui I.	2017	Adult	X	
			Uk.		
Tashu	Ponui I.	2017	Adult	X	
			Uk.		
Tomasina	Ponui I.	2017	Adult	X	
			Uk.		
Vaughan	Ponui I.	2017	Adult	X	
			Uk.		
PURANG1	Purerua	2019	Adult		X - Northern
			Uk.		
PURLAN19	Purerua	2019	Adult		X - Northern
			Uk.		
PURLAN20	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAL1	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAL2	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAL3	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAL4	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAL5	Purerua	2019	Adult		X - Northern
			Uk.		
PURMAT1	Purerua	2019	Adult		X - Northern

RUAMAY10	Moturua	2019	Uk. Adult	X - Northern
RUAMAY12	Moturua	2019	Uk. Adult	X - Northern
RUAMAY13	Moturua	2019	Uk. Adult	X - Northern
RUAMAY14	Moturua	2019	Uk. Adult	X - Northern
RUAMAY16	Moturua	2019	Uk. Adult	X - Northern
RUAMAY17	Moturua	2019	Uk. Adult	X - Northern
RUAMAY18	Moturua	2019	Uk. Adult	X - Northern
RUAMAY19	Moturua	2019	Uk. Adult	X - Northern
RUAMAY20	Moturua	2019	Uk. Adult	X - Northern
RUAMAY21	Moturua	2019	Uk. Adult	X - Northern
RUANAT4	Moturua	2019	Uk. Adult	X - Northern

*Rakaumangamanga or Cape Brett

**Based on weight and size, these juveniles were estimated to be 3 to 9 months old and hence and average of 0.5 years was used herein.

4.1.2.2 Factors

All measuring, handling, and sampling occurred in accordance with the Kiwi Best Practice Manual (Robertson & Colbourne 2017) and all samplers, measures, and handlers were accredited kiwi handlers or supervised by accredited trainers. All handling occurred with permission from the Massey University Animal Ethics committee (permit numbers: 18/82, 18/83 and 16/92) and the Department of Conservation (permit numbers: 70875-RES, 70826-CAP, and 50249-FAU). When blood samples were collected, additional data on weight, bill length, tarsus length, and tarsus width were also collected for all populations. From the blood, haemoglobin concentration, glucose level, total protein, packed cell volume, and white blood cell ratio were determined (for further detail see Table 4.1.3).

At any given year since 2004, about 30 to 50 birds in the Ponui Island population have been fitted with radio transmitters so that the same individual could be followed over time. These birds had the listed morphometric factors measured every year together with the annual transmitter replacement. Additional data from these birds utilised herein were downloaded activity data from the transmitters (see further Chapter 2.2), and proportional time spent in different habitat types based on Dixon (2015). Due to lack of among bird variation, only two of the habitat types analysed by Dixon (2015) were utilised here: forest and scrub. The year 2013 was extraordinarily dry, hence extra consideration was given to the loss of weight in that year and the recovery the following year as an indicator of stress exposure and tolerance. Together, these data resulted in 17 factors to which the relative telomere length of adult Ponui Island birds in 2017 could be compared (Table 4.1.2). For the birds of known and unknown age from other populations, morphometric data were only available for the year of sampling. Hence no measures related to growth or changes over time were analysed for these birds.

Table 4.1.3. The morphometric, molecular, and haematologic factors compared to relative telomere length of adult birds from Ponui Island.

Factor	Definition
Bill Length	Bill length measured in 2017. All length measurements taking with callipers.
Bill/TL Ratio	Bill length measured in 2017 divided by tarsus length measured in 2017
Weight/TL Ratio	Weight measured in 2017 divided by tarsus length measured in 2017
Weight 2017	Weight measured in 2017. All weights were taken using Pesola hanging scale in accordance with the Kiwi Best Practice Manual. Weight are in grams.
Body Condition	Calculated based on weight in 2017 and tarsus width in 2017 following Taborsky and Taborsky (1999).

Bill Growth	Average annual rate of bill growth
Tarsus Growth	Average annual rate of tarsus width growth
Weight Change Rate	Average annual rate of weight change
Weight Loss 2012-2013	Weight in the drought year of 2013 minus 2012
Recovery 2014/2012	Weight 2014 minus 2013 divided by weight 2013 minus 2012 times -100
Packed Cell Volume	Within eight hours of sample collection, haematocrits were centrifuged in a micro haematocrit centrifuge at 10,000 rotations per minute (rpm) for five minutes and packed cell volume (PCV) was measured as a percentage of the entire sample volume
Total Plasma Protein	The plasma from the centrifuged samples was used to measure total plasma proteins using a refractometer (Atago® Hand-held Refractometer)
Haemoglobin Conc.	Haemoglobin was measured on site using EasyTouch® GHB.
White Blood Cell Ratio	Two blood smears were generated just after sampling for each bird. Smears were airdried and then fixed in 70% ethanol in the field. In the laboratory they were stained with Giemsa stain and heterophil to lymphocyte ratio (H:L) was counted using a compound microscope.
Activity	Average number of hours spent active per night during the 30 consecutive days with the highest average
Time Spent in Forest	Proportion of time tracked found in forest (higher quality habitat)
Time Spent in Scrub	Proportion of time tracked found in scrub (lower quality habitat)

4.1.2.3 DNA purification

DNA was extracted from 10 µl thawed whole *A. mantelli* blood using a High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The manufacturer's instructions were followed with the exception that the DNA was eluted twice using 50µl of elution buffer for each centrifugation round. The DNA extraction success and quality were validated using agarose gel electrophoresis (1% (w/v) agarose in 1x TAE buffer) and

the concentration of DNA was measured using the Qubit 2.0 fluorometer and the dsDNA High Sensitivity assay (Life Technologies, CA, USA).

4.1.2.4 qPCR

For telomere amplification, I used the protocol developed by Cawthon (2002) and adapted for birds by Criscuolo *et al.* (2009). The generic (and consistently used) bird telomere primers tel1b (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT) and tel2b (GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT; Criscuolo *et al.*, 2009) were used. The single-copy control or housekeeping gene used was GAPDH (Glyceraldehyde 3-phosphate dehydrogenase). The primer set used for the control gene was a combination of a previously published reverse primer developed in Zebra finch (GAPDHR: CCATCAGCAGCAGCCTTCA; Criscuolo *et al.* 2009), and a specifically developed forward primer (GAPDHF_kiwi_n2: CTTGCACAGCTGACACAATTTG) based on the published *A. mantelli* GAPDH sequence (NCIB accession number XM_013958036; Le Duc *et al.* 2015). Primers were manufactured by Integrated DNA Technologies (Ames, IA, USA). Primer function and efficiencies were verified using standard PCR.

For telomere analysis, 1 ng of template DNA per reaction was amplified under the following conditions: 1x Hot FIREPol EvaGreen qPCR Supermix (Solis, Biodyne, Tartu, Estonia), 250 nM of each primer, made to a total volume of 20 µl with nuclease-free water. For GAPDH analysis, 4 ng of template DNA per reaction was amplified under the following conditions: 1x Hot FIREPol EvaGreen qPCR Supermix (Solis, Biodyne, Tartu, Estonia), 200 nM of each primer, made to a total volume of 20 µl with nuclease-free water. Amplification for both primer sets occurred in a LightCycler 480 II (Roche, Basel, Switzerland) with the following program: Pre-incubation at 95°C for 12

minutes, then 40 rounds of denaturation at 95°C for 15s, annealing at 60°C for 30s, and elongation at 72°C for 30s, followed by a melting curve step. Samples were run as triplicates. Each plate included a four set 2x standard curve and a water control. The DNA amounts in the dilution series were 4, 2, 1, and 0.5 ng per reaction on the telomere plates and 8, 4, 2, and 1 ng on the GAPDH plates, using DNA from one of the samples. To align with previous studies (for example Kim & Velando 2015, Watson *et al.* 2015, Eastwood *et al.* 2018, Wood & Young 2019), priority was given to keeping plate position consistent per individual, thus telomere and GAPDH primers were run on separate plates. For details on the development of the qPCR protocol see appendix A.3.

4.1.2.5 RTL calculations and analyses

To calculate relative telomere length, I used the formula developed by Pfaffl (2001), and modified by (Eastwood *et al.* 2018). This formula combines the threshold cycle (C_t), the individual qPCR efficiency (E), and a golden sample (or gold standard) for both the telomere and the GAPDH run of the individual sample (Equation 1). Values for C_t and E was obtained using LinRegPCR (Academic Medical Center, Amsterdam, NL). Averages among replicates were used, but a replicate was only included if meeting a threshold E, and the difference in Ct being sufficiently small, following Eastwood *et al.* (2018). The value for the golden sample was extracted by reading the 'ideal' C_t value for the DNA concentration used (1ng and 4ng per reaction, respectively) as indicated by the slope of the plate-specific efficiency curve based on the plate-specific dilution series. This slope was generated on software from the LightCycler. Correlations between RTL and all factors described in Table 4.1.3 plus known age was done through individual linear models in R (R core team version 3.6.2).

$$RTL = \frac{E_{Tel}^{(Gold\ standard_{Tel} - Ct_{Tel})}}{E_{Control}^{(Gold\ standard_{Control} - Ct_{Control})}}$$

Equation 4.1.1. Calculation of relative telomere length (RTL) based on the threshold cycle (C_t), the individual qPCR efficiency (E) and a golden sample value (Gold standard) extracted by reading the 'ideal' C_t value for the DNA concentration used as indicated by the slope of the plate-specific efficiency curve, and comparing this for telomeres (Tel) and GAPDH (control) for each sample (Eastwood *et al.* 2018).

4.1.3 Results

Overall, the relative telomere length (RTL) for 91 *Apteryx mantelli* was determined. Variation in length between individuals was large, ranging from 0.35 to 1.66 with a mean relative telomere length of 0.96. Based on the 26 birds of known age, no correlation could be detected between chronological age and RTL ($F = 1.22$, $df = 24$, $R^2 = 0.05$, $p = 0.28$; Figure 4.1.1). Looking at the adult birds from Ponui Island, none of the parameters relating to size or growth were found to correlate with RTL (Table 4.1.4). Neither was there any detectable relationship between RTL and drought stress, recovery, habitat utilisation, or activity (Table 4.1.4).

Table 4.1.4. Results from linear models of the relationship between relative telomere length in adult *Apteryx mantelli* from Ponui Island and 17 factors of morphometrics, stress response, activity, and habitat. For definitions see Table 4.1.3. Bold p-values highlights significance ($p<0.05$) and italics indicate $0.05<p>0.1$. For significant correlations, the intercept and the slope of the relationship are included.

Factor	**F**	**P**	**R^2**	**adj R^2**	***n***	**Intercept**	**Slope**
Bill Length	0.72	0.40	0.02	-0.01	44		
Bill/TL Ratio	0.01	0.43	0.01	-0.01	44		
Weight/TL Ratio	0.86	0.36	0.02	0.00	42		
Weight 2017	0.14	0.71	0.00	-0.02	42		
Body Condition	0.23	0.63	0.01	-0.02	42		
Bill Growth	0.57	0.45	0.02	-0.01	38		
Tarsus Growth	0.33	0.57	0.01	-0.02	38		
Weight Change Rate	1.49	0.23	0.04	0.01	39		
Weight Loss 2012-2013	0.14	0.71	0.01	-0.03	29		
Recovery 2014/2012	0.34	0.56	0.01	-0.02	30		
Packed Cell Volume	5.13	**0.03**	0.12	0.10	39	1.79	-0.02
Total Plasma Protein	0.02	0.88	0.00	-0.03	38		
Haemoglobin Conc.	5.58	**0.02**	0.14	0.12	35	1.67	-0.04
White Blood Cell Ratio	3.12	*0.09*	0.08	0.05	38		
Activity	0.35	0.56	0.01	-0.02	35		
Time Spent in Forest	0.02	0.90	0.00	-0.04	29		
Time Spent in Scrub	0.19	0.67	0.01	-0.03	29		

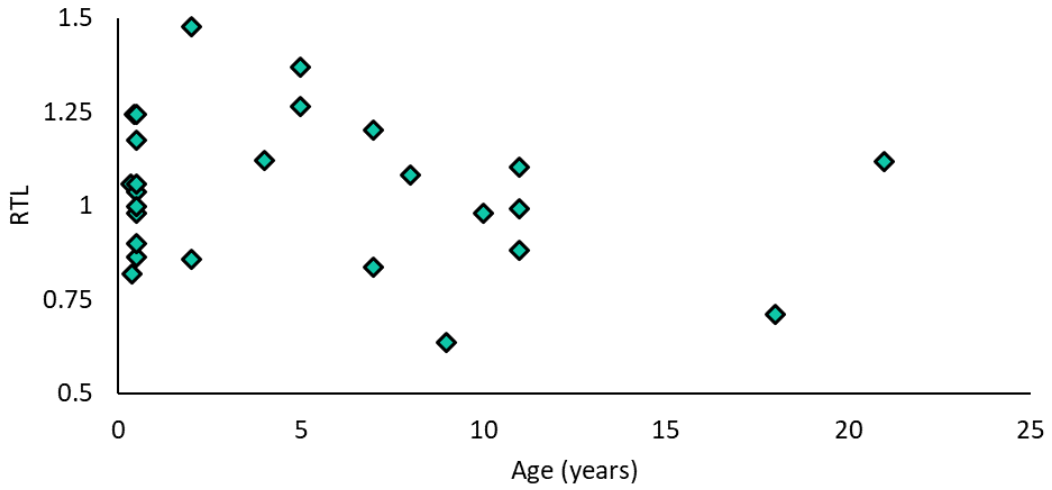


Figure 4.1.1. Scatterplot illustrating the lack of correlation between known age and relative telomere length (RTL) in *Apteryx mantelli*.

For haematological parameters, packed cell volume (PCV) as well as haemoglobin concentration (HB) were both significantly negatively correlated with RTL in adult *A. mantelli* from Ponui Island (Table 4.1.4; Figure 4.1.2a and b). The remaining haematological parameters showed no correlation (Table 4.1.4). Correlations between RTL and/or known age to PCV and HB was hence the focus of the third step of the study. No correlation was found between HB and RTL for Northern or Southern adults ($F = 1.61$, $df = 18$, $R^2 = 0.08$, $p = 0.22$, $n = 11$, and $F = 2.59$, $df = 9$, $R^2 = 0.22$, $p = 0.14$, $n = 11$, respectively; Figure 4.1.2c). PCV showed no correlation with relative telomere length for adult birds from the Northern populations ($F = 0.05$, $df = 18$, $R^2 = 0.003$, $p = 0.83$; Figure 4.1.2d), while for the Southern populations, a positive correlation was found ($F = 6.70$, $df = 9$, $R^2 = 0.43$, $p = 0.03$; Figure 4.1.2d). When looking at HB and PCV compared to known age, a strong, negative correlation was found with HB ($F = 11.43$, $df = 23$, $R^2 = 0.33$, $p = 0.002$; Figure 4.1.2e) but no correlation was detected with PCV ($F = 1.56$, $df = 23$, $R^2 = 0.06$, $p = 0.22$; Figure 4.1.2f).

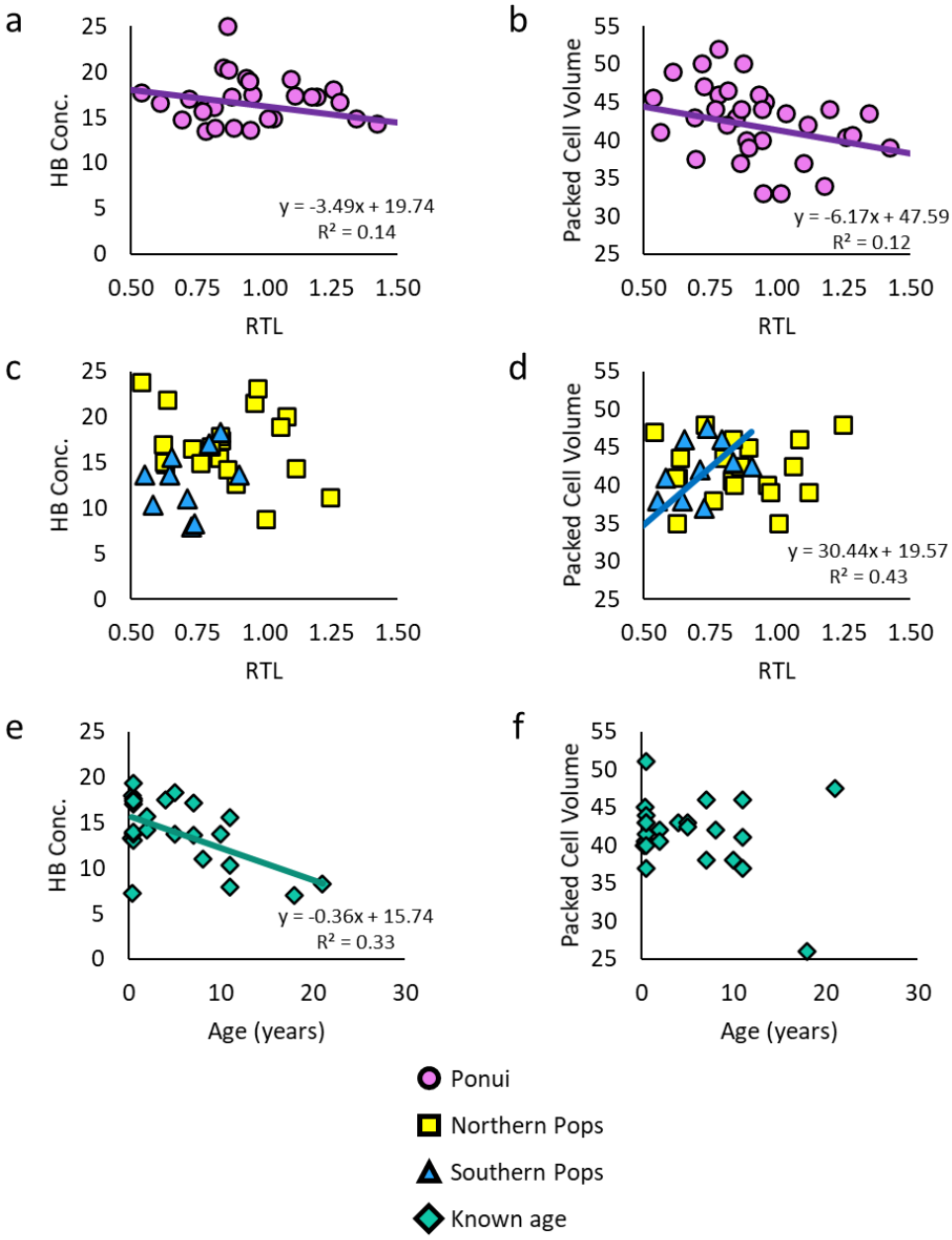


Figure 4.1.2. Scatterplots of relationship between relative telomere length (RTL; a-d), age (e-f), haemoglobin concentration (or HB; a, c, e), and packed cell volume (or PVC; b, d, f). Trendlines indicate significant correlations with associated line equations and R^2 values indicated. See Table 4.1.2 for details on sample origins and splits into sample sets.

4.1.4 Discussion and Conclusion

To the author's knowledge, this is the first ever exploration of telomere dynamics in *Apteryx*. Even though the telomeres of many bird species have been shown to shorten over time, I did not find any correlation between relative telomere length and age in *A. mantelli*. While this result was unexpected, there are a few other studies of long-lived birds for which cross-sectional studies have also failed to find any relationship between telomeres and age, for instance, the kakapo (*Strigops habroptilus*; Horn *et al.* 2011) and the wandering albatross (*Diomedea exulans*; Hall *et al.* 2004). While such findings have often been dismissed as measurements errors, alternative explanations to lacking correlation between age and telomere length exits such as (1) one study found evidence that long-lived but not short-lived birds retained telomerase activity in their somatic cells (Haussmann *et al.* 2004), (2) a more recent study has suggested that it might be due to the presence of so-called ultra-long telomeres on some chromosomes and that the vast differences in length and attrition rate between different chromosomes masks any overall trend (Atema *et al.* 2019), (3) along similar lines, there is a chance that the amount of interstitial telomeric sequences (i.e., areas within chromosomes with the same sequence repeat as telomeres that will also be picked up by qPCR; Lai *et al.* 2018) differs enough between individuals to mask overall trends (Foote *et al.* 2013). The latter might be of extra concern in ratites such as *Apteryx* since this group has been found to have extensive stretches of interstitial telomeric sequence (Nanda *et al.* 2002).

In addition to youth, longer telomeres have also more generally been associated with good health, longevity and lower mortality both when compared across and within species (Haussmann *et al.* 2003, Haussmann *et al.* 2005, Pauliny *et al.* 2006, Haussmann *et al.* 2007, Bize *et al.* 2009, Salomons *et al.* 2009, Barrett *et al.* 2013, Sudyka *et al.* 2016, Tricola *et al.* 2018, Wilbourn *et al.* 2018). Another widely used

indicator of health and good body condition in birds is haemoglobin concentration (Kaliński *et al.* 2015, Minias 2015, Glądalski *et al.* 2016). Hence it was puzzling to find a negative correlation between these two parameters in adult *A. mantelli* from Ponui Island. This could hint at an interesting trade-off between these two measures of fitness and aligns with Bauer *et al.* (2018) who suggested that telomere length is a 'better indicator of remaining life span rather than current state'. However, an equivalent negative correlation was not found among adults in the other analysed populations. On the contrary, there was a clear negative correlation between HB and age in the known-age dataset. Most other studies relating HB to age in birds have focused on changes from hatchling to fledgeling in passerines or to one year of age in long-lived precocial birds (Samour *et al.* 2011, Minias 2015, Obese *et al.* 2018). The only study that, to the author's knowledge, explored HB over the entire lifespan of a bird species found that in rock pigeons (*Columba livia*), HB decreased with age but only after a certain number of years (Prinzinger & Misovic 2010). More investigations of the relationship between these two markers of good health are warranted, in particular considering the possibility that HB is an indicator of current health while telomere length could possibly act as a more long-term indicator.

The inconsistency of the correlations with RTL and other factors between the sample sets analysed herein suggests that variation between populations might be large enough to make correlations with individual factors difficult when data from multiple populations are combined. Such population differences have been found in other studies of long-lived birds as well (Young *et al.* 2013). Unfortunately, Ponui Island was the only population from which our samples size was large enough for population-level comparisons. Hence, it is still possible that telomeres shorten in a predictable way in *A. mantelli*, but from this study, I was unable to determine which factors would be linked

to such change. Regardless of the cause and the relationship with age, population-wide short telomeres could be seen as an indicator of issues that might warrant active management, due to the well supported association between shorter telomeres and reduced longevity or increased mortality. However, to effectively plan conservation management of such populations deemed at risk, identifying exactly which stressors and/or habitat components cause the accelerated attrition would be strongly beneficial since this would hopefully allow conservation practitioners to limit translocations to areas where such stressors are at effect or to reduce the issue prior to initiation of translocation efforts.

The most promising ways to further our understanding of telomere dynamics in *A. mantelli* would be to study larger sample sets within populations or to study birds under otherwise equivalent conditions. Ideally, such birds should be of known and varied age. This could be done in multiple datasets, the effects of individual environmental factors could be investigated to allow for accounting for these statistically in future population comparisons (Dugdale & Richardson 2018). One study system with high potential is the rather large captive population of *A. mantelli* in New Zealand. Another key study would be a longitudinal study quantifying the change of telomere length over time, preferably in individuals from the same population and with as much background data as possible. In particular, since some authors argue that the rate of change rather than telomere length is the more informative marker (Boonekamp *et al.* 2014). The Ponui Island population utilised herein is likely one of the best suited study systems for such research. A third highly informative study would be measuring telomerase enzyme activity in somatic *Apteryx* cells. Considering the extraordinarily lifespan of *Apteryx*, such research would have wide ecological as well as evolutionary significance.

Chapter 4.2

The role of DNA integrity in opportunistic longitudinal telomere studies.

This Chapter has been submitted to Ecology and Evolution. All supervisors provided feedback on one or more drafts of this chapter. The expertise of Kristene Gedye, the help from Brianna Nelson and Trish McLenachan, and the hard work of previous members of the Ponui Island team were crucial for this chapter. See further Appendix 6.

Abstract

Determining both current and future prospects for individuals and populations are at the heart or studies of conservation biology, and therefore there is growing interest in molecular markers indicating so-called biological age. One such marker is telomere length. Most studies exploring the relationship between age, mortality, longevity, and telomere length have been cross-sectional, but such studies face problems with large individual variation and the selective loss paradox. Thus, interest is growing rapidly for turning to longitudinal studies. In this study, the rate of telomere loss was analysed for the extraordinarily long-lived North Island brown kiwi, *Apteryx mantelli*. Relative telomere length (RTL) was quantified using qPCR from blood from four separate sampling occasions of the same individuals across 14 years. Uniquely, the analysis of RTL was combined with microfluidic capillary electrophoresis fragment analyser analysis of genomic quality for a subset of the samples to acquire numerical values of DNA integrity. The analysis of RTL suggested about 5 % annual increase in A. mantelli telomere length. However, RTL was found to be highly correlated with DNA integrity, indicating that the perceived elongation of telomeres was likely a result of DNA quality differences between sampling time-points. Notably, the observed, positive correlation remained significant even when analysing only samples classified as being of high DNA quality. Previous work has highlighted the potential impact of sample storage differences on RTL. However, to our knowledge, this is the first study to suggest that even small differences in DNA integrity between samples can impact the results of telomere studies. These findings are of great importance since already available samples – especially for long-lived threatened species – constitute a tempting resource for opportunistic, ‘after-the-fact’ longitudinal telomere studies. For such studies, we suggest that analysis of DNA quality with higher precision than traditional gel

electrophoresis – for instance using microfluidic capillary electrophoresis – is needed to generate reliable results of telomere dynamics and that further studies are needed into how to account for this source of error statistically and/or methodologically.

4.2.1 Introduction

Telomeres are repetitive nucleotide sequences at the end of all linear, eukaryotic chromosomes (Allsopp *et al.* 1995). Due to the so-called 'end-copy-problem', the telomeric sequence shortens during each cycle of DNA replication giving telomere length the potential to act as a molecular clock (Olovnikov 1996, Shay & Wright 2000, Bize *et al.* 2009, Smith *et al.* 2016). Consequently, many studies have explored the relationship between telomere attrition and ageing (see for instance Allsopp *et al.* 1995, Olovnikov 1996, López-Otín *et al.* 2013), and found widespread support for telomere length being a promising marker for estimating induvial age and population age distribution (Juola *et al.* 2006, Pauliny *et al.* 2006, Bize *et al.* 2009, Salomons *et al.* 2009, Heidinger *et al.* 2012, Angelier *et al.* 2013, Barrett *et al.* 2013, Mizutani *et al.* 2013, Aydinonat *et al.* 2014, Boonekamp *et al.* 2014, Bebbington *et al.* 2016).

However, others argue that telomere length or rate of attrition is likely to be a better indicator of biological age rather than chronological age. Biological age (or functional age) refers to a measurable stage or status that can be compared among conspecifics and is based on one or several traits linked to phenotype, function, and/or estimated remaining life expectancy (Jackson *et al.* 2003, Jylhävä *et al.* 2017). Telomere length has high potential as a meaningful indicator of biological age, firstly because shorter telomere length or faster telomere attrition has been found to correlate with mortality risk (Salomons *et al.* 2009, Geiger *et al.* 2011, Angelier *et al.* 2013, Watson *et al.* 2015), life expectancy (Pauliny *et al.* 2006, Heidinger *et al.* 2012, Boonekamp *et al.* 2014), or

both (Bize *et al.* 2009, Barrett *et al.* 2013), often more strongly than chronological age. Secondly, a growing number of studies have connected telomere dynamics to a plethora of external factors that could affect the biological age of individuals, for instance, environmental or physical stress (Monaghan 2014, Ibáñez-Álamo *et al.* 2018, Seeker *et al.* 2018, Spurgin *et al.* 2018).

The relationship between biological, as well as chronological, age and telomere length has mostly been explored through cross-sectional studies analysing known-age individuals sampled at one point in time (see for example Haussmann *et al.* 2003, Horn *et al.* 2011, Tricola *et al.* 2018, Wilbourn *et al.* 2018). Two problems potentially compromising the accuracy of such studies are (1) large individual variation and/or low correlation between chronological age and telomere length for adult individuals (Haussmann *et al.* 2003, Horn *et al.* 2011, Dantzer & Fletcher 2015, Sudyka *et al.* 2016), and (2) the so-called selective loss paradox. The selective loss paradox (or selective disappearance problem) refers to the finding that shorter telomeres are associated with a higher risk of mortality (Wilbourn *et al.* 2018). Therefore, old individuals available for study will represent a biased sample towards 'long telomere individuals' resulting in an absent or artificially flat correlation between age and telomere length (Haussmann & Mauck 2008, Dantzer & Fletcher 2015, Salmon *et al.* 2016). These two problems have led to significant interest in moving to longitudinal studies of telomere dynamics, i.e., studies that utilize multiple samples of the same individuals over time.

The prospect of a readily quantifiable marker that is as or more informative than chronological age has recently brought attention to telomere research in a broad number of biological research fields. For example, within ecology and conservation biology, researchers and practitioners are hoping to utilize this marker as an indicator of

individual fitness or population viability. Potentially, telomere length could for instance replace or complement chronological age as a factor for population modelling (McCleery *et al.* 2008, Demongeot 2009, Wolfe *et al.* 2010, Martin 2015), in particular for long-lived species where accuracy of modelling is currently limited by difficultly to determine chronological age beyond broad categories such as 'juvenile', 'subadult', and 'adult' (Holmes & Martin 2009, Wolfe *et al.* 2010, Töpfer 2018, Edwards *et al.* 2019). Just like other ecological studies of long-term series and/or change over time, such studies are likely to rely partly or fully on an after-the-fact approach and/or opportunistic access to samples. By default, this means researchers have little or no impact over collection method, storage conditions, and handling of tissue (including blood) and extracted DNA for such studies. Furthermore, these features risk being different between points of sampling (Haskins *et al.* 2021), in particular if study species are long-lived, threatened, and/or difficult to sample (Hailer *et al.* 2006, Archie *et al.* 2007, Chiyo *et al.* 2011, Hoban *et al.* 2014, Dures *et al.* 2019), when best practice and/or methods available have changed over time or differ between areas (Johnson *et al.* 2004, Tison *et al.* 2015, Moussy *et al.* 2018), or when different sampling methods have been deemed appropriate for sampling of juveniles compared to adults (Østergaard *et al.* 2003, Hall *et al.* 2004, Plot *et al.* 2012).

These methodological differences could render a challenging scenario because collection method, storage conditions, and handling are all factors affecting the rate and extent of DNA decay (Seutin *et al.* 1991, Freed & Cann 2006, Shabihkhani *et al.* 2014, Rahikainen *et al.* 2016). High DNA quality is crucial for accurate assays of absolute telomere length (southern hybridization-based assays) as well as relative telomere length (RTL) quantitative real-time polymerase chain reaction (qPCR) assays (Fleige *et al.* 2006, Fernandez-Jimenez *et al.* 2011). In line with this, Eastwood *et al.* (2018) as

well as Reichert *et al.* (2017) found that sample handling (specifically storage media) had a significant effect on RTL in samples from wandering albatross (*Diomedea exulans*; Reichert *et al.* 2017), zebra finch (*Taeniopygia guttata*; Reichert *et al.* 2017), and purple-crowned fairy-wren (*Malurus coronatus*; Eastwood *et al.* 2018). The authors of both studies suggested that the results of their studies were due to DNA integrity differences. However, to the authors' knowledge, no study has so far tested this theory or attempted to determine the scale at which reduced or differing DNA integrity becomes a possible issue for achieving comparable RTL measurements.

Herein we explore these potential issues of after-the-fact longitudinal telomere studies using the extraordinarily long-lived species brown kiwi (Apteryx mantelli) as an exemplar. Blood samples collected in a wild A. mantelli population on four discrete occasions across 14 years were analyzed. These samples had been exposed to different and somewhat unknown handling, partly due to being collected for unrelated studies, and partly due to changes to best practice and available field equipment. We compared relative telomere length (RTL), individual efficiency, and DNA integrity to explore the annual telomere attrition rate for adult A. mantelli, and more widely investigate the possibility of telomere length as an informative marker of individual and/or population-level viability in this species. To our knowledge, this is the first study ever to combine a longitudinal telomere length study using qPCR with high resolution quantification of DNA integrity. Specifically, we quantified Genomic Quality Score (GQS) using microfluidic capillary electrophoresis. While this study is crucial for the understanding of A. mantelli telomere dynamics, quantifying the role of DNA integrity in longitudinal telomere studies will have far-reaching implications for this research area which is rapidly branching into ecology, evolutionary biology, behavioural biology, and conservation research (Ringsby *et al.* 2015, Monaghan *et al.* 2018, Tricola *et al.* 2018).

4.2.2 Materials and Methods

4.2.2.1 Blood samples

This study opportunistically utilized brown kiwi (*A. mantelli*) blood samples that were available thanks to four separate studies. Samples came from Ponui Island in the Hauraki Gulf along the eastern coast of New Zealand's North Island [36 55' S, 175 11' E], and sample collection took place in 2004, 2006-2008, 2010, and 2017-2018, respectively. The sampling in 2004 was done in association with the establishment of a long-term study using birds fitted with radio transmitters. These transmitters allowed for resampling of the same individuals in subsequent years.

All blood samples were frozen within hours of collection and transferred to a -80^{o}C freezer within one week. Samples from 2004 were frozen in no media. Samples from 2006-2008 were stored in a combination of Queen lysis buffer (Seutin buffer) and 95% alcohol. In 2010, centrifuged red blood cells were stored in heparinized tubes, and in 2017-2018 whole blood was stored in heparinized tubes. All samples had subsequently been thawed to take sub-samples for downstream analysis but the time the samples spent defrosted before being returned to long-term storage at -80^{o}C was unknown. We assumed that equivalent handling and storage had applied to samples from the same study, thus sampling year (2004, 2006-2008, 2010, or 2017-2018) was used as a factor indicative of handling scheme as well as age. In total, we analyzed the relative telomere length of 67 samples from 23 adult kiwi of unknown age. Each bird was represented by between two and four samples. Previous analyses had led to that limited sample amounts were available for this study, which restricted sample size for the study of DNA integrity to 20 birds evenly spread across years.

4.2.2.2 DNA purification

DNA was extracted in 2019 from 10 µl thawed whole *A. mantelli* blood (2004, 2006-2008, and 2017 samples), or red blood cells (2010 samples) using a High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The manufacturer's instructions were followed with the exception that the DNA was eluted twice using 50µl of elution buffer for each centrifugation round. The DNA extraction success and quality were validated using agarose gel electrophoresis (1% (w/v) agarose in 1XTAE buffer) and the concentration of DNA was measured using the Qubit 2.0 fluorometer and the dsDNA High Sensitivity assay (Life Technologies, CA, USA).

4.2.2.3 qPCR

See section 4.1.2

4.2.2.4 Longitudinal RTL comparison

See Chapter 4.1.2 with the addition that the longitudinal samples from the same individual were run on separate plates to keep plate position constant. However, to quantify repeatability, a subset of longitudinal samples was run in replicates both within the sample plate and across plates to allow for comparison.

4.2.2.5 Genomic Quality Score

All samples utilized were deemed high quality based on agarose gel electrophoresis (1% (w/v) agarose in 1XTAE buffer). As a complement to this quantification, a subset of 20 samples was chosen at random. However, the choice was somewhat restricted due to sample volume availability, but representation was maximizing from each year of sampling and inclusion of pairs of samples from the same individuals was prioritized. The gDNA integrity of these 20 samples was analyzed using the Perkin Elmer LabChip® GX Touch HT (Perkin Elmer, Waltham, MA, USA) microfluidic capillary

electrophoresis fragment analyzer and the Hi Sensitivity LabChip at the Massey Genome Service (Massey University, Palmerston North, New Zealand). The fragment analyzer presents the results as an electropherogram, a virtual gel, and a genomic quality score (GQS) for each sample and includes a ladder consisting of known length fragments for reference (Figure 4.2.1). The GQS is calculated based on the size distribution of the genomic DNA (gDNA) fragments. The maximum score is '5' corresponds to intact gDNA and the lowest is '0' corresponding to 'highly degraded' gDNA (Figure 4.2.1). Although relying on similar principals as gel electrophoresis, gDNA quality assessment using microfluidic capillary electrophoresis provides higher reproducibility, higher accuracy with less impact of variation in the amount of DNA input (within a specified concentration span), and a meaningful way to convert DNA integrity into a number for between-sample comparison (Henry 2006, Tetala & Vijayalakshmi 2016, Ragab & El-Kimary 2020).

4.2.2.6 Statistics

Statistical analyses were conducted in R (R core team version 3.6.2). Regression analysis (R core team) of year of sampling against RTL was used to determine the average annual change in *A. mantelli* telomere length. Linear modelling (lm; R core team) was used to quantify and differences in RTL (one value per sample) or qPCR efficiency (all values for each sample including within and between plate comparisons) between the four sampling events and thus between the four types of storage and handling. For the subset of sample for which GQS was quantified, a mixed model was used to explore the effect of the continuous variable GQS, the categorical variable (4 steps) sample occasion, and the interaction between the two, on RTL. Tukey test was used for *post hoc* testing of significant effect (de Mendiburu 2020).

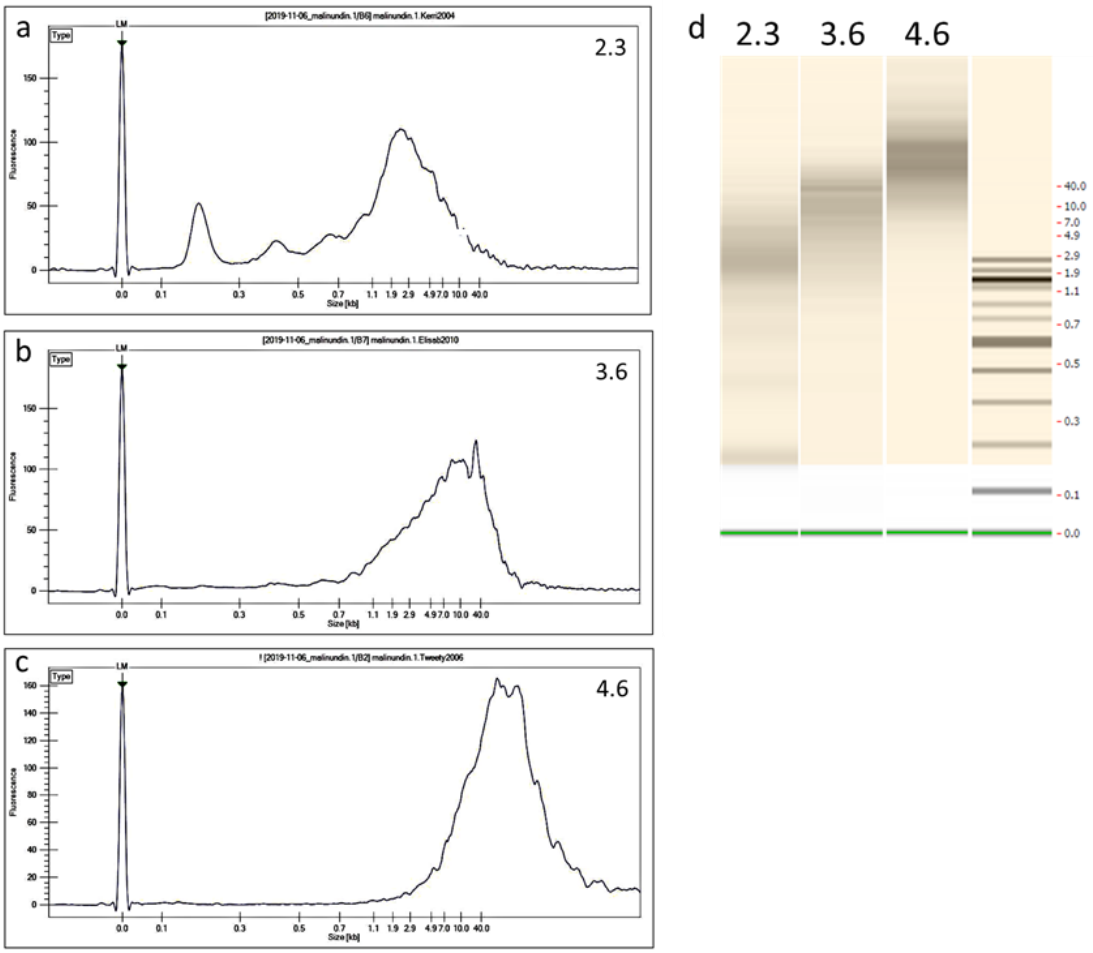


Figure 4.2.1. DNA fragment size distribution illustrated by electropherograms (a-c) and virtual gel (d). DNA fragment size distribution as visualized in two ways by the Perkin Elmer LabChip GX Touch HT microfluidic capillary electrophoresis fragment analyzer. Results are displayed as trace files (a, b & c) and comparative gel electrophoresis image (d). Results for three representative samples from our study, exemplifying partially degraded DNA (2004 sample; Genome Quality Score (GQS) 2.3; panel a and d far left), good quality DNA (2010 sample; GQS 3.6; b and d middle) and essentially intact DNA (2006 sample; GQS 4.6; c and d right; maximum GQS is 5).

4.2.3 Results

Based on the overall dataset of 67 samples from 23 birds, our results suggested that relative telomere length (RTL) increased by about 5% per year in adult *A. mantelli*. These results remained consistent both based on regression analyses for all samples combined (4.7 % annual increase, $R^2 = 0.25$; Figure 4.2.2a & b) and on averaging individual rates of change (5.5% annual increase STD 5.52).

However, a significant cohort effect for RTL was found (f = 14.55, df = 62, p-value < 0.001; Figure 4.2.3a) with samples from 2004 and 2010 (red blood cells in heparin) having shorter telomeres than those from 2006-2008 (Seutin buffer and 95% EtOH), and 2017-2018 (whole blood in heparin). However, there was no cohort effect in terms of individual qPCR efficiency (f = 0.67, df = 82, p-value = 0.57; Figure 4.2.3b), supporting consistency of the qPCR process. In addition, the subset of longitudinal samples run within as well as between plates to control for repeatability, supported that this was a true cohort effect and not caused by inter-plate differences in qPCR success (Figure 4.2.4).

Table 4.2.1. Statistical result for modelling of relative telomere length (RTL) based on year of sampling and genomic quality score (GQS). 'Year' refers to the categorical variable, 2004, 2006-2008, 2010, or 2017-2018, respectively.

	f	df	p
GQS	9.66	1	0.006
Year	7.90	3	0.001
GQS*Year	0.387	3	0.764

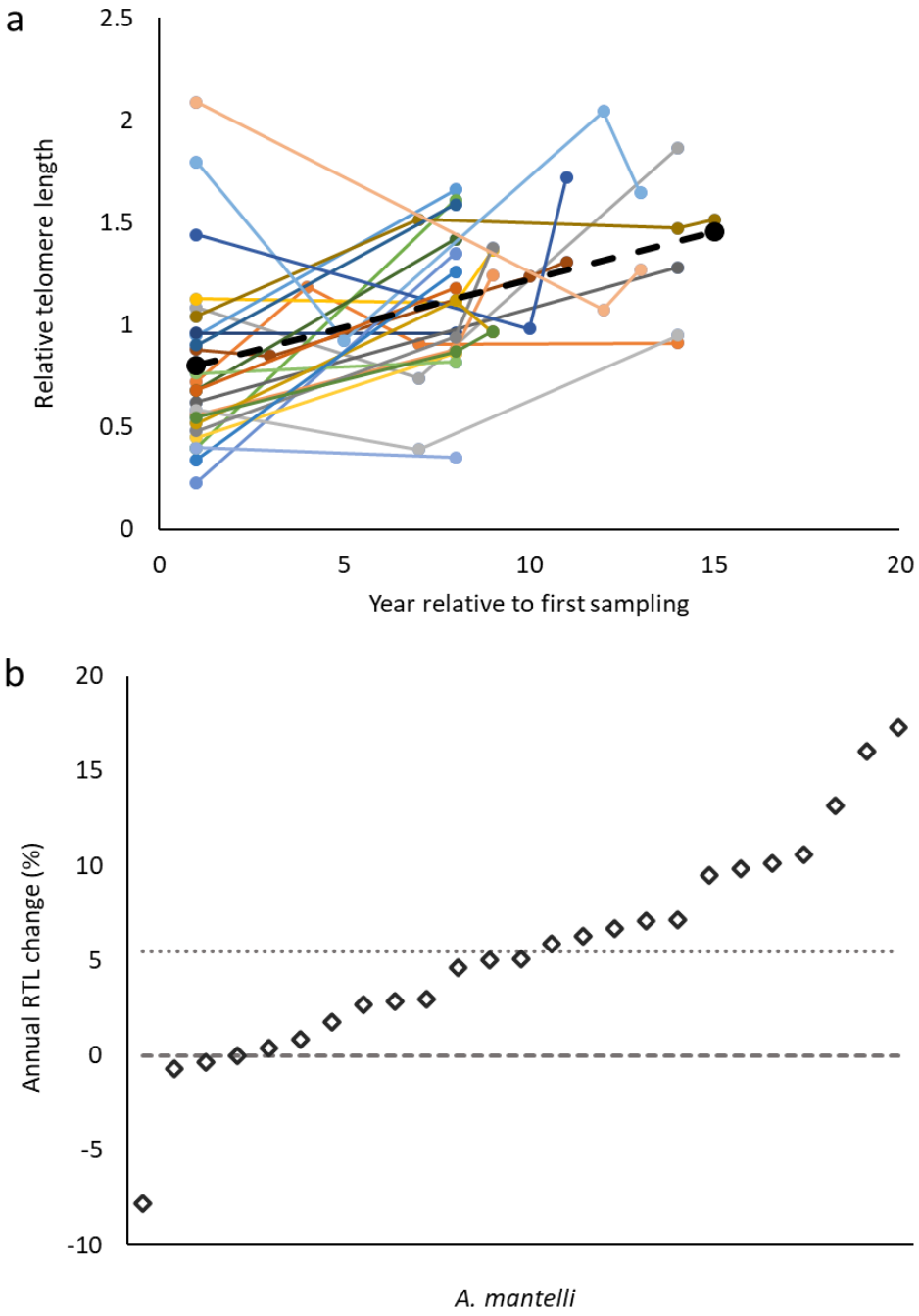


Figure 4.2.2. Longitudinal change in relative telomere length (RTL) for *Apteryx mantelli* samples. In panel (a) individual trends for all 23 analysed birds with the first year of sampling set to year one (this was year 2004, 2006, 2007, 2008, or 2010 depending on bird). Black dotted line indicates overall trend of 4.7% yearly increase. In (b) the annual change in RTL for each bird is graphed in order of rate change from fastest loss (far left) to greatest annual increase (far right). Three samples fall below the zero-change line (darker dotted line) indicating annual telomere shortening. Light grey dotted line indicates the average annual change across all sampled birds: 5.5% yearly increase.

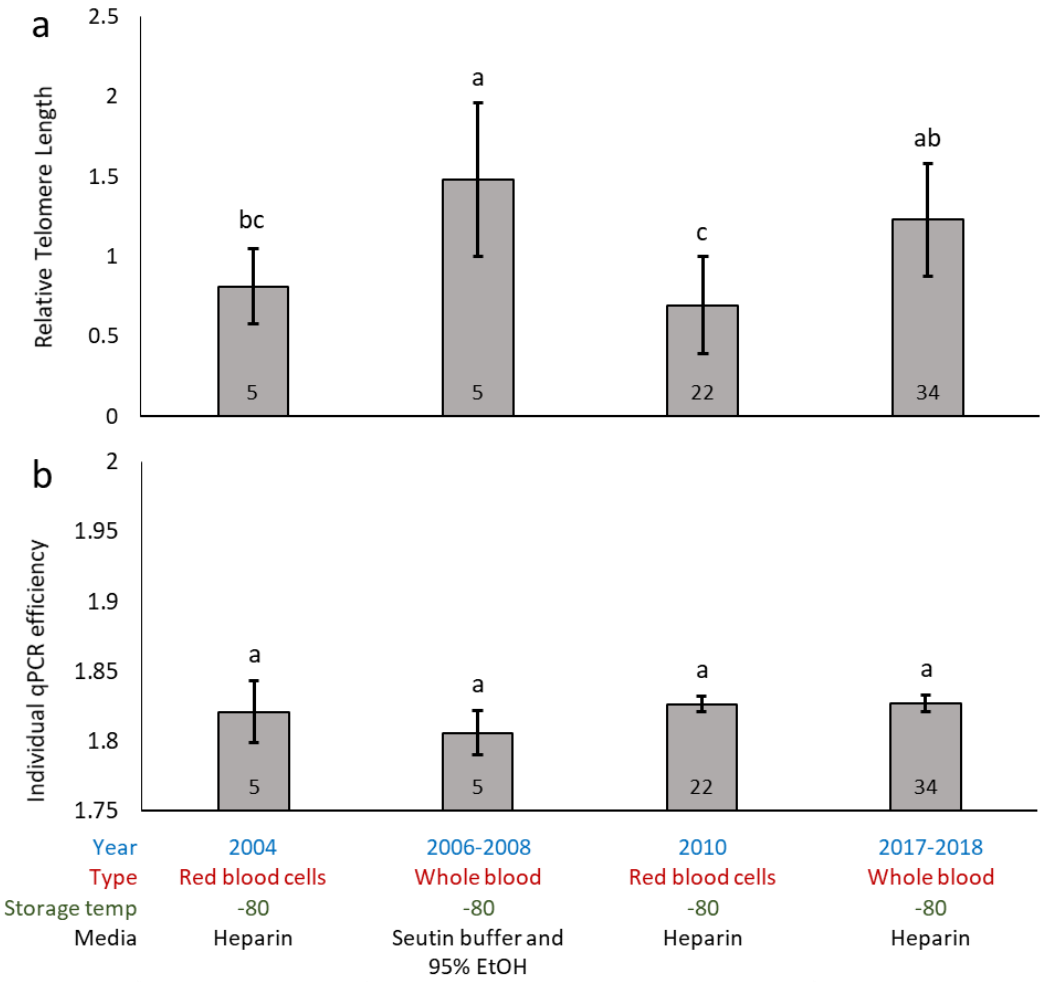


Figure 4.2.3. Comparison of relative telomere length and individual qPCR efficiency between the four sample points. A cohort effect was evident for relative telomere length (a), but not for individual qPCR efficiency for the telomere protocol (b). Bars indicate average, error bars indicate standard deviation, numbers indicate sample size and different lowercase letters above bars indicate significant difference.

Focusing on the subset of samples for which both genomic quality score (GQS) and relative telomere length (RTL) were quantified, there was a significant effect of year (and thus sample handling), as well as an independent effect of DNA integrity (Table 4.2.1). Genomic Quality Score differed among years, with 2006-2008 samples having higher and 2004 samples lower DNA integrity than other cohorts ($f = 15.36$, $df = 23$, p-value < 0.001), i.e., neither the GQS (Figure 4.2.5a) nor the RTL (Figure 4.2.3a) changed gradually with increasing sample age. Instead, the significant relationship between GQC and RLT (Table 4.2.1) was a positive correlation ($y = 0.40x – 0.50$; $R^2 = 0.41$; Figure 4.2.5b). This correlation remained similar even after excluding the three samples of lowest DNA quality ($y = 0.62x – 1.37$; $R^2 = 0.40$). The latter was done to focus only on samples with a GQS higher than 3, which is an accepted cut off for a level of quality ‘good enough for NGS library prep and sequencing’ (X. Lin pers. comm.). In addition, pairwise comparisons of longitudinal samples within the subset showed that increasing RTL was, in all cases except one, associated with higher GQS for the new samples. *Vice versa*, lower GQS for the newer sample was associated with decreasing or, in one case, unchanged RTL (Figure 4.2.6).

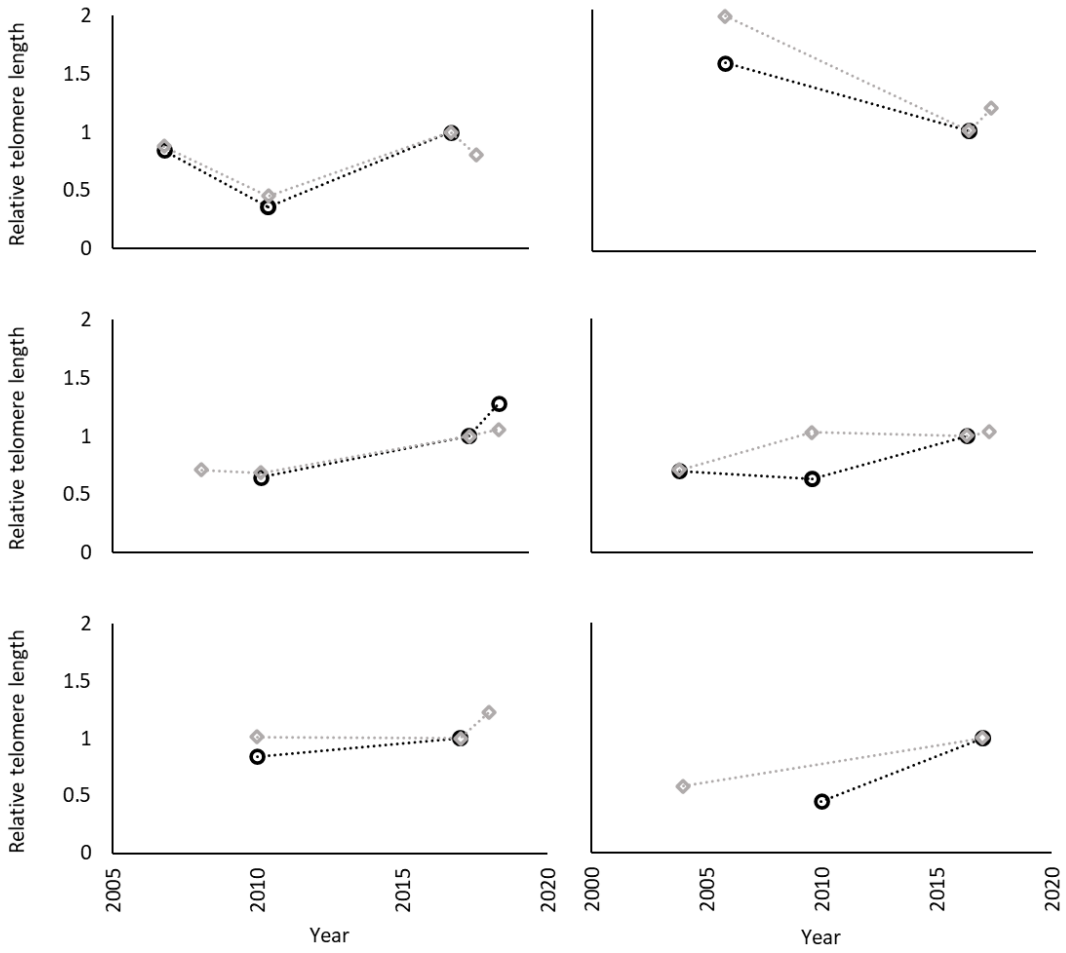


Figure 4.2.4. Comparison of estimated telomere attrition rate based on running longitudinal samples within the same or across several qPCR plates. Change in relative telomere length (RLT) between samples of different years from the same bird comparing the results of between-plate runs with consistent plate position (black line and circles) to within-plate runs (grey line and circles). RTL in 2017 has been normalised to one for clarity.

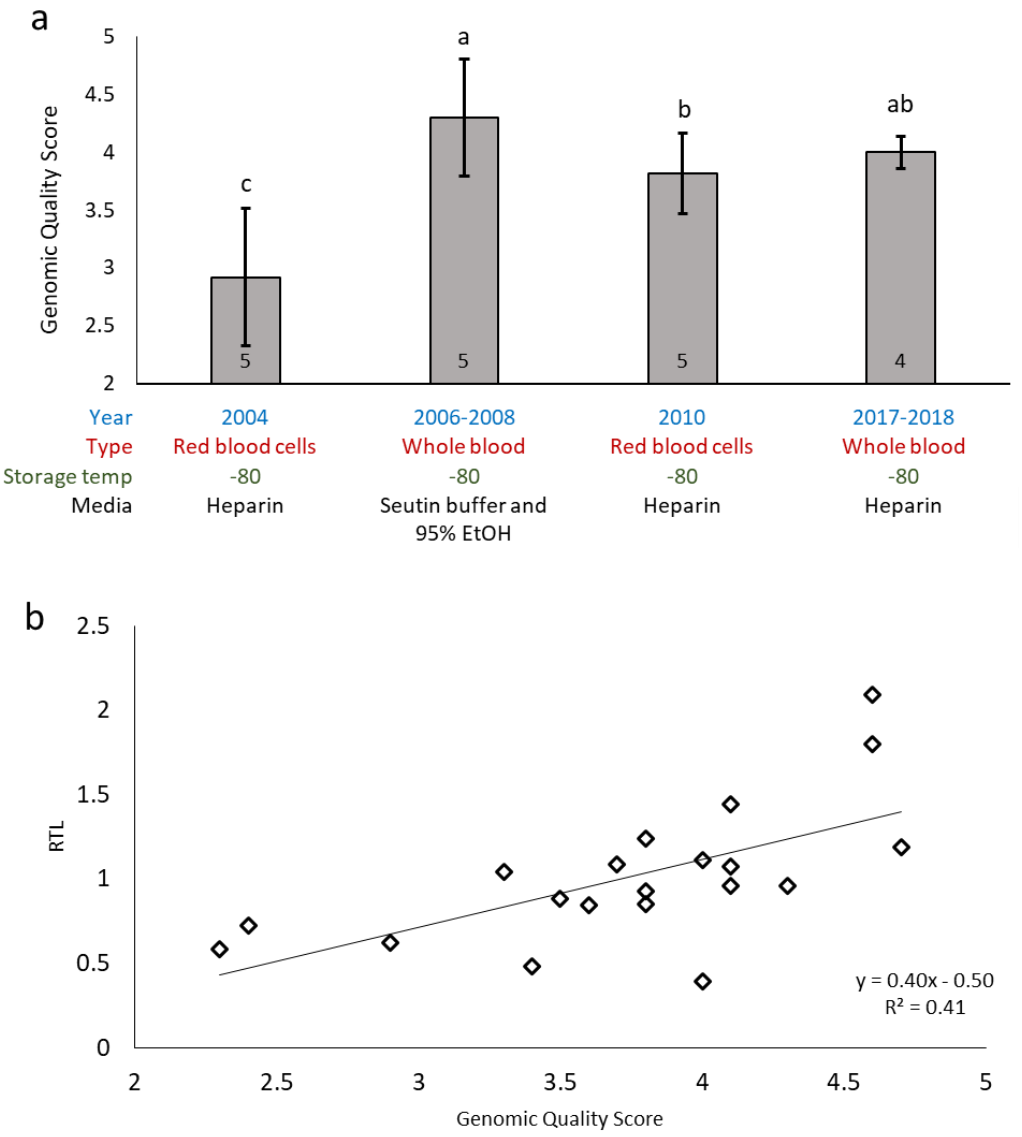


Figure 4.2.5. Comparison of relative telomere length (RTL) and year of sampling to genomic quality score (GQS). GQS was found to differ between sampling points (a), and to be positively correlated with RTL independently of this cohort effect (b). A higher GQS indicates higher DNA integrity with a GQS of 5 implying no detectable degradation. Bars in (a) indicate average, error bars indicate standard deviation, numbers indicate sample size and different lowercase letters above bars indicate significant difference.

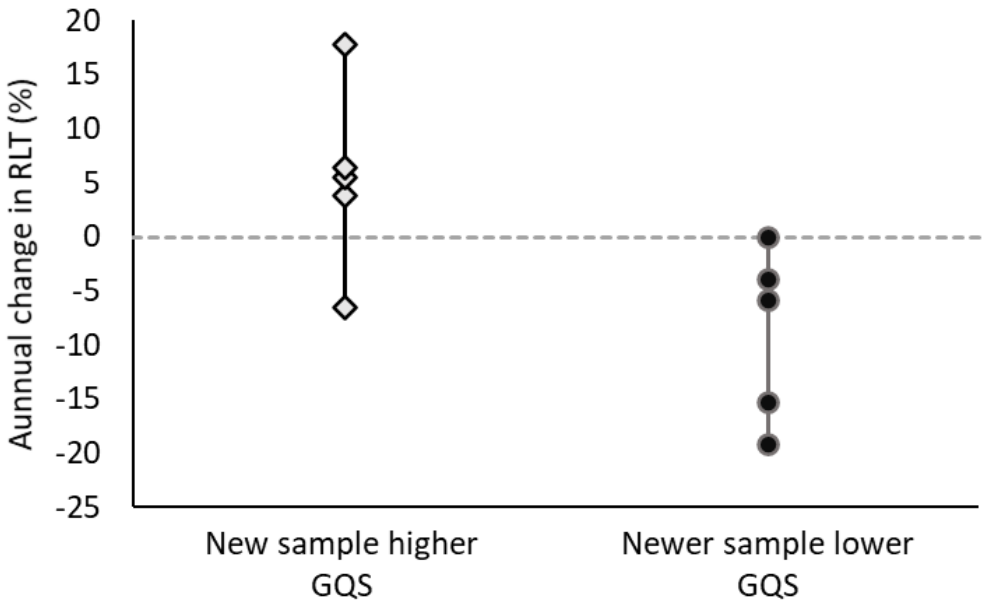


Figure 4.2.6. Annual change in relative telomere length (RTL) between pairwise longitudinal samples relative to genomic quality score. Each point indicates one pairwise comparison (*n* = 10). Comparisons are grouped by whether the newer sample had a higher (left) or lower (right) genomic quality score (GQS) and are connected for clarity. Negative y-axis values indicate telomere attrition (shortening) and dotted line highlights the transition from attrition to increasing telomere length over time.

4.2.4 Discussion

In this paper, we show for the first time that even very small amounts of DNA decay can affect qPCR-based measurements of relative telomere length (RTL) enough to impact the overall results of after-the-fact longitudinal telomere studies. Our analyses of *A. mantelli* telomeres suggested a 5% annual increase in RTL. However, we found (1) a strong positive correlation between genomic quality score (GQS) and RTL and (2) that increasing RTL between samples from the same bird, in all cases except one, was associated with a higher GQS of the newer sample. Most noticeably, this association between GQS and RTL was significant even when only samples with a GQS above 3, suggesting ‘high-quality genomic DNA’, were considered.

To our knowledge, this represents the first published analysis of *Apteryx* telomeres, and the results presented herein do not necessarily rule out annual telomere increase in *A. mantelli* – this has been observed in a few other long-lived species (see for instance Leach's storm petrel, *Oceanodroma leucorhoa*, Haussmann *et al.* 2003, edible dormouse, *Glis glis*, Hoelzl *et al.* 2016, *Eurasian oystercatcher, Haematopus ostralegus*; Tricola *et al.* 2018). However, the quality differences between our samples suggest that our results are likely to represent quantification of differences in DNA integrity rather than in number of cell divisions (and thus age).

Telomere studies for ecological and conservation purposes is a steadily growing field (Monaghan *et al.* 2018, Monaghan & Ozanne 2018, Wilbourn *et al.* 2018, Louzon *et al.* 2019), and we are convinced this will become an even more utilised marker for individual quality as well as population comparisons. With the results presented herein, we do not wish to discourage from such studies or to suggest that differences in DNA integrity must make analyses of RTL impossible. On the contrary, we wish to highlight that differences in DNA integrity is one of many important factors (growth, stress, diet, resource availability, breeding status etc.) to consider when analysing and interpreting RTL, and that this warrants more study.

Samples from threatened species need to be treated with respect, both due to the stress sampling imposes on the individual, but also to show appreciation for the important relationship between researchers and other stakeholders. Not least, people with traditional guardianship, rights, and/or relationships linked to the species. One way of showing such respect is by (with appropriate consent) maximising the information gained from the samples. Collection, storage, and handling of samples that minimize decay is likely a near universal goal in biological research. However, especially for long-lived species, studies of telomere attrition over time are likely to rely partly or

fully on opportunistic utilization of samples already collected over which the researchers will have limited control.

Consequently, we call for extensive and rigorous study of the relationships between genomic integrity (such as genomic quality score), relative telomere length, and other key features of this methodology, such as qPCR repeatability. A key focus of such studies would be how GQS, and thus RTL, is affected by factors such as sample handling and storage (Reichert *et al.* 2017, Eastwood *et al.* 2018), and the goal should be to directly quantify these parameters to facilitate statistically or methodologically accounting for them in future analyses and study design. Such ability to account for differences between samples would widen the possibilities for future utilisation for opportunistically available samples from threatened species and would thus enable informative studies with direct impact on, for instance, threatened species management.

We appreciate that most molecular labs will regularly quantify DNA quality prior to analyses such as qPCR. Conducting such quality control using microfluidic capillary electrophoresis has the benefit of generating a numerical value that can be used for comparison and analyses. While it is more expensive to perform this type of analysis of DNA integrity (NZ$5.5 per sample *versus* an estimated NZ$1 per sample for conventional gel electrophoresis) we argue that the potential additional benefits of (1) allowing more samples to be included, and (2) increasing accuracy and study outcomes out-weighs this cost.

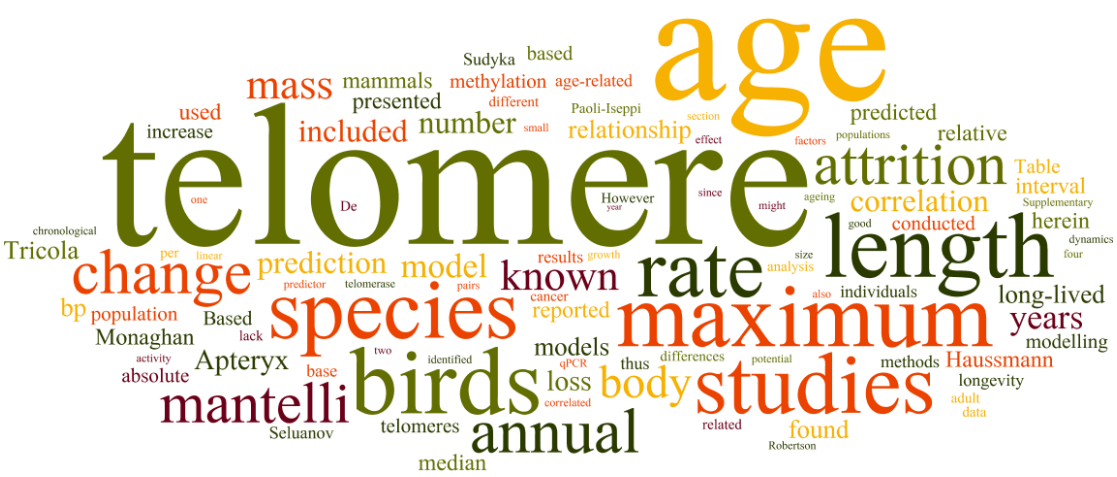
4.2.5 Conclusion

To our knowledge, this is the first study to suggest that differences in DNA decay between sample points can be big enough to directly influence the calculated annual change in telomere length. In fact, we suggest that qPCR measurements of telomeres may under such conditions, and without compensation, act more as a measure of DNA integrity than of true relative telomere length. Based on our results and others, sample storage and handling are likely to play a critical role in DNA integrity and how it may differ between sample cohorts (Reichert *et al.* 2017, Eastwood *et al.* 2018). Consequently, we suggest that more research is needed into the relationships between GQS and RTL and, for instance, which aspects of storage and handling affect DNA quality in a way that impacts RTL measurement. We suggest that this would be performed through an extension of the work of Eastwood *et al.* (2018) by intentionally exposing aliquots of the same samples to different settings after which GQS and RTL should be analysed and compared. This would enable the identification of an ideal protocol for accurate telomere quantification using qPCR in two ways: (1) one in which DNA integrity differences can be avoided, and (2) one in which differences can be quantified and accounted for statistically.

Our results can have wide implications since we have observed a significant increase in telomere studies using qPCR over the last few years, and telomere length and attrition rate is discussed more and more frequently as a tool for everything from evaluating habitat quality, population health, and age distribution, to answering big questions about the evolution of cancer prevention mechanisms. Thus, such protocols are urgently needed and could, for instance, have direct impact on the future management of iconic, but long-lived and elusive, taxa like *Apteryx*

Chapter 4.3

Should we even expect telomere shortening with age in *Apteryx mantelli*?



All supervisors provided feedback on one or more drafts of this chapter. The expertise of Doug Armstrong was crucial for this chapter.

Abstract

A large number of studies have found a negative correlation between telomere length and age. Many of these studies have been conducted on birds. Consequently, telomere length has great potential to act as an informative marker for bird population evaluation, especially in long-lived species that lack other ways of determining age. One such species is North Island brown kiwi, *Apteryx mantelli*. Herein, data from 23 bird species were used to model the relationships between telomere length, attrition rate, longevity, and size, to make predictions about expected *A. mantelli* telomere dynamics. The model with the highest explanatory power was predicting the annual rate of telomere change based on maximum known age. This model predicted the average annual change of *A. mantelli* telomeres to be centred around zero age-related change (95% prediction interval -282 to +286 base pairs per year). This suggests that telomere length might not be a good marker for determining chronological age in *A. mantelli* and other long-lived bird species. However, this still leaves telomere length as a marker with great potential for population comparisons related to stress and health, population viability and thus conservation requirements and outcomes given further studies.

4.3.1 Introduction

Ways of estimating the age of individuals and the age distribution within populations has been identified as an increasingly important tool for evaluating and studying wild populations (Noon & Sauer 1992, Danchin *et al.* 1995, Müller *et al.* 2007, Margalida *et al.* 2011, Brillinger *et al.* 2012). This is because age distribution directly links to recruitment success and mortality, and thus holds important information about populations' past and future prospects (Noon & Sauer 1992, Müller *et al.* 2007, Margalida *et al.* 2011, Brillinger *et al.* 2012). In addition, the ability to accurately age

individuals is important for accurate population viability analyses, since it enables categorising animals based on age/stage-specific demographic parameters (McCleery *et al.* 2008, Wolfe *et al.* 2010, Martin 2015). Hence, methods for ageing, especially adult individuals, are highly valuable for planning and evaluating conservation interventions such as translocations (Noon & Sauer 1992, Margalida *et al.* 2011). Telomeres' length and their shortening over time belong to the potential molecular age markers receiving the most attention and a striking number of telomere studies have been conducted on birds (Wilbourn *et al.* 2018).

Birds are well represented in telomere studies thanks to their wide range of sizes and life-expectancies and the high prevalence of long-term banding projects rendering known-age individuals in this group (Monaghan *et al.* 2018). Telomere length and/or rate of attrition has been found to be correlated with actual (chronological) age in many of these bird species (Haussmann *et al.* 2003, Blasco 2007, Salomons *et al.* 2009, Heidinger *et al.* 2012, Barrett *et al.* 2013, Sudyka *et al.* 2016, Tricola *et al.* 2018). However, a smaller number of studies of long-lived birds, for instance, kakapo (*Strigops habroptilus*; Horn *et al.* 2011) and wandering albatross (*Diomedea exulans*; Hall *et al.* 2004) have notably not found any such correlation.

The proximate reason for telomere attrition is the so-called 'end replication problem'; the DNA polymerase machinery is incapable of copying DNA strands to the very end and hence a short section of chromosome sequence is lost during each round of cell division (Olovnikov 1996, Blasco 2007, Monaghan *et al.* 2018). The attrition of telomeres is thought to be one of the main determinants of the 'Hayflick limit' whereby somatic cells enter senescence after a certain number of divisions (Shay & Wright 2000). The selective advantage of the Hayflick limit, and thereby of (somatic) telomere

shortening, is thought to be reduced cancer risk (Blasco 2007, Monaghan *et al.* 2018, Tian *et al.* 2018).

Since its discovery, a discussion has been ongoing as to whether overcoming the Hayflick limit would achieve increased longevity (Shay & Wright 2000, Foley *et al.* 2018). Potential support for this idea is that in birds and mammals, the rate of telomere loss has been found to negatively correlate with maximum age (Haussmann *et al.* 2003, Sudyka *et al.* 2016, Tricola *et al.* 2018, Whittemore *et al.* 2019). In mammals, there is also an established relationship between lower body mass (independent of lifespan) and higher telomerase activity, with telomerase activity being inversely proportional to the rate of telomere loss (Gorbunova & Seluanov 2009). The selective benefit of this mammalian trend is suggested to be that higher body mass is directly correlated with a larger number of cells and that this, in turn, is correlated with a higher cancer risk per individual, resulting in a greater need of a cancer prevention mechanism such as cell senescence in larger species (Seluanov *et al.* 2018). To the author's knowledge, the relationship between size (body mass) and rate of telomere attrition has not been explored in birds.

Apteryx mantelli is a species in need of conservation management (Robertson & de Monchy 2012, Robertson *et al.* 2016, Germano *et al.* 2018) and that lacks an accurate way to age individuals (Robertson & Colbourne 2017). All five extant *Apteryx* species are long-lived, for instance, the oldest known North Island brown kiwi, *A. mantelli* is currently about 42 and the oldest reported Tokoeka, *A. australis*, was 35 years old (genomics.senescence.info/species/; Barlow 2011). However, maximum age is a measure limited by the length of monitoring and it has been suggested that *A. mantelli and A. australis* should be able to live for over 80 years (Robertson & Colbourne 2004, Moorad *et al.* 2012, Ronget & Gaillard 2020). The results presented in Chapters 4.1 and

4.2, suggest that in order to quantify the relationship between age and telomere length in *Apteryx*, excluding other factors such as population origin and sample quality is crucial. To further facilitate such further studies, I herein utilised data from 23 bird species on their rate of telomere loss, maximum age, and body weight, to model the expected annual change in the length of *A. mantelli* telomeres.

4.3.2 Material and Methods

Based on two multi-species studies and one previous review on the topic of telomere loss and bird longevity, I collected data on median telomere length, yearly telomere attrition in base pairs (bp; absolute change), yearly telomere attrition in percentage (relative change), adult body weight, and maximum known age for 23 bird species (Table 4.3.1; Haussmann *et al.* 2003, Sudyka *et al.* 2016, Tricola *et al.* 2018).

From Tricola *et al.* (2018) and Haussmann *et al.* (2003), median telomere length was extracted from provided graphs featuring all analysed data points. The extracted median was then combined with the reported mean absolute attrition rate to calculate a relative attrition rate. From Sudyka *et al.* (2016) values for mean telomere length and for absolute and relative attrition rate were extracted from the associated supplementary data table in combination with the original studies cited (Table 4.3.1; Hall *et al.* 2004, Pauliny *et al.* 2006, Salomons *et al.* 2009, Barrett *et al.* 2013, Bauch *et al.* 2013).

Four studies included by Sudyka *et al.* (2016) were excluded from this analysis because the reported attrition rate was based on quantitative real-time polymerase chain reaction (qPCR) while the other studies were all conducted using terminal restriction fragment analyses (TRF). Previous studies have highlighted the potential issues with combining results from these two methods (Aviv *et al.* 2011, Lai *et al.* 2018, Wilbourn *et al.* 2018), and in support of this, three of the four attrition rates acquired using qPCR were indeed

outliers compared to the rates reported from TRF studies. Furthermore, qPCR provides no value for telomere length (but see Barrett *et al.* 2012). The thick-billed murre study by Young *et al.* (2013) included in Sudyka *et al.* (2016) was also excluded since this study was a longitudinal study across one single year and it reported substantially different rates of telomere change for the four sampling sites included. When the same species were included in more than one source paper, the average reported rate of telomere loss and telomere length was used. Maximum reported age was derived from the animal ageing and longevity database (genomics.senescence.info/species) and Tricola *et al.* (2018).

4.3.2.1 Statistical analyses

General linear models were fitted to evaluate whether differences in telomere length and rate of telomere attrition (loss) among species were related to maximum age and/or $\log_{10}$ body mass. This analysis was conducted twice, once with absolute attrition rate and once with relative attrition rate as the response variable, respectively. These models were then used to predict the annual rate of telomere change for *A. mantelli* using the values body weight 2500 g (average of adult males and females; Robertson & Colbourne 2017) and maximum age of 42 years. In addition, the prediction was run for a theoretical maximum age ranging from 1 to 100 years.

Table 4.3.1. Species included in general linear models of telomere length, attrition rate, body mass, and maximum known age.

Reference	Common name	Latin Name
Sudyka *et al.* 2016	Dunlin	*Calidris alpina*
Sudyka *et al.* 2016	European shag	*Phalacrocorax aristotelis*
Sudyka *et al.* 2016	Jackdaw	*Coloeus monedula*
Sudyka *et al.* 2016	Seychelle warbles	*Acrocephalus sechellensis*
Tricola *et al.* 2018	Barn swallow	*Hirundo rustica*
Tricola *et al.* 2018	White-rumped munia*	*Lonchura striata*
Tricola *et al.* 2018	Black guillemot	*Cepphus grylle*
Tricola *et al.* 2018	Common ruff	*Calidris pugnax*
Tricola *et al.* 2018	Florida scrub-jay	*Aphelocoma coerulescens*
Tricola *et al.* 2018	Great frigatebird	*Fregata minor*
Tricola *et al.* 2018	Great tit	*Parus major*
Tricola *et al.* 2018	Mangrove swallow	*Tachycineta albilinea*
Tricola *et al.* 2018	Mexican jay	*Aphelocoma ultramarina*
Tricola *et al.* 2018	Northern fulmar	*Fulmarus glacialis*
Tricola *et al.* 2018	Oystercatcher	*Haematopus longirostris*
Tricola *et al.* 2018	Red-footed booby	*Sula sula*
Tricola *et al.* 2018	Savannah sparrow	*Passerculus sandwichensis*
Tricola *et al.* 2018	Thick-billed murre	*Uria lomvia*
Tricola *et al.* 2018; Haussmann *et al.* 2003	Adelie penguin	*Pygoscelis adeliae*
Tricola *et al.* 2018; Haussmann *et al.* 2003	Leach's storm petrel	*Oceanodroma leucorhoa*
Tricola *et al.* 2018; Haussmann *et al.* 2003	Tree swallow	*Tachycineta bicolor*
Tricola *et al.* 2018; Haussmann *et al.* 2003	Zebra finch	*Taeniopygia guttata*
Tricola *et al.* 2018; Sudyka *et al.* 2016; Haussmann *et al.* 2003	Common tern	*Sterna hirundo*

*Referred to as Bengalese finch in Tricola *et al.* (2018)

4.3.3 Results

The generated models supported an effect of maximum known age on annual change of telomere length (Table 4.3.2; Figure 4.3.1). Body mass had no apparent effect on the rate of telomere attrition once the effect of maximum age had been taken into account (Table 4.3.2). Body mass and maximum age were also found to be highly correlated (R^2 = 0.43; Supplementary Figure 4.3.1A). Neither maximum age nor body mass was a good predictor of median telomere length (t = -0.50, df = 21, p-value = 0.62 and t = -0.10, df = 21, p-value = 0.33 respectively; Supplementary Figure 4.3.1B). Based on this, only maximum age was used for the presented prediction of *A. mantelli* telomere change, and no prediction of *A. mantelli* median telomere length was attempted.

Table 4.3.2. Results of general linear models of relationships between annual rate of telomere loss and maximum age (years; 'Age'), log body mass (grams; 'M'), or both. The first analysis included three alternative models for absolute annual telomere change in base pairs ('Abs. Loss') and the second analysis included three alternative models for relative annual telomere change as a percentage ('Rel. Loss').

								p-value	
Model	AIC	α	STD	β_M	STD	β_{Age}	STD	Mass	Age
Abs. Loss =									
$\alpha + \beta_M$*log(M)	303	-414.4	93.6	105.2	14.3	na	na	**0.02**	na
$\alpha + \beta_{Age}$*Age	292	-448.7	60.3	na	na	10.8	2.3	na	**<0.001**
$\alpha + \beta_M$*log(M) + β_{Age}*Age	294	-449.3	77.0	0.59	45.8	11.8	3.2	0.99	**<0.001**
Rel. Loss =									
$\alpha + \beta_M$*log(M)	94	-3.62	1.01	0.83	0.2	na	na	*0.08*	na
$\alpha + \beta_{Age}$*Age	85	-4.21	0.67	na	na	0.10	0.03	na	**0.001**
$\alpha + \beta_M$*log(M) + β_{Age}*Age	87	-3.98	0.85	-0.22	0.51	0.11	0.04	0.65	**0.002**

Based on a maximum age of 42 years, the absolute annual change of *A. mantelli* telomere length was predicted to be an increase of 3 bp per year but this prediction was very uncertain (95% CI -100 to 106; 95% prediction interval ranging from -282 to increase of 286 bp; Figure 4.3.1). Based on the same maximum age, relative annual change was predicted to be a loss of 0.1% (95% CI -1.2 to 1.1%; prediction interval from -3.2% to 3.1%). For maximum age of 80, the predicted absolute annual change was a 408 bp increase (95% prediction interval 30 to 788 bp increase), and the predicted relative annual change was a 3.7% increase (95% prediction interval 0.6% loss to 7.9% increase).

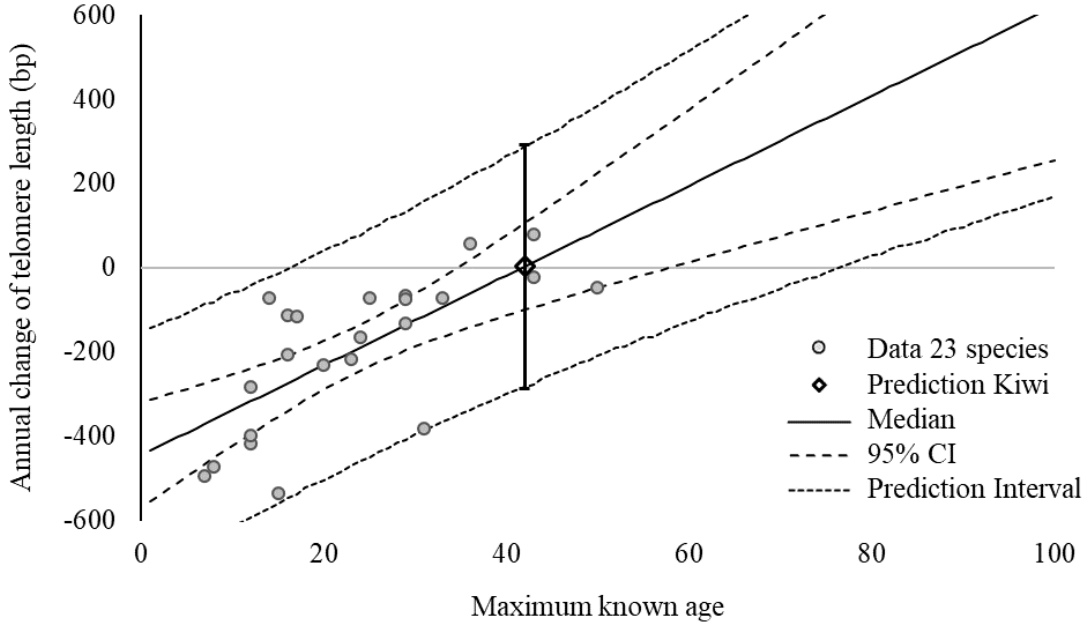


Figure 4.3.1. A linear model of the relationship between annual telomere length and maximum known age in 23 bird species. For species names see Table 4.3.1. The indicated predicted annual change for *A. mantelli* is based on maximum age 42 years.

4.3.4 Discussion

The modelling conducted here suggests that maximum known age is a better predictor of the annual rate of telomere change in birds compared to body mass. Furthermore, the dataset analysed suggested that maximum age is not a good predictor for telomere length *per se*. For instance, none of the four species with the highest reported maximum age was in the top five in terms of median telomere length. Instead, the four species with the highest reported known age in this modelling included (a) the two species with the slowest annual attrition (Northern fulmar and great frigatebird; Juola *et al.* 2006, Tricola *et al.* 2018) and (b) the only two species for which telomere had been observed to elongate with age (Leach's storm petrel and Eurasian oystercatcher; Haussmann *et al.* 2003, Tricola *et al.* 2018).

Based on a maximum known age of 42 years, the model with the strongest predictability presented herein predicted a median annual telomere gain of 3 base pair (bp) for *A. mantelli* with a prediction interval between loss of 282 and gain of 286 bp and a 95% confidence interval from -100 to +106 bp. In other words, the model predicted the possibility of no change with increasing age of *A. mantelli* telomeres. This is in line with the results presented in Chapter 4.1. Intriguingly, when the maximum age was changed from 42 to 80 years in our preferred model, the resulting prediction was noticeable annual telomere growth, with the entire prediction interval falling above zero. However, such extrapolation is highly speculative since the oldest birds used to build the model had a maximum known age of 50 years (Northern fulmar).

Interestingly, results from studies of certain species, such as Leach's storm petrel (*Oceanodroma leucorhoa*; Haussmann *et al.* 2003, Tricola *et al.* 2018), Eurasian oystercatcher (*Haematopus ostralegus*; Tricola *et al.* 2018) and edible dormouse (*Glis*

glis; Hoelzl *et al.* 2016), and of certain individuals within other species such as blue tits (*Cyanistes caeruleus*; Sudyka *et al.* 2019), Seychelles warbler (*Acrocephalus sechellensis*; Spurgin *et al.* 2018, Wood & Young 2019), European storm petrel (*Hydrobates pelagicus*; Watson *et al.* 2015), alpine swift (*Tachymarptis melba*; Bize *et al.* 2009), and zebra finch (*Taeniopygia guttata*; Heidinger *et al.* 2012) indicate elongating telomeres over time. Such results are often dismissed as measuring errors or the result of selective loss that skews data (see introduction; Bateson & Nettle 2017, Tricola *et al.* 2018). However, some authors have found convincing statistical support for that increasing telomere length should not be dismissed so quickly, but rather that lengthening can be expected to occur in a small proportion of the population (Bateson & Nettle 2017, Spurgin *et al.* 2018). Along similar lines, several studies of other long-lived bird species, such as Leach's storm petrel (Haussmann *et al.* 2003, Tricola *et al.* 2018), thick-billed murre (*Uria lomvia*; Young et al. 2013), kakapo (*Strigops habroptilus*; Horn *et al.* 2011), European shag (*Phalacrocorax aristotelis*) and wandering albatross (Hall *et al.* 2004) have not found support for shortening telomeres with increasing age, in particular among adult individuals, however, there are exceptions such as great frigatebird (*Fregata minor*; Juola *et al.* 2006). Lastly, there are ways that telomeres can elongate. The most common mechanism in animals is through the activity of the enzyme telomerase (Greider & Blackburn 1985), but other mechanisms also occur even in vertebrates (see for instance Foley *et al.* 2018).

Due to the strong correlation between maximum known age and bird body mass observed, the relationship between body mass and rate of telomere change was found to be positive, i.e., larger birds lose their telomeres at a slower annual rate than smaller species. To my knowledge, this is the first time the relationship between body mass and telomere attrition rate has been investigated in birds. This positive correlation contrasts

with the findings in mammals where smaller mammals have been found to retain more telomerase activity and thus lose telomeres slower, irrespective of lifespan (Seluanov *et al.* 2007, Gorbunova & Seluanov 2009, Seluanov *et al.* 2018, however, see Whittemore *et al.* 2019). It is plausible that this difference is related to the fact that birds and mammals generally have a drastically different growth pattern with most birds experiencing rapid growth during very early life stages while many mammals generally have later growth spurts (Monaghan & Ozanne 2018). Another, perhaps more intriguing, option is that these differences in telomere dynamics hint towards differences in selection pressures for evolving cancer prevention mechanisms between mammals and birds (Seluanov *et al.* 2018, Tian *et al.* 2018).

There were limits to the models presented herein. The number of species included was only 23, the majority of which had a substantially shorter life-span and lower body mass than *A. mantelli*. qPCR has seemingly completely taken over the studies of telomere length in birds, thus it was not possible to increase the number of species in the modelling without having to combine results from different telomere measuring methods. In addition, several of the included species are rather closely related, and Tricola *et al.* (2018) found a phylogenetic effect in telomere attrition rate. Because of this observation, phylogenetically close species might effectively represent pseudo-replication, which the sample size here was too small to effectively account for. Maximum known age was used as a parameter in this study for consistency with other studies. Even though the models suggest that it is a good predictor of telomere change, maximum age is known to depend on study length and research effort and can be assumed to represent only a small proportion of a population or species (the tail end distribution; Moorad *et al.* 2012, Ronget & Gaillard 2020). Lastly, in order to be comparable, the maximum age for different species must have been obtained under

equally benign (or harsh) conditions (Ronget & Gaillard 2020). Other more informative parameters to base a model for telomere dynamics on might be annual survival rate, since this would represent the species' longevity better than the maximum age, or metabolic rate since this would arguably have a closer connection to number of cell divisions and prevalence of other telomere damaging factors such as reactive oxygen species (ROS; Reichert & Stier 2017, Monaghan & Ozanne 2018). To the author's knowledge, such modelling has not been conducted. Interestingly, *Apteryx* species have a high annual survival as an adult (in absence of introduced mammalian predators; Robertson *et al.* 2011, Robertson & de Monchy 2012) and comparatively low O_2 consumption and thus a low metabolic rate (Calder & Dawson 1978, Sales 2005).

4.3.5 Conclusion

The strong correlation between maximum age and telomere attrition rate presented herein support the idea of a crucial role of telomere maintenance in birds' longevity (Haussmann *et al.* 2003, Haussmann & Marchetto 2010, Monaghan *et al.* 2018, Tricola *et al.* 2018, Whittemore *et al.* 2019). As seen in Chapter 4.1, the relative telomere length has not been observed to correlate with age in *A. mantelli*. As described above, studies of other long-lived bird species such as kakapo (*Strigops habroptilus*; Horn et al. 2011) and wandering albatross (*Diomedea exulans*; Hall *et al.* 2004; either not included in our modelling) have shown similar results. This lack of correlation could be because age-related change is exceedingly small for birds with these lifespans while environmental factors are stronger determinants of individual telomere length. In turn, this could be related to a retained telomeres activity in somatic cells of long-lived birds (Haussmann *et al.* 2004). Consequently, the results presented herein combined with Chapter 4.1 and previous studies in other species indicates that telomere length might be a poor

candidate for indicating actual (chronological) age in long-lived birds such as *A. mantelli* and its congeners.

However, a lack of correlation with chronological age is not reason enough to rule out telomere length as an informative marker for population evaluations. On the contrary, if telomere change due to age is small, observed differences in telomere length have greater potential to be diagnostic of other stressors such as sub-ideal habitat conditions or disease (Bize *et al.* 2009, Haussmann & Marchetto 2010, Herborn *et al.* 2014, Monaghan 2014, Bateson 2016, Salmon *et al.* 2016, Louzon *et al.* 2019). More studies of other factors affecting telomere length and dynamics are called for, as well as further studies testing the herein modelled lack of correlation between telomere length and age in long-lived birds including *Apteryx*. Based on the results presented in 4.1 and 4.2, such studies need to take care to focus on comparison within populations and of samples exposed to comparable conditions after collection. Additionally, our notion that the correlation between size and rate of telomere attrition is diametrically opposite in birds and mammals triggers interesting questions about cancer prevention and opens up for a large number of interesting studies in this exciting field. A starting point for such studies would be investigating the expression and activity of the telomere elongating enzyme telomerase in somatic tissue of birds of different lifespans, in particular of long-lived birds such as *A. mantelli*.

Are there other promising molecular markers that bring hope of finding a way to age adult *Apteryx* individuals? A rapidly increasing number of studies are finding connections between epigenetic changes and fitness differences (Kawanabe *et al.* 2016, Lauss *et al.* 2018, Soulsbury *et al.* 2018). For the last 10 years, studies of the relationship between epigenetics, specifically DNA methylation, and age have received substantial attention (De Paoli-Iseppi *et al.* 2017). Most of these studies have revolved

around humans and model organisms such as mice (De Paoli-Iseppi *et al.* 2017). These methods have now been applied to wild populations, perhaps most famously by Polanowski *et al.* (2014) who developed epigenetic markers for ageing humpback whales based on loci previously identified to be age-related in humans and mice. Attempts to modify this protocol to make it suitable for other whale species currently underway (Tanabe *et al.* 2020). As in genetics, methods for epigenetic analysis are fast developing. Initially limited to quantifying a total amount of genome-wide (or 'global') methylation, methods are now being used to target tens of thousands of specific loci per individual and diagnose them methylated or not (Hansen *et al.* 2012, Jelinek & Madzo 2016, Trucchi *et al.* 2016, van Gurp *et al.* 2016, Zhang *et al.* 2018).

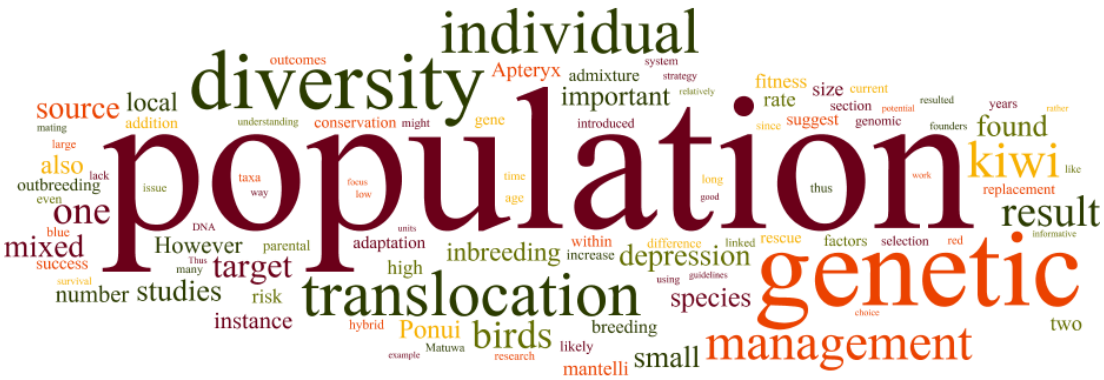
Most of the work on age-related methylation in birds has focused on chicken (Gryzinska *et al.* 2013, De Paoli-Iseppi *et al.* 2017). However, recently, the first study ever of age-related methylation changes in wild birds was conducted in short-tailed shearwaters (*Ardenna tenuirostris*; De Paoli-Iseppi et al. 2019). This study used digital restriction enzyme analysis of methylation (DREAM) and identified seven loci for which methylation was age-related (Jelinek & Madzo 2016). A model was developed that estimated age and identified ageing with encouraging accuracy (De Paoli-Iseppi *et al.* 2019). This protocol is a useful starting point for others wanting to explore age-related methylation in long-lived birds such as *Apteryx* species.

PART V – DISCUSSION

In this last part of my thesis, I bring my results together and discuss them from the perspectives of identifying suitable source and target populations, and the potential genetic outcomes of *Apteryx* reinforcement translocations. I also directly address selected segments from the current Kiwi Recovery Plan and make suggestions for future editions based on my research. Finally, I outline important studies that could further improve our ability to perform predictable and successful kiwi translocations that result in diverse, long-lasting populations.

Chapter 5.1

General discussion on maintaining, spreading, and understanding *Apteryx* genetic diversity



All supervisors provided feedback on one or more drafts of this chapter.

Abstract

Genetic management is complex and identifying the issues limiting effective implementation of it is pivotal. Based on my research, I suggest that the main issue for genetic management of *Apteryx* is not that historic translocations have generated undesirable populations. On the contrary, the hybrid origin combined with mixed mating after the translocations have made the Ponui Island population remarkably diverse despite being founded with as few as 14 birds. Meanwhile, large mainland populations were found to not necessarily harbour high diversity. Thus, if the main goal is increasing genetic diversity, neither small populations nor ones with mixed or unclear origin should be considered undesirable as sources or targets for future translocations. Instead, I suggest that two pressing issues for genetic management of kiwi are: not knowing the extent of local adaptation in *Apteryx* diversification, and the unclear relationships between population density, mate choice, admixture, genomic diversity, and fitness measures such as fecundity or population growth rate. A third issues is the lack of informative indicators of age and viability – my studies suggested that telomere length is not the marker to use. I show that these issues cannot be solved be re-analyses of existing data. However, fortunately, *A. mantelli* populations, and their variety of management histories, constitutes a promising study system for resolving these issues. One promising marker type to fucus such studies of is DNA methylation.

5.1.1 General discussion

Translocations are drastic and complex management interventions that are becoming increasingly common in New Zealand and globally (Bouzat *et al.* 2008, IUCN/SSC 2013, Batson *et al.* 2015, Shier 2015, Flanagan *et al.* 2018, Armstrong *et al.* 2019, Parker *et al.* 2020). A long list of factors must be considered when developing general

guidelines and before launching a specific translocation. These factors stretch from biological parameters such as habitat and disease, ethical issues such as the capture and transport of animals, logistical challenges such as travel and timing, resource questions such as securing long-term funding, and human resource topics such as consultations with indigenous people with traditional authority and other stakeholders. An often-neglected part is developing a framework for the incorporation of Tikanga Māori and other cultural protocols. Templates for such frameworks are emerging, and key features are the identification of three to five core values – for example, respect, acknowledging spirituality, acknowledging the people of the land, and/or working together as one – and development of a protocol that specifies how these values will be incorporated at each step of the translocation process. All these things and more need to feature in a national strategy for kiwi translocations. However, the two critical factors I will focus this discussion on are (1) the identification of suitable source and target populations, and (2) the genetic diversity and identity of the target population once a reinforcement translocation has taken place.

5.1.2 Targets and sources

Reinforcement translocations are conservation interventions that involve moving individuals from one or several source populations into a target population. Increasingly, this is not only done to increase the number of individuals, but also add genetic diversity and alleviate inbreeding, commonly referred to as genetic rescue (Ingvarsson 2001, Frankham 2015, Bell *et al.* 2019). The key question is how to identify suitable sources and targets, especially in a scenario - such as for *Apteryx* - where (1) so many populations are small, bottlenecked, and isolated that not all can be targets, and (2) so few candidate source populations are ‘pristine’. In addition, intense management of invasive predators is currently necessary for *Apteryx* population growth,

making predator-free sites, such as islands and fenced areas, tempting translocation targets. However, these sites are completely isolated from immigration and gene flow. Taken together, choosing sources and target sites will inevitably include a number of trade-offs.

While a small population size usually warrants conservation attention, past population size, and historic access to gene flow are other crucial factors that determine the size of a gene pool, the rate at which drift and inbreeding are diminishing it and, thus, the risk of extinction (Figure 5.1.1; Reed 2010, Lopez *et al.* 2020). In adition, as I found for brown kiwi on the Purerua Peninsula, large population size is not synonymous with high genetic diversity and low inbreeding (Angeloni *et al.* 2011). Similarly, as I found in the Ponui Island population, a small number of founders is not necessarily synonymous with an immediate extinction risk and/or extensive homozygosity. Lastly, regardless of size and gene flow, the effect of a small gene pool will depend on several factors, perhaps most importantly the environment with which it interacts (Milot *et al.* 2007, Johnson *et al.* 2009, Reed 2010). This means that even very small populations with high levels of inbreeding, might not manifest symptoms of inbreeding depression (i.e., negative fitness impacts linked inbreeding) until environmental change makes a lack of adaptability or plasticity apparent (Crnokrak & Roff 1999). Inbreeding depression usually affects traits linked to reproduction such as sperm quality, clutch size, and hatching rate (DeRose & Roff 1999, Jamieson & Ryan 2000, Briskie & Mackintosh 2004, White 2013, Taylor 2014). This generates an additional complication for the management of long-lived animals like kiwi since recruitment failure can go unnoticed for many years if success is defined as census population size, especially if the this is estimated using methods such as call count surveys. Consequently, we need indicators of viability that are complementary to population size to identify target

populations in need of help as well as healthy source populations that can withstand the 'harvest' of a sufficient number of individuals.

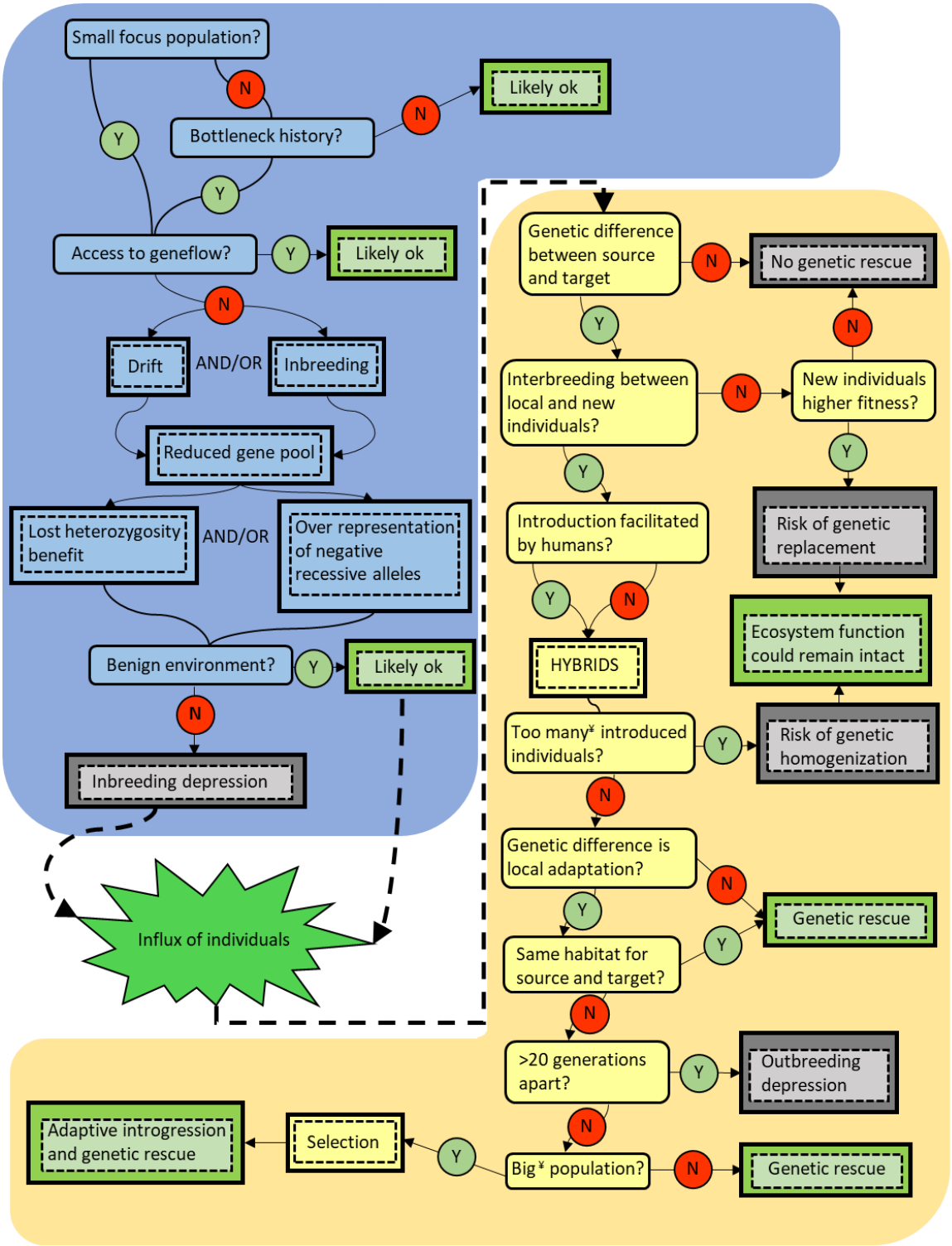


Figure 5.1.1. Flow chart illustrating translocation premisses and possible outcomes simplified into yes-no dichotomies. This simplistic framework aims to capture multiple issues regarding

the variety of factors impacting how a small population does before (blue area) and after (yellow area) reinforcement translocations. Each solid line box includes a question to which the answer is either yes (Y) or no (N). Arrows and dotted boxes indicate the outcomes associated with each answer. Many answers are highly species- and context-dependent, for instance, the cut-offs for quantities like 'too many' or 'big' (indicated by '¥'). In addition, what constitutes a 'benign' environment, or the 'same' habitat will also be context-dependent. These uncertainties, and the difficulty to account for them in current population genetic models, is part of the reason why predicting translocation outcomes is challenging.

Box 1. Genetic management issues specified in the Kiwi Recovery Plan

The Kiwi Recovery Plan 2018-2028 (Germano *et al.* 2018) lists 10 recovery principles for kiwi. One of these is '*Rerenga ā-ira – Genetic diversity: The genetic diversity and distribution of each species or subspecies will be maintained or enhanced as much as is feasible*.' This is further specified under the topic 'Genetic management'. Below I provide my thoughts around six of the seven issues listed under this topic (leaving out issue 6 since it focuses specifically on *A. rowi*)

To first address issues 2, 4, and 5: '*The results of genetic research are not always publicly available, clear or incorporated into management and education.*', '*There is a lack of clear records, consistency or coordination of kiwi genetic samples, which are spread across various locations and organisations.*' and '*There is a need to increase research on non-invasive genetic techniques and genomic approaches for the conservation management of kiwi.*'

I agree that these are important issues. In response to the first two, I collated all published research done so far on *Apteryx* genetics in a review (Chapter 1.2). This review showed that as many as 41 studies, based on samples from about 2000 individuals, have featured *Apteryx* genetics. However, I found that combining all these data provided little new information of value for genetic management, for instance, even the largest combinable dataset –

mitochondrial sequence from 488 birds – did not provide any answers about diversity within or between Apteryx species and management units. Thus, I concluded that the issue is not lacking access to existing data, but that studies have addressed unrelated questions and used uncompilable markers, making their results of limited interest for genetic management. Thus, further studies, rather than reanalyses of previous data, are urgently needed.

One drawback with the existing genetic data is the uneven geographical spread of kiwi sampling. Part of resolving this will likely be developing protocols for less invasive sampling. Herein, I mainly relied on blood samples since I was worried that other sources would not provide DNA of high enough quality to give confidence to the results. However, the small number of feathers I used gave promising results (see Appendix A1) and developing an optimal protocol for feather storage and labelling should be considered a high priority. There is also interesting ongoing work on extracting DNA from faecal samples (Carroll *et al.* 2018, Ramón-Laca *et al.* 2018). A recent realignment of the published *A. mantelli* genome will further help to make less optimal DNA usable for genomic analyses. Less invasive methods and detailed records of already collected samples will reduce stress on both birds and scientists and will allow more studies to be conducted despite the cumbersome permitting systems which currently, and unfortunately, are slowing down crucial research. Finally, the discussion on sample storage and accurate records requires a Tiriti of Waitangi perspective, luckily, this might soon become easier thank to the newly founded Indigenous Genomics Institute ®.

Moving on to issues 1 and 3 '*A lack of knowledge and understanding of the importance of genetics for long-term kiwi conservation has resulted in some poor management actions (e.g., the creation of genetic bottlenecks and hybrid populations)*.' and '*There is confusion about how to manage hybrid and mixed provenance individuals, as well as individuals and populations that are suspected to contain deleterious alleles/genes*.'

Historic kiwi translocations have created several severely bottlenecked populations. Such small, isolated populations will inevitably lose diversity over time. Growing evidence support the benefits of augmented gene flow into such populations (Sutherland *et al.* 2019), preferably from sources that maximise post-translocation genetic diversity (Frankham 2015, Whiteley *et al.* 2015, Ralls *et al.* 2020). Thus, it is intuitive to focus *Apteryx* management on large mainland populations and use these as sources for translocations. According to my results, the Trounson Kauri Park harbours one such desirable population with large genetic diversity combined with high density and long-running management. However, my results showed that being a large mainland population was no guarantee for having more genetic diversity than a small island population with only 14 local founders (Purerua Peninsula *versus* Moturua). In fact, the hybrid population on Ponui Island was the most diverse in my dataset despite having received only 14 founders, suggesting that the mixing of taxa may have counteracted the effect of this small founder size. This finding is at odds with the recommendation letter sent to DOC in 2016 in which it was assumed that the tight bottleneck had resulted in high inbreeding depression, while the mixed origin had also resulted in outbreeding depression, making the Ponui Island birds doubly undesirable for conservation efforts. On the contrary, I suggest that the Ponui Island population is likely to be the site in New Zealand where the most kiwi diversity could be sourced in the shortest amount of time and at the lowest economic cost.

The lack of a simple correlation between the number of founders, diversity, and success indicates that the current guidelines of using 40 unrelated birds to found a new *Apteryx* population might need reconsideration. In fact, a previous study has concluded that a founder size of 40 kiwi will require an influx of about one bird every two years to avoid diversity loss and concerning levels of inbreeding (Weiser *et al.* 2013). In addition, the number of birds released is of less importance compared to the number of genome equivalents that contribute to the population which will always be lower due to relatedness and uneven success among surviving birds (Kardos *et al.* 2018). For instance, as suggested in Chapter 3.1, random

catching of birds in a confined population (such as an island or a kōhanga site) is unlikely to result in 40 unrelated birds. Encouragingly, studies have found that mixing several small and inbred populations can also result in genetic rescue and diverse, successful populations (Hedrick & Fredrickson 2010, Frankham 2015). As mentioned in Chapter 3.1, the issue with utilising inbred individuals as a source is that they will contribute with less diversity per individual. It is not that they harbour or risk spreading deleterious alleles. All populations will contain deleterious alleles. Why this comes up in the discussion of management of small populations is because (1) the smaller the effective population size, the more unlikely it is that selection will successfully remove deleterious alleles from the population, and (2) inbreeding increases the risk of inheriting the same allele from both parents, which is the reason why recessive deleterious alleles will affect the phenotype more regularly in smaller populations. Thus, I suggest that even movement of birds between small populations (for instance island-bound populations that are the result of historic translocations) could have great positive effects.

Directly linked to the prospect of using hybrid populations as sources for translocation, is issue 7 '*Confirmation is needed about the appropriate kiwi taxa / evolutionarily significant units to manage to retain remaining genetic diversity.'*

I strongly agree with this statement. The lack of understanding of local adaptation in *Apteryx* has resulted in the current division into management units risk being suboptimal. Furthermore, the current approach where managing *Apteryx* diversity has been interpreted as keeping areas with discernible genetic differences (often quantified as isolation-by-distance) separate, risk being counterproductive and result in reinforcing differences caused by loss of connectivity (Senn *et al.* 2014, Weeks *et al.* 2016). The frequent interbreeding between individuals form separate MUs at Ponui Island, Remutaka Forest Park, and Pūkaha National Wildlife Centre, combined with other known occurrences of fertile hybrids between kiwi species, could indicate relatively low levels of local adaptation separating these taxa.

Meanwhile, it is worth noting that the lower-than-expected representation of LBI genotypes in the current Ponui Island population could be a sign of outbreeding depression. This in turn could be the result of local adaptation of either or both of Ponui Island's parental taxa. However, the small number of founders on Ponui Island and the bottleneck history of LBI, suggest that it is highly likely that the real cause of the low LBI representation is random chance. See box 2 for comparison. Further studies are urgently needed to quantify the extent and significance of local adaption for kiwi diversification and among populations that are nowadays disjunct. These questions can be formalised as testing the 'pied' and 'rainbow' hypothesis outlined in Chapter 1.2 of this thesis. The dataset utilised herein (which is still expanding) can be used as part of such investigations by looking for signal consistent with positive or negative selection (so-called outlier analyses; Milano *et al.* 2014).

One informative indicator of population viability is age distribution. Previous studies have shown that the ratio between adults and juveniles in *A. mantelli* populations can be accurately determined with dog surveys (Robertson & Fraser 2009). I found no difference between the sample sets caught using dogs during the day *versus* encounter catching at night (Appendix A2), suggesting that either method will likely give a good estimate of the adult to juvenile ratio. However, this ratio provides limited information and determining the age of the adults would add important data on population dynamics and the expected future development in terms of growth and size. Unfortunately, I found that telomere length is unlikely to be the long-sought-after marker that would allow us to determine individual kiwi age and population age distributions (Chapter 4.1); this was, perhaps, an expected outcome following the results of studies of telomeres in other long-lived birds (Chapter 4.3).

Identifying informative age and fitness markers is important for understanding population viability and the consequences of inbreeding. However, I would argue that a diverse gene pool is such an important resource that augmenting geneflow through translocations should be considered as a key part of genetic management for long-lived species even if symptoms of inbreeding depression have not been quantified (see further Ralls *et al.* 2018). The current Kiwi Recovery Plan (Germano *et al.* 2018) follows a worldwide pattern where genetic diversity and management are listed as important, but how to achieve or evaluate this is rarely specified in greater detail (Shafer *et al.* 2015, Pierson *et al.* 2016, Wennerström *et al.* 2017). Goals in these plans are generally focused on maintaining genetic diversity, which tends to be interpreted as keeping taxa, populations, or management units separate for a fear that mixing will reduce overall diversity (Bouzat *et al.* 2008, IUCN/SSC 2013, Todesco *et al.* 2016, Love Stowell *et al.* 2017). This attitude has resulted in that two of the most widely used criteria for identifying suitable source and target populations are minimising (1) genetic difference and (2) geographic distance between them; sometimes even within recognised management units (see further Box 1; Craig *et al.* 2011, Scrimgeour & Pickett 2011).

Personally, I find the focus on minimising difference an unfortunate paradox, in fact, introducing individuals with a very similar genetic makeup to the target population is unlikely to result in genetic rescue. Particularly unfortunate and surprising is that this hesitation towards mixing populations that are ‘too different’ is supported by the current IUCN guidelines for translocations (IUCN/SSC 2013). While these guidelines highlight the importance of genetic diversity, the authors spend about three times more words on advocating for the risks of mixing lineages and creating hybrids, compared to on the potential benefits of doing so. The guidelines state that the safest method is introducing individuals as similar as possible to the target population and refer to risks associated

with larger differences being incompatibilities and maladaptation. The few words said in favour of deliberate admixture names this a '*more radical*' strategy but acknowledges that '*Taking individuals from multiple populations can increase the genetic diversity and decrease the risk of inbreeding depression in the translocated population. This is appropriate if outbreeding depression and/or (for animals) behavioural differences between the populations are considered unlikely*'. This hesitation for mixing relates to a widespread and pervasive view that hybridization represents something unnatural (Piett *et al.* 2015, Love Stowell *et al.* 2017). However, on the contrary, hybrids have been detected in wild populations of at least 9% of all bird species, and in certain groups – such as ducks, grouse, birds of paradise, new world warblers, and tits – 20-25% of species hybridise (a rate on par with famously hybridisation-prone vascular plants; Mallet 2005). This high inter-specific rate of hybridisation in birds suggests that mixture of individuals from distinct lineages within species should be at least as common if the rate of interbreeding is assumed to increase with similarity (Grant & Grant 2014, Ottenburghs 2019).

In addition, the IUCN guidelines repeatedly refer to the need to introduce '*adequate*' genetic diversity, which is vague and underlines the problematic lack of knowledge that has been highlighted repeatedly in this thesis. If there is such a thing as adequate diversity, this will depend on what the desired outcome for the translocations is. Unfortunately, translocations plans are often missing details about what desired outcomes are and how to quantify and evaluate them (Shier 2015, Pierson *et al.* 2016, Parker *et al.* 2020).

5.1.3 Outcomes

Once source(s) and target populations have been chosen and birds have been translocated, the outcome depends on several factors (Figures 5.1.1 & 5.1.2; see for example Armstrong *et al.* 2020). In terms of genetic composition and amount of genetic diversity post translocations, the most influential factors can essentially be boiled down to two questions: (1) At what rate are original and introduced individuals interbreeding? (2) What is the nature of the genetic difference between the source and the target populations?

I addressed the first question by assessing mate-choice and the breeding system *of A. mantelli.* The breeding system of a species will have a large impact on translocation outcomes. For instance, if parentage is highly skewed towards a small number of individuals (of either or both sexes), such as in wolves and lek-breeding birds, a small number of introduced individuals can rapidly spread their genetic material if they become successful breeder (Adams *et al.* 2011, Mussmann *et al.* 2017). However, these breeding systems can also drastically reduce effective population size which increases the risk of inbreeding. On the other hand, in some species, a polygamous mating system results in parentage varying considerably between years which can result in a rapid admixture of the two parental genomes (Thavornkanlapachai *et al.* 2019; see Box 2).

I found no evidence for assortative mate choice. On the contrary, mixed mating was found to be common in the present-day Ponui Island population based on relatedness among mates (Chapter 2.2). Mixed mating had also been occurring over time, as was evident from the structure analyses that found birds of essentially all possible proportional combinations of the two parental genomes (Chapter 3.1). The long lifespan of kiwi makes studying certain aspects of the fitness consequences of mixing *versus*

taxon separation challenging. Luckily, a few other comparable mixed populations of more fast-living species exist (see Box 2) and results from such model systems suggest a promising potential for achieving genetic rescue through supplementary kiwi translocations.

However, the longer the lifespan and the higher the monogamous pair fidelity is, the more important the proportion of initial mixed mating after translocation will be for diversification and genetic rescue. For instance, I found that 10 to 12 years after the translocation of LBI birds to Pūkaha and Remutaka, many *A. mantelli* at these sites are inbred despite the high overall diversity in the combined population. This delayed diversification was due to the fact that (1) 95% of the supplemented LBI birds bred with each other rather than mixed with the local birds and (2) mating between close relatives among the local birds exaggerated the effect of this separation. There are ways to reduce the risk of this happening, for instance, by introducing individuals of only on sex, and the birds at Pūkaha and Remutaka are now interbreeding. In addition, as I outlined in Chapter 2.1, there is theoretical support for *A. mantelli* not being as monogamous as previously assumed. In Chapter 2.3 I, indeed, found that about one fifth of breeding units in a high-density *A. mantelli* population included more than two individuals. These observations of group breeding warrant future study into how common this strategy is across *A. mantelli*, and how parentage is distributed within groups. Mixed parentage would result in faster genetic shifts following translocation than predicted based on long-term monogamy, and thus in an increased potential for genetic rescue (Weiser *et al.* 2013). My results combined with those presented by Vieco-Gálvez (2019), indicated that mixed parentage might be more common than previously appreciated and this may have played a role in the success of the *A. mantelli* population on Ponui Island.

Box 2. The Matuwa burrowing bettongs: another mixed origin translocation success

The mixed origin of the Ponui Island kiwi population makes it rare but not unique. One interesting case for comparison is the burrowing bettong (or boodie, *Bettongia lesueur*) population at Matuwa. Like kiwi, burrowing bettongs are the odd-one-out in their group by being nocturnal and burrow-dwelling (Sander *et al.* 1997, Short & Turner 1999). Another similarity is that predation by invasive mammals is a main cause of their decline, and thus that islands, predator-proof fencing, and translocations are key parts of their conservation management. Normally, one source population per translocation has been used for *B. lesueur*; Matuwa is the only example where two taxa (sometimes referred to as two subspecies) have been mixed. In addition, one of the sources is an island population that in turn was the result of an earlier translocation, comparable to LBI. Like Western and Northland *A. mantelli*, the parental taxa of Matuwa are believed to have been separated for long enough to allow local adaption (Donaldson *et al.* 2017). They also differ in size and breeding timing to an even larger extent than *A. mantelli* from LBI and Trounson Kauri Park respectively (Sander *et al.* 1997, Short & Turner 1999). Encouragingly, the outcome of these translocations has been similar, with both Ponui Island and Matuwa experiencing extensive admixture, rapid population growth, transgressive segregation for some traits, and higher genetic diversity than parental populations and single-origin translocated populations (Rick *et al.* 2019, Thavornkanlapachai *et al.* 2019).

However, there are some important differences. First, while the Ponui Island population was established with 14 birds, 154 burrowing bettongs were released at Matuwa (Rick *et al.* 2019). Second, admixture of the two genomes was very rapid at Matuwa for three reasons: (1) size matching was found to be common, resulting in an overrepresentation of mixed pairs with males from the morphometrically smaller and females from the larger source population, (2) a harem like breeding system resulting in high variation in parentage between years, (3) a

rapid life cycle with sexual maturity at 7-8 months and a lifespan of about 3 years (Rick *et al.* 2019, Thavornkanlapachai *et al.* 2019).

This rapid admixture enables research that would be highly informative but difficult and time-consuming in kiwi, such as studies of survival rate and lifetime reproductive success. Such studies in the Matuwa population have found no support for outbreeding depression in terms of survival rate; individuals of all levels of admixture had equal or higher survival rate than pure individuals of either parental population (Rick *et al.* 2019). In fact, the survival rate was found to peak for F1 hybrids, which is consistent with heterosis (or hybrid vigour; Duvick 1999). For reproductive rate, the picture is more complex, Rick *et al.* (2019) found that 50-100% ancestry from the previously bottlenecked island population (hereafter 'DRY') was associated with a lower recruitment rate. This scenario could lead to genomic representation from this source diminishing over time. Indeed, after five years, no sampled individuals had a pure DRY identity. However, the high survival of individuals of all degrees of mixtures, and the fact that individuals with less than 50% DRY had equal or higher recruitment success as purebred individuals from the second source population could counteract such reduction. Indeed, from the fifth year onwards, the proportional representation of DRY remained relatively steady. In addition, the breeding strategy of burrowing bettongs suggests that facilitated immigration, i.e., supplementing with more DRY individuals, would likely be a successful strategy for ensuring long term representation of DRY genetics.

As I have shown in this thesis, hybridization is primarily a way of increasing variation. There are many examples of this in the literature, with one famous one involving the Darwin finches of the Galapagos Islands (Grant & Grant 2008, Grant & Grant 2014, Lamichhaney *et al.* 2016, Lamichhaney *et al.* 2018). In saying that, the hybridised

genomes can combine in many different ways, thus the diversity in a population after translocation is rarely equal to the additive diversity of the two parental populations. Part of why predicting outcomes remain challenging is the continuous referencing to two competing theories for heterosis and inbreeding depression published in 1908 (Davenport 1908, East 1908). I argue in section 1.3 that the reason we have not agreed that one hypothesis best explains available data is because neither is particularly good. This lack of theoretical predictability feeds into the hesitation described above of causing outbreeding depression, homogenisation, or replacement and thus loss of diversity, which has resulted in that inaction has often been considered the safer option from a conservation perspective. I and many others (Hoffmann *et al.* 2015, Hamilton & Miller 2016, Weeks *et al.* 2016, Frankham *et al.* 2017, Liu *et al.* 2017, Ralls *et al.* 2018, Taylor & Larson 2019) argue that this fear is unjustified and harmful.

The scenario in which lower than expected post-translocation diversity is most likely to happen is when differences between source and target population are due to local adaptation to different environments, since this can result in outbreeding depression and replacement. Luckily, studies have found that if taxa have evolved in similar enough environments, thousands of generations apart might still not cause outbreeding depression upon admixture (Edmands 1999, Rundle *et al.* 2000), and even when evolving in environments that differ significantly in terms of selection pressures, incompatibility and, thus, outbreeding depression is predicted to take 20 generations or more to develop (Frankham *et al.* 2011). In addition, if the environment at the target site is expected to change, source populations with local adaptation to a different environment might even be desirable.

Another factor that increases the risk of lower than expected post-translocation diversity is that target populations often suffer from inbreeding which has resulted in some level of negative fitness effects (inbreeding depression). Thus, it is likely that the introduced individuals or the first-generation (F1) have superior fitness compared to 'pure' target population individuals. The larger this difference in fitness, the larger is the risk of genetic replacement (Pritchard & Edmands 2013, Todesco *et al.* 2016, Lipshutz *et al.* 2019). Similarly, if F1s have lower fitness than the target population (generally referred to as outbreeding depression) replacement can also occur. However, a more likely scenario is that the post-translocation population contains no 'purebred' target individuals, but that most individuals have a mixed genomic identity, especially if fitness differences for individual traits counteract each other (see for example the burrowing bettong example in Box 2). In both the case of borrowing bettongs at Matuwa (Box 2) and brown kiwi on Ponui Island, the parental taxon that was found to be underrepresented post translocations (but not fully replaced) was the one with the most drastic bottleneck history (assuming that Waipoua is the true second parental population of Ponui Island). These observations might appear discouraging since the aim of reinforcement translocations will often be to rescue bottlenecked target populations. However, it is important to consider that both Ponui Island and Matuwa represent mixed introductions (not reinforcements) and that the number introduced individuals from either parental taxon was about half-and-half. In a reinforcement scenario, the number of individuals representing the bottlenecked or inbred taxa (the target population) will most likely be substantially larger than the number of individuals supplemented from the comparatively outbred source population. In addition to keeping the number of introduced individuals low, other strategies for reducing the homogenization and replacement risk are introducing individuals of only one sex,

removing offspring of one sex, and/or removing the introduced individuals after breeding (Johnson *et al.* 2010, Bateson *et al.* 2014, Mussmann *et al.* 2017). Another strategy is removing individuals from the target population if it is deemed close to carrying capacity (for instance in the case of small islands like Moturua). Timing of the supplementation is also likely to be important since this will influence the likelihood of mixed pairing in many species.

For some very small taxa with a high risk of extinction, surviving as partial genetic material following hybridisation might be the only viable option (Bennewitz *et al.* 2008, Mallet 2008, Becker *et al.* 2013, Prüfer *et al.* 2014, Sankararaman *et al.* 2014, Taylor & Larson 2019). Facilitating hybridisation for this reason has, for instance, been identified as the preferred management strategy for the last remaining Italian red-legged partridge (*Alectoris rufa rufa*) population (Hamilton & Miller 2016, Forcina *et al.* 2020). In addition, how undesirable homogenisation or replacement is, also depends on the goal of conservation management. For instance, if the goal of management is maintaining rich and functional ecosystems, perhaps homogenization and genetic replacement are of less concern (Hunter *et al.* 2013, Lunt *et al.* 2013, Stronen & Paquet 2013, Janes & Hamilton 2017, vonHoldt *et al.* 2018, Matzek *et al.* 2019). For some parts of New Zealand, the original kiwi taxa are now extinct, thus genetic replacement is the only option if *Apteryx* spp. is decided to be a desirable feature of the ecosystem, for instance, in the most southern part of the North Island and along the east coast of the South Island (Shepherd *et al.* 2012, Weir *et al.* 2016, Germano *et al.* 2018).

Providing that the target and source individuals successfully interbreed, selection and genetic drift will be acting on the combined genome and further change the post-translocation diversity. The respective rate and strength of these two forces will cause

mixed populations to sit along a gradient between random and highly structured allelic associations where certain alleles from either parental population are overrepresented. Random associations and over representation are consistent with genetic drift being the dominating force shaping allele frequencies while directional selection will result in structured patterns of over- or underrepresentation (Figure 5.1.2). In sufficiently large populations, selection can, for instance, lead to the introgression of particular adaptive traits from the source population, while the rest of the target population's genome can remain relatively unchanged (Fitzpatrick *et al.* 2010, Song *et al.* 2011, Pardo-Diaz *et al.* 2012, Smith & Kronforst 2013, Hoffmann *et al.* 2017). In a smaller population, drift will be the dominating force which over time will result in loss of diversity and random allele fixation and introducing diversity will reduce the rate of fixation. In other words, the specific traits present in the source population and the size of the target population post translocation will both affect the future genetic profile.

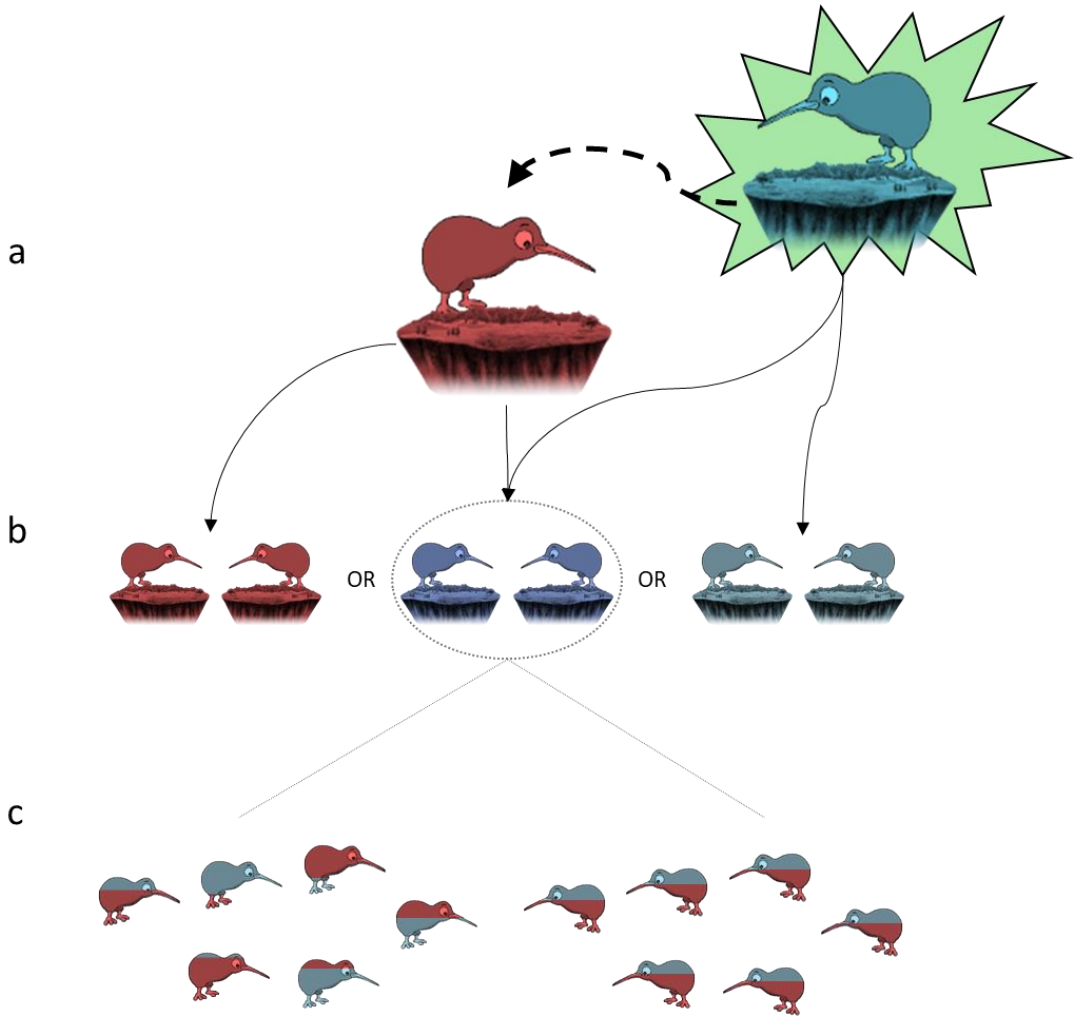


Figure 5.1.2. Schematic illustration of possible population-level outcomes of reinforcement translocations from a source (blue) into a target population (red; a). After sufficient time, there are three basic options for the genetic makeup of the population (b): (left) an all 'red' population indicates that the introduced individuals failed to establish, or went extinct, for instance, through low fitness of hybrid offspring combined with exclusively mixed mating of the 'blue' birds. (right) if the introduced individuals are superior enough to outcompete the original individuals and any mixed offspring, genetic replacement is possible, resulting in a 'blue' population. (middle) if none of these extremes occurs, the genomic makeup will be mixed. Upon closer investigation, the mixed population can fall along a spectrum. At one end of this spectrum is a hybrid swarm with seemingly random individual makeups of 'red' and 'blue' genomics. This is consistent with high compatibility and low influence of selection. At the other end are populations in which particular 'red' or 'blue' alleles are overrepresented, this suggests the presence of selection and that the genetic difference between 'red' and 'blue' is likely to be partly caused by local adaptation. Two special cases along the spectrum might warrant concern. First, if the genetic identity of each individual is 100% 'red' or 'blue', this can suggest that pre-

or post-zygotic barriers prevent either mixed mating from happening or from producing viable offspring. Under such conditions, genetic rescue will not occur. The second case is an extension of the first where a large percentage consists of fifty-fifty 'red' and 'blue' makeups which suggests that mix breeding has occurred but that the F1 hybrids are not reproducing, possibly as a result of outbreeding depression.

Lastly, I suggest that the genetic outcomes of translocations need to be put into a wider context. As long as populations remain cut off from geneflow and translocations are seen as independent events designed to help individual target sites, diversity will continue to be lost and the effect of genetic rescue may be large shortly after a translocation but will diminish over time. Noting that this kind of temporary effect for kiwi will last many years, probably decades, additional translocations will be needed eventually. However, there is another option, which is using translocations as a way of restoring connectivity and creating opportunity for reoccurring gene flow through unassisted dispersal of birds between population within reach. A detailed example of how translocations have been used to successfully increase diversity, connectivity, and population growth in Scandinavian Arctic foxes is provided in Box 3. The Kiwi Recovery Plan states that 'Genetic principles must be applied when managing kiwi', and that genetic diversity is 'an important factor' and 'critical' for longer- and shorter-term success and has done so in some form since the first edition from 1991 (Butler & McLennan 1990, Germano *et al.* 2018). However, to my surprise, the importance of increasing connectivity is mentioned nowhere. For instance, while the level of diversity within *A. mantelli* is mentioned to be 'good', there is no strategy for increasing access to this diversity within or between currently recognised management units.

Box 3. Managing fragmented populations, and arctic fox example

The arctic fox (*Vulpes lagopus*) is a small canine native to the area around the arctic circle (Fuglei & Ims 2008). At the southern end of its range, it is confined to the high alpine zone, which is a naturally fragmented habitat. Thus, the distribution of arctic fox in central Scandinavia would historically have been better described as a metapopulation system rather than continuous. During the 1800s and early 1900s, the arctic fox population was severely reduced and fragmented by hunting for fur, taking the Scandinavian population down to 40-60 individuals (Nyström *et al.* 2006, Elmhagen *et al.* 2017). This resulted in legal protection of the species that has now been in place for over 80 years. However, despite an inherent potential to grow rapidly through increased litter size during favourable conditions, the expected population increase did not happen (Angerbjörn *et al.* 1995, Tannerfeldt & Angerbjörn 1998, Elmhagen *et al.* 2017). Seemingly, population numbers, connectivity, diversity, or a combination of these, had gone below a crucial threshold value. In addition, new threats appeared mainly the spread north of the larger and more competitive red fox (*Vulpes vulpes*), and the disruption of the four years rodent cycles (Frafjord *et al.* 1989, Angerbjörn *et al.* 1995, Kausrud *et al.* 2008). Both these threats are likely partly linked to climate change (Kausrud *et al.* 2008).

To support this iconic species, a triple edged approach was launched in the late 1990s/early 2000s: culling of red foxes, supplementary feeding, and reestablishment (through releases) of populations in strategic areas of local extinction (Elmhagen *et al.* 2017). This was highly successful, with numbers of foxes, as well as fox populations, increasing, resulting in restored metapopulation dynamics between previously isolated areas. The increased connectivity boosted population growth (Hasselgren *et al.* 2018, Hemphill *et al.* 2020), suggesting that low genetic diversity was holding the populations back. In other words, migration resulted in genetic rescue and reversal of inbreeding depression. While this geneflow increased diversity within, it also reduced genetic difference between populations; a desirable result since

differences were deemed to represent founder effects and random drift rather than local adaptation.

This management has been a success story. A specific example is the population at Helags (Hasselgren *et al.* 2018). This population was deemed to genetically stem from only six founders, and effectively all breeding individuals were half-siblings. Luckily, after the establishment of a population 250km away, three male foxes immigrated to Helags. The F1 offspring between immigrants and local females were found to have higher survival and breeding success, resulting in rapid population growth, and increased genetic diversity. However, genetic diversity did not increase as much as might be expected for several reasons (1) the tight bottleneck of 40-60 individuals has likely led to the total artic fox gene pool in Scandinavia being relatively small, (2) the natural, food availability linked, fluctuations in this species means that effective genetic population size is even smaller, reducing the gene pool even further, (3) reproductive success at Helags was skewed towards two of the three immigrants, and lastly, (4) the two successful immigrants were full brothers (Nyström *et al.* 2006, McEachern *et al.* 2011, Hasselgren *et al.* 2018, Hemphill *et al.* 2020). Considering this, it is likely that the genetic rescue and reduced inbreeding will be a rather short-term effect unless further immigration happens. Thus, going forward, maintaining the re-established metapopulation dynamics will be a key focus for arctic fox management.

5.1.4 What is next?

It is established that genetic diversity is important for, for instance, creating resilient populations that can rebound once other threats, such as introduced predators, have been removed (Jamieson 2007, Smyser *et al.* 2013). Meanwhile, new threats are emerging, in particular climate change, and genetic diversity will be crucial for allowing, especially, populations of long-lived species with low dispersal capacity to respond to this (Hoban

et al. 2020, Young & Duchicela 2020). Thus, managing genetic diversity should be thought of as a way of ensuring that all the hard work put in by volunteers and conservationist across New Zealand into pest-control was worthwhile. However, to establish an optimal genetic management plan that can maintain genetic, species, and ecosystem diversity, there are still a number of studies that should be conducted, many of which could have implications far beyond *Apteryx* conservation.

Globally, many studies of inbreeding and genetic diversity have focused on species such as little spotted kiwi and kakapo that do not have large, genetically diverse populations available for comparison (Bouzat 2000, White 2013, Taylor 2014, Taylor *et al.* 2017c). Luckily, *A. mantelli* still has a wide spectrum of populations that range from diverse, mixed-origin populations like on Ponui Island and small populations with known history like on Moturua, to relatively pristine mainland populations such as in Trounson and mainland populations that are recovering from bottlenecks such as those on the Purerua Peninsula and on Rakaumangamanga. In addition, the sample collection over the last several years by Isabel Castro's group should have generated confidence that blood sampling of kiwi is not as challenging as previously thought. Furthermore, Angelia Hura's ongoing work has produced promising, preliminary results suggesting that feathers may be used for high resolution genomic analyses (Appendix A1). Furthermore, my analyses show that night and day catching should give comparable sample subsets (Appendix A2), which allow future studies to adjust their catching method to the conditions of each site and still be confident in their results. Together these things make *A. mantelli* a promising study system for further research in this field.

I suggest that priority should be given to comparative studies that are utilising the span of populations to investigate of inbreeding levels and symptoms of depression.

Preferably, these should be designed to identify informative markers of fitness that avoid the need to measure reproductive success over decades. One candidate indicator is sperm quality, which has been investigated in relation to inbreeding in many other species (White 2013, Opatová *et al.* 2016). Another candidate is genome-wide or gene-specific DNA methylation (see below). Dense and thriving populations like the one on Ponui Island should be considered for well-designed experimental studies. Such studies could, for instance, involve moving a known number, with known genetic makeup, off Ponui Island to other islands, to study population growth rate of diverse birds relative to individual level of admixture. A similar set up would also be ideal for elucidating the role of density on population growth, mate choice, and prevalence of mixed parentage. In addition to such experiments, I suggest that taking genetic samples from all birds prior to translocations should be considered best practice, since this will allow for future study how diversity changes over time.

Additional study of mate choice and pairing after introductions and reinforcement translocations would be important to advice on strategies for optimising admixture and diversity. Based on the results and discussion in Chapters 2.1 and 2.2, studies of mate choice should focus on factors for kin recognition and the role of MHC diversity and its relationship to fitness would be highly informative. These studies are of particular importance since reduced immune competence is one of the main concerns for bottlenecked and inbreed populations (Hale & Briskie 2007), such research could resolve whether the surprisingly high MHC diversity in Rowi is linked to disassortative mate choice and heterozygosity benefits. In addition, MHC diversity or identity has been found to be an informative marker for adaptive diversity in other species (Manlik *et al.* 2019), and this potential should be investigated in kiwi.

In saying that, I think that the most important outstanding question for advancing *Apteryx* genetic management is the role of local adaptation for diversification within and between kiwi species. Based on the results of Rundle *et al.* (2000), Edmands (1999), and Frankham *et al.* (2011), such studies should focus on historic records and habitat features of importance to kiwi to determine if the amount of time populations have spent isolated, and the environmental conditions they experience, are long and different enough to expect divergent local adaptation. If the conclusion of such investigations is that local adaptation to different habitats is likely, genetic support for this hypothesis should be examined. One reason such examinations has not gotten the attention it deserves is that until recently, quantification of genetic divergence has been based on a few loci with limited correlation to fitness, management needs, and translocation outcomes (Bonin *et al.* 2007, Putman & Carbone 2014, Jeffries *et al.* 2016). With genomic tools, we finally have the ability to link genetic difference to fitness and local adaptation. A good start would be using the dataset generated for this thesis to determine (1) where the Ponui Island population fall along the spectrum illustrated in Figure 5.1.2 and (2) what the genes with a signal for selection (if any) code for. After that, studies of selection and adaptation should include an increasing spread of populations. Preferably, all collected samples for these and other Apteryx studies should become part of a national genetic survey with uniform sampling and storage guidelines that can enable easy and respectful future use of them. This sample collection can, for instance, prove important since the investigation of genomic sequence in unlikely to provide all the answers.

5.1.4.1 Epigenetics

It is a longstanding paradox in evolutionary biology and taxonomy that the observed levels of genetic divergence between two lineages frequently correlates poorly with

their levels of phenotypic difference, plasticity, adaptive potential, and persistence (Nussey *et al.* 2005, Valladares *et al.* 2006, Milot *et al.* 2007, Johnson *et al.* 2009, Reed 2010, Rheindt *et al.* 2013, Merilä & Hendry 2014, Senn *et al.* 2014, Forsman 2015, O'Dea *et al.* 2016, Seneviratne *et al.* 2016, Branch *et al.* 2017). A leading hypothesis is that this discrepancy is explained by difference in expression levels of genes rather than in sequence identity. This hypothesis suggests that analyses of transcriptomes would be highly informative (Mason & Taylor 2015, Jax *et al.* 2018). Some investigations into kiwi transcriptomics have been done, but mainly to explore differences compared to chicken and thus elucidate questions around avian evolution (Subramanian *et al.* 2010, Ramstad *et al.* 2016). Thus, further study would be needed in order to address questions about local adaptation.

However, transcriptome analyses are expensive, and RNA is unstable, thus, another marker type of great interest is epigenetic modifications of the DNA sequence (Miryeganeh & Saze 2019, West-Eberhard 2019; Figure 5.1.3). Epigenetic modifications can increase or decrease the activity of promoter regions and regulatory elements, or control the physical folding of the DNA, all of which alters the exposure of genes to transcription and, ultimately, the transcriptome (Johnston *et al.* 2019). Studies in plants have found that patterns of epigenetic diversity combine and change in a non-additive way after hybridisation, which has been linked to observed hybrid phenotypes and heterosis (Vergeer *et al.* 2012, Groszmann *et al.* 2013, Kawanabe *et al.* 2016, Lauss *et al.* 2018, Sinha *et al.* 2020). Hybridisation can thus potentially not only contribute with increase genetic diversity in strict terms, but also with biodiversity on an epigenetic level that has a direct effect on phenotype, fitness, resilience to change, and survival (Cheptou & Donohue 2013, Biémont & Vieira 2014, O'Dea *et al.* 2016). We also know that epigenetic changes can be inherited between generations in birds

(Guerrero-Bosagna *et al.* 2018). Lastly, age evaluation in long-lived animals is also increasingly turning towards epigenetics (De Paoli-Iseppi *et al.* 2017, De Paoli-Iseppi *et al.* 2019); one example is an ongoing project at Otago university which is assessing the relationship between age and DNA methylation in Kakapo (after a previous study found no correlation between age and telomere length; Horn *et al.* 2011).

Consequently, studies of epigenetic diversity and the causally linked transcriptomes in kiwi populations before and after translocations, as well as between areas and management units, are likely to provide crucial information for understanding current diversity and its implications for management. One of the most common and researched types of epigenetic modifications is DNA methylation, and the prevalence of this type of modification can be quantified genome-wide or at specific genetic loci (Jelinek & Madzo 2016, Trucchi *et al.* 2016, Lam *et al.* 2020). Developing protocols for DNA methylation investigations and their interpretation in *Apteryx* has the potential to be revolutionary and could potentially alter the field of conservation genetics in a similar way that the introduction of genomic methods has done over the last decade (Sepers *et al.* 2019, Rey *et al.* 2020).

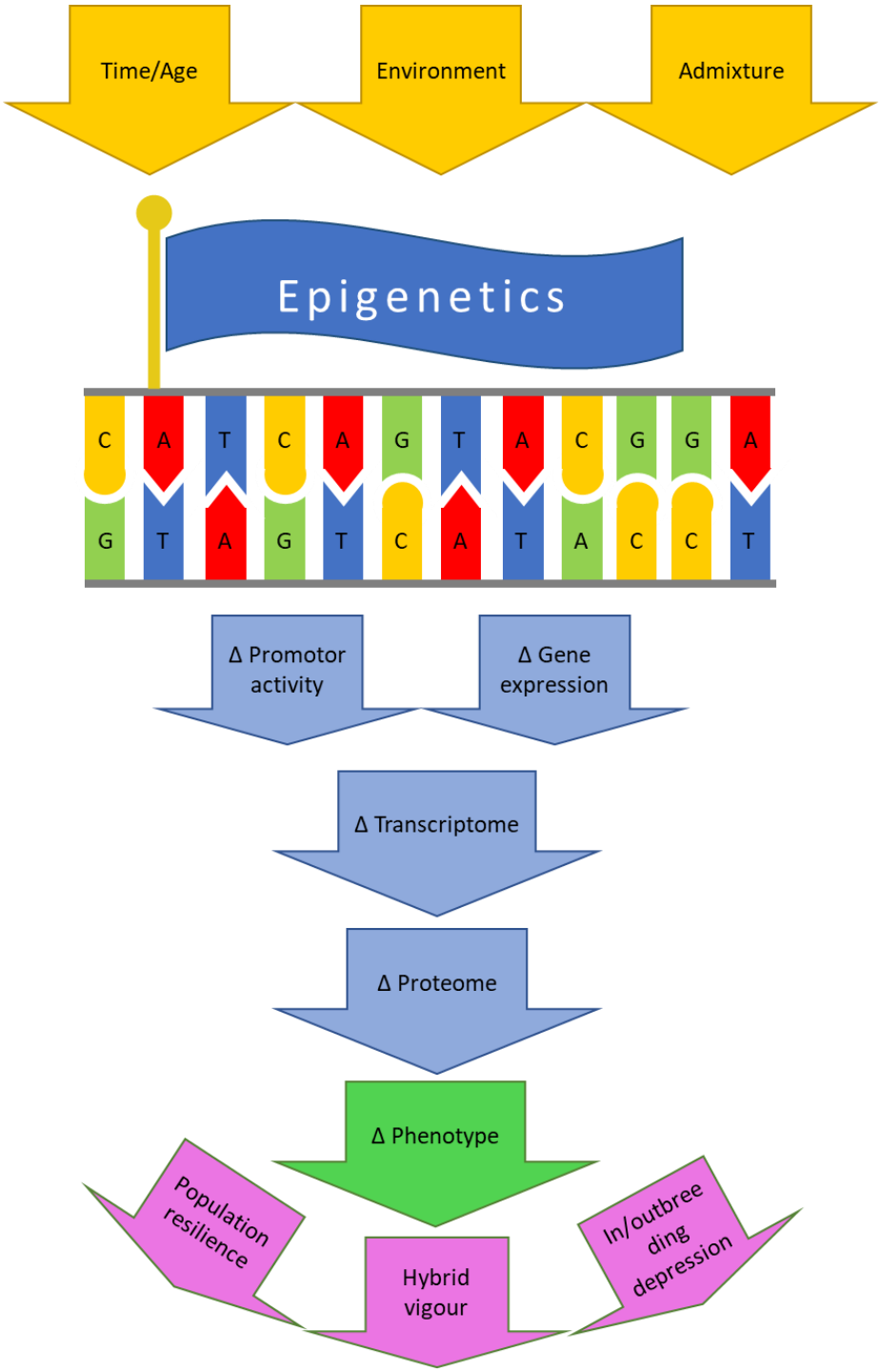


Figure 5.1.3. Schematic illustration of three key factors discussed in this thesis (yellow arrows) and the pathways through which they affect the epigenome (the epigenetic modifications of the genome, such as methylation and chromatin packaging). Modifications of the epigenome lead to alterations (blue arrows) that govern the phenotype of an individual and its plasticity, which affect properties of a population directly linked to conservation outcomes (pink arrows).

5.1.5 Concluding remarks

At the start of this thesis, I listed four research goals. First, I hoped to critically review our knowledge about *Apteryx* genetics, genetic rescue, inbreeding, and outbreeding depression, and highlight how lack of data and widespread misconceptions affect conservation management and translocation policy. This work resulted in two peer-reviewed publications that both emphasis the potential of further *Apteryx* studies to fill crucial knowledge gaps.

My second goal was to examine breeding strategies and mate choice in *A. mantelli* and investigate potential implications for translocation outcomes, outbreeding depression, and genetic rescue of these behaviours. The key take-home message was that brown kiwi do interbreed, are not as strictly monogamous as the current literature claim, and that both these factors suggest that reinforcement translocations are likely to result in steady but slow admixture followed by genetic rescue.

Thirdly, I aimed to examine whether introducing genetic variation through mixed source translocations could (a) out-weigh the risk of causing outbreeding depression or homogenisation, and (b) generate suitable source populations for further translocations. My results suggest that the Ponui Island population could be a diverse and accessible source, which is in stark contrast to previous claims of these birds being of 'no genetic value whatsoever for use in restoration'. In addition, the diverse and highly successful Ponui Island birds arguably constitute a study system of international interest.

Lastly, I took the first ever look at *Apteryx* telomeres to evaluate if their length could be an informative indicator for evaluating translocation needs and outcomes as well as determine the age of adult kiwi. While I identified critical factors for future telomere

studies of long-lived species, the main take-home message was that telomere length was not a good marker for kiwi age.

By addressing these goals, I was ultimately hoping to have a direct impact on *Apteryx* conservation management. For instance, I believe my work contributes towards two objectives and one action listed in the 2018-2028 Kiwi Recovery Plan: Objective 3.3 '*To clarify the uncertainties in the taxonomy of kiwi and the roles of hybrid and mixed provenance populations for kiwi management*,' Objective 3.2 '*To ensure managers and practitioners have an understanding of the importance and practical application of genetic principles in kiwi management'*, and Action 10.3 '*Develop a national kiwi translocation strategy to guide future releases, including where translocations would be an inappropriate tool.*' I hope my work inspires some revision of future national kiwi recovery plans as well as the individual taxon plans. In particular, I suggest that the new or adjusted issues listed under genetic management should be:

1. The unclear meaning of the statements that (a) current levels of genetic diversity is 'sufficient' within *A. rowi* and *A. australis* Haast is, and 'good' within *A. mantelli*, *A. haastii*, and remaining *A. australis*, and (b) the management should maintain genetic diversity 'as much as is feasible', are too vague to set useful goal for genetic management and raises questions around how and on what scale diversity should be quantified.
2. The lack of understanding concerning the extent and significance of local adaptation for *Apteryx* diversification is currently limiting our ability to (a) constructively delimit management units, (b) evaluate the risk of causing outbreeding depression, and (c) develop translocation guidelines with specific recommendations on how to choose source populations.

3. Due to a lack of comparative studies between mixed, inbred, and relatively pristine populations, it remains impossible to predict (a) when inbreeding depression will take effect and which traits will be affected, as well as (b) how supplementary translocations should be conducted to optimise chances of genetic rescue.
4. Insufficient focus has been given to the low connectivity within and between current management units.
5. Population evaluations are currently limited by a lack of informative markers needed to assess individual and population-level fitness.
6. The regular use of threshold numbers for founder size and the number of offspring harvested as a proxy for retaining genetic diversity is imprecise and risks causing unnecessarily narrow genetic bottlenecks, for instance when moving birds to and from kōhanga sites.
7. The current Department of Conservation research-permitting system is very slow moving and risks delaying, and discouraging, pivotal studies that would improve *Apteryx* conservation management.

There will be no one silver bullet for future *Apteryx* management; on the contrary, a highly integrative approach is needed (Taylor & Dizon 1999, Smyser *et al.* 2013, Gaggiotti *et al.* 2018, Young & Duchicela 2020) that include the perspective of for instance PF2050 (Parker *et al.* 2020), Te Tiriti o Waitangi, habitat restoration, public awareness, genetic diversity, and research. Many questions remain unanswered. However, the spectrum of *A. mantelli* populations with different sizes, diversity, backgrounds, management, and taxon identities is a promising model system for answering questions of importance to this and countless other species in need of clearly defined genetic management policies (Pierson *et al.* 2016). Yet, no one person or

project can hope to find every answer needed for predictable, fool-proof translocations. We must not let this discourage us. I had the honour to attend a talk by Te Rangitākuku Kaihoro on the topic of Māori world views. In this talk he conveyed that the only thing that any one person can aspire to is identifying measures that will take us closer to the solutions needed, even when you know that there is no way the problem will be solved within your (life) time. Rather than failure, taking steps towards a solution you cannot reach, must be thought of as paving the way for, and empowering, your successors – whether it be your grandchildren's grandchildren or the next postgraduate student – so that they main solve the problem and/or live to see the benefits the solutions bring. Thus, if my research has contributed to identification of important research routes to take from here, and in providing helpful recommendations for kiwi management, then my project will have been successful.

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Part 6 – Appendixes

Appendix 1

A.1 When what we have is what we get, comparison between old, but available, and newly collected samples for genomic analyses of *Apteryx mantelli*

Abstract

Ecology and conservation research of threatened species frequently includes weighing costs and risks against benefits when establishing sampling regimes. Sometimes the trade-off is between utilising already available and collecting new samples. Genomic analyses readily call for high-quality DNA and hence, including old samples is only a viable option if they can meet such standards. Herein the extraction and GBS library prep success of newly collected blood samples were compared to that of samples collected up to 15 years prior as well as to 9-year-old DNA. The results suggest that if the DNA concentration met the cut-off value and the gel electrophoresis showed a high molecular weight band, the performance of old and new samples in GBS library preparation was comparable. There are other considerations for including older samples in conservation genomic studies, however, if purely considering DNA quality we conclude that 0-15 years of storage in a -80C freezer need not be a problem for genomic analyses of *Apteryx mantelli* samples.

Introduction

Faced with the 6th mass extinction, conservationists are in desperate need of more knowledge about the ever-increasing number of threatened species to enable development of scientifically grounded and successful management strategies. A

growing number of scientists and managers recognizes that such strategies will require, or at least benefit greatly from, genetic or even genomic information (Allendorf *et al.* 2010, Hoffmann *et al.* 2015, Garner *et al.* 2016, vonHoldt *et al.* 2018, Bell *et al.* 2019). However, threatened species are rare and/or occur in remote areas, accredited samplers are often few, and many species are surrounded by extensive permitting requirements (see for instance Cardador *et al.* 2018). In addition, there is a need to recognize that sampling is often costly and invasive (Carroll *et al.* 2018, Swift *et al.* 2018) and thus that samples ought to be utilized as much as possible once collected. Consequently, it is tempting, often seemly wise, and sometimes truly the only option, to use already available samples for research (hereafter referred to as "old samples").

Potential issues with utilizing old but available samples can be linked to differences in DNA quality and/or quantity, which risk altering result as well as their interpretation (Seutin *et al.* 1991, Rawlence *et al.* 2009, Vilahur *et al.* 2013, Rahikainen *et al.* 2016). For instance, lower quality DNA increases the risk of so-called allelic drop out which can mistakenly be interpreted as homozygosity which in turn can result in overestimations of, for instance, inbreeding or population differentiation (Miller *et al.* 2002, Wang *et al.* 2012). Extensive allelic drop out also lowers the overall number of loci available for comparison which risk reducing accuracy in analyses and limit what questions can be addressed with confidence (Stenglein *et al.* 2010, Carpenter & Dziminski 2017). Although molecular and analytical methods are steadily improving to overcome problems in this category, they can only ever partly account for missing information (Bi *et al.* 2013, Russello *et al.* 2015). Meanwhile, high DNA quality is arguably becoming more crucial thanks to the increased use of modern genetic and genomic analyses methods (Elshire *et al.* 2011, Shafer *et al.* 2015, van Gurp *et al.* 2016).

Old samples can, of course, be utilised successfully, for instance for studies of phylogenetic relationship and analyses of changes over time (Shepherd & Lambert 2008, Hartnup *et al.* 2011, Shepherd *et al.* 2012, Mitchell *et al.* 2014). However, a second potential issue with including old samples, or a mixture of old and contemporary samples, it that this risk providing an outdated, biased, or in other ways incorrect picture in some scenarios. For instance, if comparing genetic diversity among populations, and the older and newer samples are from different geographical areas, identified population difference might represent temporal rather than spatial patterns (Lacy 1987, Navascues *et al.* 2010). This is not an implausible scenario. On the contrary, the opportunity to increase the geographical spread and/or to enable new sampling to focus in previously unvisited areas are leading arguments for including old samples in studies of threatened species. At what point age difference between samples risk affect results and their interpretation is likely related to the generation time of the organism, the population trends, and how the threat situation has changed over time (Hailer *et al.* 2006, Lippe *et al.* 2006, Kuussaari *et al.* 2009), hence deciding what is "too old" can be complex.

Including old samples in genetic studies of kiwi (*Apteryx* spp.) has been more rule than exception (for example Baker *et al.* 1995, Herbert & Daugherty 2002, Burbidge *et al.* 2003, Weir *et al.* 2016; see further section 1.2). In some cases, this has rendered up to around 30 years difference between the collection of the newest and oldest samples included in the same study, and in the cases when studies have included museum samples the difference has been thousands of years (Baker *et al.* 1995, Herbert & Daugherty 2002, Burbidge *et al.* 2003, Shepherd *et al.* 2012, Weir *et al.* 2016). One consequence of this is that samples are combined for which DNA has been extracted using vastly different techniques. Supplementary figure S1.2.4 highlights that many of the mitochondrial haplotypes identified in *Apteryx* have only been found in museum

samples. It is tempting to interpret this as a sign of vast loss of diversity over time, but a plausible alternative explanation that warrants further investigations is that new and old samples might not be directly comparable (Navascues *et al.* 2010).

Here I qualitatively and quantitatively compare the results of DNA extraction and reduced representation sequencing (genotype-by-sequencing; GBS) between three categories of freezer-stored blood samples from North Island brown kiwi (*Apteryx mantelli*): New blood (collected 0-2 years before extraction and analyses), Old blood (collected 9 to 15 years before extraction and analysis), and old DNA (extracted 9 years before analyses from blood collected 0-4 years prior to that). In addition, the analyses include DNA extracted from feathers. I compare these sample types in terms of extraction- and sequencing success and whether success differences could impact the results of diversity and relatedness comparisons within as well as between populations. The main objective of this was to justify (or dismiss) the utilisation of these different sample types for the different analyses presented in this thesis.

Methods and Materials

For samples collection and site, DNA extraction protocols, and GBS library preparation, see other chapters.

Descriptions and definitions of the sample types compared herein can be found in table A1.1.

Pre plating DNA evaluation and GBS performance

DNA was extracted from 10-50 µl thawed whole blood using a High Pure PCR template preparation kit (Roche). The exception was samples from 2010, which were stored as bed blood cells rather than whole blood. Manufacturer's instructions were followed with the exception that elution was done in two rounds with 50µl of elution buffer each

centrifugation round. The DNA extraction success and quality were validated using agarose gel electrophoresis (1-2% agarose in TAE buffer) and the concentration of DNA was measured using the Qubit dsDNA High Sensitivity assay (Life Technologies, CA, USA). The DNA was then diluted to a concentration of 20-100 ng per microliter, plated with 30 µl per sample and submitted for genotype-by-sequencing (GBS) library prep by the Elshire group (Elshire *et al.* 2011).

The GBS library was generated following the Elshire *et al.* (2011) method and included the following changes: 100 ng of genomic DNA were used, 1.44 ng of total adapters were used, the genomic DNAs were restricted with EcoT22i enzyme and the library was amplified with 18 PCR cycles. Based on the resulting library, call rate, read count, and read depth was compared using Anova (R package car; Fox & Weisberg 2019), combined with Tukey tests (R packages multcomp; Hothorn *et al.* 2008, and agricolae; de Mendiburu 2020) for post-hoc analyses. Samples were compared both with relation to sample type, sampling year, and extraction year. Lastly, comparison was done between sample reaching and those not reaching the requested minimum DNA concentration and/or a high molecular band visible through gel electrophoresis.

Effects on genetic analyses

SNP calling was carried out in the STACKS 2.5 pipeline. Based on the stacks output combined with reassembly of the published *A. mantelli* genome (Le Duc *et al.* 2015) allelic similarity was generated through using KDG (Dodds *et al.* 2015). After LD pruning 51 691 variable sites were utilised in the KDG analyses and the resulting unscaled allelic similarity matrix was used for analyses herein. This matrix was used to compare pairwise relatedness of biological replicates and to compare overall relatedness within Ponui Island and between the Ponui Island population and the Moturua population, depending on sample type was included in the analyses. Lastly, it was used

to examine grouping of samples based on sample type using the principal component analyses (PCA) in the online-based Embedding Projector Tensorflow (Abadi *et al.* 2015).

Table A1.1. Description of the sample types and qualities compared in this appendix.

Sample type	Description
New blood	Blood collected in 2017-2019 and extracted in 2019
Old blood	Blood collected in 2004-2010 and extracted in 2019
Old DNA	DNA extracted in 2010 from blood collected in 2004-2010
Feathers	Feathers collected in 2017-2019 and extracted in 2019

Sample quality	Description
Good samples	Samples that met the requested DNA concentration of < 20ng/µl and the requested gel electrophoresis band integrity (Elshire *et al.* 2011).
Bad samples	Samples of any type and/or age that failed to live up to either the requested DNA concentration of < 20ng/µl, the requested gel electrophoresis band integrity, or both.*

*Twelve 'bad' samples were included in the GBS library prep despite their substandard quality.

Results and Discussion

Pre plating DNA evaluation

DNA extraction from New blood and Old blood (Table A1.1) was found to equally often result in adequate DNA quantity and quality for GBS library preparation (i.e., being "Good samples"; Table A1.1; minimum 77 and 74 % of New and Old samples respectively; Figure A1.1). Sufficiently high DNA concentrations and high molecular weight bands were also achieved with similar frequency for the three cohorts within the Old blood samples (74 to 83% per cohort; Figure A1.1B), although it should be noted that sample size for the older cohorts was small. In addition, the Old DNA samples had maintained sufficient DNA concentration to a remarkably high degree (72 out of 73 samples; Figure A1.1), however, the original DNA concentration of these samples was unknown and thus the loss over time could not be quantified. The success rate from

feather was lower with only 26 % of the extractions resulting in both sufficient DNA quality and quantity (“Good samples”; Figure A1.1). In addition, the connection between high quality and sufficient quantity was relatively low for feathers, resulting in more samples being classified as bad when one but not both criteria were met. By contrast, for new and old blood, the same samples tended to underperform both in quality and quantity, resulting in overall fewer samples being classified as bad samples from these categories.

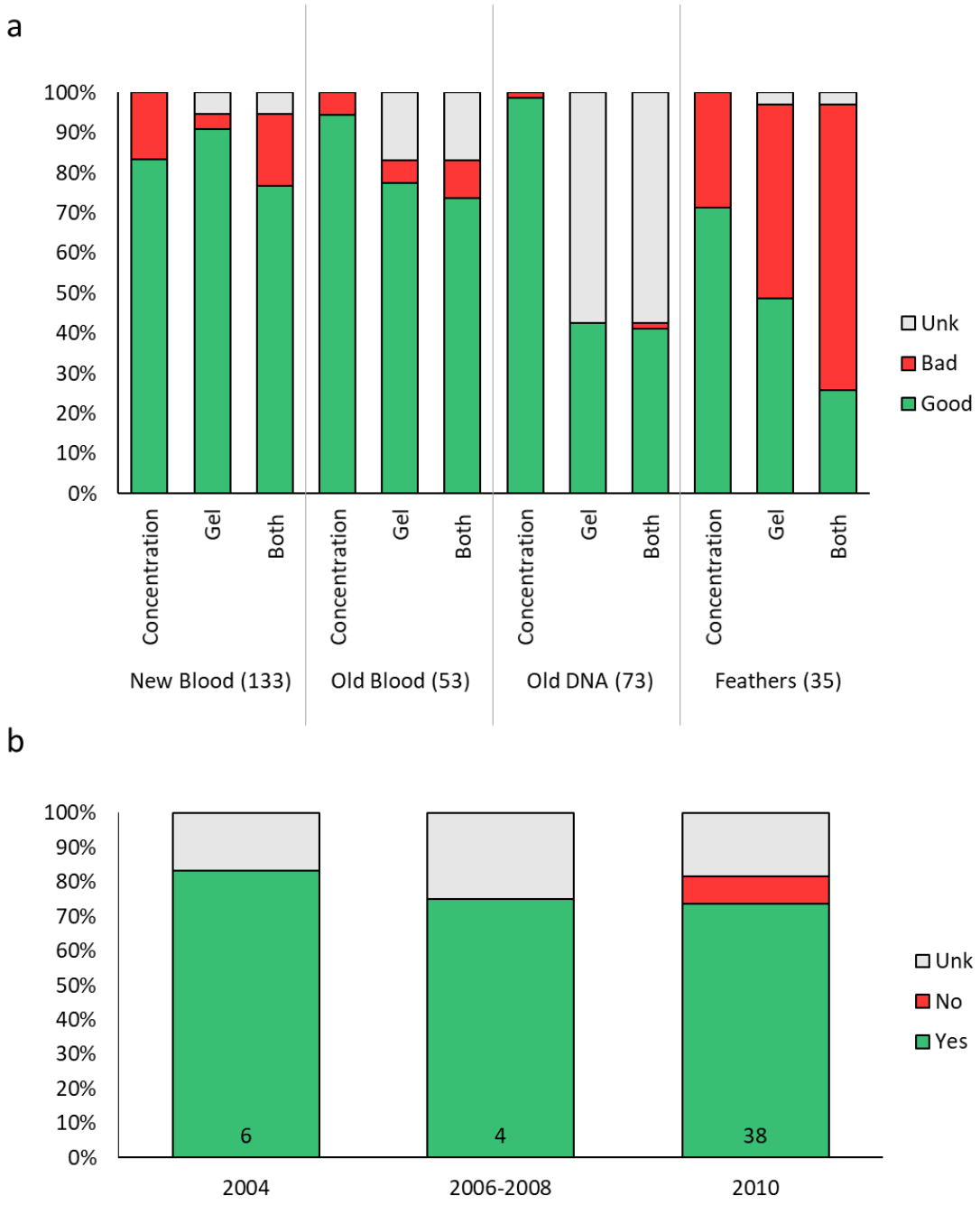

Figure A1.1. Comparison of DNA quantity and quality relative to sample type. (a) shows the proportion of extracted samples that lived up (green) or did not live up to (red) the requested DNA concentration for GBS library prep by the Elshire group (>20ng/µl; left bar in each group), gels showing a high molecular band, and (3) both the requested concentration and gel result (right bar in each group). "Unk" refers to samples for which a gel was never run and thus we do not know their quality (grey). The categories compared are "New Blood" referring to blood samples collected 0-2 years prior to extraction and analyses, "Old Blood" collected 9-15 years prior to extraction and analyses, "Old DNA" extracted nine years prior to analyses from blood collected 0-6 years prior to extraction, and "Feathers" refers to DNA extracted from

feather shafts of feathers collected 0-2 years prior to extraction and analyses. Numbers in brackets after each category state sample size. Panel (b) breaks down the "Old Blood" further into the sample cohorts it represents. Numbers inside bars state sample sizes.

GBS performance

The read count, call rate, and read depth for all 187 analysed samples were found to be highly correlated, thus the focus herein lies on the sample depth since this parameter is of greatest importance for analyses of performance (Beissinger *et al.* 2013, Loureiro *et al.* 2020). Firstly, Good samples performed better than Bad samples in terms of read depth ($F = 6.921$, $df = 1$, $p = 0.009$; see table A1.1 for sample type definitions). Looking at Good samples only, New blood resulted in significantly greater depth than DNA extracted in 2010 as well as DNA extracted in 2019 from feathers ($F = 8.364$, $df = 3$, $p < 0.001$; Figure A1.2a). Old blood samples performed intermediate although not statistically significantly different from any other type (Figure A1.2). Samples collected in different years produced slightly different depths ($F = 4.633$, $df = 1$, $p = 0.033$; Figure A1.2B), however, Post-hoc analyses failed to discriminate which years differed significantly from which suggesting such differences have weak support. The main difference was found to be linked to the year of extraction, with the DNA stored in a -80C freezer for 9 years performing less well than the DNA extracted in the year of analyses ($F = 23.1$, $df = 2$, $p < 0.001$; Figure A1.2c). However, the Old DNA samples still performed significantly better than the Bad samples ($F = 25.19$, $df = 1$, $p = <0.001$; Figure A1.2c).

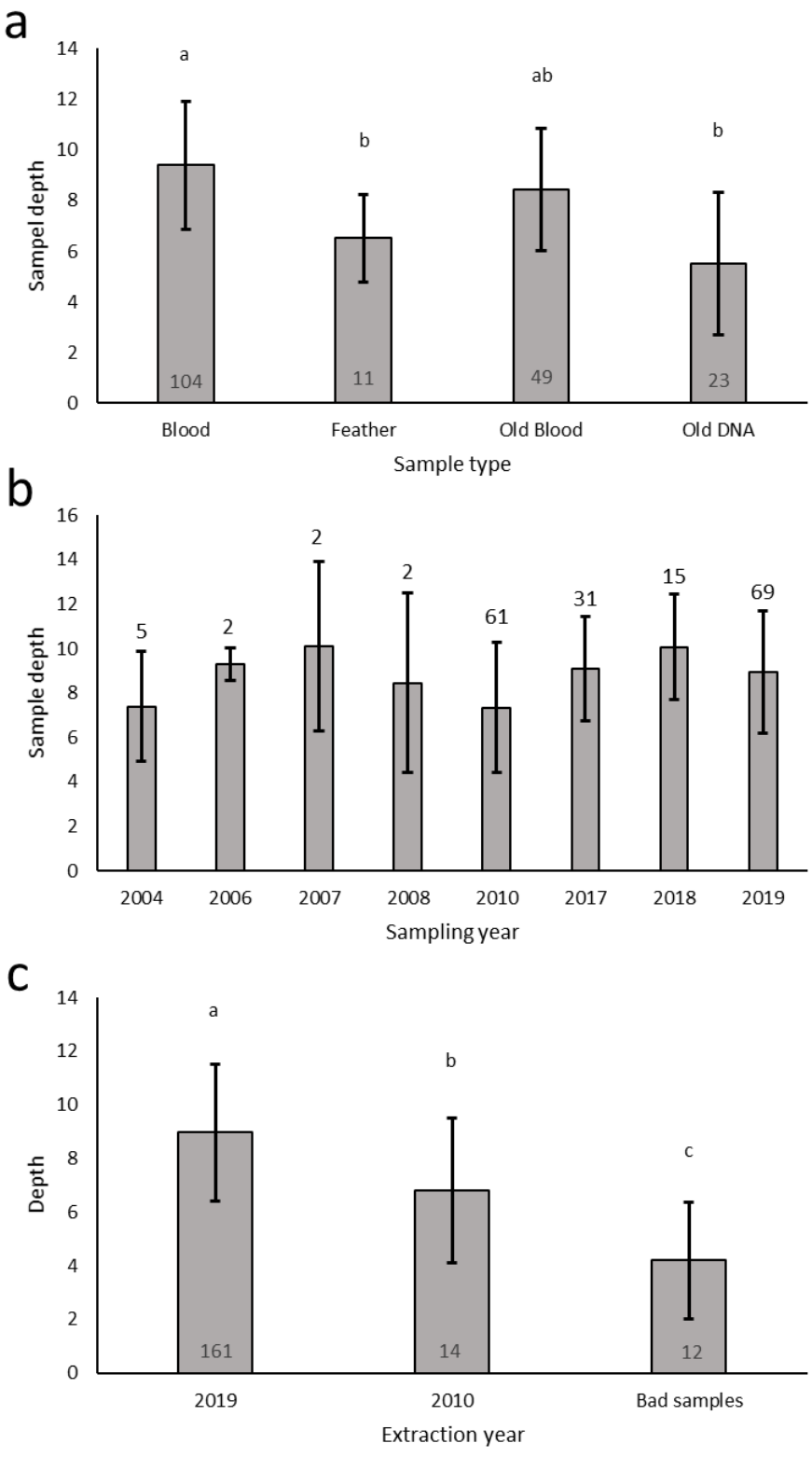

Figure A1.2. Comparison of GBS read depth with regards to sample type (a), sample year (b), and DNA extraction year (c). Sample types refer to: blood collected 0-2 years before extraction and analyses as "Blood", feather as "Feather", blood collected 9 to 15 years before extraction and analysis as "Old Blood" and DNA extracted 9 years before analyses from blood collected 0-

4 years prior to that as “Old DNA”. (c) also includes sample depth for samples with suboptimal DNA concentration and gel band integrity (“bad samples”) for comparison. Error bars represent standard deviation, numbers above bars represent sample size and different letters above bars represent significantly different groups (linear model anova).

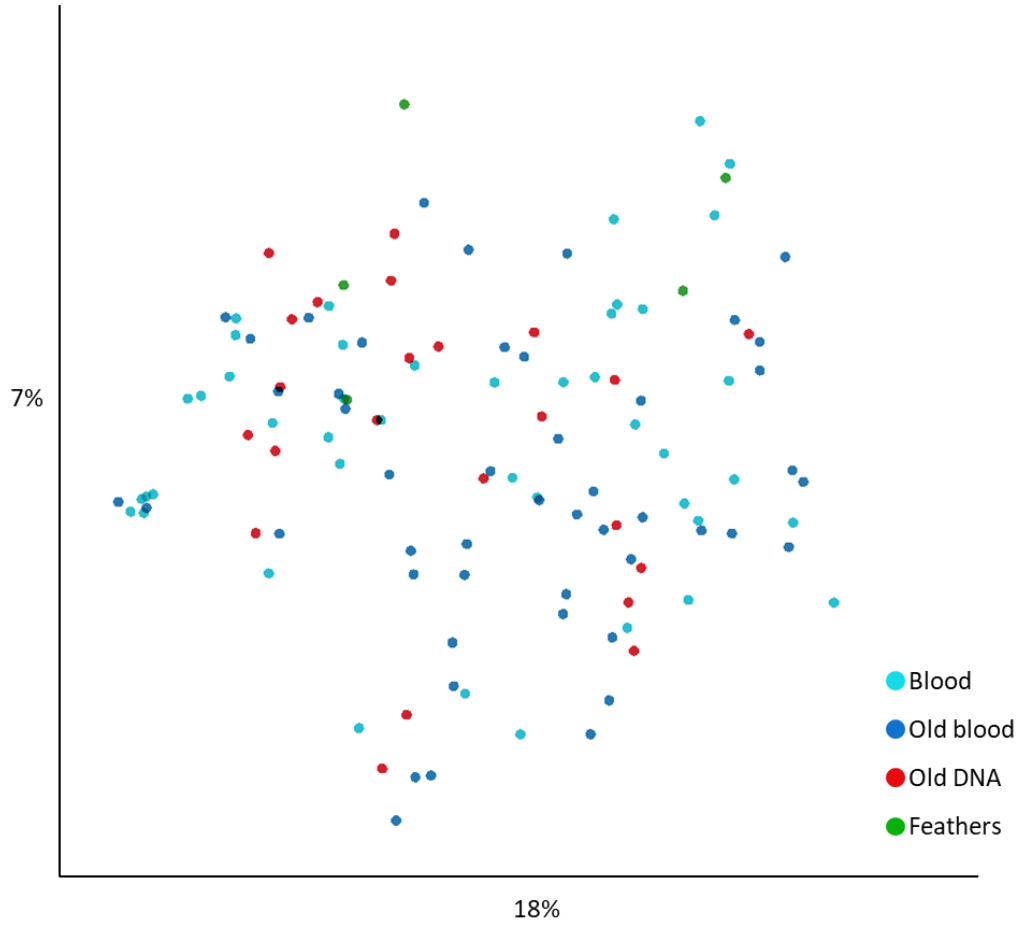

Figure A1.3. Principal component analyses (PCA) comparing allelic similarity between samples of different types in two dimensions. Twenty-six percentage of total variance is covered. Illustrating that there was go grouping among samples based on sample type or age. Dots represent the 120 analyses samples from Ponui Island, with colours representing the 4 sample types (see further Table A1.1).

Effects on genetic analyses

Looking only at the 120 samples from Ponui Island, there was no evidence for genetic structuring linked to sample type (Figure A1.3). In total, 18 samples occurred as biological duplicates in the GBS library and one as a triplicate. These repeated samples fell in five categories: one blood sample and one feather sample from the same bird; two new blood samples; one new and one old blood sample; an old sample with both newly extracted DNA and old DNA; and two different but both old blood samples. For all five categories, pairwise relatedness (allelic similarity) between duplicates was high (level of identical twins; Figure A1.4a). Furthermore, the average relatedness among Ponui Island birds (n = 85, using only the 2 GBS plates run in 2019), was remarkably similar when calculated based on New blood samples only compared to on all remaining sample types (Figure A1.4b). Similarly, the relatedness between Ponui Island birds and birds from another island population, Moturua, was found to be the same whether calculated based only on New blood samples from Ponui Island or on the other sample types (Figure A1.4c).

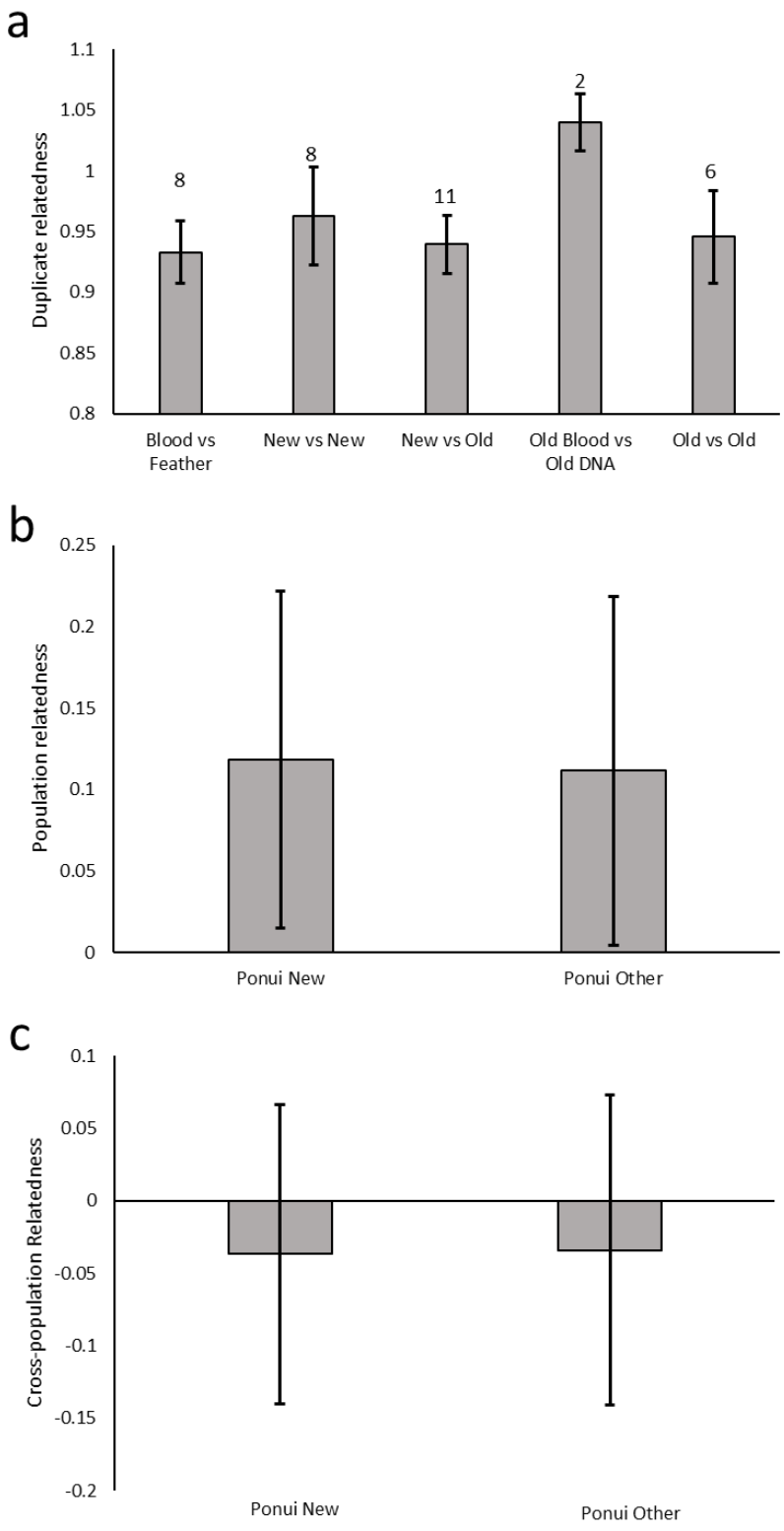

Figure A1.4. Comparisons of the effect of sample type on the estimation of average pairwise relatedness (allelic similarity) within and between populations. (a) Shows the high pairwise allelic similarity for all five categories of biological replicates i.e., two samples from the same bird. Numbers above bars in panel indicate sample sizes. (b) Shows the average apparent

relatedness among birds on Ponui Island calculated based on only New blood samples (left) or on a combination of the three remaining good sample types (see table A1.1 for definitions). (c) Shows the apparent relatedness between birds on Ponui Island and birds on Moturua calculated based on only New blood samples (left) or on a combination of the three remaining good sample types. Error bars indicate standard deviations.

Conclusion

We found that high DNA quality and quantity, as evaluated by qubit measured concentration and gel electrophoresis quantified quality, ensured good and comparable GBS library preparation results regardless of the age and the type for our samples. In terms of their basic performance during the establishment of a GBS library (sample depth), old blood samples did essentially as well as newly sampled blood, but old extracted DNA stored in a freezer for the last 9 years did not perform as well. However, the old DNA did perform significantly better than samples not reaching the recommended standards in terms of DNA concentration and gel band integrity; and even the less well-performing samples had a depth high enough for further analyses according to other studies (Elshire *et al.* 2011, Fumagalli 2013). Regardless of sample type combination, all paired repeats from the same bird (biological replicates) came out as genetically similar enough to be identical twins. In addition, analyses of both within and between population parameters of diversity and separation gave comparable results when based on new blood samples only or on other sample types.

Based on this we argue that despite some quantitative differences for some sample types, we did not detect qualitative differences large enough to affect population-level comparison. In other words, the sample type used would seemingly not have affected, for instance, what conservation management suggestions would be made based on these data. Consequently, I used the old sample for my thesis. I also encourage others to use old samples either in the form of DNA or blood, especially if this can increase sample

size/resolution/spread for conservation genomic analyses. It is, however, important to note that the age span of our samples was only 15 years, which is substantially shorter than the lifespan of *Apteryx mantelli*. I do call for caution, noticing that the generation time and how the threat situation of a species has changed over time, could set an upper limit beyond which including older samples in diversity analyses risks providing an old picture. While this does not necessarily need to discourage utilizing old samples, it highlights a need to be aware. Sample quality is likely the main limiting factor for including old samples even though we did not find such an effect here. However, the risk of a slightly less accurate analysis due to lower or mixed sample quality must be weighed against the stress, harm and cost imposed by additional sampling. It is particularly encouraging that feathers seem to do well enough to potentially allow for less invasive sampling in future studies, even though the success rate in the extraction step was lower for this sample type and further protocol optimisation is warranted.

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Appendix 2

A.2 It is not like night and day! Capture method or time of sampling did not bias *Apteryx mantelli* sample set or blood parameters

Abstract

Sampling bias can have dire consequences for research. One potential source of bias is differences in sampling methods. If preferred sampling method is linked to site features such as population density and terrain, sampling-method-effects risk altering the outcomes in population comparisons. Here we compare two methods for catching *Apteryx mantelli*, North Island brown kiwi: night-time encounter catching *versus* daytime catching using a certified kiwi finding dog. Sixty-five and 62 birds were caught with each method and despite the diametrical difference between them, the results suggest that both methods captured a comparable subset of a population. Furthermore, no physiological effects were evident in haematological analyses despite the differences in time of day of the blood collection. Hence, we recommend that either method or a combination of both should be considered for future studies and used in a way that maximises sample size and spread. Notably, we found that night-time encounter catching had a far superior success rate in very high-density populations. Since this method also bypasses the dependency on our limited number of certified dogs, we suggest that benefits could be gained by increasing the utilisation of night-time encounter catching in *A. mantelli* research.

Introduction

The risk of sampling bias is an ever-present worry in ecology research. Some authors even argue that, as long as sampling is not a total population census, bias is inevitable (for instance (Stuber *et al.* 2013). A specific scenario of concern is when results are gathered with two or more sampling methods and combined in the same analyses. Utilising more than one method can, for instance, be of bias-concern if (1) they differ in what subset of the population they capture, potentially resulting in a sex-, age-, personality-, social status- or health status- bias (Weatherhead & Greenwood 1981, Borràs & Senar 1986, Domènech & Senar 1997, Stuber *et al.* 2013, Michelangeli *et al.* 2015, Camacho *et al.* 2017), (2) they differently affect the parameters of interest, for instance by being conducted at a different time of day or year, or causing different levels of stress (Wilson & Wilson 1989, Romero & Romero 2002, Angelier *et al.* 2010, Michelangeli *et al.* 2015), or (3) they are associated with different level of success, either in the sampling itself or in sample processing (Marion *et al.* 1981, Davis 2005, Ronconi *et al.* 2010, Benítez-López *et al.* 2011).

Sampling method differences can be hard to avoid; if method choice is related to habitat, accessibility, and/or density, the optimal method might naturally differ between populations and/or sites (DeGraaf *et al.* 1991, Buckland *et al.* 2008, Gottschalk & Huettmann 2011). Consequently, plausible method-induced sampling bias can be particularly detrimental when the studies' purpose is to compare populations (Faanes & Bystrak 1981, Domènech & Senar 1997, Lyra-Jorge *et al.* 2008, Pacheco *et al.* 2013). Another scenario when method differences can be practically unavoidable is when it is desirable to combine or compare older and newer samples in the same analyses, and common practice, technology, and/or method recommendations have changed over time (Ronconi *et al.* 2010, Benítez-López *et al.* 2011).

For instance, *Apteryx* (kiwi birds) can be caught with two common but diametrically different capturing methods: daytime catching using a certified kiwi dog that finds roosting birds by scent, and night-time catching that relies on humans spotting birds while they are out foraging and/or attracting birds using playback calls and then catching them either by grabbing their legs or by lowering a net over them (Robertson 2017). What determines which method is used is mainly the population density, the habitat accessibility, resources available, and to some extent personal preference (Robertson & Fraser 2009, Robertson 2017). The large difference between the methods suggested that comparing them side by side is warranted. Firstly, these methods could be suspected to differ in terms of bird detectability, and detectability, in turn, could plausibly differ between sexes, sizes, and/or age groups (Colbourne & Kleinpaste 1983, Halterman 2009, Alves *et al.* 2017). Previous work has shown that trained Kiwi dogs *find* a subset well representing the age composition of a population (Robertson & Fraser 2009). However, the difference in habitat preference/utilisation between age groups and sexes potentially causes a difference in extractability and consequently in sampling accessibility – deep burrows are for instance preferred by adults while juveniles are more commonly found on the surface and females are more frequently found in open pasture than males (Wilson 2014, Dixon 2015, Jamieson *et al.* 2016). Previous work has raised concern about night catching leading to a female-biased sample due to sex differences in catchability, relating to sex differences in behaviour such as running pattern and weariness (Colbourne & Kleinpaste 1983). Such behavioural differences, if present, would unlikely come into play during daytime catching, which again could result if differences between the two methods. In addition to sex, it is plausible that night-time catchability relates to health status, potentially causing a bias towards either healthier or less healthy individuals (Weatherhead & Greenwood 1981, Gorney *et al.*

1999, Bisi *et al.* 2011). Lastly, it is possible that the difference in timing of the two methods affects the physiology of the birds at capture. Other studies have, for instance, found haematologic differences linked to activity level, temperature, and time since last feeding, which all could lead to an effect of time of sampling for such parameters (Jenni-Eiermann & Jenni 1997, Downs *et al.* 2010, Lill 2011).

Many questions remain unsolved regarding the biology, health, behaviour, and management of *Apteryx*, and since the successful conservation of this iconic genus receives much attention, we deem it likely that many more future *Apteryx* studies will be underway. For instance, ongoing research on North Island brown kiwi, *Apteryx mantelli*, is done to evaluate population differences in levels of genetic diversity and signs of inbreeding- or outbreeding depression (Undin et al. in prep, Hura et al. in prep). For these studies to be accurate, it is crucial to avoid or account for sampling bias in terms of age, sex, fitness, and health status (Danchin *et al.* 1995, Blanckenhorn *et al.* 1999, Kidd *et al.* 2015). Thus, herein we present the first comparison of what sample sets are captured using daytime catching with a certified dog compared to night catching without a dog. Furthermore, to elude whether the time of day affects the quantification of haematology-parameters and thus the estimation of the individual- as well as the population level health, haemoglobin concentration (HB), packed cell volume (PCV), glucose concentration, and total protein level were compared between the birds caught daytime and night-time as described above. For the latter comparison, a smaller number of birds captured daytime thanks to them being fitted with radio transmitters were also included. To facilitate making recommendations for future *Apteryx* research projects, we also compared the two capturing methods with respect to success rate and related success to population density.

Methods and Materials

Study species and sites

A total of 146 North Island brown kiwi were captured during the no-breeding season (Jan to May) in 2019 and 2020 from nine populations: Motuarohia, Moturoa, Moturua, the Puketi Forest, the Purerua Peninsula, Rakaumangamanga (also known as Cape Brett), Trounson Kauri Park, the Pukaha National Wildlife Centre, and the Remutaka Forest. All except the last two of these populations belong to the *A. mantelli* Northland taxon (Germano *et al.* 2018), while the birds from Remutaka and Pukaha are of mixed taxon origin (see further chapter X).

Blood sampling was done from the metatarsal vein in accordance with the Kiwi Best Practice Manual and our granted permits form the Massey University Animal Ethics Committee (permit 18/83 and 18/84) and Department of Conservation (permit 70875-RES and 70826-CAP). Birds were found, extracted, handled, and released following the Kiwi Best Practice Manual for (1) daytime catching utilising a certified kiwi tracing dog-and-handler pair, (2) night-time encounter capture, (3) night-time catching via attracting birds through sound recordings and/or whistle.

Blood sampling and analyses

Immediately after capture, blood sampling was initiated, followed by the collection of six body measurements. Weight was measured using a 2.5kg or 5kg Pesola® precision scale. Bill length, tarsus depth, tarsus width, and tarsus length were all measured using manual or digital Vernier stainless steel callipers with 3 replicates per measure. We calculated Taborsky's body condition (BC) using equation 1 (Taborsky & Taborsky 1999).

$$BC = \frac{Weight^X}{TW}$$

$$X = \frac{1}{k}$$

$$\log Weight = k * \log TW + m$$

Equation 1. Calculation of Taborsky's body condition (BC) where weight refers to the body weight in kilograms, TW to the tarsus width in millimetres and X refers to the reciprocal of the slope (k) found by relating log weight to log TW.

The sampled blood was used to measure total serum protein level, packed cell volume (PCV), white blood cell volume, haemoglobin concentration (HB), and glucose level. Glucose and HB were measured on site in direct connection to sample collection using an EasyTouch® GHb Dual-function Monitoring System (Nephrocare©, Germany). PCV was measured within 8 h of sampling using blood stored in heparinised haematocrits (capillary tubes) that were centrifuged for 5 minutes at 10000 rpm. Total serum protein level was then measured from the plasma in the haematocrits after centrifugation using a hand-held refractometer (Atago®, Tokyo, Japan).

PCR sexing and defining age groups

It is not possible to sex *Apteryx* with confidence based on morphology or behavior before they have reached full size, and even then, it can remain challenging unless the same bird is tracked over multiple years. Hence, a molecular, polymerase changing reaction (PCR) based protocol for sexing has been developed by Huynen et al. (2002) and further improved by Huynen et al. (2003). In short, DNA was extracted from 5-50 µl thawed whole *A. mantelli* blood using a High Pure PCR template preparation kit (Roche, Basel, Switzerland). Manufacturer's instructions were followed with the exception the DNA was eluted twice using 50µl of elution buffer for each

centrifugation. For amplification, the primers w5 (5'-AAT CAC CCT TTA AAC AAG CTG TTA AAG CAA-3') and w7 (5'-CCT TTC TCA AAT CTC TCT TTT GTT CTA GAC AC-3') published by Huynen et al. (2003) were used. The amplified DNA was then analyzed using agarose gel electrophoresis (1% agarose in 1X TAE buffer - 40mM Tris, 20mM Acetate and 1mM EDTA pH8.6). This fragment size separating step results in that for female *Apteryx*, two amplification products are made visible on the gel: one of about 350 bp and one of about 200 bp. The shorter fragment represents a site on the female-defining W chromosome. For male *Apteryx*, only the 350 bp product is amplified since males lack a W chromosome.

Based on the sex, combined with bill length, tarsus length, and weight, each bird was assigned to an age group. All birds < 1000 g were considered juveniles. Birds were assigned adults if they were female and had a weight > 2000 g, or a TW > 11 mm plus a weight over 1700 g, or a bill > 113 mm and a weight over 1700 g; and if they were male and had a weight > 1700 g, or a TL > 90 mm plus a weight over 1400 g, or a bill > 90 mm plus a weight over 1400 g. All birds falling in neither the juvenile nor the adult category were classes as sub-adults. The weight limits of 1400 g and 1700 g respectively are low but were justified by the dry and harsh conditions affecting the birds in 2020 but still only used in combination with measurements of size.

Effort and success

Catching success was defined as the number of birds caught per team per day. Eight populations were used for this comparison; Pukaha was excluded since all sampling there relied on birds being previously fitted with transmitters and/or being held in an enclosure. Populations were grouped into three categories: “very high”, “high”, and “medium” density based on the relative density of brown kiwi experienced during catching. No sampling was conducted in populations that could be referred to as “low”

density relative to all known *A. mantelli* populations (McLennan & Potter 1992, Robertson & de Monchy 2012, Germano *et al.* 2018). To compare sampling success, I also considered in the analyses the amount of blood successfully taken (up to a maximum allowed 0.5ml) and how easy was it to obtain the sample on a scale from 1 (easy) to 4 (hard) as experienced by the sampler and noted directly after sample collection.

Statistical analyses

To compare the distribution of sexes and age-groups, I conducted Chi-square tests using R (version 3.6.2; R core team). I conducted a general linear model (glm) to examine the effect of time of day (actual time as well as categorical "night" and "day"), population (Motuarohia, Moturoa, Moturua, Pukaha National Wildlife Centre, Puketi Forest, Purerua Peninsula, Rakaumangamanga, Remutaka Forest Park, and Trounson Kauri Park), the month of sampling (January, February, March, and May) and year of sampling (2019 and 2020) and their interactions using R (version 3.6.2; R core team). P-values from the analyses were then corrected for multiple testing using Holm Bonferroni with all tests considered one family.

Results and Discussion

Sex, age and morphometrics

Overall, 84 birds were caught during the daytime and 62 at night. Of the 84 birds caught during the day, 19 were found thanks to their own or their partners' transmitter, leaving 65 birds caught with the assistance of a trained dog for comparison of sample composition (Table A2.1). The sex as well as the age group composition turned out to be remarkably similar between dog-birds and night-birds. Based on PCR sexing, 57 % and 58 % were identified as females, respectively (Figure A2.1a). Based on size and

weight, 72 % of dog-birds and 66 % of night-birds were identified as adults, and 18 and 29% sub-adults respectively and the rest as juveniles (Figure A2.1b). These differences were not found to be statistically significant (sex: χ-sq. = 0.45, df = 2, p-value = 0.80; age group: χ-sq. = 2.54, df = 2, p-value =0.28).

Table A2.1. Definitions of how *A. mantelli* samples were subdivided for comparisons of bias and catching success.

Referred to as	**Definition**	***n***	**Included in**
"night-birds"	All birds caught at night	62	Both composition and blood parameter comparison
"day-birds"	All birds caught during the day	84	Both
"dog-birds"	Sub-set of day-birds found using a trained kiwi dog	65	Composition
"tx-birds	Sub-set of day-birds found by tracking their own or their partners radio transmitter	19	Blood parameter comparison*

*excluded from composition comparison since these represent known birds and thus does not reflect the comparison of catching birds at night versus with a dog.

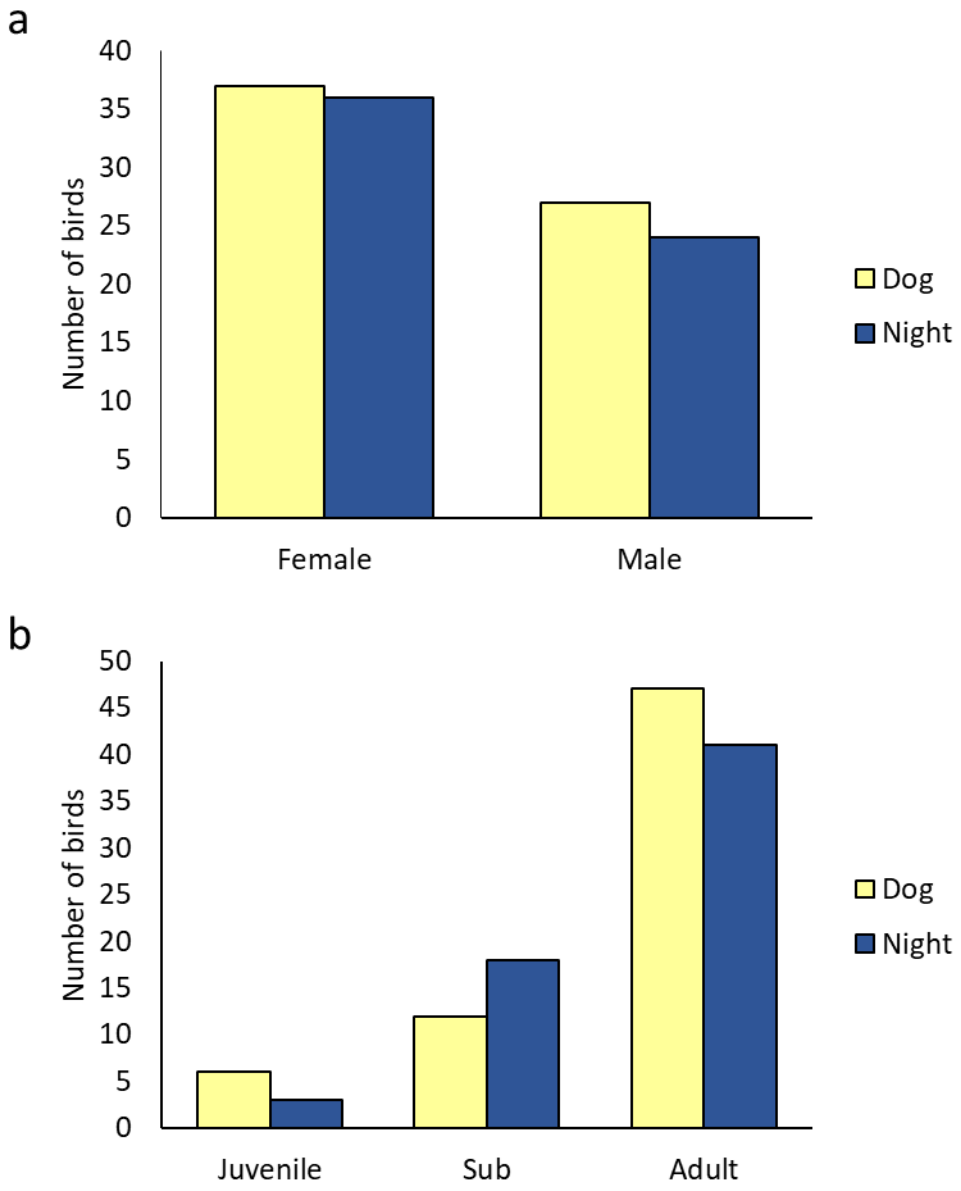


Figure A2.1. Sex- and age-group composition among birds caught in the daytime using a licensed kiwi finding dog ("Dog"; *n* = 65) versus at night without a dog ("Night"; *n* = 62). Sexing was based on PCR (Huynen *et al.* 2002, Huynen *et al.* 2003). Age group was estimated based on a combination of sex, bill length, weight, and tarsus length.

The distribution of the six morphometrical characteristics quantified was found to not vary significantly between dog-birds and night-birds independent of assigned sex and age class; tarsus length (Table A2.2; Figure A2.2). Taken together this suggests that there was no age, sex, or size bias induced by using the different catching methods.

Robertson and Fraser (2009) have previously concluded that searching with a certified dog resulted in a sample set representative of the true sex and age composition of a population. Thus, we suggest that night catching is also likely to generate results representative in this way. Consequently, we suggest that overall, the sampled population had a female bias and that, on average, about two thirds of a population's individuals are adults.

Table A2.2. Comparison of six morphometric parameters between *Apteryx mantelli* caught in the daytime using certified dogs or using night-time encounter catching.

Factor	Chisq	DF	P-value*
Bill length	1.61	1	> 0.05
Tarsus Length	3.50	1	> 0.05
Tarsus Depth	6.76	1	> 0.05
Tarsus Width	4.87	1	> 0.05
Weight	2.76	1	> 0.05
Taborsky's BC	0.23	1	> 0.05

*Adjusted using Holm's Bonferroni correction.

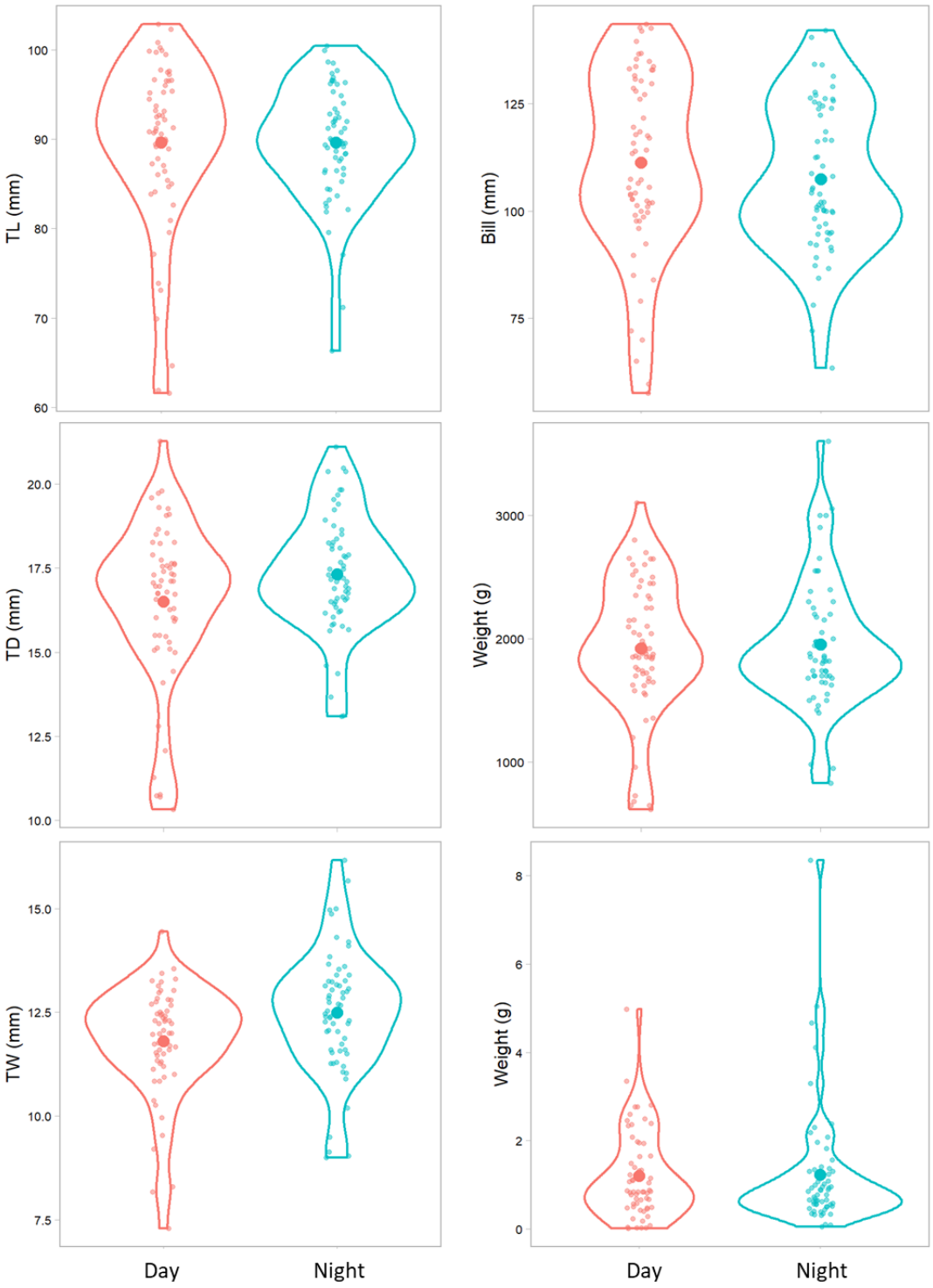


Figure A2.2. Comparison of morphometrics between birds caught daytime and night-time. Specifically, distributions of tarsus length, weight, tarsus width, bill length, tarsus depth, and Taborsky's body condition were compared between daytime and night-time caught birds respectively. The shape of the violin plots represents the distribution of obtained values, the small dots represent each individual bird, and the larger dot represents the average value for each parameter. None of the distributions were found to differ significantly.

Success

Catching success (defined as birds caught per team per day) was noticeably related to population density with success rate being over eight times higher in the most compared to the least dense population sampled (Figure A2.3). Overall, mean catching success was about 50% higher at night than during the day, however, this was related to population density with night catching being substantially more effective in “very high” and “medium” density but less effective in “high” density populations (Figure A2.3; no “low” density populations were sampled). For “very high” density, the higher success rate at night was even more explicit when only accounting for the populations were both methods were used (Figure A2.3A). Once caught, sampling success was found to not differ between day-birds and night-birds, neither in terms of reported ease of bleeding (Chisq = 2.80, df = 1, p-value > 0.05) or blood volume collected (Chisq = 1.00, df = 1, p-value > 0.05).

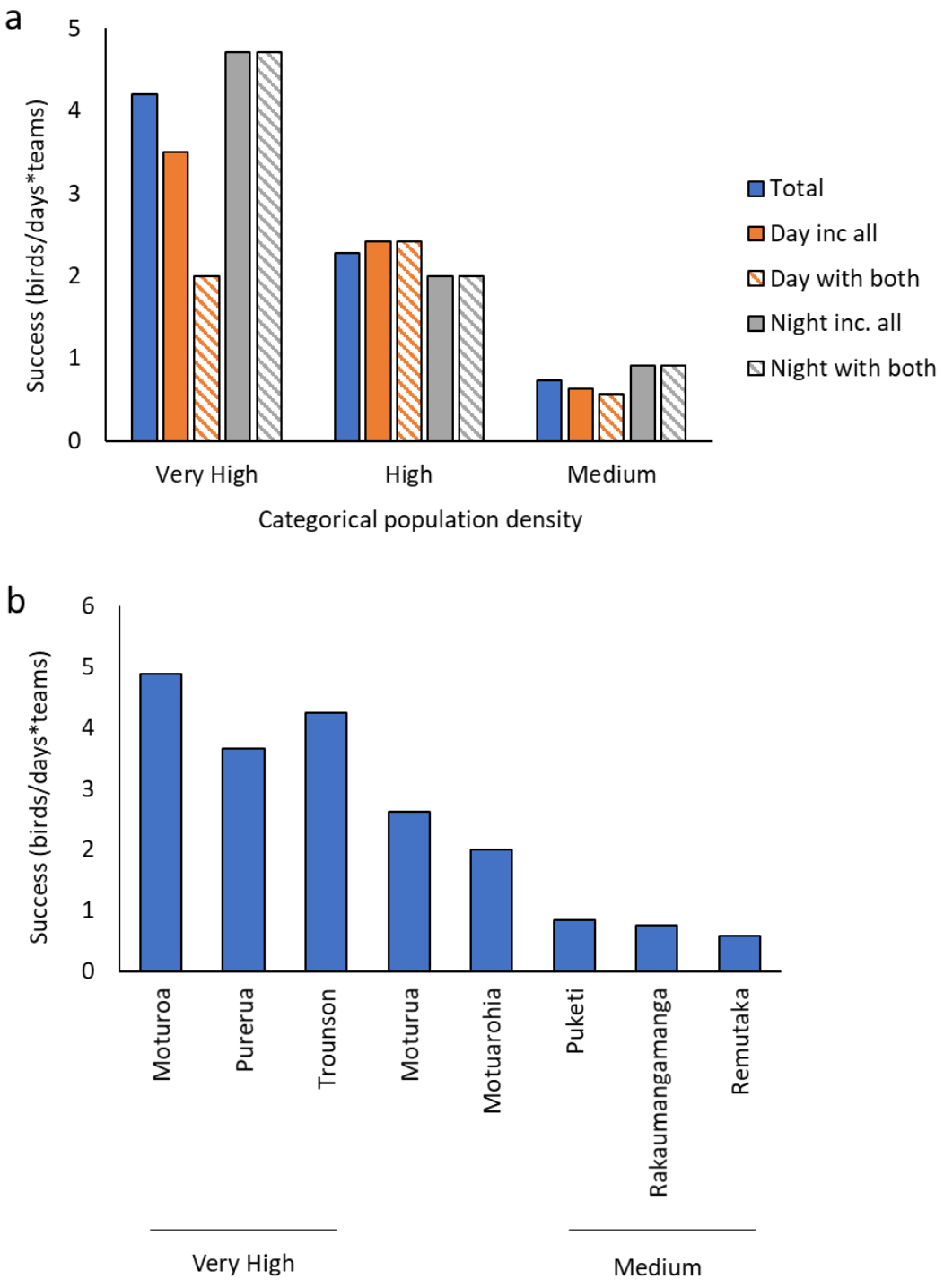


Figure A2.3. Comparison of catching success – total as well as daytime and night-time separately – with respect to categorical *A. mantelli* density. Density categories are named "very high", "high", and "medium" respectively to highlight that none of the populations sampled would be considered low density when taking the full range of *A. mantelli* population densities national into account. (A) illustrates catching success measured as the number of birds caught per day per catching team overall (blue bars), daytime and night-time when including all populations (filled orange and grey bars respectively), and daytime and night-time when only

including populations where both methods were used (striped orange and grey bars respectively). (B) illustrates total success broken down by population. Populations are ordered by density with the highest density to the left. Lines under the graph indicate which populations make up which category in (A) with unmarked population making up the “high” density category.

Blood parameters

None of the four measured haematological parameters were found to differ significantly between day-birds and night-birds; packed cell volume (PCV; Chisq = 1.58, df = 1, p-value > 0.05), total protein content (Chisq = 0.01, df = 1, p-value > 0.05), glucose concentration (Chisq = 0.77, df = 1, p-value > 0.05) and haemoglobin concentration (HB; Chisq = 0.02, df = 1, p-value > 0.05; Figure A2.4). Neither was there any relationship found between each parameter and actual time of sampling (Supplementary figure A2.S1).

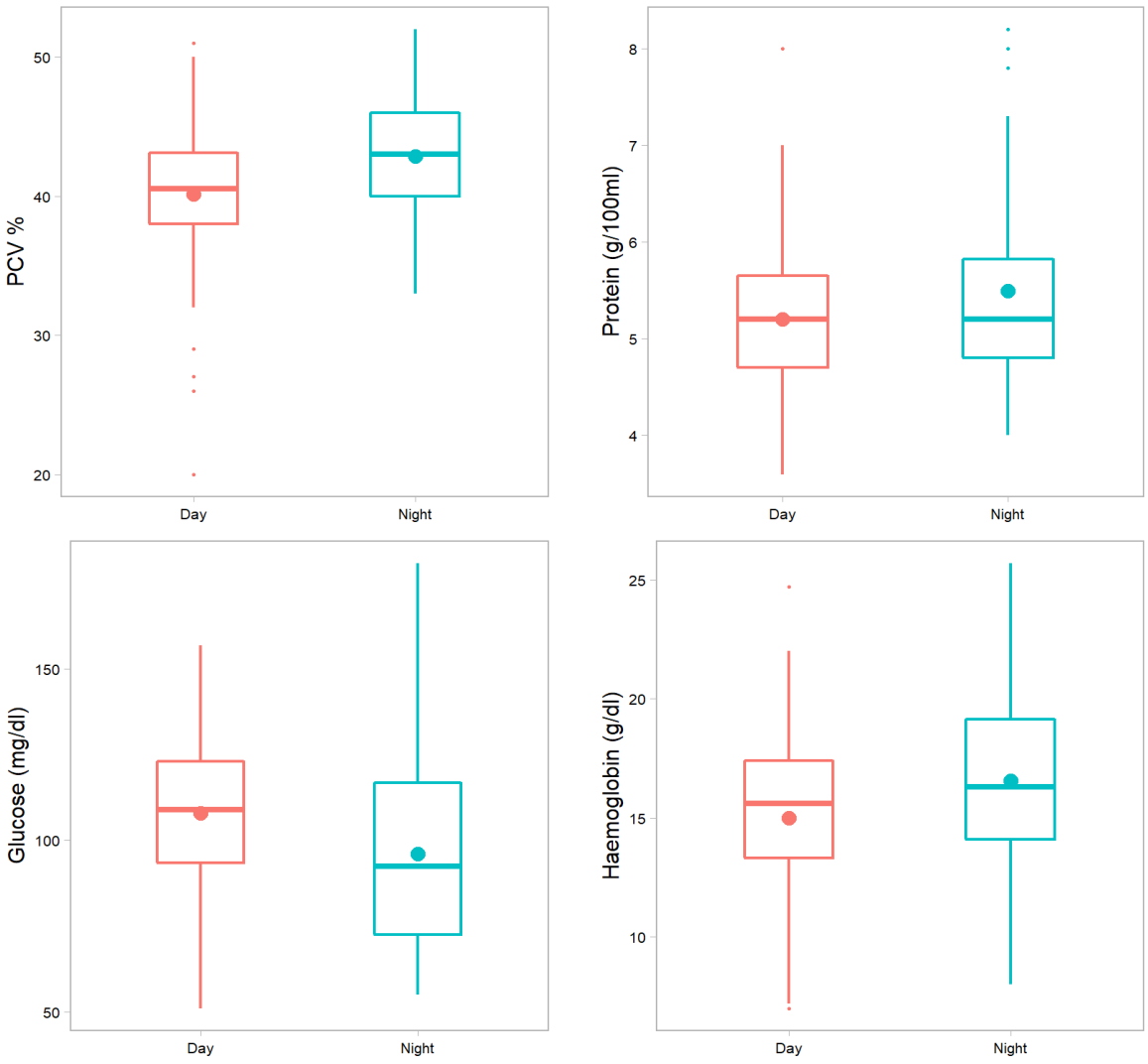


Figure A2.4. Comparisons of haematological parameters between *A. mantelli* caught daytime (red) and night-time (blue). Specifically, packed cell volume, total blood protein, blood glucose level, and haemoglobin concentration, respectively. Central lines indicate median and circles the mean values, the boxes indicated the inter quartile range. None of the parameters were found to differ significantly.

The apparent, but non-significant, difference for glucose concentration (Figure A2.4) was found to be linked to a population difference (Chisq = 34.46, df = 8, corrected p-value = 0.002; Figure A2.5). In addition, the difference in the timing of sampling in different populations (Table A2.3) was deemed responsible for an apparent effect of

time of sampling. Blood glucose is a rather rarely used measurement in birds since it does not have equivalent interpretations as mammalian blood glucose level (Braun & Sweazea 2008). However, studies have found glucose concentration differences linked to habitat (Kaliński *et al.* 2014) and diet (more specifically protein poor diet; Machın *et al.* 2004), supporting our finding of differences between populations. Contrary to these findings, other studies have found differences based on time of day and/or temperature in other bird species (Downs *et al.* 2010, Lill 2011).

Table A2.3. Table of populations, methods of capture, and the year(s) and month(s) of sampling. Sample size in brackets.

Population	**Night (N)**	**Day (N)**	**Year**	**Month**
Motuarohia	(0)	Dog (20)	Both	Jan; Feb
Moturoa	Encounter (18)	Dog (4)	2020	Jan
Moturua	Encounter (12)	Dog (9)	2019	Jan; May
Pukaha	(0)	Tx (7)*	2020	Feb; March
Puketi	(0)	Dog (5)	2019	March
Purerua	Encounter (22)	(0)	2019	May
Rakaumangamanga	Whistle and Encounter (9)	Dog (6)	Both	Jan; Feb
Remutaka	Whistle and Encounter (1)	Tx (5); Dog (4)	2020	Feb; March
Trounson	(0)	Tx (3); Dog (17)	2020	Feb

*4 of these birds were held in an enclosure and not actually fitted with transmitters, however, they were still included in the tx-birds subset for this chapter.

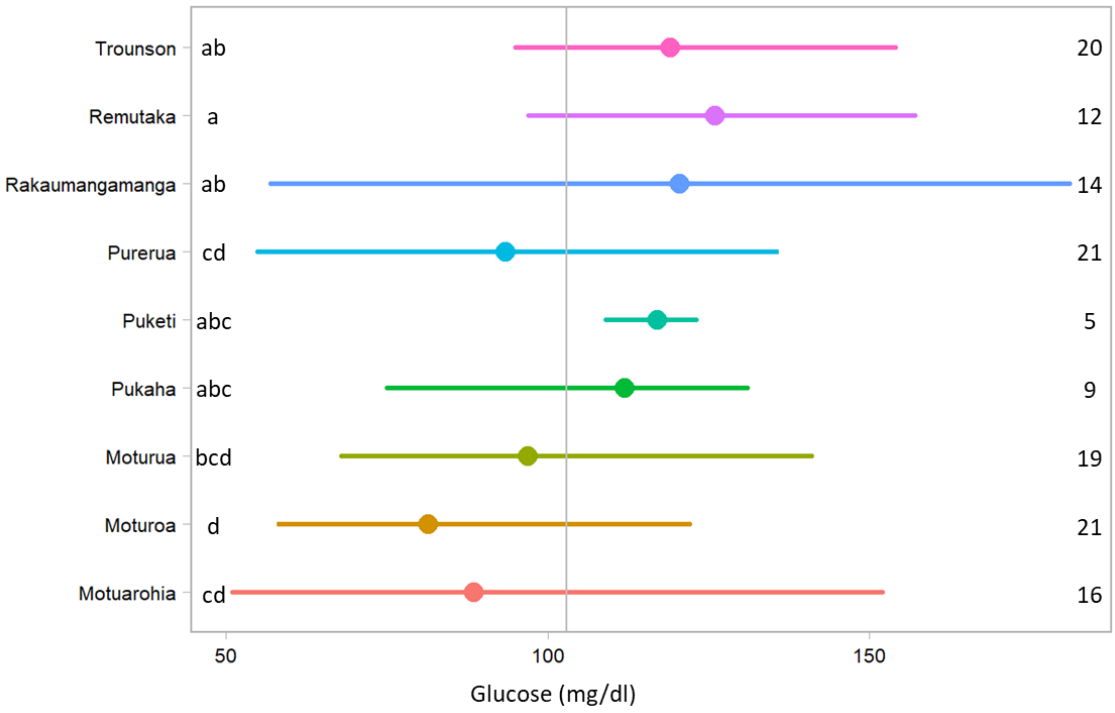


Figure A2.5. Comparison of blood glucose concentration between nine *A. mantelli* populations. Nighttime and daytime samples combined. Coloured lines indicate the spread from the highest to the lowest recorded values and circles indicate the mean for each population. The vertical grey line indicates the overall mean across all populations. Different letters indicate significant difference. Number to the right indicate sample size.

Conclusions and recommendations

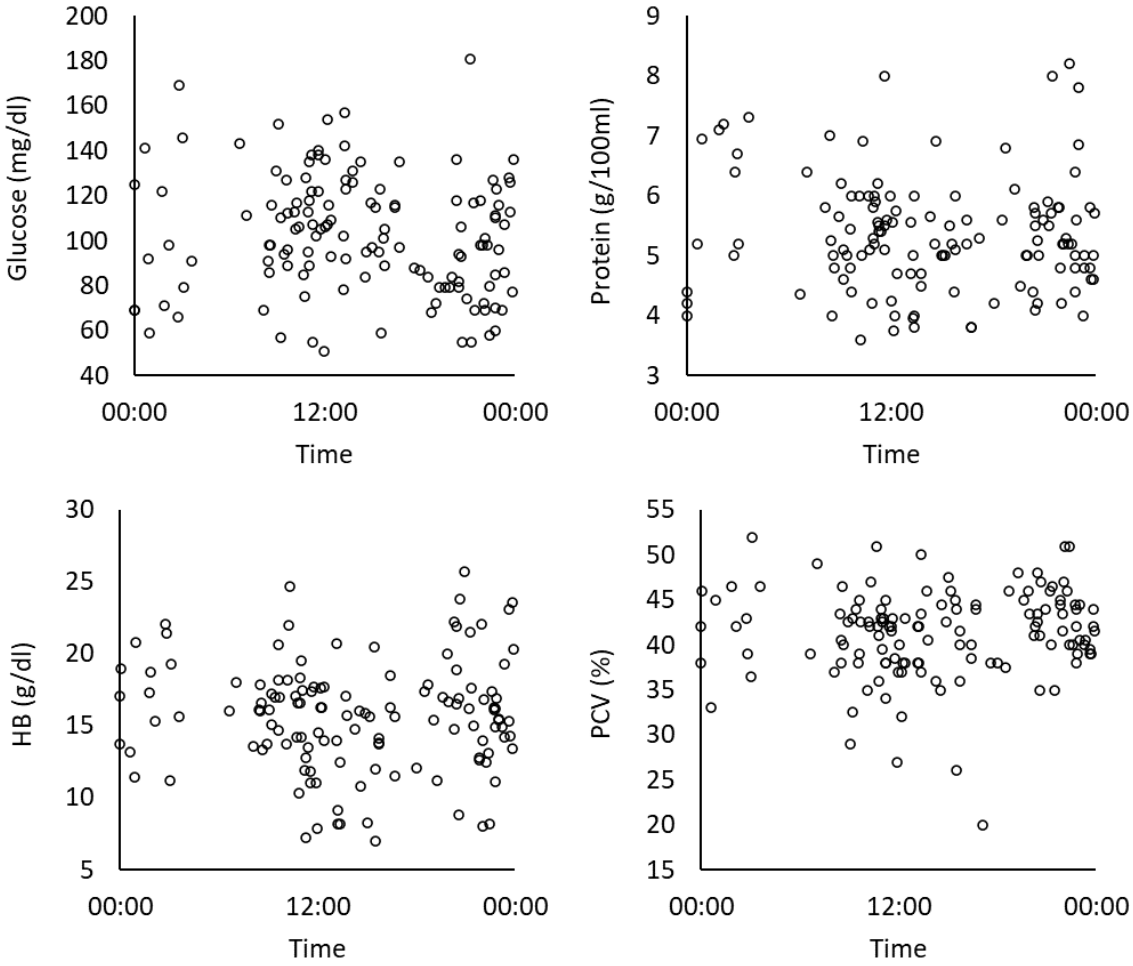
The results presented herein, demonstrate that an *A. mantelli* sample set is very unlikely to be affected by any sample bias due to the utilisation of the two diametrically different techniques night-time- and daytime catching, respectively. This was found to be true for sex-, age-, size-, and health status composition. In addition, we found no evidence for that the time of day affected any of the four blood parameters analysed here: packed cell volume (PCV), total protein content, glucose concentration and haemoglobin concentration (HB). Furthermore, we found that blood extraction was equally successful at night and during the day.

In many situations, population studies will benefit from maximising samples size, thus sampling efficiency and success rate should be considered when choosing methodology (Marion *et al.* 1981, Kritzer *et al.* 2001, Benítez-López *et al.* 2011). We found that catching success was substantially higher at night than daytime, in particular in very high and medium *A. mantelli* densities. The two main reasons for our lower success rate daytime are arguably that birds tended to utilise the more accessible areas such as open grassland and/or tracks at night, while during the day the more difficult terrain around the roost sites and/or the layout of the roost itself resulted in more time "wasted" combating the terrain. In addition, daytime catching relies on access to certified kiwi conservation dogs which is a limiting factor that can be avoided through night-time catching.

Our main way of night-catching was encounter catching rather than attracting birds via whistling or playback calls. This was simply because our success with calls was very low. It is, however, possible that some birds, in particular in the Rakaumangamanga population, might have been caught through a combination of the two approaches, i.e., that the playing of calls increased our success for encounter catching because it made birds move in closer. Previously, it has been discussed if playback has risks causing sex and age bias (Robertson 2017). Unfortunately, we cannot examine this with our data due to the small sample size birds for sure caught thanks to playback calls. Our lack of success with calling birds in could have been an effect of time of year. Time of year has been found to be crucial for calls to be a successful method of catching Tokoeka, *A. australis* (Kirkman pers. comm., 2020). Thus, we suggest that more research is needed to identify if there is a time window for which call catching is more effective for *A. mantelli* as well.

We suggest that based on bias alone, either or a combination of both daytime catching with certified kiwi dogs and night-time encounter catching can be recommended for future studies. However, based on catching success, night-time encounter catching should be the recommended method for maximising sample size, with the caveat that this is limited to sites where moving around, catching, and handling birds in the dark can happen in a safe way for birds as well as practitioners. We do also recognise that there are other factors to consider when deciding on methodology and we suggest more studies are needed to compare the methods with respect of stress minimisation and that results of such studies should be incorporated in future recommendation. Perhaps the most uplifting outcome of our results is that combining new and old samples collected using these two different methods should not be an issue. Hence, our results could be paving the way for future population comparisons with larger sample sizes from more population. Such increased sampling resolution would arguably be the best way to learn more about the elusive *A. mantelli* and how to ensure long-term sustainable management of this iconic species.

Supplementary figures



Supplementary figure A2.S1. Scatterplots illustrating the lack of relationships between time of blood sampling (hh:mm) and blood glucose level, total protein level, haemoglobin concentration, and packed cell volume (PCV) for *Apteryx mantelli*. *n* = 128 to 133 birds.

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Appendix 3

A.3 Extended methods: development of qPCR protocol

Evaluation and development of primers

The telomere sequence is very conserved (Allsopp *et al.* 1995). Thanks to this, all studies to my knowledge use the same set of telomere primers, tel1b and tel2b (Criscuolo *et al.* 2009), for qPCR based measurement of bird telomeres. These primers were confirmed to work for *A. mantelli* using standard PCR.

Table A3.1. Primers evaluated and chosen for the amplification of telomeres and the control gene, GAPDH, in *Apteryx mantelli* samples.

GENE	NAME	SEQUENCE	DEVELOPER	FINAL
TEL.	tel1b	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTT TGGGTT	Criscuolo *et al.* (2009)	Yes
TEL.	tel2b	GGCTTGCCTTACCCTTACCCTTACCCTTACCCT TACCCT		Yes
GAPDH	GAPDH-R	CCATCAGCAGCAGCCTTCA		Yes
GAPDH	GAPDH-F	AACCAGCCAAGTACGATGACAT		No
GAPDH	GAPDHF_kiwi	CTCAGGCCAAGTATGATGACAT	Our lab	No
GAPDH	GAPDHF_kiwi_n1	CTTGCACAGCTGACACAATTTG	Our lab	No
GAPDH	GAPDHF_kiwi_n2	CTTGCACAGCTGACACAATTTG	Our lab	Yes
GAPDH	GAPDHR_kiwi1	ATGCCAGGATGCCCTTCAA	Our lab	No
GAPDH	GAPDHR_kiwi2	CTGTGTATGCCAGGATGCC	Our lab	No

A commonly used single-copy control gene for calculating relative telomere length is GAPDH (see for instance Bize *et al.* 2009, Heidinger *et al.* 2012, Barrett *et al.* 2013,

Aydinonat *et al.* 2014, Herborn *et al.* 2014, Kim & Velando 2015, Bebbington *et al.* 2016). Previously published GAPDH primers (GAPDH-R and GAPDH-F; Criscuolo *et al.* 2009) were first evaluated *in silico*. The reverse primer was deemed a good candidate, but the forward primer's binding site did not match the published *A. mantelli* genome (Le Duc *et al.* 2015; scaffold XM_013958036 (NCBI) featuring the GAPDH gene), hence a new primer was developed (GAPDHF_kiwi). However, *in situ*, this primer pair rendered unwanted amplification in addition to the expected band. Thus, four additional GAPDH primers were developed (Table A3.1). All primers were evaluated using standard PCR and temperature gradient PCR. The preferred primer combination was identified to be the original reverse primer (GAPDH-R) and one of the new forward primers (GAPDHF_kiwi_n2; Table 1).

Table A3.2. Parameters evaluated during optimisation and the protocol chosen for qPCR assay of relative telomere length in *A. mantelli* samples. Optimisation for 96 well plates containing Telomere and GAPDH primers respectively (Table A3.1) and *Apteryx mantelli* DNA.

FACTOR	TESTED	USED (BOTH PRIMER SETS)
QPCR ENZYME KIT	LightCycler® 480 SYBR Green I Master (Roche), 5x HOT FIREPol® EvaGreen®qPCR Supermix, and 5x HOT FIREPol® SolisGreen qPCR Supermix (both Solis BioDyne)	4µl 5x HOT FIREPol® EvaGreen®qPCR Supermix
PRE-INCUBATION	As recommended per kit	95°C for 12 min
ANNEALING TEMPERATURE	56, 58, and 60°C	60°C
AMPLIFICATION TIME	10s to 20s	30s
ELONGATION TIME	10s and 30s	30s at 72°C
DENATURATION TIME	10s and 15s	15s at 95°C
ROUNDS	40	40
PRIMER CONCENTRATION	50 to 500 nM	Telomeres 250 nM GAPDH: 200 nM
DNA AMOUNT PER REACTION	0.004 to 100ng	Telomeres: 1 ng GAPDH: 4 ng
TOTAL REACTION VOLUME	10 and 20 µl	20 µl
PURIFICATION COLUMN BEFORE AMPLIFICATION	Used or no used	Not used

qPCR optimisation

Temperature, concentration, and time spans to evaluate were chosen based on the recommendations for each master-mix and protocols used in previous publications (Bize *et al.* 2009, Criscuolo *et al.* 2009, Beaulieu *et al.* 2011, Geiger *et al.* 2011, Barrett *et al.* 2013, Aydinonat *et al.* 2014, Herborn *et al.* 2014, Becker *et al.* 2015, Kim & Velando 2015, Watson *et al.* 2015, Eastwood *et al.* 2018; Table A3.2). Telomere and GAPDH primers were run on separate plates to keep the plate position constant for each sample between the two primer sets for consistency with other studies (who often have used different protocols for the primers, see for instance Bize *et al.* 2009, Heidinger *et*

al. 2012, Barrett *et al.* 2013, Aydinonat *et al.* 2014, Herborn *et al.* 2014, Kim & Velando 2015, Bebbington *et al.* 2016).

To render reliable, repeatable RTL values based on the equation developed by Pfaffl (2001) and refined by Eastwood *et al.* (2018), high plate efficiency and a consistent individual efficiency are the most important characters. (Individual efficiency is the extent at which an individual well lives up to the expected doubling of fluorescence for each round of amplification.) The overall, as well as individual efficiency, achieved using the LightCycler® 480 SYBR Green I Master (Roche), was not satisfactory. Hence, thanks to great support from dnature™ (www.dnature.co.nz), a side-by-side evaluation of 5x HOT FIREPol® EvaGreen®qPCR Supermix, and 5x HOT FIREPol® SolisGreen qPCR Supermix (both Solis BioDyne) was carried out. For this trial, three samples were selected based on their comparatively high and even success with LightCycler® 480 SYBR Green I Master (Roche) protocol. The results of this side by side comparison are summarized in table A3.3 and figure A3.1 respectively. In short, both 5x HOT FIREPol® EvaGreen®qPCR Supermix, and 5x HOT FIREPol® SolisGreen qPCR Supermix (both Solis BioDyne) performed higher and more consistent efficiencies than LightCycler® 480 SYBR Green I Master (Roche). In addition, this high and constant efficiency was achieved with about one order of magnitude less DNA per reaction. I concluded that 5x HOT FIREPol® EvaGreen®qPCR Supermix was the more suitable kit for my study. This was because, even though individual efficiencies were higher when using 5x HOT FIREPol® SolisGreen qPCR Supermix, the efficiencies were more similar for the two primer sets when using 5x HOT FIREPol® EvaGreen®qPCR Supermix.

Once the optimal protocol had been developed (Table A3.2), samples were run as triplicates and each plate included a 4 set 2x dilution series and water control (also in

triplicates). This enabled running 27 samples for each set of two 96 well plates. DNA was normalised to a concentration of 0.5 ng so that 2µl and 8µl could be added per reaction on the telomere and the GAPDH plate, respectively. The DNA amounts in the dilution series were 4, 2, 1, and 0.5 ng per reaction on the telomere plates and 8, 4, 2, and 1 ng on the GAPDH plates. Stock solution for each concentration was prepared beforehand and the same stock was used for all plates.

Table A3.3. Results of comparison between 5x HOT FIREPol® EvaGreen®qPCR Supermix and 5x HOT FIREPol® SolisGreen qPCR Supermix for amplifying telomere sequence and the GAPDH gene from *A. mantelli* blood samples. The table includes C_t values for the highest and lowest concentration used for the standard curve.

Mix	Gene	Primer conc.	An. temp.	Mean C_t 4 ng DNA	Mean C_t 4 pg DNA	Efficiency	Ind. efficiency
EvaGreen	Tel	250 nM	60	16.829	26.321	2.063	1.815
SolisGreen	Tel	250 nM	60	9.339	18.780	2.050	2.024
EvaGreen	GAPDH	100 nM	60	29.162	39.392	N/A*	1.859
SolisGreen	GAPDH	100 nM	60	26.583	38.083	N/A*	1.888
EvaGreen	Tel	250 nM	58	38.151[£]	22.613	N/A*	1.849
SolisGreen	Tel	250 nM	58	9.803[¥]	19.207	1.997	1.994
EvaGreen	GAPDH	100 nM	58	29.631	39.089	2.130	1.807
SolisGreen	GAPDH	100 nM	58	28.227[¥]	38.546	1.709	1.890

* The LightCykler 480 II, defaults all Ct values > 35 to 35 and thus efficiency cannot be correctly calculated

[¥]Based on one value.

[£]This calculated C_t value is an artefact. The high noise level makes the software unable to accurately calculate the value, for all other 3 steps in the elution curve results are sufficient.

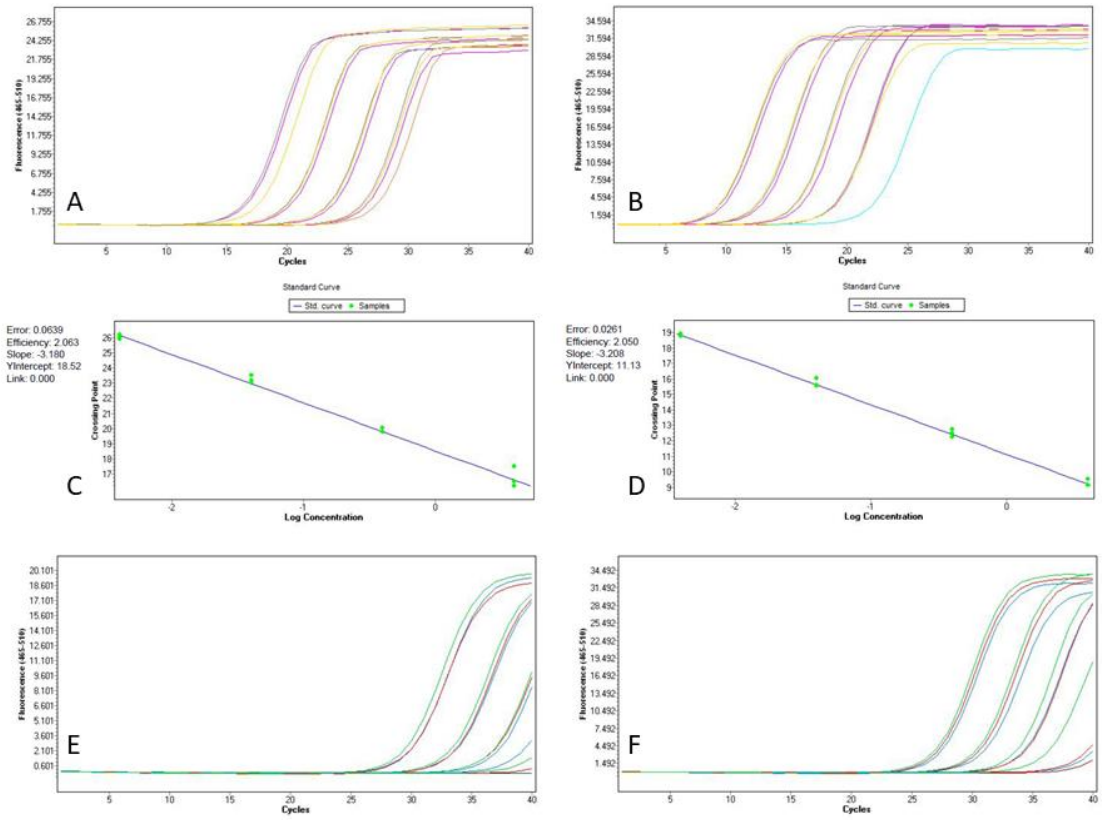

Figure A3.1. qPCR results visualizing the dilution series comparing between EvaGreen and SolisGreen qPCR Supermixes. Based on 3 samples (not three replicates of the same sample) of the trial plates run at 60°C. Ct values (A) and efficiency curve (C) for 5x HOT FIREPol® EvaGreen®qPCR Supermix and telomere primers. Ct values (B) and efficiency curve (D) for 5x HOT FIREPol® SolisGreen qPCR Supermix and telomere primers. Ct values for 5x HOT FIREPol® EvaGreen®qPCR Supermix and GAPDH primers (E). Ct values for 5x HOT FIREPol® SolisGreen qPCR Supermix and GAPDH primers (F).

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Appendix A4 – Supplementary tables and figures

Supplementary table S1.2.1. List of all 41 studies featuring *Apteryx* genetics included in the review.

Reference	Title	Categorical topic	Brief description /result /method	Focus species	Marker types used	Specific markers used	Sample size	Year
Baker at al. 1995	Flightless brown kiwis of New Zealand possess extremely subdivided population structure and cryptic species like small mammals.	Phylogeny - *Apteryx*	First published study of *Apteryx* genetics, confirms high degree of population identity and that Brown Kiwi should be slit in several species.	All / Brown Kiwi	Allozymes mtDNA	Cytochrome B	146 for Allozymes 61 mtDNA	1995
Binney 2007	The major histocompatibility complex (MHC) of the kiwi (*Apteryx* spp.)*	Diversity	Characterise the diversity in the immune system MHC genes	*A. mantelli, A. rowi and A. owenii*	Nuclear DNA	MHC genes	36	2007
Burbidge et al. 2003	Molecular and other biological evidence supports the recognition of at least three species of brown kiwi	Phylogeny - *Apteryx*	First detailed genetic study of Brown kiwi suggesting a split in 3 species: *A. australis, A. mantelli* and *A. rowi*	Brown Kiwi	mtDNA	Control region, Cytochrome B and ATPase	60	2003

Cooper et al. 1992	Independent origins of New Zealand moas and kiwis.	Phylogeny - Ratites	First genetic study suggesting *Apteryx* and Moa are not forming a monophyletic group	All	mtDNA	12S rRNA	3	1992
Cooper et al. 2001	Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution	Phylogeny - Ratites	Based on the entire mt-genome of 2 Moa; *Apteryx* and Moa are not forming a monophyletic group	*A. mantelli*	mtDNA	Mitochondrial protein coding region	1	2001
Crimp 2010	The mitochondrial genome of the little spotted kiwi	Diversity	Characterisation of the mitochondrial genome of *A. owenii*	*A. owenii*	mtDNA	Complete mitochondrial genome	1	2010
Dawson et al. 2015	A marker suitable for sex-typing birds from degraded samples.	Applied: Sex determination	Development of a sexing method based on degraded DNA such as scats	*A. australis*	Nuclear DNA	Z chromosome	14	2015

de Boer 1980	Do the chromosomes of the kiwi provide evidence for a monophyletic origin of the ratites?	Phylogeny - Ratites	The karyogram of *Apteryx australis* has 40 pairs of chromosomes, 6 macro and 34 micro pairs, just like ostrich, rhea, cassowary and emu, supporting monophyly of all ratites.	*A. australis*	Nuclear DNA	Karyotype	1	1980
Grueber et al. 2013	Primers for amplification of innate immunity toll-like receptor loci in threatened birds of the Apterygiformes, Gruiformes, Psittaciformes and Passeriformes.	Diversity	Characterisation of primers of amplification of innate immunity toll-like receptor genes	*A. mantelli*	Nuclear DNA	Toll-like receptors (TLR)	2	2013
Grueber et al. 2015	Toll-like receptor diversity in 10 threatened bird species: relationship with microsatellite heterozygosity.	Diversity	Comparison of diversity analyses using microsatellites and innate immunity toll-like receptor genes	*A. mantelli*	Nuclear DNA	Toll-like receptors (TLR)	20	2015

Hackett et al. 2008	A phylogenomic study of birds reveals their evolutionary history.	Phylogeny - Aves	Using coding nuclear DNA to generate Aves wide phylogeny	*A. australis*	Nuclear DNA	20 genes	1	2008
Haddrath & Baker 2001	Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis	Phylogeny - Ratites	Based on the entire mt-genome of 2 Moa; *Apteryx* and Moa are not forming a monophyletic group	*A. haastii*	mtDNA	Complete mitochondrial genome	1	2001
Haddrath & Baker 2012	Multiple nuclear genes and retroposons support vicariance and dispersal of the palaeognaths, and an Early Cretaceous origin of modern birds.	Phylogeny - Ratites	Analyses of coding nuclear sequences confirms that flight have been lost multiple times within the ratites	All	Nuclear DNA	27 genes	5	2012
Harshman et al. 2008	Phylogenomic evidence for multiple losses of flight in ratite birds.	Phylogeny - Ratites	Analyses of coding nuclear sequences confirms that flight have been lost multiple times within the ratites	*A. australis*	Nuclear DNA	20 genes	1	2008

Hartnup et al. 2011	Ancient DNA recovers the origins of Māori feather cloaks.[+]	Phylogeny - Historic diversity and distribution	Genetic analyses of the origin of Apteryx feathers in traditional Kakahu (or Kahu Kiwi; Maori feather cloaks)	*A. mantelli*	mtDNA	Control region	1042	2011
Herbert & Daugherty 2002*	Genetic variation, systematics and management of kiwi (*Apteryx* spp.).	Phylogeny - *Apteryx*	Summarising work done in 80s and 90s regarding geographic patterns of diversity and support for current taxonomy	All	Allozymes		245	2002
Huynen et al. 2002	A DNA test to sex ratite birds	Applied: Sex determination	First developed sexing test that works for ratites	*A. mantelli*	Nuclear DNA	W chromosome	21	2002

Huynen et al. 2003	A DNA test for sex assignment in kiwi (*Apteryx* spp.)	Applied: Sex determination	Further development of the ratite sex determination test to ensure it also works on *A. australis*	*A. mantelli*	Nuclear DNA	W chromosome	17	2003
Jensen et al. 2008	Isolation and characterization of microsatellite loci in the North Island brown kiwi, *Apteryx mantelli*.	Diversity	Identification of 8 polymorphic microsatellites that can be used in further studies	*A. mantelli*	Nuclear DNA	Microsatellites (8 loci)	79	2008
Le Duc et al. 2015	Kiwi genome provides insights into evolution of a nocturnal lifestyle.	Diversity	Sequencing of the whole *A. mantelli* genome	*A. mantelli*	Nuclear DNA	Whole genome	3	2015
Liu et al. 2017	The complete mitochondrial genome of North Island brown kiwi (*Apteryx mantelli*).	Diversity	Sequencing and characterisation of the whole mt genome for *A. mantelli*	*A. mantelli*	mtDNA	Complete mitochondrial genome	3	2017

McLennan & McCann 2002	Genetic variability, distribution and abundance of great spotted kiwi (Apteryx haastii).	Diversity	Summary of current knowledge including genetics about *A. haastii* to evaluate conservation status	*A. haastii*			35	2002
Miller et al. 2011	Characterisation of class II B MHC genes from a ratite bird, the little spotted kiwi (*Apteryx owenii*).	Diversity	Characterisation of immune system class II B MHC genes in little spotted kiwi (*Apteryx owenii*).	*A. owenii*	Nuclear DNA	MHC genes	14	2011
Mitchell et al. 2014	Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies ratite bird evolution.	Phylogeny - Ratites	First study to include enough mtDNA sequence from elephant birds to confirms is close relationship with *Apteryx*	Spotted Kiwi	mtDNA	Complete mitochondrial genome	2	2014

Philips et al. 2009	Tinamous and moa flock together: mitochondrial genome sequence analysis reveals independent losses of flight among ratites.	Phylogeny - Ratites	Analyses of whole mt genome sequences confirms that flight have been lost multiple times within the ratites	All	mtDNA	Complete mitochondrial genome	2	2009
Powlesland 1988	Kiwi Research and Conservation: An Account of a N.Z. Wildlife Service Workshop 20-21 May 1986[++]	Diversity	Two pilot studies on genetic diversity and methods for detecting it, suggesting large detectable variation within Brown Kiwi (at the time on species today split into *A. australis, A. rowi and A. mantelli*)	*A. mantelli*	Allozymes		47	1988
Ramon-Laca et al. 2018	Extraction of DNA from captive-sourced feces and molted feathers provides a novel method for conservation management of New Zealand kiwi (*Apteryx spp.*).	Diversity	Characterisation of polymorphic microsatellites using non-invasive samples such as feathers and scats	All	Nuclear DNA	Microsatellites (23 loci)	233	2018

Ramstad et al. 2010	Fourteen microsatellite loci cross-amplify in all five kiwi species (*Apteryx* spp.) and reveal extremely low genetic variation in little spotted kiwi (*A. owenii*)	Diversity	Identification of 14 polymorphic microsatellites across all *Apteryx* to quantify diversity within *A. owenii*	*A. owenii*	Nuclear DNA	Microsatellites (14 loci)	124	2010
Ramstad et al. 2013	Genetic consequences of a century of protection: serial founder events and survival of the little spotted kiwi (*Apteryx owenii*)	Diversity	Using microsatellites to characterise diversity within modern *A. owenii*	*A. owenii*	Nuclear DNA	Microsatellites (15 loci)	24	2013
Ramstad et al. 2016	Sixteen kiwi (*Apteryx* spp) transcriptomes provide a wealth of genetic markers and insight into sex chromosome evolution in birds.	Diversity	Identification of genetic markers using sequencing of transcriptomes	*A. owenii + A. rowi*	Transcriptomes		16	2016

Sackton et al. 2019	Convergent regulatory evolution and loss of flight in paleognathous birds	Phylogeny - Ratites	Mapping genetic origin of loss of flight	All	Nuclear DNA	Whole genome	6	2019
Shepherd & Lambert 2006	Nuclear microsatellite DNA markers for New Zealand kiwi (*Apteryx* spp.)	Diversity	Identification of first 5 polymorphic microsatellites for *A. mantelli* that can be used in further studies	*A. mantelli*	Nuclear DNA	Microsatellites (5 loci)	66	2006
Shepherd & Lambert 2008	Ancient DNA and conservation: lessons from the endangered kiwi of New Zealand	Phylogeny - Historic diversity and distribution	First study if diversity in museum and other ancient samples	All	mtDNA	Control region and cytochrome B	88	2008

Shepherd & Lambert 2013	Using ancient DNA to enhance museum collections: A case study of rare kiwi (*Apteryx* spp.) specimens.	Applied	Using mtDNA to determine the species identity of 6 *Apteryx* skeletons of unknown origin	All	mtDNA		6	2013
Shepherd et al. 2009	Selection of a neotype for Apteryx mantelli Bartlett, 1852, with the support of genetic data.	Applied	Characterisation of neotype for *A. mantelli*	*A. mantelli*			1	2009
Shepherd et al. 2012	Ancient DNA analyses reveal contrasting phylogeographic patterns amongst kiwi (*Apteryx* spp.) and a recently extinct lineage of spotted kiwi.	Phylogeny - Historic diversity and distribution	Genetic analyses of past distribution of *A. owenii*	*A. owenii*	mtDNA	Control region, cytochrome B and ATPase	115	2012

Subramanian et al. 2010	Next generation sequencing and analysis of a conserved transcriptome of New Zealand's kiwi	Phylogeny - Aves	Comparison of Kiwi and Chicken sequence and gene expression level to confirm evolutionary distance between them	*A. mantelli*	Transcriptomes		1	2010
Taylor 2014	Detecting inbreeding depression in a severely bottlenecked, recovering species: the little spotted kiwi (*Apteryx owenii*).**	Applied	Using microsatellites to characterise diversity within modern A*. owenii*	*A. owenii*	Nuclear DNA	Microsatellites	155	2014
Weir et al. 2016	Explosive ice age diversification of kiwi.	Phylogeny - *Apteryx*	Most comprehensive study so far of genetic diversity and phylogeny of Apteryx.	All	mtDNA, Nuclear DNA	mtDNA and GBS	203 mtDNA 97 GBS	2016

White et al. 2018	Novel genetic variation in an isolated population of the nationally critical Haast tokoeka (*Apteryx australis* 'Haast') reveals extreme short-range structure within this cryptic and flightless bird.	Diversity	Detailed study of diversity within A. australis from Haast	*A. australis*	mtDNA		66	2018
Ziesemann 2011	The social organisation and mating system of the Brown Kiwi (*Apteryx mantelli*)**	Applied	Genetic characterisation of relationships and kinship among *A. mantelli* on Ponui island	*A. mantelli*	Nuclear DNA	Microsatellites (12 loci)	233	2011

*Master thesis
**PhD thesis
[+]of which 849 individual cloak feathers not necessarily for as many individuals
[++]Department of Conservation report, not published in peer reviewed journal

Supplementary table S1.2.2. Sample sizes per area, taxon, and species for each of the Apteryx genetic studies featuring in the review.

	Total	Waitangi	Purua	Waipoua	Tangiteroria	Whangarei	Northland unspecified	Coromandel	Whanganui	Taranaki	Okara	Tongariro	Ruapehu	Little Barrier Island	Ponui	Kaweka
		Northland							Western					Eastern		
Baker 1995	178	36								5				10		
Binney 2007	38					12										
Burbidge 2003	61	3			1				2	5				6		
Cooper 1992	3															
Cooper 2001	1				1											
Crimp 2010	1															
Dawson 2015	14															
de Boer 1980	1															
Grueber 2013	2		2													
Grueber 2015	20		20													
Hackkett 2008	1															
Haddrath & Baker 2001	1															
Haddrath & Baker 2012	1															
Harshman 2008	1															
Hartnup 2011	192	3	14		1	2	3	15	15	25		10	11	6		4
Herbert & Daugherty 2002	250	36		7	31				1	5				8		1
Huynen 2002	21															
Huynen 2003	32															
Jensen 2008	79							35								
Le Duc 2015	3						1									
Liu 2017	3						1									
McLennan 2002	40															

Miller 2011	13															
Mitchell 2014	1															
Phillips 2009	1															
Powlesland 1988	47	5			5											
Ramon-Laca 2018	286			3		33		55	4	2		14				55
Ramstad 2010	124															
Ramstad 2013	167															
Ramstad 2016	16															
Sackton 2019	6						1									
Shepherd 2006	67				1		35									
Shepherd 2008	88	3			1				4	5			1	6		
Shepherd 2009	1									1						
Shepherd 2012	125	3			1		2		4	5			1	6		
Shepherd 2013	7				1		3									
Subramanian 2010	1															
Taylor 2014	155															
Weir 2016	220	2	5	8		1		9	5	1	4	4				
White 2018	66															
Ziesemann 2011	225					145									80	
Total	2559	91	41	18	43	193	46	114	35	54	4	28	13	42	80	60

	Wairoa	Hawkes Bay	Whakatane/Ohope/Houpoto	Rotorua/Te Puke/Omataroa	Gisborne	Otamatuna	Waikaremoana	Whirnaki	Maungataniwha	Hinepukohurangi	Ruahine Ranges	Brown Kiwi unspecified	Okarito	Rowi unspecified	Extinct population: Hawkes bay	Extinct population: Tasman
						Eastern								Rowi		
Baker 1995	3		22										10			
Binney 2007													18			

Burbidge 2003	3		2	1								9				
Cooper 1992																
Cooper 2001																
Crimp 2010																
Dawson 2015																
de Boer 1980																
Grueber 2013																
Grueber 2015																
Hackkett 2008																
Haddrath & Baker 2001																
Haddrath & Baker 2012																
Harshman 2008																
Hartnup 2011	3	3	22	5	1		8	14	17	5	5					
Herbert & Daugherty 2002	3		4	1	1	2	1		1			16	10			
Huynen 2002												21				
Huynen 2003												14	2			
Jensen 2008			30									14				
Le Duc 2015							2									
Liu 2017							2									
McLennan 2002																
Miller 2011																
Mitchell 2014																
Phillips 2009																
Powlesland 1988												37				
Ramon-Laca 2018		1	1	1		3	4		11		3		9			
Ramstad 2010												6		6		
Ramstad 2013																
Ramstad 2016													8			
Sackton 2019							2						1			
Shepherd 2006													9			
Shepherd 2008	3		2	1									9		1	5
Shepherd 2009																
Shepherd 2012	3		2	1									10		1	5
Shepherd 2013																
Subramanian 2010												1				

	Total	Extinct population: Martinborough	Haast	Upper Arawhata	Clinton Valley	Thomson	Murchison/Anau	Awe Burn ('Central' Fiordland)	Deep Cove	Cavendish	Resolution Island	Supper Cove	Fiordland unspecified	Mason Bay	Rakiura unspecified	Tokoeka unspecified	Extinct population: Mt. Cookson
Taylor 2014																	
Weir 2016		2	1	1	1		7	9	5					18		1	5
White 2018																	
Ziesemann 2011																	
	Total	20	5	86	11	2	12	28	19	29	5	8	109	113	6	3	15

	Haast		North Fiordland	South Fiordland	Rakiura	
Baker 1995	7	1	2	5	36	
Binney 2007						
Burbidge 2003	8	2	2	5	9	
Cooper 1992						1
Cooper 2001						
Crimp 2010						
Dawson 2015						14
de Boer 1980						1
Grueber 2013						
Grueber 2015						
Hackkett 2008						1
Haddrath & Baker 2001						
Haddrath & Baker 2012						
Harshman 2008						1
Hartnup 2011						
Herbert & Daugherty 2002	3		1	4	38	
Huynen 2002						

Huynen 2003			5														
Jensen 2008																	
Le Duc 2015																	
Liu 2017																	
McLennan 2002																	
Miller 2011																	
Mitchell 2014																	
Phillips 2009																	
Powlesland 1988																	
Ramon-Laca 2018			41	6		4		4					5	2	10		
Ramstad 2010																6	
Ramstad 2013																	
Ramstad 2016																	
Sackton 2019																	
Shepherd 2006																	5
Shepherd 2008		3	8	2			4		5					9			5
Shepherd 2009																	
Shepherd 2012		3	8	2			4		5					9			5
Shepherd 2013															1		
Subramanian 2010																	
Taylor 2014																	
Weir 2016		2	14	1	6	4	5	12	4	1	2	6		14			5
White 2018			36	7		3		4					4	2	10		
Ziesemann 2011																	
	Total	8	130	21	6	11	18	20	28	1	2	6	9	45	95	24	20

	Extinct population: Castle Rock	Extinct population: Mt. Somers	Mercery Island	Kapiti Island	Durville Island	Long Island	Tiritiri Matangi	Zelandia	Little Spotted Kiwi unspecified	Extinct population: Waitomo	Extinct population: NW Nelson	Extinct population: Castle Rock	Extinct population: Mt. Cookson	Extinct population: West coast	Extinct population: South Cantebury	Extinct population: South Fiordland
Little spotted kiwi																
Baker 1995									33							
Binney 2007			8													
Burbidge 2003									1							
Cooper 1992									1							
Cooper 2001																
Crimp 2010									1							
Dawson 2015																
de Boer 1980																
Grueber 2013																
Grueber 2015																
Hackkett 2008																
Haddrath & Baker 2001																
Haddrath & Baker 2012				1												
Harshman 2008																
Hartnup 2011																
Herbert & Daugherty 2002				31	2											
Huynen 2002																
Huynen 2003				9												
Jensen 2008																
Le Duc 2015																
Liu 2017																

McLennan 2002																	
Miller 2011					10		3										
Mitchell 2014																	
Phillips 2009										1							
Powlesland 1988																	
Ramon-Laca 2018					5				1								
Ramstad 2010					100												
Ramstad 2013				27	99		14	27									
Ramstad 2016					5		2		1								
Sackton 2019					1												
Shepherd 2006		5	1							2							
Shepherd 2008		5	1		1												
Shepherd 2009																	
Shepherd 2012		5	1		3	1				3	2	5	2	1	3	2	1
Shepherd 2013										2							
Subramanian 2010																	
Taylor 2014								43	112								
Weir 2016		5	1		6	1					2	5	2	1	3	2	1
White 2018																	
Ziesemann 2011																	
	Total	20	4	35	271	4	19	70	114	44	4	10	4	2	6	4	2

	Extinct population: Te Mimi	Extinct population: Coonoor	Extinct population: Hawkes Bay	Northwest Nelson	Westport	Paparoa Range	Arthur's Pass	Great Spotted unspecified	Extinct population: Mt. Arthur	A. owenii x A. mantelli (?) Franz Josef	A. mantelli x A. australis (?) Kapiti Island
					Great Spotted Kiwi						
Baker 1995								8			
Binney 2007											
Burbidge 2003								2			
Cooper 1992								1			
Cooper 2001											
Crimp 2010											
Dawson 2015											
de Boer 1980											
Grueber 2013											
Grueber 2015											
Hackkett 2008											
Haddrath & Baker 2001								1			
Haddrath & Baker 2012											
Harshman 2008											
Hartnup 2011											
Herbert & Daugherty 2002				23		4	7	6		1	2
Huynen 2002											
Huynen 2003							2				
Jensen 2008											
Le Duc 2015											

Liu 2017												
McLennan 2002					23		4	7	6			
Miller 2011												
Mitchell 2014									1			
Phillips 2009												
Powlesland 1988												
Ramon-Laca 2018							3	6				
Ramstad 2010									6			
Ramstad 2013												
Ramstad 2016												
Sackton 2019								1				
Shepherd 2006									9			
Shepherd 2008							2		1	1		
Shepherd 2009												
Shepherd 2012			1	1	6		3	3	1	1		
Shepherd 2013												
Subramanian 2010												
Taylor 2014												
Weir 2016		1	1	1	8		6	8		1		
White 2018												
Ziesemann 2011												
	Total	1	2	2	60	0	22	34	42	3	1	2

Supplementary table S1.2.3. Geographic origin and genotype identity for all 512 kiwi with full or partial sequencing of the mitochondrial DNA and which publication(s) they have featured in. Equivalent to published online material 3 but split into 3 parts.

Sample Identification

Sample/Bird I.D.	Species	Lineage	Locality	Locality group
nib.75	A. mantelli	Northland	Northland	Unspecified
88bp	A. mantelli	Northland	Purua	Purua
5ffRr	A. mantelli	Northland	Rarewarewa	Purua
ob1Mo	A. mantelli	Northland	Rarewarewa	Purua
198H	A. mantelli	Northland	Hodges, kamo	Purua
R44928	A. mantelli	Northland	Rarewarewa	Purua
R44968	A. mantelli	Northland	Rarewarewa	Purua
2dbRp	A. mantelli	Northland	Riponui	Purua
624Rp	A. mantelli	Northland	Riponui	Purua
FdaH	A. mantelli	Northland	Hodges, kamo	Purua
22aM	A. mantelli	Northland	Marlow	Purua
3a2M	A. mantelli	Northland	Marlow	Purua
R45936	A. mantelli	Northland	Purua	Purua
099Bh	A. mantelli	Northland	Rarewarewa	Purua
157Mo	A. mantelli	Northland	Rarewarewa	Purua
424Rr	A. mantelli	Northland	Rarewarewa	Purua
9e9D	A. mantelli	Northland	Rarewarewa	Purua
R44973	A. mantelli	Northland	Purua	Purua
891Rr	A. mantelli	Northland	Rarewarewa	Purua
c22 IDA6A6F	A. mantelli	Northland	Rarewarewa	Purua
T456	A. mantelli	Northland	Tangiteroria	Tangiteroria
TK164	A. mantelli	Northland	Trounson	Waipoua
TK86	A. mantelli	Northland	Trounson	Waipoua
R35015	A. mantelli	Northland	Waipoua	Waipoua

TK153	A. mantelli	Northland	Trounson	Waipoua
TK85	A. mantelli	Northland	Trounson	Waipoua
R35012	A. mantelli	Northland	Waipoua	Waipoua
R35016	A. mantelli	Northland	Waipoua	Waipoua
R35017	A. mantelli	Northland	Waipoua	Waipoua
CD1995	A. mantelli	Northland	Waitangi	Waitangi
CD1997	A. mantelli	Northland	Waitangi Forest	Waitangi
CD1996	A. mantelli	Northland	Waitangi Forest	Waitangi
?	A. mantelli	Northland	Waitangi	Waitangi
RA 0161	A. mantelli	Northland	Glenbervie	Whangarei
O9d11	A. mantelli	Northland	Whangarei	Whangarei
4e6Bh	A. mantelli	Northland	Bream Head	Whangarei
BMNH 1842.5.17.2	A. mantelli	Northland	"presented by Miss Rebecca Stone" (have Northland haplotype)	Unspecified
BMNH 1838.5.12.102	A. mantelli	Northland	"presented by the Earl of Derby" (have Northland haplotype)	Unspecified
K86	A. mantelli	Northland	Tangiteroria (?)	Tangiteroria
FT2988	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
FT2989	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
FT2990	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
LBK001	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
LBK004	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
FT2986	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
FT2987	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
LBK002	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
LBK003	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
?	A. mantelli	Little Barrier Island	Little Barrier Island	Little Barrier Island
alex	A. mantelli	Coromandel	Coromandel	Coromandel

aru	A. mantelli	Coromandel	Coromandel	Coromandel
Bubbles	A. mantelli	Coromandel	Coromandel	Coromandel
Guy	A. mantelli	Coromandel	Coromandel	Coromandel
Homer	A. mantelli	Coromandel	Coromandel	Coromandel
Marenui	A. mantelli	Coromandel	Coromandel	Coromandel
Moneypenny	A. mantelli	Coromandel	Coromandel	Coromandel
palo	A. mantelli	Coromandel	Coromandel	Coromandel
Percy	A. mantelli	Coromandel	Coromandel	Coromandel
Rewarewa	A. mantelli	Coromandel	Coromandel	Coromandel
Simo	A. mantelli	Coromandel	Coromandel	Coromandel
Tango	A. mantelli	Coromandel	Coromandel	Coromandel
Taunis	A. mantelli	Coromandel	Coromandel	Coromandel
R32852	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
Minnie	A. mantelli	Coromandel	Coromandel	Coromandel
chick 12	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
chick 3	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
chick 6	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
R32860	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
R36095	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
R36099	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel

R55426	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
R55426	A. mantelli	Coromandel	Kuaotunu, Coromandel Peninsula	Coromandel
kina	A. mantelli	Coromandel	Moehau	Coromandel
Pinky	A. mantelli	Eastern	Gisbourne	Gisbourne
DM1212	A. mantelli	Eastern	Waikoau, Hawke's Bay	Hawkes Bay
49	A. mantelli	Eastern	Hawkes Bay	Hawkes Bay
Ari	A. mantelli	Eastern	Hawkes Bay	Hawkes Bay
Bracken	A. mantelli	Eastern	Westshore Wildlife Reserve, Napier, Hawkes Bay	Hawkes Bay
DM1552	A. mantelli	Eastern	Hawke's Bay	Hawkes Bay
corona	A. mantelli	Eastern	Hinepukohurangi	Maungataniwha
Hinemoa	A. mantelli	Eastern	Hinepukohurangi	Maungataniwha
Maggy	A. mantelli	Eastern	Hinepukohurangi	Maungataniwha
magnum	A. mantelli	Eastern	Hinepukohurangi	Maungataniwha
Tocha	A. mantelli	Eastern	Hinepukohurangi	Maungataniwha
Waiwai	A. mantelli	Eastern	Kawekas	Kawekas
Albert	A. mantelli	Eastern	Kawekas	Kawekas
Hariana	A. mantelli	Eastern	Kawekas	Kawekas
Pudding	A. mantelli	Eastern	Kawekas	Kawekas
Berry	A. mantelli	Eastern	Maungataniwha	Maungataniwha
Blue Gum	A. mantelli	Eastern	Maungataniwha	Maungataniwha
cookie	A. mantelli	Eastern	Maungataniwha	Maungataniwha
darwin	A. mantelli	Eastern	Maungataniwha	Maungataniwha
domino	A. mantelli	Eastern	Maungataniwha	Maungataniwha
kora	A. mantelli	Eastern	Maungataniwha	Maungataniwha
pavlova	A. mantelli	Eastern	Maungataniwha	Maungataniwha

shaggy	A. mantelli	Eastern	Maungataniwha	Maungataniwha
splash	A. mantelli	Eastern	Maungataniwha	Maungataniwha
taoka	A. mantelli	Eastern	Maungataniwha	Maungataniwha
trooper	A. mantelli	Eastern	Maungataniwha	Maungataniwha
chute	A. mantelli	Eastern	Maungataniwha	Maungataniwha
fred	A. mantelli	Eastern	Maungataniwha	Maungataniwha
bondi	A. mantelli	Eastern	Maungataniwha	Maungataniwha
Eco	A. mantelli	Eastern	Maungataniwha	Maungataniwha
rowi	A. mantelli	Eastern	Maungataniwha	Maungataniwha
Hine	A. mantelli	Eastern	Maungataniwha	Maungataniwha
CD887	A. mantelli	Eastern	Whakatane	Ohope
breeze	A. mantelli	Eastern	Ohope	Ohope
champers	A. mantelli	Eastern	Ohope	Ohope
Chitchat	A. mantelli	Eastern	Ohope	Ohope
epi	A. mantelli	Eastern	Ohope	Ohope
fig	A. mantelli	Eastern	Ohope	Ohope
koripo	A. mantelli	Eastern	Ohope	Ohope
lag	A. mantelli	Eastern	Ohope	Ohope
mako	A. mantelli	Eastern	Ohope	Ohope
Manuka	A. mantelli	Eastern	Ohope	Ohope
rangi	A. mantelli	Eastern	Ohope	Ohope
stinger	A. mantelli	Eastern	Ohope	Ohope
twinkle	A. mantelli	Eastern	Ohope	Ohope
whiturau	A. mantelli	Eastern	Ohope	Ohope
Quazi	A. mantelli	Eastern	Ohope	Ohope
diamond	A. mantelli	Eastern	Ohope	Ohope
ohiwa	A. mantelli	Eastern	Ohope	Ohope
teva	A. mantelli	Eastern	Ohope	Ohope
Wedgie	A. mantelli	Eastern	Ohope	Ohope
Bear	A. mantelli	Eastern	Ohope	Ohope

boxer	A. mantelli	Eastern	Ohope	Ohope
Wafer	A. mantelli	Eastern	Ohope	Ohope
teer	A. mantelli	Eastern	Omataroa	Omataroa
vollie	A. mantelli	Eastern	Omataroa	Omataroa
Flipper	A. mantelli	Eastern	Omataroa	Omataroa
Hydee	A. mantelli	Eastern	Omataroa	Omataroa
K311	A. mantelli	Eastern	Otamatuna, Northern Ureweras, Urewera National Park	Otamatuna
CD1295	A. mantelli	Eastern	Urewera National Park	Otamatuna
R31812	A. mantelli	Eastern	Otamatuna, Northern Ureweras, Urewera National Park	Otamatuna
R32946	A. mantelli	Eastern	Otamatuna, Northern Ureweras, Urewera National Park	Otamatuna
R59125	A. mantelli	Eastern	Otamatuna, Northern Ureweras, Urewera National Park	Otamatuna
RC01	A. mantelli	Eastern	Urewera Nat Park	Otamatuna
RC02	A. mantelli	Eastern	Urewera Nat Park	Otamatuna
48	A. mantelli	Eastern	Rotorua	Rotorua
CD890	A. mantelli	Eastern	Te Puke, Rotorua	Rotorua
Hetty	A. mantelli	Eastern	Hauotaruke Bay, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana

R43538	A. mantelli	Eastern	Parahamuti Point, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
R43517	A. mantelli	Eastern	Te Rata Bay, Lake Waikaremoana, Urewera National Park	Waikaremoana
R43572	A. mantelli	Eastern	Te Ruapohatu Stream, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
R43544	A. mantelli	Eastern	Tikitiki Point, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
B97	A. mantelli	Eastern	Waikaremoana	Waikaremoana
M97.2	A. mantelli	Eastern	Waikaremoana	Waikaremoana
Maggie	A. mantelli	Eastern	Waikaremoana	Waikaremoana
Moko	A. mantelli	Eastern	Waikaremoana	Waikaremoana
Ry97.2	A. mantelli	Eastern	Waikaremoana	Waikaremoana
R43528	A. mantelli	Eastern	Tapuaenui Stream, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
R43573	A. mantelli	Eastern	Parahamuti Point, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
a96.1	A. mantelli	Eastern	Waikaremoana	Waikaremoana

R43519	A. mantelli	Eastern	Waikopiro Stream, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
R43536	A. mantelli	Eastern	Waapu Stream, Puketukutuku Peninsula, Lake Waikaremoana, Urewera National Park	Waikaremoana
Dreads	A. mantelli	Eastern	Waikaremoana	Waikaremoana
Gonzo	A. mantelli	Eastern	Waikaremoana	Waikaremoana
CD1210	A. mantelli	Eastern	Wairoa	Wairoa
CD961	A. mantelli	Eastern	Wairoa	Wairoa
CD963	A. mantelli	Eastern	Wairoa	Wairoa
CD886	A. mantelli	Eastern	Haupoto - Houpoto??	Ohope
CD891	A. mantelli	Eastern	Whakatane	Ohope
R55414	A. mantelli	Eastern	near Whirinaki River, Whirinaki Forest, Okahu Valley	Whirinaki
R55413	A. mantelli	Eastern	near Whirinaki River, Whirinaki Forest,Okahu Valley	Whirinaki
R55402	A. mantelli	Eastern	near Minginui, Whirinaki Forest	Whirinaki
Awa	A. mantelli	Eastern	Whirinaki	Whirinaki
buster	A. mantelli	Eastern	Whirinaki	Whirinaki
claus	A. mantelli	Eastern	Whirinaki	Whirinaki
hone	A. mantelli	Eastern	Whirinaki	Whirinaki
Karaponia	A. mantelli	Eastern	Whirinaki	Whirinaki
marley	A. mantelli	Eastern	Whirinaki	Whirinaki
marmalade	A. mantelli	Eastern	Whirinaki	Whirinaki

martini	A. mantelli	Eastern	Whirinaki	Whirinaki
roimata	A. mantelli	Eastern	Whirinaki	Whirinaki
Te Wairoa	A. mantelli	Eastern	Whirinaki	Whirinaki
R55403	A. mantelli	Eastern	near Minginui, Whirinaki Forest	Whirinaki
Maroke	A. mantelli	Eastern	Whirinaki	Whirinaki
speights	A. mantelli	Eastern	Whirinaki	Whirinaki
Timata	A. mantelli	Eastern	Whirinaki	Whirinaki
NIBK001	A. mantelli	Eastern	near Minginui, Whirinaki Forest	Whirinaki
buttons	A. mantelli	Eastern	Whirinaki	Whirinaki
allbut	A. mantelli	Eastern	Ruahine Ranges	Ruahine Ranges
Rubiks	A. mantelli	Eastern	Ruahine Ranges	Ruahine Ranges
blue	A. mantelli	Eastern	Ruahine Ranges	Ruahine Ranges
Camo	A. mantelli	Eastern	Ruahine Ranges	Ruahine Ranges
mokai	A. mantelli	Eastern	Ruahine Ranges	Ruahine Ranges
54 Don	A. mantelli	Western	Aotuhia, Okara Forest	Okara
81 Emma	A. mantelli	Western	Aotuhia, Okara Forest	Okara
82 Penny	A. mantelli	Western	Aotuhia, Okara Forest	Okara
70 Cathy	A. mantelli	Western	Aotuhia, Okara Forest	Okara
DM14849	A. mantelli	Western	Retaruke Valley, Katieke	Okara
mayhem	A. mantelli	Western	Karioi Rahui	Ruapehu
Kaha	A. mantelli	Western	Ruapehu	Ruapehu
Kuraiti	A. mantelli	Western	Ruapehu	Ruapehu
Ross	A. mantelli	Western	Ruapehu	Ruapehu
DM9375	A. mantelli	Western	Ohakune	Ruapehu
Iguazu	A. mantelli	Western	Kariroi Rahui	Ruapehu

kruze	A. mantelli	Western	Kariroi Rahui	Ruapehu
Nugget	A. mantelli	Western	Kariroi Rahui	Ruapehu
Rio	A. mantelli	Western	Kariroi Rahui	Ruapehu
Libby	A. mantelli	Western	Ruapheu	Ruapehu
Putiputi	A. mantelli	Western	Ruapheu	Ruapehu
CD2021	A. mantelli	Western	New Plymouth	Taranaki
CD964	A. mantelli	Western	New Plymouth	Taranaki
Bayfield	A. mantelli	Western	Taranaki	Taranaki
Dawson	A. mantelli	Western	Taranaki	Taranaki
Ford	A. mantelli	Western	Taranaki	Taranaki
harenge	A. mantelli	Western	Taranaki	Taranaki
Ingle	A. mantelli	Western	Taranaki	Taranaki
ironman	A. mantelli	Western	Taranaki	Taranaki
kaimata	A. mantelli	Western	Taranaki	Taranaki
Mautau	A. mantelli	Western	Taranaki	Taranaki
purangi	A. mantelli	Western	Taranaki	Taranaki
red	A. mantelli	Western	Taranaki	Taranaki
Ringo	A. mantelli	Western	Taranaki	Taranaki
runner	A. mantelli	Western	Taranaki	Taranaki
Rusty	A. mantelli	Western	Taranaki	Taranaki
Solstice	A. mantelli	Western	Taranaki	Taranaki
tahuna	A. mantelli	Western	Taranaki	Taranaki
tarata	A. mantelli	Western	Taranaki	Taranaki
Tk.Ure1/vogel	A. mantelli	Western	Taranaki	Taranaki
CD1994	A. mantelli	Western	New Plymouth	Taranaki
Jaime	A. mantelli	Western	Taranaki	Taranaki
Ra	A. mantelli	Western	Taranaki	Taranaki
CD1993	A. mantelli	Western	New Plymouth	Taranaki
AWOL	A. mantelli	Western	Taranaki	Taranaki
CD962	A. mantelli	Western	New Plymouth	Taranaki

Eddie	A. mantelli	Western	Tongariro	Tongariro
Fleming	A. mantelli	Western	Tongariro	Tongariro
Haki	A. mantelli	Western	Tongariro	Tongariro
pokano	A. mantelli	Western	Tongariro	Tongariro
Robyn	A. mantelli	Western	Tongariro	Tongariro
topo	A. mantelli	Western	Tongariro	Tongariro
R36079	A. mantelli	Western	Tongariro Forest, Tongariro National Park	Tongariro
R46251	A. mantelli	Western	Tongariro Forest, Tongariro National Park	Tongariro
Smee	A. mantelli	Western	Tongariro Forest, Tongariro National Park	Tongariro
Waihaika	A. mantelli	Western	Tongariro Forest, Tongariro National Park	Tongariro
april	A. mantelli	Western	Tongariro	Tongariro
Elmo	A. mantelli	Western	Tongariro	Tongariro
Morton	A. mantelli	Western	Tongariro	Tongariro
Possum	A. mantelli	Western	Tongariro	Tongariro
76	A. mantelli	Western	Whanganui	Whanganui
6	A. mantelli	Western	Jerusalem	Whanganui
frankin	A. mantelli	Western	Waimarino	Whanganui
gold	A. mantelli	Western	Waimarino	Whanganui
Mataritki	A. mantelli	Western	Waimarino	Whanganui
Moony	A. mantelli	Western	Waimarino	Whanganui
myrrh	A. mantelli	Western	Waimarino	Whanganui
Niglet	A. mantelli	Western	Waimarino	Whanganui
pea	A. mantelli	Western	Waimarino	Whanganui
Rudolph	A. mantelli	Western	Waimarino	Whanganui
Tanekaha	A. mantelli	Western	Waimarino	Whanganui

Aroha	A. mantelli	Western	Waimarino Forest, near Whanganui National Park	Whanganui
Tia	A. mantelli	Western	Waimarino Forest, near Whanganui National Park	Whanganui
Tua	A. mantelli	Western	Waimarino Forest, near Whanganui National Park	Whanganui
Zinger	A. mantelli	Western	Waimarino Forest, near Whanganui National Park	Whanganui
Sirius	A. mantelli	Western	Wanganui	Whanganui
Kowhai	A. mantelli	Western	Waimarino	Whanganui
R36085	A. mantelli	Western	Waimarino Forest, near Whanganui National Park	Whanganui
DM10276	A. mantelli	Western	Waverley	Whanganui
DM13551	A. mantelli	Western	Waitotara Valley, whanganui	Whanganui
DM2135 or OR.2435	A. mantelli	Unknown		Unspecified
OR.1148	A. mantelli	Unknown	?	Unspecified
?	A. mantelli	Unknown	?	Unspecified
OR.1152	A. mantelli	Unknown		Unspecified
R35003	A. rowi	Okarito	Okarito	Okarito
AV800	A. rowi	Okarito	Okarito	Okarito
CD832	A. rowi	Okarito	Okarito	Okarito
R34152	A. rowi	Okarito	Okarito	Okarito
R34153	A. rowi	Okarito	Okarito	Okarito
R34155	A. rowi	Okarito	Okarito	Okarito
R34156	A. rowi	Okarito	Okarito	Okarito
R35002	A. rowi	Okarito	Okarito	Okarito
R43461	A. rowi	Okarito	Okarito	Okarito

R43477	A. rowi	Okarito	Okarito	Okarito
R43493	A. rowi	Okarito	Okarito	Okarito
R46314 Scooter	A. rowi	Okarito	Okarito	Okarito
R55120	A. rowi	Okarito	Okarito	Okarito
R55135	A. rowi	Okarito	Okarito	Okarito
R55139	A. rowi	Okarito	Okarito	Okarito
R55313	A. rowi	Okarito	Okarito	Okarito
R55393	A. rowi	Okarito	Okarito	Okarito
R34151	A. rowi	Okarito	Okarito	Okarito
R34154	A. rowi	Okarito	Okarito	Okarito
R43474 Maxine	A. rowi	Okarito	Okarito	Okarito
R43495	A. rowi	Okarito	Okarito	Okarito
R55133	A. rowi	Okarito	Okarito	Okarito
R55136	A. rowi	Okarito	Okarito	Okarito
R55312	A. rowi	Okarito	Okarito	Okarito
R55357	A. rowi	Okarito	Okarito	Okarito
R55366	A. rowi	Okarito	Okarito	Okarito
?	A. rowi	Okarito	Okarito	Okarito
S.009401	A. rowi	Extinct Population	Poukawa	Hawkes Bay
DM7869	A. rowi	Extinct Population	Martinborough, North Is.	Martinborough
DM7900	A. rowi	Extinct Population	Martinborough, North Is.	Martinborough
DM7896	A. rowi	Extinct Population	Martinborough	Martinborough
AV16697	A. rowi	Extinct Population	Kiwi Hole, Caanan, Takaka, tasman	Tasman
S.23211	A. rowi	Extinct Population	Takaka	Tasman
1.S.24355.1 or S.24355.1	A. rowi	Extinct Population	Perini Creek, Buller Gorge, Tasman	Tasman
2.S.24355.2 or S.24355.2	A. rowi	Extinct Population	Perini Creek, Buller Gorge, Tasman	Tasman

THW collection	A. rowi	Extinct Population	Takaka Fossil Cave, Takaka, tasman	Tasman
R55342	A. australis	Haast	Branch Creek, North Slip, Haast	Haast
R55341	A. australis	Haast	Branch Creek, South Slip, Haast	Haast
RA 0930	A. australis	Haast	Haast (Alpine)	Haast
19_02_93	A. australis	Haast	Haast (Range)	Haast
R34157	A. australis	Haast	Lake Greaney	Haast
R34158	A. australis	Haast	Lake Greaney	Haast
R34159	A. australis	Haast	Lake Greaney	Haast
R34160	A. australis	Haast	Lake Greaney	Haast
RA0921	A. australis	Haast	Lake Greaney	Haast
RA 0936	A. australis	Haast	Lake Greaney	Haast
RA 0996	A. australis	Haast	Slippery Spur, Haast Range (Waiatoto?)	Haast
RA 0940	A. australis	Haast	Thirsty Ridge Camp, Haast	Haast
RA0937	A. australis	Haast	Thirsty Ridge Mid, Haast	Haast
RA0938	A. australis	Haast	Thirsty Ridge Mid, Haast	Haast
TFCK	A. australis	Haast	Tuning Fork Creek	Haast
R55343	A. australis	Haast	Waiatoto River, Haast	Haast
RA 0992	A. australis	Haast	Waiatoto, Haast	Haast
RA 0994	A. australis	Haast	Waiatoto, Haast	Haast
S310_1	A. australis	Haast	Haast	Haast
S344_1	A. australis	Haast	Haast	Haast
33992	A. australis	Haast	Haast	Haast
33997	A. australis	Haast	Haast	Haast
AR	A. australis	Haast	Arawhata	Haast
AV	A. australis	Haast	Arawhata	Haast

BH	A. australis	Haast	Arawhata	Haast
CF	A. australis	Haast	Waiatoto	Haast
FT	A. australis	Haast	Arawhata	Haast
HB	A. australis	Haast	Hindley	Haast
HI	A. australis	Haast	Hindley	Haast
MG	A. australis	Haast	Arawhata	Haast
NH1	A. australis	Haast	Waiatoto	Haast
OS	A. australis	Haast	Arawhata	Haast
RS	A. australis	Haast	Waiatoto	Haast
SC	A. australis	Haast	Waiatoto	Haast
SL	A. australis	Haast	Waiatoto	Haast
TC	A. australis	Haast	Alpine	Haast
TR	A. australis	Haast	Alpine	Haast
WD	A. australis	Haast	Hindley	Haast
CS	A. australis	Haast	Arawhata	Haast
DP	A. australis	Haast	Arawhata	Haast
HH	A. australis	Haast	Hindley	Haast
JV	A. australis	Haast	Arawhata	Haast
TI	A. australis	Haast	Arawhata	Haast
BE	A. australis	Haast	Alpine	Haast
Wisneski	A. australis	Haast	Arawhata	Haast
Whitewash	A. australis	Haast	Hindley	Haast
NH2	A. australis	Haast	Waiatoto	Haast
CC	A. australis	Haast	Haast	Haast
WO	A. australis	Haast	Waiatoto	Haast
MW	A. australis	Haast	Haast	Haast
BH2	A. australis	Haast	Arawhata	Haast
TZ	A. australis	Haast	Arawhata	Haast
WW	A. australis	Haast	Hindley	Haast
HF	A. australis	Haast	Hindley	Haast

BR	A. australis	Haast	Waiatoto	Haast
TR	A. australis	Haast	Alpine	Haast
WT	A. australis	Haast	Hindley	Haast
LM	A. australis	Haast	Upper Arawhata	Upper Arawhata
UC	A. australis	Haast	Upper Arawhata	Upper Arawhata
R35021	A. australis	Haast	McArthur Creek, Haast	Upper Arawhata
RA0991	A. australis	Haast	Joe Creek	Upper Arawhata
GG	A. australis	Haast	Upper Arawhata	Upper Arawhata
JC	A. australis	Haast	Upper Arawhata	Upper Arawhata
LC	A. australis	Haast	Upper Arawhata	Upper Arawhata
UM	A. australis	Haast	Upper Arawhata	Upper Arawhata
UM	A. australis	Haast	Upper Arawhata	Upper Arawhata
R31551	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0601	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0603	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0730	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0743	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0823	A. australis	N. Fiordland	Clinton Valley, Milford Track, Fiordland	Clinton Valley
RA 0731	A. australis	N. Fiordland	Doubtful Is, ex Murchison Mountains, Fiordland	Murchison

RA 0738	A. australis	N. Fiordland	Doubtful Is, ex Murchison Mountains, Fiordland	Murchison
CD1141	A. australis	N. Fiordland	Murchison Mtns	Murchison
CD1151	A. australis	N. Fiordland	Murchison Mtns	Murchison
RA 0740	A. australis	N. Fiordland	Te Anau Is, ex Murchison Mountains, Fiordland	Murchison
AV588	A. australis	N. Fiordland	Te Anau, Fiordland	Te Anau
AV589	A. australis	N. Fiordland	Te Anau, Fiordland	Te Anau
RA 0708	A. australis	N. Fiordland	Lake Thomson, Fiordland	Thomson
RA 0741	A. australis	N. Fiordland	Lake Thomson, Fiordland	Thomson
RA 0707	A. australis	N. Fiordland	Lake Thomson, Fiordland	Thomson
RA 0742	A. australis	N. Fiordland	Lake Thomson, Fiordland	Thomson
RA 0781	A. australis	C. Fiordland?	Awe Burn	Awe Burn
RA 0784	A. australis	C. Fiordland?	Awe Burn	Awe Burn
RA 0785	A. australis	C. Fiordland?	Awe Burn	Awe Burn
RA 0746	A. australis	C. Fiordland?	Lake Norwest	Awe Burn
RA 0748	A. australis	C. Fiordland?	Lake Norwest	Awe Burn
RA 0788	A. australis	C. Fiordland?	Freeman Burn	Awe Burn
chick 52	A. australis	C. Fiordland?	Lake Norwest	Awe Burn
RA 0773	A. australis	N. Fiordland	Camelot river	Camelot river
RA 0774	A. australis	N. Fiordland	Camelot river	Camelot river
RA 0775	A. australis	N. Fiordland	Camelot river	Camelot river
CD1150	A. australis	S. Fiordland	Wilmot Pass	Wilmot Pass
RA 0278	A. australis	N. Fiordland	Iris Burn	Iris Burn
RA 0396	A. australis	N. Fiordland	Iris Burn	Iris Burn

RA 0202	A. australis	S. Fiordland	Cavendish River, Fiordland	Cavendish
DC3	A. australis	S. Fiordland	Deep Cove, Fiordland	Deep Cove
DC1	A. australis	S. Fiordland	Deep Cove, Fiordland	Deep Cove
DC2	A. australis	S. Fiordland	Deep Cove, Fiordland	Deep Cove
DC4	A. australis	S. Fiordland	Deep Cove, Fiordland	Deep Cove
RA 0208	A. australis	S. Fiordland	Loch Maree, Dusky Track, Fiordland	Loch Maree
RA 0209	A. australis	S. Fiordland	Loch Maree, Dusky Track, Fiordland	Loch Maree
RA 0143	A. australis	S. Fiordland	Cormorant River, Resolution Is, Fiordland	Resolution Island
RA 0224	A. australis	S. Fiordland	Resolution Is, Fiordland	Resolution Island
TOKSC1	A. australis	S. Fiordland	Burt Flat, Supper Cove, Fiordland	Supper Cove
TOKSC4	A. australis	S. Fiordland	Supper Cove, Fiordland	Supper Cove
TOKSC2	A. australis	S. Fiordland	Burt Flat, Supper Cove, Fiordland	Supper Cove
TOKSC5	A. australis	S. Fiordland	Bridge, Supper Cove, Fiordland	Supper Cove
OR.26398	A. australis	Stewart Is.	Stewart Is. (haplotyope)	Unspecified
RA 0152	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0154	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0894	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0896	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island

RA 0903	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0923	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0881	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0902	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0890	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0925	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0927	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA002	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0868	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0882	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0887	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0883	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0888	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA 0924	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0865	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0885	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
RA0886	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island
?	A. australis	Stewart Is.	Mason Bay, Stewart Is/Rakiura	Stewart Island

AV12651	A. australis	Extinct Population	Mt. Somers Quarry	Mt. Somers Quarry
3.S.33369.3 or S.33369.3	A. australis	Extinct Population	Mt. Cookson	Mt. Cookson
5.S.33369.5 or S.33369.5	A. australis	Extinct Population	Mt. Cookson	Mt. Cookson
1.S.33369.1 or S.33369.1	A. australis	Extinct Population	Mt. Cookson	Mt. Cookson
2.S.33369.2 or S.33369.2	A. australis	Extinct Population	Mt. Cookson	Mt. Cookson
4.S.33369.4 or S.33369.4	A. australis	Extinct Population	Mt. Cookson	Mt. Cookson
DM6498	A. australis	Extinct Population	Castle Rocks (Groups with S. Fiordland)	Castle Rocks
DM6557	A. australis	Extinct Population	Castle Rocks (Groups with S. Fiordland)	Castle Rocks
DM6555	A. australis	Extinct Population	Castle Rocks (Groups with S. Fiordland)	Castle Rocks
DM6489	A. australis	Extinct Population	Castle Rocks (Groups with S. Fiordland)	Castle Rocks
S.34531	A. australis	Extinct Population	Castle Rocks (Groups with S. Fiordland)	Castle Rocks
?	A. australis	Unknown	?	Unspecified
?	A. owenii	Kapiti Island	?	Kapiti Island
0-20599 M	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
O 20557	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
O 20600	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
O-20575	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
CD1206	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
CD899	A. owenii	Kapiti Island	Kapiti Island	Kapiti Island
WS1764	A. owenii	Kapiti Island	Durville Island	Kapiti Island
DM6672	A. owenii	Extinct Population	Castle Rocks	Castle Rocks
AV32392B	A. owenii	Extinct Population	Cave at Springhill	Castle Rocks

S.24478	A. owenii	Extinct Population	Coonoor	Coonoor
AV17079	A. owenii	Extinct Population	Hawkes Bay	Hawkes Bay
S.33365	A. owenii	Extinct Population	Holocene Cave, Mt. Cookson	Mt. Cookson
NM23036 or OR.23036	A. owenii	Extinct Population	Banjo Creek, Westhaven Inlet, NW Nelson	NW Nelson
S.27784.1 and S.27784.2	A. owenii	Extinct Population	Earl Grey cave, Takaka Hill	NW Nelson
S.001164	A. owenii	Extinct Population	Nelson	NW Nelson
S.1174 or OR.1174	A. owenii	Extinct Population	Otututu or Rogh River	NW Nelson
AV16713	A. owenii	Extinct Population	Canaan, Takaka Hill	NW Nelson
AV25301	A. owenii	Extinct Population	Kings Cave, South Canterbury	South Canterbury
AV12648C	A. owenii	Extinct Population	Somers Quarry	South Canterbury
S.2069 or OR.2069	A. owenii	Extinct Population	Lake Manapouri	South Fiordland
O 20596	A. owenii	Extinct Population	Te Mimi	Te Mimi
AV22817	A. owenii	Extinct Population	Helectite Hole, Raglan	Waitomo
WO255	A. owenii	Extinct Population	Anns Cavern, Waitomo	Waitomo
S.22007 or OR.22007	A. owenii	Extinct Population	Karangarua River	West Coast
S.23043 or OR.23043	A. owenii	Extinct Population	Smyth River	West Coast
AV25141	A. owenii	Extinct Population	West Coast	West Coast
NMNZ s.n.	A. owenii	Unknown	?	Unspecified
AV32392A	A. owenii	Unknown	?	Unspecified
OR.1145	A. owenii	Unknown	?	Unspecified
OR.966	A. owenii	Unknown	?	Unspecified
DM6663	A. owenii	Unknown	?	Unspecified
?	A. owenii	Unknown	?	Unspecified

GS10	A. haastii	Arthur's Pass	Deception River, Arthurs Pass	Arthur's Pass
GS11	A. haastii	Arthur's Pass	Deception River, Arthurs Pass	Arthur's Pass
RA 0133	A. haastii	Arthur's Pass	Hurunui	Arthur's Pass
RA 0135	A. haastii	Arthur's Pass	Hurunui	Arthur's Pass
RA 0136	A. haastii	Arthur's Pass	Hurunui	Arthur's Pass
RA 0137	A. haastii	Arthur's Pass	Hurunui	Arthur's Pass
RA 1024	A. haastii	Arthur's Pass	Hurunui	Arthur's Pass
GS21	A. haastii	Arthur's Pass	Taramakau River, Arthurs Pass	Arthur's Pass
S.34491	A. haastii	Extinct Population	Mount Arthur	Lost
S.25792	A. haastii	NW Nelson	Oparara, West Coast	NW Nelson
GS14	A. haastii	NW Nelson	Ugly River, Nelson	NW Nelson
M1B	A. haastii	NW Nelson	along Heaphy track, Saxon	NW Nelson
MP	A. haastii	NW Nelson	along Heaphy track, Saxon	NW Nelson
M3	A. haastii	NW Nelson	Heaphy, NW Nelson	NW Nelson
FT2920	A. haastii	NW Nelson	Kahurangi, Buller dist, NW Nelson	NW Nelson
FT2921	A. haastii	NW Nelson	Kahurangi, Buller dist, NW Nelson	NW Nelson
GS12	A. haastii	NW Nelson	Ugly River, Nelson	NW Nelson
GS13	A. haastii	NW Nelson	Ugly River, Nelson	NW Nelson
GS17	A. haastii	NW Nelson	Ugly River, Nelson	NW Nelson
GS02	A. haastii	Paparoa	Ohikanui River, Paparoa Range	Paparoa
GS06	A. haastii	Paparoa	Ohikanui River, Paparoa Range	Paparoa
GS09	A. haastii	Paparoa	Ohikanui River, Paparoa Range	Paparoa
FT2922	A. haastii	Paparoa	Big River	Paparoa

AV19163	A. haastii	Paparoa	Charleston	Westport?
S.23187	A. haastii	Paparoa	Charleston	Westport?
?	A. haastii	Unknown	?	Unknown
?	A. haastii	Unknown	?	Unknown
?	A. haastii	Unknown	?	Unspecified
?	A. owenii	Unknown	Otorohanga Kiwi House	Unspecified
CD1204	A. haastii	Unknown	Otorohanga Kiwi House	Unspecified
73	A. mantelli	Northland	?	Unspecified
AT5	A. mantelli	Eastern	Waikaremoana	Waikaremoana
16-12'	A. mantelli	Eastern	Waikaremoana	Waikaremoana

Genotype

Sample/Bird I.D.	Haplotype in this paper	mt Genome	Control Region	Cytochrome b OR 12S Ribosomal RNA	ATPase 1	GBS Data
nib.75	H6					
88bp	H14					
5ffRr	H14					
ob1Mo	H14					
198H	H15					
R44928	H15		KX578296	KX578461		
R44968	H15		KX578293	KX578458		
2dbRp	H15					
624Rp	H15					
FdaH	H5					
22aM	H5					
3a2M	H5					

R45936	H5	KX578304	KX578469	SAMN05520635
099Bh	H5			
157Mo	H5			
424Rr	H5			
9e9D	H5			
R44973	H6	KX578301	KX578466	SAMN05520634
891Rr	H6			
c22 IDA6A6F	H6	KX578303	KX578468	
T456	H14	AY150611	AAU28707	
TK164	-	Missing	KX668257	SAMN05520631
TK86	H15	KX578295	KX578460	
R35015	H15	KX578294	KX578459	
TK153	H6	KX578297	KX578462	
TK85	H6	KX578302	KX578467	SAMN05520636
R35012	H6	KX578298	KX578463	
R35016	H6	KX578299	KX578464	SAMN05520633
R35017	H6	KX578300	KX578465	
CD1995	H14	AY150611	AAU28708	
CD1997	H5	KX578305/AY150609	KX578470	SAMN05520632
CD1996	H6	AY150610	AAU28707	SAMN05520629
?		Missing		
RA 0161	-	Missing	KX668256	SAMN05520630
O9d11	H5			
4e6Bh	H5			

BMNH 1842.5.17.2	H15		DQ295830	
BMNH 1838.5.12.102	H15		DQ295829	
K86	H5	GU071057 AY016010	GU071057, AY016010	GU071057, AY016010
FT2988	-		Missing	AAU28701
FT2989	-		Missing	AAU28702
FT2990	-		Missing	AAU28702
LBK001	H11		AY150618	AAU28701
LBK004	H11		AY150618	AAU28700
FT2986	H13		AY150617	AAU28702
FT2987	H13		AY150617	AAU28702
LBK002	H13		AY150617	AAU28702
LBK003	H13		AY150619	AAU28702
?			Missing	
alex	H10			
aru	H10			
Bubbles	H10			
Guy	H10			
Homer	H10			
Marenui	H10			
Moneypenny	H10			
palo	H10			
Percy	H10			
Rewarewa	H10			
Simo	H10			
Tango	H10			
Taunis	H10			

R32852	H10	KX578306	KX578471	SAMN05520640
Minnie	H6			
chick 12	H6	KX578309	KX578474	SAMN05520639
chick 3	H6	KX578313	KX578478	SAMN05520637
chick 6	H6	KX578308	KX578473	SAMN05520638
R32860	H6	KX578312	KX578477	
R36095	H6	KX578314	KX578479	SAMN05520641
R36099	H6	KX578307	KX578472	SAMN05520642
R55426	H6	KX578310	KX578475	
R55426	H6	KX578311	KX578476	SAMN05520643
kina	H6			
Pinky	H3			
DM1212	H10	AY713315		
49	H2			
Ari	H2			
Bracken	H2	KX578320	KX578485	
DM1552		Missing	AY749106	
corona	H2			
Hinemoa	H2			
Maggy	H2			
magnum	H2			
Tocha	H2			
Waiwai	H1			
Albert	H10			
Hariana	H10			

Pudding	H10		
Berry	H1		
Blue Gum	H1		
cookie	H1		
darwin	H1		
domino	H1		
kora	H1		
pavlova	H1		
shaggy	H1		
splash	H1		
taoka	H1		
trooper	H1		
chute	H2		
fred	H2		
bondi	H4		
Eco	H4		
rowi	H4		
Hine	H7		
CD887	-	Missing	AAU28709
breeze	H1		
champers	H1		
Chitchat	H1		
epi	H1		
fig	H1		
koripo	H1		
lag	H1		
mako	H1		
Manuka	H1		
rangi	H1		
stinger	H1		

twinkle	H1			
whiturau	H1			
Quazi	H2			
diamond	H3			
ohiwa	H3			
teva	H3			
Wedgie	H3			
Bear	H4			
boxer	H4			
Wafer	H4			
teer	H1			
vollie	H1			
Flipper	H4			
Hydee	H4			
K311	H1	KX578338	KX578503	
CD1295	H1	KX578330	KX578495	
R31812	H4	KX578326	KX578491	
R32946	H4	KX578327	KX578492	SAMN05520646
R59125	H4	KX578329	KX578494	SAMN05520650
RC01	H4	KX578323	KX578488	SAMN05520651
RC02	H4	KX578324	KX578489	
48	H2	AY150612	AAU28711	
CD890	H3	KX578316	KX578481	
Hetty	H1	KX578334	KX578499	
R43538	H1	KX578332	KX578497	
R43517	H1	KX578335	KX578500	SAMN05520647
R43572	H1	KX578331	KX578496	

R43544	H1	KX578333	KX578498	
B97	H1			
M97.2	H1			
Maggie	H1			
Moko	H1			
Ry97.2	H1			
R43528	H2	KX578321	KX578486	SAMN05520649
R43573	H3	KX578319	KX578484	
a96.1	H3			
R43519	H3	KX578318	KX578483	SAMN05520648
R43536	H4	KX578328	KX578493	
Dreads	H4			
Gonzo	H4			
CD1210	H1	AY150616	AAU28712	
CD961	H1	AY150616	AAU28712	
CD963	H4	AY150615	AAU28711	
CD886	H2	AY150613	AAU28711	
CD891	H1	AY150614	AAU28710	
R55414	H1	KX578337	KX578502	
R55413	H1	KX578336	KX578501	SAMN05520645
R55402	H2	KX578322	KX578487	
Awa	H2			
buster	H2			
claus	H2			
hone	H2			
Karaponia	H2			
marley	H2			
marmalade	H2			

martini	H2			
roimata	H2			
Te Wairoa	H2			
R55403	H3	KX578317	KX578482	SAMN05520644
Maroke	H3			
speights	H3			
Timata	H3			
NIBK001	H4	KX578325	KX578490	
buttons	H7			
allbut	H7			
Rubiks	H7			
blue	H9			
Camo	H9			
mokai	H9			
54 Don	H11	KX578339	KX578504	
81 Emma	H11	KX578348	KX578513	SAMN05520653
82 Penny	H11	KX578340	KX578505	SAMN05520654
70 Cathy	H6	KX578315	KX578480	SAMN05520652
DM14849		Missing	AY749107	
mayhem	H11			
Kaha	H11			
Kuraiti	H11			
Ross	H11			
DM9375	H12	AY713335	AY713368	
Iguazu	H13			
kruze	H13			
Nugget	H13			

Rio	H13			
Libby	H8			
Putiputi	H8			
CD2021	H11	AY150621	AAU28700	
CD964	H11	AY150618	AAU28698	
Bayfield	H11			
Dawson	H11			
Ford	H11			
harenge	H11			
Ingle	H11			
ironman	H11			
kaimata	H11			
Mautau	H11			
purangi	H11			
red	H11			
Ringo	H11			
runner	H11			
Rusty	H11			
Solstice	H11			
tahuna	H11			
tarata	H11			
Tk.Ure1/vogel	H11			
CD1994	H12	AY150620	AAU28700	
Jaime	H12			
Ra	H12			
CD1993	H13	KX578342/AY150617	KX578507	SAMN05520656
AWOL	H13			
CD962	H9	AY150623	AAU28707	

Eddie	H11			
Fleming	H11			
Haki	H11			
pokano	H11			
Robyn	H11			
topo	H11			
R36079	H11	KX578341	KX578506	
R46251	H11	KX578346	KX578511	SAMN05520658
Smee	H11	KX578345	KX578510	
Waihaika	H11	KX578344	KX578509	
april	H8			
Elmo	H8			
Morton	H8			
Possum	H8			
76	H11	AY150618	AAU28701	
6	H11	AY150622	AAU28700	
frankin	H11			
gold	H11			
Mataritki	H11			
Moony	H11			
myrrh	H11			
Niglet	H11			
pea	H11			
Rudolph	H11			
Tanekaha	H11			
Aroha	H11	KX578349	KX578514	SAMN05520655
Tia	H11	KX578347	KX578512	
Tua	H11	KX578350	KX578515	SAMN05520659

Zinger	H11	KX578351	KX578516	
Sirius	H11			
Kowhai	H13			
R36085	H13	KX578343	KX578508	SAMN05520657
DM10276	H13	AY713332	AY713367	
DM13551	H6	AY713336	AY713369	
DM2135 or OR.2435	H11	JQ904618	JQ904612	
OR.1148	H11	JQ904620	JQ904611	
?	H6	JQ968612		
OR.1152	H7	JQ904619	JQ904610	
R35003	H22	KX578352/AY150626	KX578517	SAMN05520661
AV800	H36	AY713319		
CD832	H36	AY150624		
R34152	H36	AY150624		
R34153	H36	AY150624		
R34155	H36	AY150624		
R34156	H36	AY150625		
R35002	H36	KX578364/AY150625	KX578529	SAMN05520660
R43461	H36	KX578365	KX578530	SAMN05520662
R43477	H36	KX578360	KX578525	SAMN05520664
R43493	H36	KX578366	KX578531	
R46314 Scooter	H36	KX578361	KX578526	SAMN05520666
R55120	H36	KX578362	KX578527	SAMN05520667
R55135	H36	KX578367	KX578532	SAMN05520669

R55139	H36	KX578368	KX578533	
R55313	H36	KX578369	KX578534	SAMN05520672
R55393	H36	KX578370	KX578535	SAMN05520673
R34151	H44	AY150627		
R34154	H44	AY150628		
R43474 Maxine	H44	KX578353	KX578518	SAMN05520663
R43495	H44	KX578354	KX578519	SAMN05520665
R55133	H44	KX578355	KX578520	SAMN05520668
R55136	H44	KX578356	KX578521	SAMN05520670
R55312	H44	KX578357	KX578522	SAMN05520671
R55357	H44	KX578358	KX578523	
R55366	H44	KX578359	KX578524	
?		Missing		
S.009401	H26	AY713316	AY713366	
DM7869	H42	AY713341	AY713345	
DM7900	H42	AY713340	AY713343	
DM7896	H42	AY713342	AY713351	
AV16697	H27	AY713320	AY713359	
S.23211	H28	AY713314	AY713370	
1.S.24355.1 or S.24355.1	H50	AY713337	AY713348	
2.S.24355.2 or S.24355.2	H50	AY713339	AY713349	
THW collection	H50	AY713338	AY713365	
R55342	H34	KX578372	KX578537	SAMN05520681

R55341	H34	KX578381	KX578546	SAMN05520680
RA 0930	H34	KX578385/MF481176	KX578550/MF481129	SAMN05520674
19_02_93	H34	KX578383	KX578548	
R34157	H34	KX578379/AY150630	KX578544	
R34158	H34	KX578384/AY150629	KX578549	SAMN05520679
R34159	H34	AY150629		
R34160	H34	AY150629		
RA0921	H34	AY150629		
RA 0936	H34	KX578373	KX578538	
RA 0996	H34	KX578378	KX578543	
RA 0940	H34	KX578376	KX578541	SAMN05520676
RA0937	H34	KX578374	KX578539	SAMN05520675
RA0938	H34	KX578375	KX578540	
TFCK	H34	AY150629		
R55343	H34	KX578382	KX578547	SAMN05520682
RA 0992	H34	KX578377	KX578542	SAMN05520677
RA 0994	H34	KX578371	KX578536	SAMN05520678
S310_1	H34	MF481178	MF481131	
S344_1	H34	MF481179	MF481132	
33992	H34	MF481149	MF481102	
33997	H34	MF481150	MF481103	
AR	H34	MF481151	MF481104	
AV	H34	MF481152	MF481105	
BH	H34	MF481154	MF481107	

CF	H34	MF481158	MF481111
FT	H34	MF481161	MF481114
HB	H34	MF481163	MF481116
HI	H34	MF481166	MF481119
MG	H34	MF481171	MF481124
NH1	H34	MF481173	MF481126
OS	H34	MF481175	MF481128
RS	H34	MF481177	MF481130
SC	H34	MF481180	MF481133
SL	H34	MF481181	MF481134
TC	H34	MF481182	MF481135
TR	H34	MF481184	MF481137
WD	H34	MF481190	MF481143
CS	H34	MF481159	MF481112
DP	H34	MF481160	MF481113
HH	H34	MF481165	MF481118
JV	H34	MF481168	MF481121
TI	H34	MF481183	MF481136
BE	H34	MF481153	MF481106
Wisneski	H34	MF481192	MF481145
Whitewash	H34	MF481191	MF481144
NH2	H34	MF481173 or 74??	MF481126
CC	H34	MF481157	MF481110
WO	H34	MF481193	MF481146
MW	H34	MF481172	MF481125
BH2	H34	MF481155	MF481108
TZ	H34	MF481186	MF481139
WW	H34	MF481194	MF481148
HF	H34	MF481164	MF481117

BR	H34	MF481156	MF481109	
TR	H34	MF481184 or 85??	MF481137	
WT		n/a	MF481147	
LM	H33	MF481170	MF481123	
UC	H33	MF481187	MF481140	
R35021	H34/H33	KX578380/AY150631	KX578545	
RA0991	H43	AY150632		
GG	H43	MF481162	MF481115	
JC	H43	MF481167	MF481120	
LC	H43	MF481169	MF481122	
UM	H43	MF481188	MF481141	
UM	H43/H33	MF481188 or 89??	MF481141	
R31551	H20	KX578398	KX578563	SAMN05520692
RA 0601	H23	KX578393	KX578558	SAMN05520683
RA 0603	H23	KX578390	KX578555	SAMN05520684
RA 0730	H23	KX578389	KX578554	SAMN05520686
RA 0743	H23	KX578391	KX578556	SAMN05520690
RA 0823	H23	KX578392	KX578557	SAMN05520691
RA 0731	H20	KX578397	KX578562	
RA 0738	H20	KX578407	KX578572	
CD1141	H20	AY150635		
CD1151	H20	AY150636		
RA 0740	H38	KX578387	KX578552	SAMN05520687
AV588	H20	AY713317	AY713352	

AV589	H39	AY713318	AY713355	
RA 0708	H20	KX578396	KX578561	
RA 0741	H38	KX578388	KX578553	SAMN05520688
RA 0707	H39	KX578394	KX578559	SAMN05520685
RA 0742	H39	KX578395	KX578560	SAMN05520689
RA 0781	H20	KX578406		
RA 0784	H20	KX578400		
RA 0785	H20	KX578401		
RA 0746	H20	KX578404		
RA 0748	H20	KX578403		
RA 0788	H35	KX578423		
chick 52	H35	KX578416		
RA 0773	H20	KX578405		
RA 0774	H20	KX578408		
RA 0775	H20	KX578399		
CD1150	H35	AY150633		
RA 0278	H20	KX578402		
RA 0396	H38	KX578386		
RA 0202	H45	KX578413	KX578578	SAMN05520696
DC3	H25	KX578411/AY150637	KX578576	
DC1	H35	KX578420/AY150634	KX578585	SAMN05520693
DC2	H35	KX578421/AY150634	KX578586	
DC4	H35	KX578417/AY150633	KX578582	SAMN05520694
RA 0208	H35	KX578418	KX578583	SAMN05520697

RA 0209	H35	KX578419	KX578584	SAMN05520698
RA 0143	H46	KX578414	KX578579	SAMN05520695
RA 0224	H46	KX578415	KX578580	SAMN05520699
TOKSC1	H25	KX578409	KX578574	
TOKSC4	H25	KX578410	KX578575	
TOKSC2	H35	KX578422	KX578587	SAMN05520700
TOKSC5	H45	KX578412	KX578577	SAMN05520701
OR.26398	H16	JQ904617	JQ904609	
RA 0152	H17	KX578424	KX578589	SAMN05520702
RA 0154	H17	KX578425	KX578590	SAMN05520703
RA 0894	H17	KX578426	KX578591	
RA 0896	H17	KX578427	KX578592	SAMN05520707
RA 0903	H17	KX578429	KX578594	SAMN05520709
RA 0923	H17	KX578430	KX578595	SAMN05520710
RA0881	H17	AY150640		
RA0902	H17	KX578428/AY150640	KX578593	SAMN05520708
RA 0890	H18	KX578433	KX578598	
RA 0925	H18	KX578432	KX578597	
RA 0927	H18	KX578434	KX578599	SAMN05520712
RA002	H18	AY150638		
RA0868	H18	AY150638		
RA0882	H18	AY150638		

RA0887	H18	KX578431/AY150638	KX578596	SAMN05520705
RA 0883	H19	KX578435	KX578600	SAMN05520704
RA 0888	H19	KX578436	KX578601	SAMN05520706
RA 0924	H19	KX578437	KX578602	SAMN05520711
RA0865	H19	AY150639		
RA0885	H19	AY150639		
RA0886	H19	AY150639		
?		Missing		
AV12651	H29	AY713323	AY713361	
3.S.33369.3 or S.33369.3	H30	AY713327	AY713354	
5.S.33369.5 or S.33369.5	H30	AY713328	AY713363	
1.S.33369.1 or S.33369.1	H41	AY713326	AY713362	
2.S.33369.2 or S.33369.2	H41	AY713325	AY713353	
4.S.33369.4 or S.33369.4	H41	AY713324	AY713357	
DM6498	H51	AY713322	AY713356	
DM6557	H51	AY713321	AY713360	
DM6555	H52	AY713313	AY713347	
DM6489	H53	AY713333	AY713346	
S.34531	H53	AY713334	AY713344	
?			X67626	
?	H55	AY150642		
0-20599 M	H55	KX578290	KX578455	
O 20557	H55	KX578292	KX578457	SAMN05520627

O 20600	H55	KX578288	KX578453		
O-20575	H55	KX578289	KX578454		SAMN05520626
CD1206	H55/H56	KX578291/FJ820104	KX578456/FJ820082, FJ820059	JQ219166	SAMN05520628
CD899	H56	FJ820115	FJ820081, FJ820058	JQ219165	
WS1764	H58	FJ820105	FJ820078, FJ820055	JQ219175	
DM6672	H55	FJ820109	FJ820063, FJ820086	JQ219167	
AV32392B	H55	FJ820099	FJ820052, FJ820075	JQ219169	
S.24478	H57	FJ820092	FJ820045, FJ820068	JQ219176	
AV17079	H31	FJ820112			
S.33365	H55	FJ820108	FJ820062, FJ820085	JQ219172	
NM23036 or OR.23036	H55	FJ820095	FJ820048, FJ820071	JQ219164	
S.27784.1 and S.27784.2	H55	FJ820094	FJ820047, FJ820070	JQ219162	
S.001164	H55	JQ219189	JQ219195	JQ219183	
S.1174 or OR.1174	H55	FJ820107	FJ820061, FJ820084	JQ277702	
AV16713	H58	FJ820090	FJ820043, FJ820066	JQ219168	
AV25301	H55	FJ820110	FJ820064, FJ820087	JQ219161	
AV12648C	H56	FJ820098	FJ820051, FJ820074	JQ219173	
S.2069 or OR.2069	H55	FJ820106	FJ820060, FJ820083	JQ219170	
O 20596	H55	KX578287	KX578452		

AV22817	H31	FJ820096	FJ820049, FJ820072	JQ219178	
WO255	H54	FJ820089	FJ820042, FJ820065	JQ219177	
S.22007 or OR.22007	H55	FJ820093	FJ820046, FJ820069	JQ219174	
S.23043 or OR.23043	H55	FJ820091	FJ820044, FJ820067	JQ219163	
AV25141	H55	FJ820097	FJ820050, FJ820073	JQ219171	
NMNZ s.n.	H47	FJ820100			
AV32392A	H55	FJ820111			
OR.1145	H55	JQ904615	JQ904613		
OR.966	H55	JQ904616	JQ904614		
DM6663	H58	FJ820113			
?			X67628		
GS10	H47	JQ219191	JQ219200	JQ219186	
GS11	H47	JQ219190	JQ219199	JQ219185	
RA 0133	H47	KX578282	KX578447		SAMN05520621
RA 0135	H47	KX578283	KX578448		SAMN05520622
RA 0136	H47	KX578284	KX578449		SAMN05520623
RA 0137	H47	KX578286	KX578451		SAMN05520625
RA 1024	H47	KX578285	KX578450		SAMN05520624
GS21	H47	FJ820102	FJ820077, FJ820054	JQ219180	
S.34491	H48	AY713312	AY713350	No	
S.25792	H47	AY713329			

GS14	H47	FJ820114	FJ820080, FJ820057	JQ219182	
M1B	H49	KX578273	KX578438		
MP	H49	KX578276	KX578441		SAMN05520620
M3	H49	FJ820101	FJ820076, FJ820053	JQ219179	
FT2920	H49	KX578274/JQ219192	KX578439/JQ219196	JQ219187	
FT2921	H49	KX578275/ FJ820103	KX578440/FJ820079, FJ820056	JQ219187	SAMN05520616
GS12	H49	KX578280	KX578445		
GS13	H49	KX578281	KX578446		
GS17	H49	JQ219194	JQ219198	JQ219184	
GS02	H47	KX578277	KX578442		SAMN05520617
GS06	H47	KX578278	KX578443		SAMN05520618
GS09	H47	KX578279	KX578444		SAMN05520619
FT2922	H49	JQ219193	JQ219197	JQ219188	
AV19163	H47	AY713330	AY713358	No	
S.23187	H47	AY713331	AY713364	No	
?	H47	AY150641			
?					
?			X67627		
?	GU071057, NC_013806				
CD1204	AF338708,				

	NC_002782
73	KU695537
AT5	KU695537
16-12'	KU695537

	Represented in																
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
nib.75				Y													
88bp				Y													
5ffRr				Y													
ob1Mo				Y													
198H				Y													
R44928						Y											
R44968						Y											
2dbRp				Y													
624Rp				Y													
FdaH				Y													
22aM				Y													
3a2M				Y													
R45936						Y											
099Bh				Y													
157Mo				Y													
424Rr				Y													
9e9D				Y													
R44973						Y											
891Rr				Y													
c22																	
IDA6A6F						Y											
T456		Y	Y	Y	Y												
TK164						Y											
TK86						Y											
R35015						Y											
TK153						Y											
TK85						Y											
R35012						Y											
R35016						Y											
R35017						Y											
CD1995	Y	Y	Y	Y	Y												
CD1997	Y	Y	Y	Y	Y	Y											
CD1996	Y	Y	Y	Y	Y	Y											
?	Y																
RA 0161						Y											
O9d11				Y													
4e6Bh				Y													
BMNH 1842.5.17.2				Y	Y												

BMNH 1838.5.12. 102				Y	Y												
K86											Y	Y					
FT2988	Y																
FT2989	Y																
FT2990	Y																
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
LBK001	Y	Y	Y	Y	Y												
LBK004	Y	Y	Y	Y	Y												
FT2986	Y	Y	Y	Y	Y												
FT2987	Y	Y	Y	Y	Y												
LBK002	Y	Y	Y	Y	Y												
LBK003	Y	Y	Y	Y	Y												
?	Y																
alex				Y													
aru				Y													
Bubbles				Y													
Guy				Y													
Homer				Y													
Marenui				Y													
Moneypenny				Y													
palo				Y													
Percy				Y													
Rewarewa				Y													
Simo				Y													
Tango				Y													
Taunis				Y													
R32852						Y											
Minnie				Y													
chick 12						Y											
chick 3						Y											
chick 6						Y											
R32860						Y											
R36095						Y											
R36099						Y											
R55426						Y											
R55426						Y											
kina				Y													
Pinky				Y													
DM1212				Y													
49				Y													

Ari				Y													
Bracken						Y											
DM1552																	
corona				Y													
Hinemoa				Y													
Maggy				Y													
magnum				Y													
Tocha				Y													
Waiwai				Y													
Albert				Y													
Hariana				Y													
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
Pudding				Y													
Berry				Y													
Blue Gum				Y													
cookie				Y													
darwin				Y													
domino				Y													
kora				Y													
pavlova				Y													
shaggy				Y													
splash				Y													
taoka				Y													
trooper				Y													
chute				Y													
fred				Y													
bondi				Y													
Eco				Y													
rowi				Y													
Hine				Y													
CD887	Y																
breeze				Y													
champers				Y													
Chitchat				Y													
epi				Y													
fig				Y													
koripo				Y													
lag				Y													
mako				Y													
Manuka				Y													
rangi				Y													
stinger				Y													

twinkle				Y												
whiturau				Y												
Quazi				Y												
diamond				Y												
ohiwa				Y												
teva				Y												
Wedgie				Y												
Bear				Y												
boxer				Y												
Wafer				Y												
teer				Y												
vollie				Y												
Flipper				Y												
Hydee				Y												
K311						Y										
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
CD1295						Y										
R31812						Y										
R32946						Y										
R59125						Y										
RC01						Y										
RC02						Y										
48		Y	Y	Y	Y											
CD890						Y										
Hetty						Y										
R43538						Y										
R43517						Y										
R43572						Y										
R43544						Y										
B97				Y												
M97.2				Y												
Maggie				Y												
Moko				Y												
Ry97.2				Y												
R43528						Y										
R43573						Y										
a96.1				Y												
R43519						Y										
R43536						Y										
Dreads				Y												
Gonzo				Y												
CD1210		Y	Y	Y	Y											

CD961	Y	Y	Y	Y	Y												
CD963	Y	Y	Y	Y	Y												
CD886		Y	Y	Y	Y												
CD891	Y	Y	Y	Y	Y												
R55414						Y											
R55413						Y											
R55402						Y											
Awa				Y													
buster				Y													
claus				Y													
hone				Y													
Karaponia				Y													
marley				Y													
marmalade				Y													
martini				Y													
roimata				Y													
Te Wairoa				Y													
R55403						Y											
Maroke				Y													

Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
speights				Y													
Timata				Y													
NIBK001						Y											
buttons				Y													
allbut				Y													
Rubiks				Y													
blue				Y													
Camo				Y													
mokai				Y													
54 Don						Y											
81 Emma						Y											
82 Penny						Y											
70 Cathy						Y											
DM14849					Y												
mayhem				Y													
Kaha				Y													
Kuraiti				Y													
Ross				Y													
DM9375			Y	Y	Y			Y	Y								
Iguazu				Y													
kruze				Y													

Nugget				Y												
Rio				Y												
Libby				Y												
Putiputi				Y												
CD2021	Y	Y	Y	Y	Y											
CD964	Y	Y	Y	Y	Y											
Bayfield				Y												
Dawson				Y												
Ford				Y												
harenge				Y												
Ingle				Y												
ironman				Y												
kaimata				Y												
Mautau				Y												
purangi				Y												
red				Y												
Ringo				Y												
runner				Y												
Rusty				Y												
Solstice				Y												
tahuna				Y												
tarata				Y												
Tk.Ure1/vogel				Y												
CD1994	Y	Y	Y	Y	Y											

Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
Jaime				Y													
Ra				Y													
CD1993	Y	Y	Y	Y	Y	Y											
AWOL				Y													
CD962	Y	Y	Y	Y	Y												
Eddie				Y													
Fleming				Y													
Haki				Y													
pokano				Y													
Robyn				Y													
topo				Y													
R36079						Y											
R46251						Y											
Smee						Y											
Waihaika						Y											
april				Y													

Elmo				Y													
Morton				Y													
Possum				Y													
76		Y	Y	Y	Y												
6		Y	Y	Y	Y												
frankin				Y													
gold				Y													
Mataritki				Y													
Moony				Y													
myrrh				Y													
Niglet				Y													
pea				Y													
Rudolph				Y													
Tanekaha				Y													
Aroha						Y											
Tia						Y											
Tua						Y											
Zinger						Y											
Sirius				Y													
Kowhai				Y													
R36085						Y											
DM10276			Y	Y	Y												
DM13551			Y	Y	Y												
DM2135 or OR.2435								Y									
OR.1148 ?								Y									
OR.1152								Y									
R35003	Y	Y	Y		Y	Y											

Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
AV800					Y												
CD832	Y	Y	Y		Y												
R34152	Y	Y	Y		Y												
R34153	Y	Y	Y		Y												
R34155	Y	Y	Y		Y												
R34156	Y	Y	Y		Y												
R35002	Y	Y	Y		Y	Y											
R43461						Y											
R43477						Y											
R43493						Y											
R46314 Scooter						Y											

R55120						Y											
R55135						Y											
R55139						Y											
R55313						Y											
R55393						Y											
R34151	Y	Y	Y		Y												
R34154	Y	Y	Y		Y												
R43474 Maxine						Y											
R43495						Y											
R55133						Y											
R55136						Y											
R55312						Y											
R55357						Y											
R55366						Y											
?	Y																
S.009401			Y		Y	Y											
DM7869			Y		Y	Y											
DM7900			Y		Y	Y											
DM7896			Y		Y												
AV16697			Y		Y	Y											
S.23211			Y		Y	Y											
1.S.24355.1 or S.24355.1			Y		Y	Y											
2.S.24355.2 or S.24355.2			Y		Y	Y											
THW collection			Y		Y	Y											
R55342						Y											
R55341						Y											
RA 0930						Y					Y						
19_02_93						Y											
R34157	Y	Y	Y		Y	Y											
R34158	Y	Y	Y		Y	Y											
R34159	Y	Y	Y		Y												
R34160	Y	Y	Y		Y												
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
RA0921		Y	Y		Y												
RA 0936						Y											
RA 0996						Y				Y							
RA 0940						Y											

RA0937	Y	Y	Y		Y	Y	
RA0938	Y	Y	Y		Y	Y	
TFCK	Y	Y	Y		Y		
R55343						Y	
RA 0992						Y	
RA 0994						Y	Y
S310_1							Y
S344_1							Y
33992							Y
33997							Y
AR							Y
AV							Y
BH							Y
CF							Y
FT							Y
HB							Y
HI							Y
MG							Y
NH1							Y
OS							Y
RS							Y
SC							Y
SL							Y
TC							Y
TR							Y
WD							Y
CS							Y
DP							Y
HH							Y
JV							Y
TI							Y
BE							Y
Wisneski							Y
Whitewash							Y
NH2							Y
CC							Y
WO							Y
MW							Y
BH2							Y
TZ							Y
WW							Y

Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
HF										Y							
BR										Y							
TR										Y							
WT										Y							
LM										Y							
UC										Y							
R35021		Y	Y		Y	Y											
RA0991	Y	Y	Y		Y												
GG										Y							
JC										Y							
LC										Y							
UM										Y							
UM										Y							
R31551						Y											
RA 0601						Y											
RA 0603						Y											
RA 0730						Y											
RA 0743						Y											
RA 0823						Y											
RA 0731						Y											
RA 0738						Y											
CD1141	Y	Y	Y		Y												
CD1151	Y	Y	Y		Y												
RA 0740						Y											
AV588			Y		Y	Y											
AV589			Y		Y	Y											
RA 0708						Y											
RA 0741						Y											
RA 0707						Y											
RA 0742						Y											
RA 0781																	
RA 0784																	
RA 0785																	
RA 0746																	
RA 0748																	
RA 0788																	
chick 52																	
RA 0773																	
RA 0774																	
RA 0775																	
CD1150	Y	Y	Y		Y												

Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
RA 0278																	
RA 0396																	
RA 0202						Y											
DC3	Y	Y	Y		Y	Y											
DC1	Y	Y	Y		Y	Y											
DC2	Y	Y	Y		Y	Y											
DC4	Y	Y	Y		Y	Y											
RA 0208						Y											
RA 0209						Y											
RA 0143						Y											
RA 0224						Y											
TOKSC1						Y											
TOKSC4						Y											
TOKSC2						Y											
TOKSC5						Y											
OR.26398								Y									
RA 0152						Y											
RA 0154						Y											
RA 0894						Y											
RA 0896						Y											
RA 0903						Y											
RA 0923						Y											
RA0881	Y	Y	Y		Y												
RA0902	Y	Y	Y		Y	Y											
RA 0890						Y											
RA 0925						Y											
RA 0927						Y											
RA002	Y	Y	Y		Y												
RA0868	Y	Y	Y		Y												
RA0882	Y	Y	Y		Y												
RA0887	Y	Y	Y		Y	Y											
RA 0883						Y											
RA 0888						Y											
RA 0924						Y											
RA0865	Y	Y	Y		Y												
RA0885	Y	Y	Y		Y												
RA0886	Y	Y	Y		Y												
?	Y																
AV126513.S.33369.3 or S.33369.3			Y		Y	Y											

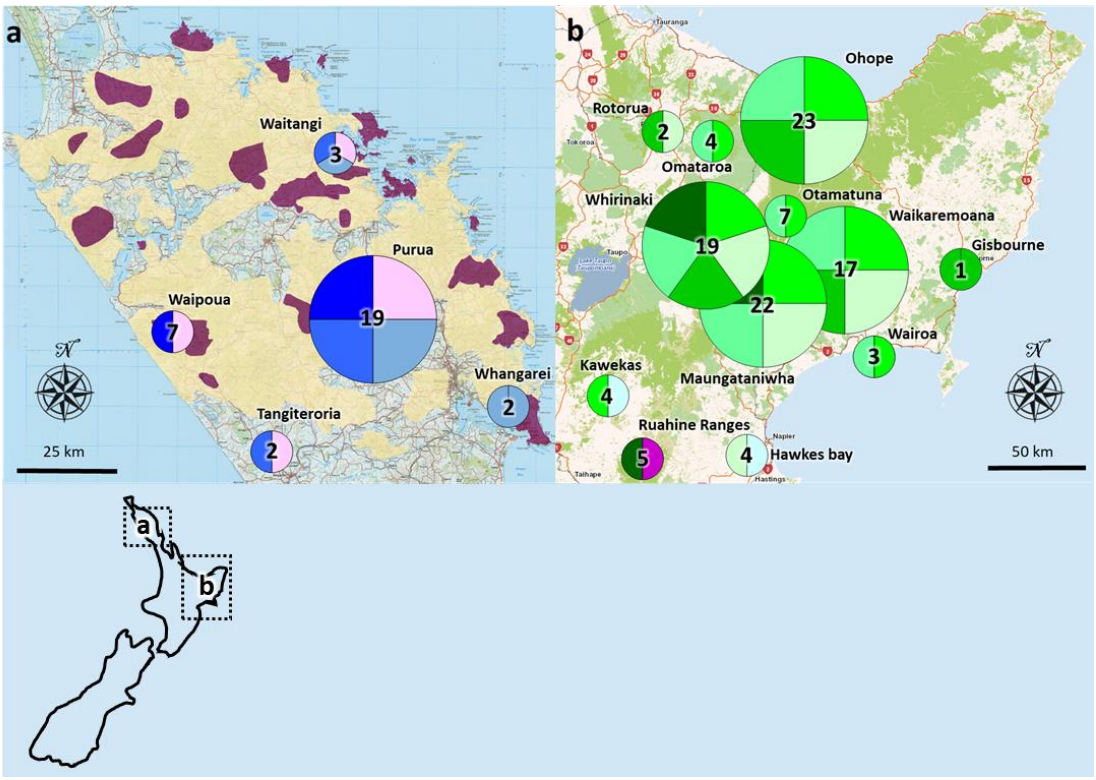
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
5.S.33369.5 or S.33369.5			Y		Y	Y											
1.S.33369.1 or S.33369.1			Y		Y	Y											
2.S.33369.2 or S.33369.2			Y		Y	Y											
4.S.33369.4 or S.33369.4			Y		Y	Y											
DM6498			Y		Y	Y											
DM6557			Y		Y	Y											
DM6555			Y		Y	Y											
DM6489			Y		Y	Y											
S.34531			Y		Y	Y											
?																	
?	Y	Y	Y		Y												
0-20599 MO 20557						Y											
O 20600						Y											
O-20575						Y											
CD1206					Y	Y	Y										
CD899					Y	Y											
WS1764					Y	Y											
DM6672					Y	Y											
AV32392B					Y	Y											
S.24478					Y	Y											
AV17079					Y												
S.33365					Y	Y											
NM23036 or OR.23036					Y	Y											
S.27784.1 and S.27784.2					Y	Y											
S.001164					Y	Y											
S.1174 or OR.1174					Y	Y											
AV16713					Y	Y											
AV25301					Y	Y											
AV12648C					Y	Y											

S.2069 or OR.2069				Y	Y											
O 20596					Y											
AV22817				Y	Y											
WO255				Y	Y											
S.22007 or OR.22007				Y	Y											
S.23043 or OR.23043				Y	Y											
AV25141				Y	Y											
NMNZ s.n.				Y												
AV32392A				Y												
OR.1145								Y								
OR.966								Y								
DM6663				Y												
?																

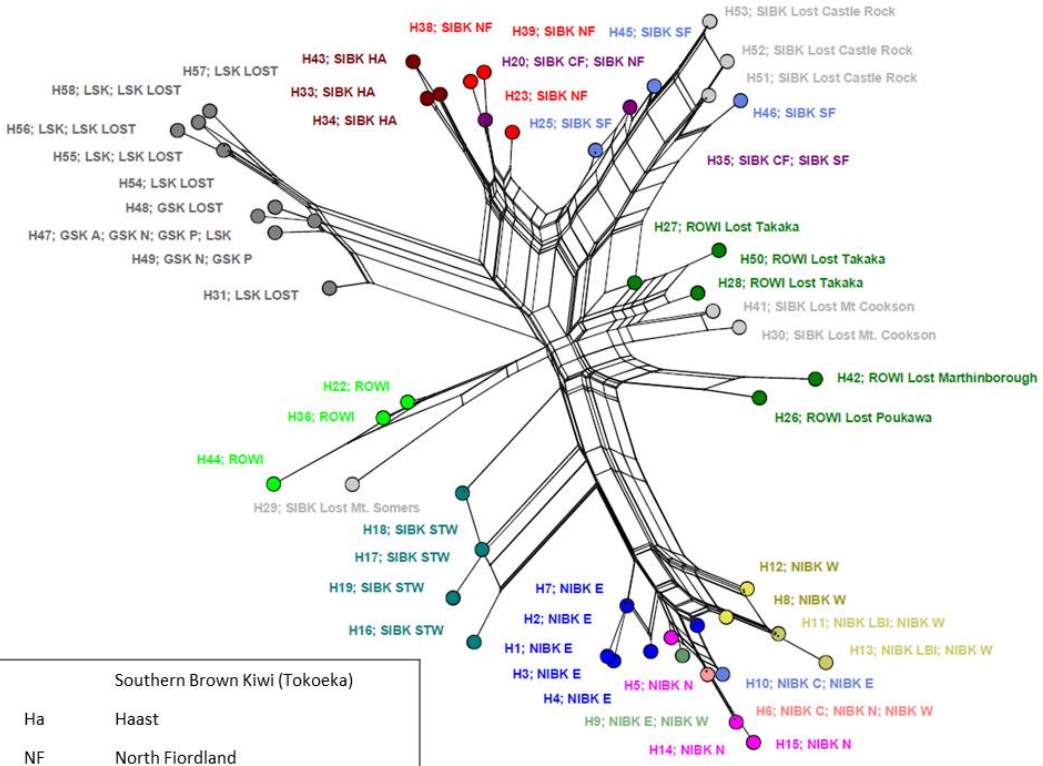
Sample/Bird I.D.	Baker 1995	Burbidge 2003	Shepherd 2008	Hartnup 2011	Shepherd 2012	Weir 2016	Haddrath 2012	Shepherd 2013	Shepherd 2009	White 2018	Cooper 2001	Phillips 2009	Crimp 2010	Haddrath 2001	Liu 2016	Le Duc 2015	Mitchell 2014
GS10					Y	Y											
GS11					Y	Y											
RA 0133						Y											
RA 0135						Y											
RA 0136						Y											
RA 0137						Y											
RA 1024						Y											
GS21					Y	Y											
S.34491			Y		Y	Y											
S.25792					Y												
GS14					Y	Y											
M1B						Y											
MP						Y											
M3					Y												
FT2920					Y	Y											
FT2921					Y	Y											
GS12						Y											
GS13						Y											
GS17					Y	Y											
GS02						Y											
GS06						Y											
GS09						Y											
FT2922					Y	Y											
AV19163			Y		Y	Y											
S.23187			Y		Y	Y											

?	Y	Y	Y		Y												
?	Y																
?																	
?												Y	Y				
CD1204														Y	Y		Y
73															Y	Y	
AT5															Y	Y	
16-12'															Y	Y	

Supplementary figure S1.2.1. Schematic illustration of the optional population histories behind the genetically distinct and isolated *A. mantelli* populations observed today. Many kiwi populations today are isolated and genetically distinct from neighboring areas (a). However, it is not yet determined whether genetic differences are due to (b) a reduction of larger populations that have become adapted to different local conditions (the ‘pied’ scenario) or (c) whether the distribution use to form a gradient or continuum that has been fragmented due to habitat disturbance which led to ‘speciation by extinction’ when intermediate forms have disappeared (the ‘rainbow’ scenario).

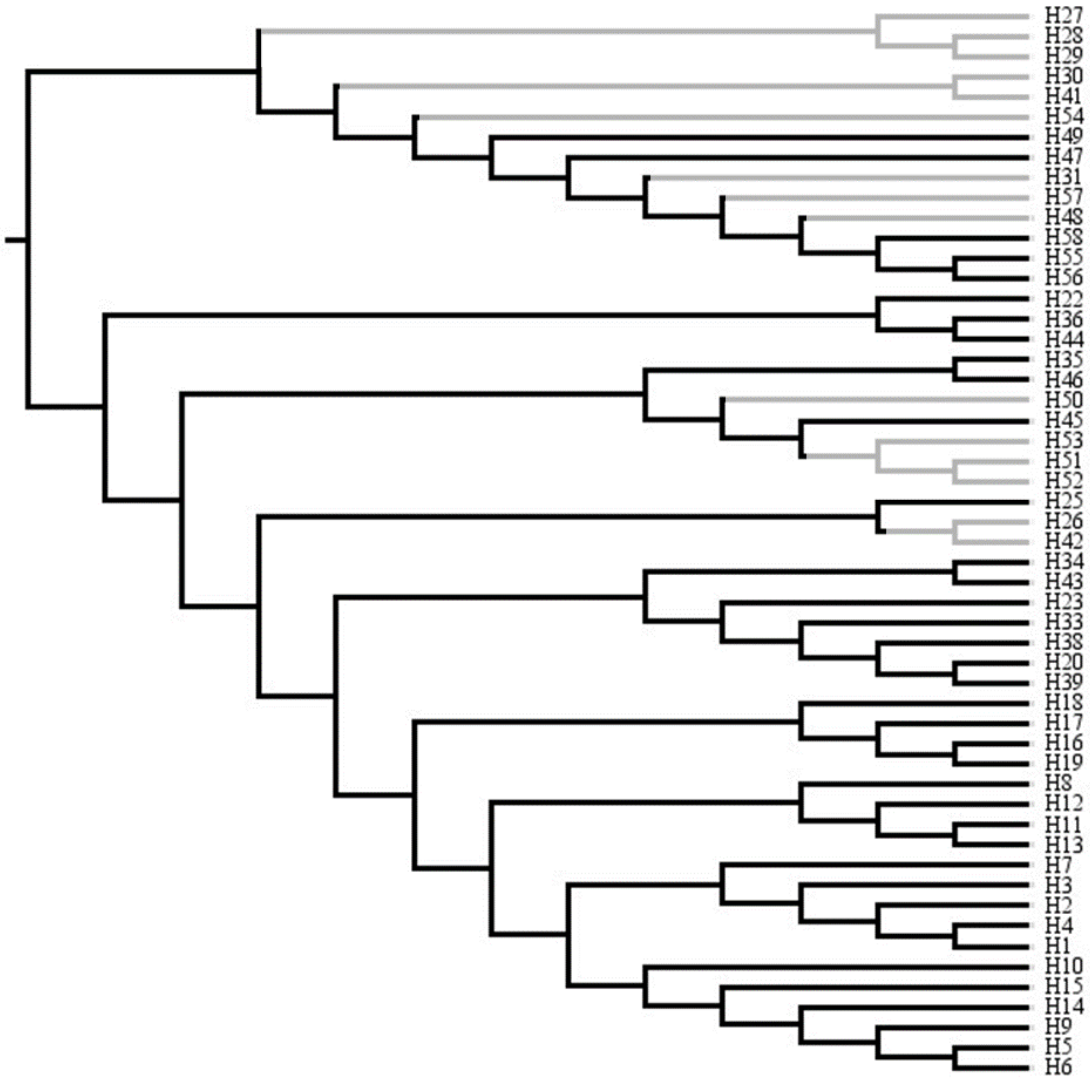


Supplementary figure S1.2.2. Geographical distributions of haplotypes for a 175 bp sequence of the kiwi mitochondrial control region. This figure represents a zoom in on 2 geographical areas (compare Figure 4a in main text). (a) North Island Brown Kiwi Northland (background map from DOC GIS Team/R. Fuchs, unpubl. 2019, where purple areas represent denser present kiwi populations and yellow areas with scattered kiwi observations). (b) North Island Brown Kiwi Eastern, which is the taxon that has the highest number of samples analysed (background map from topomap.co.nz). Sizes of pie charts represent relative sample size and numbers represent exact sample size. Inset map indicate which parts of New Zealand are covered.

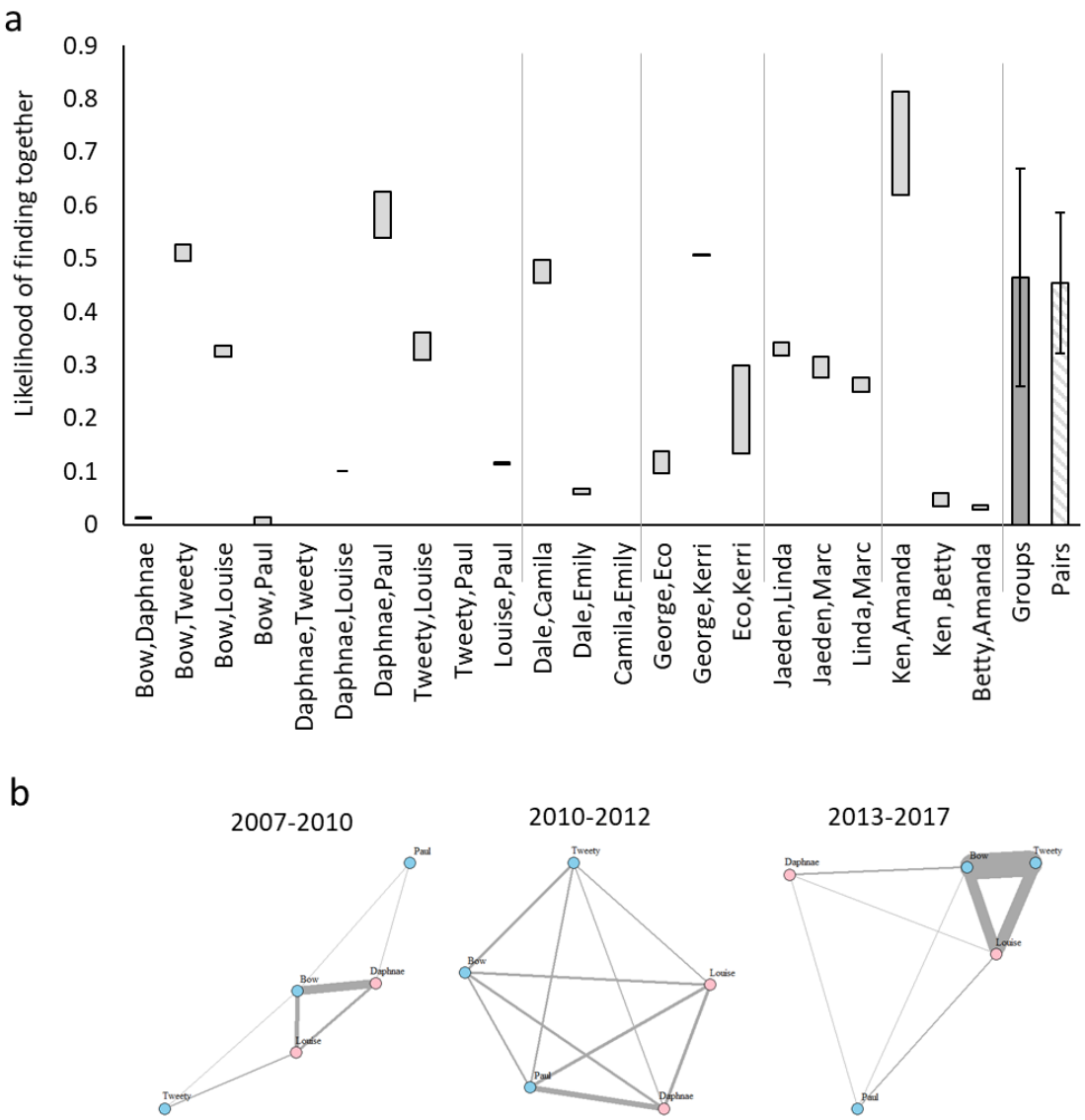


SIBK		Southern Brown Kiwi (Tokoeka)
	Ha	Haast
	NF	North Fiordland
	CF	Central Fiordland
	SF	South Fiordland
	STW	Stewart Island
NIBK		North Island Brown Kiwi
	E	Eastern
	W	Western
	N	Northland
	C	Coromandel
	LBI	Little Barrier Island
ROWI		Okarito Brown Kiwi
LSK		Little Spotted Kiwi
GSK		Great Spotted Kiwi

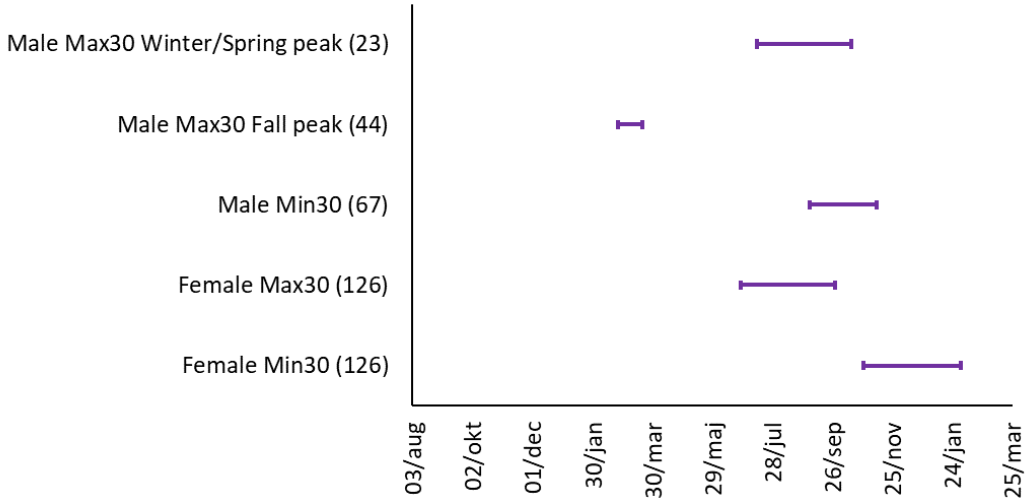
Supplementary figure S1.2.3. Splits tree of the 53 haplotypes representing 488 samples for a 175 bp sequence fragment of the kiwi mitochondrial control region. This represents the longest possible fragment across the largest possible dataset available. The resulting splits tree illustrates that the short fragment analysed does not provide enough information to resolve important relationships within this genus, neither on species, nor on taxon level due to significant incompatibility of site patterns – indicated by 'boxes' rather than simple branches connecting haplotypes. Labels indicate the taxon/taxa in which the respective haplotype was found. Colours connect labels to the correct nodes. 'Lost' in this case refers to museum samples, some of which represent areas where kiwi are now locally extinct. When known, more detailed information on location has been provided in the labels of the museum samples. 'Central Fiordland' refers to the area where the Southern and Northern Fiordland taxa seemingly overlap.



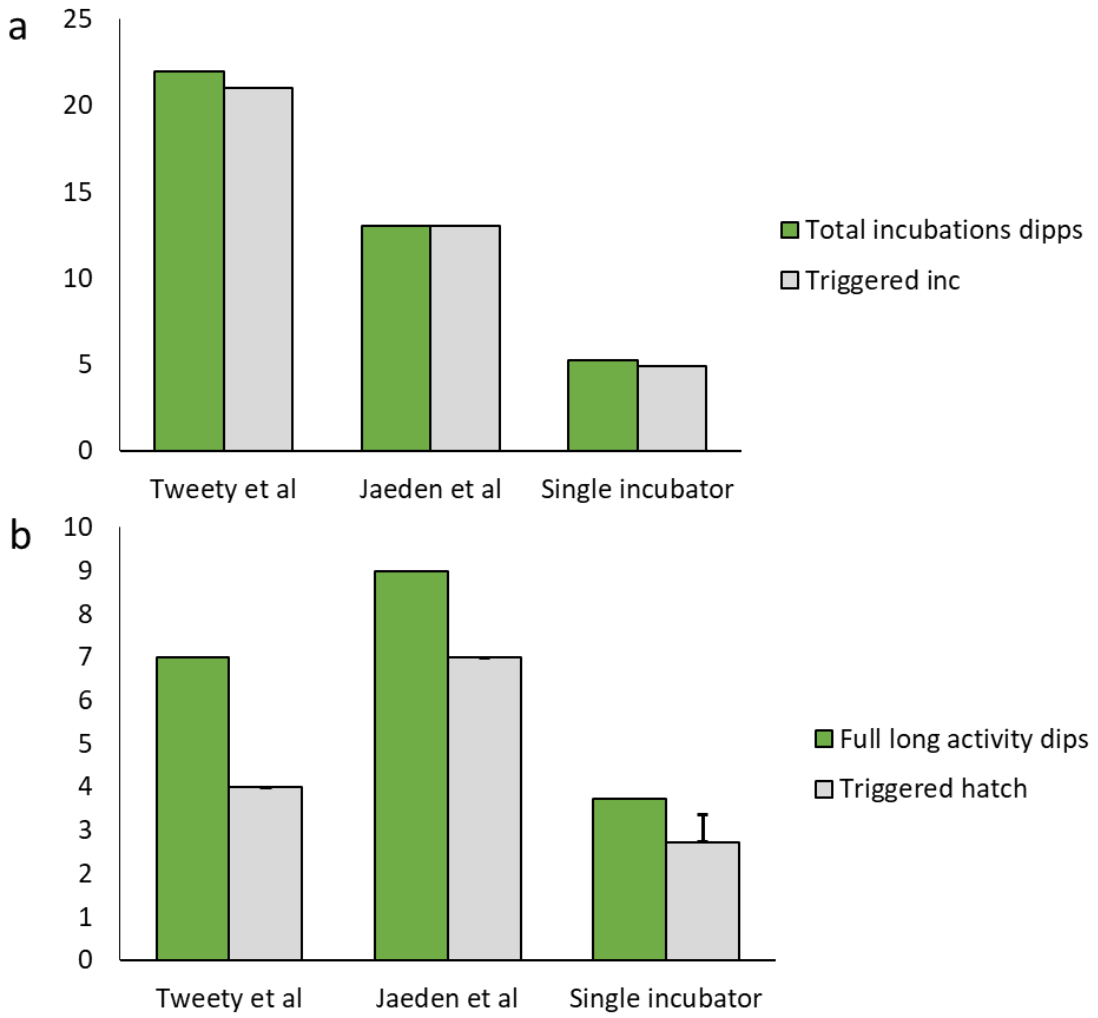
Supplementary figure S1.2.4. Phylogenetic tree illustrating the evolutionary relationships with the strongest statistical support among 53 identified haplotypes. The tree represents 488 birds from all five kiwi species inferred from phylogenetic analysis of a 175 base pair fragment of the mitochondrial control region. Sixteen of these haplotypes (light grey branches) have currently only been found in museum samples and/or in birds that were sampled from areas outside the present-day distribution of kiwi.



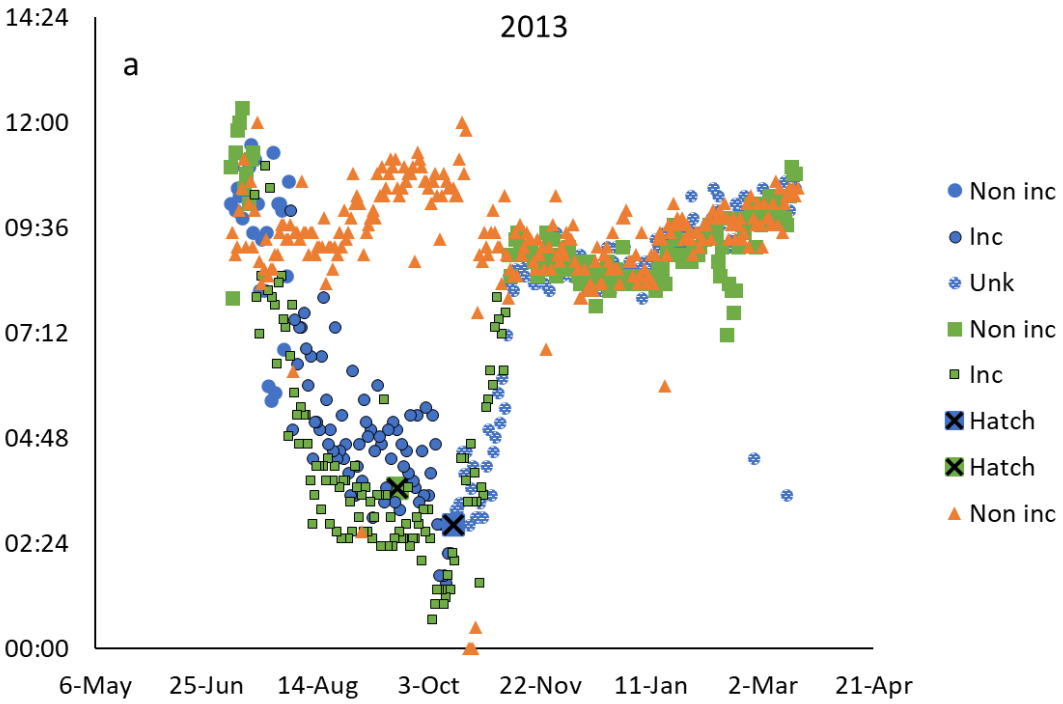
Supplementary figure S2.3.1. (a) Likelihood of finding birds from the same breeding unit together when tracked during the daytime. Bars to the right demonstrate the overall likelihood of finding a group bird with any other member of its breeding unit (grey) and a paired bird with their mate (striped; *n* = 10 pairs). Error bars indicating STD. Light grey boxes indicate the likelihood of finding two specific members together. Boxes span from the lower to the higher likelihood in cases where birds were not tracked the same number of times. Grey vertical lines split the 5 included groups for clarity. N was 3-73 times tracked per bird with a median of 61 times. Data from 2013-2017. Likelihoods for individual years were averaged. (b) how this likelihood has changed over time in the quintet. Thickness of network edges indicate likelihood. Node colour indicate bird sex.

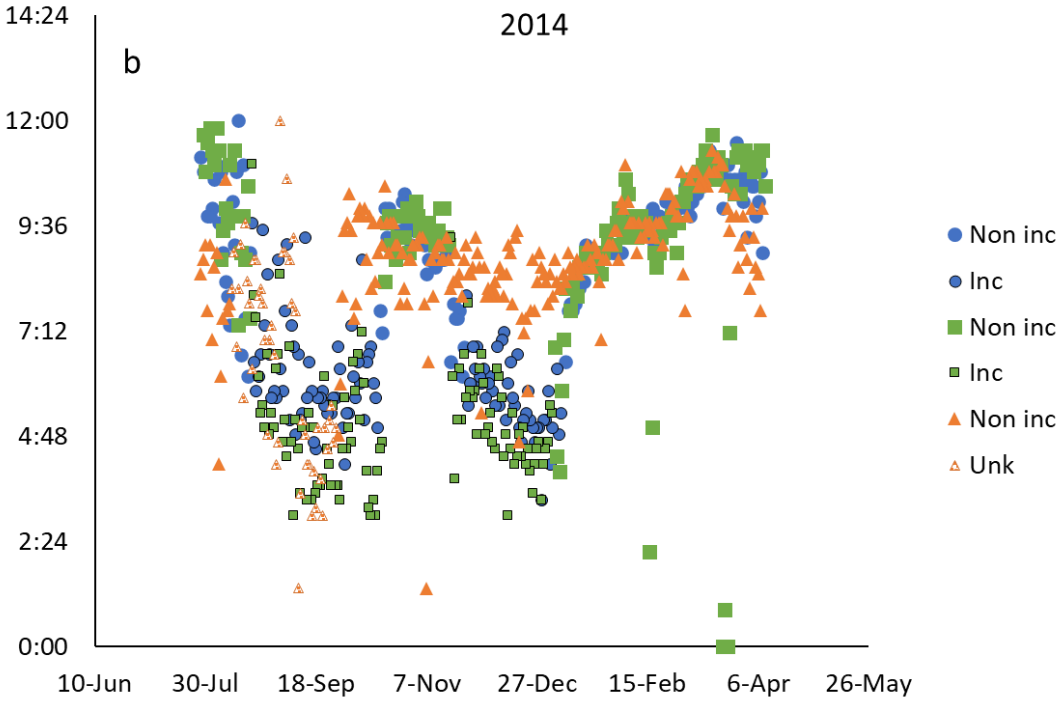


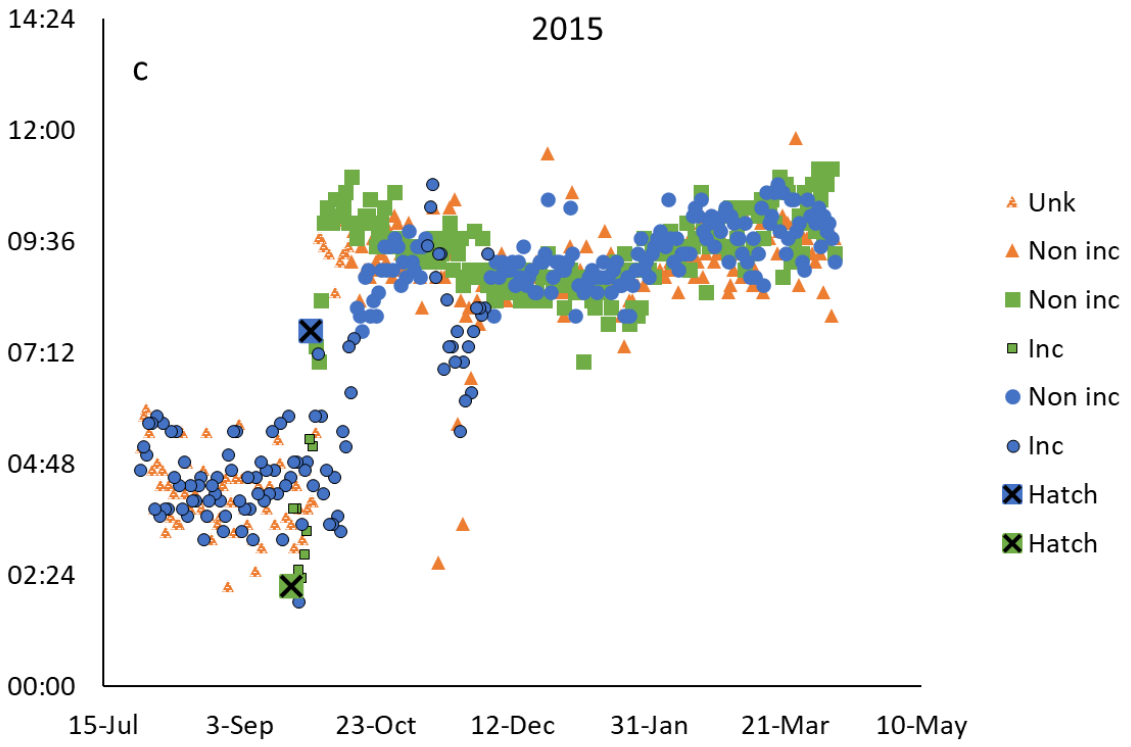
Supplementary figure S2.3.2. Timing of lowest and highest activity for male and female *A. mantelli*. Timing is represented as **s**tandard deviations around the mean date for the 30 consecutive days of lowest (Min30) and highest activity (Max30), respectively. Only data from years with one full-long incubation bout were included for males. Data from the start of July to the end of March from the 2013-2017 breeding seasons was used. Male Max30 is split in two groups since there was a strong dichotomy in this category. Numbers in brackets indicate N in birds-years.



Supplementary figure S2.3.3. Comparison of the sensitivity and accuracy of the widely used Chick timer transmitters™ (wildtech.co.nz/Kiwi.aspx) for nests with shared and single male incubation. Comparison spans the 2013 to 2017 breeding seasons. The two groups with shared incubation are presented separately with Tweety *et al.* referring to the group in which incubation is usually shared between Tweety, and Bow but about half of the time with Paul as well (see Figure 3 and Supplementary figure 6). Jaeden *et al.* refer to the group in which Jaeden and Marc essentially always share incubation (see Figure 3 and Supplementary figure 7). The single incubator bar is an average for 11 other males. (a) illustrates how many of the observed activity dips resulted in transmitters switching to incubation mode, and (b) how often full-long incubations triggered the hatching mode. The grey bar for single incubators in (b) represents certain triggered hatches and the error bar indicates the maximum number (accounting for the 6 cases where hatching trigger activation was unknown).


2013
a
14:24
12:00
09:36
07:12
04:48
02:24
00:00
6-May
25-Jun
14-Aug
3-Oct
22-Nov
11-Jan
2-Mar
21-Apr
Non inc
Inc
Unk
Non inc
Inc
Hatch
Hatch
Non inc


2014
b
14:24
12:00
9:36
7:12
4:48
2:24
0:00
10-Jun
30-Jul
18-Sep
7-Nov
27-Dec
15-Feb
6-Apr
26-May
Non inc
Inc
Non inc
Inc
Non inc
Unk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23-Oct
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31-Jan
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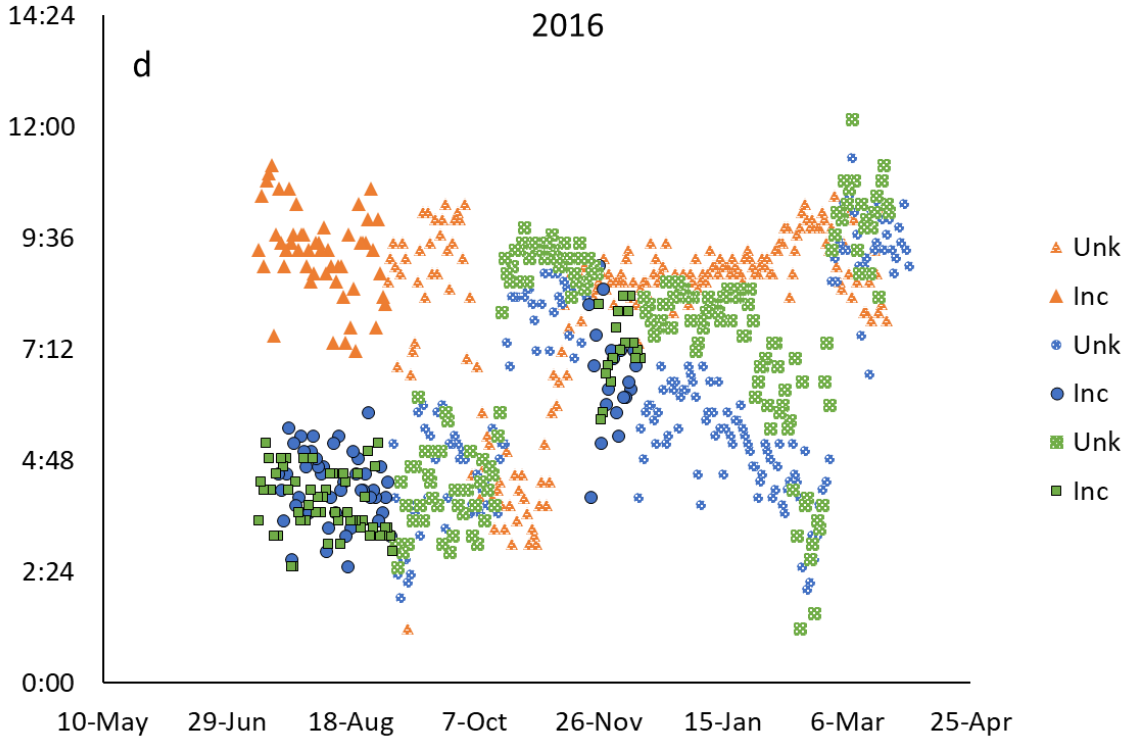
10-May
Unk
Non inc
Non inc
Inc
Non inc
Inc
Hatch
Hatch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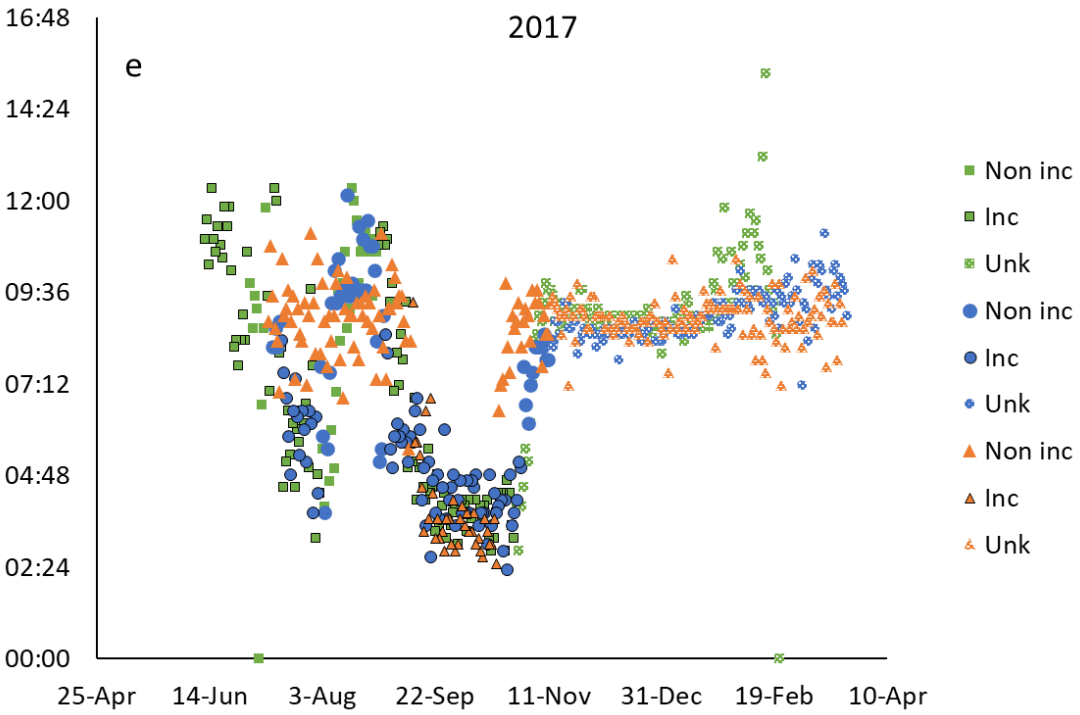
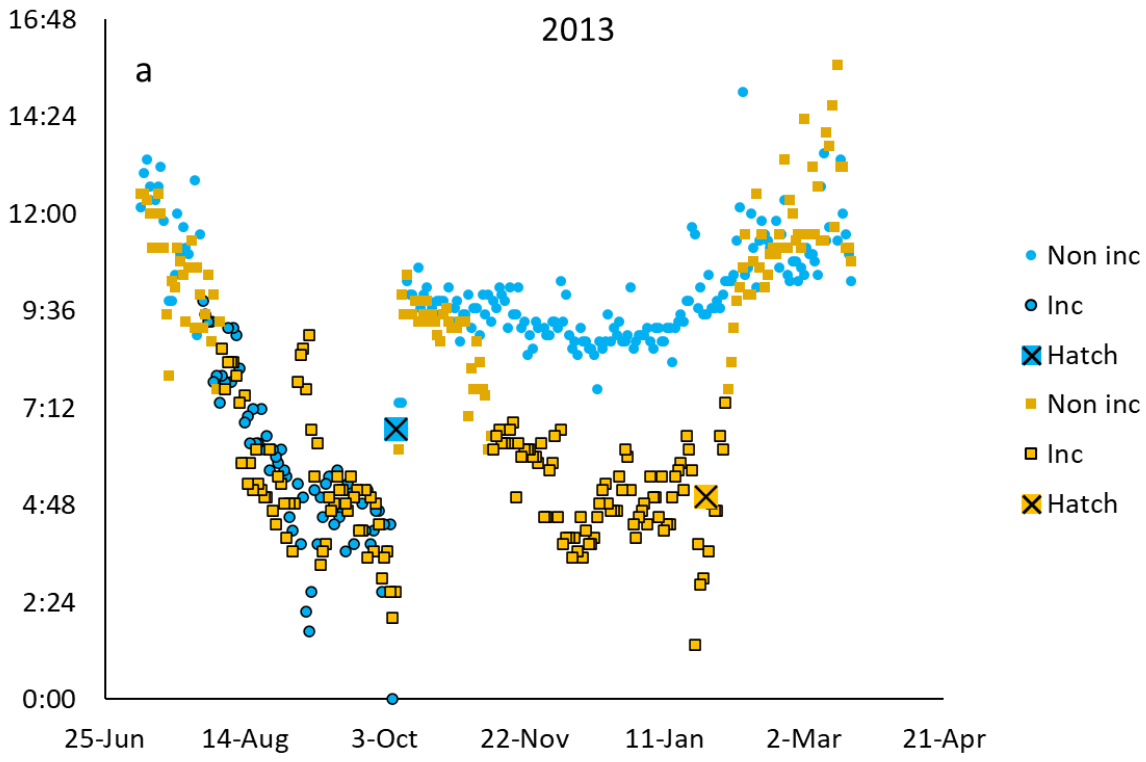
6-Mar
25-Apr
Unk
Inc
Unk
Inc
Unk
Inc



Supplementary figure S2.3.4. Overlays of the activity for three *A. mantelli* males form the same breeding unit (Bow, Tweety, and Paul) from the years 2013 to 2017. Overlays of the activity for Bow in green/squares, Tweety in blue/circles, and Paul in orange/triangles. Symbols indicate when the Chick timer transmitters™ signaled non-incubation (Non inc), incubation (Inc), and hatching (Hatch) respectively, and when the mode of the transmitter was unknown (Unk). Each bird has one activity value per night but start and finish date differ depending on when the individual transmitters were replaced.


2013
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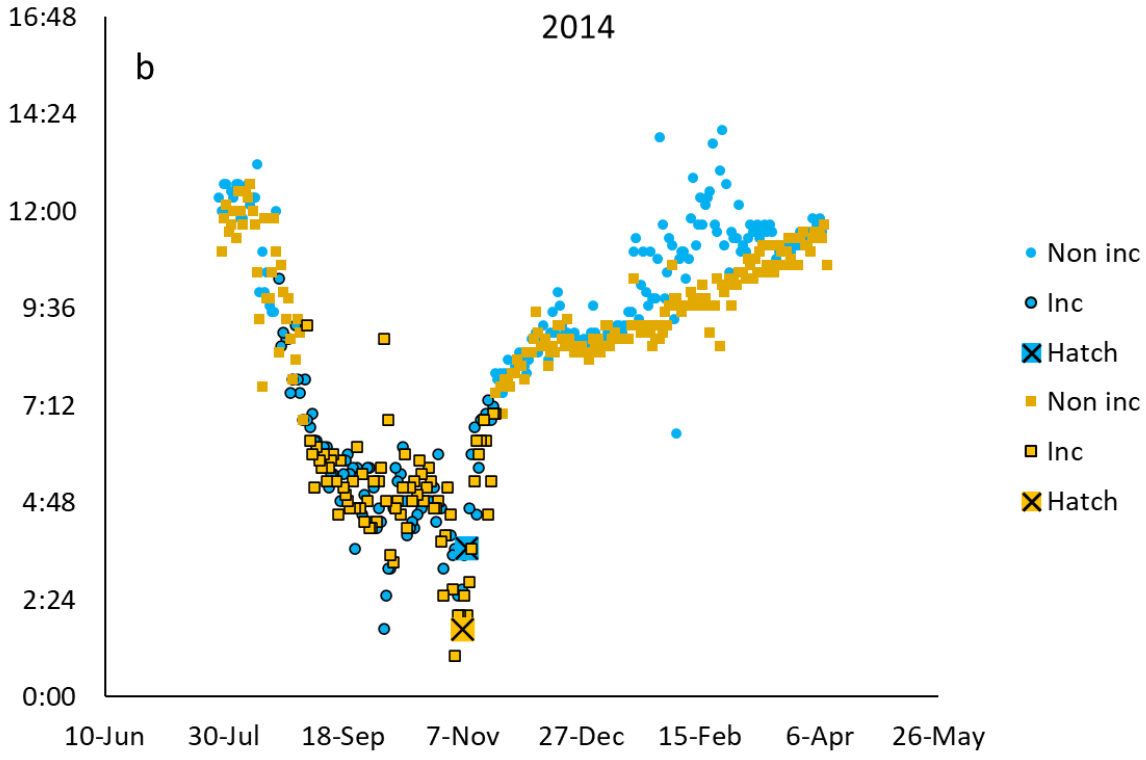
25-Jun
14-Aug
3-Oct
22-Nov
11-Jan
2-Mar
21-Apr
Non inc
Inc
Hatch
Non inc
Inc
Hatch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9:36
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4:48
2:24
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10-Jun
30-Jul
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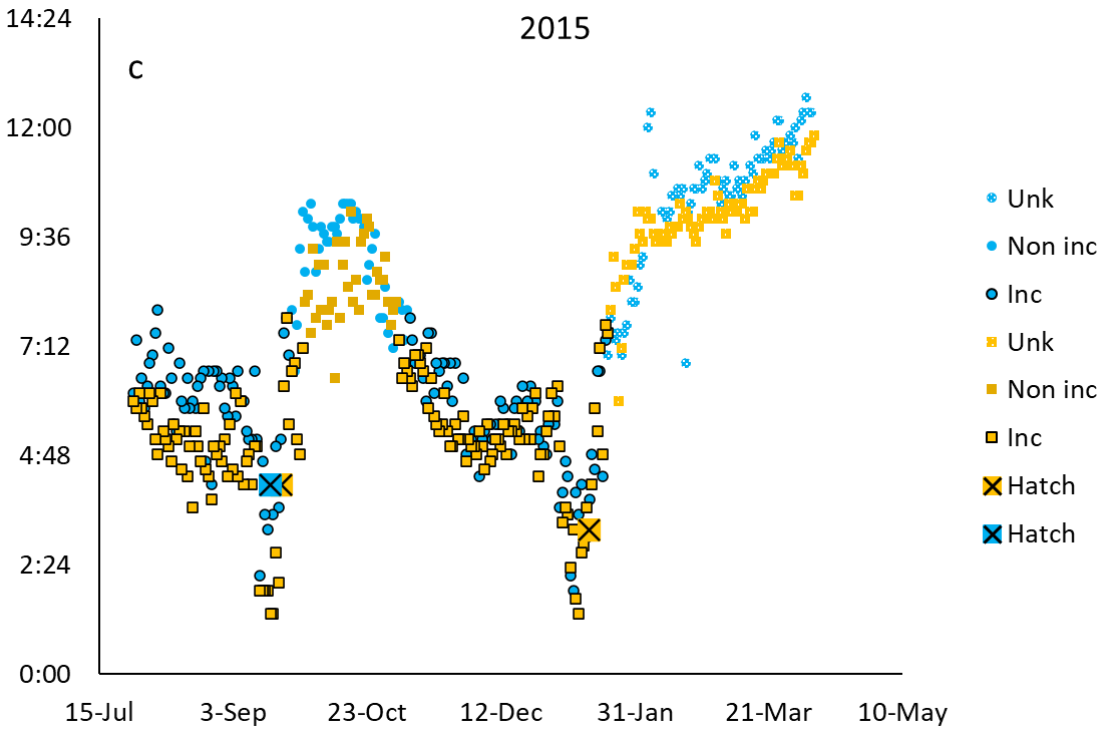
27-Dec
15-Feb
6-Apr
26-May
Non inc
Inc
Hatch
Non inc
Inc
Hatch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Non inc
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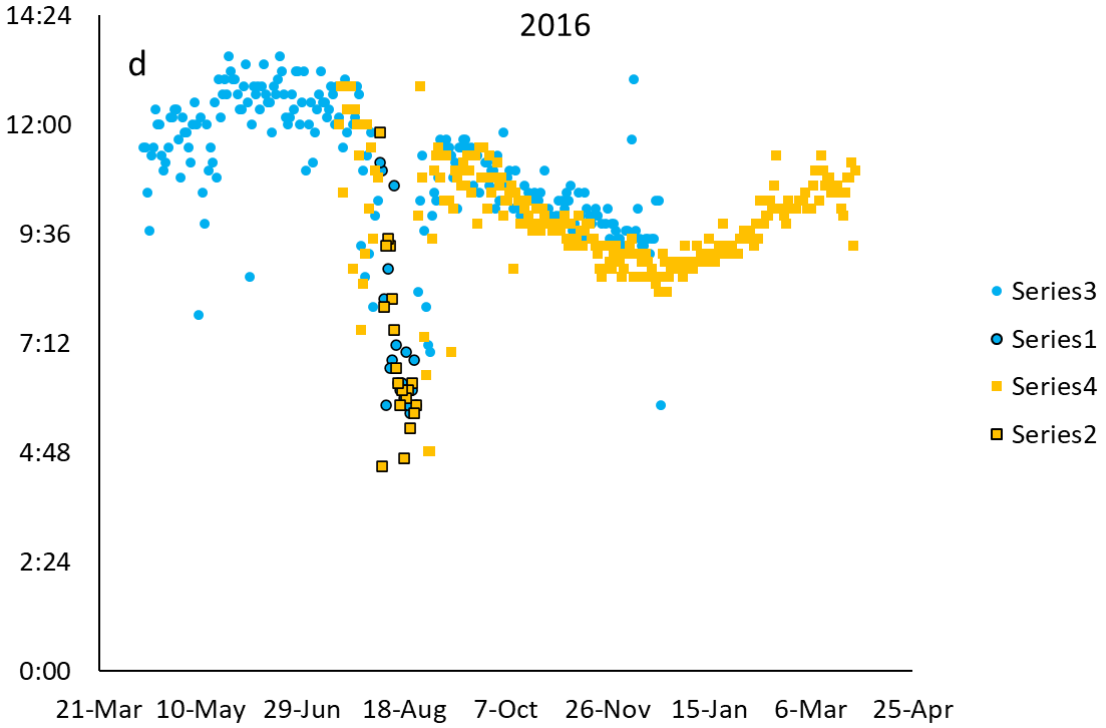
2016
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25-Apr
Series3
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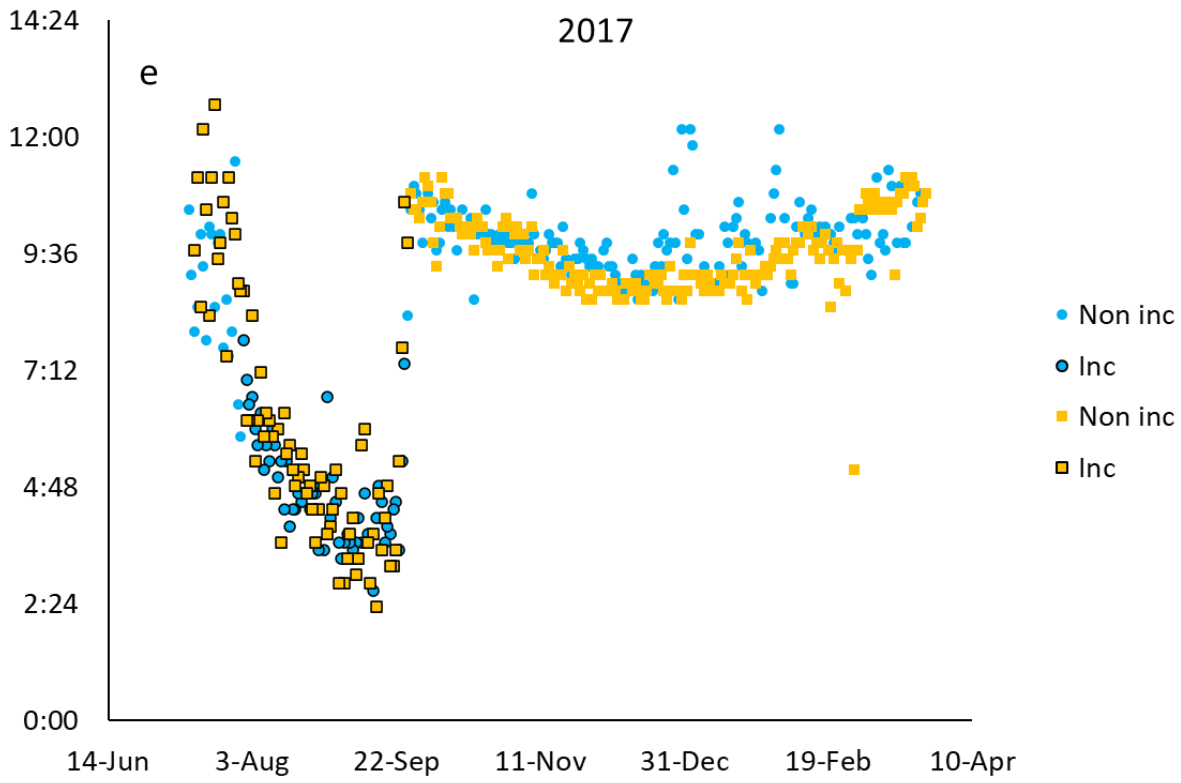
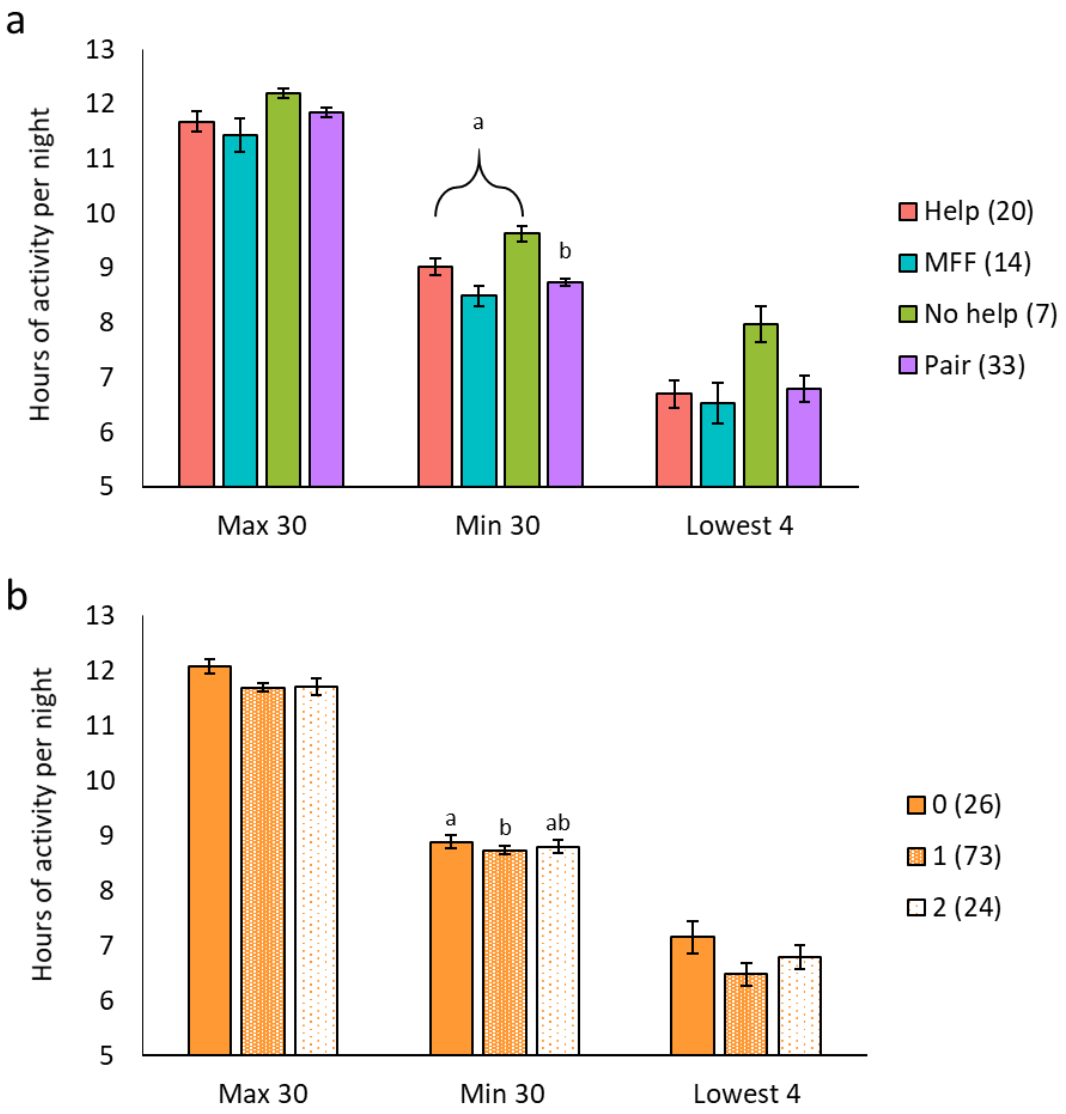
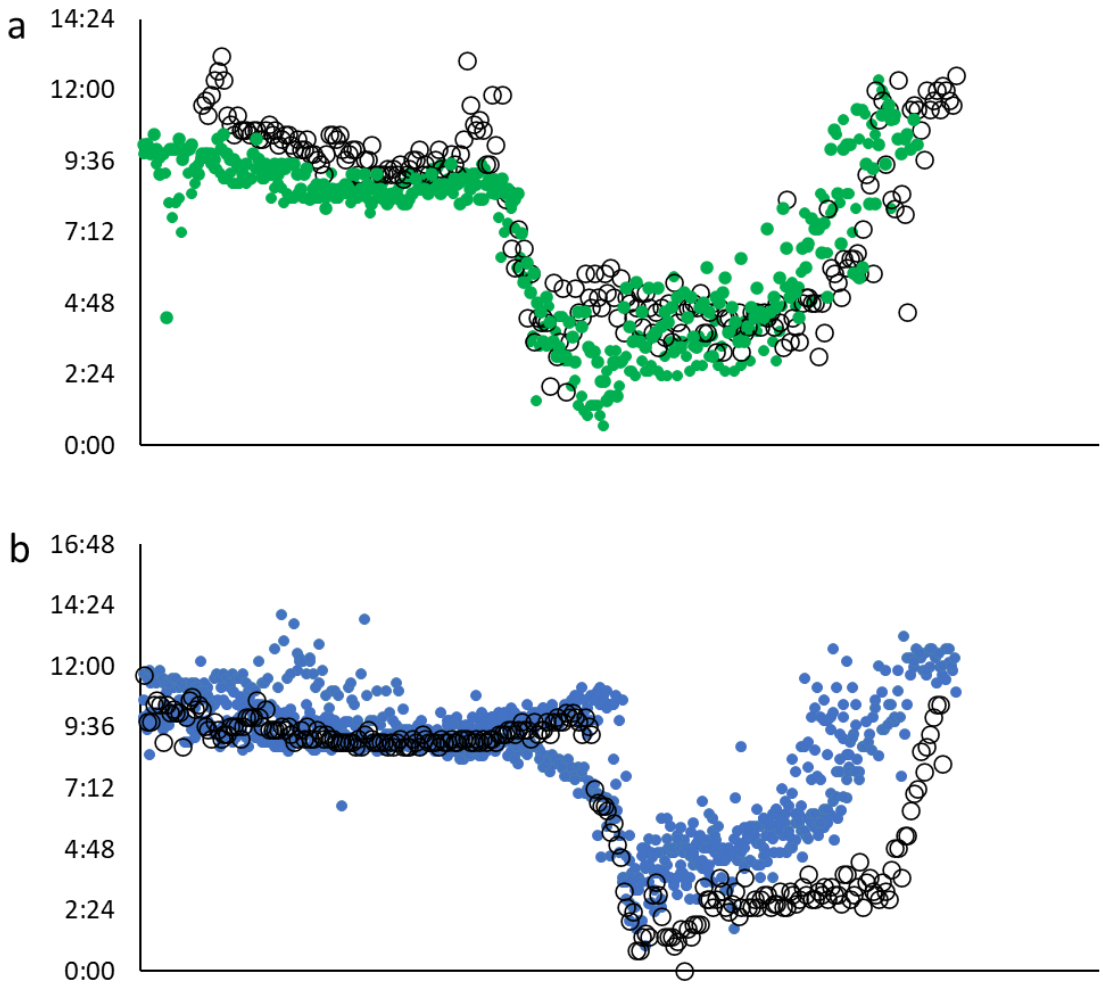
Series2



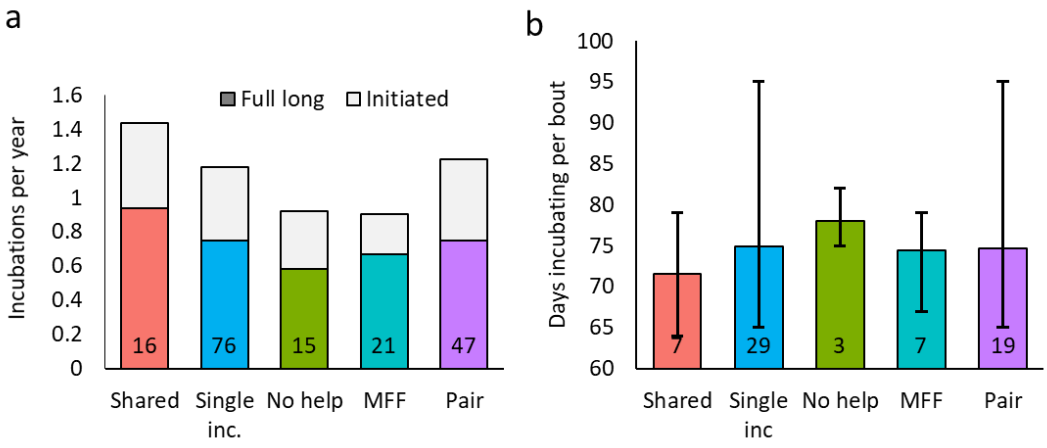
Supplementary figure S2.3.5. Overlays of the activity for two *A. mantelli* males form the same breeding unit (Jaeden and Marc) from the years 2013 to 2017. Jaeden in blue/circles and Marc in yellow/squares. Symbols indicate when the Chick timer transmitters™ signaled non-incubation (Non inc), incubation (Inc), and hatching (Hatch) respectively, and when the mode of the transmitter was unknown (Unk). Each bird has one activity value per night but start and finish date differ depending on when the individual transmitters were replaced.



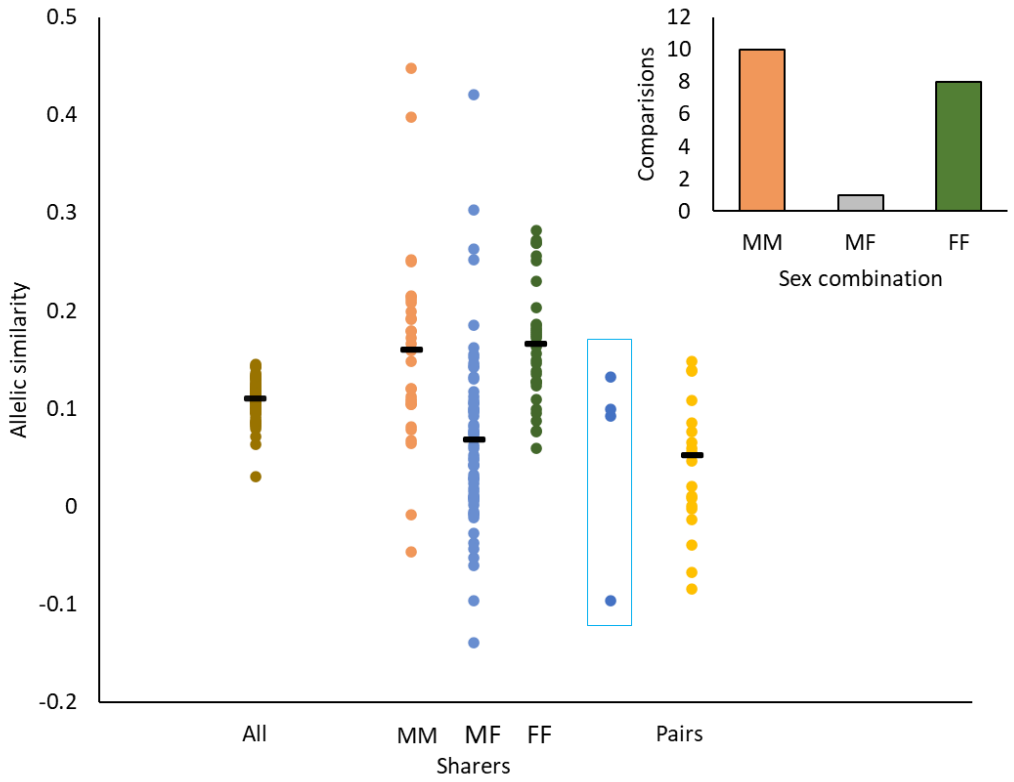
Supplementary figure S2.3.6. Bar graphs illustrating female activity in relation to the breeding strategy of her associated male(s; a) and the number of incubations initiated by her associated male(s; b) as a proxy for number of eggs laid. Lower case letters indicate statistically significant difference (see further Table 4 in main text). For (a) the statistical analyses only split the data as pairs *versus* groups (due to insufficient sample sizes) hence the statistical significance is indicated using a bracket across the bars making up the category "group".



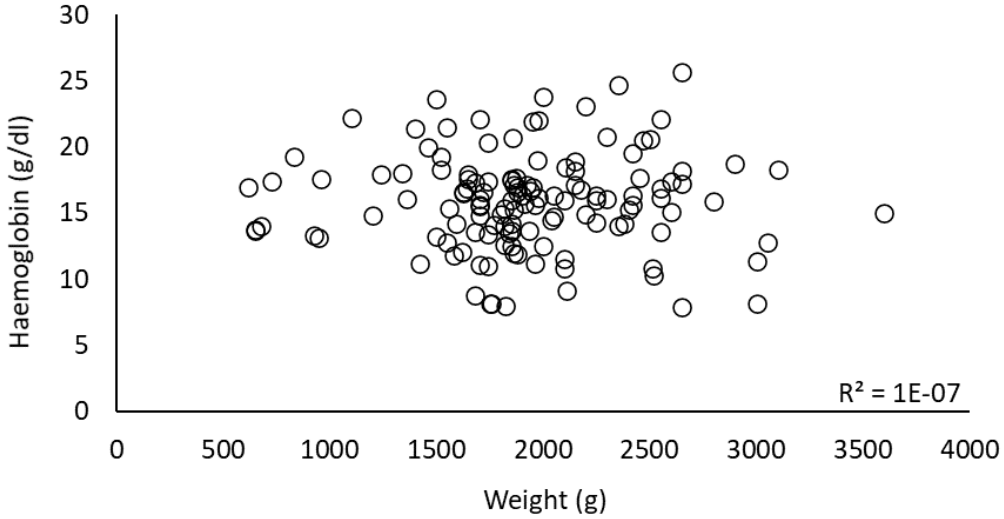
Supplementary figure S2.3.7. Graphic comparison of incubation length and level of activity between shared and nonshared *A. mantelli* incubations.Example (a) shows the activity of Tweety and Bow sharing incubation in 2013 (in green) overlaid with Martin's single incubation activity in 2012 (black circles). Example (b) shows the activity of the sharing males Jaeden and Marc from both 2014 and 2017 (in blue) overlaid with the activity of the single incubating male named Ponui Island in 2017 (black circles). The dates of the initial incubation dip have been aligned independent of the actual date for clarity.

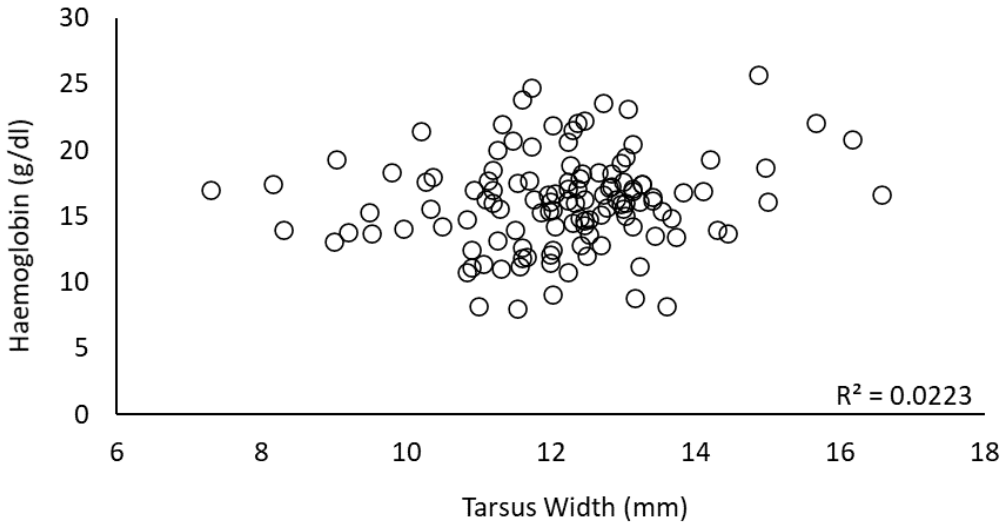


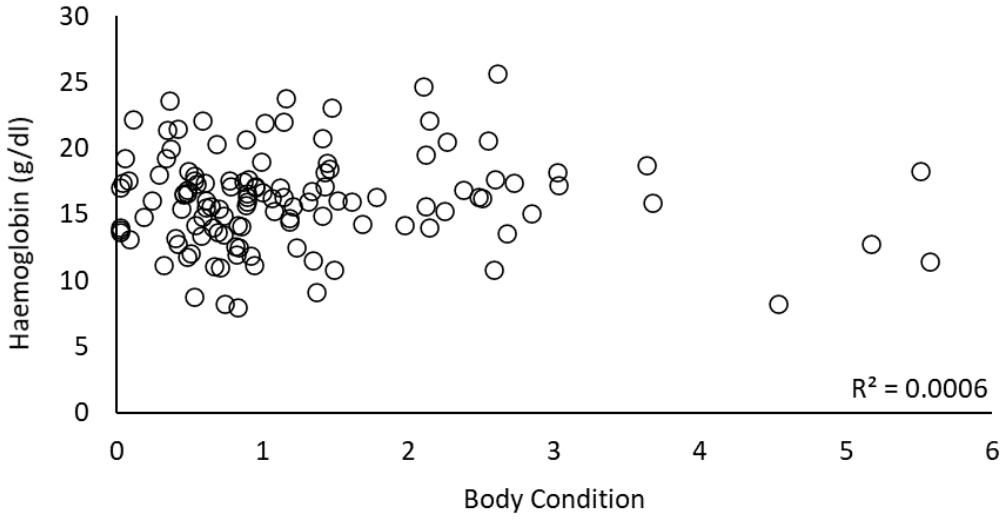
Supplementary figure S2.3.8. Comparison of the number of initiated and full-long incubations per year (a), and the average length of the full-long incubation bouts (b) with respect to mating strategy. In (a) average number of full long incubations per year is indicated in colour and additional initiated but not finished incubations are displayed in light grey in a stacked fashion. See figure 4 and table 3 in main text for category specifications. Error bars in (b) stretch from the minimum to the maximum number of days observed. Outcomes of the nest were unknown. No statistically significant differences were found. Numbers denote sample size in birds-years.



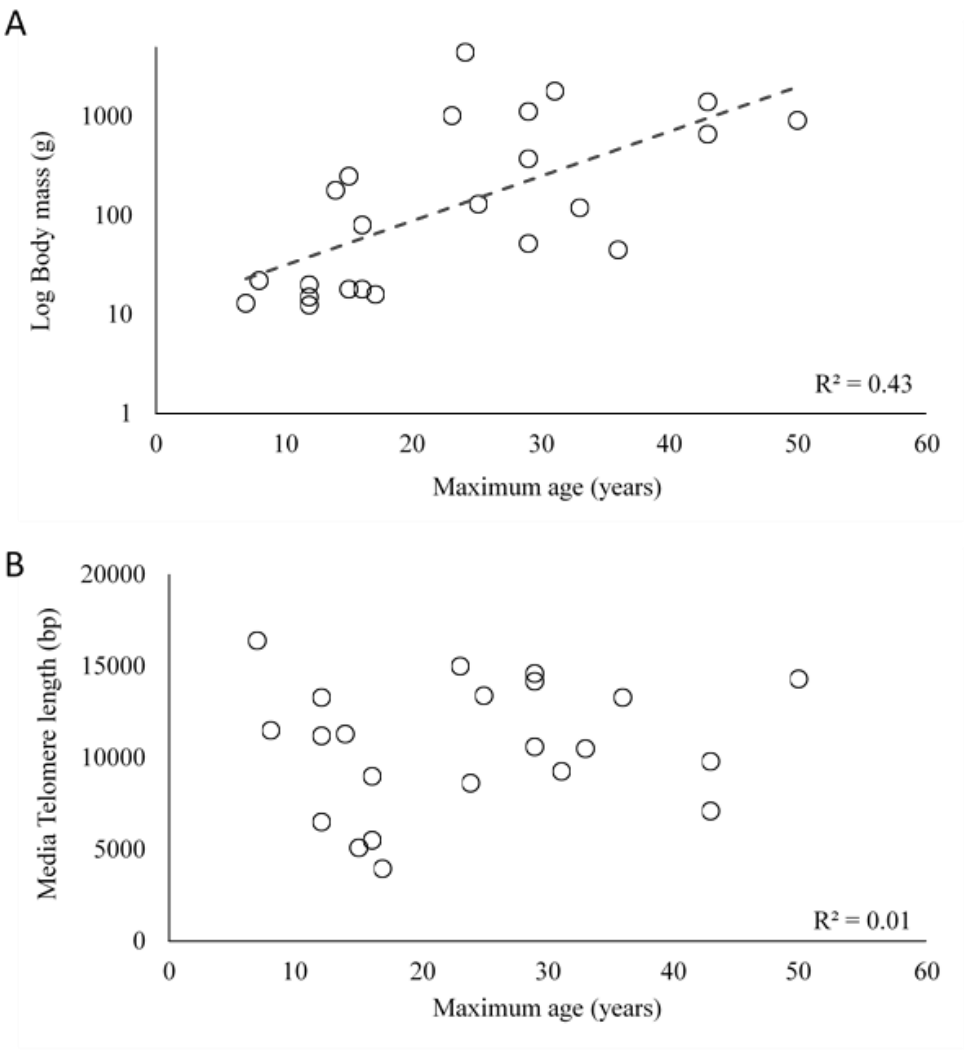
Supplementary figure S2.3.9. Pairwise relatedness between birds with overlapping home ranges ("Sharers") compared to overall relatedness in the population ("All") and to paired birds. Relatedness among shares are further split into Male-Male, Male-Female, and Female-Female comparisons. Black lines denote averages for each group. The dots in the blue square represent a repeat of four pairwise overlaps of birds found together in burrows at the same time. The inset illustrates the sex-composition of the highest pairwise relatedness value when comparing overlapping groups and/or pairs; birds of same sex (male-male or male-female) were found to be closer related than sharers of opposite sex (see further table 4 in the main text).







Supplementary figure S3.1.1. Correlations between haemoglobin concentration, weight, tarsus length, body condition, respectively, in *A. mantelli*. For visibility, an outlier value of a Taborsky´s body condition of 9.3 is not shown.



Supplementary figure S4.3.1. Scatter plots illustrating (A) the positive correlation between body mass and the maximum known age, and (B) the lack of correlation between known maximum age and median telomere length for 23 bird species. *Parus major*, although used in the modelling, was excluded from (B) for scaling purposes due to having exceptionally long telomeres of 49 000 bp.

Appendix A5 - Research Permits

Department of Conservation
Te Papa Atawhai

9 January 2019

Richard Witehira & Isabel Castro
C/- Ecology Institute of Natural Resources
Massey University
PO Box 11222
Palmerston North 4410

Re: WILDLIFE ACT AUTHORITY APPLICATION 70826-CAP APPROVAL

I am pleased to advise you that your application for a Wildlife Act Authority has been approved and I am now able to offer you an authority outlining the terms and conditions of this approval. Please find the authority enclosed.

This document contains all the terms and conditions of your authorisation to undertake the activity and represents the formal approval from the Department for Richard Witehira and Isabel Castro to carry out the activity.

Please read the terms carefully so that you clearly understand your obligations.

Please note, the Russell Forest (Pt Northland Conservation Park) has been excluded as an Authorised location as the Russell Forest roopu wish to establish a formal governance structure before providing support for the research, and a clear mandate for governance is yet to be obtained form hapu. Once governance is established, if you still wish to include this location, you may apply for a variation to the Authorisation.

No fee is payable for processing this application.

Yours sincerely

Sue Reed-Thomas
Director, Operations
Northern North Island Region

Department of Conservation
Te Papa Atawbai

Wildlife Act Authority for wildlife located on public conservation land [and other land]

Authorisation Number: 70826-CAP

THIS AUTHORITY is made this 9th day of January 2019

PARTIES:

The Director-General of Conservation and where required the Minister of Conservation (the Grantor)

AND Richard Witehira (the Authority Holder)

AND Isabel Castro (the Authority Holder)

BACKGROUND:

A. The Director-General of Conservation is empowered to issue authorisations under the Wildlife Act 1953.

B. Where the authorisation applies to wildlife located on public conservation land a further authorisation is required, depending upon the legislation applying to the public conservation land, from either the Director-General of Conservation or the Minister of Conservation.

C. The Authority Holder wishes to exercise the authorisation issued under the Wildlife Act 1953 and where applicable the authorisation issued under the relevant legislation applying to the public conservation land subject to the terms and conditions of this Authority.

OPERATIVE PARTS:

In exercise of the Grantor's powers the Grantor **AUTHORISES** the Authority Holder under Section 53 of the Wildlife Act 1953, clause 38 of the Wildlife Regulations 1955, Section 38 of the Conservation Act 1987 subject to the terms and conditions contained in this Authority and its Schedules.

SIGNED on behalf of the Grantor by Sue Reed-Thomas
Director, Operations, Northern North Island Region
acting under delegated authority in the presence of:

J. A. McLeish
Witness Signature

Witness Name: Jenny McLeish

Witness Occupation: Personal Assistant

Witness Address: 17 Kirikiri Rd, Whangarei 0110

A copy of the Instrument of Delegation may be inspected at the Director-General's office at 18-32 Manners Street, Wellington.

SCHEDULE 1

1.	**Authorised activity (including the species, any approved quantities and collection methods)** (Schedule 2, clause 2)	a. Activity i. to catch alive North Island Brown kiwi (*Apteryx mantelli*) for the purpose of species management and research ii. to mark North Island Brown kiwi (*Apteryx mantelli*) for the purpose of distinguishing the wildlife b. Quantity – 20 per location c. Method – i. catch alive by hand ii. mark using a. metal bands b. PIT tags
2.	**The Land** (Schedule 2, clause 2)	a. Public Conservation Land as per the map contained in Schedule 4 and subject to Schedule 3.29 of this Authority: i. Moturua Island Scenic Reserve ii. Opua Forest (Pt Northland Conservation Park) iii. Puketi Forest (Pt Northland Conservation Park) iv. Warawara forest (Pt Northland Conservation Park) v. Motuarohia Island Recreation Reserve vi. Manawahuna Scenic Reserve vii. Te Toroa Scenic Reserve viii. Whangamumu Scenic Reserve b. Other land: Private land at the following locations as per the map contained in Schedule 4 and subject to Schedule 2.2 of this Authority i. Purerua Peninsula ii. Waimate North iii. Waitangi Forest iv. Motuarohia v. Rakamangamanga (Cape Brett)

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		vi. Moturoa Island
3.	**Personnel authorised to undertake the Authorised Activity** (Schedule 2, clause 3)	a. catch alive i. Richard Witehira ii. Isabel Castro iii. Rana Rewha iv. Alvin Rewha v. Karen Mason vi. Natasha Bansal vii. Kathryn Strang viii. Emily Thompson ix. Hinemoana Black x. Monika Nowicki xi. Simon Hills xii. Doug Armstrong xiii. Pete Lockhart xiv. David Vieco xv. Ange Hura xvi. Steve McManus b. Mark by banding i. Isabel Castro c. Mark by PIT Tagging i. Isabel Castro ii. Lesley Baigents
4.	**Term** (Schedule 2, clause 4)	Commencing on and including 13 January 2019 and ending on and including 31 December 2022
5.	**Authority Holder's address for notices** (Schedule 2, clause 8)	The Authority Holder's address in New Zealand is: Massey University AgHort Building, A-wing, Office 1.42 Riddett Road Palmerston North 4410 Email:blandy@gmail.com/i.c.castro@massey.ac.nz
6.	**Grantor's address for notices**	The Grantor's address for all correspondence is: Level 4 73 Rostrevor Street Hamilton 3204 permissionshamilton@doc.govt.nz

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SCHEDULE 2

STANDARD TERMS AND CONDITIONS OF THE AUTHORITY

1. Interpretation

1.1 The Authority Holder is responsible for the acts and omissions of its employees, contractors or, agents. The Authority Holder is liable under this Authority for any breach of the terms of the Authority by its employees, contractors or agents as if the breach had been committed by the Authority Holder.

1.2 Where obligations bind more than one person, those obligations bind those persons jointly and separately.

2. What is being authorised?

2.1 The Authority Holder is only allowed to carry out the Authorised Activity on the Land described in Schedule 1, Item 2.

2.2 Any arrangements necessary for access over private land or leased land are the responsibility of the Authority Holder. In granting this authorisation the Grantor does not warrant that such access can be obtained.

2.3 The Authority Holder must advise the Department of Conservation's local Operations Manager(s) prior to carrying out the Authorised Activity in the District (where possible, one week prior), when the Authority Holder intends to carry out the Authorised Activity.

2.4 The Authority Holder and Authorised Personnel must carry a copy of this Authority with them at all times while carrying out the Authorised Activity.

2.5 The Authority Holder must comply with any reasonable request from the Grantor for access to any wildlife.

2.6 The Authority Holder may publish authorised research results.

2.7 The Authority Holder must immediately notify the Grantor of any taxa found which are new to science. In addition, the Authority Holder must lodge holotype specimens and a voucher specimen of any new taxa with a recognised national collection.

3. Who is authorised?

3.1 Only the Authority Holder and the Authorised Personnel described in Schedule 1, Item 3 are authorised to carry out the Authorised Activity, unless otherwise agreed in writing by the Grantor.

4. How long is the Authority for - the Term?

4.1 This Authority commences and ends on the dates set out in Schedule 1, Item 4.

5. What are the obligations to protect the environment?

5.1 The Authority Holder must not cut down or damage any vegetation; or damage any natural feature or historic resource on any public conservation land being part of the

Land; or light any fire on such public conservation land; or erect any structure such public conservation land without the prior consent of the Grantor.

5.2 The Authority Holder must ensure that it adheres to the international "Leave No Trace" Principles at all times (www.leavenotrace.org.nz).

5.3 The Authority Holder must not bury:

(a) any toilet waste within 50 metres of a water source on any public conservation land being part of the Land; or
(b) any animal or fish or any part thereof within 50 metres of any water body, water source or public road or track.

6. What are the liabilities?

6.1 The Authority Holder agrees to exercise the Authority at the Authority Holder's own risk and releases to the full extent permitted by law the Grantor and the Grantor's employees and agents from all claims and demands of any kind and from all liability which may arise in respect of any accident, damage or injury occurring to any person or property arising from the Authority Holder's exercise of the Authorised Activity.

6.2 The Authority Holder must indemnify the Grantor against all claims, actions, losses and expenses of any nature which the Grantor may suffer or incur or for which the Grantor may become liable arising from the Authority Holder's exercise of the Authorised Activity.

6.3 This indemnity is to continue after the expiry or termination of this Authority in respect of any acts or omissions occurring or arising before its expiry or termination.

7. What about compliance with legislation and Grantor's notices and directions?

7.1 The Authority Holder must comply with all statutes, bylaws and regulations, and all notices, directions and requisitions of the Grantor and any competent authority relating to the conduct of the Authorised Activity. Without limitation, this includes the Conservation Act 1987 and the Acts listed in the First Schedule of that Act and all applicable health and safety legislation and regulation.

8. Are there limitations on public access and closure?

8.1 The Authority Holder acknowledges that the public conservation land being part of the Land is open to the public for access and that the Grantor may close public access to that public conservation land during periods of high fire hazard or for reasons of public safety or emergency.

9. When can the Authority be terminated?

9.1 The Grantor may terminate this Authority at any time in respect of the whole or any part of the Land, and/or the whole or any part of the Authorised Activity if:

(a) the Authority Holder breaches any of the conditions of this Authority; or

(b) in the Grantor's opinion, the carrying out of the Authorised Activity causes or is likely to cause any unforeseen or unacceptable effects.

9.2 If the Grantor intends to terminate this Authority in whole or in part, the Grantor must give the Authority Holder such prior notice as, in the sole opinion of the Grantor, appears reasonable and necessary in the circumstances.

10. How are notices sent and when are they received?

10.1 Any notice to be given under this Authority by the Grantor is to be in writing and made by personal delivery, by pre paid post or email to the Authority Holder at the address, fax number or email address specified in Schedule 1, Item 5. Any such notice is to be deemed to have been received:

(a) in the case of personal delivery, on the date of delivery;

(b) in the case of post, on the 3rd working day after posting;

(c) in the case of email, on the date receipt of the email is acknowledged by the addressee by return email or otherwise in writing.

10.2 If the Authority Holder's details specified in Schedule 1, Item 5 change then the Authority Holder must notify the Grantor within 5 working days of such change.

11. What about the payment of costs?

11.1 The Authority Holder must pay the standard Department of Conservation charge-out rates for any staff time and mileage required to monitor compliance with this Authority and to investigate any alleged breaches of the terms and conditions of it.

12. Biosecurity

12.1 The Authority Holder must take all precautions to ensure weeds and non-target species are not introduced to the Land; this includes ensuring that all tyres, footwear, gaiters, packs and equipment used by the Authority Holder, its staff and clients are cleaned and checked for pests before entering the Land.

13. Are there any Special Conditions?

13.1 Special conditions are specified in Schedule 3. If there is a conflict between this Schedule 2 and the Special Conditions in Schedule 3, the Special Conditions will prevail.

14. Can the Authority be varied?

14.1 The Authority Holder may apply to the Grantor for variations to this Authority.

SCHEDULE 3

SPECIAL CONDITIONS

1. This Authorisation gives the Authority Holder the right to hold absolutely protected wildlife in accordance with the terms and conditions of the Authorisation, but the wildlife remains the property of the Crown. This includes any dead wildlife, live wildlife, any parts thereof, any eggs or progeny of the wildlife, genetic material and any replicated genetic material.

2. Unless expressly authorised by the Grantor in writing, the Authority Holder must not donate, sell or otherwise transfer to any third party any wildlife, material, including any genetic material, or any material propagated or cloned from such material, collected under this Authority.

3. If any wildlife should die as a result of undertaking the Authorised Activities, the Authority Holder must:
    a. inform the Grantor (whangarei@doc.govt.nz) within 24 hours;
    b. chill the body if it can be delivered within 24 hours, or freeze the body if delivery will take longer than 24 hours;
    c. send, at the Authority Holders costs, the body to Massey University Wildlife Post Mortem Service for necropsy, along with details of the animal's history;
    d. pay for any costs incurred in investigation of the death; and,
    e. If required by the Grantor, cease the Authorised Activity for a period determined by the Grantor.

4. Within one month of completion of the Authorised Activity or termination of this Authority, the Authority Holder must forward a report electronically to the Grantor to permissionshamilton@doc.govt.nz. This report must contain the following:
    a. the Authority Number [70826-CAP];
    b. the number of birds caught at each location
    c. the number of blood samples taken
    d. the results of all blood analysis
    e. a summary of findings;
    f. any implications for conservation management; and

    The Authority Holder acknowledges that the Grantor may provide copies of these reports to tangata whenua and the general public if requested.

5. Only persons registered with the Grantor as Accredited Kiwi Handlers must catch the wildlife unsupervised.

6. All persons who are not registered with the Grantor as Accredited Kiwi Handler must catch the wildlife under the supervision of an Accredited Kiwi Handler.

7. The Authority Holder must submit an annual report to the Grantor identifying each person's skill level.

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8. Isabel Castro, a Level 3 bander, certified under the New Zealand National Bird Banding Scheme, must oversee and be accountable for the Authorised Activity. Level 2 banders may operate without direct supervision, but must operate under the general direction of a Level 3 Certified bander. Level 1 banders must be directly supervised by a Level 3 Certified bander.

9. The Authority Holder must undertake the Authorised Activity in accordance with the application received and the most recent edition of the New Zealand National Bird Scheme Bird Bander's Manual (**"the Bird Banding Manual"**).

10. Only metal bird bands supplied by the Department of Conservation, New Zealand are to be used, except where other marking techniques are authorised.

11. The Authority Holder must have approval of the Banding Office to trial new band sizes. Any changes to recommended band sizes must be notified in writing to the Banding Office as soon as practicable.

12. If a band is taken off a bird for any reason, it must NOT be used on another bird.

13. The Authority Holder must supply the Banding Office with electronic copies of all banding schedules used to record newly banded or re-banded birds, plus a completed copy of the band stock-take sheet by 1 April each year. Standard electronic templates will be supplied by the Banding Office.

14. Band recoveries for dead birds and any birds re-banded are mandatory. Band recoveries must be submitted on the standard recovery format templates in electronic or paper form. Other recapture data can be submitted on these forms or on electronic spreadsheets.

15. The Authority Holder must comply with all guidelines and notices issued by the Kauri Dieback Programme to prevent and avoid the spread of the pest organism Kauri Dieback Disease (*Phytophthora taxon agathis*) as specified on the website http://www.kauridieback.co.nz/. This includes ensuring that all vehicles, personal items and equipment are thoroughly cleaned of all visible soil and is sprayed with SteriGENE (formally known as Trigene) solution before entering and when moving between areas where there are kauri.

16. The Authorised Holder is permitted to catch the wildlife with the assistance of dogs provide both dogs and handler have been fully certified under the Department of Conservation approved Conservation Dogs Programme

17. All wildlife must be caught from day time roosts outside the breeding season by experienced Kiwi handlers.

18. All wildlife must be caught according to the Kiwi Best Practice Manual

19. Blood samples may be taken from the wildlife.

20. Only persons registered with the Grantor as qualified to take blood samples must do so unsupervised

21. All persons who are not registered with the Grantor as qualified to take blood samples must conduct blood sampling under the supervision of a qualified person.

22. Blood collection must be undertaken according to the methodologies set out in the Department of Conservation Avian Blood/Feather Sampling and Reptilian Tissue Collection Standard Operating Procedure, provided to the Authority Holder by the Grantor.

23. All blood samples must be taken for a vein in the animal's leg and shall be no greater than 0.5 millilitres in volume

24. Blood samples must only be sent to Massey University, Palmerston North, for analysis and all analysis and research is restricted to:

    a. analysing extracted DNA

    b. measuring:
        i. genetic distance both for neutral loci and genes under selection

        ii. the level of intermixture and introgression of genes

        iii. presence and abundance of genotypes

        iv. length of telomeres.

25. For the avoidance of doubt, no blood collected under this Authorisation shall be used for or made available for any other research apart from that stipulated at Schedule 3.24 and research for any other purpose requires a separate Authorisation.

26. The Authority Holder must use best endeavours to ensure that the Authorised Activity is not undertaken within sight of the public.

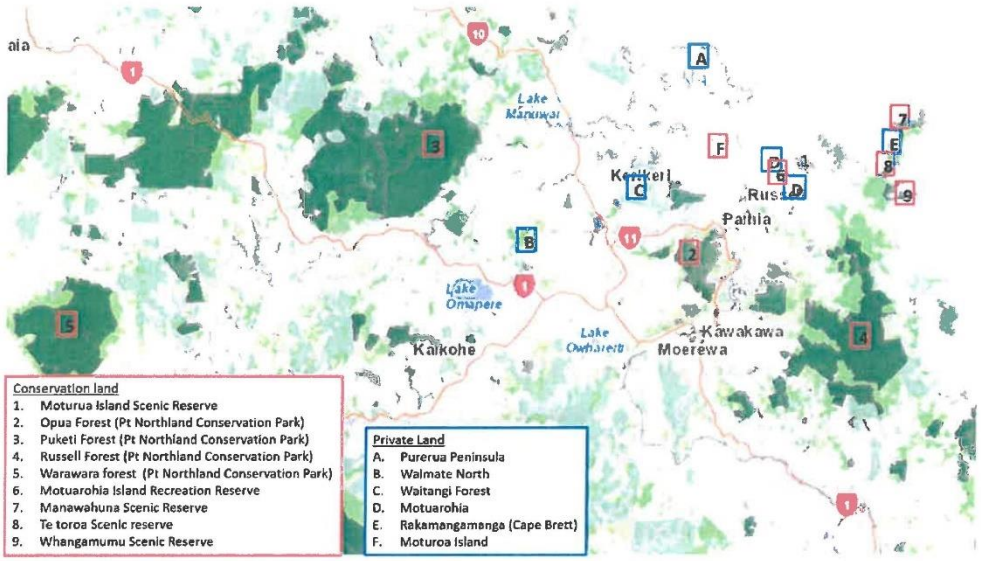
27. While undertaking the Authorised Activity on public conservation land, the Authority Holder must not exclude or impede the public from accessing any sites, tracks or facilities.

28. If approached by members of the public while carrying out the Authorised Activity, the Authority Holder shall provide an explanation of why the Authorised Activity is taking place.

29. For the avoidance of doubt, the Russel Forest (Pt Northland Conservation Park) identified as location number 4 on the map under Schedule 4 of this Authority is excluded from this Authority as an Authorised location.

30. A new clause 7.1 (c) is added to Schedule 2, to read as follows:
"Or for any other reason that the Grantor may decide".

SCHEDULE 4


Lake Kanuwai
Kerikeri
Russell
Pahia
Lake Omapere
Kaikohe
Lake Owhareiti
Kawakawa
Moerewa
Conservation land
1. Moturua Island Scenic Reserve
2. Opua Forest (Pt Northland Conservation Park)
3. Puketi Forest (Pt Northland Conservation Park)
4. Russell Forest (Pt Northland Conservation Park)
5. Warawara forest (Pt Northland Conservation Park)
6. Motuarohia Island Recreation Reserve
7. Manawahuna Scenic Reserve
8. Te toroa Scenic reserve
9. Whangamumu Scenic Reserve
Private Land
A. Purerua Peninsula
B. Waimate North
C. Waitangi Forest
D. Motuarohia
E. Rakamangamanga (Cape Brett)
F. Moturoa Island

Department of Conservation
Te Papa Atawbai

8 March 2019

Richard Witehira & Isabel Castro
C/- Ecology Institute of Natural Resources
Massey University
PO Box 11222
Palmerston North 4410

Re: VARIATION TO AUTHORITY 70826-CAP - APPROVAL

I am pleased to advise your application for a variation to the above Authority has been approved. Please find the variation enclosed.

Except to the extent to which it is amended by this variation, the provisions expressed and implied in the Authority continue to apply.

Please affix the enclosed signed variation to your current Authority.

No fee is payable for processing this application

Yours sincerely

Sue Reed-Thomas
Director, Operations
Northern North Island Region

Department of Conservation
Te Papa Atawhai

Variation to a Wildlife Act Authority under the Wildlife Act 1953

Authorisation Number: 70826-CAP

THIS DEED OF VARIATION OF AN AUTHORITY is made this 8th day of March 2019

PARTIES:

The Director General of Conservation, and where required, the Minister of Conservation (the Grantor)

AND Richard Witehira (the Authority Holder)

AND Isabel Castro (the Authority Holder)

BACKGROUND

A. By an Authorisation dated the 9th day of January 2019 the Director-General of Conservation granted an Authority under the Wildlife Act 1953 to the Authority Holder upon the terms and conditions expressed and implied in the Authority.

B. The Grantor hereby varies that Authority.

NOW BY THIS DEED the Grantor authorises as follows:

1. **Variation**

   In exercise of the Grantor's powers under the Wildlife Act the Grantor varies the Authority as follows:

   (i) To Schedule 1.3.a add "*Malin Undin*"

2. **Confirmation of other Authority Covenants**

   Except to the extent to which they are amended by this Variation the provisions expressed and implied in the Authority continue to apply.

SIGNED on behalf of the Grantor
by Sue Reed-Thomas, Director, Operations, acting under delegated authority
in the presence of:

Witness Signature — Witness name: Lorrein Meyers

Witness Occupation: DOC - Administrator — Witness Address: 53 Church Rd, Kaitaia

A copy of the Instrument of Delegation may be inspected at the Director-General of Conservation's office at 18-32 Manners Street, Wellington.

Department of Conservation
Te Papa Atawhai

File Ref: 70875-RES

29 April 2019

Massey University
Private Bag 11222
Tennent Drive
Palmerston North 4442
New Zealand

Dear Richard Witehira

Re: WILDLIFE ACT AUTHORITY APPLICATION 70875-RES APPROVAL

I am pleased to advise you that your application for a Wildlife Act Authority has been approved. Please find the authority enclosed.

This document contains all the terms and conditions of your authorisation to undertake the activity and represents the formal approval from the Department for you to carry out the activity.

Please read the terms carefully so that you clearly understand your obligations.

Please also find attached the following supporting documents for your information and use:

1. Captive Health Care Workbook 2004
2. Wildlife Health Management - Standard Operating Procedure
3. Sampling Avian Blood and Feathers, and Reptilian Tissue - Standard Operating Procedure

Yours sincerely,

Grant Balsom
Permissions Advisor
Hamilton

Email: gbalsom@doc.govt.nz

Department of Conservation
Te Papa Atawbai

Wildlife Act Authority for wildlife located on public conservation land

Authorisation Number: 70875-RES

THIS AUTHORITY is made this 17th day of April 2019

PARTIES:

The Director-General of Conservation and where required the Minister of Conservation (the Grantor)
AND
Massey University (National Holder) (the Authority Holder)

BACKGROUND:

A. The Director-General of Conservation is empowered to issue authorisations under the Wildlife Act 1953.

B. Where the authorisation applies to wildlife located on public conservation land a further authorisation is required, depending upon the legislation applying to the public conservation land, from either the Director-General of Conservation or the Minister of Conservation.

C. The Authority Holder wishes to exercise the authorisation issued under the Wildlife Act 1953 and where applicable the authorisation issued under the relevant legislation applying to the public conservation land subject to the terms and conditions of this Authority.

OPERATIVE PARTS:
In exercise of the Grantor's powers the Grantor:

AUTHORISES the Authority Holder under Section 53 (Taking or Killing of Wildlife for Certain Purposes) of the Wildlife Act 1953;

SIGNED on behalf of the Grantor by Andrew Baucke, Operations Director acting under delegated authority

in the presence of:

Witness Signature

Witness Name: Olivia Keane

Witness Occupation: Ranger, Community

Witness Address: 24 Wellesley St West AKL

A copy of the Instrument of Delegation may be inspected at the Director-General's office at 18-32 Manners Street, Wellington.

SCHEDULE 1

1.	**Authorised activity (including the species, any approved quantities and collection methods)** (Schedule 2, clause 2)	a) Activity: i. to catch alive North Island Kiwi (*Apteryx mantelli*) for the purposes of species management and research ii. to mark North Island Kiwi (*Apteryx mantelli*) for the purposes of distinguishing the wildlife b) Species: North Island Brown Kiwi (*Apteryx mantelli*) c) Quantity: Ninety (90) in total d) Method: i. catch alive by hand ii. mark using metal bands
2.	**The Land** (Schedule 2, clause 2)	Public Conservation Land: a. Remutaka Forest Park b. Waipoua Forest (Pt Northland Conservation Park) c. Trounson Kauri Park d. Kawau Island Scenic Reserve e. Te Hauturu-o-Toi/Little Barrier Island Nature Reserve f. Pukaha/Mount Bruce Scenic Reserve
3.	**Personnel authorised to undertake the Authorised Activity** (Schedule 2, clause 3)	a. Richard Witehira b. Isabel Castro c. Simon Hills
4.	**Term** (Schedule 2, clause 4)	Commencing on and including 15th April 2019 and ending on and including 31st May 2022.
5.	**Authority Holder's address for notices** (Schedule 2, clause 8)	The Authority Holder's address in New Zealand is: Massey University AgHort Building, A-wing, Office 1.42 Riddett Road Palmerston North 4410 New Zealand Phone: 0800 627739 / 021 678915 Email: blandyw@hotmail.com
6.	**Grantor's address**	The Grantor's address for all correspondence is:

	for notices	Department of Conservation Hamilton Permissions Team Level 4 73 Rostrevor Street Hamilton 3204 Phone: 07 858 1000 Email: permissionshamilton@doc.govt.nz

SCHEDULE 2

STANDARD TERMS AND CONDITIONS OF THE AUTHORITY

1. Interpretation

1.1 The Authority Holder is responsible for the acts and omissions of its employees, contractors or, agents. The Authority Holder is liable under this Authority for any breach of the terms of the Authority by its employees, contractors or agents as if the breach had been committed by the Authority Holder.

1.2 Where obligations bind more than one person, those obligations bind those persons jointly and separately.

2. What is being authorised?

2.1 The Authority Holder is only allowed to carry out the Authorised Activity on the Land described in Schedule 1, Item 2.

2.2 Any arrangements necessary for access over private land or leased land are the responsibility of the Authority Holder. In granting this authorisation the Grantor does not warrant that such access can be obtained.

2.3 The Authority Holder must advise the Department of Conservation's local Operations Manager(s) prior to carrying out the Authorised Activity in the District (where possible, one week prior), when the Authority Holder intends to carry out the Authorised Activity.

2.4 The Authority Holder and Authorised Personnel must carry a copy of this Authority with them at all times while carrying out the Authorised Activity.

2.5 The Authority Holder must comply with any reasonable request from the Grantor for access to any wildlife.

2.6 The Authority Holder may publish authorised research results.

2.7 The Authority Holder must immediately notify the Grantor of any taxa found which are new to science. In addition, the Authority Holder must lodge holotype specimens and a voucher specimen of any new taxa with a recognised national collection.

3. Who is authorised?

3.1 Only the Authority Holder and the Authorised Personnel described in Schedule 1, Item 3 are authorised to carry out the Authorised Activity, unless otherwise agreed in writing by the Grantor.

4. How long is the Authority for - the Term?

4.1 This Authority commences and ends on the dates set out in Schedule 1, Item 4.

5. What are the obligations to protect the environment?

5.1 The Authority Holder must not cut down or damage any vegetation; or damage any natural feature or historic resource on any public conservation land being part of the

Land; or light any fire on such public conservation land; or erect any structure such public conservation land without the prior consent of the Grantor.

5.2 The Authority Holder must ensure that it adheres to the international "Leave No Trace" Principles at all times (**www.leavenotrace.org.nz**).

5.3 The Authority Holder must not bury:

(a) any toilet waste within 50 metres of a water source on any public conservation land being part of the Land; or
(b) any animal or fish or any part thereof within 50 metres of any water body, water source or public road or track.

6. What are the liabilities?

6.1 The Authority Holder agrees to exercise the Authority at the Authority Holder's own risk and releases to the full extent permitted by law the Grantor and the Grantor's employees and agents from all claims and demands of any kind and from all liability which may arise in respect of any accident, damage or injury occurring to any person or property arising from the Authority Holder's exercise of the Authorised Activity.

6.2 The Authority Holder must indemnify the Grantor against all claims, actions, losses and expenses of any nature which the Grantor may suffer or incur or for which the Grantor may become liable arising from the Authority Holder's exercise of the Authorised Activity.

6.3 This indemnity is to continue after the expiry or termination of this Authority in respect of any acts or omissions occurring or arising before its expiry or termination.

7. What about compliance with legislation and Grantor's notices and directions?

7.1 The Authority Holder must comply with all statutes, bylaws and regulations, and all notices, directions and requisitions of the Grantor and any competent authority relating to the conduct of the Authorised Activity. Without limitation, this includes the Conservation Act 1987 and the Acts listed in the First Schedule of that Act and all applicable health and safety legislation and regulation.

8. Are there limitations on public access and closure?

8.1 The Authority Holder acknowledges that the public conservation land being part of the Land is open to the public for access and that the Grantor may close public access to that public conservation land during periods of high fire hazard or for reasons of public safety or emergency.

9. When can the Authority be terminated?

9.1 The Grantor may terminate this Authority at any time in respect of the whole or any part of the Land, and/or the whole or any part of the Authorised Activity if:

(a) the Authority Holder breaches any of the conditions of this Authority; or

(b) in the Grantor's opinion, the carrying out of the Authorised Activity causes or is likely to cause any unforeseen or unacceptable effects.

9.2 If the Grantor intends to terminate this Authority in whole or in part, the Grantor must give the Authority Holder such prior notice as, in the sole opinion of the Grantor, appears reasonable and necessary in the circumstances.

10. How are notices sent and when are they received?

10.1 Any notice to be given under this Authority by the Grantor is to be in writing and made by personal delivery, by pre paid post or email to the Authority Holder at the address, fax number or email address specified in Schedule 1, Item 5. Any such notice is to be deemed to have been received:

(a) in the case of personal delivery, on the date of delivery;

(b) in the case of post, on the 3rd working day after posting;

(c) in the case of email, on the date receipt of the email is acknowledged by the addressee by return email or otherwise in writing.

10.2 If the Authority Holder's details specified in Schedule 1, Item 5 change then the Authority Holder must notify the Grantor within 5 working days of such change.

11. What about the payment of costs?

11.1 The Authority Holder must pay the standard Department of Conservation charge-out rates for any staff time and mileage required to monitor compliance with this Authority and to investigate any alleged breaches of the terms and conditions of it.

12. Biosecurity

12.1 The Authority Holder must take all precautions to ensure weeds and non-target species are not introduced to the Land; this includes ensuring that all tyres, footwear, gaiters, packs and equipment used by the Authority Holder, its staff and clients are cleaned and checked for pests before entering the Land.

13. Are there any Special Conditions?

13.1 Special conditions are specified in Schedule 3. If there is a conflict between this Schedule 2 and the Special Conditions in Schedule 3, the Special Conditions will prevail.

14. Can the Authority be varied?

14.1 The Authority Holder may apply to the Grantor for variations to this Authority.

SCHEDULE 3

SPECIAL CONDITIONS

1. This Authorisation gives the Authority Holder the right to hold absolutely protected wildlife in accordance with the terms and conditions of the Authorisation, but the wildlife remains the property of the Crown. This includes any dead wildlife, live wildlife, any parts thereof, any eggs or progeny of the wildlife, genetic material and any replicated genetic material.

2. Unless expressly authorised by the Grantor in writing, the Authority Holder must not donate, sell or otherwise transfer to any third party any wildlife, material, including any genetic material, or any material propagated or cloned from such material, collected under this Authority.

3. The Authority Holder must comply with any reasonable request from the Grantor for access by the Grantor or the Grantor's nominee to any collected species or material.

4. This authority does not confer any right over any private land; or public conservation land leased by the Grantor (unless specified in the Authorised Activity). Any arrangements necessary for access over private land or leased land are the responsibility of the Authority Holder. In granting this authority the Grantor does not warrant that such access can be obtained.

5. All records of the Authority Activity shall be made available for inspection at reasonable times by officers of the Grantor.

6. Upon completion of the research or revocation of this Activity, the Authority Holder shall forward a copy of the research findings, reports and publications to the Grantor within one month of the final report being completed. The final report shall be forwarded electronically to permissionshamilton@doc.govt.nz citing Authority number 70875-RES. The Authority Holder acknowledges that the Grantor may provide copies of these findings to tangata whenua.

7. A new clause 9.1(c) is added to Schedule 2, to read as follows: "Or for any other reason that the Grantor may decide"

8. A Level 3 bander, certified under the New Zealand National Bird Banding Scheme, must oversee and be accountable for the Authorised Activity. Level 2 banders may operate without direct supervision but must operate under the general direction of a Level 3 Certified bander. Level 1 banders must be directly supervised by a Level 3 Certified bander.

9. The Authority Holder must undertake the Authorised Activity in accordance with the application received and the most recent edition of the New Zealand National Bird Scheme Bird Bander's Manual ("the Bird Banding Manual").

10. Any person can carry out this activity provided they hold a level 3 banding authority or are supervised by Isabel Castro (or person holding a level 3 banding authority).

11. Only metal bird bands supplied by the Department of Conservation, New Zealand are to be used, except where other marking techniques are authorised.

12. Any injuries or deaths of birds resulting from implementation of the Authorised Activity must be reported to the Grantor as soon as possible after the incident but at least within one week.

13. The Authority Holder must have approval of the Banding Office to trial new band sizes. Any changes to recommended band sizes must be notified in writing to the Banding Office as soon as practicable.

14. If a band is taken off a bird for any reason, it must not be used on another bird.

15. The Authority Holder must supply the Banding Office with electronic copies of all banding schedules used to record newly banded or re-banded birds, plus a band stock-take by 1 April each year. Standard electronic templates will be supplied by the Banding Office.

16. Band recoveries for dead birds and any birds re-banded are mandatory. Band recoveries must be submitted on the standard recovery format templates in electronic or paper form. Other recapture data can be submitted on these forms or on electronic spreadsheets.

17. All wildlife must be caught from day time roosts outside the breeding season by experienced Kiwi handlers.

18. All wildlife must be caught according to the Kiwi Best Practice Manual.

19. Blood samples may be taken from the wildlife.

20. Only persons registered with the Grantor as qualified to take blood samples must do so unsupervised.

21. All persons who are not registered with the Grantor as qualified to take blood samples must conduct blood sampling under the supervision of a qualified person.

22. Blood collection must be undertaken according to the methodologies set out in the Department of Conservation Avian Blood/Feather Sampling and Reptilian Tissue Collection Standard Operating Procedure, provided to the Authority Holder by the Grantor.

23. All blood samples must be taken from a vein in the animal's leg and shall be no greater than 0.5 millilitres in volume.

24. Blood samples must only be sent to Massey University, Palmerston North, for analysis and all analysis research is restricted to:

    a. analysing extracted DNA
    b. measuring:

i. genetic distance both for neural loci and genes under selection
ii. the level of intermixture and introgression of genes
iii. presence and abundance of genotypes
iv. length of telemores

25. For the avoidance of doubt, no blood collected under this Authorisation shall be used for or made available for any other research apart from that stipulated at Schedule 3.24 and research for any other purpose requires a separate Authorisation.

26. The Authority Holder must use best endeavours to ensure that the Authorised Activity is not undertaken within sight of the public.

27. While undertaking the Authorised Activity on public conservation land, the Authority Holder must not exclude or impede the public from accessing any sites, tracks or facilities.

28. If approached by members of the public while carrying out the Authorised Activity, the Authority Holder shall provide an explanation of why the Authorised Activity is taking place.

29. The Authority Holder must comply with all guidelines and notices issued by the Kauri Dieback Programme to prevent and avoid the spread of the pest organism Kauri Dieback Disease (Phytophthora taxon agathis) as specified on the website http://www.kauridieback.co.nz/. This includes ensuring that all vehicles, personal items and equipment are thoroughly cleaned of all visible soil and is sprayed with SteriGENE (formally known as Trigene) solution before entering and when moving between areas where there are kauri.

30. The Authority Holder and members of their team shall know the plants that are affected by myrtle rust, and what the rust symptoms look like. This serious fungal disease only affects plants in the Myrtle (Myrtaceae) family which includes pohutukawa, manuka, kanuka, and ramarama.
See http://www.mpi.govt.nz/protection-and-response/responding/alerts/myrtle-rust.

31. The Authority Holder and members of their team shall not park vehicles under myrtle species where vehicles can easily be contaminated while undertaking the Authorised Activity.

32. The Authority Holder shall carry large black plastic bags and ties, 2% SteriGENE spray bottle and Isopropanol wipes while undertaking the Authorised Activity on Public Conservation Land where Myrtaceae are part of the flora.

33. If the Authority Holder or any members of their team believe they have seen the symptoms of myrtle rust, they are not to touch the plant.

34. If the Authority Holder or members of their team believe they are in an infected area, all team members must decontaminate with SteriGENE as per below:

a. Call the MPI Exotic Pest and Disease Hotline immediately on 0800 80 99 66

b. If possible, take clear photographs, including the whole plant, the whole infected leaf, and a close-up of the spores/affected area of the plant

c. Do not touch or try to collect samples as this may increase the spread of the disease

d. Spray obviously contaminated clothing/hats and then place items in a large plastic bag

e. Tie and spray the outside of the bag

f. Mist spray other clothing being worn

g. Clean and spray all footwear and equipment, including packs, phones, glasses, watches etc

h. Repeat decontamination steps again at 100m from the infected area and before entering a vehicle

35. The Authority Holder and their team members shall have a hot shower and clean their hair as soon as possible to remove any spores (which may be invisible). Clothing worn while undertaking the Authorised Activity must be washed in a hot wash with detergent.

Special Condition specific to: Waipoua Forest

1. Te Roroa must be notified at least one week before any activity is undertaken in Waipoua.
   - Contact: General Manager – Snow Tane
   - Email: opm@teroroa.iwi.nz
   - Phone: 09 439 6443

Special Conditions specific to: Te Hauturu-o-Toi/Little Barrier Island

1. The Authority Holder must comply with all biosecurity and quarantining measures as advised by the Grantor.

2. The Authority Holder must obtain an entry permit a minimum of 20 working days prior to accessing the Land and provide with this a Work Plan, a Safety Plan and a summary of the relevant skills of each member of the team.

3. The Authority Holder shall make all reasonable endeavours to attend a cultural induction with Ngati Manuhiri (f.mckenzie@ngatimanuhiri.iwi.nz) prior to visiting the island.

4. Any commercial vessels to be taken to the island must hold a Pest-free Warrant and be on the biosecurity approved vessel list for the Land.

5. The Authority Holder must contact the Little Barrier Island ranger (021 399 3355 or littlebarrier@doc.govt.nz) to discuss their arrival to, and movements on the island (and to book the bunkhouse) and shall adhere to are directions given.

Special Conditions specific to: Kawau Island

1. The Authority Holder must travel to Kawau Island on a vessel which holds a valid wharf landing permit or wharf licence if using the wharf.

2. The Authority Holder must contact the island ranger (027 536 1072) at least one week prior to visiting the island and adhere to any directions given.

Department of Conservation
Te Papa Atawhai

File Ref: 70875-RES

04/11/2020

Massey University (National Holder)
Private Bag 11222
Tennent Drive
Palmerston North 4442
New Zealand

Dear Isabel Castro,

Re: WILDLIFE ACT AUTHORITY APPLICATION 70875-RES APPROVAL

I am pleased to advise you that your Wildlife Act Authority Variation Application has been approved. Please find the authority enclosed.

This document contains all the terms and conditions of your variation authorisation to undertake the activity and represents the formal approval from the Department for you to carry out the varied activity.

Please read the terms of your authorisation carefully so that you clearly understand your obligations.

Yours sincerely,

Grant Balsom
Permissions Advisor
Hamilton

Department of Conservation
Te Papa Atawhai

Variation to a Wildlife Act Authority under the Wildlife Act 1953

Authorisation Number: 70875-RES

THIS DEED OF VARIATION OF AN AUTHORITY is made this 2nd day of November 2020

PARTIES:

The Director General of Conservation, and where required, the Minister of Conservation (the Grantor)

AND

Massey University (National Holder) (the Authority Holder)

BACKGROUND

A. By an Authorisation dated the 17th day of April 2019 the Director-General of Conservation granted an Authority under the Wildlife Act 1953 to the Authority Holder upon the terms and conditions expressed and implied in the Authority.

B. By the same Authorisation, the Director-General of Conservation or the Minister of Conservation granted an Authority under the legislation applying to the public conservation land to the Authority Holder upon the terms and conditions expressed and implied in the Authority.

C. The Grantor hereby varies that Authority.

NOW BY THIS DEED the Grantor authorises as follows:

1. Variation

In exercise of the Grantor's powers under the Wildlife Act the Grantor varies the Authority as follows:

(i) Clause 1, Schedule 1 is deleted and replaced with the following wording:

a) Activity:
i. to catch alive North Island Brown Kiwi (*Apteryx mantelli*) for the purposes of species management and research.
ii. to mark North Island Brown Kiwi (*Apteryx mantelli*) for the purposes of distinguishing wildlife.

b) Species: North Island Brown Kiwi (*Apteryx mantelli*).

c) Quantity:
Ninety (90) in total for locations labelled a. – f. (inclusive) in Clause 2, Schedule 1, and;
A minimum of eight (8) and a maximum of twenty (20) for locations labelled g. – h. (inclusive) in Clause 2, Schedule 1.

d) Method:
i. catch alive by hand
ii. mark using metal bands

(ii) Clause 2, Schedule 1 is amended to include the following locations:
g. Purangi Scenic Reserve
h. Rotokare Scenic Reserve
as new locations that have been added to the Authority.

(iii) Clause 3, Schedule 1 is amended to include the following names:
d. Malin Undin
e. Karen Mason
f. Natasha Bansal
g. Monika Nowicki
h. Stephen Marsland
i. Lesley Baigent

2. Confirmation of other Authority Covenants

Except to the extent to which they are amended by this Variation the provisions expressed and implied in the Authority continue to apply.

3. Costs

The Authority Holder must pay the costs of and incidental to the preparation and completion of this Variation.

SIGNED on behalf of the Grantor by Daniel Heinrich, Director Operations, Hamilton acting under delegated authority

in the presence of:

Witness Signature

Witness Name: Penny Loomb

Witness Occupation: Personal Assistant

Witness Address: 73 Rostrevor Street, Hamilton

A copy of the Instrument of Delegation may be inspected at the Director-General of Conservation's office at 18-32 Manners Street, Wellington.

Appendix A6 - DRC 16 forms

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Undin, M., Hills, S. F. K., Lockhart, P. J., & Castro, I. (2021). Gaps in genetic knowledge affect conservation management of kiwi (Apteryx) species. Ibis doi: 10.1111/ibi.12951	
In which Chapter is the Manuscript /Published work:	Ch 1.2
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	85
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collated and analysed all the data, drafted the paper for submission, addressed the reviewers feedback, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.20 09:54:09 +12'00'
Date:	20/4/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.16 20:09:03 +12'00'
Date:	16/04/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

GRS Version 4– January 2019

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Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Undin, M., Lockhart, P. J., Hills, S. F. K., & Castro, I. (2021). Genetic rescue and the plight of Ponui hybrids. Frontiers in Conservation Science 1, 10.3389/fcosc.2020.622191	
In which Chapter is the Manuscript /Published work:	Ch 1.3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collated all the data, drafted the paper for submission, addressed the reviewers feedback, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.20 09:56:11 +12'00'
Date:	21/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.16 20:10:04 +12'00'
Date:	16/04/2021

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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Mixed mating in multi-origin population suggests high potential for genetic rescue in North Island brown kiwi, Apteryx mantelli	
In which Chapter is the Manuscript /Published work:	Ch 2.2
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collected and analysed all the data, drafted the paper for submission, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
Frontiers in Conservation Science	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.20 09:55:37 +12'00'
Date:	20/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.16 20:13:11 +12'00'
Date:	16/04/2021

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Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Access to resources, rather than kin-selection, is the likely driver of group breeding and shared incubation in Apteryx mantelli	
In which Chapter is the Manuscript /Published work:	2.3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	70
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collated and analysed the data, drafted the paper for submission, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
IBIS	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.21 10:22:20 +12'00'
Date:	21/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.21 10:12:45 +12'00'
Date:	21/04/2021

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Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
What is a kiwi hybrid?	
In which Chapter is the Manuscript /Published work:	Ch 3.1
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collected and analysed the data, drafted the paper for submission, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
Genes	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.21 10:21:24 +12'00'
Date:	21/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.21 10:07:33 +12'00'
Date:	21/04/2021

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MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

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Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Undin, M., Gedye, K. R., Hills, S. F. K., & Castro, I. The role of DNA integrity in opportunistic longitudinal telomere studies. Manuscript ID: ECE-2021-03-00530. Ecology and Evolution	
In which Chapter is the Manuscript /Published work:	Ch 4.2
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	85
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collected and analysed all the data, drafted the paper for submission, addressed the reviewers feedback, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.20 09:54:57 +12'00'
Date:	20/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.16 20:13:59 +12'00'
Date:	16/04/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

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DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

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Name of candidate:	Malin Undin
Name/title of Primary Supervisor:	Assoc. Prof. Isabel Castro
Name of Research Output and full reference:	
Day and night are not like night and day; capturing method found to not induce sampling bias in A. mantelli	
In which Chapter is the Manuscript /Published work:	Appendix 2
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	80
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
The candidate collected and analysed the data, drafted the paper for submission, and collaborated with co-authors.	
For manuscripts intended for publication please indicate target journal:	
New Zealand Journal of Ecology	
Candidate's Signature:	Malin Undin Digitally signed by Malin Undin Date: 2021.04.21 10:21:54 +12'00'
Date:	21/04/2021
Primary Supervisor's Signature:	Isabel Castro Digitally signed by Isabel Castro Date: 2021.04.21 10:08:24 +12'00'
Date:	21/04/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

Appendix A7 - Conference abstracts

NZVA Wildlife Society Annual Conference* in Nelson 27 – 29 November 2020. *Kiwi of one feather does not flock together*

Reinforcement translocations are increasingly utilised with the hope of achieving genetic rescue. Less desired results of such crossing between populations are genetic homogenisation or replacement. The rate of intermixture between original and introduced individuals is one factor determining translocation outcomes. Mate choice will be one of the key mechanisms controlling this rate. Here we study mate preference and its consequences over time in an *Apteryx mantelli* population founded by translocations from two arenas that are now recognised as belonging to two separate taxa. We found evidence for morphological size preference and for that re-mating often led to a better size match between male and female. Our genetic results suggest that kiwi of one feather does not flock together, but rather that mate choice results in pairing between individuals less related than expected by random. Despite this disassortative mating, 50 years has not been enough to homogenise this hybrid population. We suggest that these results are promising for the future utilisation of genetic rescue in kiwi conservation. However, we also suggest that reconsideration might be warranted around the restrictive policies around translocations in place for these iconic birds.

Wildbase Symposium in Palmerston North 3 of September 2020. *There is more than one way: the previously unappreciated flexibility in the mating system of North Island brown kiwi*

Over four decades ago, theoretical works started to challenge the prevailing view that long-term, stable monogamy dominates the bird world. Ever since, the list of species for which the mating system has been reclassified keeps increasing, not the least thanks to the rapid evolution of genetic methods enabling evaluation of parentage and relatedness. Within the kiwi genus *Apteryx*, tokoeka and rowi both practice shared incubation and partly breed in groups. The closely related North Island brown kiwi, however, has only been reported to breed in monogamous pairs with male only incubation, until now. Here we utilised data collected during 16 years on Ponui Island and found several alternative breeding unit compositions as well as at least four distinctive male breeding strategies: (1) multi-male groups with shared incubation, (2) multi-male groups with a single incubator, (3) female-female-male groups and (4) "traditional" monogamous pairs. Only one case was found where kin selection seemed a plausible driver for this group behaviour, instead we suggest that access to beneficial home range habitat could play a key role. Anthropogenic habitat alterations have severely affected kiwi population density and distribution. Thus, the fact that group breeding has only been reported from the uniquely dense population of Ponui Island could indicate that these alterations have in fact changed the breeding behaviour of North Island brown kiwi. This observation highlights the importance to consider that most studies of wild organisms are confined to populations experiencing habitat, population and selection conditions severely altered by human activities.

School of Agriculture and Environment Annual Symposium in Palmerston North 14-15 November 2019*. *Is Kiwi the Benjamin Button Bird?*

Telomere shortening (attrition) has been identified as one of nine 'hallmarks of aging' because (1) it is a natural part of aging, (2) increased rate of attrition accelerates aging and (3) experimental elongation of telomeres seemingly results in slowed aging. The fact that telomeres shorten with increasing age makes their length a potentially very informative biological marker. My study measured relative telomere length (RTL) using qPCR for Brown kiwi (*Apteryx mantelli*) samples collected from the same birds (n = 23) over a period of 14 years. Surprisingly, my results suggest ~ 5% yearly increase in telomere length. Does this mean Kiwi are the Benjamin Buttons of Birds, who grow younger over time? It is known that the enzyme telomerase has the ability to elongate telomeres and other studies have occasionally detected telomere length increase over time, however, the activity of telomerase is often restricted to stem cells and gonad tissues. This is believed to be a cancer prevention mechanism. When reviewing the rate of telomere loss for 24 other bird species, I confirmed that this is correlated to maximum lifespan. In addition, I found a correlation with body mass. Interestingly, based on body mass, *A. mantelli's* expected yearly telomere change is a loss of 0.8% or 60bp per year. However, based on maximum age, the expected value is 0-5% yearly telomere elongation. I will discuss what this means for *Apteryx*; what it can teach us about longitudinal telomere studies in general and what it suggests about cancer prevention in birds.

Palmy Bioinfo Monthly Meeting in Palmerston North 25 October 2019: *Whakapapa of Brown kiwi Part one, GBS data on the way, what do we do now?* **

Australasian Ornithological Conference in Darwin 3-5 July 2019: *Kiwi telomeres and evaluation of age, health and translocation success*

Assessing the conservation status of many long-lived bird species requires determining the age of adult individuals. One novel approach for measuring age involves examining telomere length and/or the rate of telomere attrition (shortening). A growing number of studies connect bird telomere length with ageing, survival, life expectancy and exposure to stress. It has been suggested that telomeres provide a more appropriate measure of "biological age" for population modelling than does actual (chronological) age, because telomere shortening appears closely correlated with survival and reproductive success. This talk will present preliminary findings on the first ever study of telomeres in the genus *Apteryx*. Previous studies of long-lived birds such as Leach's Storm Petrel (*Oceanodroma leucorhoa*), Thick-billed Murre (*Uria lomvia*) and Kakapo (*Strigops habroptilus*) have found no correlation between age and telomere length. However, these studies have been cross-sectional or longitudinal across a single year. Our work is unique in that it is longitudinal over many years (3-14) and is based on multiple (2-4) blood samples from over 30 wild, adult *A. mantelli*. With this setup we aim to evaluate the relationship between telomere length and ageing. Our next step will be to measure relative telomere length in captive birds of known chronological age. The third step will be to analyse telomere length distribution in wild *A. mantelli* populations, focusing on correlating telomere data to translocation history, population founder number, time and provenance, inbreeding and (loss of) genetic diversity. This comparison and the information it provides will hopefully generate a model protocol for how telomere length distribution can be used to help improve the (genomic) outcomes

of past as well as future translocations of *A. mantelli*. This model should also be useful for assessing the conservation status of other age-elusive birds facing a fragmented future.

Wildbase Symposium in Palmerston North 26 of June 2019: *Kiwi, age, telomeres and methylation*

Age and age-distribution are important markers of population viability, and thus important for wildlife monitoring and conservation. However, determining the age of adult individuals in long-lived species is a worldwide challenge. One such long-lived taxon is the Kiwi genus, *Apteryx*. The Kiwi Recovery Group has highlighted that solving the "age question" is one of the keys to long-term success of future *Apteryx* conservation. My PhD will investigate two possible biological age markers in *Apteryx*: telomere length and DNA methylation. This talk presents preliminary results of qPCR studies that I am undertaking to measure telomere length and the rate of telomere attrition (shortening) in *Apteryx mantelli*. It also outlines my plans for investigating genome wide methylation patterns in *Apteryx* and how these patterns differ between populations and how they change over time with age. A growing number of studies connect bird telomere length with ageing, survival, life expectancy and exposure to stress. However, longitudinal studies are few and some studies of long-lived species have produced ambiguous results. To date, studies of DNA methylation as an indicator of age have been conducted largely in humans and other mammals. However, a recent methylation study on Short-tailed shearwaters (*Ardenna tenuirostris*) and new methods for methylation profiling combining next generation sequencing (NGS) with restriction endonuclease enzyme cutting patterns provide encouragement for extending methylation studies to *Apteryx*. In my project, age markers (telomeres and methylation patterns) will be studied in three sets of samples: (1) a longitudinal set where the same birds (N≈30) have been sampled 2-4 times over 3-14 years - to study how the markers change over time; (2) a set of captive birds of known age - to see how the markers relate to chronological age; and (3) a set of wild birds from different populations - to correlate genetic patterns to factors including habitat, geographic and taxon origin, translocation history, inbreeding and genetic (genomic) diversity. This work forms part of the "kiwi whakapapa research program", whose overall goal is to develop guidelines for future *Apteryx* translocations.

My confirmation in Palmerston North 4 March 2019: *The Whakapapa of North Island Brown Kiwi*

The future of *Apteryx mantelli* conservation will almost inevitably include translocations. With the Whakapapa of Brown Kiwi project, myself, Angelia Hura and the rest of our team are determined to study the results of historic translocations in order to draw conclusions that allow us to model the potential success of future translocations. This is done in a project very closely integrated with tangata whenua who exercise kaitiakitanga over our *A. mantelli* sampling areas. My work focuses on what can be learnt about genetic diversity and the risks of outbreeding, by studying so called 'hybrid' populations, which is populations where historic translocations have resulted in admixture of birds that nowadays are classified as belonging to separate taxa. My second focus is on investigating whether telomere length is an informative measurement for translocation outcomes and population sustainability. My progress to date is that I am finishing off the writing of two literature reviews, working in the lab on measuring

telomeres, have started the field sampling of blood, have presented my project orally on several occasions and have successfully secured significant funding. This Confirmation Report includes the background to the project, the outline of my work with tangata whenua thus far, a plan listed chapter by chapter towards the completion of a PhD thesis, progress to date, a time line for the remaining of my PhD, and a risk analysis.

Annual New Zealand Phylogenomics Meeting in Napier 10-14 February 2018 during an extension catered towards teachers: *The Whakapapa of Apteryx mantelli – for a future with hapu lead conservation and genetically diverse Kiwi***

NZVA Wildlife Society Annual Conference 2018 in Dunedin the 23-25 of November 2018: *The Whakapapa of North Island Brown Kiwi (*Apteryx mantelli*) - genetics for sustainable translocations*

How do we as a conservation community ensure that Kiwi (*Apteryx* spp) populations are sustainable in the long term? This project seeks to answer this question by combining Genotype-by-Sequencing (GBS) data analyses, population modelling, local knowledge and Kaitiakitanga. We argue that maintenance of high genetic diversity is key for sustainability, since loss of diversity is strongly linked to reduced disease resistance, decreased fitness and lowered evolutionary potential – all major threats, especially in a changing world. The most widely used tool for maintaining, restoring, introducing and/or saving genetic diversity involves translocation of birds between localities. For translocations to be successful the decision of whether or not to move which birds where and when needs to be informed by knowledge of historical population inbreeding and outbreeding. How do we approach the trade-off between preserving distinctive population identity and increasing the risk of inbreeding depression? Can we successfully separate genetic erosion from local adaptation? Where is the point of balance between introducing genetic diversity through population intermixing and causing genetic homogenization through hybridization? Since the 1960s, people have repeatedly founded Brown Kiwi populations with small (but known) numbers of individuals of known provenance on predator-free offshore islands. We will conduct detailed GBS studies of such populations in Ipipiri (Eastern Bay of Islands) and the Hauriki gulf, and compare their genetic diversity to that of mainland founder populations. What can genomics tell us about the Whakapapa (genealogy) of these birds? An additional, and important aim of this project is to investigate telomere length as an indicator for the biological age of individual Kiwi - since telomere length is thought to be an indicator for not only age but also health and population sustainability. This project is spearheaded by the tangata whenua (people of the land) of Ipipiri who want to use this information to fulfil their Kaitiakitanga (guardianship) role for Brown Kiwi. Working with Northland hapu/iwi, our goal is to develop a model approach for assessing population sustainability and translocation outcomes for Brown Kiwi. We also see the potential for such a model in the management of other taonga species throughout New Zealand.

Wildbase Symposium in Palmerston North the 28 of June 2018: *Why do we need to know more about Kiwi genetics?*

Research on Kiwi (*Apteryx* spp.) took a large step forwards when genetics entered the scene some 30 years ago. Thanks to this we are now able to sex juveniles and we know that not all *Apteryx* are strictly monogamous. Perhaps most strikingly Rowi has been identified as its own species, and the Elephant bird as the closest relative

to Apteryx. Despite 34 published papers based on samples from around 2000 birds, we argue that we still don't know enough about Kiwi genetics. DNA technology is evolving fast, enabling identification of more subtle genetic differences, generating inflation in the number of distinct taxa identified – currently we are at eleven taxa and five species within the *Apteryx* genus. However, the analysis methods and the sample resolution used to date prevent us from saying anything about the nature of the genetic differences between and within these taxa. This makes the current knowledge insufficient for informed decision making in an area where this field has huge potential: translocations. Translocations has been part of *Apteryx* conservation for a long time, first as emergency moves of threatened birds to predator free island and later mainly of eggs and chicks to safe crèches and later back to the wild. Modern genotype-by-sequencing (GBS) methods can help develop models for more long-term sustainable translocations that retain genetic diversity and evolutionary potential while minimizing inbreeding and out breeding by identifying suitable source populations and populations in need of genetic rescue; potentially even individual birds suitable (or unsuitable) for translocation.

Birds New Zealand Annual Conference in Waitangi 2-4 of June 2018: *What do we really know about Kiwi genetics?*

Research on Kiwi (*Apteryx* spp.) took a large step forwards when genetics entered the scene 30 years ago. Thanks to this we are now able to sex juveniles, we know that not all *Apteryx* species are strictly monogamous and most strikingly Rowi has been identified as its own species. DNA technology is evolving fast, enabling identification of more subtle genetic differences, generating inflation in the number of distinct taxa identified. Genetics will have a crucial role in *Apteryx* conservation, however here we want to highlight issues that impact findings: (i) how, where and when *Apteryx* spp. are sampled, (ii) how results are compared, (iii) what detecting genetic differences really mean, and (iv) how to best interpret genetic information. One of the main concerns is making decisions about translocations. Modern genetics can aid in identifying suitable source populations and populations in need of genetic rescue; potentially even in identifying individual birds suitable for translocation or birds that carry unwanted genes or pathogens. For informed decision making there is a need to (a) quantify genetic diversity, inbreeding and its consequences on a finer geographical scale and (b) increase our understanding of the underlying causes of genetic differences among populations. Until we have this information it will be impossible to predict the outcome of any *Apteryx* translocations. Because of this we stress the need for patience; mistakes we commit today can have irreversible effects, and long lived species like *Apteryx* spp. have time to wait for us to do this right.

*Where I was awarded a certificate for best oral presentation

** No abstract