TE KUNENGA KI PŪREHUROA | MASSEY UNIVERSITY

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Mate choice and pair fidelity in a translocated population of North Island brown kiwi

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To Leo,

whose constant encouragement turned challenges into milestones

Thesis abstract

Aotearoa New Zealand is renowned for its high levels of endemism, yet it has also suffered profound species declines and extinctions following human settlement and the arrival of invasive species. Many species that once dominated New Zealand's landscape are now confined to small, isolated populations with limited gene flow. Conservation translocations (the intentional movement and release of an organism inside its indigenous range from which it has disappeared) of kiwi (*Apteryx* spp.) are among the most common and increasingly important practices in New Zealand kiwi recovery programs. The success of translocations depends on animals securing food and shelter, avoiding predators, and, critically, finding mates to reproduce. Consequently, post-release mate choice, pair fidelity, and divorce are key considerations, yet the roles of behavioural, genetic, and morphological traits in shaping these processes in translocated kiwi populations remain unclear. My thesis addresses this gap using a dense, translocated population of North Island (NI) brown kiwi (*Apteryx mantelli*) on Ponui Island as a study system. For this, I combine long-term behavioural monitoring, morphometric analyses, next-generation sequencing of MHC genes and Bayesian statistical modelling. My major findings indicate that: (1) fidelity probability increased with male incubation effort, such that the more days a male incubated, the greater the likelihood of the pair remaining together; (2) shared incubators in polygamous groups tend to invest less in nest attendance than single incubators, suggesting benefits linked to nest relief; (3) NI brown kiwi exhibits the highest allelic diversity and sequence polymorphism at MHC class IIβ yet reported for any ratite (4) The high MHC class IIβ in kiwi on Ponui Island likely reflect its mixed ancestry; (5) MHC IIβ exon 2 features did not appear to influence the formation of social pairings, yet males with higher MHC diversity showed a weak trend toward greater pair retention; (6) Morphological traits showed no evidence of assortative mating, and weak size-based correlations were largely explained by residual covariance rather than true mate choice. Collectively, this body of research offers valuable insights into how behavioural, genetic, and morphological factors interact to shape reproductive strategies in reintroduced populations, providing an empirical foundation for managing genetic health and pair dynamics in conservation translocations.

Keywords

Aotearoa New Zealand, *Apteryx mantelli*, breeding system, conservation translocation, divorce, inbreeding depression, incubation effort, kiwi, major histocompatibility complex, mate choice, mating strategy, monogamy, outbreeding depression, pair bond status, pair familiarity, phenotypic features, polygamy, Ponui Island.

Declaration by author

The research carried out for my doctoral thesis has been used in whole or in part for this qualification only. The research is my original work, except as indicated by appropriate attribution in the text and/or acknowledgements; quotation marks have been used where required; and I take responsibility for the content and quality of this thesis. The "Statement of Contribution Doctorate with Publications/Manuscripts", has been completed for each research chapter within the thesis, and is included at the start of each chapter.

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Publications during candidature

Article submitted for publication in peer-reviewed journals, included in this thesis:

Ramos, E., Gibb, G., & Castro, I. Pair bond status is influenced by male incubation effort in a translocated population of North Island brown kiwi. *Animal Behaviour*, accepted.

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Coastal forest–grassland mosaic on Ponui Island (Hauraki Gulf, Aotearoa New Zealand North Island), home to a translocated population of North Island brown kiwi (*Apteryx mantelli*).

Chapter One

"And this leads me to say a few words on what I call Sexual Selection. This depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring."

(Darwin, 1859, p. 87)

Thesis introduction: Breeding systems and mating decisions in kiwi conservation translocations

Conservation translocation in kiwi

Humans have become a prominent evolutionary force driving elevated extinction rates (Dirzo et al., 2014; Ceballos et al., 2015). Among vertebrates, extinction rates over the past century have risen to 100 times preindustrial levels (Ceballos et al., 2015), while global wildlife populations have declined by an average of 73% in the last 50 years (WWF, 2024). Conservation translocation—human-mediated movement of organisms between sites for conservation benefit—has become an essential tool to mitigate the loss and depletion of imperilled species and populations (Seddon et al., 2014; Hoffmann et al., 2015; Berger-Tal et al., 2020). The first documented conservation translocation occurred in 1895, when kākāpō (*Strigops habroptilus*) were released onto offshore islands in New Zealand (Lloyd and Powlesland, 1994) to safeguard surviving individuals and establish insurance populations (Morris et al., 2021). Since then, translocations have emerged as a cornerstone of conservation practice worldwide, with the number of species translocated increasing substantially in the past 20 years (Seddon and Redford, 2025). Translocations have underpinned some of conservation's most celebrated successes—such as rescuing the Arabian oryx (*Oryx leucoryx*) and the California condor (*Gymnogyps californianus*) from the brink of extinction—yet they are also marked by a long record of less celebrated failures (reviewed in Morris et al., 2021).

Kiwi (*Apteryx* spp.) are flightless ratites (Palaeognathae) endemic to New Zealand whose numbers and distributions have declined following human arrival (McLennan et al., 1996; Shepherd et al., 2012; Shepherd et al., 2021). Currently, five kiwi species are recognised, which diverged from one another between 4 and 13 million years ago, depending on the study (Weir et al., 2016; Grealy et al., 2017; Yonezawa et al., 2017). Kiwi species fall into two phenotypic groups based on plumage differences (Tennyson et al., 2003; Shepherd et al., 2021): the brown coloured kiwi, comprising North Island (NI) brown kiwi or kiwinui (*Apteryx mantelli*), rowi (*Apteryx rowi*), and tokoeka (*Apteryx australis*); and the spotted kiwi, comprising little spotted kiwi or pukupuku (*Apteryx owenii*) and great spotted kiwi or roroa (*Apteryx haastii*). Before human arrival, these iconic birds were widespread across New Zealand, but are now restricted to small, isolated populations that depend on intensive conservation management for their persistence (Innes et al., 2015; Germano et al., 2018b; Jahn et al., 2022). This is reflected in their conservation with four species classified as 'vulnerable' and the little spotted

kiwi as ‘near threatened’, the latter largely supported by strongholds on managed offshore islands (IUCN, 2025).

The management of kiwi over the past century illustrates the evolution of conservation translocation in New Zealand, shifting from early ‘trial-and-error’ efforts with little or no planning to recent programmes guided by rigorous planning, science inputs, and advances in predator control (Jahn et al., 2022). Interestingly, while early mainland translocations were largely unsuccessful, with most birds dying or dispersing (Miskelly and Powlesland, 2013; Jahn et al., 2022), many island populations established through early translocations have persisted to the present (Colbourne and Robertson, 2000; Jahn et al., 2022). This contrast raises a central question: why did some poorly planned early introductions apparently succeed in establishing self-sustaining populations, while others failed? Unfortunately, the scarcity of published information on kiwi translocations and their outcomes prevents us from fully addressing this question, thus limiting our ability to evaluate effectiveness and refine the role of translocations in kiwi recovery management (Jahn et al., 2022). Predicting translocation outcomes is particularly challenging due to the complex interactions between genotype, phenotype, behaviour, and environment (Undin et al., 2021b). These factors add complexity to translocations, especially when mixing organisms from genetically distinct populations, raising concerns about the conservation value and fate of such human-settled populations (Undin et al., 2021c).

Kiwi are the most frequently translocated bird species in New Zealand (Cromarty and Alderson, 2013; Miskelly and Powlesland, 2013), and with the number of new projects steadily increasing (Jahn et al., 2022), it is essential to identify the factors that distinguish successful from failed attempts to improve outcomes. This is especially true for the NI brown kiwi, the most frequently translocated kiwi species, yet the one with the least impact on improving conservation status (Jahn et al., 2022). Extant NI brown kiwi populations remain small and isolated, raising concerns of potential inbreeding depression (Undin et al., 2021b). Small populations with eroded genetic diversity are unlikely to possess the genotypic scope to respond to natural selection under rapid environmental change (Bowler et al., 2020). Hence, contemporary kiwi translocations have brought genetic management to the table to maximise their success (Miller et al., 2011; Undin et al., 2021b; Jahn et al., 2022). The breeding system and mating decisions

of a species also play an important role (Quader, 2005; Bradley et al., 2014; Kretzschmar et al., 2020), and these factors can directly affect the demography and genetics of translocated kiwi populations (Undin and Castro, 2022). It is therefore surprising that these factors remain largely neglected in kiwi conservation and management (Undin and Castro, 2022).

Leveraging breeding behaviour in kiwi translocations

Translocations can alter key factors shaping breeding systems and mating strategies by placing individuals into novel selective contexts, where changes in resource distribution, demographic structure, and social environments may shift reproductive tactics (Pribil and Searcy, 2001; Quader, 2005; Griffith et al., 2010; Undin and Castro, 2022). Breeding systems are highly sensitive to the spatial and temporal distribution of resources and mates (Emlen and Oring, 1977; Undin and Castro, 2022), and empirical work has shown that socially monogamous birds can shift to polygamy under conditions of habitat saturation (e.g., Carrete et al., 2006a). Shifts in sex ratios may favour polygyny, either after mate loss or when populations contain an excess of breeding females (e.g., Smith et al., 1982; Schlicht and Kempenaers, 2021). In small or low-density translocated populations (including those established by few founders), mate shortages may limit opportunities to sample optimal partners before making mating decisions (Quader, 2005). In such cases, divorce or infidelity can act as mechanisms to replace suboptimal mates and boost reproductive success (Choudhury, 1995; Culina et al., 2015). Alterations in mate choice following translocation, particularly shifts in assortative mating, are critical to consider because they can reshape pairing dynamics and influence both reproductive outcomes and genetic structure (Bradley et al., 2014; Engler et al., 2019; Undin et al., 2021b). This is especially relevant when mixing individuals from multiple sources that may differ in reproductive behaviour and genetic makeup (Bradley et al., 2014; Undin and Castro, 2022).

The potential effects of translocations on breeding systems and mating strategies are multifarious, and a full review is beyond the scope of this introduction, as they have been addressed elsewhere (see Quader, 2005; Undin and Castro, 2022; Miller et al., 2024). Most importantly, any potential change in breeding behaviour remains critical for

conservation translocation, since such changes can shape individual reproductive success, genetic admixture, population growth, and ultimately population viability (Anthony and Blumstein, 2000; Quader, 2005; Bradley et al., 2014; Koenig et al., 2019; Undin and Castro, 2022). Critically, conservation translocation often involves moving specific sex and age classes from wild populations, with the potential to disrupt breeding systems and mating strategies in both source and recipient populations (Komdeur et al., 1997; Quader, 2005), further complicating the already difficult task of predicting outcomes. When embedded in translocation planning, interventions targeting breeding behaviours to maximise reproductive output can substantially improve success. Yet the knowledge base required to design such interventions remains limited for most species (Kretzschmar et al., 2020). Therefore, improving knowledge of mating and reproductive behaviour is essential for the effective conservation of species whose populations depend on active management to increase numbers (e.g., Quader, 2005; Bradley et al., 2014; Kretzschmar et al., 2020), such as kiwi (Undin and Castro, 2022).

Kiwi are cryptic and nocturnal birds that nest in subterranean or tree cavities and abandon incubation when disturbed (Taylor et al., 2014), which poses challenges for studying their breeding ecology. Leg-fitted radio transmitters equipped with an algorithm that can register and interpret activity patterns (wildtech.co.nz/Kiwi.aspx) have been crucial in overcoming these challenges (Colbourne, 2002; Taylor et al., 2014; Colbourne et al., 2020; Undin and Castro, 2022). Among the five extant kiwi species, NI brown kiwi has been highlighted as a promising study system for advancing knowledge of breeding behaviour and integrating it into conservation management, owing to (1) its wide range of population sizes and densities, (2) a well-documented history of translocations with known founder sizes, source populations, and establishment times, and (3) established methods and infrastructure for pre- and post-release monitoring (Undin and Castro, 2022). Moreover, this is the most common and best-studied kiwi species, and some aspects of its breeding behaviour have been investigated (e.g., McLennan, 1988; Taborsky and Taborsky, 1999; Colbourne, 2002; Ziesemann et al., 2011; Vieco-Gálvez, 2019; Undin, 2021; Undin et al., 2021a), although many remain poorly understood. Successful translocation outcomes rely on kiwi being able to secure mates to reproduce (see Bell, 2016; Greggor et al., 2025). Consequently, post-release mate choice, pair fidelity, and divorce represent key factors to evaluate and form the central focus of this thesis.

A translocated kiwi population on Ponui Island as a study system

Aotearoa New Zealand is renowned for its high levels of endemism, yet it has also suffered profound species declines and extinctions since human settlement and the introduction of invasive species (Gibbs, 2006). Most kiwi translocations have established populations beyond their historical ranges, particularly on offshore islands and other mainland areas (Jahn et al., 2022). This is the case of NI brown kiwi (*Apteryx mantelli*) on Ponui Island (1,770 ha), a population settled in 1964 through two translocations of eight founder birds from Waipoua Forest and six from Hauturu-o-Toi (Little Barrier) Island (Colbourne, 2005; Craig et al., 2011; Undin et al., 2021b). While anecdotal evidence suggests an uncertain or hybrid origin for kiwi on Hauturu-o-Toi (Baker et al., 1995; Burbidge et al., 2003), genetic data indicate a single Taranaki origin (Undin, 2021). Given its translocation history, kiwi on Ponui Island are deemed as hybrids (Figure 1.1a), resulting from admixture between two geographically separated and genetically distinct management units: Northland and Western (Craig et al., 2011; Scrimgeour and Pickett, 2011; Germano et al., 2018a; Undin et al., 2021b).

The population size of kiwi on Ponui Island remains uncertain, yet rough numbers suggest a high density of about one bird per hectare, despite predation by feral cats (*Catus domesticus*; Castro and Cunningham, pers. obs.). This estimate is based on more than two decades of monitoring kiwi in forested gullies encompassing ~200 ha of mixed habitat (native forest, wetlands, and pasture) that is broadly representative of the Ponui Island landscape (Miles and Castro, 2000; Figure 1.1b). However, beyond this estimate, the sex ratio and the number of breeding males and females within the study area remain unknown. Territoriality in NI brown kiwi varies among populations, ranging from little or no territorial defence and widely overlapping home ranges to aggressive defence of territory boundaries (Potter, 1990; McLennan & Potter, 1992; Taborsky & Taborsky, 1999; Ziesemann, 2011). This variation appears to reflect, at least in part, differences in population numbers, with birds at low densities occupying larger, less overlapping home ranges and exhibiting stronger territoriality than those at high densities (McLennan et al., 1987; McLennan, 1988; Potter, 1989; Taborsky & Taborsky, 1992). Consistent with this, kiwi on Ponui appear to have relatively small, overlapping home ranges during both breeding and non-breeding seasons (Ziesemann, 2011), with breeding usually occurring from June to January (Colbourne, 2002; Ziesemann et al., 2011).

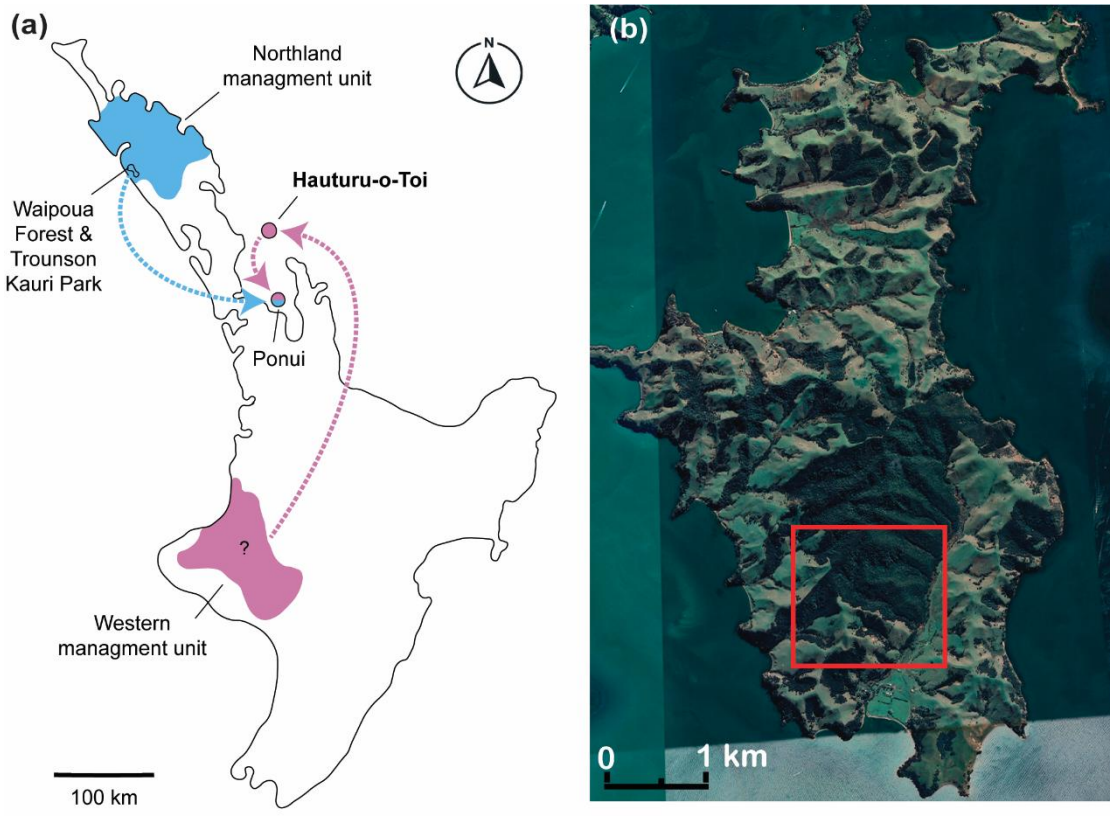

Figure 1.1. Map of North Island (New Zealand) showing (a) the translocation history of North Island brown kiwi on Ponui Island (redrawn from Undin et al. 2021a), and (b) the gullies where monitored birds occur (red box, image from Google Earth). Ponui Island's source populations, and the origin of Hauturu-o-Toi population are indicated with arrows. Translocation from the Western management unit (also known as Taranaki) to Hauturu-o-Toi Island is shown with a question mark to highlight the disputed origin of the kiwi population on that island (Baker et al., 1995; Burbidge et al., 2003; Colbourne, 2005).

The apparently high density implies a growth rate comparable to that of populations in predator-free sanctuaries or in areas where juvenile mortality is reduced through egg or chick rescue programs (Undin, 2021). Rapid population growth on Ponui Island over just 4–6 kiwi generations has raised concerns about inbreeding depression, given the small founder population (14 birds), and outbreeding depression from mixing birds adapted to different local conditions (Undin, 2021; Undin et al., 2021b; Undin et al., 2021c) originating from source units that could have diverged 100–220 thousand years ago (kya; Weir et al., 2016). Recent genomic SNP comparisons suggest that kiwi on Ponui Island constitute a successful hybrid swarm with high genetic diversity. Additionally, there is significantly lower-than-expected relatedness between pairing birds, indicative of kin recognition to reduce inbreeding and increase diversity (Undin et al., 2021b). The mechanisms underlying such kin-recognition ability are intriguing and may involve

detecting compatibility through behaviour, phenotype, or genotype to ensure offspring fitness (Kopp et al., 2018; Undin et al., 2021b).

Scope of the dissertation

The success of translocations depends on animals being able to secure food and shelter, avoid predators, and, most importantly, find mates to reproduce (Bell et al., 2019; Greggor et al., 2025). Therefore, post-release mate choice, pair fidelity and divorce are key aspects to consider. How behavioural, genetic, and morphological traits shape mating decisions in translocated kiwi populations remains unclear. In this thesis, I address this question using the Ponui Island kiwi population as a study system. The translocation of NI brown kiwi to Ponui Island in 1964 unintentionally established a hybrid population that has since thrived in its island refuge (Undin et al., 2021a). The long-term and sustained monitoring of kiwi on Ponui Island, coupled with intensive research efforts, has generated an extensive body of behavioural, genetic, and morphological data. This wealth of information makes kiwi on Ponui Island an exceptional system for investigating mate choice and pair fidelity following translocation into a novel environment. Understanding the determinants of mating and breeding behaviour is crucial not only for assessing the potential benefits of long-term socially monogamous partnerships, but also for evaluating how translocation influences breeding systems and mating strategies.

The overarching goal of this work is to advance our understanding of the behavioural, genetic, and morphological determinants of mate choice, pair fidelity, and divorce in translocated kiwi populations, using *Apteryx mantelli* on Ponui Island as a study system. This thesis is written as a collection of publications and is divided into six chapters. This introduction (Chapter One) briefly elaborates the theoretical framework from which my research derives and presents the scope and outline of the doctoral thesis. Four research chapters (Chapters Two to Five) compose the main body of this thesis and are written as independent publishable research articles. Chapter Two was published in the Q1 Journal *Animal Behaviour,* and Chapters Three, Four and Five are intended to be submitted for publication in scientific journals. As papers are co-authored, I use “we” and “our” in these chapters to be inclusive of co-authors (see “Declaration by Author” on page iv).

My proportional contribution is indicated in the Statement of Contribution form at the beginning of each chapter. The final chapter (Chapter Six) summarises the major findings of this research, placing them in a broader evolutionary and conservation context, and outlines promising avenues for future investigation.

Thesis Outline

Translocations may influence key factors shaping breeding systems and mating strategies, such as population density, resource availability, and mate access (Komdeur et al., 1995; Pribil and Searcy, 2001; Carrete et al., 2006a; Undin and Castro, 2022). Kiwi birds often form socially monogamous pairs that endure across multiple breeding seasons (McLennan, 1988; Taborsky and Taborsky, 1999), yet recent reports from NI brown kiwi on Ponui Island suggest a more flexible strategy with the potential for polygamy with non-kin co-breeders (Undin and Castro, 2022). Divorce may also help to avoid inbreeding by fixing initial 'errors' in mate choice that result in incestuous pairings (Speelman et al., 2024), and this is particularly relevant for translocated kiwi populations founded by a small number of birds. My second chapter used long-term monitoring of kiwi on Ponui Island to describe breeding systems and investigate the factors influencing partner fidelity and divorce. This chapter aims to (1) investigate whether reproductive, social, and mate quality factors influenced the occurrence of divorce, (2) assess the benefits of pair familiarity on reproductive performance, and (3) explore the benefits of forming polyandrous breeding units. Understanding the determinants of breeding and mating behaviour is not only important for exploring the possible benefits that both males and females gain from establishing and maintaining socially monogamous partnerships, but also for predicting outcomes of translocation (Anthony and Blumstein, 2000; Sigg et al., 2005; Renan et al., 2018; Undin and Castro, 2022).

The major histocompatibility complex (MHC) is a highly polymorphic, gene-dense region in vertebrate genomes that encodes cell-surface molecules essential for adaptive immune responses (Klein, 1986; Eizaguirre and Lenz, 2010; Strandh et al., 2012; Santos et al., 2016). Recent studies suggest that sexual selection via mate choice may contribute to MHC diversity (Knafler et al., 2012; Strandh et al., 2012), a consideration particularly relevant for translocated kiwi populations where inbreeding risk is high and/or genetic

kin recognition may be required in the absence of social cues to avoid inbreeding (Undin et al., 2021b). My third chapter examined the MHC IIβ structure and diversity of NI brown kiwi on Ponui Island and its two parental units using next-generation sequencing (NGS). This chapter aims to (1) quantify MHC IIβ diversity in kiwi on Ponui Island and compare it with its source populations, (2) assess the relative contribution of each parental MHC genome to the genetic makeup of the hybrid Ponui Island population, and (3) investigate the role of selection in shaping MHC diversity in the studied populations. Understanding the structure, diversity, and selection signatures at functional loci of adaptive significance, such as the MHC, is essential for informing kiwi translocations. This knowledge is crucial for assessing adaptive potential, disease resistance, and long-term population viability (Ramstad and Dunning, 2021).

Mate choice is particularly important in long-lived species with biparental care, such as kiwi that form long-term breeding bonds (Undin et al., 2021b). Genes of the MHC are considered a key target of mate choice, as they may confer immunological advantages to offspring by enhancing pathogen recognition and defence (Landry et al., 2001; Mays and Hill, 2004; Setchell and Huchard, 2010). Mate choice mediated by MHC genes is expected in long-lived bird species with well-developed olfactory capabilities (Zelano and Edwards, 2002), including kiwi (Undin et al., 2021b). This mechanism may also be relevant to translocated populations, where founder effects and limited mate availability can disrupt natural mate-choice patterns (Ramstad et al., 2013; Undin, 2021). Moreover, the potential role of MHC in alternative mating strategies, such as divorce, has received little attention. In my fourth chapter, I investigate whether MHC IIβ exon 2 variation influences mate choice and pair-bond stability in the kiwi population on Ponui Island. This chapter examines whether kiwi prefer mates with (1) greater MHC diversity, (2) greater or optimal MHC dissimilarity, and (3) whether pair stability depends on MHC compatibility between partners. Understanding whether MHC variation underlies mate choice or divorce can provide key insights into the genetic and behavioural mechanisms that maintain diversity and reproductive success in translocated kiwi populations.

Assortative mating refers to the process in which ‘like mates with like’, expressed as a positive correlation between male and female phenotypes in mated pairs (Jiang et al., 2013; Rueger et al., 2016; Wang et al., 2019). While this correlation is usually attributed to non-random mate choice based on phenotypic similarity, it may also arise from shared

environmental effects creating within-pair residual covariance that causes ‘mates to become alike’ (Class et al., 2017). In socially monogamous species with slow breeding and low reproductive success, such as kiwi, mate compatibility is critical, and phenotype-based assortative mating may evolve to facilitate optimal partnerships (Rueger et al., 2016; Kvarnemo, 2018; Undin et al., 2021b). This mechanism is also thought to play a key role during secondary contact by promoting reproductive isolation of populations that have diverged in allopatry (Servedio, 2016; Janicke et al., 2019). In my fifth, chapter I use repeated morphological measurements from kiwi pairs collected over two decades and combine correlative approaches with mixed-effects models to assess the role of assortative mating in kiwi on Ponui Island, following human-mediated secondary contact between two allopatric populations that differ in body size (Northland and Taranaki). This chapter investigates whether (1) measurement repeatability and (2) inter-operator effects differ between labile and fixed traits; and (3) whether assortative mating occurs in kiwi on Ponui Island, and if so, the mechanisms underlying it. Uncovering intrinsic assortative mating correlations (i.e., ‘like mates with like’), or lack thereof, is challenging but essential for making reliable inferences about post-translocation mating patterns and their evolutionary implications for species of conservation concern.

As argued across these chapters, the major findings of this thesis are not limited to the kiwi study population (or kiwi, for that matter), but may have broader implications for understanding how translocations influence breeding systems, mate choice, and mating strategies in wildlife populations more generally. Because kiwi translocations often involve removing individuals from natural populations (Jahn et al., 2022), demographic and genetic consequences may also arise within the source populations themselves. Understanding mating systems and mate choice could therefore provide important practical information for conservation management. For example, selective removal of particular sexes, age classes, socially dominant individuals, or highly preferred mates could alter operational sex ratios, disrupt pair bonds, reduce breeding opportunities, or influence reproductive success in both source and receiving populations. Likewise, translocations that fail to consider mate compatibility, familiarity, behavioural syndromes, or mating preferences may reduce pair formation, increase stress or dispersal, delay breeding, or lower reproductive output after release. This may be particularly important if mate choice is associated with genetic compatibility, such as selection linked

to Major Histocompatibility Complex (MHC) diversity, as suggested in kiwi and other vertebrates. Ignoring these mechanisms could inadvertently reduce adaptive genetic variation or disrupt naturally occurring mate-selection processes associated with immune function and offspring fitness. Incorporating behavioural, social, and genetic aspects of mating systems into translocation planning could therefore improve establishment success, genetic diversity retention, social stability, disease resilience, and long-term population viability, while simultaneously reducing unintended impacts on the populations from which individuals are sourced.

Understanding how reproductive behaviour operates within translocated kiwi populations is fundamental for interpreting both social stability and the evolutionary processes shaping reproductive success. Given the importance of pair formation, pair retention, and incubation behaviour in kiwi reproductive ecology, the first chapter of this thesis examines behavioural patterns associated with pair bonds and reproductive investment in the Ponui Island population.

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A wild North Island brown kiwi (*Apteryx mantelli*) chick on Ponui Island (Hauraki Gulf, Aotearoa New Zealand North Island).

Chapter Two

Pair bond status is influenced by male incubation effort in a translocated population of North Island brown kiwi

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Eliana Ramos Pallares
Name and title of main supervisor:	Isabel Castro
In which chapter is the manuscript/published work?	Chapter 2

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Data curation, formal analysis, and visualization were conducted by Eliana Ramos with assistance from Isabel Castro and Gillian Gibb. Conceptualization was primarily undertaken by Eliana Ramos and Isabel Castro, with additional contributions provided by Gillian Gibb. Writing the original draft, as well as reviewing and editing, was led by Eliana Ramos and supported by Isabel Castro and Gillian Gibb. All authors read and approved the final manuscript.

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Abstract

Long-lived bird species often form socially monogamous pairs that endure across multiple breeding seasons, and the establishment and maintenance of such long-term bonds can provide reproductive benefits, thereby enhancing overall success. Here, we used long-term monitoring data from a translocated, high-density population of North Island brown kiwi (*Apteryx mantelli*) to describe patterns of divorce and partner fidelity. This flightless species breeds primarily as long-term socially monogamous pairs and secondarily as non-kin polygamous units, both with male-only incubation. Some of these pairs and groups divorce, and we used Bayesian statistical models to investigate whether reproductive, social, and mate quality factors influenced the occurrence of divorce and explored the relationship between pair familiarity and reproductive success. We also explored the benefits of forming polyandrous breeding units by testing for differences in daily incubation effort between males sharing incubation in breeding groups against males incubating alone in pairs. We found a low average annual divorce rate for this brown kiwi population. Fidelity probability increased with increasing incubation effort, indicating that the more days a male incubated, the higher the likelihood of staying together. Polygamous breeding units consisted of single or multiple males that may help (shared incubation) or not help incubating (single incubation). We found a tendency for shared incubators to invest less in nest attendance than single incubators, suggesting benefits linked to nest relief. We suggest that mating flexibility expressed as divorce represents a secondary strategy to compensate for suboptimal partnerships and boost reproductive outputs. The inter-annual variability in incubation investment provides a fruitful field for investigating external covariates of divorce such as demographic trends and environmental factors. Unlike most studies on divorce and mating systems, we provide rare insights into the social and reproductive behaviours of a bird with cryptic reproductive habits. Our findings have potential implications for kiwi conservation.

Keywords: divorce, incubation effort, *Apteryx mantelli*, pair bond status, pair familiarity, non-kin polygamy

Introduction

Social interactions are essential for integration and cooperation, and often lead to communal benefits such as reduced predation and increased resource acquisition, but can also have fitness consequences (Silk et al., 2003; Archie et al., 2014; Sánchez-Macouzet et al., 2014; Griffith, 2019; Maldonado-Chaparro et al., 2021). For example, establishing and maintaining social bonds between reproductive pairs has been linked to benefits in reproductive components such as fecundity, breeding success, and offspring survival (Fowler, 1995; Silk et al., 2003, Silk, 2007; Archie et al., 2014; Culina et al., 2020; Maldonado-Chaparro et al., 2021). Birds provide major examples of this reproductive behaviour with up to 80% of species forming socially monogamous pair bonds (Cockburn, 2006; Sánchez-Macouzet et al., 2014; Crino et al., 2017). In such pairings, two birds of the opposite sex establish an exclusive and secure relationship, also known as a pair bond (Fowler, 1995; Bales et al., 2021). These relationships can ensure successful reproduction and reap fitness benefits (Forslund & Larsson, 1991; Wiley & Ridley, 2018; Maldonado-Chaparro et al., 2021). Socially monogamous birds, however, display varying degrees of mate retention or partner fidelity over consecutive breeding attempts (Black, 1996; Griffith, 2019), with some pairs establishing long-term partnerships and others favouring single-mating relationships (Ens et al., 1996; Sánchez-Macouzet et al., 2014; Culina et al., 2015; Crino et al., 2017; Maldonado-Chaparro et al., 2021).

Although bond duration may logically be interrupted by the death of one partner (widowing), pair-bonded birds often face the choice of staying with their mate or separating (getting divorced) to form a new bond (Choudhury, 1995; Mercier et al., 2021). Divorce is considered a key aspect of the mating system in socially monogamous bird species with long-lasting relationships, as both staying with a suboptimal partner or divorcing to find a better mate can have strong fitness consequences (Choudhury, 1995; Black, 1996; Ens et al., 1996; Culina et al., 2015; Adkins-Regan & Tomaszycki, 2007). For instance, partner retention may reduce the energetic costs of finding a new mate without affecting reproductive success (Real, 1990; Bried & Jouventin, 2002; Adkins-Regan & Tomaszycki, 2007; Mercier et al., 2021) and reinforce pair bonds by better coordinating behaviours that boost reproductive output (e.g., resource acquisition and offspring attendance), as predicted by the ‘pair familiarity effect’ (Black, 1996;

Desrochers & Magrath, 1996; Gill, 2012; Griffith, 2019). In some cases, however, the fitness benefits of divorce may surpass those of partner retention, particularly in pairs with poor breeding success, as divorce allows an individual to break free from suboptimal partnerships to find a better mate with higher reproductive potential (Choudhury, 1995; Black, 1996; Bried & Jouventin, 2002; Culina et al., 2015; Mercier et al., 2021). Therefore, to better understand the evolutionary significance of these opposing behaviours, both the costs and benefits of establishing and maintaining socially monogamous partnerships should be considered (Sanchez-Macouzet et al., 2014).

Factors explaining pair-bond dynamics in long-lived socially monogamous birds often correlate with life-history traits (e.g., breeding phenology, site fidelity), social environment (e.g., age structure and sex ratio), and individual features such as reproductive experience and body condition (e.g., Cézilly et al., 2000; Bried et al., 2003; Jeschke & Kokko, 2008; Griffith, 2019; Halimubieke et al., 2020). Long-lasting pairs, for instance, may capitalise on mate fidelity by breeding early in the season and/or securing reproductive success (Black, 1996; Bried & Jouventin, 2002; Gill, 2012; Griffith, 2019). In contrast, site fidelity is expected to have little, if any, influence on divorce rates when pairs maintain some degree of association outside the breeding season (Cézilly et al., 2000). The social environment is also known to influence divorce rates. For instance, divorce rates can increase when there is a higher proportion of older, more experienced unpaired birds in the population (Black, 1996; Woodman et al., 2024). Likewise, skewed sex ratios can contribute to higher divorce rates by increasing high-quality mate availability (e.g., more fertile) for the rarer sex, or when the rarer sex does not invest in mate maintenance (e.g., territorial behaviour) permitting partner usurpation (Black, 1996; Ens et al., 1996; Taborsky & Taborsky, 1999; Liker et al., 2014). In the latter case, body condition may confer some advantages in pairing status as individuals in good shape might be more successful at ejecting intruders (e.g., Robb et al., 1992).

Mating systems are also shaped by density-dependent changes in demographic parameters, and long-lived monogamous species can shift mating strategies in response to habitat saturation (Carrete et al., 2006a,b). This is particularly relevant when considering species of conservation concern, such as flightless kiwi *Apteryx* species (Palaeognathae), whose populations rely on active management to boost population numbers (Colbourne, 2005; Innes et al., 2015; Germano et al., 2018; Jahn et al., 2022).

Here we analysed long-term monitoring data from a translocated, high-density population of North Island (NI) brown kiwi *Apteryx mantelli* to describe patterns of divorce and partner fidelity, investigate the influence of intrinsic factors (specific to the mating units) on pair bond maintenance, and explore the benefits of pair familiarity on reproductive performance. This species has traditionally been considered long-term socially monogamous with females producing energy-rich eggs that are incubated solely by males (McLennan, 1988; Taborsky & Taborsky, 1999), yet earlier observations (Falla, 1979; McLennan, 1988; Taborsky & Taborsky, 1999) and recent reports from a high-density translocated population suggest a more flexible strategy with the potential for polygamy with non-kin co-breeders (Undin & Castro, 2022). Translocations may influence key factors shaping breeding systems and mating strategies, such as population density, resource availability, and mate access (Komdeur et al., 1995; Pribil & Searcy, 2001; Carrete et al., 2006ab; Griffith et al., 2010; Undin & Castro, 2022). Consequently, understanding the determinants of breeding and mating behaviour is not only important for exploring the possible benefits that both males and females gain from establishing and maintaining socially monogamous partnerships, but also for predicting outcomes of translocation (Anthony & Blumstein, 2000; Sigg et al., 2005; Renan et al., 2018; Undin & Castro, 2022).

While pair bonding has been widely studied in neoaves, studies on paleognaths remain scarce. Kiwi are cryptic and nocturnal birds that nest in subterranean or tree cavities and abandon incubation when disturbed (Taylor et al., 2014), which poses challenges for studying their behavioural ecology. The long mean lifespan of NI brown kiwi (~40 years; Robertson & de Monchy, 2012) suggests long-term pair-bonding (e.g., Taborsky & Taborsky, 1999), so we expected to find low divorce rates as seen in other long-lived birds (e.g., Jeschke & Kokko, 2008; Botero & Rubenstein, 2012; Liker et al., 2014). We used Bayesian mixed-effects modelling to investigate how intrinsic factors (specific to the mating pairs) influenced the probability of pair fidelity. We predicted that pairs with males in better body condition, and thus likely investing more in reproduction, would be less prone to divorce (Table 2.1). As reproductive effort depends on age, we also expected divorce to covary with breeding experience and pair-bond tenure (Pyle et al., 2001; Sánchez-Macouzet et al., 2014; Culina et al., 2020; Speelman et al., 2024). We then investigated the influence of pair bond duration, testing the predictions that long-lasting pairs (1) start breeding earlier in the reproductive season and (2) obtain greater

reproductive success (e.g., Gill, 2012; Sánchez-Macouzet et al., 2014; Griffith, 2019). We also explored the potential consequences of differing mating strategies (monogamy or polygamy) on incubation effort. The prolonged incubation period in NI brown kiwi imposes significant costs on males, affecting their body condition (McLennan, 1988) and limiting their time to engage in other vital activities, such as self-maintenance (Undin, 2021). We predicted that shared incubator males in polygamous breeding units should invest less time incubating compared with single incubator males in socially monogamous pairs, such that cooperative polygamy helps to reduce the trade-off between parental investment and increasing mating opportunities or self-maintenance (Covas & Griesser, 2007; Undin, 2021). Finally, we discuss the potential implications of conservation efforts on kiwi mating strategies and pair-bond stability.

Material and Methods

Study site and population monitoring

Ponui Island, New Zealand (1,770 ha), houses a human-translocated population of NI brown kiwi settled in 1964 through two translocations of eight founder birds from Waipoua Forest and six from Hauturu-o-Toi (Little Barrier) Island (Miles & Castro, 2000; Colbourne, 2005; Undin et al., 2021a). Birds in these source locations belong to the *A. mantelli* Northland (Waipoua) and Western (Hauturu-o-Toi) management units, although the origin of the latter population has been regarded as 'hybrid' or uncertain (Burbidge et al., 2003; Colbourne, 2005; Undin et al., 2021a). While the actual population size of kiwi on Ponui Island remains uncertain, rough estimates indicate a density of around one bird per hectare (Cunningham & Castro, 2011), suggesting that brown kiwi have thrived in this environment. Rapid population growth on Ponui Island over 4–6 kiwi generations has raised concerns about both inbreeding and outbreeding depression due to the small founder population (14 birds) and the mixing of birds likely adapted to different local conditions, respectively (Undin et al., 2021ab). However, recent genomic comparisons using single-nucleotide polymorphisms (SNPs) indicate that the kiwi population on Ponui Island formed a successful hybrid swarm, characterised by high genetic diversity and lower-than-expected relatedness among breeding pairs. This pattern

suggests the presence of a kin-recognition mechanism that helps reduce inbreeding and maintain genetic diversity (Undin et al., 2021b).

Table 2.1. Potential factors influencing pair status in North Island (NI) brown kiwi and their predicted effect on divorce propensity based on previous research in kiwi and other socially monogamous species.

Variable	Prediction	Background
Breeding status	Kiwi birds paired with mates that breed each reproductive season divorce less.	Securing mates to avoid missing reproductive seasons may be favoured (see Johnston & Ryder, 1987; Choudhury, 1995) since wild NI brown kiwi have high hatching failure rates (McLennan, 1988; Undin & Castro, 2022).
Incubation effort	Male kiwi investing more in incubation are less prone to divorce.	Retaining mates with high reproductive potential may be advantageous (see Burley, 1988; McNamara & Forslund, 1996), given the high costs of egg production for female kiwi (Calder et al. 1978).
Nesting success	Kiwi pairs with low nesting success are more prone to divorce.	Wild NI brown kiwi experience high hatching failure rates (McLennan, 1988; Undin & Castro, 2022), and faithful pairs with successful nesting males may enhance reproductive prospects (see Dubois & Cézilly, 2002; Culina et al., 2015).
Nesting attempts	Male kiwi that incubate more than once in a breeding season divorce less.	Female NI brown kiwi normally lay two clutches per year and may re-lay eggs when the first clutch fails early in incubation (McLennan, 1988), and faithful pairs with re-incubating males can compensate for early hatching failures.
Pair familiarity	Long-lasting kiwi pairs are less likely to divorce.	Fidelity may be favoured as familiar pairs can coordinate behaviours to enhance breeding success and/or defend territory and mates (see Griffith, 2019; Culina et al., 2020; Sánchez-Macouzet et al., 2014; Speelman et al., 2024).
Age	Young kiwi birds divorce more.	Young, first-time breeding males of NI brown kiwi often desert nest shortly after starting incubation (McLennan, 1988), so staying with experienced breeders can be advantageous (see Pyle et al., 2001; Speelman et al., 2024).
Body condition	Kiwi birds paired with mates in poor body condition divorce more.	Wild NI brown kiwi in better body condition are more likely to be paired (Taborsky & Taborsky, 1999), thus divorce can be a mechanism to release suboptimal mates and boost reproductive success (see Choudhury, 1995).

Previous research on Ponui Island (2005–2006) using radio transmitters (Ziesemann et al., 2011), alongside video monitoring of nests and roosts (2008–2009 and 2012) and regular monitoring using video cameras and radiotelemetry data from 2009 onwards, identified distinct breeding units (Castro, unpubl. data): pairs (two birds) and groups (more than two birds). Network analyses using radiotelemetry data (2004–2017) further enhanced confidence in identifying and delineating these breeding units (Undin, 2021). As a result, we now have data on individual and unit features, such as relative age, mate identities, and nesting sites for 5–14 pairs (10.23 ± 3.04 SD) and 4–5 groups (4.45 ± 0.52 SD) per year. Adulthood was determined based on morphological traits: females weighing more than 1.6 kg and having bills longer than 108 mm, and males weighing more than 1.4 kg with bills up to 99 mm long (Cunningham & Castro, 2011). Most paired birds were caught as adults (92%, 34 of 37), so their actual ages remain unknown. To estimate their relative ages, we added the average age at which birds of each sex stop growing to the year they were first caught and tagged: males 4.4 years and females 7.5 years (estimated from five birds caught as chicks and two chicks born to monitored pairs, tracked until they stopped growing). We acknowledge our age estimates might be underestimated, especially if birds from the first generations after translocation are present in our dataset. Bird sex was determined based on morphological features, as females are larger and have longer bills than males (Colbourne et al., 2020). On Ponui Island, female kiwi bills are on average 30% longer than male bills, with no overlap in bill length between the sexes (Cunningham & Castro, 2011), so birds with bills longer than 108 mm were deemed females. Additionally, DNA sexing using feathers or blood samples has been used to determine the sex of males and young females with bills shorter than 108 mm (Castro, unpubl. data).

Data collection and analyses

Only socially monogamous, incubating adult males with a verified (tagged) partner were included in our analysis. Pair bond status (hereafter pair status) was categorised annually as follows: fidelity, when both members of a pair in year t were observed together again during the breeding season in year t + 1; divorce, when both individuals were alive and present in the study area but did not form a pair in year t + 1; and widowing, when the death of one partner was confirmed. Annual divorce rate was calculated as the number

of divorces divided by the total number of monitored pairs each year. An overall divorce rate was then estimated as the mean of annual rates across the study period.

We monitored North Island brown kiwi in three forested gullies, Kauri, Red Stony Hill, and Pipe, spanning approximately 200 ha of mixed habitat, including native forest, wetlands, and pasture (Miles & Castro, 2000), representative of the broader Ponui Island landscape. Since 2004, between 30 and 50 adult kiwi have been monitored annually (mean ± SD: 46.2 ± 4.6), using a combination of radiotelemetry (e.g., Jamieson et al., 2016), camera traps (e.g., Fan, 2023), handheld infrared video (e.g., Cunningham & Castro, 2011), and camcorders deployed at roost and nest sites (e.g., Wilson, 2013). Monitoring intensity and frequency varied depending on research goals, but baseline efforts included annual captures for health checks and transmitter replacement (once annually from 2004–2009; twice annually from 2010 onward), monthly radiotelemetry to locate marked individuals, and annual nest location for all marked males and the monitoring of some nests to assess chick hatching success.

From 2004 to 2010, we used conventional VHF transmitters. From 2010 onward, these were replaced by Chick Timer™ transmitters (CTs; wildtech.co.nz/Kiwi.aspx), which enabled consistent long-term tracking and improved reproductive monitoring. CTs incorporate motion-sensing technology to remotely detect incubation behaviour, record activity patterns, and log nest entry and exit times, facilitating nest detection with minimal disturbance (Taylor et al., 2014). CT data were retrieved in two ways. First, temporary data were accessed monthly in the field using a handheld receiver and antenna. These included indicators of breeding status (e.g., incubating or not), number of days since incubation onset or termination, estimated hatch date, male nest desertion (typically post-hatching), and summaries of recent activity over the last 24 hours, 48 hours, and four-day average (recorded in 10-minute increments). Incubation status was always verified in the field by locating each nest, and in most years, eggs were candled to assess developmental stage and determine nesting success. Nesting sites were marked each season and grouped according to their location within one of the three gullies. Additionally, CTs stored long-term daily activity data on onboard loggers for approximately nine months. These data, downloaded annually in March during transmitter replacement, covered, for most males, the full breeding season, which typically spans from mid-June to January for Ponui Island kiwi (Ziesemann et al., 2011;

Bansal, 2020). We created a long-term database recording the reproductive activity of tagged individuals derived from daily activity recorded by CT transmitters.

To investigate factors influencing pair status, we extracted seven variables expected to affect partner fidelity from CT-derived data (Table 2.1). Breeding status, codified as a binary variable, indicated whether a male was breeding (1) or not (0) in a given year. Male kiwi reduce activity levels when they are incubating, as they spend more time in the nest. We identified incubation onset as a consistent decline in activity, while a sustained return to higher levels marked the end of incubation. A male was considered incubating if his daily activity fell to 6.5 hours or less, based on observed differences between incubating and non-incubating males in our dataset. Incubation effort was defined as the number of days a male spent incubating during a season. Nesting success, a proxy for hatchling success (Figure S2.1), was inferred when incubation lasted longer than 75 days, consistent with a full incubation bout (Potter, 1989; McLennan, 1988; Colbourne, 2002). Breeding attempts were measured as the number of times a male initiated incubation during the breeding season, regardless of outcome. Pair familiarity was estimated as the number of consecutive years a bird nested with the same partner, using combined data from VHF telemetry and CT transmitters (2004–2020). Age in years (estimated as described above) was used as a proxy for breeding experience (Curio, 1983; Orel et al., 1994; Pyle et al., 2001). Body condition, a potential indicator of mate quality in kiwi (Taborsky & Taborsky, 1999), was estimated using the scaled mass index (Peig & Green, 2009), which adjusts body mass relative to a linear body measurement (Figure S2.2) and reflects relative energy stores.

To assess whether partner retention influenced reproductive success, as predicted by the pair familiarity effect (Black, 1996; Desrochers & Magrath, 1996; Gill, 2012; Wiley & Ridley, 2018; Griffith, 2019), we examined two response variables: nesting success (as defined above) and breeding onset, estimated as the calendar day when a male's activity declined to ≤ 6.5 hours. Finally, to test whether incubation effort differed between monogamous males and those in breeding groups (i.e., polygamous associations with shared incubation), we extracted two variables: (i) daily incubation effort, defined as the number of hours per day an incubating male spent at the nest during a complete bout (at least 75 days); and (ii) proportional incubation day, calculated as the fraction of the species' typical incubation period already completed. This was based on an average

incubation period of 82.5 days, derived from the typical 75–90 day range reported for North Island brown kiwi (Potter, 1989; McLennan, 1988; Colbourne, 2002), following Bulla et al. (2017).

Ethical Note

All research and monitoring activities on Ponui Island adhered to strict ethical and legal guidelines to ensure the well-being of the kiwi involved. Specifically, protocols followed the Kiwi Best Practice Manual (Colbourne et al., 2020), a government document outlining the recommended methods for safely handling kiwi in the wild. These procedures were carried out under the ethical approval of the Massey University Animal Ethics Committee permit 20/68 and with the authorisation of the Department of Conservation Wildlife permit (38796-RES). In summary, kiwi were located using radio telemetry and manually captured at their roosting sites during daytime by accredited handlers. To minimise stress and struggle, the birds' heads were covered with a pillowcase during handling, and everyone involved in the process remained as quiet as possible. Transmitters were attached to the leg (tibiotarsus) using a hospital identification band carefully covered with electrical tape. The band was adjusted to allow free rotation around the leg while ensuring it was not loose enough to slip over, nor too tight to restrict movement (see Miles & McLennan 1998; Colbourne et al., 2020). To further reduce handling time, two people were always involved, one to hold the bird and the other to attach the transmitter. After handling, kiwi were released at the same location where they were captured to minimise disruption to their natural behaviour.

Statistical analyses

We fitted Bayesian generalised linear mixed models (B-GLMMs) using the R (R Core Team, 2023) package 'brms' 2.20.4 (Bürkner, 2017). To boost model performance and interpretability, continuous predictor variables were standardised by centering and dividing them by two standard deviations (Gelman, 2008). We used weakly informative priors [normal (0, 2)] for fixed effects and brms's default priors for the remaining parameters, so priors' influence is expected to be negligible (Bürkner, 2017). Models used four chains of 30,000 iterations, discarding the first half as burn-in, and saving every 10th sample, and adapt-deltas of 0.999. Model convergence was checked by examining trace plots of posterior samples and making sure that diagnostic Rhat ($\hat{R}$) values for each

parameter were close to 1.0, indicative of proper mixing of chains and reliable parameter estimation (Gelman & Rubin, 1992). We also assessed the effective posterior sample size (ESS) to determine how many independent (or anticorrelated) samples the chain effectively provided (Gelman et al., 2013), with values above 1,000 indicating reliability (Muth et al., 2018). Model fit was assessed by examining graphical posterior predictive checks and Pareto-*k* values estimated from a Pareto-smoothed importance sampling leave-one-out cross-validation approach (Vehtari et al., 2017, 2022) using the R package 'loo' 2.7.0 (Vehtari et al., 2024). We reported posterior medians, an estimate of the central tendency of the posterior distribution; median absolute deviations (MAD), a measure quantifying the dispersion of parameter estimates around the median; and 95% Bayesian high-density intervals (HDI), a metric of uncertainty indicating the probable range for model parameters (Gelman et al., 2013). We considered predictors to have a meaningful effect if the 95% HDI did not overlap zero. Bayesian indices of effect existence (probability of direction, pd) and significance (region of practical equivalence, ROPE) were estimated using the R package 'bayestestR' 0.12.1 (Makowski et al., 2019). The pd indicates the certainty related to the most likely direction of an effect (positive or negative), while the ROPE informs whether a parameter is linked or not to a meaningful change in the outcome (Makowski et al., 2019). Here, pd ≥ 99% were inferred as evidence of the existence of an effect (Makowski et al., 2019), and parameter estimates were considered meaningful when ≥ 1% of the 95% HDI fell within the ROPE (Kruschke & Liddell, 2018).

Factors explaining fidelity probability

To investigate the factors explaining fidelity probability, we used a dataset including 113 data points representing the social pair bond history of 21 breeding pairs over multiple years. We fitted B-GLMMs to test whether pair status, modelled as a binary variable (fidelity = 1, divorce = 0) with a binomial distribution and logit link, was explained by the predictors listed in Table 2.1. To examine the potential influence of female body condition and age on pair status, these variables were incorporated separately for males and females in our modelling approach. Although the dataset contained 113 data points, we recognised that the number of statistically independent units, individual breeding pairs, was limited to 21, reducing statistical power and increasing the risk of overfitting. To address this, we adopted a projection predictive feature selection approach (PPFS)

with the R package 'projpred' 2.8.0 (Piironen et al., 2023; Pavone et al., 2023). This method fits a flexible full model and then projects its predictive performance onto simpler submodels with fewer parameters. Importantly, the full model is not used for inference but serves as a reference to guide principled variable selection. PPFS offers an excellent balance between model complexity and predictive accuracy, particularly when the goal is to identify a minimal set of informative predictors (Pavone et al., 2023).

As a first step, we fitted a Bayesian multilevel reference model including all nine fixed effects and three random effects: (i) pair identity, to account for repeated measures and potential variation in pair quality (e.g., Bried & Jouventin, 2002; Sánchez-Macouzet et al., 2014; Mercier et al., 2021); (ii) year, to capture annual variations in abiotic (climatic fluctuations) and biotic (resources, predation) conditions (e.g., Botero & Rubenstein, 2012; Mercier et al., 2021); and (iii) nesting gully, as a proxy of site-level differences in habitat quality (e.g., Massaro et al., 2001; Mercier et al., 2021). The full model took the form:

pair status ~ breeding status + incubation effort + nesting success + breeding attempts + pair familiarity + male quality + male experience + female quality + female experience + (1| pair identity) + (1|year) + (1| nesting gully)

However, this structure consistently led to high Pareto *k*-values (> 0.7), indicating influential observations and potential model misspecification (Vehtari et al., 2022). We therefore refitted a simplified reference model, excluding the random effect of gully and the fixed effects for male and female quality, which did not substantially improve predictive performance (see Figure S2.3 for details).

Next, we applied latent PPFS, as recommended for binomial models (Catalina et al., 2021), to identify the smallest submodel with predictive performance comparable to the simplified reference model. We assessed model performance using mean log predictive density (MLPD) and geometric mean predictive density (GMPD). Projection predictive modelling provides a robust framework for simplifying complex or parameter-rich models while minimising overfitting risk (Piironen et al., 2020). To reduce computational time, the forward selection process was halted at a submodel size of three. We retained the smallest submodel that produced results consistent with the reference model, and used this final submodel, for interpretation and inference. Random effects were delayed during

the feature selection process to improve predictor ranking, following best-practice recommendations (see Table S2.1 for additional methodological details). The final submodel retained three predictors: incubation effort, breeding success, and familiarity. These variables consistently ranked highest in predictive importance and accounted for most of the explanatory power of the reference model. This simplified model was used for all subsequent inference and interpretation. The retained predictors are biologically meaningful and align with prior research suggesting that previous breeding experience, current reproductive engagement, and pair familiarity may influence pair bond stability (e.g., Black, 1996; Gill, 2012; Griffith, 2019).

Mate familiarity effect

To test the influence of pair familiarity on reproductive success (i.e., nesting success) and breeding onset (i.e., incubation onset date), we fitted two separate B-GLMMs using a dataset including 112 data points representing the social partnerships of 21 breeding pairs. These models included familiarity as a fixed effect and to reliably separate the effects of pair familiarity from those of breeding experience (Sánchez-Macouzet et al., 2014), we also added a two-way interaction term between pair familiarity and male age (as a proxy of breeding experience). Female age was not considered here as this variable did not influence pair status as indicated by our variable selection approach (see Results). Models in this section used pair identity and year as random variables (as defined above). We used appropriate error distribution families depending on the response variable being modelled: for our binary response variable (nesting success) we used a Bernoulli distribution (link = logit), while for the count response variable (breeding onset) we used a negative binomial distribution (link = logit).

Effects of different mating strategies on incubation

To test for differences in daily incubation effort between male kiwi sharing incubation in breeding groups against males incubating alone in pairs we used a dataset including 8,280 data points for daily nest attendance from all nests incubated by males in our dataset over the course of 11 years ($n_{\text{shared incubators}}$ = 1,585 incubation days from 21 nests incubated by five males, $n_{\text{single incubators}}$ = 6,695 incubation days from 80 nests incubated by 17 males). We fitted a model including incubation effort as the response variable, and incubation type (single = one incubator, shared = more than one incubator) and

proportional incubation day as predictors. This model included a two-way interaction term: incubation type × proportional incubation day (see Bulla et al., 2017). This model used a Gaussian distribution (link = identity) and included random effects of year (as in previous models) and male identity to account for the lack of independence in daily incubation effort estimates from the same male.

Results

Factors explaining fidelity probability

Social monogamy was the dominant mating strategy for kiwi on Ponui Island with 78% (21 of 27) of pairs, although polygamous units occurred at a notable rate of 22% (6 of 27). Within polygamous groups, polyandrous (one female and two males, n = 3), polygynous (two females and one male, n = 2), and a polygynandrous unit (two females and three males, n = 1) were observed (Figure 2.1a). Divorce rates for kiwi pairs varied over time, and divorces were observed in six out of 11 study years (Figure 2.1b). Divorce occurred in 10 kiwi pairs, with annual divorce rates ranging from 0.0% to 33.3%, averaging 8.1% over the 10-year monitoring period (2010–2020). Of the monogamous study birds, only four (20%) had more than one mate (2–3) during the study period, two changed partners due to divorce and two due to widowhood. After divorcing, the time required to establish a new relationship (n = 6) varied between 1 and 5 years.

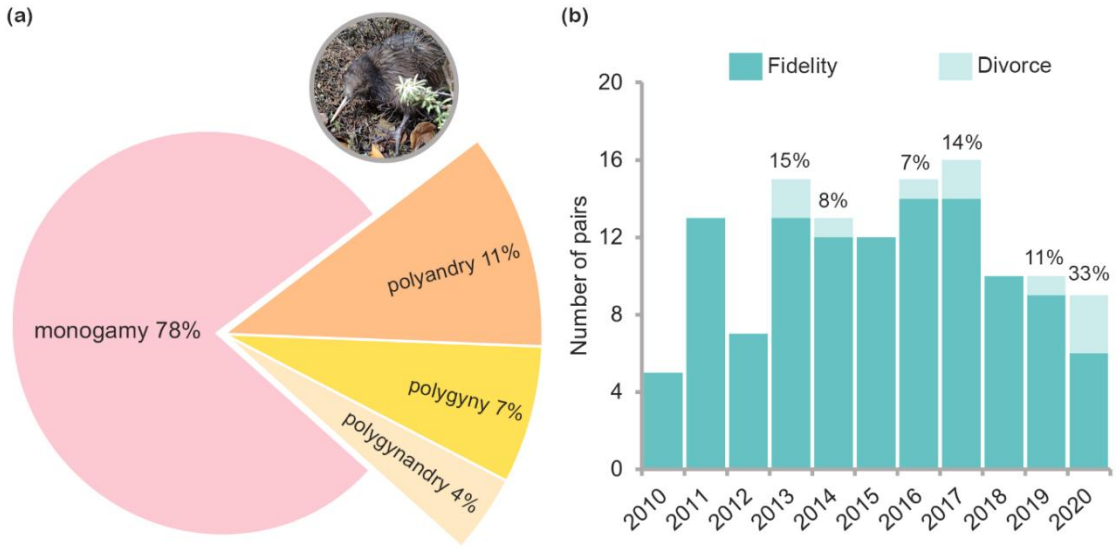

Figure 2.1. Breeding strategies and divorce rates for North Island brown kiwi (inset photo) on Ponui Island (2010–2020). (a) Frequency of breeding units in each mating system found on the island during the study period ($n = 27$). Polygamous groups are classified as polyandry, polygyny, and polygynandry. (b) Number of faithful and divorced pairs over the course of 11 years. Percentages (%) indicate the annual divorce rate per year.

The latent projection predictive feature selection identified incubation effort as the sole predictor necessary to achieve predictive performance comparable to the simplified reference model (Figure 2.2). Incubation effort ranked highest and was selected as relevant 90% of bootstrap iterations and ranked highest in predictive importance (Table S2.1). The inclusion of additional predictors, such as nesting success and familiarity did not improve model performance. Thus, we projected the reference model onto a submodel including only incubation effort (i.e., the highest-ranked predictor) to derive the final model used for inference. This submodel revealed that incubation effort had a positive (pd = 100%) and significant effect (ROPE = 0.00%) on fidelity. The median estimated effect size was 3.82, with a 95% credible interval between 2.03 and 6.63, indicating that greater incubation effort was consistently associated with higher pair fidelity (Figure 2.3; Table S2.2).

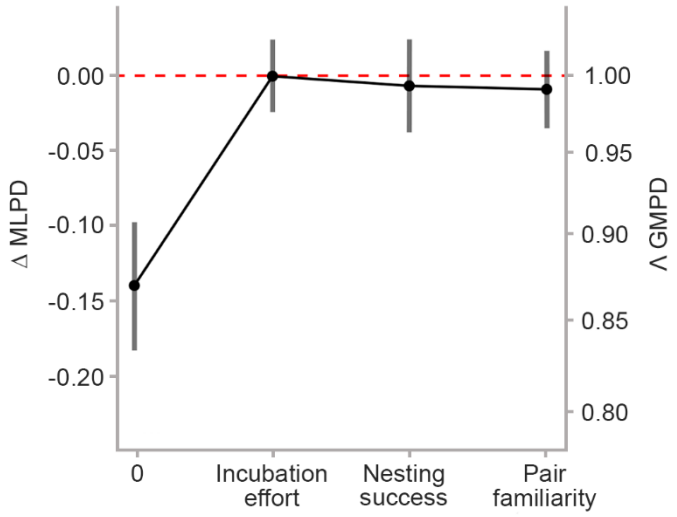

Figure 2.2. Submodel size plot (number of predictor terms) from the latent projection predictive feature selection. The x-axis shows the number of predictors during the forward search. Note that only one predictor (incubation effort) was sufficient to obtain a predictive performance close to that of the final reference model (dashed red line), which is by definition 0 (left y-axis) and 1 (right y-axis). The left y-axis (ΔMLPD) represents the submodel mean log predictive density (MLPD) minus the reference model MLPD. The right y-axis (ΛGMPD) denotes the ratio of the submodel geometric mean predictive density (GMPD) to the reference model GMPD). The uncertainty bars here indicate ±1 standard error of the ΔMLPD estimator.

Mate familiarity effect

Models testing the familiarity effect ran smoothly without post-sampling warnings, diagnostic parameters indicated convergence (Table S2.3), and posterior predictive checks and Pareto k-values < 0.7 indicated a good fit. We found that familiarity had a positive and significant effect on nesting success (pd = 99.05%, ROPE = 0.00%). The median estimated effect size was 2.23, with a 95% credible interval ranging from 0.21 to 4.30 (Figure 2.4). Other terms in the model were not meaningful (Table S2.3a), indicating no significant effect of male experience on nesting success, either as a main effect or in interaction with pair familiarity. Likewise, we did not find any evidence of a familiarity effect on incubation onset as indicated by pd ≤ 90.63% and ROPEs ≥ 75.72% for all fixed effects (Table S2.3b).

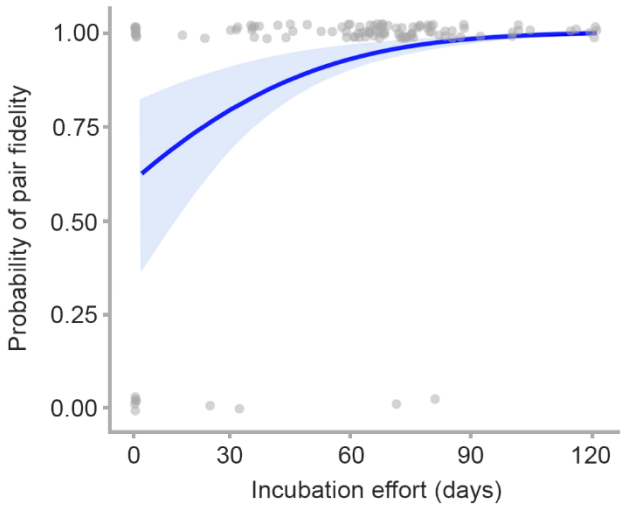

Figure 2.3. Effects of incubation effort and pair familiarity on pair status, that is, the probability of staying together (divorce = 0, fidelity = 1) in North Island brown kiwi in Ponui Island. The dark blue line shows the average predicted probabilities, and the faded blue band indicates 95% uncertainty (projected posterior) intervals. Raw data points (grey dots) were jittered slightly to reduce overlap and enhance clarity.

Effects of different mating strategies on incubation

Models testing for differences in daily incubation effort between male kiwi sharing incubation in breeding groups and males incubating alone in pairs ran efficiently without post-sampling warnings, diagnostic parameters indicated convergence (Table S2.4), and posterior predictive checks and Pareto k-values < 0.7 indicated good fit. Daily incubation effort was similar across the incubation period in single and shared incubators, but single incubators tended to invest more in daily nest attendance than those in polygamous groups that shared incubation (Figure 2.5a). Despite this, we did not find an effect of incubation type on daily incubation effort (median = −0.03, 95% HDI [−0.56, 0.48], pd = 55.48%, ROPE = 38.00%), indicating no statistical difference in incubation effort between single and shared incubators (Figure 2.5b, Table S2.4). Contrary, we found that proportional incubation day had a positive (pd = 100%) and strong effect (ROPE = 0.00%) on daily incubation effort (median = 0.69, 95% HDI [0.56, 0.81]), indicating that incubation investment tends to increase over the incubation period (Figure 5).

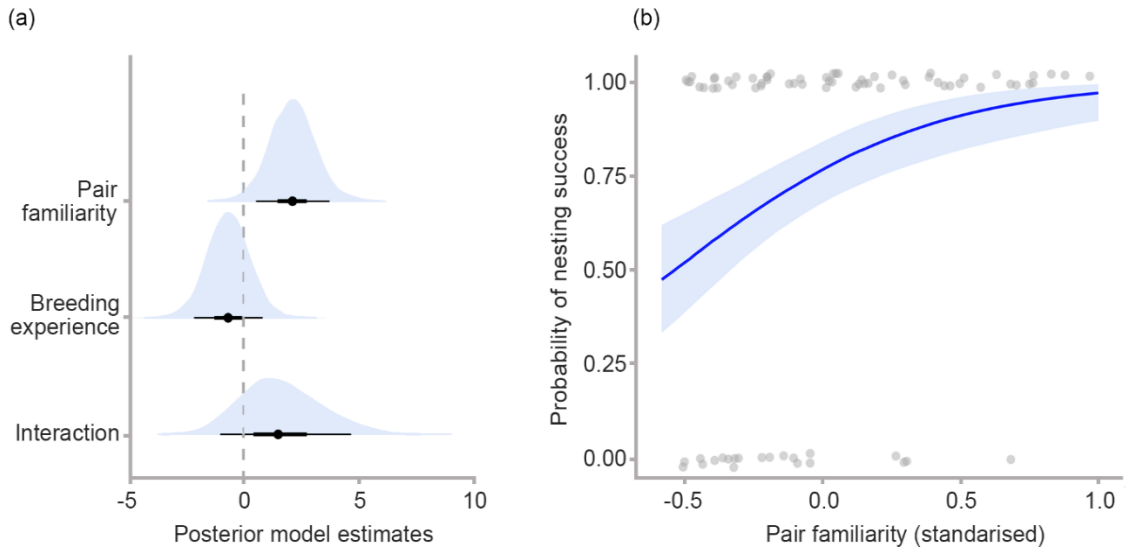

Figure 2.4. Effect of pair familiarity on nesting success (failure = 0, success = 1) in North Island brown kiwi on Ponui Island. (a) Posterior projected probabilities indicating the effect of pair familiarity on nesting success. The thick black bars indicate 95% Bayesian high-density intervals (HDI), with the black circles representing median estimates. Variables with 95% HDI overlapping zero are inferred not to affect nesting success. (b) Projected effect of pair familiarity on nesting success. The dark line shows the average predicted probabilities, and the faded blue band indicates 95% uncertainty (projected posterior) intervals. Raw data points (grey dots) were jittered slightly to reduce overlap and enhance clarity.

Discussion

We found that social monogamy was the most common mating system in NI brown kiwi on Ponui Island, although polygamous groups composed of three or more birds also occur (see Undin & Castro, 2022). Our models indicate that the probability of pair fidelity (i.e., staying together) increased with incubation effort, highlighting male reproductive investment as a critical factor influencing long-term pair cohesion. We also found that pair familiarity had a positive effect on nesting success, indicating the potential benefits of extended pair-bond tenure for reproductive outcomes. Males sharing incubation in polygamous groups tended to invest less in nest attendance than single incubators in socially monogamous pairs, suggesting benefits linked to nest relief, which can be understood as the temporary replacement of one incubating male by another during the incubation period.

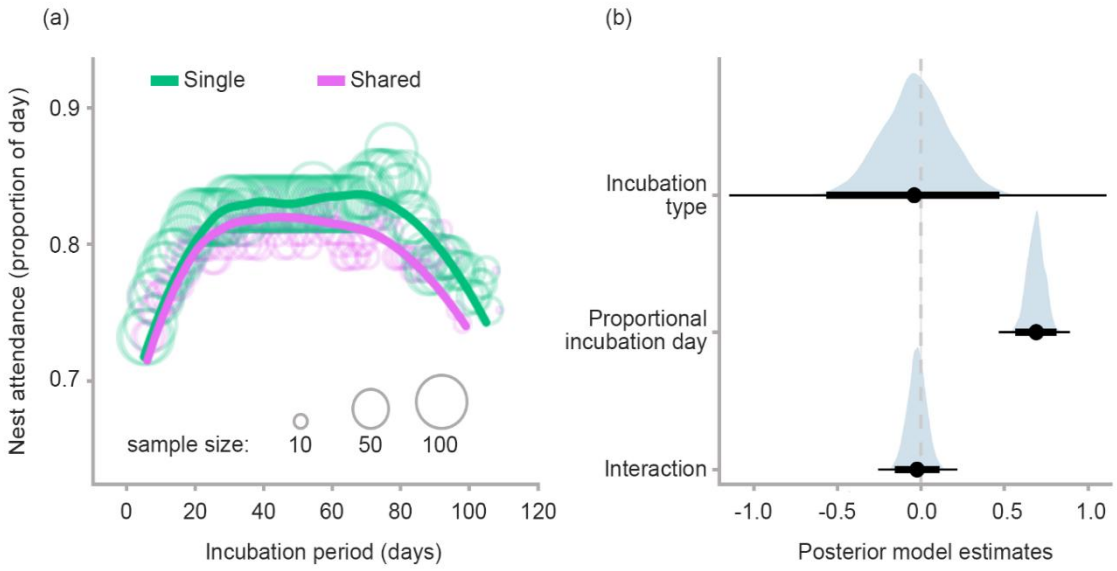

Figure 2.5. Daily incubation effort between male incubation type (single and shared) for North Island brown kiwi on Ponui Island. (a) Change in daily nest attendance over the incubation period for single and shared incubators. Each circle represents the mean daily nest attendance from all nests incubated by males in our dataset over the course of 11 years (dot size reflects sample size: $n_{\text{shared incubators}}$ = 1,585 incubation days from 21 nests incubated by five males, $n_{\text{single incubators}}$ = 6,695 incubation days from 80 nests incubated by 17 males). Lines represent locally weighted scatterplot smoothing. (b) Posterior projected probabilities indicating the effects of incubation type (single and shared) and proportional incubation day on daily incubation effort. The thick black bars indicate 95% Bayesian high-density intervals (HDI), with the black circles representing median estimates. Variables with 95% HDI overlapping zero are inferred not to affect daily incubation effort.

The low mean divorce rate (8.1%) in the NI brown kiwi on Ponui Island is largely consistent with those from other conspecific populations (Taborsky & Taborsky, 1999) and mirrors findings from other long-lived socially monogamous birds (e.g., seabirds, geese, swans), where divorce rates are generally below 15% (Culina et al., 2015; Mercier et al., 2021). Short-term monitoring of four NI brown kiwi populations also reported low divorce rates (~5%) in all but one population, where half of all pairs divorce each year (Potter, 1989; Taborsky & Taborsky, 1999). Pairs in this population split regardless of their reproductive output in the previous season (Potter, 1989), with no mate improvement after divorce (Taborsky & Taborsky, 1999), suggesting divorce was not

adaptive (see Choudhury, 1995; Black, 1996; Dubois & Cézilly, 2002; Speelman et al., 2024). Interestingly, this population was relatively dense with a strongly female-biased sex ratio and no territorial behaviour, suggesting divorce may be 'forced' due to a surplus of high-quality females (Taborsky & Taborsky, 1999), creating low-cost opportunities for mate replacement (e.g., Ens et al., 1993; Cézilly & Nager, 1995; Black, 1996; Liker et al., 2014). While the sex ratio for kiwi on Ponui Island is unknown, interannual divorce rates were usually low despite shared home ranges (Ziesemann, 2011), close proximity during nesting and roosting (Dixon, 2015), and lack of territorial behaviour (Castro, pers. obs.). This challenges the idea that kiwi territoriality is essential for mate retention (Taborsky & Taborsky, 1999) and suggests that other factors may be influencing pair stability in our system.

Indeed, divorce rates are often related to intrinsic factors such as phenotypic quality (e.g., Gousy-Leblanc et al., 2023) and extrinsic environmental factors like mate availability (e.g., Taborsky & Taborsky, 1999) and thus may be specific to each kiwi population. The low mean between-season divorce rate (8.1%) indicates that divorce represents a secondary mating strategy in our system, likely in response to changing environmental conditions and/or individual reproductive success, where birds switch partners to improve their overall fitness. While interannual divorce rates were generally low, we found a high divorce rate in 2020 (33%), which correlated with a severe drought that caused the death of approximately a quarter of the monitored birds on Ponui Island, particularly those in poor body condition (Castro et al., 2020). Such mass mortalities may have created new vacancies for better-quality mates, therefore boosting divorce rates as observed in other species (Ens et al., 1993; Cézilly & Nager, 1995). Divorce may also help to avoid inbreeding by fixing initial 'errors' in mate choice that result in incestuous pairings (Speelman et al., 2024), and this is particularly relevant for translocated kiwi populations founded by a small number of birds, where inbreeding can become cryptic due to overlapping generations and the reproductive success of a handful of founders (see Taylor et al., 2017). Nevertheless, divorces could also have occurred for various non-adaptive reasons, and we cannot rule out the possibility that some splits were opportunistic or accidental (Ens et al., 1993; Heg et al., 2003; Gousy-Leblanc et al., 2023).

Despite this, kiwi pairs with males investing more in incubation were less likely to divorce, suggesting that some divorces might be triggered by low breeding performance. Indeed, incubation effort in the studied system is directly linked to nesting success, which serves as an informative proxy for hatching success. This aligns with the findings of a meta-analysis, indicating that divorce in socially monogamous birds can be an adaptive behavioural strategy to escape poor reproductive outputs (Culina et al., 2015). Divorce can also be triggered by other adaptive factors, such as avoiding poor coordination of parental duties (Choudhury, 1995; Sánchez-Macouzet et al., 2014; Speelman et al., 2024) and/or poor-quality mates (Black, 1996; Dubois & Cézilly, 2002). However, variables related to pair familiarity, mate quality (e.g., body condition) and experience (e.g., age) in our dataset were not informative. Therefore, nest attendance (expressed as incubation effort) seems to be the feature that females assess when retaining partners, suggesting their role as the 'choosy' sex (Bried & Jouventin, 2002; Cézilly et al., 2000; Ventura et al., 2021). Thus, retaining mates with high reproductive potential may be advantageous (see Burley, 1988; McNamara & Forslund, 1996), given the high costs of egg production for female kiwi (Calder et al., 1978). However, caution is needed, as the low incidence of divorce in our study resulted in a limited sample size for divorce events, which may have influenced the analysis. Further research using a larger and longer-term data set is required to better understand whether kiwi birds choose or retain their partners based on attributes signalling increased reproductive outputs in future breeding seasons.

Although we did not find an effect of pair familiarity on incubation onset, we found a positive effect on nesting success, indicating that kiwi pairs in long-term partnerships are more likely to complete incubation bouts than newly bonded pairs. This benefit of pair familiarity seems independent of breeding experience, which also covaries with age (Forslund & Pärt, 1995; Ens et al., 1996; Sánchez-Macouzet et al., 2014; Speelman et al., 2024). However, caution is advised as we used an indirect metric to account for breeding experience (i.e., relative age), which could bias model inferences; thus, disentangling the potential confounding effect of pair familiarity from those of breeding experience in the studied population remains a challenge. Kiwi on Ponui Island seemed to differ from other long-lived socially monogamous birds, in which pair familiarity is influential for breeding timing (e.g., Black, 2001; van de Pol et al., 2006; Naves et al., 2007; Gabriel & Black, 2013; Sanchez-Macouzet et al., 2014). Therefore, we anticipate other factors not incorporated in our models (e.g., environmental variation) may be influential for the

reproductive performance of kiwi pairs on Ponui Island. Indeed, inter-annual variability in daily incubation effort suggests that variations in abiotic (climatic fluctuations) and biotic (food resources, predation) factors may influence reproductive investment, and therefore, divorce rates. This is particularly true of species where incubation relies on males, such as kiwi, as they are more likely to reduce parental effort under unfavourable conditions to conserve energy (Wiebe, 2008; Spencer et al., 2010). To our knowledge, no studies have directly quantified the energetic costs of male incubation in kiwi, although observed male weight loss during incubation suggests such costs may be significant (McLennan, 1988).

The reasons why some kiwi on Ponui Island form non-kin polygamous groups are unclear. Social polygamous units of genetically unrelated individuals are rare and difficult to explain via kin selection, where individuals boost their inclusive fitness by aiding genetic relatives (Riehl, 2013). Polygamous groups on Ponui Island can be broadly categorised into (i) cooperative and (ii) non-cooperative breeding systems. Avian life-history theory predicts that not all helping behaviour is kin-selected, and non-kin members in polygamous units must invest in costly tasks to remain within the group and potentially secure breeding opportunities later in life (the 'pay-to-stay' hypothesis; Gaston, 1978; Kokko et al., 2002). Incubation is energetically costly (Nord & Williams, 2015) but a key aspect of avian parental care (Matysioková & Remeš, 2010; Amininasab et al., 2017), and this is particularly true of precocial species such as kiwi (Vleck et al., 1979; McLennan, 1988). While not statistically significant, shared incubators in our dataset tended to invest less in nest attendance, suggesting that cooperative breeding might partly help to remedy the trade-off between self-maintenance and the thermal needs of the embryos (Amininasab et al., 2017). Thus, helpers in cooperative breeding units on Ponui Island could have adopted a pay-to-stay strategy by assisting in the incubation of unrelated chicks (Carrete et al., 2006b; Cockburn, 2006). Relatedness data support this, showing that known kiwi pairs on Ponui Island are socially and genetically monogamous, with no extra-pair offspring (Ziesemann, 2011). However, caution is warranted, as our study system is largely socially monogamous, and therefore, we had a small sample of shared incubators that might have influenced the analysis.

The existence of polygamous breeding units suggests that demographic and environmental pressures might also play a role in shaping variation in the mating system

of the studied system (see Davies & Hartley, 1996; Heg & Treuren, 1998; Carrete et al., 2006b). For example, long-lived monogamous birds can shift mating systems in response to habitat saturation, breeding monogamously at low densities and polygamously at high densities (e.g., Carrete et al., 2006b). Polygamy can also be a response to environmental deterioration or variability (Carrete et al., 2006b; Rubenstein & Lovette, 2007; Jetz & Rubenstein, 2011). In such cases, some individuals may be forced to enter already occupied high-quality territories as tolerated intruders, since the high energetic costs of agonistic interactions may deter eviction by territory holders (Bertran & Margalida, 2002; Carrete et al., 2006b; Clutton-Brock & Parker, 1995; Hamilton & Taborsky, 2005; Carrete et al., 2006b). Such birds might be making the 'best of a bad job' and gaining by group living rather than going without access to critical resources (see Koenig et al., 1992). Even if investing in any kind of cooperative behaviour, the net fitness effect of subordinates on dominant breeders is expected to be negative (Hamilton & Taborsky, 2005; Carrete et al., 2006b). For instance, polygamous breeding might be a strategy for males to increase their fitness prospects by queuing a female or a high-quality territory (Carrete et al., 2006b). The seemingly high density of kiwi on Ponui Island may indeed suggest a limitation in the availability of high-quality territories. Further research is needed to test the relationship between territory quality and population density in this context. While the reason why kiwi on Ponui Island form polygamous groups is unknown, the existence of polygamy has conservation implications, especially concerning the need to consider density-dependent processes in conservation actions (e.g., translocations) to better predict their outcomes.

Although behavioural mechanisms appear to contribute substantially to pair stability and reproductive outcomes, these patterns may also be shaped by underlying genetic processes. In particular, genes associated with immune function, such as those within the major histocompatibility complex (MHC), can influence individual fitness and reproductive success, and may contribute to patterns of genetic compatibility. The following chapters therefore extend the behavioural focus of this chapter by examining immunogenetic variation and mate-pairing patterns in North Island brown kiwi. Specifically, Chapter 4 explores patterns of MHC diversity and selection, while Chapter 5 examines whether immunogenetic and phenotypic characteristics are associated with pair formation and pair stability.

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Appendix

Figure S2.1. Relationship between incubation effort and hatching success. We fitted a Bayesian generalised linear model using the R (R Core Team 2023) package 'brms' 2.20.4 (Bürkner, 2017) to investigate whether incubation effort (in days, as defined in the main text) was a good predictor of hatching success (i.e., nest survival from incubation onset to chick hatching; Ziesemann et al., 2011). For this, we used a dataset collected by Ziesemann et al. (2011), which consisted of systematic data on incubation effort (as defined in the main text) and hatching success collected from 2006 to 2007, plus additional opportunistic data from 2005, 2010, and 2013 (Castro et al., unpubl. data). Hatching success was coded as a binary variable (failure = 0, success = 1), thus the model used a Bernoulli distribution (link = logit). The model was run using the same settings as in the models in the main text, following the basic syntax: hatching success ~ incubation effort. This model ran smoothly without post-sampling warnings, diagnostic parameters indicated good performance, and posterior predictive checks and Pareto k-values < 0.7 indicated a good fit. We found that incubation effort had a positive (pd = 100%) and strong effect (ROPE = 0.00%) on the probability of hatching success (median = 11.65, 95% CI [6.89, 18.07]). (a) Posterior projected probabilities showing the effect of incubation effort on hatching success. The thick black bars indicate 95% Bayesian high-density intervals (HDI). Dark points on the bars show median estimates. Note that 95% HDI did not overlap zero, indicating a meaningful effect. (b) Projected effect of incubation effort on hatching success. The dark line shows the average predicted probabilities, and the faded blue band indicates 95% uncertainty (projected posterior) intervals. Raw data points (grey dots) were jittered slightly to reduce overlap and enhance clarity. Raw data points (grey dots) were jittered slightly to reduce overlap and enhance clarity. When incubation effort exceeds 75 days (vertical red line), which is consistent with a full incubation bout (Potter 1989; McLennan 1988; Colbourne 2002), the probability of hatching success is close to 1. Therefore, we considered nesting success (measured as complete incubation bouts) as an informative proxy of hatching success in North Island brown kiwi.

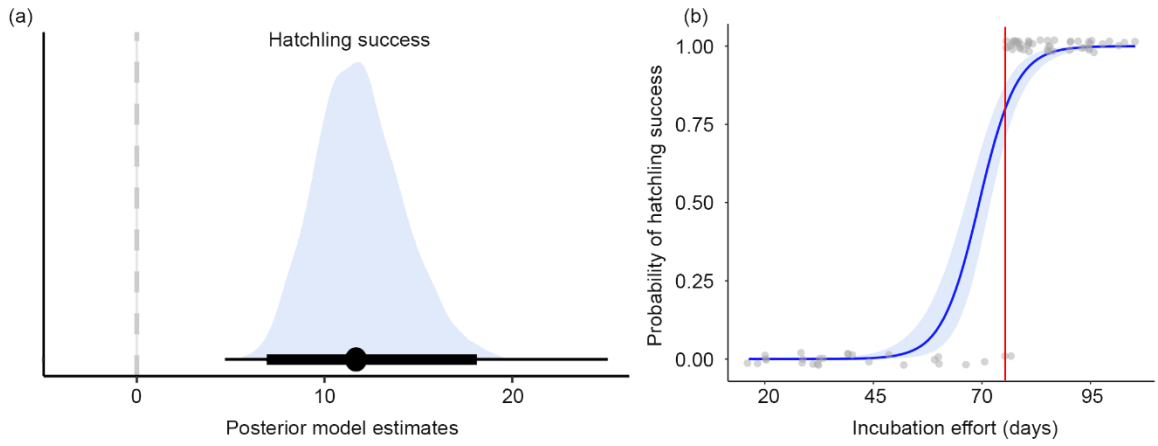

Figure S2.2. Relationships between different linear measures of body size and body weight to select the linear variable for the scaled mass index (SMI). This index accounts for covariation between body size and body mass components by scaling mass against a relative linear body dimension. Here we used tarsus width as a body length proxy, as this metric is more closely related to body mass when compared to other body dimensions (e.g., bill length, tarsus length, and tarsus depth), this linear dimension is expected to best explain the fraction of mass associated with structural size (see Peig & Green 2009). Pairwise Pearson correlation coefficients between the natural logarithm (ln) of body size metrics for males (n = 58) and females (n = 62). Significant ($p < 0.001$) correlations are denoted by ***.

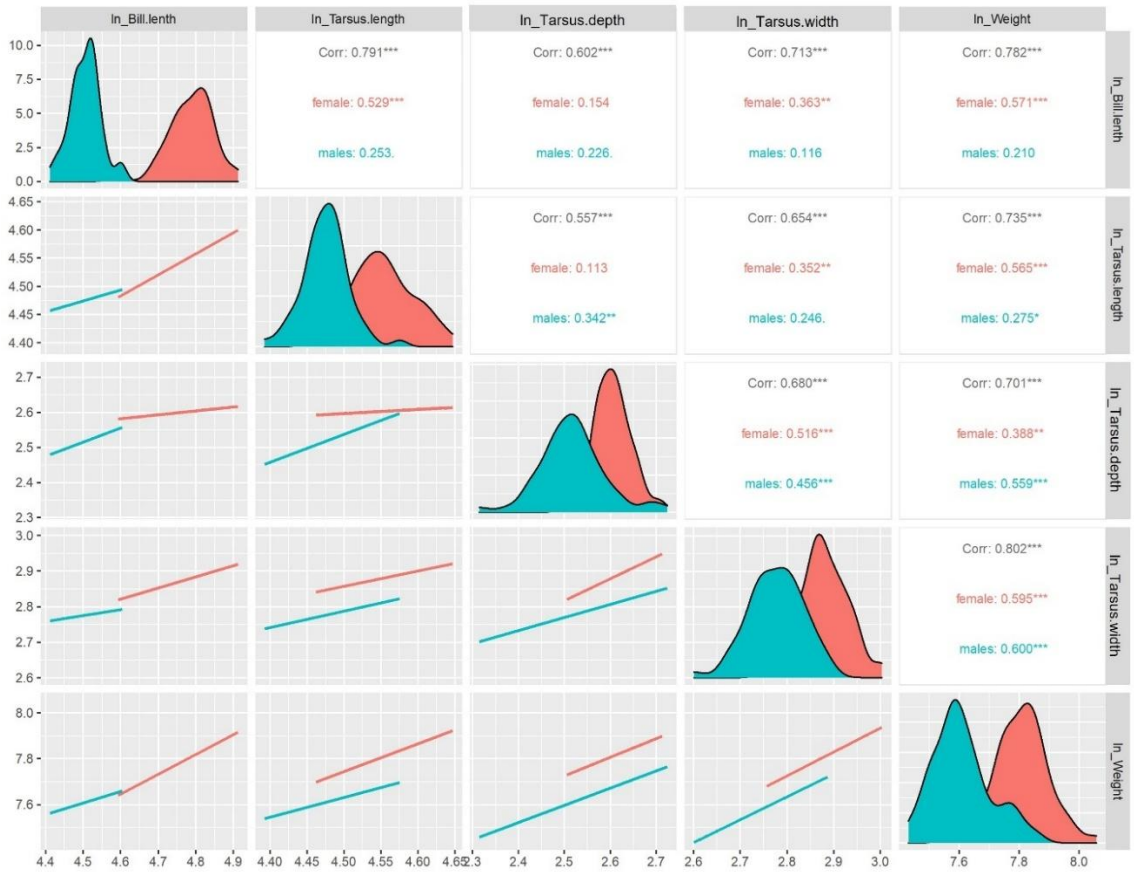

Figure S2.3. Reference model fit and analysis for the latent projection-predictive feature selection. We first fitted an additive Bayesian multilevel reference model including all nine predictors plus the three random variables as defined in the main text. This initial reference model took the form: pair status ~ breeding status + incubation effort + nesting success + breeding attempts + pair familiarity + male quality + female quality + male age + female age + (1| pair identity) + (1|year) + (1| nesting gully). This model ran smoothly without post-sampling warnings, and posterior predictive checks showed a good fit. However, about 11.5% of the observations (n = 113) had high Pareto k-values (> 0.7), indicating influential data points and potential model misspecification (Vehtari et al., 2022). We fitted a new reference model with a simpler structure. Specifically, we removed the random effect of nesting gully and the fixed effects of male and female quality, as these variables did not contribute meaningful information to improve predictive performance. This final reference model took the form: pair status ~ breeding status + incubation effort + nesting success + breeding attempts + pair familiarity + male age + female age + (1| pair identity) + (1|year). This model ran smoothly without post-sampling warnings, and posterior predictive checks and Pareto k-values indicated a good fit. We then used latent projection-predictive feature selection (PPFS) as recommended for binomial models (Catalina et al. 2021) to find the submodel with fewer predictors that showed similar predictive performance as the reference model. To save computation time, we halted the forward search at submodel size three (see Table S2.1).

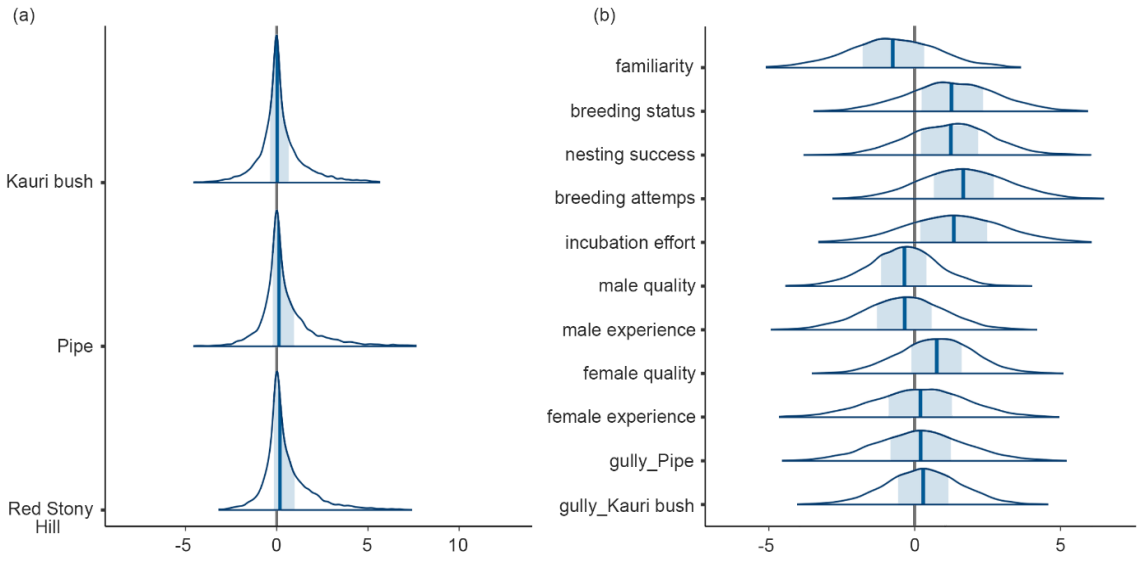

Table S2.1. Predictor ranking for pair status (divorce = 0, fidelity = 1) based on latent projection predictive feature selection. The order of the last three columns (i.e., the predictors) followed the full-data predictor ranking. For each submodel size *i* (first column), the values from the predictor columns (i.e., the last three columns) indicated the proportions of cross-validation (CV) folds that had the predictor from the respective column at position *i* of their forward search's predictor ranking. Since the search was terminated at size 3, which was less than the total number of predictors in our dataset, proportions did not need to sum to 1 in all cases. Note that incubation effort ranked first 90% of the time.

Submodel size	Incubation effort	Breeding success	Familiarity
1	0.9	0.0	0.0
2	0.1	0.3	0.3
3	0.0	0.4	0.3

Table S2.2. Diagnostics and posterior summary statistics for the projected pair status (fidelity = 1, divorce = 0) submodel with the top-ranked predictor (i.e., incubation effort) that had a predictive performance close to that of the final reference model, as indicated by the latent projection predictive feature selection. We reported posterior medians (median), median absolute deviation (mad), 95% Bayesian high-density intervals (95% HDI), Rhat values, effective posterior sample size (ESS), and the Bayesian indices of effect existence (probability of direction = pd%) and significance (region of practical equivalence = ROPE%), with meaningful values indicated with an asterisk (*).

Parameter	Median	mad	95% HDI	Rhat	ESS	Pd%	ROPE%
intercept	5.01	1.326	[3.10, 8.17]	1.00	5,990	100*	0.00*
incubation effort	3.82	1.501	[2.03, 6.63]	1.00	6,007	100*	0.00*

Table S2.3. Diagnostic and posterior summary of the estimated parameters of the models investigating the familiarity effect on nesting success (a) and incubation onset (b). We reported posterior medians (median), median absolute deviation (mad), and 95% Bayesian high-density intervals (95% HDI) as measures of variance for the estimated parameters. Rhat values, effective posterior sample size (ESS), and Bayesian indices of effect existence (probability of direction = pd%) and significance (region of practical equivalence = ROPE%) are also reported. Meaningful values for Bayesian indices are indicated with an asterisk (*). Estimates for random effects are reported as standard deviations.

Parameter	Median	mad	95% HDI	Rhat	ESS	Pd%	ROPE%
(a) nesting success ~ pair familiarity × breeding experience + (1|pair identity) + (1|year)							
intercept	1.20	0.438	[0.35, 2.21]	1.00	6132	99.65*	0.00*
familiarity	2.23	0.981	[0.21, 4.30]	1.00	5970	99.05*	0.00*
male experience	−0.69	0.935	[−2.60, 1.20]	1.00	5958	76.85	12.23
familiarity × male experience	1.58	1.790	[−1.46, 5.53]	1.00	6166	82.95	5.95
Random effects							
couple identity	0.85						
year	0.51						
(b) breeding onset ~ pair familiarity × breeding experience + (1|pair identity) + (1|year)							
intercept	5.45	0.027	[5.39, 5.50]	1.00	5694	100*	0.00*
familiarity	−0.06	0.054	[−0.17, 0.05]	1.00	5723	85.52	78.91
male experience	0.07	0.049	[−0.04, 0.17]	1.00	5890	90.63	75.72
familiarity × male experience	0.01	0.063	[−0.12, 0.13]	1.00	6177	55.90	93.05
Random effects							
couple identity	0.07						
year	0.04						

Table S2.4. Diagnostic and posterior summary of the estimated parameters of the models investigating differences in daily incubation effort between male kiwi sharing incubation in breeding groups and incubating alone in pairs. We reported posterior medians (median), median absolute deviation (mad), 95% Bayesian high-density intervals (95% HDI), Rhat values, effective posterior sample size (ESS), and the Bayesian indices of effect existence (probability of direction = pd%) and significance (region of practical equivalence = ROPE%), with meaningful values indicated with an asterisk (*). Estimates for random effects are reported as standard deviations.

Parameter	Median	mad	95% HDI	Rhat	ESS	Pd%	ROPE%
daily incubation effort ~ incubation type × proportional incubation day + (1|male identity) + (1|year)							
intercept	19.64	0.237	[19.17, 20.12]	1.00	6,169	100*	0.00*
incubation type	−0.04	0.252	[−0.56, 0.48]	1.00	6,049	55.48	38.00
proportional day	0.69	0.064	[0.56, 0.81]	1.00	6,211	100*	0.00*
incubation type × proportional day	−0.02	0.069	[−0.16, 0.11]	1.00	6,071	63.17	90.72
Random effects							
male identity	0.51						
year	0.24						
residual	1.09						

Close-up of Jaeden, a North Island brown kiwi (*Apteryx mantelli*), showing the soft, hair-like texture of the plumage

Chapter Three

Diversity and selection signatures within the major histocompatibility complex class IIB of North Island brown kiwi

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Abstract

Translocations are key tools for species recovery, but their genetic outcomes remain poorly understood. The North Island brown kiwi (*Apteryx mantelli*) population on Ponui Island offers a unique case to examine the effects of admixture following a hybrid translocation between two genetically distinct source populations. By sequencing the MHC class IIβ exon 2 region, we assessed functional genetic diversity and selection in 136 individuals spanning the Ponui Island receiver population and its two source populations, Trounson and Hauturu-o-Toi. We identified 96 functional alleles distributed across at least ten loci—representing the highest MHC IIβ diversity reported for any ratite—and typical features of vertebrate MHC genes, including conserved residues and extensive duplication. Despite the small founder size, kiwi on Ponui Island retained substantial diversity, with 26% of alleles being private to the island population, suggesting that admixture contributed to maintaining immune variation. However, nucleotide and amino acid diversity were slightly lower in Ponui Island than in the parental populations, consistent with mild genetic erosion after establishment. Phylogenetic networks revealed no population-specific clustering, but instead strong signals of trans-species polymorphism and recombination, indicating that balancing selection has preserved ancestral MHC variation across kiwi lineages. Signatures of positive selection were found at 26 codon sites, many corresponding to peptide-binding residues known in other vertebrates, highlighting the adaptive relevance of these loci. Overall, our results demonstrate that hybrid translocation can help maintain functional immunogenetic diversity even in small, recently founded populations, and that monitoring adaptive loci such as MHC genes is essential for assessing the long-term viability of managed kiwi populations.

Keywords

Hybrid populations, major histocompatibility complex, North Island brown kiwi, translocations

Introduction

Translocations—the human-mediated movement of organisms between sites—are widely used by wildlife managers to recover populations and reduce extinction risk (Seddon et al., 2014; Hoffmann et al., 2015; Berger-Tal et al., 2020). The most common types of translocations are reintroductions, where organisms are released into areas from which conspecific populations have been extirpated, and reinforcements, where organisms are released into areas with existing conspecifics to boost population viability (Berger-Tal et al., 2020; Undin et al., 2021b). Translocations are a common conservation and management tool but require sound science, a deep understanding of species biology, and monitoring of outcomes within an adaptive framework (Renan et al., 2018; Berger-Tal et al., 2020; Undin et al., 2021b). The low success of many past translocation efforts can be attributed to the failure to align reintroduction science with good planning (Germano et al., 2014; Taylor et al., 2017a; Berger-Tal et al., 2020). Predicting translocation outcomes is particularly challenging due to the complex interactions between genotype, phenotype, behaviour, and environment (Renan et al., 2018; Undin et al., 2021b). These factors add complexity to translocations, especially when mixing organisms from genetically distinct populations, raising concerns about the conservation value and fate of such human-settled populations (Undin et al., 2021c).

These issues are illustrated by translocations of highly threatened flightless, nocturnal kiwi *Apteryx* species (Palaeognathae: Apterygiformes: Apterygidae) endemic to Aotearoa New Zealand. These iconic long-lived birds were once common and widespread but today are confined to small and isolated populations that rely on active conservation management to persist (Innes et al., 2015; Germano et al., 2018), as evidenced by the growing number of translocations since the 19th century (Jahn et al., 2022). Most kiwi translocations have focused on establishing populations in areas outside of their historical ranges, such as offshore islands and other mainland areas, and to a lesser extent on reinforcing existing populations and reintroducing birds following local extinctions (Jahn et al., 2022). Early translocations overlooked demographic and genetic factors crucial for long-term success (Craig et al., 2011; Germano et al., 2018; Undin et al., 2021c; Jahn et al., 2022), which may explain their general failure (Jahn et al., 2022). Extant kiwi populations remain small and isolated, raising concerns of potential inbreeding depression (Undin et al., 2021c). Small populations with eroded genetic

diversity are unlikely to have the genotypic scope to respond to natural selection arising directly from rapid environmental change (Bowler et al., 2020; Meza-Joya et al., 2023). Hence, today kiwi translocations bring genetic management to the table to maximise their success (Miller et al., 2011; Undin et al., 2021c; Jahn et al., 2022).

The North Island (NI) brown kiwi *Apteryx mantelli* offers opportunities to study the genetic outputs of translocations into novel environments, given a long and well-known history of introductions on offshore islands with known founder population size, establishment time and source populations (Undin et al., 2021c). This is the case of kiwi on Ponui Island (1,770 ha), a population that was established in 1964 by translocating eight founder birds from Waipoua Forest and six from an introduced population on Hauturu-o-Toi Island (Colbourne, 2005; Craig et al., 2011). While anecdotal data suggest an unknown or hybrid origin for kiwi on Hauturu-o-Toi (Baker et al., 1995; Burbidge et al., 2003), genetic data imply a Taranaki origin (Undin, 2021). Kiwi on Ponui Island are deemed hybrids (Figure 3.1) resulting from admixture between two geographically separated and genetically distinct management units, Northland and Western (Craig et al., 2011; Scrimgeour and Pickett, 2011; Germano et al., 2018; Undin et al., 2021b). These source units could have diverged about 100–220 kiloyears ago (Weir et al., 2016), and this could have allowed selection or genetic drift and restricted gene flow to contribute to population differentiation, although the relative roles of these processes remain uncertain (Undin et al., 2021b; Undin et al., 2021c).

Kiwi on Ponui Island are thought to number around one bird per hectare despite predation by feral cats (Castro and Cunningham, pers. obs.; Strang, 2018). Although the accuracy of this estimate is unclear, this apparent high density suggests a population growth rate comparable to that of populations in predator-free sanctuaries and/or areas where juvenile mortality is reduced through egg or young chick rescue programs (Undin et al., 2021b). Rapid population growth in Ponui Island over only 4–6 kiwi generations has raised concerns about potential inbreeding depression given the small founder population (14 birds) and outbreeding depression from mixing birds adapted to different local conditions (Undin et al., 2021b; Undin et al., 2021c). This is of particular concern for long-lived species with overlapping generations, such as kiwi, as long generation times can mask inbreeding depression for many decades despite positive population growth (Taylor et al., 2017b).

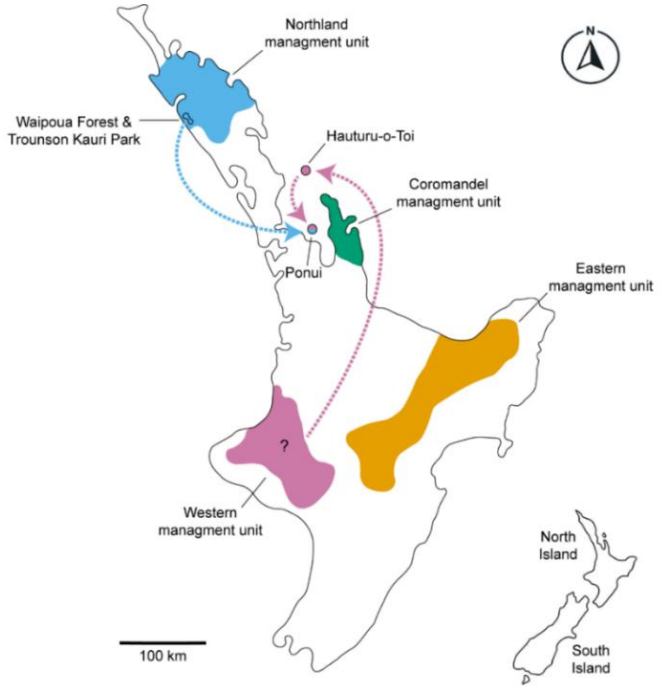

Figure 3.1. Map of North Island (New Zealand) illustrating the approximate distribution of the four geographically disjunct and genetically distinct management units within North Island brown kiwi (Baker et al., 1995; Burbidge et al., 2003; Colbourne, 2005). The location of Ponui Island, its source populations, and the origin of the Hauturu-o-Toi population are indicated with arrows. Translocations from the Western management unit (also known as Taranaki) to Hauturu-o-Toi Island are shown with a question mark to highlight the disputed origin of the kiwi population on that island (Baker et al., 1995; Burbidge et al., 2003; Colbourne, 2005). Redrawn from (Undin et al., 2021c).

Likewise, kiwi populations with current large census sizes may still experience genetic erosion from serial founder effects, as seen in little spotted kiwi *Apteryx owenii* on Kapiti Island (Ramstad et al., 2013). Despite this, recent genomic SNP comparisons suggest that kiwi on Ponui Island constitute a successful hybrid swarm with high genetic diversity and significantly lower-than-expected relatedness between pairing birds, indicative of a kin-recognition mechanism to reduce inbreeding and increase diversity (Undin et al., 2021b; Undin et al., 2021c).

The major histocompatibility complex (MHC) is a highly polymorphic, gene-dense region in vertebrate genomes that encodes cell-surface molecules essential for adaptive immune responses to parasites and pathogens (Eizaguirre and Lenz, 2010; Strandh et al.,

2012; Klein & Figueroa, 1986; Santos et al., 2016). There are two main types of MHC molecules, the MHC class I and II, which are primarily associated with defence against intracellular (e.g., viruses) and extracellular parasites and pathogens (e.g., bacteria), respectively (Piertney and Oliver, 2006; Klein, 1986). Within these types, the highest polymorphism is found in exon 3 of the MHC class I genes and in exon 2 of the MHC class IIβ genes (Eizaguirre and Lenz, 2010). Such extraordinary MHC polymorphism is thought to be mainly driven by pathogen-mediated balancing selection, and therefore MHC genes have become the marker of choice for assessing functional, fitness-related genetic variation in natural populations (Piertney and Oliver, 2006; Ujvari and Belov, 2011). Recent studies suggest that sexual selection via mate choice may contribute to MHC diversity (Knafler et al., 2012; Strandh et al., 2012), and this is relevant when inbreeding is likely, or a genetic kin-recognition system is needed in the absence of social recognition (Jordan and Bruford, 1998).

The characterisation of genetic diversity at functional loci of adaptive significance, such as MHC, is a priority to inform kiwi intensive management (Ramstad and Dunning, 2021). Studies on MHC diversity in kiwi have focused on the two species that experienced the most severe bottleneck histories (Binney, 2007; Miller et al., 2011) and have revealed intriguing outputs. Translocated populations of little spotted kiwi (*A. owenii)* have extremely low diversity, as expected from populations established from at most five founders (Miller et al., 2011). In contrast, the remnant natural population of Okarito brown kiwi (*Apteryx rowi*) has higher-than-expected diversity, despite a small current size (300 – 600 birds; (Germano et al., 2018) and recent bottleneck (Binney, 2007). Here we examined the MHC IIβ structure and diversity of NI brown kiwi on Ponui Island and its two parental units using next-generation sequencing (NGS). We hypothesised that MHC IIβ diversity in brown kiwi on Ponui Island is higher than in its source populations due to the admixture of two parental MHC genomes as found in genomic SNP data (Undin, 2021). We also examined the relative contribution of the parental MHC genomes to the genetic makeup of the hybrid Ponui Island population. In addition, we incorporated MHC IIβ sequences from other kiwi species (Binney, 2007; Miller et al., 2011) into a phylogenetic network to compare interspecific relationships. Finally, we evaluated the role of selection in driving MHC diversity.

Methods

Sampling and DNA extraction

We used blood samples previously collected in four separate cohorts: in 2004, 2006–2008, 2010, 2017–2019 and 2022 (see Undin et al. 2021b) to characterise the MHC IIβ structure and diversity of NI brown kiwi on Ponui Island and its source populations. Samples from Ponui Island (n = 102) were collected between 2004–2022, including blood (n = 84) and feather (n = 12) samples taken from transmitter-marked birds, and eggshell membranes (n = 6) from eggshell retrieved from completed nests. Unfortunately, we were not able to include samples directly from the source populations (i.e. Waipoua Forest and Hauturu-o-Toi Island), thus, samples collected in 2020 and 2021 from Trounson Kauri Park, Remutaka Forest and Pūkaha National Wildlife Centre (Mount Bruce) were used to represent the genetic makeup of these parental populations (see Undin et al., 2021b). Blood samples from Trounson Kauri Park (n = 19) were used to represent the genetic makeup of Waipoua Forest population as kiwi in this area has been declining since the early 1990s (Craig et al., 2011). Trounsen was continuous with Waipoua prior to human disturbance from Mid-1800s – Early 1900s. Likewise, blood samples from kiwi translocated from Hauturu-o-Toi Island to Remutaka Forest and Pūkaha National Wildlife Centre (n = 15) were used as a representative of kiwi from Hauturu-o-Toi Island (Figure 3.1). Tissue collection was granted by the Department of Conservation Wildlife permits AK-14969-RES, AK-21519-FAU, 50249-FAU and 70875-RES. Genomic DNA (gDNA) was extracted from 136 samples using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland), following the manufacturer's instructions. The quality and quantity of the extracted gDNA were assessed by three methods: agarose gel electrophoresis (1% w/v agarose in 1X TAE buffer) for assessing gDNA purity and integrity; Nanodrop (Thermo Fisher Scientific) for evaluating gDNA purity (OD 260/280 ratio); and Qubit 2.0 fluorometer (Thermo Fisher Scientific) for quantifying gDNA concentration.

MHC IIβ primer design, library preparation and Illumina sequencing

Primer design targeted the MHC IIβ exon 2 genes, which are directly involved in pathogen recognition and exhibit high levels of amino acid diversity (Radwan et al., 2020). Primer design used NI brown kiwi sequences from the National Center for

Biotechnology Information (NCBI) sequence read archive (SRA BioProject PRJNA745563) and whole genome sequencing (WGS BioProject PRJEB6383) datasets, along with MHC IIβ sequences from another congeneric species, *A. owenii* (Miller et al., 2011; GenBank accession numbers HQ639688–96). We retrieved 27 and nine MHC IIβ sequences from the *A. mantelli* and *A. owenii* datasets, respectively. Sequences were then aligned using MAFFT (Katoh and Standley, 2013) with default settings in Geneious Prime® 2019.1.1 (Kearse et al., 2012). The *A. owenii* forward primer (LSKMHC2F1-GC; Miller et al., 2011) was modified to amplify the same region in the NI brown kiwi along with a new reverse primer. The novel primer set sequences are: NIBK_MHCIIβe2-F 5'-TCTCTCTGCACAACAGGGTATTTCC-3' and NIBK_MHCIIβe2-R 5'-CTCTCCCGTGCACTCACCTCTCC-3'. This primer set amplifies a conserved region at the intron–exon 2 boundaries, as indicated by Sanger-sequenced PCR products using an ABI 3730 Genetic Analyzer (Applied Biosystems Inc) and BLAST searches in the NCBI database (https://blast.ncbi.nlm.nih.gov). The resulting amplicons covered approximately 94.4% of the exon 2 length (~260 base pairs; bp).

Library preparation used a dual polymerase chain reaction (PCR) protocol for 136 samples, plus 10% as replicates (n = 14), totalling 150 samples. During the first PCR, fragments were amplified using our primer set (NIBK_MHCIIβe2-F and NIBK_MHCIIβe2-R) with 5' overhang Illumina adapters (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-NIBK_MHCIIβe2-F and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-NIBK_MHCIIβe2-R) under the following PCR conditions: initial denaturation at 95 °C for 4 min, 30 cycles of denaturation at 95 °C for 30 sec, annealing at 57 °C for 30 sec, extension at 72 °C for 30 sec, and final extension at 72 °C for 5 min. The resulting PCR products were cleaned using AMPure XP Beads (Beckman Coulter) following the manufacturer's protocol. The quality of the purified amplicons was checked on 2% agarose gel, and DNA concentration was measured using a Qubit® 3.0 fluorometer (Thermo Fisher Scientific). During the second PCR, unique barcodes for dual indexing were attached to the purified amplicons containing Illumina overhangs using the Nextera XT DNA index kit (Illumina) according to the manufacturer's instructions under the following PCR settings: initial denaturation at 95 °C for 3 min, 8 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for 30 sec, and final extension at 72 °C for 5 min. The indexed PCR products were cleaned using AMPure XP Beads, and the final size of

the libraries was determined on a Bioanalyzer DNA 1000 Chip (Agilent). Library preparation and Illumina MiSeq sequencing were performed by the Massey Genome Service (Palmerston North, New Zealand).

MHC IIβ exon 2 genotyping

Raw MHC IIβ exon 2 paired-end reads were analysed using the genotyping pipeline ACACIA: Allele CAlling proCedure for Illumina Amplicon sequencing data (Gillingham et al., 2021). We first assessed raw reads quality using the FastQC tool (Andrews, 2010), trimmed low-quality read ends, and checked FastQC quality scores of the trimmed sequences. The paired-end reads that passed this first filter were merged using FLASh (Magoč and Salzberg, 2011) with a minimum overlap of 50 bp and a maximum overlap of 250 bp. Then, primer sequences were trimmed, and a second quality filter based on sequence mean phred quality ($q = 30$) and percentage scores ($p = 90$) was performed. We removed singletons (i.e. sequences that appear one single time) to reduce randomly generated artefactual sequences (Quail et al., 2012) and chimeras (i.e. sequences derived from two or more distinct parental sequences) using the vsearch tool (Rognes et al., 2016). The remaining sequences were blasted against MHC IIβ exon 2 sequences from other paleognaths (ratites and allies) as well as fowls (ducks, chickens and allies) to eliminate unspecific PCR priming products. Sequences that passed this filter were aligned using MAFFT, then the oligotyping tool (Eren et al., 2013) was used to call candidate alleles. The final allele calling included the removal of unique allele variants and the retention of 'true' amplicon variants by adjusting two parameters: 'absolute number of reads' (abs_nor) and 'lowest proportion of reads' (low_por). We tested distinct abs_nor values: 0–50 reads, at 10-read intervals, with low_por set at 0, and low_por scores: 0–10%, at 0.5% intervals, with abs_nor set at 10 (Gillingham et al., 2021). This procedure indicated that for a variant to be retained as 'true', the optimal abs_nor and low_por scores were 10 and 1.5%, respectively.

We considered our genotyping to be reliable using the criteria (1) high similarity to known MHC IIβ exon 2 alleles from other bird species (MegaBLAST hits with e-values $< 1e^{-5}$ and > 85% sequence identity), (2) high sequencing repeatability (98%; $n = 14$), calculated as the average percentage of alleles called in both replicates across samples (Gillingham et al., 2021); and (3) congruent patterns of putative alleles segregation

between related individuals (i.e. parents with known offspring; n = 10) in our dataset (Gaigher et al., 2016).

Diversity and inference of selection in the MHC IIβ exon 2

MHC IIβ exon 2 sequences for NI brown kiwi were aligned using the MAFFT plugin in Geneious with default settings, and the pairwise sequence identity was estimated in the same software. Sequence polymorphism was assessed as the number of polymorphic sites, the average number of nucleotide differences (k), and nucleotide diversity (π) using DNAsp 6.12.03 (Rozas et al., 2017). Amino acid p-distances with uniform rates were estimated in MEGA 11 (Tamura et al., 2021). Phylogenetic relationships among sequences from NI brown kiwi and other kiwi species were inferred with Neighbor-Net networks Splitstree4 (Huson and Bryant, 2005). This approach is well-suited to the MHC system, which is known to evolve by gene duplication and recombination (Gaigher et al., 2023).

We looked for signatures of positive selection by comparing rates of non-synonymous substitutions (dN) to synonymous substitutions (dS) in amino acid sites (ω = dN/dS ratio) in PAML 4.10.6 (Yang, 2007). To infer codon sites undergoing positive selection, we used the maximum likelihood site-models in CodeML as implemented in PAML. We compared four models: M1a (nearly neutral), M2a (positive selection), M7 (β : ω ratio varies among sites according to a β distribution) and M8 (β + ω: similar to M7 but with an additional site class that allows ω > 1). Trees used as input for CodeML analysis were inferred with MrBayes (Ronquist and Huelsenbeck, 2003) with K2 + Γ as the best-supported substitution model inferred by MEGA 11. Analyses used four chains on two runs for 10^6 generations, with a sampling frequency of 10^3 generations and a burn-in of 0.10. Convergence of the posterior distribution parameters was examined by monitoring the effective sample size and trace plots in Tracer v1.6 (Rambaut et al., 2018). Analyses were performed separately on alleles of different lengths (no insertion, 3 bp insertion and all alleles). Because positive selection was detected in all datasets, positively selected codons were identified using the Bayes empirical Bayes approach (Yang et al., 2005) implemented in PAML.

We also tested for positive selection at each codon site separately using two complementary approaches implemented in Datamonkey 2.0 (Weaver et al., 2018):

FUBAR (fast, unconstrained Bayesian approximation; Murrell et al., 2013) and MEME (mixed-effects model of evolution; Murrell et al., 2012). FUBAR posterior probabilities > 0.9 were considered indicative of strong selection (Murrell et al., 2013), and the significance threshold for MEME was set to $P < 0.05$. The strength of selection was estimated by calculating the average dN/dS ratio across all sequence pairs using codon-based Z-tests of positive selection (probability dN > dS) in MEGA 11, with 1,000 bootstraps for variance estimation. This analysis was run for the entire exon 2 using the Nei-Gojobori model with Jukes-Cantor correction for multiple substitutions (Nei and Gojobori, 1986). As positive selection is expected to be strongest at peptide-binding sites (PBS), we ran the analysis using the set of codons of humans (Brown et al., 1993) and chicken PBS (Zhang et al., 2020), as well as those codons recently identified as positively selected in non-passerines (Minias et al., 2018).

Results

Protein-coding MHC IIβ exon 2 sequences were successfully obtained from 147 out of 150 samples, which yielded 8,919,035 pairs of raw 250 bp-long reads, with an average of 59,460 ± 13,259 s.d. (standard deviation) reads per sample. The genotyped region produced fragments of 85–86 amino acid residues, covering approximately 94% of the full exon 2 encoding for the beta 1 domain of the MHC receptor. Following quality control and primer trimming, and after removing chimeras and BLAST hits, we retained 3,346,057 reads, with an average of 22,762 ± 4,959 s.d. reads per sample, which were used to define alleles. We found no relationship between the number of alleles and read depth per sample ($R^2 = 0.013$, $F_{(1,145)} = 0.198$, $p = 0.657$), indicating that our genotyping results are not biased by read depth (Figure S3.1). Several structural features typical of vertebrate MHC IIβ exon 2 were found in NI brown kiwi (Figure 3.2), including two canonical cysteines involved in disulfide bonds (Cβ15, Cβ79), two N-linked glycosylation sites (Nβ19, Tβ21), and two peptide-binding residues important for establishing hydrogen bonds (Wβ61, Hβ82), as well as other conserved characteristics and residues (see Kaufman et al., 1994). Despite this, variation in some conserved residues was detected in a small number of sequences (Figure 3.2). Sequences from the

NI brown kiwi showed high similarity to those previously described for the little spotted kiwi (Miller et al., 2011).

Genomic organisation of MHC IIβ loci

We reconstructed the genomic organisation of the MHC IIβ region for NI brown kiwi using the reference genome bAptMan1, assembled at the chromosome level (NCBI BioProject PRJNA1061176, assembly accession GCF_036417845.1). We identified ten different MHC IIβ loci spread across a ~100 kbp region on chromosome 32 (3.13 Mbp). All loci but one were oriented in the same direction (Figure S3.2). For each locus, we retrieved the exons encoding for the signal peptide, the peptide-binding domain (β1), the immunoglobulin superfamily domain (β2), the connecting piece, the transmembrane domain, and the cytoplasmic tail. These exons adhered to the expected organisation of vertebrate MHC IIβ genes.

MHC allelic diversity

We identified 96 putative alleles for MHC IIβ exon 2 (Figure 3.2) after discarding allele sequences that showed signs of non-functionality, such as stop codons or frameshift mutations (n = 7). The number of alleles per individual ranged from 4 to 20, with an average of 12.82 ± 3.28 (s.d.), indicating the amplification and genotyping of at least 10 homologous loci, consistent with the findings from the reference genome. A small number of individuals (n = 6) had fewer than 8 alleles (Figure 3.3a). Notably, 25 putative alleles include a three-nucleotide insertion when compared to the other sequences. This insertion has also been identified in: *A. owenii* and *A. rowi* (Binney, 2007; Miller et al., 2011). Alleles with this insertion appear functional, as no premature stop codons were detected. All individuals had alleles without indels (range = 2–16, average = 9.63 ± 2.84 s.d.), and 97.8% of the individuals carried alleles with the 3 bp insertion (range = 1–7, average = 3.05 ± 1.51 s.d.). Alleles without the insertion were more polymorphic (π = 0.147) than alleles with the 3 bp insertion (π = 0.133).

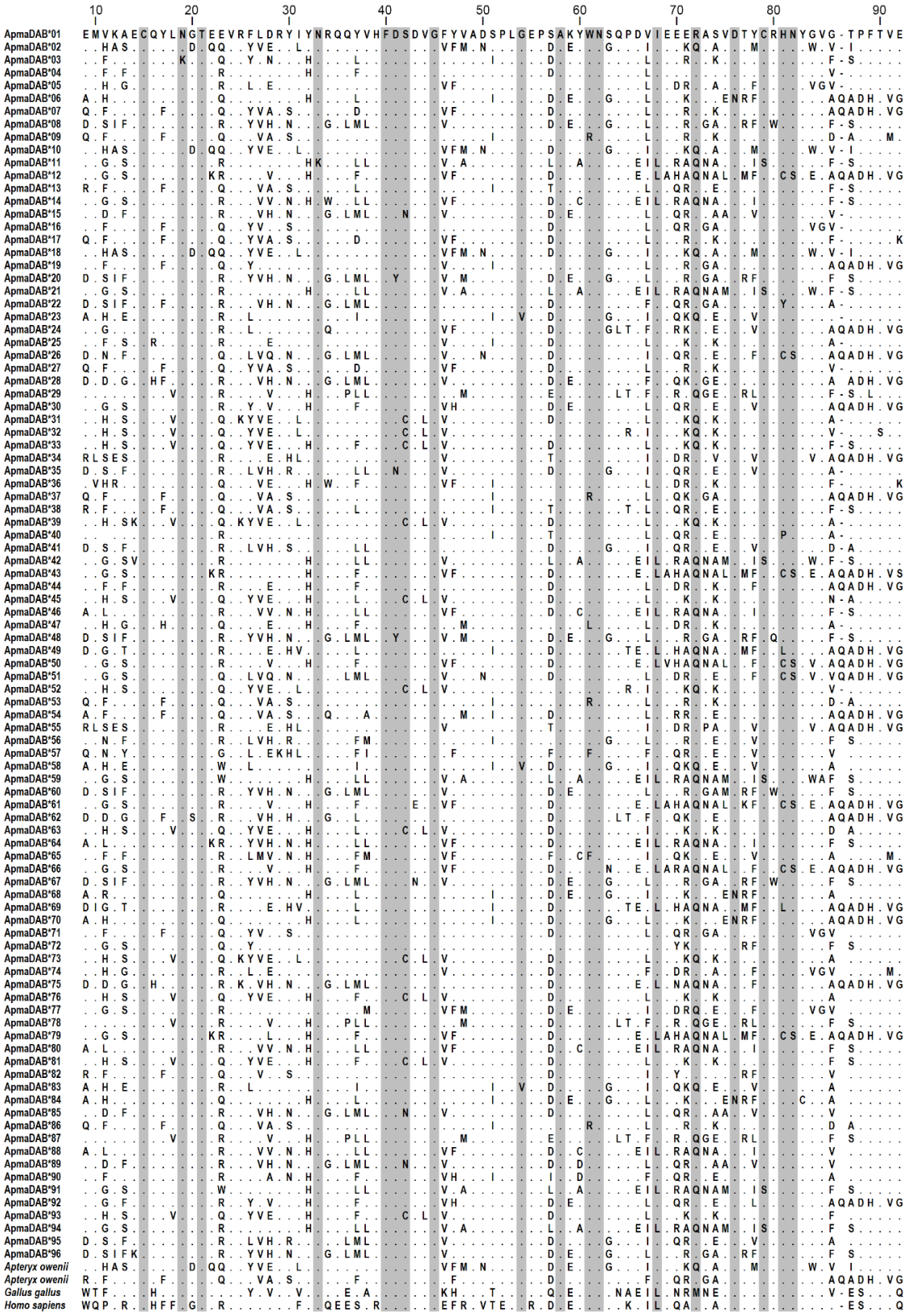

Figure 3.2. Amino acid alignment of NI brown kiwi MHC IIβ exon 2 sequences (ApmaDAB). Dots indicate identity with the ApmaDAB*001 sequence (used as reference), and dashes indicate gaps. Conserved residues found in classical MHC IIβ exon 2 molecules are shaded (see Kaufman

et al. 1994). *Apteryx owenii* (HQ639683.1, HQ639686.1 respectively), *Gallus gallus* (BAF62996) and *Homo sapiens* (NP_002115.2) sequences were included for comparison. Sequences were named in decreasing order of abundance (AmanDAB*01–96) following standard nomenclature (Klein et al. 1990).

The populations with the largest sample sizes displayed the highest number of alleles, with 67 in Ponui Island, in contrast to 54 in Trounson and 39 in Hauturu-o-Toi (Table 3.1). Likewise, the number of polymorphic sites was higher in Ponui Island (S = 160) than in Trounson (S = 151) and Hauturu-o-Toi (S = 142). The larger sample size from Ponui Island likely allowed for a more comprehensive capture of allelic diversity, yet allele saturation was not reached in the other two populations (Figure S3.3), indicating that the number of alleles in these populations is under-represented. Despite its smaller sample size, nucleotide diversity (π) and average number of pairwise differences (k) were higher in Hauturu-o-Toi (π = 0.173; k = 44.05), compared to Trounson (π = 0.170; k = 43.43) and Ponui Island (π = 0.169; k = 43.02). Similarly, amino acid pairwise distance values were higher in Hauturu-o-Toi (0.287) than in Trounson (0.285) and Ponui Island (0.284), indicating relatively higher levels of genetic diversity.

Table 3.1. Genetic diversity at the North Island brown kiwi MHC IIβ exon 2. Sample size (*n*), number of polymorphic sites (*S*), average number of pairwise differences (*k*), nucleotide diversity and standard deviation (π, s.d.), amino acid pairwise distance and standard error (p-distance, s.e.).

Population	***n***	**Number of alleles**	**Number of sites**	***S***	***k***	**π (s.d.)**	**p-distance (s.e.)**
Hauturu-o-	15	39	255–258	142	44.05	0.173	0.287 (0.028)
Trounson	19	54	255–258	151	43.43	0.170	0.285 (0.027)
Ponui Island	102	67	255–258	160	43.02	0.169	0.284 (0.028)
Total	136	96	255–258	166	42.97	0.169	0.284 (0.027)

The MHC IIβ exon 2 of the three populations was composed of shared and unique alleles (Figure 3.3b). Overall, between 6% and 25% of the alleles were unique to each population, with Ponui Island having the largest proportion of private alleles (26%), compared to Trounson (13.5%) and Hauturu-o-Toi (6.3%). Ponui Island shared the largest proportion of alleles with Trounson (32.3%; 24% with Hauturu-o-Toi). A small proportion of alleles (12.5%) were shared by the three populations.

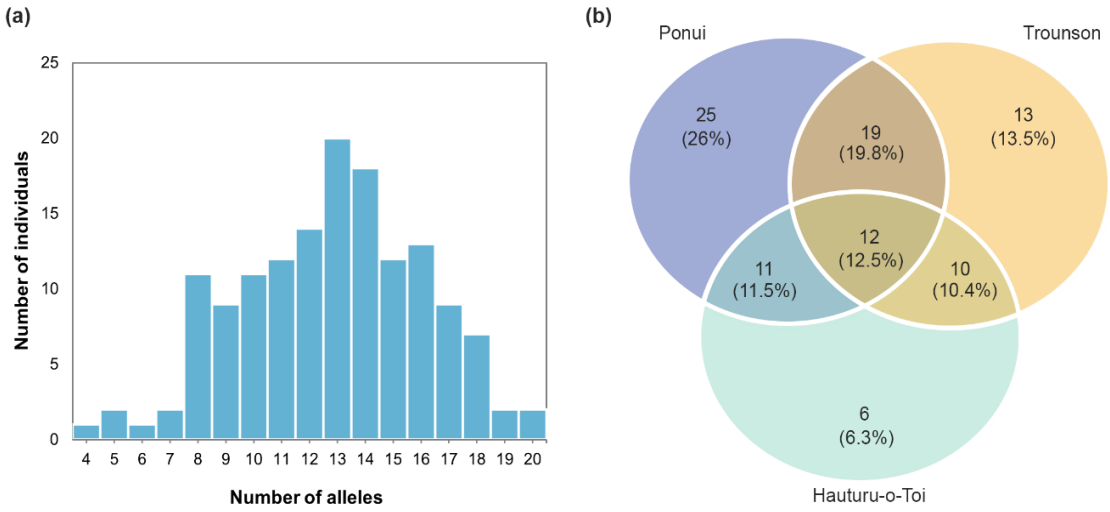

Figure 3.3. Distributions of MHC IIβ exon 2 alleles in North Island brown kiwi. (A) Number of alleles per individual. (B) Venn diagram of unique and shared alleles among populations with percentages based on the total number of alleles.

Phylogenetic networks of the NI brown kiwi MHC IIβ exon 2 did not reveal any clustering separating alleles by populations (Figure 3.4a). This lack of clustering is also observed when including all kiwi species for which sequences are available: great spotted kiwi *Apteryx haastii*, *A. owenii* and *A. rowi* (Figure 3.4b). Here, sequences from different species appeared to be more like each other than to sequences within species, suggesting a trans-species evolutionary model. The numerous conflicting signals in our networks indicate substantial recombination between sequences. Interestingly, in both networks, alleles with a 3-bp insertion were consistently grouped in a single cluster, suggesting genetic similarity and shared ancestry.

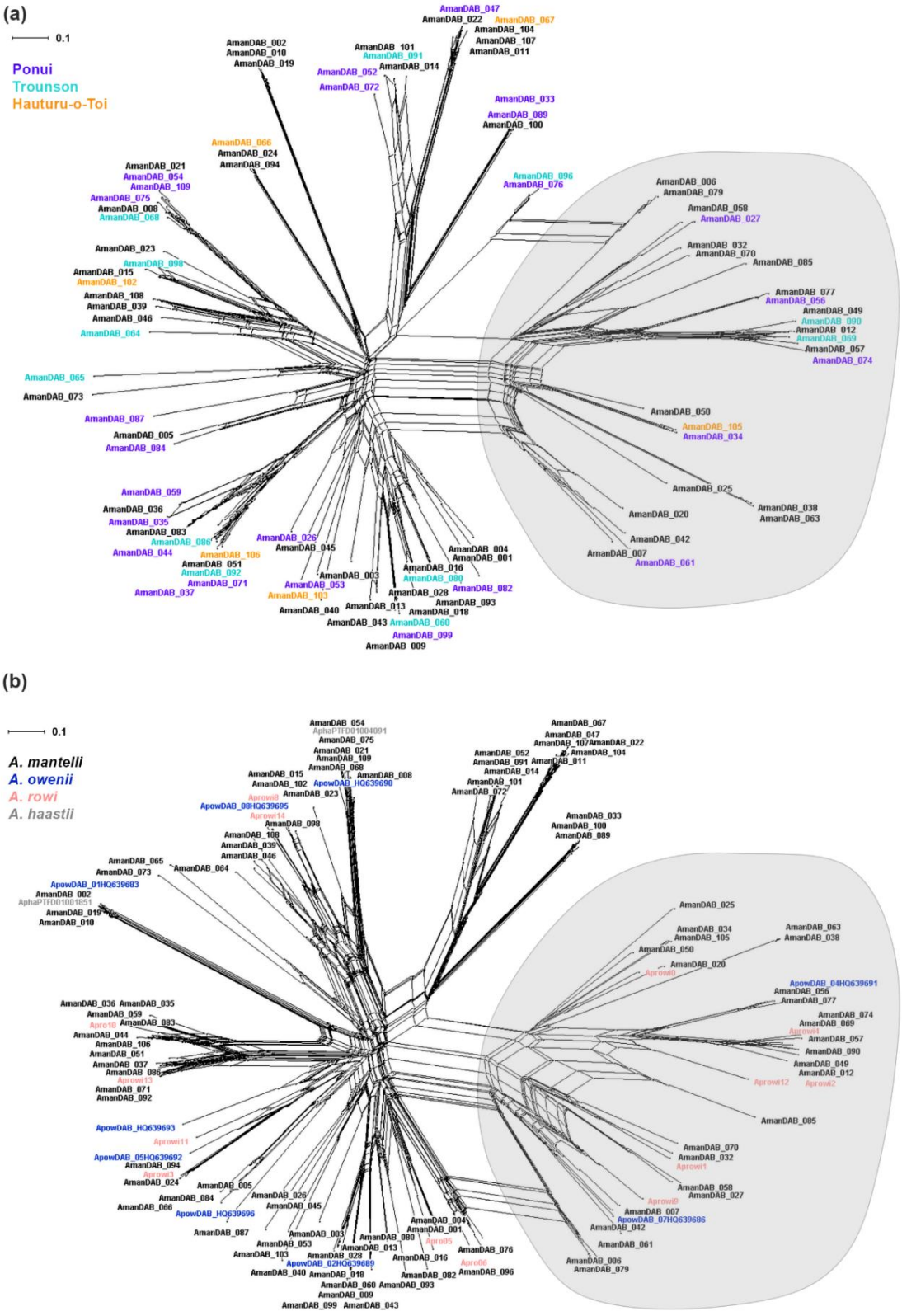

Figure 3.4. Phylogenetic networks comparing North Island brown kiwi MHC IIβ exon 2 alleles from three different populations (a) and from other kiwi species (b). In panel (a), alleles shared between populations are denoted in black, while unique alleles are colour-coded according to their population of origin. Grey shading highlights alleles with a 3-bp insertion.

Inference of selection

We found signatures of positive selection at 26 sites (27.4% of codons) in NI brown kiwi MHC IIβ exon 2 (Table 3.2). Each of the alternative methods for positive selection (FUBAR, MEME, CodeML) inferred several codons showing signatures of positive selection. The method detecting episodic selection (MEME) identified a higher percentage of codons under positive selection (20%; 19 codons) than the method identifying pervasive selection (FUBAR = 14.7%, 14 codons). Additionally, FUBAR identified 19 sites under negative selection (Table S3.1), which generally exhibited no polymorphic alleles. Among the four codon evolution models assessed by CodeML, the positive selection models M8 and M2a had the strongest support (Table S3.2). These models detected a similar percentage of codons under positive selection (14.7% [14 codons] and 13.7% [13 codons], respectively). Seven codons were consistently identified by all methods as being under positive selection, representing 7.4% of codons. Of the 26 sites identified as positively selected, 16 corresponded to peptide-binding sites in humans (Brown et al., 1993), 14 to the peptide-binding sites in chicken (Zhang et al., 2020), and 17 to positively selected sites across non-passerines (Minias et al., 2018).

Table 3.2. Positively selected sites in North Island brown kiwi MHC IIβ exon 2 inferred by mixed-effects model of evolution (MEME), fast, unconstrained Bayesian approximation for inferring selection (FUBAR), and CodeML (best-supported models M8 and M2a). Numbers represent amino acid positions; underlining denotes codons that correspond to positively selected sites of non-passerine (Minias et al., 2018); asterisks show chicken peptide-binding sites (Zhang et al., 2020); bold type indicates peptide-binding sites in humans (Brown et al., 1993).

Method		Positively selected sites
FUBAR		**11*** 26* 27 **28*** **30*** **32*** 46 57* **65** **70*** **71*** 84 **86*** **88**
MEME		**11*** 14 27 **28*** **30*** 46 57* **65** **68** **70*** **71*** 73 **78*** **81*** 84 **86*** **89** 90 91
CodeML	M8	**9*** **11*** **13*** 23 26* **28*** **37*** 57* **65** **70*** **71*** 73 **78*** **86***
	M2a	**9*** **11*** **13*** 23 26* **28*** **37*** 57* **70*** **71*** 73 **78*** **86***

Measuring the strength of selection as the ratio of non-synonymous and synonymous substitutions per site (dN/dS) also revealed that the NI brown kiwi MHC IIβ exon 2 is under positive selection (Table 3.3). As expected, the dN/dS ratio was higher for PBS residues compared to all sites overall, reflecting the selective pressure exerted on these residues. Supporting this, the Z-test consistently indicated significant positive selection for non-passerine and human PBS in all data partitions ($Z > 2.17$, $P < 0.012$). However, chicken PBS were only significant for alleles with a 3 bp insertion ($Z = 2.960$, $P = 0.002$). These significant associations indicate that human and non-passerine PBS are good predictors of positively selected sites in the NI brown kiwi MHC IIβ exon 2.

Table 3.3. Average rates of non-synonymous substitutions (dN) and synonymous substitutions (dS) at all sites and putative peptide-binding sites (PBS) according to human (Brown et al., 1993) and chicken peptide-binding sites (Zhang et al., 2020), as well as positively selected sites (PSS) in non-passerines (Minias et al., 2018). Standard errors (s.e.) are shown. Significant ($P < 0.05$) Z-test values for positive selection (HA: dN > dS) are highlighted in bold.

Partition	dN (s.e.)	dS (s.e.)	dN/dS	*Z*	*P*
All alleles					
PSS non-passerines	0.446 (0.070)	0.220 (0.072)	2.026	2.748	**0.003**
PBS chicken	0.409 (0.071)	0.281 (0.086)	1.456	1.343	0.091
PBS human	0.407 (0.068)	0.217 (0.078)	1.875	2.277	**0.012**
All sites	0.112 (0.020)	0.247 (0.052)	0.455	-2.469	1.000
Non-insertion alleles					
PSS non-passerines	0.404 (0.077)	0.178 (0.055)	2.264	3.011	**0.002**
PBS chicken	0.396 (0.079)	0.263 (0.079)	1.502	1.385	0.084
PBS human	0.356 (0.077)	0.161 (0.056)	2.208	2.617	**0.005**
All sites	0.095 (0.016)	0.213 (0.048)	0.446	-2.308	1.000
Insertion alleles					
PSS non-passerines	0.383 (0.076)	0.118 (0.050)	3.246	3.719	**0.000**
PBS chicken	0.389 (0.068)	0.202 (0.068)	1.925	2.361	**0.010**
PBS human	0.354 (0.074)	0.150 (0.051)	2.357	2.960	**0.002**
All sites	0.086 (0.015)	0.164 (0.044)	0.521	-1.644	1.000

Discussion

Previous studies of kiwi MHC class II are based on genomic cloning (Binney, 2007; Miller et al., 2011). Here, we provide a detailed characterisation and assessment of the genetic diversity of the MHC IIβ exon 2 in a translocated population of NI brown kiwi, along with its two parental units, using NGS. We successfully reconstructed the genomic MHC IIβ region, validated a genotyping protocol for amplifying exon 2, and identified typical features of functional MHC genes, including high polymorphism across multiple loci and signatures of positive selection. Remarkably, we found the highest allelic diversity and sequence polymorphism ever reported in any ratite bird, a group known for its low MHC IIβ diversity (see Miller et al., 2011; Minias et al., 2019). This likely reflects the increased sensitivity in picking up alleles using NGS methods, plus the effectiveness of our primer design.

The accurate reconstruction of the genomic architecture of complex gene families with duplicated loci, such as the MHC, relies on high-quality reference genome sequences (O'Connor et al., 2019; He et al., 2022). Using the high-quality genome for NI brown kiwi (bAptMan1, BioProject PRJNA1061176) as a reference, we mapped ten MHC IIβ exon 2 gene copies to chromosome 32. This finding challenges the expectation of a low number of MHC IIβ copies in non-passerine species, which range from 1 to 8 gene copies, with a mean of 2.04 ± 0.08 s.e. (Minias et al., 2019). This number is even lower in ratites, ranging from 1 to 5 gene copies, with a mean of 3.2 ± 1.62 s.e. (estimated from data by (Binney, 2007; Miller et al., 2011; Minias et al., 2019). Indeed, previous studies have reported a limited number of MHC IIβ gene copies in kiwi, including five copies in *A. owenii* (Miller et al., 2011) and *A. rowi*, and three copies in NI brown kiwi (Binney, 2007). The number of MHC IIβ copies varies widely, even between congenerics, as seen in *Luscinia* passerines, where *L. luscinia* has three and *L. svecica* has 11 copies (Anmarkrud et al., 2010; Minias et al., 2019). The ten gene copies found here sharply contrast with the three reported by Binney (2007). This discrepancy may reflect our larger sample size (134 vs. 10 birds) and differing methodologies (genotyping by NGS vs. genomic cloning). Indeed, sample sizes greater than 100 individuals are often required to achieve sampling completeness of MHC IIβ alleles (Gaigher et al., 2023), while MHC genotyping by NGS enables higher resolution and accuracy compared to genomic cloning (O'Connor et al., 2019).

We characterised MHC IIβ exon 2 variation in an extensive sampling of NI brown kiwi and identified 96 putative alleles, with individual kiwi carrying between 4 and 20 alleles, on average about 14 alleles. This indicates that MHC IIβ genes are extensively duplicated in this species, and the number of alleles varies significantly among individuals. Most kiwi (97.8%) from the studied species (*A. mantelli*, *A. owenii*, and *A. rowi*) have alleles with a three-nucleotide insertion (Miller et al., 2011), suggesting these alleles existed in the common ancestor of kiwi, around 5.96 to 13.43 million years ago (Weir et al., 2016; Grealy et al., 2017; Yonezawa et al., 2017). Several studies of the avian MHC have documented short insertions or deletions (indels) in the exons that code for PBS (e.g., Bollmer et al., 2010; Karlsson and Westerdahl, 2013), but deletions seem to be more prevalent than insertions (Minias et al., 2018). While it remains uncertain whether indels play an adaptive role in MHC-based pathogen recognition in birds (Minias et al., 2018), in silico models predict that allelic variants with indels may possess unique peptide-binding specificity in some avian species (Follin et al., 2013). Despite this, alleles without indels exhibited greater polymorphism compared to alleles with the 3 bp insertion. Thus, kiwi with a greater number and diversity of allelic variants are likely to have enhanced resistance to a broader range of pathogens (see Borghans et al., 2004; Follin et al., 2013).

Depletion of MHC diversity can render small managed populations more susceptible to disease and thus more prone to extinction (Hughes, 1991). Here we found that the number of MHC IIβ exon 2 alleles and the number of polymorphic sites were higher in kiwi on Ponui Island, compared to its parental populations (Table 3.1). This aligns with inferences from genomic SNP data, suggesting that admixture has retained genetic diversity from both parental populations (Undin et al., 2021b). Indeed, 63% of MHC alleles detected in Ponui Island were also present in the parental populations, while the remaining alleles were private (Figure 3.3b). However, caution is warranted, as allele saturation was not reached in the Trounson and Hauturu-o-Toi populations. This indicates that our limited sample sizes (19 and 15 birds, respectively) are insufficient to fully characterise the diversity of MHC alleles in those populations (see Gaigher et al., 2023). Interestingly, a substantial proportion of alleles existing in the source populations (30.2%) were absent in Ponui Island, suggesting that these sequences were either not present in the founders or less likely, mutated from parental sequences after translocation over only 4–6 kiwi generations. This is relevant from a conservation genomics perspective as alleles unique to source populations may increase the immunological

defence against local pathogen pools (Borghans et al., 2004). Nevertheless, allele variants unique to Ponui Island (26%) are likely present in the source populations and will become apparent with future sampling efforts.

Despite having reached allele saturation, kiwi on Ponui Island had the lowest diversity as measured by nucleotide diversity, average number of pairwise differences, and amino acid pairwise distances. This contrasts with genomic SNP data suggesting that kiwi birds on Ponui Island have higher genetic diversity than their source populations (Undin, 2021), yet the accuracy of these estimates is unclear given uneven sampling sizes (Ponui Island = 117 birds, source populations $\leqslant$ 22 birds). There is ample evidence for balancing selection shaping MHC diversity in species with otherwise limited neutral marker diversity (reviewed in Sommer, 2005), which may explain the higher MHC but lower genomic SNP diversity found in our source populations. Notably, avian MHC diversity is often significantly reduced in populations that have undergone artificial genetic bottlenecks, such as translocated populations founded with a small number of birds. For example, extremely low MHC diversity has been found in translocated little spotted kiwi on Kapiti Island and Long Island, whose populations were established with at most five birds (Miller et al., 2011). Likewise, South Island robins (*Petroica australis australis*) translocated to Motuara Island (five founders) have lower MHC diversity than their source population on Nukuwaiata Island (Miller and Lambert, 2004). Reduced MHC diversity is also common in natural bottlenecked populations on small islands, such as the Seychelles warbler *Acrocephalus sechellensis* (Richardson and Westerdahl, 2003) and the Chatham Island black robin *Petroica traversi* (Miller and Lambert, 2004). Therefore, the lower MHC diversity in kiwi on Ponui Island, compared to its source populations, may stem from its small founder population (14 birds; Colbourne, 2005; Craig et al., 2011) and fluctuations in size caused by devastating drought events (Castro et al., 2020).

Interestingly, the relict inland population of Okarito brown kiwi has higher-than-expected MHC diversity (Binney, 2007), despite tight past range contractions (Shepherd and Lambert, 2008) and small current size (300–600 birds) from ~150 birds in the mid-1990s (Holzapfel et al., 2008). While this finding suggests that over a hundred kiwi are needed to maintain high MHC diversity, historical factors (e.g., past distribution and population size) may underpin current patterns of MHC variation (Binney, 2007). Thus,

the number of founder individuals and the demographic history of the source population(s) are crucial for future translocations (e.g., Zeisset and Beebee, 2013). The genomic admixture and rapid population growth of kiwi on Ponui Island over 50 years has been interpreted as indicative of the potential for successful genetic rescue in future reinforcement translocations (Undin et al., 2021a; Undin et al., 2021b). However, caution is warranted as our MHC IIβ exon 2 dataset shows that a significant proportion of alleles are private to the source populations and that kiwi on Ponui Island have comparatively lower diversity in most metrics. Moreover, there is evidence that even at large current sizes, growing kiwi populations may still experience reduced fitness and cryptic inbreeding depression due to overlapping generations and the reproductive success of a handful of founder pairs (Ramstad et al., 2013; Taylor et al., 2017b).

The phylogenetic network analysis of MHC IIβ exon 2 sequences from translocated and founder populations of NI brown kiwi revealed the absence of allele clustering. This suggests that either the MHC IIβ exon 2 genes duplicated too recently for alleles to show orthologous clustering, or that alleles did not evolve independently following gene duplication (Miller et al., 2011). Indeed, highly similar alleles can be shared across loci due to gene conversion, leading to homogenisation via concerted evolution (Goebel et al., 2017; Minias et al., 2023), which aligns well with the recombination signatures observed here. Concerted evolution has been proposed to play a predominant role in shaping diversity at MHC genes in kiwi (Miller et al., 2011) and birds in general (review in Goebel et al., 2017; Minias et al., 2023). In agreement with previous findings (Miller et al., 2011), the phylogenetic network for kiwi revealed that alleles do not cluster by species, suggesting trans-species evolution where duplications predate speciation events and allelic lineages are maintained across species (Klein, 1987; Figueroa et al., 1988). This pattern is characteristic of MHC genes that are under pathogen-mediated balancing selection, which preserves allelic diversity over time (Těšický and Vinkler, 2015; Winternitz et al., 2023). However, we cannot rule out the possibility of convergent sequence evolution driven by similar selective pressures (e.g., pathogens), which can also lead to allele sharing between independently evolved lineages (Srithayakumar et al., 2012; Slade et al., 2019; Winternitz et al., 2023). Interestingly, alleles with a 3-bp insertion consistently grouped in a single cluster (see also Miller et al., 2011), suggesting they may have a common evolutionary origin.

Multiple structural characteristics commonly found in vertebrate MHC IIβ exon 2 were detected in the NI brown kiwi (Figure 3.2), including conserved features at key functional residues (e.g., Wβ61, Hβ81, Nβ82), suggesting peptide presentation (see Winternitz et al., 2023). We detected signatures of positive selection shaping MHC IIβ exon 2 diversity at 26 sites (27.4% of codons) in NI brown kiwi, yet the number of codons inferred to be under selection varied across methods (FUBAR, MEME, CodeML). Most sites evolving under positive selection were found at PBS in humans (Brown et al., 1993) and chicken (Zhang et al., 2020), as well as positively selected sites across non-passerines (Minias et al., 2018). Signatures of past positive selection at MHC IIβ PBS are commonly observed in birds and jawed vertebrates in general and are thought to be mediated by selective pressures imposed by pathogens (see Spurgin and Richardson, 2010; Minias et al., 2018; Gaigher et al., 2023). Despite this, variation was observed in a few sequences at conserved residues of functional importance, hinting at the possibility of functional divergence (Biedrzycka et al., 2017; Marmesat et al., 2017; Winternitz et al., 2023).

As is typical in MHC studies of non-model avian species, we were unable to assign alleles to specific loci, which prevented us from assessing allele sharing across loci or detecting copy number variation (e.g., Gillingham et al., 2016; Sallaberry-Pincheira et al., 2016; Slade et al., 2019; Winternitz et al., 2023). This limitation could introduce biases in our estimates of selection signatures. Despite this, pooling alleles across loci is likely to lead to conservative estimates of selection rather than false positives, as distinct selection patterns across pooled sites should reduce the overall selection signal (Gillingham et al., 2016; Marmesat et al., 2017). Further studies sequencing larger MHC IIβ regions (e.g., Llaurens et al., 2012), generating long-read genomic data (e.g., He et al., 2020), or examining allele segregation patterns in families (e.g., Gaigher et al., 2016; Gaigher et al., 2018) could help overcome this limitation and confirm genomic rearrangements, improve allele scoring and determine the extent of copy number variation in the studied populations (Gaigher et al., 2023; Winternitz et al., 2023).

Genomics is a young field and there are still numerous gaps to be addressed to inform successful kiwi management strategies aimed at increasing population numbers without compromising genetic diversity (Undin et al., 2021a). While we found that kiwi on Ponui Island generally have lower MHC IIβ exon 2 diversity than their source populations, the potential effects of such reduced variability on this population's viability are unclear,

given its recent origin. Lower MHC diversity does not imply this population is facing imminent extinction, as previous research shows that most monitored pairs breed annually (Undin and Castro, 2022; Chapter 2) and encounters with juveniles and subadults in the field suggest recruitment (see Ramstad et al., 2013). However, it may result in poor fitness that may become apparent under stressful environmental conditions (e.g., Ramstad et al., 2013), due to a reduced ability to respond effectively to disease challenges (e.g., Miller et al., 2011). Predicting successful kiwi translocation outcomes that involve genetic rescue requires detailed information about the genetic and epigenetic variation (Reviewed in Undin et al., 2021a), as well as robust estimates of the current effective population size of the target as well as the source populations (see Ramstad & Dunning, 2021). A deeper understanding of how pathogens vary across populations is also essential to elucidate the mechanisms maintaining MHC diversity (Gillingham et al., 2017) and its impact on individual fitness-related traits (Gaigher et al., 2023).

The substantial MHC diversity observed in kiwi on Ponui Island indicates that immunogenetic variation has been retained within this translocated population. However, the functional or behavioural consequences of this diversity remain uncertain. Determining whether kiwi preferentially associate or maintain pair bonds based on MHC characteristics is therefore necessary to better understand the relationship between immunogenetic diversity and reproductive behaviour. The high MHC diversity observed in kiwi on Ponui Island may have important implications for reproductive behaviour and mate choice within this translocated population. If kiwi are capable of assessing genetic compatibility through behavioural, olfactory, or other phenotypic cues, as suggested in other vertebrates, MHC variation could potentially influence pair formation, pair retention, or reproductive success. These possibilities are explored further in the following chapter.

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Appendix

Table S3.1. Negatively selected sites in North Island brown kiwi MHC IIβ exon 2 inferred by fast, unconstrained Bayesian approximation for inferring selection (FUBAR).

Method	**Negatively selected sites**
FUBAR	12 15 16 17 19 21 24 29 39 40 42 43 45 47 49 55 58 62 77

Table S3.2. Likelihood values and parameter estimates for the four candidate models of codon evolution assessed by CodeML. The positive selection models M8 and M2a had the strongest support, both models allowing positive selection (i.e., M2a and M8).

Model	Parameter estimates	ln L	2ΔlnL	P-value	AIC
M1a	p: 0.647 0.352 w: 0.121 1.000	-4590.33	89.768	3.21E-20	9520.65
M2a	p: 0.592 0.222 0.184 w: 0.161 1.000 2.622	-4545.44			**9434.88**
M7	p = 0.353 q = 0.503	-4589.52	94.476	3.06E-21	9519.04
M8	p0 = 0.807 p = 0.587 q = 1.228 (p1 = 0.192) w = 2.293	-4542.28			**9428.56**

Figure S3.1. MHC-IIB exon 2 allele count per amplicon shows no statistically significant relationship with read depth, indicating that allele discovery and final genotypes were unbiased by amplicon read coverage. Summary of results from the linear regression model: R2 = 0.013, F(1,145) = 0.198, p = 0.657.

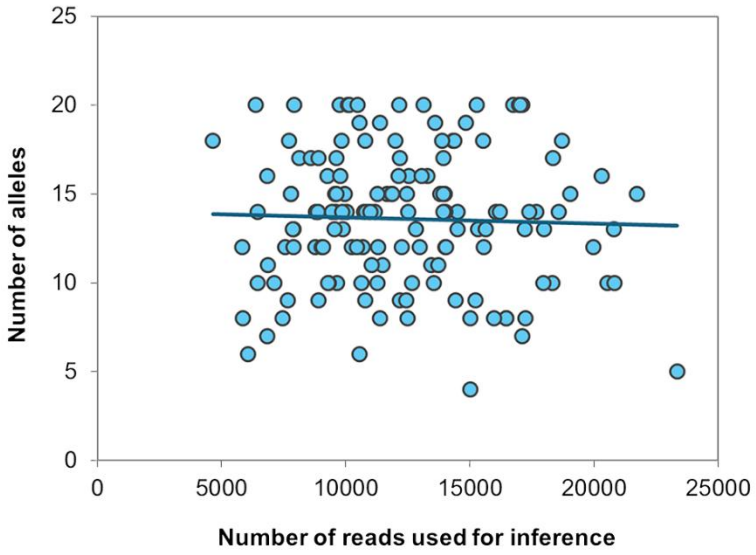


Figure S3.2. Schematic illustration of the genomic organisation of MHC class IIβ region using the North Island brown kiwi, *Apteryx mantelli* reference genome bAptMan1, assembled at the chromosome level (NCBI BioProject PRJNA1061176, assembly accession GCF_036417845.1). We identified ten different MHC IIβ loci spread across a ~100 kbp region on chromosome 32 (3.13 Mbp). Coloured triangles indicate the orientation of the MHC IIβ loci. For each locus, we retrieved the exons encoding for: SP, signal peptide; β1, peptide-binding domain; β2, immunoglobulin superfamily domain; CP, connecting piece; TM, transmembrane domain; CY, cytoplasmic tail. These exons adhered to the expected organisation of vertebrate MHC IIβ genes.

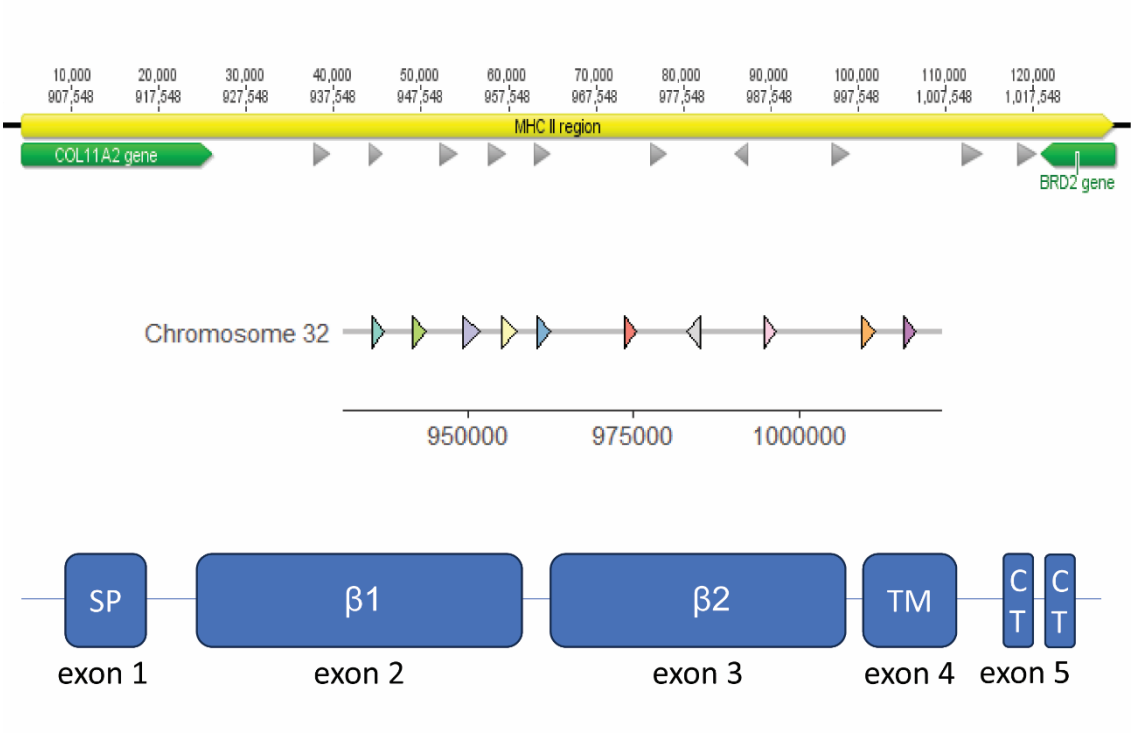
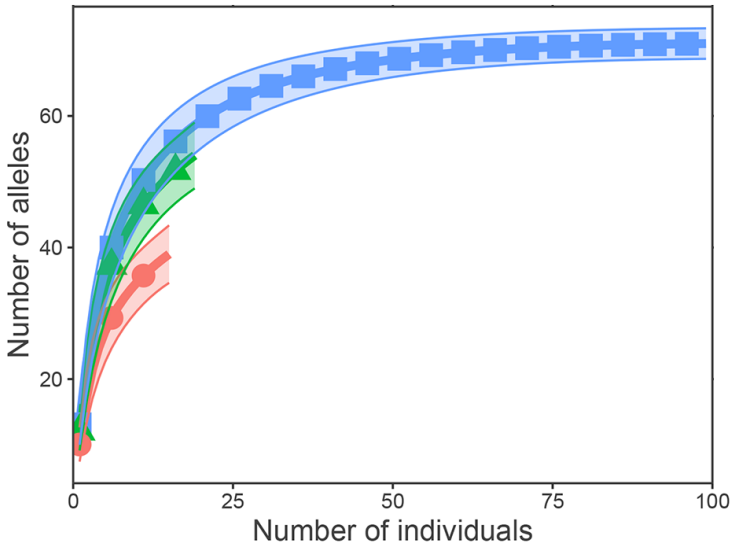


Figure S3.3. Accumulation curves suggest that sampling effort likely captured the allelic diversity for Ponui Island, but not for the other two location Trounson and Hauturu-o-Toi, where no plateau was reached. Ponui Island (blue), Trounson (green) and Hauturu-o-Toi (red).



Jaeden, a male North Island brown kiwi (*Apteryx mantelli*) from Ponui Island (Hauraki Gulf, Aotearoa New Zealand North Island).

Chapter Four

Assessing the existence of major histocompatibility complex-based mate choice in a translocated population of North Island brown kiwi

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Data generation, analysis, and visualization were conducted by Eliana Ramos with assistance from Isabel Castro and Gillian Gibb. Conceptualization was equally undertaken by Eliana Ramos, Isabel Castro, and Gillian Gibb. The original draft was written by Eliana Ramos. The final document was edited and approved by Isabel Castro and Gillian Gibb.

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Abstract

Mate choice represents a major component of individual fitness among sexually reproducing species. Genes of the major histocompatibility complex are considered a key target of mate choice, as they may confer immunological advantages to offspring by enhancing pathogen recognition and defence. While MHC dissimilarity between mates has been suggested to promote mate choice, empirical support remains inconsistent. Moreover, the potential role of MHC in alternative mating strategies, such as divorce, has received little attention. Here, we investigated the role of the MHC IIβ exon 2 in mate choice and pair-bond stability in a translocated population of North Island brown kiwi (*Apteryx mantelli*) on Ponui Island, New Zealand. We analysed MHC data from 80 genotyped individuals, including 33 pairs, using Monte Carlo randomisation tests and Bayesian generalised linear mixed models. Because the sexes differ markedly in reproductive energy investment in kiwi, we analysed males and females separately. Contrary to our expectations, we found no evidence that MHC IIβ exon 2 characteristics influenced the formation of social pairings, regardless of whether females or males were presumed to exert mate choice. Similarly, MHC diversity showed no meaningful effect on pair-bond stability when considering features from either sex, although we detected a weak marginal trend in which males with higher MHC diversity were somewhat more likely to retain pair bonds. Overall, our findings suggest that MHC IIβ exon 2 variation either does not influence mate choice and pair-bond stability in kiwi on Ponui Island or that any such effects are too subtle to be detected with the current dataset. These findings underscore the need to investigate additional MHC regions and increase sample sizes to clarify whether MHC variation influences mate choice and pair-bond retention. Besides this, alternative mechanisms should be considered, as other mating criteria—such as inbreeding avoidance or traits associated with reproductive performance—may supersede the role of MHC in guiding mate selection.

Keywords: *Apteryx mantelli*, major histocompatibility complex, mate choice, pair-bond stability, translocated populations

Introduction

Mate choice—the non-random allocation of reproductive investment by individuals (Paul, 2002; Edward, 2014; Brandies et al., 2018)—is perhaps the most influential decision in animal species that reproduce sexually, and is a powerful catalyst for both natural and sexual selection on reproductive, social and phenotypic traits (Darwin, 1871; Rosenthal, 2017; Neelon et al., 2019). However, how such mate preferences evolved in the first place is not clear (Jones and Ratterman, 2009; Sawada et al., 2020). Since Darwin, evolutionary biologists have documented extensive evidence of sexual selection across animal taxa, with both phenotypic (e.g., body ornaments) and behavioural traits (e.g., courtship displays) often emerging as key targets of mate choice (Andersson and Simmons, 2006; Sawada et al., 2020). Moreover, growing evidence suggests that genetic features, particularly those linked to fitness and compatibility, can also be direct targets of mate selection (for an overview of recent publications on this topic, see Brandies et al., 2018). From an evolutionary standpoint, mate choice driven by sexual selection for genetic benefits plays a critical role in shaping and maintaining gene pools within animal populations (Jennions, 1997; Tregenza and Wedell, 2000; Mays and Hill, 2004; Ferrandiz-Rovira et al., 2016), as exemplified across a wide range of vertebrate taxa (Forsberg et al., 2007; Bos et al., 2009; Miller et al., 2009; Ferrandiz-Rovira et al., 2016; Sawada et al., 2020).

The mechanisms underlying mate selection based on genetic traits are often explained by three non-mutually exclusive hypotheses (Mays and Hill, 2004; Setchell and Huchard, 2010; Kamiya et al., 2014): quantity-of-alleles (good genes as heterozygosity), genetic-compatibility-between-mates (disassortative mating), and advantage-of-particular-alleles (good genes *per se*). Under the quantity of alleles hypothesis, individuals gain a fitness advantage by mating with partners with high genetic diversity, particularly at immune-related loci (Landry et al., 2001), or with elevated heterozygosity across multiple loci, which can enhance offspring competitiveness and mating success (Brown, 1997; Mays and Hill, 2004). The genetic-compatibility-between-mates hypothesis states that mate choice based on genetic dissimilarity driven by the choosier genotype may confer fitness advantages through enhanced offspring genetic diversity and reduced inbreeding risk (Brown, 1997; Setchell and Huchard, 2010; Brandies et al., 2018). Finally, the advantage-of-particular-alleles hypothesis predicts that mate choice favours

individuals carrying specific alleles or genotypes that enhance host adaptation to rapidly evolving antigenic targets, thereby increasing offspring resistance to pathogens (Apanius et al., 1997; Bonneaud et al., 2005; Setchell and Huchard, 2010). Evidence of these mechanisms can be found at the genomic level but may also be linked to specific gene regions, such as those in the major histocompatibility complex (Brandies et al., 2018; Farquharson et al., 2020)

The major histocompatibility complex (MHC) is a highly polymorphic, gene-dense region in vertebrate genomes that plays a crucial role in mediating adaptive immune responses to parasite and pathogen resistance (Klein and Figueroa, 1986; Eizaguirre and Lenz, 2010; Strandh et al., 2012; Santos et al., 2016). The MHC gene family includes two main subgroups of immunologically active molecules. The MHC class I is primarily associated with defence against intracellular pathogens such as viruses, while the MHC class II is related to immune response derived from extracellular parasites and pathogens such as bacteria (Klein, 1986; Klein and Figueroa, 1986; Piertney and Oliver, 2006). Consistent with their immunological function, MHC genes are among the most variable genes described in vertebrate species across a broad taxonomic range (Klein, 1986; Piertney and Oliver, 2006). While the extraordinary polymorphism within this gene cluster is thought to be primarily driven by pathogen-mediated balancing selection, accumulating evidence suggests that sexual selection through mate choice can also contribute to the generation and maintenance of MHC diversity (Milinski, 2006; Knafler et al., 2012; Strandh et al., 2012; Jan Ejsmond et al., 2014; Winternitz et al., 2023). However, high MHC diversity may elevate the risk of autoimmune disease and reduce T-cell receptor diversity (Migalska et al., 2019; Radwan et al., 2020). Therefore, intermediate diversity can, in some cases, represent the optimum for fitness and mate choice (Wegner et al., 2003).

Mate choice driven by MHC genes is expected under conditions where inbreeding risk is high and/or where genetically based kin recognition is required in the absence of social cues (Jordan and Bruford, 1998). Such mechanisms have also been proposed for long-lived bird species with well-developed olfactory capabilities (Zelano and Edwards, 2002). Since kiwi (*Apteryx* spp.) possess a highly developed and functional olfactory system (Wenzel, 1968; Cunningham et al., 2009), they represent promising candidates for investigating MHC-based mate choice (Undin et al., 2021). Social monogamy is the

dominant mating system in brown kiwi, with pairs usually establishing long-term relationships lasting for multiple breeding seasons (Taborsky and Taborsky, 1999; Vieco-Gálvez, 2019; Chapter 1), thus the selection of optimal mates is a critical choice to their lifelong reproductive success. Here, we extend our previous work on the structure and diversity of MHC IIβ exon 2 (Chapter 3) to investigate the role of this genomic region in mate choice and pair-bond stability in a human-translocated population of North Island (NI) brown kiwi (*Apteryx mantelli*) on Ponui Island, New Zealand. Kiwi on Ponui Island (1,770 ha) provide a convenient study system due to their well-documented translocation history. Established in 1964 with eight founders from Waipoua Forest and six from an introduced Hauturu-o-Toi population (Colbourne, 2005; Craig et al., 2011), this population is considered hybrid owing to admixture between two geographically and genetically distinct lineages (Craig et al., 2011; Scrimgeour and Pickett, 2011; Germano et al., 2018; Undin et al., 2021).

The small and heterogeneous founder population on Ponui Island (14 birds from separated lineages) certainly indicates that parental birds faced restricted mate choice and were likely forced into suboptimal pairing. Yet current estimates indicate a rough density of one bird per hectare across the island, even under ongoing pressure from invasive predators (Cunningham et al., 2007). Such rapid growth over only 4–6 generations has raised concerns about inbreeding, given the small founder population, and outbreeding from mixing birds adapted to different local conditions (Undin et al., 2021). These risks are compounded by kiwi's long generation times, which can mask inbreeding depression for decades (Taylor et al., 2017), and by the potential for genetic erosion in large populations founded by few individuals (Ramstad et al., 2013). Despite this, genomic SNP data indicate that kiwi on Ponui Island constitute a successful hybrid swarm with high genetic diversity and lower-than-expected relatedness among pairs, indicative of a kin-recognition mechanism to reduce inbreeding and maximise offspring diversity (Undin, 2021; Undin et al., 2021). While kiwi on Ponui Island are largely socially monogamous, divorce does occur at low rates (8.1%). This phenomenon likely serves as a secondary strategy to address suboptimal pairings and improve reproductive success. Research indicates that females assess nest attendance (incubation effort) when selecting or retaining their partners (Chapter 2). This implies the existence of a certain form of mate 'incompatibility' that can result in breeding failure, thereby creating costs that favour the decision to divorce.

In this study, we first assessed whether MHC-based mate choice occurs in kiwi on Ponui Island. Our previous work on MHC IIβ exon 2 revealed high sequence polymorphism and allelic diversity, with 67 alleles identified and individual birds carrying 4–20 alleles (Chapter 3). Accordingly, kiwi with more allelic variants are likely to exhibit broader pathogen resistance and improved survival prospects (Chapter 3), suggesting that MHC IIβ may play an important role in mate choice in this population. According to the quantity-of-alleles mechanism (good genes as heterozygosity), we expect kiwi to prefer mates with a high number of MHC IIβ variants. Alternatively, under the genetic-compatibility between mates hypothesis, we predict kiwi to pair with maximally or optimally dissimilar mates. We specifically tested three non-mutually exclusive hypotheses of mate choice in kiwi: (1) preference for mates with greater MHC diversity (i.e., good genes as heterozygosity); (2) preference for mates more dissimilar at the MHC than themselves (i.e., disassortative mating); and (3) preference for males with intermediate MHC dissimilarity (i.e., intermediate optimum). Finally, we tested whether pair-bond stability depends on MHC dissimilarity between mates. If divorce enables brown kiwi to escape suboptimal pairings, we expected partner retention to increase with greater MHC dissimilarity. In brown kiwi, sexes differ markedly in the way they invest energy in reproduction, with females producing energy-rich eggs that are incubated solely by males (McLennan, 1988; Taborsky and Taborsky, 1999). We therefore tested these predictions separately for males and females, using their respective MHC features to evaluate potential sex differences in mating decisions.

Methods

Kiwi sampling and MHC genotyping

Ponui Island hosts a translocated population of NI brown kiwi that has been intensively monitored since 2004. Although kiwi occur throughout the island, monitoring has focused on three forested gullies (Kauri, Red Stony Hill, and Pipe), spanning around 200 ha of mixed habitat, including native forest, wetlands, and pasture (Miles and Castro, 2000), representative of the broader Ponui Island landscape (Chapter 2). Monitoring initially relied on conventional VHF transmitters (2004–2010), complemented by video surveillance of nests and roosts (2008–2009 and 2012), and from 2010 onwards, has

incorporated Chick Timer™ transmitters (CTs; wildtech.co.nz/Kiwi.aspx) radiotelemetry data, which enabled consistent long-term tracking and improved reproductive monitoring (see Chapter 1 for details). As a result, we now have data on social monogamous pairs, such as mate identities, relative age, and nesting sites for 5–14 kiwi pairs (10.23 ± 3.04 SD) per year (Chapter 2). We focused on the MHC IIβ exon 2, which exhibits stronger selection than the MHC class I in non-passerine birds (Minias et al. 2018) and is highly diverse in the studied kiwi population (Chapter 3). Here, we used 80 of the 102 Ponui Island birds previously genotyped (Chapter 3), including 33 marked pairs. Tissue samples (blood and feathers) were collected from 2004 to 2022 under Department of Conservation wildlife permits AK-14969-RES, AK-21519-FAU, 50249-FAU, and 70875-RES.

Genomic DNA extraction, primer design, library preparation, Illumina sequencing, and genotyping were performed as described in Chapter 3. Briefly, genomic DNA was extracted and quality checked. Primer design targeted the MHC IIβ exon 2 genes, which are directly involved in pathogen recognition and exhibit high levels of amino acid diversity (Radwan et al., 2020), and used available conspecific sequence and whole genome data, along with congeneric sequences MHC IIβ sequences from *A. owenii* (Miller et al., 2011). The resulting novel primer set (NIBK_MHCIIβe2-F and NIBK_MHCIIβe2-R) amplifies a conserved intron–exon 2 region, covering around 94.4% of the exon 2 length (~260 base pairs; bp). Libraries were prepared with a dual PCR protocol, indexed, cleaned, and quality-checked before sequencing on an Illumina MiSeq platform at Massey Genome Service (Palmerston North, New Zealand). Raw paired-end reads from MHC IIβ exon 2 were processed using the ACACIA genotyping pipeline (Allele CAlling proCedure for Illumina Amplicon sequencing data; Gillingham et al., 2021), which included quality control, trimming, merging, removal of artefacts and chimeras, and filtering against reference sequences from closely related species (ratites and allies) and fowls (ducks, chickens and allies). Candidate alleles were identified through alignment and oligotyping, with final allele calls based on optimised thresholds for read number and proportion. For methodological details, see Chapter 3.

Genetic indices for MHC mate choice

Our genotyping was considered highly reliable as supported by high similarity to known avian MHC IIβ exon 2 alleles from other bird species, strong repeatability across

replicates, and consistent segregation patterns among related individuals in the full dataset (see Chapter 3). Accordingly, we used the resulting allele calls to calculate eight genetic indices for assessing female MHC-based mate choice. These indices have been previously applied in this context (Wetton et al., 1987; Landry et al., 2001; Santos et al., 2016; Sawada et al., 2020) and are defined as follows:

(1) Male allele dissimilarity (MalDis): number of MHC alleles carried by a male that are not shared with its partner (i.e., the number of alleles "unknown" to the female). (2) Male allele diversity (MalDiv): number of different MHC alleles in each male. (3) Male amino acid diversity (MaaDiv): the sum of amino acid distances among MHC alleles in each male. (4) Couple allele dissimilarity (CalDis): total number of non-shared MHC alleles in each couple. (5) Mean amino acid dissimilarity (μaaDis): mean amino acid distance (measured as the number of amino acid differences) among non-shared MHC alleles in each couple. (6) MHC share (MHCshare): proportion of MHC alleles shared between partners. (7) Peptide Binding Region amino acid distance (PBRaaDis): mean pairwise amino acid distance at PBR sites between all MHC alleles of the two partners. (8) Non-Peptide Binding Region amino acid distance (non-PBRaaDis): mean pairwise amino acid distance at non-PBR sites between all MHC alleles of the two partners. Since functional differences among alleles depend on amino-acid sequence, the last two indices are expected to capture more functional variation than MHCshare (Sawada et al. 2020).

While five of these indices measure MHC features of kiwi pairs (CalDis, μaaDis, MHCshare, non-PBRaaDis, and PBRaaDis), three are inherently related to each assessed male (MalDis, MalDiv, MaaDiv). Therefore, to test for male MHC-based mate choice, we derived the corresponding female indices using the same procedures as before: female allele dissimilarity (FalDis), female allele diversity (FalDiv), and female amino acid diversity (FaaDiv). All genetic indices were calculated in base R 4.3.2 (R Core Team, 2025), except the last two, which were computed in MEGA 11 (Tamura et al., 2021).

Mate choice tests

We used the two-step approach described by Santos et al. (2016), applying Monte Carlo randomisation tests (MCRTs) and generalised linear mixed models (GLMMs) to test whether kiwi chose their mating partners depending on MHC IIβ exon 2 features. Because females invest heavily in egg production and males are solely responsible for

incubation, we analysed males and females separately to explore potential sex differences in mating decisions

We first tested whether kiwi chose partners in a way that differed significantly from randomness by comparing the observed means of each MHC index to randomised means obtained by Monte Carlo simulations (Sin et al. 2015). All MCRT were run in base R as follow: (i) calculating the observed mean genetic index of the real pairs; (ii) randomly assigning a male to each female to simulate random mating; (iii) calculating the mean genetic index of the simulated pairs; (iv) repeating steps (ii) and (iii) 10,000 times to generate a null distribution; and (v) calculating two-tailed p-values as the proportion of times the simulated means were more extreme than the observed mean. For this, in step (i) we randomly assigned males to females without constraints, as kiwi on Ponui Island move freely among the monitored gullies and appear to face no spatial or temporal barriers to mate access. We calculated *p*-values as the proportion of times in which the simulated means deviated significantly from the observed mean, in either direction. Statistical significance was assessed using *q*-values derived from *p*-value for false discovery rate (FDR) correction (Benjamini and Hochberg, 1995). When no evidence was found for preference for MHC-dissimilar mates (i.e., disassortative mating), we tested whether individuals preferred mates with optimal (intermediate) MHC dissimilarity. For this, we calculated the variance in MHCshare, non-PBRaaDis, and PBRaaDis for mated pairs within the population and compared it with that from randomised mating pairs (Forsberg et al., 2007). Because individuals are expected to prefer mates that bring them closer to the intermediate MHC-IIB optimum, we expected that the observed variance in MHC dissimilarity would be significantly smaller than the random variance.

Alternatively, we used GLMM to test whether the probability of being chosen as a mate is predicted by any of the eight MHC indices described above (Santos et al., 2016). Each observed couple was treated as the outcome of a mate choice event, and we calculated all genetic indices for the actual partner (i.e., actual female choice) as well as for each potential partner (i.e., potential female choice). Due to collinearity among some genetic indices (Figure S4.1), we excluded MalDiv and CalDis from models of female mate choice, and FalDiv and CalDis from models of male mate choice to avoid redundancy. We then defined a binary response variable (choice) as one (1) or zero (0), depending on

whether the couple was real or not, respectively. Each genetic index was included separately as a fixed effect, and the identities of males and females were included as random effects, as both were involved in several 'choice' events that were not necessarily independent. The final dataset consisted of 33 observed pairs (i.e., chosen = 1) and 664 simulated pairs (i.e., chosen = 0). We evaluated mate choice using three alternative models testing (1) whether females tend to choose males based on MHC features: choice ~ MalDis + MaaDiv + (1|male id) + (1|female id); (2) whether males tend to choose females based on MHC features: choice ~ FalDis + FaaDiv + (1|male id) + (1|female id); and (3) whether mate choice is predicted by MHC compatibility between mates: choice ~ μaaDis + MHCshare + PBRaaDis + non-PBRaaDis + (1|male id) + (1|female id).

We fitted Bayesian GLMM (B-GLMMs) using the R package 'brms' 2.20.4 (Bürkner, 2017) using a binomial distribution and cloglog link function. To boost model performance and interpretability, fixed effects were standardised by centering and dividing them by two standard deviations (Gelman, 2008). We used weakly informative priors [normal (0, 2)] for fixed effects and brms's default priors for the remaining parameters, so priors' influence is expected to be negligible (Bürkner, 2017). Models were run with four chains of 20,000 iterations, discarding the first half as burn-in, thinning every 5th sample, and using an adapt-delta of 0.99. Convergence was assessed by inspecting trace plots of posterior samples and confirming that Rhat ($\hat{R}$) values for all parameters were close to 1.0, indicating proper chain mixing and reliable parameter estimation (Gelman and Rubin, 1992). We also evaluated the effective posterior sample size (ESS) to quantify the number of effectively independent samples (Gelman et al., 2013), with values >1,000 considered reliable (Muth et al., 2018). Predictors were deemed meaningful when the 95% HDI did not overlap zero. Bayesian indices of effect existence (probability of direction, pd) and practical significance (region of practical equivalence, ROPE) were estimated using the R package bayestestR 0.12.1 (Makowski et al., 2019). The pd reflects the certainty of an effect's direction (positive or negative), whereas the ROPE assesses whether a parameter is associated with a meaningful change in the outcome (Makowski et al., 2019). We inferred evidence for an effect when pd ≥ 99% and considered parameter estimates meaningful when ≥ 1% of the 95% HDI fell within the ROPE (Kruschke and Liddell, 2018).

Pair stability tests

We used Bayesian GLMMs (as described before) to test whether pair-bond stability depends on MHC diversity between mates, based on the subset of genotyped individuals with divorce information available (i.e., 18 breeding pairs). Females and males were tested separately. Pair bond status (hereafter pair status) was categorised annually (2011–2021) as follows: fidelity, when both members of a pair in year t were observed together again during the breeding season in year t + 1; divorce, when both individuals were alive and present in the study area but did not form a pair in year t + 1. We fit two GLMMs to test whether pair status, modelled as a binary variable (fidelity = 1, divorce = 0) with a binomial distribution and logit link, was explained by MaaDiv (or FaaDiv). Models took the form: pair status ~ MalDiv + (1|male identity) and pair status ~ FalDiv + (1|female identity). The final dataset consisted of 97 data points, yet the number of statistically independent units (i.e., pairs) was limited to 18.

Results

MHC genotyping

From a total of 80 samples, we identified 67 MHC IIβ exon 2 alleles (Figure 4.1a), most of which were present at low levels (< 50%) in the genotyped birds (Figure 4.1b), with only three alleles occurring in more than 50% of the individuals. Remarkably, 18 putative alleles contain a three-nucleotide insertion when compared to the other sequences, which has also been identified in other kiwi species (see Chapter 3). Alleles with this insertion appear to be functional, as no premature stop codons were detected. All individuals had alleles without indels (range = 3–16, average = 10.09 ± 2.75 standard deviation; s.d.) and 96.5% of the individuals carried alleles with the 3 bp insertion (range = 1–6, average = 3.04 ± 1.45 s.d.). The number of alleles per individual ranged from 5 to 20, with an average of 13.03 ± 3.42 (s.d.), indicating the amplification and genotyping of at least 10 homologous loci. However, a small number of individuals (n = 12) had fewer than 10 alleles (Figure 4.1c). There was no difference in the total allele number of males and females (male mean = 13.24, SD = 3.35; female mean = 12.51, SD = 3.20; Welch's t test $_{t77.1}$ = –0.78, p = 0.43).

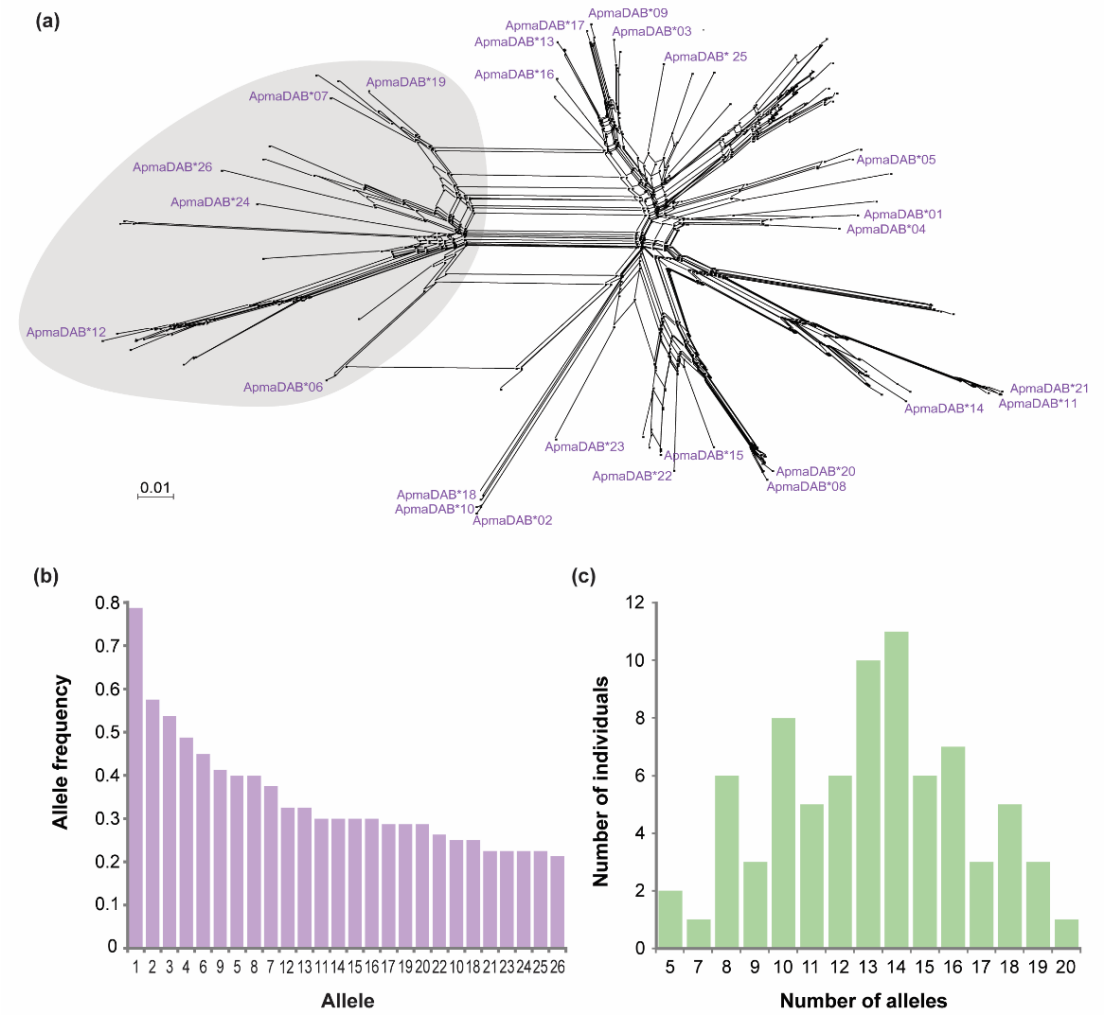

Figure 4.1. MHC IIβ exon 2 allele characteristics in North Island brown kiwi on Ponui Island, based on 80 genotyped individuals. (a) Phylogenetic relationships among MHC IIβ exon 2 alleles detected in the population, inferred using Neighbour-Net networks in SplitsTree4 (Huson & Bryant 2005). Grey circle highlights the alleles with a 3-bp insertion. For clarity, only alleles with frequencies above 20% are labelled. (b) Allele frequency in the population (as a percent of 80 birds), indicating that only three alleles occurred in more than 50% of the individuals. For clarity, only alleles with frequencies above 20% are shown in decreasing order, with allele names abbreviated as numeric identifiers (e.g., ApmaDAB*01 = 1). (c) Number of alleles detected per genotyped individual. Although allele counts varied (5–20), most individuals carried more than eight alleles.

Mate-choice tests

We compared the actual values of the mean genetic index to the null distribution generated from a simulation of random mating by MCRTs and found no evidence for (dis-)assortative mating. In all cases the empirical means of the genetic indices lay well within the central range of the null distributions generated under random mating (Figure 4.2), and none differed significantly between observed and simulated pairs (p-values > 0.335; q-values = 0.735), indicating that female kiwi do not appear to select mates based on MHC IIβ exon 2 variation (Table S4.1). Randomisations assuming males as the 'choosy' sex yielded similar results, leaving the conclusions unchanged (Table S4.1). Likewise, we found no evidence that kiwi mated with significantly more intermediately dissimilar partners than expected under random mating for any of the examined indices (MHCshare, p-value = 0.367; PBRaaDis, p-value = 0.389; non-PBRaaDis, p-value = 0.240).

Models testing whether mate choice probability varied with MHC class IIβ exon 2 features ran smoothly without post-sampling warnings, diagnostic parameters indicated convergence (Table 4.1), and posterior predictive checks indicated a good fit. We found no significant associations between any examined indices and pairing outcomes, regardless of whether male (MalDis: pd = 74.96%, ROPE = 25.22%; MaaDiv: pd = 50.39%, ROPE = 29.45%), female (FalDis: pd = 76.94%, ROPE = 25.86%; FaaDiv: pd = 58.19%, ROPE = 31.59%), or pair-level MHC features (μaaDis: pd = 59.94%, ROPE = 28.41%; MHCshare: pd = 70.94%, ROPE = 25.92%; PBRaaDis: pd = 55.43%, ROPE = 28.10%; non-PBRaaDis: pd = 56.39%, ROPE = 31.60%) were included as fixed effects. These results suggest that neither individual MHC profiles nor pairwise MHC compatibility influences mate choice decisions in this population. By applying two distinct statistical approaches that address related, but not identical questions (MCRTs and Bayesian GLMMs), our results suggest that mate choice in kiwi on Ponui Island is unlikely to be driven by MHC-dependent preferences, at least at the examined region.

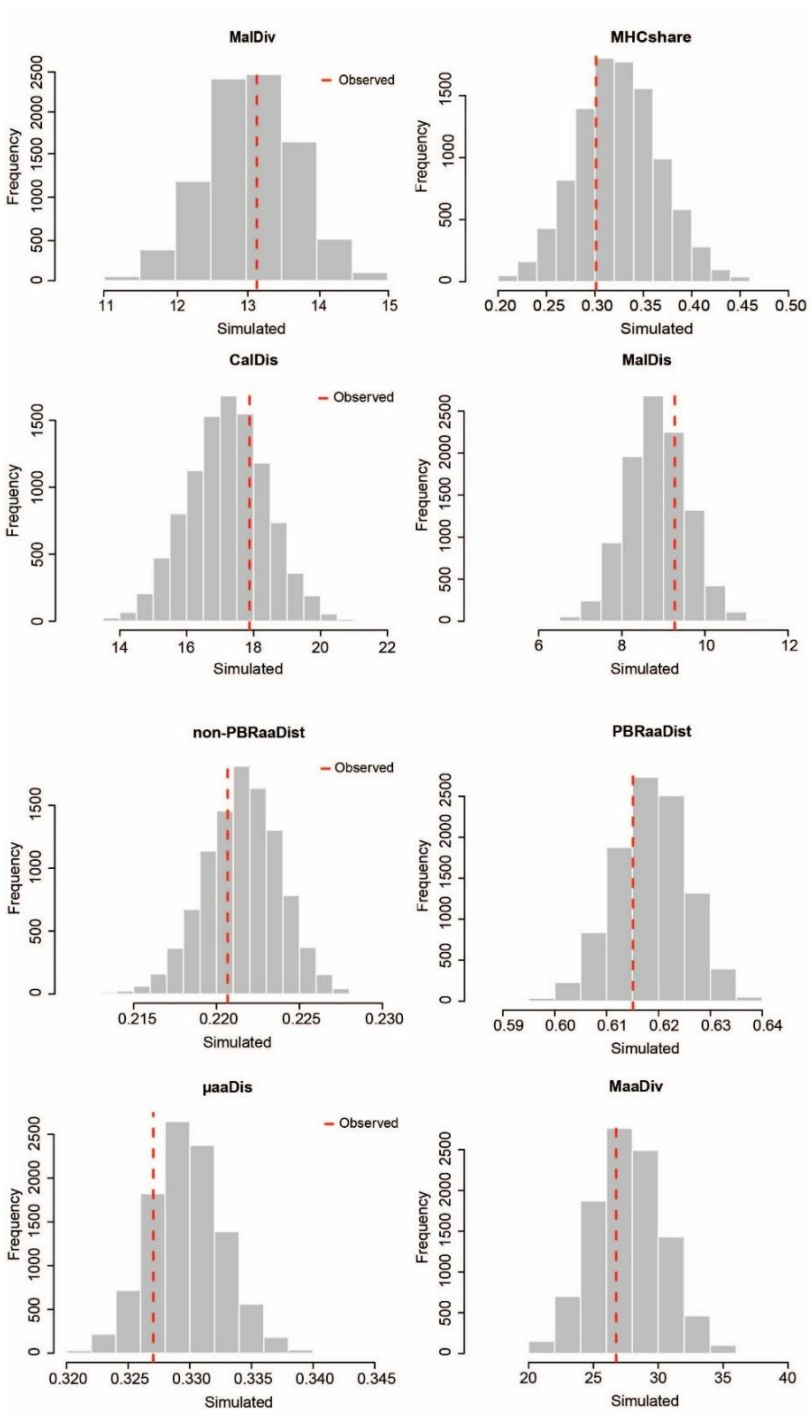

Figure 4.2. Frequency distributions from randomisation tests of (dis-)assortative mating in North Island brown kiwi on Ponui Island, based on eight genetic indices capturing distinct features of MHC class IIβ exon 2. Distributions show mean values generated from 10,000 permutations of random pairing. Dashed lines show empirical means from observed pairings. Abbreviations: male allele dissimilarity (MalDis), male allele diversity (MalDiv), male amino acid diversity (MaaDiv), couple allele dissimilarity (CalDis), mean amino acid dissimilarity (μaaDis), MHC share (MHCshare), Non-Peptide Binding Region amino acid distance (non-PBRaaDis), and Peptide Binding Region amino acid distance (PBRaaDis).

Table 4.1. Summary statistics of Bayesian generalised linear mixed models testing the effect of MHC-related indices on the probability of being chosen as a mate in North Island brown kiwi on Ponui Island. Models assuming females (a) and males (b) as the 'choosy' sex are presented. We reported posterior medians (median), standard errors (SE), and 95% Bayesian high-density intervals (95% HDI) as measures of variance for the estimated parameters. Rhat values, effective posterior sample size (ESS), and Bayesian indices of effect existence (probability of direction = pd%) and significance (region of practical equivalence = ROPE%) are also reported. Rhat values near 1.0 and effective sample sizes (ESS) exceeding 1,000 indicate proper chain mixing and reliable parameter estimation (see Gelman & Rubin 1992; Muth et al. 2018). Meaningful values for Bayesian indices are indicated with an asterisk (*).

Parameter	**Median**	**SE**	**95% HDI**	**Rhat**	**ESS**	**Pd%**	**ROPE%**
(a) choice ~ MalDis + MaaDiv + (1|male id) + (1|female id)							
intercept	−3.07	0.19	[−3.45, −2.71]	1.00	7738	100*	0.00*
MalDis	0.32	0.48	[−0.58, 1.29]	1.00	8146	74.96	25.22
MaaDiv	−0.01	0.48	[−0.98, 0.89]	1.00	8001	50.39	29.45
Random effects							
male identity	0.18						
female identity	0.16						
(b) choice ~ FalDis + FaaDiv + (1|male id) + (1|female)							
intercept	−3.06	0.19	[−3.47, −2.71]	1.00	7954	100*	0.00*
FalDis	0.31	0.45	[−0.52, 1.22]	1.00	7890	76.94	25.86
FaaDiv	−0.09	0.44	[−0.97, 0.74]	1.00	7948	58.19	31.59
Random effects							
male identity	0.18						
female identity	0.17						
(c) choice ~ μaaDis + MHCshare + PBRaaDis + non-PBRaaDis + (1|male id) + (1|female id)							
intercept	−3.11	0.20	[−3.53, −2.73]	1.00	7836	100*	0.00*
μaaDis	−0.13	0.49	[−1.17, 0.72]	1.00	8005	59.94	28.41
MHCshare	−0.26	0.48	[−1.25, 0.65]	1.00	7572	70.94	25.92
PBRaaDis	0.07	0.49	[−0.90, 1.06]	1.00	8198	55.43	28.10
non-PBRaaDis	0.08	0.46	[−0.78, 1.02]	1.00	8161	56.39	31.60
Random effects							
male identity	0.19						
female identity	0.17						

Pair stability tests

Models testing whether pair-bond stability depends on MHC-diversity between mates ran smoothly without post-sampling warnings, diagnostic parameters indicated convergence (Table S4.2), and posterior predictive checks indicated a good fit. We found no meaningful effect of MHC diversity (MaaDiv and FaaDiv) on pair-bond stability, when considering either females (MaaDiv: pd = 93.88%, ROPE = 5.36%) or males (FaaDiv: pd = 52.41%, ROPE = 16.99%) as the 'choosy' sex (Table S4.2). Despite overall null effects, models assuming females as the 'choosy' sex revealed a positive but non-significant trend, suggesting that males with higher MHC diversity were somewhat more likely to maintain pair bonds (i.e., less likely to divorce).

Discussion

The MHC genes are widely regarded as important targets of mate choice because of the potential genetic benefits they confer to offspring (Brown, 1997; Landry et al., 2001; Mays and Hill, 2004; Bonneaud et al., 2005; Setchell and Huchard, 2010). However, it remains unclear whether selection favours preferences for genetic compatibility to boost immunocompetence or avoid inbreeding risk (Sepil et al., 2015). Here, we investigated for the first time the role of MHC IIβ exon 2 in mate-choice decisions and pair-bond stability in kiwi, using a translocated population on Ponui Island as a study system. We predicted that mating decisions in this population may reflect selection pressures favouring either maximal MHC diversity in offspring or an intermediate optimum that balances immunogenetic benefits with broader lifetime fitness outcomes (Thel et al., 2025). Contrary to our expectations, we found no evidence that MHC IIβ exon 2 characteristics influenced the formation of social pairings, regardless of whether females or males were presumed to exert mate choice.

Indeed, MCRTs showed that mate choice did not differ significantly from randomness, while Bayesian GLMMs indicated that the probability of being chosen as a mate was not predicted by any of the examined MHC indices. The absence of any link between MHC IIβ exon 2 features and female pairing preferences suggests that MHC-dependent mate choice is unlikely to occur in this population, at least within the region analysed. This contrasts with recent research reporting genome-wide disassortative mating in the same

kiwi population, where females tended to pair with genetically dissimilar males (Undin et al., 2021). Although unexpected, the lack of MHC-dependent mate choice in kiwi on Ponui Island is consistent with findings from other avian systems, including passerine (Westerdahl, 2004; Sepil et al., 2015; Wright et al., 2015) and non-passerine species (Ekblom et al., 2004; Dearborn et al., 2016; Stervander et al., 2020). One potential explanation is that the high MHC IIβ exon 2 diversity already present in the studied population (see also Chapter 3) reduces selective pressure for females to further maximise diversity through mate choice, as existing variation may already provide broad pathogen resistance. Alternatively, because the Ponui Island population was established only in 1964 (equivalent to roughly 4–6 kiwi generations), there may not have been sufficient time for MHC-based mate-choice behaviours to evolve (e.g., Stervander et al., 2020). Together, these findings suggest that behavioural and ecological factors, including incubation investment and pair stability mechanisms identified in Chapter 2, may play a stronger role in shaping kiwi reproductive associations than MHC similarity alone.

We acknowledge that our sample size (33 pairs) may have limited the power to detect (dis)assortative mating. However, given the position of our empirical mean relative to the distribution generated by randomisations (Figure 4.2), a substantially larger sample would be likely required to detect a potential association, if one exists. Indeed, studies with sample sizes comparable to ours have identified MHC-dependent patterns in wild populations from other systems (Bonneaud et al., 2006; Juola and Dearborn, 2012) and even smaller datasets (20 pairs) have detected genome-wide disassortative mating in the studied kiwi population (Undin et al., 2021). Reanalysis of our dataset using the same kiwi pairs as in Undin et al. (2021) did not alter our overall conclusion but revealed a weak trend suggesting that females may prefer males with dissimilar alleles. However, this pattern lacks statistical power and may be confounded by the inclusion of socially bonded pairs in our dataset. Clarifying whether this trend reflects MHC-based mate choice linked to inbreeding avoidance will require a larger sample of genetically profiled kiwi pairs with confirmed reproductive status. The small founder population of kiwi on Ponui Island has raised concerns about inbreeding depression, which could compromise long-term population persistence (Undin, 2021; Undin et al., 2021). Evidence of genome-wide disassortative mating (Undin et al., 2021), coupled with random mating at MHC loci (MHC IIβ exon 2), suggests that selection may favour strategies that reduce inbreeding and increase genome-wide heterozygosity, rather than preferences for MHC-

based genetic compatibility to enhance offspring immunocompetence (see Sepil et al., 2015).

Another possible explanation for the absence of MHC-dependent mate choice is that female kiwi may be unable to discriminate the MHC profiles of potential mates via olfactory cues. While experimental work on other vertebrate systems (e.g., mammals and fish), has offered compelling evidence that females use olfactory cues to evaluate the MHC genotype of their prospective mating partners (Reusch et al., 2001; Milinski et al., 2005; Radwan et al., 2008), the role of MHC-related odour signals in avian mate choice remains poorly understood (Sepil et al., 2015; Leclaire et al., 2017). Notably, studies on monogamous bird species with highly developed olfaction have revealed potential for MHC-based mate choice via olfactory cues (e.g., Strandh et al., 2012) and offer strong evidence for odour-mediated assessment of MHC dissimilarity (Leclaire et al., 2017). Although kiwi possess a highly developed and functional olfactory system (Castro et al., 2010), no mechanism for self-recognition or MHC compatibility assessment has yet been demonstrated. Thus, the absence of MHC-dependent mate choice suggests that kiwi may not use MHC cues to recognise kin or avoid inbreeding. Instead, they may rely on olfactory information in other ecological or social contexts, such as food location and foraging (reviewed in Castro et al., 2010). Experimental studies examining the link between MHC-based mate choice and individual odour profiles are therefore warranted and could clarify whether kiwi use scent cues to assess the MHC genotype of potential mates.

Likewise, we found no meaningful effect of MHC diversity on pair-bond stability when considering either females or males as the 'choosy' sex, suggesting that MHC variation does not influence the maintenance of social bonds in this population. Despite this, the model assuming females as the 'choosy' sex indicated a weak yet non-significant trend whereby males with higher MHC diversity were somewhat more likely to retain pair bonds (i.e., less likely to divorce). This pattern aligns with previous findings on MHC-dependent drivers of divorce in human-translocated populations of Seychelles warblers introduced to previously unoccupied islands (Wright et al., 2015) and raises questions about the role of MHC variation in shaping post-pairing dynamics. However, this apparent trend should be interpreted with caution, as our dataset is skewed toward stable pairings (89 stable vs. 8 divorced), reflecting the low divorce rate in the Ponui Island

population (see Chapter 2). Furthermore, it remains unclear whether pair-bond dissolution in this population reflects active mate choice to leave or male–male competition, whereby some males may be less capable of defending mates or territories against intruding rivals (Chapters 2). While the potential link between kiwi pair stability and male MHC diversity is intriguing, any inference remains tentative and warrants further investigation using larger, longitudinal datasets with comprehensive genotypic profiles.

Overall, our findings suggest that MHC IIβ exon 2 variation either does not influence mate choice and pair-bond stability in kiwi on Ponui Island or that any such effects are too subtle to be detected with the current dataset. This highlights the need to investigate additional MHC variable regions, such as the MHC I exon 3, while increasing sample sizes and incorporating measures of MHC dissimilarity and relatedness to clarify whether MHC variation itself is the target of mate choice (see Potts et al., 1994; Thoß et al., 2011; Sepil et al., 2015). Besides this, alternative mechanisms should be considered, as other mating criteria may supersede the role of MHC in guiding mate selection. For example, male nest attendance (expressed as incubation effort) seems to be the feature that females assess when retaining partners (Chapter 2) and may therefore represent a more proximate target of mate choice. Likewise, phenotypic features signalling reproductive performance may also influence pair formation, given the substantial energetic investment required to produce and incubate kiwi eggs (Ziesemann, 2011). Unlike most studies on MHC-driven mate choice and pair stability in birds, we provide rare insights into the influence of MHC features on such reproductive behaviours of a bird with cryptic reproductive habits. Although we found no evidence of MHC-based mate choice and pair stability, reporting these results is crucial to better understand the extent to which active mate choice for functional genes such as the MHC occurs (Wright et al., 2015) and support future meta-analyses aimed at clarifying its evolutionary role (Sawada et al., 2020).

While this chapter found little evidence supporting strong MHC-based mate choice, the results nevertheless highlight the complexity of reproductive interactions and the possibility that behavioural, ecological, or phenotypic factors may contribute more strongly to pair formation and maintenance. The following chapter, therefore, broadens this perspective by examining patterns of similarity and covariance within kiwi pairings to further investigate mechanisms underlying reproductive associations.

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Appendix

Figure S4.1. Pairwise Pearson's correlation coefficients between the genetic indices used to test MHC-based mate choice in North Island brown kiwi on Ponui Island. Abbreviations: male allele dissimilarity (MalDis), male allele diversity (MalDiv), male amino acid diversity (MaaDiv), female allele dissimilarity (FalDis), female allele diversity (FalDiv), female amino acid diversity (FaaDiv), couple allele dissimilarity (CalDis), mean amino acid dissimilarity (μaaDis), MHC share (MHCshare), Peptide Binding Region amino acid distance (PBRaaDis), and Non-Peptide Binding Region amino acid distance (non-PBRaaDis).

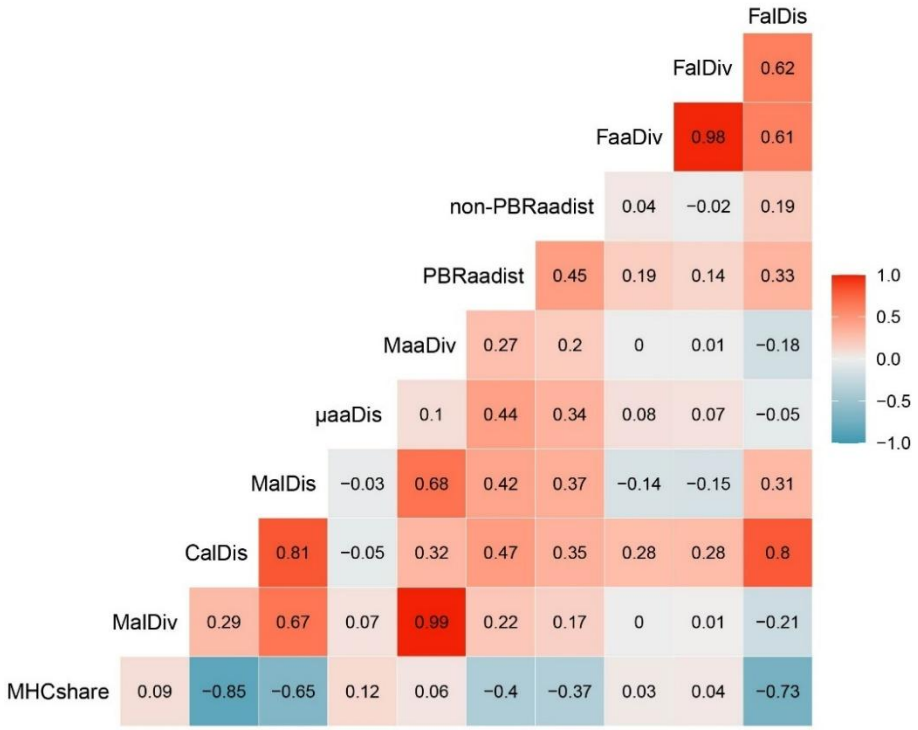
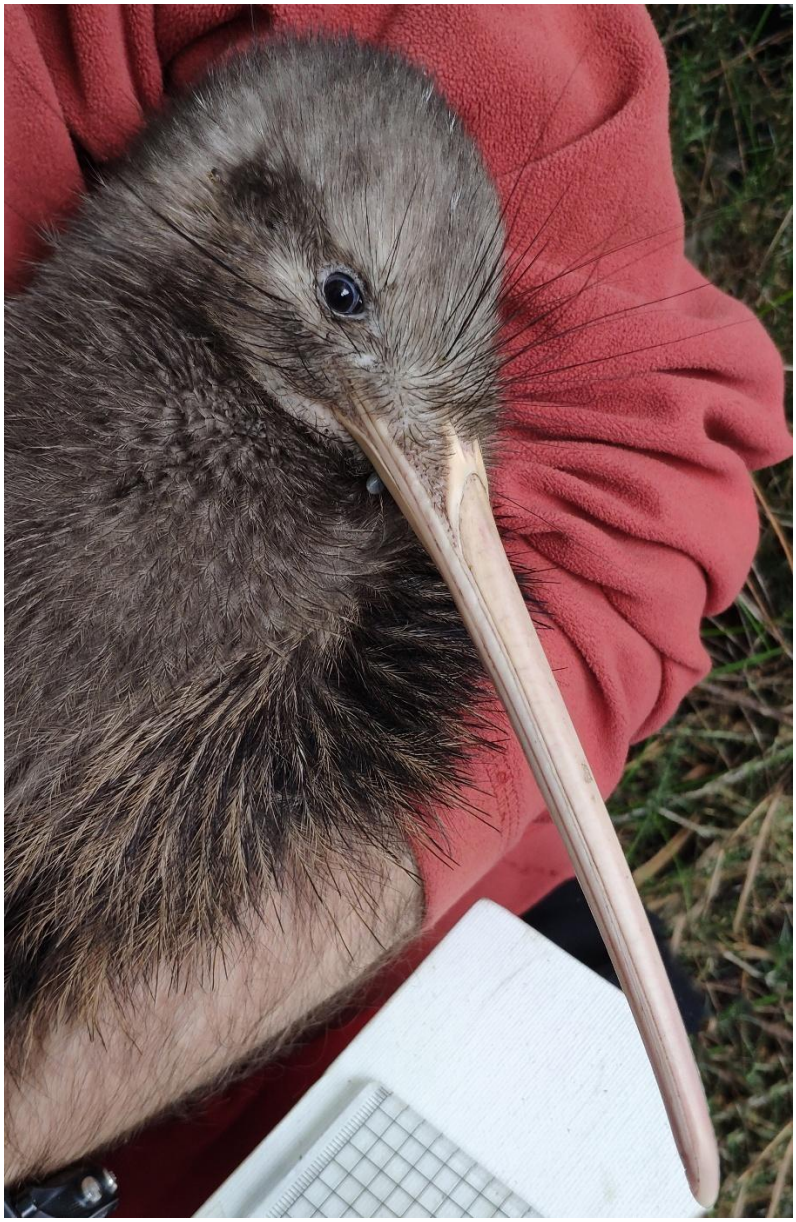


Table S4.1. Summary statistics of randomisation tests assessing disassortative mating in North Island brown kiwi on Ponui Island, based on genetic indices capturing distinct features of MHC class IIβ exon 2 for males and females. The MHC column specifies whether each index measures MHC features of kiwi males, females, or pairs. Note that none of the genetic indices differed significantly between actual and randomly simulated pairs. Significance expressed as raw *p*-values and *q*-values (false discovery rate control for multiple testing). Abbreviations: male allele dissimilarity (MalDis), male allele diversity (MalDiv), male amino acid diversity (MaaDiv), female allele dissimilarity (FalDis), female allele diversity (FalDiv), female amino acid diversity (FaaDiv), couple allele dissimilarity (CalDis), mean amino acid dissimilarity (μaaDis), MHC share (MHCshare), Peptide Binding Region amino acid distance (PBRaaDis), and Non-Peptide Binding Region amino acid distance (non-PBRaaDis).

Metric	**MHC**	**Observed**	**CI lower**	**CI upper**	***p*-value**	***q*-value**
MalDis	Male	9.273	7.455	10.273	0.369	0.872
MalDiv	Male	13.152	11.818	14.333	0.623	0.872
MaaDiv	Male	26.747	22.452	33.101	0.735	0.872
FalDis	Female	8.606	6.788	9.455	0.492	0.872
FalDiv	Female	12.485	11.333	13.364	0.793	0.872
FaaDiv	Female	23.306	19.407	27.342	0.997	0.999
CalDis	Pair	17.879	14.879	19.515	0.335	0.872
μaaDis	Pair	0.327	0.324	0.336	0.355	0.872
MHCshare	Pair	0.301	0.243	0.411	0.457	0.872
PBRaaDis	Pair	0.615	0.605	0.632	0.614	0.872
non-PBRaaDis	Pair	0.221	0.217	0.226	0.695	0.872

Table S4.2. Summary statistics of Bayesian generalized linear mixed models testing the effect of MHC-diversity on pair-bond stability assuming females (a) and males (b) as the 'choosy' sex. We reported posterior medians (median), standard errors (SE), and 95% Bayesian high-density intervals (95% HDI) as measures of variance for the estimated parameters. Rhat values, effective posterior sample size (ESS), and Bayesian indices of effect existence (probability of direction = pd%) and significance (region of practical equivalence = ROPE%) are also reported. Rhat values near 1.0 and effective sample sizes (ESS) exceeding 1,000 indicate proper chain mixing and reliable parameter estimation (see Gelman & Rubin 1992; Muth et al. 2018). Meaningful values for Bayesian indices are indicated with an asterisk (*). Estimates for random effects are reported as standard deviations. Abbreviations: male amino acid diversity (MaaDiv) and female amino acid diversity (FaaDiv).

Parameter	**Median**	**SE**	**95% HDI**	**Rhat**	**ESS**	**Pd%**	**ROPE%**
(a) pair status ~ MalDiv + (1|male identity)							
intercept	2.71	0.55	[1.85, 3.98]	1.00	7837	100*	0.00*
MaaDiv	1.33	0.98	[−0.52, 3.35]	1.00	7353	93.88	5.36
Random effects							
male identity	0.78						
(b) pair status ~ FalDiv + (1|female identity)							
intercept	2.78	0.68	[1.81, 4.52]	1.00	7278	100*	0.00*
FaaDiv	−0.05	0.92	[−1.83, 1.76]	1.00	7354	52.41	16.99
Random effects							
female identity	0.96						



Daniela, a female North Island brown kiwi (*Apteryx mantelli*) from Ponui Island (Hauraki Gulf, Aotearoa New Zealand North Island).

Chapter Five

Disentangling phenotypic estimates of assortative mating in a translocated population of North Island brown kiwi

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Name and title of main supervisor:	Isabel Castro
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Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1] Conceptualization, data curation, analysis, and visualization were conducted by Eliana Ramos with assistance from Isabel Castro and Gillian Gibb. The original draft was written by Eliana Ramos. The final document was edited and approved by Isabel Castro and Gillian Gibb.	
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Abstract

Assortative mating occurs when individuals with similar traits pair together, creating a positive correlation between male and female phenotypes in mated pairs. Although this pattern is often assumed to reflect genuine mate choice for similar traits, it can also arise when partners share environmental conditions that make them more alike over time. In such cases, within-pair residual covariance—not active mate choice—can generate the appearance of assortative mating. Assortative mating and its underlying mechanisms have been extensively studied in the wild, and interest has recently increased in conservation translocations, especially when birds are moved from multiple source populations that may differ in reproductive behaviour. Here, we investigated whether the source population influences post-translocation mating patterns in a mixed-origin population of North Island brown kiwi (*Apteryx mantelli*) on Ponui Island, Aotearoa New Zealand. We examine both labile traits (which vary over time) and fixed traits (which are more stable but still subject to measurement error). Using repeated morphological measurements from kiwi pairs collected over two decades, we combined simple correlation analyses and mixed-effects models to estimate trait repeatability, test for assortative mating, and identify the processes driving any observed patterns. We found that fixed traits showed higher repeatability and more consistent measurement than labile traits. Correlation analyses suggested no evidence of assortative mating across traits, but mixed-effects models revealed weak yet significant positive correlations for tarsus length and body weight, indicating some degree of size-assortative mating. Further analyses showed that these correlations were largely driven by residual covariance rather than intrinsic mate choice. Although the origin of this covariance is uncertain, it most likely reflects shared environmental influences acting similarly on both partners, causing them to appear more alike. Our results highlight that failing to account for residual phenotypic covariance between mates can lead to overestimates of true assortative mating. This distinction is important for understanding post-translocation pairing behaviour and for evaluating the success of conservation translocations.

Keywords: *Apteryx mantelli*, assortative mating, intrinsic assortative mating correlation, mate choice, repeatability, residual correlation

Introduction

Translocations—the human-mediated movement of organisms between sites for conservation benefit—have become an essential component of the conservation toolbox (Seddon et al., 2014; Hoffmann et al., 2015; Berger-Tal et al., 2020). Anticipating the outcome of such interventions remains challenging, in part due to the complex interplay between genotype, phenotype, environment, and its effect on post-translocation behaviour (Renan et al., 2018; Undin et al., 2021). Successful translocation outcomes, defined as the production of a viable, self-sustaining population in the wild (Griffith et al., 1989; Seddon, 2010), depend on the formation of breeding pairs and successful reproduction (Bradley et al., 2014; Bell, 2016; Greggor et al., 2025). Consequently, post-release breeding behaviour and mate choice represent key factors to evaluate given their direct influence on the effective population size following translocation (Anthony and Blumstein, 2000; Undin et al., 2021). Behavioural considerations are particularly relevant in translocations that mix individuals from genetically different source sites, a practice that is becoming increasingly common in conservation (Quader, 2005; Parker et al., 2010; Bradley et al., 2014; Undin et al., 2021), as animals from multiple locations may differ in their reproductive behaviours (Rowe and Bell, 2007; Bradley et al., 2014; Fracas et al., 2023; Reyes et al., 2024). Failure to consider such variation may foster genetic isolation due to assortative mating behaviours, reducing the effective population size of the founder group and ultimately compromising translocation success (Bradley et al., 2014; Day et al., 2021).

Assortative mating occurs when individuals with similar phenotypes tend to mate with each other (‘like mates like’) more often than expected by chance (Wright, 1921; Jiang et al., 2013; Class et al., 2017; Wang et al., 2019). This mating pattern is typically quantified as a positive correlation in the phenotypes of mated males and females (Lewontin et al., 1968; Redden and Allison, 2006; Jiang et al., 2013; Class et al., 2017) and appears to be commonly observed in the wild across many animal systems (reviewed in Jiang et al., 2013). Phenotypic correlations between partners are common in socially monogamous bird species, particularly for traits such as body size, plumage colouration, age, and behaviour (reviewed in Wang et al., 2019). However, little is known about the underlying drivers of such associations, namely whether such patterns reflect active mate choice driven by the fitness advantages of specific phenotypes, or whether they emerge

incidentally as a by-product of other processes (Class et al., 2017; Wang et al., 2019). For example, both pair members may experience shared environmental influences on traits exhibiting phenotypic plasticity, leading to apparent resemblance between mates ("mates become alike") that mimics assortative mating (Class et al., 2017; Class and Brommer, 2018; Wang et al., 2019). Likewise, similarity between mates can also result from methodological artefacts, such as measurement error (Class et al., 2017; Wang et al., 2019).

The phenotypic correlation observed between pair members reflects the combined influence of the intrinsic assortative mating (i.e., mate choice based on trait similarity) and a residual correlation, which represents phenotypic covariation arising from shared environmental conditions and/or correlated measurement error. For example, residual covariance can occur if the same observer measures both partners at the same time (Class et al., 2017; Class and Brommer, 2018). Even when individuals genuinely prefer similar mates, the resulting pattern of assortative mating may appear weak or may even disappear if the data are dominated by uncorrelated residual noise (Class et al., 2017; Class and Brommer, 2018). Labile traits, those that can fluctuate in response to internal or external influences, such as body weight, body condition and hormone levels, are particularly susceptible to residual correlation. However, even traits typically considered fixed (such as tarsus length and other skeletal measurements) can be influenced by uncorrelated residual noise due to measurement error (Class et al., 2017; Class and Brommer, 2018). As a result, the direction and strength of the residual correlation, together with trait repeatability, determine whether the observed phenotypic correlation between partners underestimates or overestimates true assortative mating (Class and Brommer, 2018). Distinguishing among these sources of variation is therefore essential when interpreting phenotypic correlations between mated pairs. Failure to do so may lead to misleading conclusions about post-translocation assortative mating and ultimately obscure accurate interpretation of translocation outcomes.

Kiwi (*Apteryx*) are highly threatened, flightless and nocturnal birds endemic to Aotearoa New Zealand, whose persistence depends on active conservation management (Germano et al., 2018), as highlighted by the growing number of translocations since the 19th century (Jahn et al., 2022). These iconic, long-lived birds are today restricted to small and isolated populations that have served as sources for translocations aimed at

establishing populations beyond their historical ranges, including offshore islands and mainland sanctuaries (Jahn et al., 2022). Here, we investigate whether source population origin influences post-translocation mate choice, using as a study system a population of North Island (NI) brown kiwi (*Apteryx mantelli*) on Ponui Island (1,770 ha) started with founders from two separate management units. This population was established in 1964 with eight birds from Waipoua Forest (Northland management unit) and six from Hauturu-o-Toi (Little Barrier) Island (mostly Western management Unit) Colbourne, 2005; Craig et al., 2011). Sixty-one years after translocation, the Ponui Island population is now presumed to have about one bird per hectare (Cunningham and Castro, 2011). Kiwi at the source sites represent distinct genetic lineages that may have diverged ~100–220 thousand years ago (Weir et al., 2016) and differ in morphology, with Northland birds being larger and having longer bills (Undin et al., 2021). Mate preferences toward certain phenotypes evolve when individuals gain benefits from choosing particular mates over others (Darwin, 1871; Halliday, 1983; Andersson and Iwasa, 1996; Kokko et al., 2003; Höbel et al., 2022), so one might expect kiwi from these source populations to prefer familiar or source-specific phenotypes (e.g., Rowe and Bell, 2007; Bradley et al., 2014; Fracas et al., 2023; Reyes et al., 2024).

Life-history theory predicts greater reproductive investment when fitness returns are high, making traits that signal such investment key targets of mate choice (Andersson and Iwasa, 1996; Harris and Uller, 2009). In many taxa, larger body size is positively associated with reproductive returns, and sexual selection often favours assortative mating based on partner size (Rollinson and Rowe, 2016). Assortative mating by size could be relevant for pair formation in kiwi given the high energetic cost of producing disproportionally large energy-rich eggs that are incubated exclusively by males (McLennan, 1988; Taborsky and Taborsky, 1999). Bill size may also be important for mate selection in kiwi. The avian bill plays roles in numerous fitness-related behaviours, including foraging, defence, thermoregulation, and communication (Darwin, 1871; Boag and Grant, 1981; Greenberg et al., 2012; Wilkins et al., 2013; Trallero et al., 2019). Bill size and shape can also affect the efficacy of pair formation behaviours (Chaine and Lyon, 2008; Ballentine et al., 2013; Olsen et al., 2013), potentially influencing reproductive isolation following translocation when differences among source populations disrupt these behaviours, alter mate preferences, and promote assortative pairing (e.g., Rowe and Bell, 2007; Bradley et al., 2014; Fracas et al., 2023). Despite this,

recent work on Ponui Island found no indication of size-assortative mating in kiwi when assessed using traditional correlation analyses (Undin et al., 2021). Here, we used a long-term morphological dataset (2004–2023) to test whether the apparent lack of post-translocation assortative mating genuinely reflects an absence of intrinsic assortative mating.

Our main aim was to examine whether phenotypic correlations between partners occur, and whether any observed patterns reflect intrinsic assortative mating or residual correlations that inflate or obscure true assortative mating (see Class et al., 2017). We used labile morphological traits as well as traits generally considered fixed but still subject to measurement error. Specifically, we asked the following questions: (1) Does measurement repeatability differ between labile and fixed traits? We predicted lower repeatability for labile traits as they can be plastically adjusted to environmental conditions between time points (Class et al., 2017). (2) Do measurer effects differ between labile and fixed traits? We expected measurer effects to be more pronounced for labile traits, as their greater variability may amplify differences among measurers during measurement. (3) Does assortative mating occur for labile and fixed traits in kiwi on Ponui Island? We predicted no phenotypic correlations between partners across traits (see Undin et al., 2021a) but hypothesised that this apparent absence may underestimate true assortative mating if the signal is obscured by uncorrelated residual noise (Class et al., 2017; Class and Brommer, 2018).

Materials and Methods

Kiwi monitoring

Kiwi on Ponui occur throughout the island, but monitoring has been concentrated in three forested gullies—Kauri, Red Stony Hill, and Pipe—encompassing about 200 ha of mixed habitat, including native forest, wetlands, and pasture (Miles and Castro, 2000), representative of the wider island landscape (Chapter 2). Kiwi are cryptic birds that nest in burrows or tree cavities, posing challenges for studying their behavioural ecology (Taylor et al., 2014), yet intensive monitoring and research on Ponui Island since 2004 (see Chapter 2 for details) have yielded extensive data on individual features and pair

identities for 5–14 pairs (10.23 ± 3.04 SD) per year. Birds in this population are largely socially monogamous (70% of known breeding units), although cooperative breeding (22%) and divorce (8.1%) occur. Divorce likely represents a secondary strategy to offset suboptimal pairings (Chapter 2). Preliminary data also suggest that extra-pair copulation does occur, but is very rare (Ziesemann, 2011). The average body mass of adult males and females are 1.926 ± 0.221 kg and 2.223 ± 0.326 kg (Cunningham and Castro, 2011), showing reversed sexual size dimorphism.

Morphometric measurements

We used a long-term (2004–2023) individual-based dataset including metric data collected following the Kiwi Best Practice Manual (Colbourne et al., 2020). Bird sex was determined using established morphological criteria, supported by DNA-based methods for ambiguous individuals. On Ponui Island, female kiwi bills are on average 30% longer than male bills, with no overlap between the sexes (Cunningham & Castro, 2011), birds with bills longer than 108 mm were deemed females. Additionally, DNA sexing using feathers or blood samples has been used to determine the sex of males and young females with bills shorter than 108 mm (Castro, unpubl. data). Most of the study birds were caught twice each year, once in March and a second time in late May-early June. Four measurements, tarsus length (TL), tarsus depth (TD), tarsus width (TW), and bill length (BL) were taken by kiwi practitioners from 38 kiwi pairs (although some pairs were widowed or divorced during this time). In short, TL measures the tarsometatarsus length, TD and TW represent its minimum depth and width, respectively, while BL measures the bill chord (Colbourne et al., 2020). Measurements were taken in triplicate during each capture to the nearest 0.1 mm using a calibrated Vernier calliper, allowing estimation of measurement repeatability.

We measured each bird's body weight (BD) once prior to release using a calibrated Pesola Spring Scale (2.5-kg scale with 20-g precision for males, 5-kg scale with 50-g precision for females), as this metric is straightforward to obtain reliably and its variation is expected to reflect both environmental factors (e.g., food availability) and measurement error due to the relatively low technical precision of the scales. Measuring all traits requires capturing individuals, which is not always feasible in the field; hence, most birds have missing morphometric data in some years. Here, we treated TD and TW

as labile traits because they are strongly correlated with body weight (Chapter 2; see also Taborsky and Taborsky, 1999) and can therefore vary over time with environmental conditions or reproductive state, resulting in differences both among and within individuals. In addition, because TD and TW are the smallest traits in our dataset, they are likely more susceptible to measurement error (e.g., Subasinghe et al., 2021). Nevertheless, TL and BL were treated as fixed traits because they represent structural body dimensions that remain stable in adulthood and are not largely influenced by environmental conditions (Canale et al., 2016), aside from potential measurement error.

Measurement repeatability

We used a linear mixed-effects model (Nakagawa and Schielzeth, 2010) as implemented in the R (R Core Team, 2025) package 'rptR' 0.9.22 (Stoffel et al., 2017), to calculate measurement repeatability (*R*). In this analysis, VG represents the variance among individuals, reflecting consistent morphological differences between birds, while VR represents within-individual (residual) variance arising from measurement error and short-term variability. Repeatability therefore describes the proportion of total variation (VP = *VG* + *VR*) that is attributable to true differences among individuals rather than measurement noise. R is expressed as the ratio of these variance components (Stoffel et al., 2017): $R = \frac{VG}{VG+VR}$. We modelled repeatability separately for each trait (TL, TW, TD, and BL) to partition total variance into among-individual variance (*VG*) and within-individual (residual) variance (*VR*). Analyses used all birds in the dataset (*n* = 30♀, 34♂), with each individual measured in triplicate for every trait during each capture event. Body weight was excluded from repeatability analyses as each bird was weighed only once per capture. Models used trait as the dependent variable; sex, year of capture, and season of collection as fixed effects to control for their effect on the phenotypic variance (Subasinghe et al., 2021); and bird identity and operator identity as random effects to estimate, respectively, the among-individual and among-measurer components of variance used to calculate trait repeatability. Confidence intervals (95%) around repeatability estimates were obtained from 1,000 parametric bootstraps, and significance was assessed using 1,000 permutations. Confidence intervals (95%) around repeatability values were derived from 1,000 parametric bootstraps, and significance from 1,000 permutations.

Assortative mating analysis

We examined assortative mating using the classical approach (correlating unique trait values across mated pairs) and two statistical alternatives proposed by Class et al. (2017) that account for different sources of variation: correlation of individual means (CIM) and bivariate linear mixed-effects models (BLMMs).

For the classical approach, we examined the correlation of the first phenotypic value collected for each male and female within each pair (Class et al., 2017; Sawada et al., 2022). By using the first phenotypic measurements, we aimed to reduce potential "mates become alike" effects that may arise due to shared environmental exposure (Sawada et al., 2022), while recognising that some convergence may already have occurred before the initial measurement. While classical correlative analyses include each individual only once, our longitudinal dataset allows the same individual to appear in multiple pairings as a result of re-mating following divorce or widowhood. The CIM approach is a straightforward extension of the classical method, which utilises mean phenotypic values instead of single measurements to account for repeated observations over time (Class et al., 2017). This approach assumes that the correlation between individual mean trait values across mated pairs approximates the intrinsic assortative mating correlation (Class et al., 2017; Class and Brommer, 2018), if measurement error and environmental effects are minimal or random. For both datasets, we used parametric Pearson correlations (R package stats v4.6.0; R Core Team, 2025), as the data showed no marked deviation from normality (Figures S1–S2), with significance assessed by two-tailed t-tests.

The BLMM approach represents a sophisticated multi-level version of the classical method (Class et al., 2017), and allows disentangling the different sources of phenotypic resemblance between paired mates (Class et al., 2017; Class and Brommer, 2018). For each trait (TL, TW, TD, BL, BW), we fitted separate Bayesian BLMMs with the R package 'MCMCglmm' 2.36 (Hadfield, 2010), using homologous male and female trait values as two response variables. Measurement date (in calendar days) was included as a continuous fixed effect to account for seasonal variation in environmental conditions and reproductive state within each year. Pair identity, operator identity, and measurement year were included as random effects. Year and operator effects were included to control for potential covariance arising when both sexes are similarly influenced by year-specific

conditions or measured in a similar way by operators. This approach partitions the total covariance between male and female traits into two components. The between-pair correlation yields an unbiased estimate of the intrinsic assortative mating correlation, reflecting true similarity in mate choice. In contrast, the within-pair (residual) covariance captures phenotypic similarity arising from other sources, most likely shared environmental conditions shared by both partners and/or correlated measurement error (Class et al., 2017). Models assumed a Gaussian error structure for all response variables and were run for 900,000 iterations, with a burn-in of 100,000 and a thinning interval of 800. To boost model performance and interpretability, continuous predictor variables were standardised by centering and dividing them by two standard deviations (Gelman, 2008). We used weakly informative priors ($\nu = 3$) for all variance–covariance components and applied parameter expansion to improve convergence and mixing during MCMC sampling. Model performance was evaluated by confirming low autocorrelation (< 0.1) and effective sample sizes exceeding 1,000. Correlations between traits were considered significant when their 95% credible intervals did not include zero.

Results

All examined traits showed substantial variation within and between sexes, with females exhibiting significantly larger mean values than males across all four linear body measurements and body weight (Figure S5.1). These results align with the female-biased sexual size dimorphism previously reported for NI brown kiwi on Ponui Island (Cunningham and Castro, 2011) and more broadly across other populations and kiwi species in general (see Colbourne et al., 2020).

Measurement repeatability varied between traits (Figure 5.1a; Table S5.1), exhibiting a moderate to high level of agreement ($R \geq 0.642$). As expected, repeatability coefficients were higher for fixed traits ($R \geq 0.892$) than for labile traits ($R \leq 0.713$). Notably, repeatability was highest for bill length ($R = 0.997 \pm 0.001$ standard error; SD), indicating consistent measurements across repeated trials and negligible measurement error across operators. These results are supported by the inter-operator variance estimates (Figure 5.1b; Table S5.2), which indicate substantially higher measurement consistency for fixed traits compared with labile traits. Specifically, bill length and tarsus length were largely

unaffected by operator identity (< 2.5% of total variance), whereas tarsus width and depth showed stronger operator effects (10% and 13% of total variance, respectively), indicating greater sensitivity to observer-related differences in measurement precision.

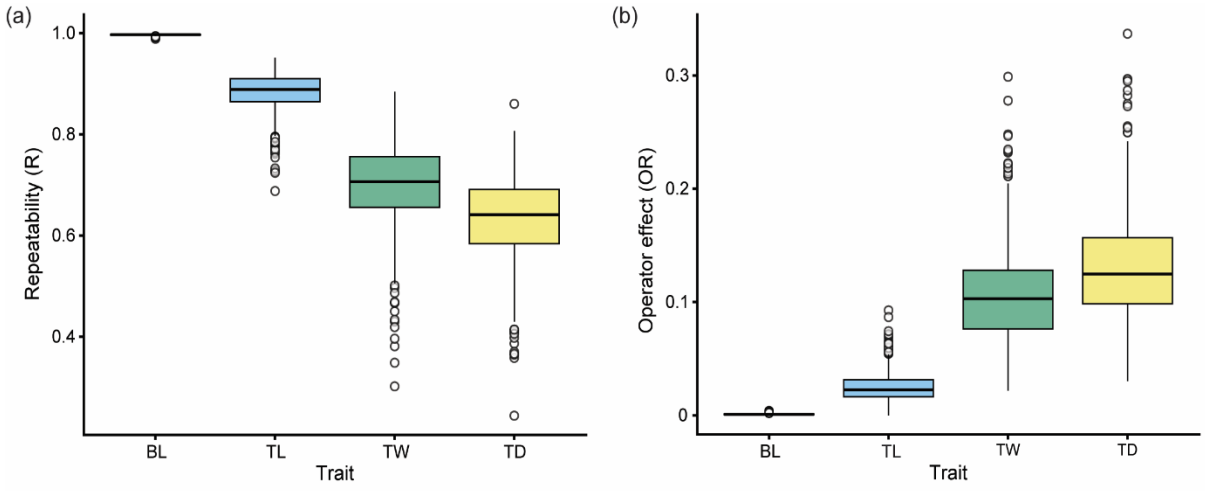

Figure 5.1. Estimates of measurement repeatability (a) and operator effects (b) for four body dimensions from paired birds in North Island brown kiwi on Ponui Island. Boxes correspond to the interquartile range, horizontal lines indicate the median, and whiskers represent the data range excluding outliers, which are shown as individual points.

Pearson's correlations between male and female trait values were generally weak and non-significant across all traits and analytical methods (Figure 5.2; Table S5.3). Correlation coefficients (r) obtained under the CA approach, using the first phenotypic value collected for each male and female within each unique pair, ranged from −0.108 (for bill length) to 0.171 (for body weight) and showed no statistical support (p-values ≥ 0.299). The CIM approach, which used mean phenotypic values to account for repeated measurements over time (Class et al., 2017), yielded similarly low correlation estimates ranging from −0.079 (for bill length) to 0.232 (for tarsus length), none of which were statistically significant (p-values ≥ 0.155; Table S5.3).

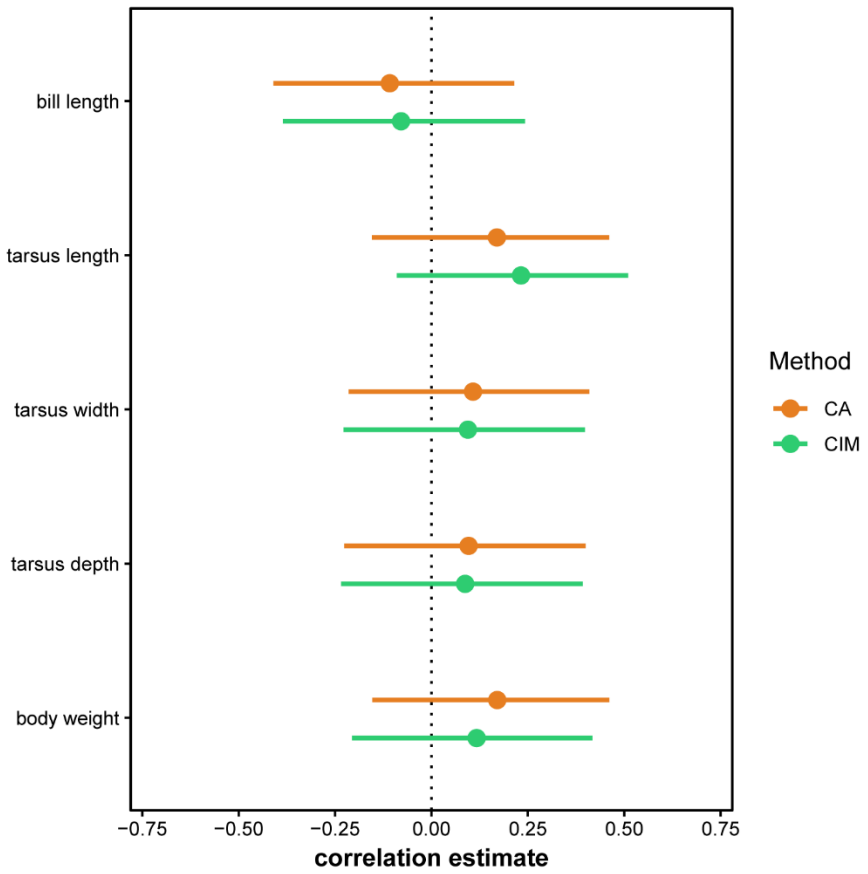

Figure 5.2. Distribution of correlation estimates (Pearson's *r* and 95% confidence intervals) between male and female trait values in paired North Island brown kiwi from Ponui Island, estimated using two analytical methods of assortative mating: the classical (CA) and the correlation of individual means (CIM) approaches.

The bivariate linear mixed-effects models (BLMMs) showed that labile traits (tarsus width, tarsus depth, and body weight) increased significantly with measurement date (Table S5.4), indicating that both sexes were heavier and that males exhibited more robust tarsi toward mid-year (around June). Models also indicated that male and female values did not correlate at the operator level, suggesting that measurement error was random and did not systematically influence the observed phenotypic correlations between paired males and females (Table S5). However, significant year-level correlations were detected for males' and females' tarsus depth (posterior mean = 0.724; 95% CI = 0.376–0.970) and body weight (posterior mean = 0.864; 95% CI = 0.670–0.995), suggesting that these traits covary across years (Table S5). Assortative mating,

as inferred from phenotypic correlations, was detected for tarsus length (posterior mean = 0.185; 95% CI = 0.006–0.363) and body weight (posterior mean = 0.167; 95% CI = 0.014–0.352), where weak but significant positive correlations indicated that males and females of similar tarsus length and body weight (which are considered useful proxies of overall body size in birds; e.g., Rising & Somers, 1989; Senar & Pascual, 1997; Subasinghe et al., 2021) are more likely to pair. However, partitioning the phenotypic correlations into their between-pair (intrinsic assortative mating) and within-pair (residual) components revealed that the apparent strength of assortative mating was overestimated and was largely driven by residual covariance (Figure 5.3).

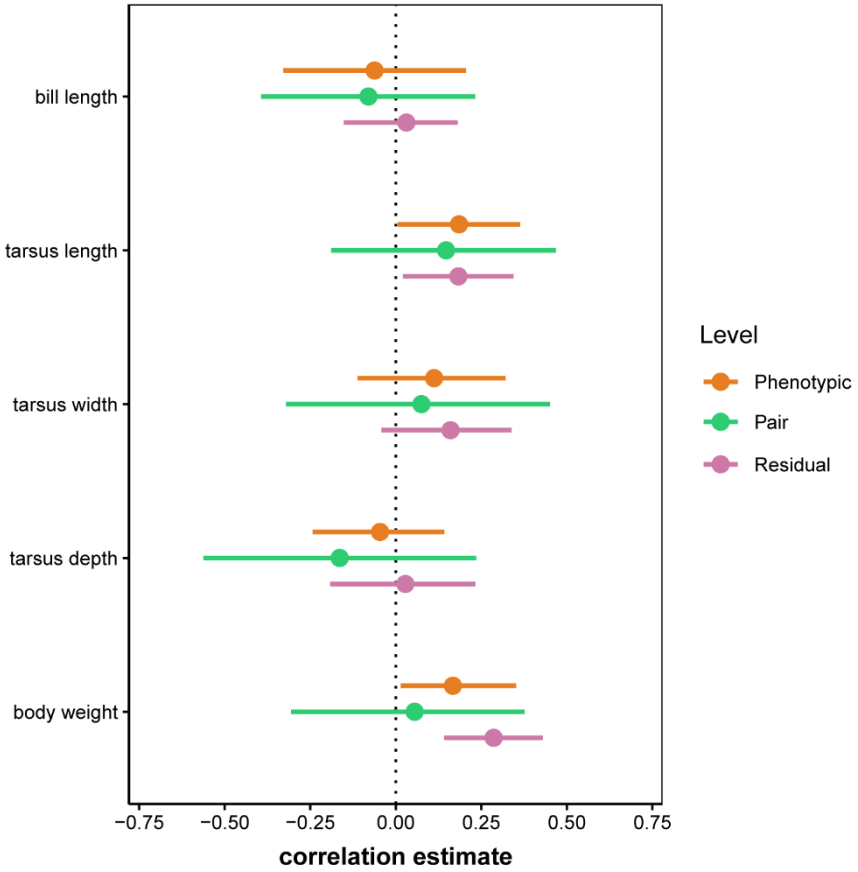

Figure 5.3. Distribution of correlation estimates (mean point estimates and 95% confidence intervals) of the phenotypic, between-pair (assortative), and residual (within-pair) correlations between male and female trait values in paired North Island brown kiwi from Ponui Island, estimated using bivariate linear mixed-effects models. Phenotypic correlations represent the combined influence of both between-pair (assortative) and within-pair (residual) components.

Discussion

Measurement repeatability is a major source of variation in phenotypic trait data and directly influences the statistical power and reliability of downstream analyses (Bailey and Byrnes, 1990; Subasinghe et al., 2021; Wylde and Bonduriansky, 2021; Meza-Joya et al., 2022). However, measurement repeatability is seldom accounted for in empirical assortative mating research (but see Class and Brommer, 2018; Common et al., 2024). We examined five morphological traits, some of which are labile and/or prone to measurement error and thus are expected to exhibit differing degrees of variability across repeated measurements. Accordingly, we found that repeatability coefficients differed among traits, with fixed traits exhibiting higher repeatability than labile traits. Notably, fixed traits (bill length and tarsus length) were also the largest, suggesting that trait size contributes to measurement consistency, and this agrees with previous findings comparing repeatability coefficients across bird species (Subasinghe et al., 2021). However, repeatability may differ even among fixed traits of comparable size owing to operator error, especially for traits with poorly defined boundaries, such as tarsus length, which is challenging to measure in kiwi (Colbourne et al., 2020). Despite these differences, fixed traits in our dataset were highly repeatable and largely unaffected by operator effects, which accounted for less than 2.5% of the total variance. In contrast, labile traits, such as tarsus width and depth, were more susceptible to operator effects, accounting for more than 10% of the total variance. This suggests that measurement repeatability in these traits reflects the combined influence of at least temporal variation, operator effects, and trait size.

Repeatability lies at the core of assortative mating research, as individuals are assumed to mate non-randomly with respect to individual-specific characteristics, such as the expression of traits that are highly repeatable or fixed (Kirkpatrick & Barton, 1997; Class et al., 2017). High repeatability minimises the risk that residual correlations between partners will bias estimates of assortative mating, even when these residual effects are pronounced (Class and Brommer, 2018). Accordingly, in our data set, the correlation between partners in bill length showed no indication of assortative mating. However, as repeatability decreases, phenotypic correlations may become increasingly influenced by residual covariance between partners, potentially biasing estimates of assortative mating (Class et al., 2017; Class and Brommer, 2018). This was the case for the weak, yet

significant phenotypic correlation observed in tarsus length in our study. Disentangling such apparent phenotypic correlation into its between-pair (intrinsic) and within-pair (residual) components revealed that the apparent strength of assortative mating was overestimated and largely driven by residual covariance. Taken together, these results highlight that even traits considered fixed and measured with relatively low error, such as tarsus length, can yield inflated estimates of assortative mating when residual covariance is not accounted for.

When considering traits with low repeatability, such as tarsus width and depth, we found no evidence of correlation between males and females at any level. This lack of association suggests that mate pairing for these traits is largely random, consistent with the results from both correlative approaches. For body weight, a labile trait that varies over time (Canale et al., 2016) and for which repeatability could not be estimated, the weak yet significant phenotypic correlation appears to be strongly influenced by residual covariance between mates rather than reflecting clear evidence of intrinsic assortative mating. As the origin of the residual covariances detected in these traits remains uncertain, we speculate that they may reflect shared environmental effects on paired individuals or correlated measurement error (see Class et al., 2017; Class and Brommer, 2018). For example, because paired kiwi often maintain their bonds beyond the breeding season and roost together for extended periods (Ziesemann, 2011), both partners are likely to experience and respond similarly to local environmental fluctuations, such as spatial and temporal variation in food availability and climate (Howard et al., 2024). These results are consistent with evidence that residual correlations between mated birds often arise in the wild, producing positive phenotypic associations even in the absence of intrinsic assortative mating (Class and Brommer, 2018).

While our findings indicate no evidence of assortative mating for the traits examined in paired kiwi on Ponui Island, further research is clearly needed to investigate the generality of this apparent pattern. Birds in this population usually form long-term relationships lasting for multiple breeding seasons (Undin et al., 2021; Chapter 2), so divorce or widowhood constitutes a critical event that can trigger extensive mate assessment before a new pair is established (Chapter 2). In this system, pair bond persistence is positively associated with male incubation effort (Chapter 2), suggesting that reproductive investment may play a role in shaping mate choice and that traits

signalling such investment could be key targets of selection (see Andersson and Simmons, 2006; Harris and Uller, 2009). The absence of strong evidence for assortative mating based on morphology parallels the limited support for MHC-based mate choice identified in Chapter Four, suggesting that kiwi pair formation on Ponui Island may depend more heavily on ecological opportunity, behavioural compatibility, or social dynamics than on strong phenotypic or genetic matching.

Our understanding of the phenotypic traits that influence mate choice in kiwi and how these traits signal reproductive potential is still emerging, offering valuable avenues for further investigation. For instance, larger body size is generally associated with greater reproductive returns, and sexual selection often favours size-assortative mating (Rollinson and Rowe, 2016). Despite this, assortment tends to be weak for structural traits, such as body dimensions, that do not accurately reflect overall body size (Jiang et al., 2013). A similar limitation may apply to body weight, as this trait is highly labile and strongly influenced by environmental conditions (Canale et al., 2016), which can obscure or weaken any signal relevant to mate choice. Nevertheless, as most kiwi were already paired at the time of the first encounter, our dataset likely does not reflect the trait values under selection during pair formation, a rather common limitation that is rarely acknowledged in empirical studies (but see (Common et al., 2024).

There are also additional possibilities suggesting that the absence of assortative mating in kiwi on Ponui Island may reflect a genuine biological pattern. Mating decisions based on phenotypic traits depend not only on preferences for specific characteristics but also on the availability of potential mates expressing those preferred phenotypes (Crespi, 1989; Wang et al., 2019; Höbel et al., 2022). The small founding kiwi population on Ponui Island (14 birds) may have restricted mate availability (Chapter 3), thereby limiting the opportunity for birds to realise their preferences—if any—for high-quality or compatible mates. In this context, the parental birds may have faced a trade-off between foregoing reproduction and accepting a suboptimal partner, with divorce possibly occurring as a behavioural mechanism to mitigate the costs of suboptimal pairing (Chapter 2). Moreover, recent genome-wide SNP data indicate that kiwi on Ponui Island form a successful hybrid swarm characterised by high genetic diversity when compared to the source populations and lower-than-expected relatedness between paired individuals (Undin et al., 2021). This pattern suggests the presence of kin-recognition

mechanisms that reduce inbreeding and promote genetic mixing, which may, in turn, weaken assortative mating based on phenotypic similarity by favouring disassortative pairing to maintain genetic diversity (Undin et al., 2021; Undin and Castro, 2022).

By using repeated measurements of kiwi pairs spanning more than two decades, our analyses reveal that phenotypic correlations between mates largely reflect residual covariation instead of true intrinsic assortative mating. The bivariate mixed models used here, allow intrinsic and residual components of assortative mating to be separated explicitly, providing a clearer understanding of the processes underlying mate similarity. Although our sample size (38 pairs) is modest and may limit the precision of some estimates, the long-term, repeated measurements for each pair considerably strengthen the reliability of our inferences (see Class et al., 2017). Further research incorporating additional pairs and broader geographic sampling would help confirm the generality of these patterns, but the present study provides strong initial evidence that residual covariance, rather than mate choice, is the primary driver of phenotypic similarity in this population. Likewise, traits not examined in this study may play a more important role in mate choice in NI brown kiwi. Kiwi possess a highly developed olfactory system (Cunningham et al., 2009; Wenzel, 1968), suggesting that chemical cues could be influential (e.g., Undin et al., 2021). Although we found no evidence that MHC-related odour cues contribute to mate choice in kiwi on Ponui Island (Chapter 4), olfactory signals may nevertheless influence other aspects of social behaviour, including partner recognition, assessment of reproductive status, or evaluation of partner quality (reviewed in Buchinger and Li, 2023). In addition, kiwi are unique in other sensory aspects, possessing poor vision but a highly developed auditory system (Corfield et al., 2011), which may be particularly important for mate choice when visual cues are limited. For example, male and female kiwi in all species have sexually dimorphic calls and produce duets (where males and females call together or respond to each other's calls; Corfield et al., 2008; Digby et al. 2014; Dent and Molles, 2015; Toy et al. 2021). In Little Spotted Kiwi (*A. oweni*), birds coordinated duets play a key role in reproductive behaviour by facilitating pair contact, resource defence, and pair bonding (Digby et al., 2014). Kiwi on Ponui Island also duet, but whether these vocalisations serve the same purpose is not yet known. Integrating acoustic data with current statistical approaches represents an exciting avenue for future research on the evolutionary ecology of assortative mating in kiwi, with broader implications for conservation and management.

In summary, the analyses in this chapter show that phenotypic resemblance between kiwi partners largely reflects residual covariance rather than intrinsic assortative mating. By clarifying the relative contributions of measurement consistency, environmental influences, and true mate choice, this study provides an important foundation for future work on kiwi mating strategies. Identifying the cues that genuinely inform mate choice—whether morphological, behavioural, olfactory or acoustic—remains a key priority for advancing our understanding of kiwi reproductive biology. Together, the behavioural and genetic findings presented throughout this and the previous chapters in this thesis reveal a complex reproductive system in which pair stability, incubation behaviour, and immunogenetic variation interact within the unique context of a translocated kiwi population. Although evidence for direct MHC-based mate choice was limited, the integration of behavioural and genetic perspectives provides a broader understanding of reproductive ecology, evolutionary processes, and conservation management in kiwi.

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Appendix

Figure S5.1. Distribution histograms for each morphological trait included in the classical correlational approach of assortative mating among paired North Island brown kiwi from Ponui Island.

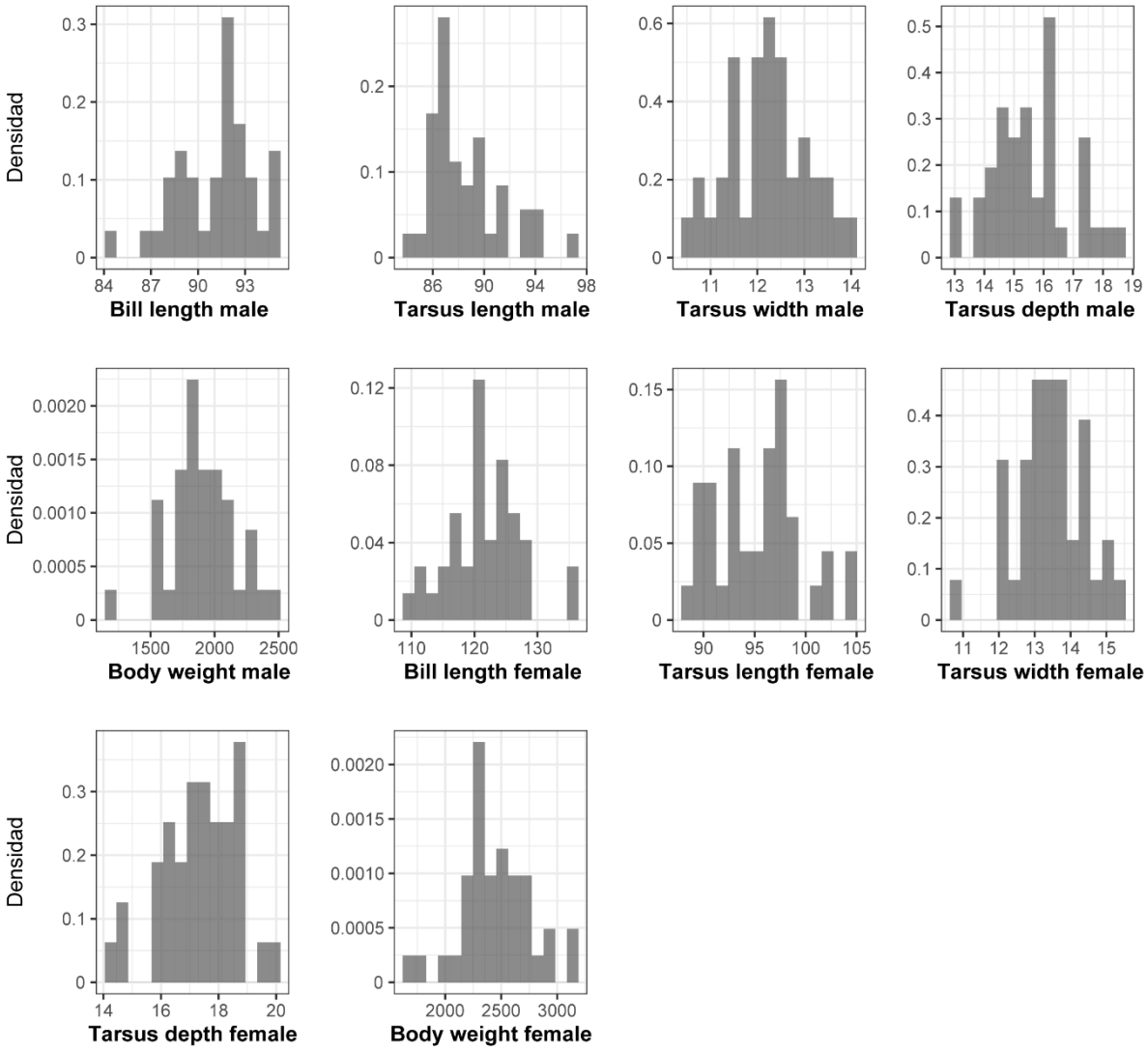

Figure S5.2. Distribution histograms for each morphological trait included in the correlation of individual means approach of assortative mating among paired North Island brown kiwi from Ponui Island.

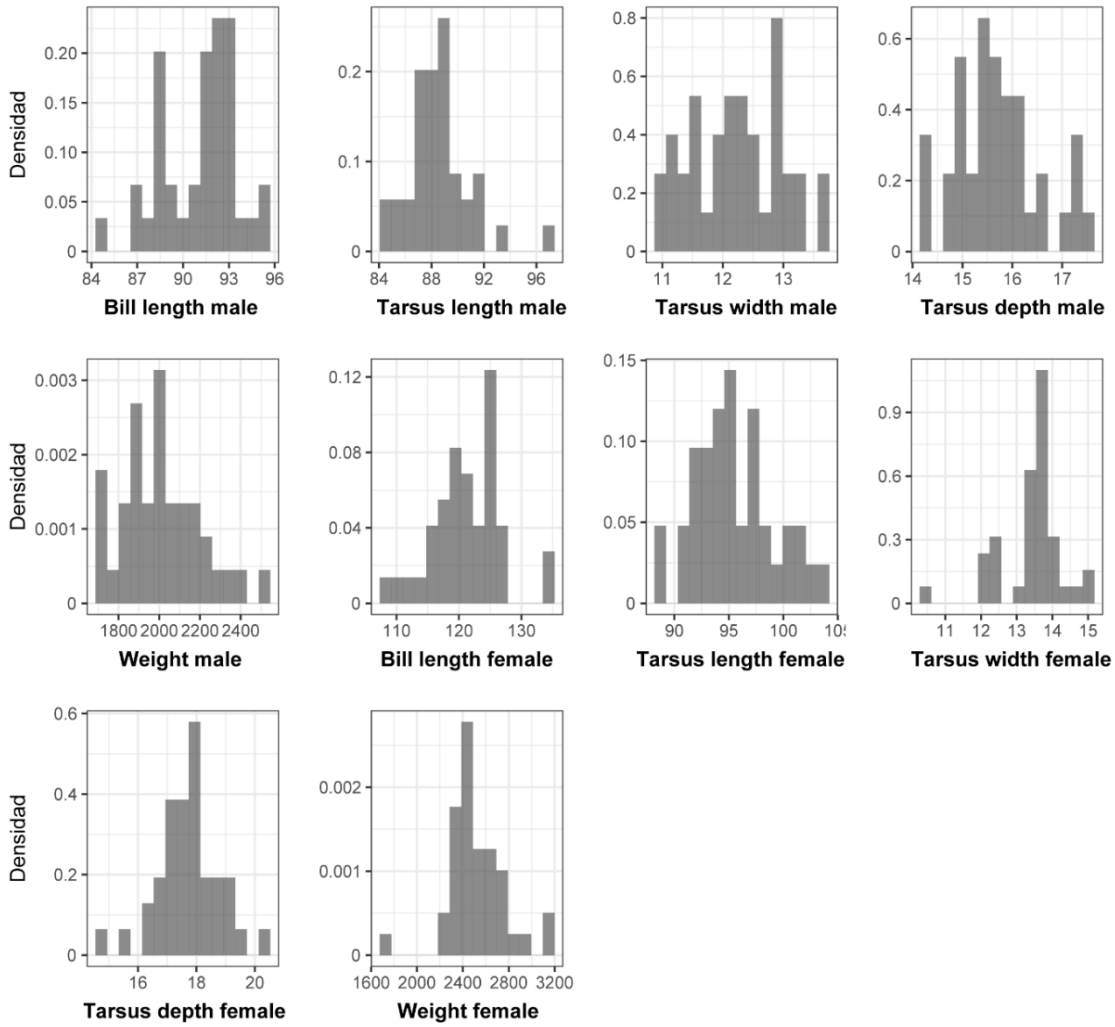

Figure S5.3. Violin plots showing morphological differences between males and females in traits used to assess assortative mating in North Island brown kiwi from Ponui Island. Note that females exhibited significantly larger mean values than males across all four linear body measurements and body weight, as determined by independent t-tests. Boxes correspond to the interquartile range, horizontal lines indicate the median, and whiskers represent the data range excluding outliers, which are shown as individual points.

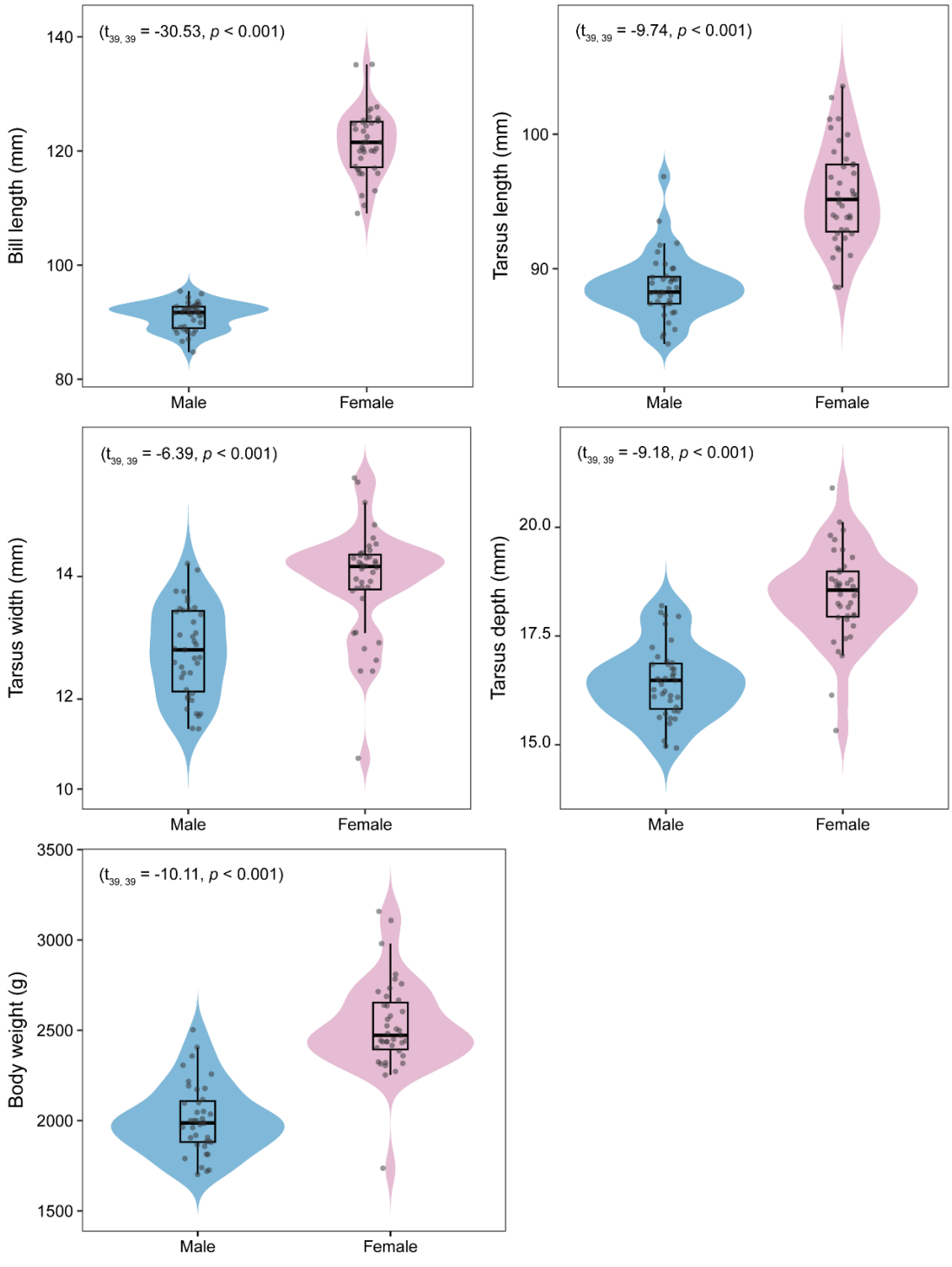

Table S5.1. Measurement repeatability of four body dimensions from paired birds in North Island brown kiwi on Ponui Island. Note that repeatability (R) coefficients were higher for fixed traits than for labile traits. Repeatability scores are presented with standard errors (SE) and 95% confidence intervals (95% CI). Traits are ordered from highest to lowest repeatability.

Trait	Type	R	SE	95% CI
bill length	fixed	0.997	0.001	[0.994, 0.998]
tarsus length	fixed	0.892	0.037	[0.794, 0.936]
tarsus width	labile	0.713	0.076	[0.543, 0.824]
tarsus depth	labile	0.643	0.081	[0.457, 0.769]

Table S5.2. Proportion of total measurement variance explained by operator effects for four body dimensions from paired birds in North Island brown kiwi on Ponui island. Note that operator effect (OR) coefficients were lower for fixed traits than for labile traits. Operator effect scores are presented with standard errors (SE) and 95% confidence intervals (95% CI). Traits are ordered from highest to lowest repeatability (see Table S5.1).

Trait	Type	OR	SE	95% CI
bill length	fixed	0.001	0.000	[0.000, 0.002]
tarsus length	fixed	0.024	0.012	[0.007, 0.056]
tarsus width	labile	0.103	0.043	[0.043, 0.190]
tarsus depth	labile	0.129	0.058	[0.058, 0.229]

Table S5.3. Correlation coefficients (Pearson's *r* and 95% confidence intervals = 95% CI) between male and female trait values in paired North Island brown kiwi from Ponui Island, estimated using two analytical methods of assortative mating: the classical and the correlation of individual means approaches. Note that none of the analysed traits showed statistical support for assortative mating (p-values ≥ 0.155).

Method	**Trait**	**Pearson's *r***	**95% CI**	***p*-value**
Classical approach	body weight	0.171	[−0.153, 0.461]	0.299
	tarsus length	0.170	[−0.154, 0.462]	0.302
	bill length	−0.108	[−0.410, 0.215]	0.512
	tarsus width	0.108	[−0.215, 0.409]	0.514
	tarsus depth	0.096	[−0.226, 0.400]	0.559
Correlation of individual means	body weight	0.117	[−0.206, 0.417]	0.478
	tarsus length	0.232	[−0.090, 0.510]	0.155
	bill length	−0.079	[−0.385, 0.243]	0.633
	tarsus width	0.094	[−0.228, 0.398]	0.567
	tarsus depth	0.087	[−0.235, 0.392]	0.597

Table S5.4. Posterior estimates for the effect of measurement date on male and female traits in North Island brown kiwi from Ponui Island. Values are posterior means, 95% credible intervals (95% CI), and Bayesian p-values (pMCMC) as estimated by the R package 'MCMCglmm' 2.36 (Hadfield, 2010). Notably, labile traits such as tarsus width, tarsus depth, and body weight increased significantly in at least one sex toward mid-year (around June), likely reflecting greater resource availability and increased energy allocation to reproduction.

Trait	**Sex**	**Mean**	**95% CI**	***p*-value**
bill length	male	−0.028	[−0.161, 0.083]	0.653
	female	−0.025	[−0.080, 0.030]	0.363
tarsus length	male	−0.205	[−0.406, 0.025]	0.072
	female	−0.065	[−0.162, 0.051]	0.256
tarsus width	male	0.159	[0.013, 0.319]	0.046
	female	0.093	[−0.032, 0.219]	0.171
tarsus depth	male	0.287	[0.048, 0.487]	0.009
	female	0.027	[−0.123, 0.177]	0.757
body weight	male	0.241	[0.148, 0.336]	< 0.001
	female	0.302	[0.213, 0.394]	< 0.001

Table S5. Correlations between male and female trait values in paired North Island brown kiwi from Ponui Island, partitioned across hierarchical levels of variation in the bivariate linear mixed-effects models. Entries are posterior mean correlations with 95% credible intervals (95% CI). Pair correlations represent the between-pair (assortative) component, whereas residual correlations reflect within-pair (residual) covariance. Phenotypic correlations represent the combined effect of both between-pair (assortative) and within-pair (residual) components. Correlations were considered significant when their 95% credible intervals did not include zero. Significant correlations are highlighted in bold.

Trait	Phenotypic	Pair	Year	Operator	Residual
bill length	−0.062	−0.080	−0.047	0.053	0.031
[95% CI]	[−0.329, 0.205]	[−0.394, 0.232]	[−0.862, 0.732]	[−0.785, 0.851]	[−0.152, 0.181]
tarsus length	**0.185**	0.147	0.152	0.095	**0.183**
[95% CI]	**[0.006, 0.363]**	[−0.189, 0.468]	[−0.665, 0.952]	[−0.767, 0.824]	**[0.021, 0.344]**
tarsus width	0.112	0.075	0.153	0.212	0.160
[95% CI]	[−0.112, 0.321]	[−0.321, 0.451]	[−0.610, 0.910]	[−0.566, 0.912]	[−0.042, 0.338]
tarsus depth	−0.046	−0.164	**0.724**	−0.095	0.028
[95% CI]	[−0.243, 0.142]	[−0.562, 0.235]	**[0.376, 0.970]**	[−0.827, 0.736]	[−0.192, 0.233]
body weight	**0.167**	0.055	**0.864**	0.277	**0.286**
[95% CI]	**[0.014, 0.352]**	[−0.306, 0.377]	**[0.670, 0.995]**	[−0.346, 0.953]	**[0.141, 0.430]**

Forest–grassland landscape with gullies on Ponui Island (Hauraki Gulf, Aotearoa New Zealand, North Island), habitat of a translocated population of North Island brown kiwi (*Apteryx mantelli*).

Chapter Six

"Such pairs would have an advantage in rearing offspring, more especially if the male had the power to defend the female during the pairing-season, as occurs with some of the higher animals, or aided in providing for the young."

(Darwin, 1871, p. 263)

Thesis discussion: Mate choice and pair fidelity in North Island brown kiwi on Ponui Island

Kiwi translocations have shifted from early 'trial-and-error' efforts with little or no planning to recent programmes guided by rigorous planning, scientific input, and advances in predator control (Jahn et al., 2022). Despite substantial advances, national and regional conservation and translocation guidelines for kiwi (Craig et al., 2011; Germano et al., 2018; Jahn et al., 2022) still pay limited attention to a key component of kiwi natural history, its breeding behaviour (Undin and Castro, 2022). Indeed, the success of translocations depends on animals being able to secure food and shelter, avoid predators, and, most importantly, find mates to reproduce with (Bell et al., 2019; Greggor et al., 2025). This thesis delivers updated and novel information about the behavioural, genetic, and morphological determinants of mate choice, pair fidelity, and divorce in a translocated population of North Island (NI) brown kiwi on Ponui Island. A central implication is that translocation outcomes may depend not only on the demographic and genetic composition of release groups, but also on how released individuals reorganise socially and reproductively after establishment. In this section, I briefly consider how our major findings might contribute to ongoing discussions about the role of breeding behaviour in future kiwi conservation translocation and management.

Translocations can alter key factors shaping breeding systems and mating strategies by placing individuals into novel selective contexts (Pribil and Searcy, 2001; Quader, 2005; Griffith et al., 2010), and this appears to be the case for kiwi on Ponui Island. While long-term social monogamy was the most common mating system in this population, birds in this population also divorced, and in 22% of cases, formed non-kin polygamous breeding units (see also Undin and Castro, 2022). Such breeding flexibility is particularly important in this context, as partner swaps and polygamous associations can increase opportunities for admixture and help reduce the risk of inbreeding depression, ultimately influencing genetic variability and population resilience (Lande, 1988; Møller and Legendre, 2001; Frankham, 2010; Kretzschmar et al., 2020; Undin and Castro, 2022). When polygamy allows individuals to mate with multiple partners, the resulting increase in gene flow can help retain genetic diversity compared with monogamy, potentially offsetting the constraints imposed by a small founder population (Undin and Castro, 2022). Monitoring breeding systems and mating dynamics following translocation may therefore offer early indications of how admixture is progressing and whether genetic mixing is being maintained, thereby informing management actions—such as

reinforcement translocations, if needed—to mitigate potential negative effects (e.g., inbreeding depression) and support long-term population viability.

Divorce in kiwi on Ponui Island occurred at low rates (8.1%) and was more likely following poor incubation effort, indicating that male reproductive investment plays a key role in maintaining long-term pair cohesion. This opens opportunities to investigate phenotypic traits that signal behaviours linked to high reproductive investment and could help inform translocation planning, but current knowledge for kiwi remains limited, underscoring the need for continued research and long-term monitoring. While low divorce rates are typical in most NI brown kiwi populations studied to date (Potter, 1989; Taborsky and Taborsky, 1999; Chapter 2), one dense population showed exceptionally high rates, likely driven by a surplus of high-quality females (Potter, 1989; Taborsky and Taborsky, 1999). This pattern suggests that divorce in NI brown kiwi is shaped by demographic and social context, emphasising the need to consider density-dependent processes in conservation planning. Nevertheless, we found that pair familiarity had a positive effect on nesting success, indicating that long-lasting partnerships may enhance reproductive outcomes. This finding is especially relevant as kiwi translocations often involve removing individuals from wild populations (Jahn et al., 2022), which may inadvertently disrupt established pair bonds and alter reproductive outcomes in source populations.

To minimise this risk, managers should be cautious about translocating only established pairs, as mated birds often do not remain bonded after translocation (see Colbourne et al., 2020). However, targeting exclusively unpaired adults may also be problematic, as some individuals may be unpaired because of reproductive inexperience, poor condition, behavioural traits, or competitive disadvantages that limit their ability to secure or maintain mates. A more appropriate approach would therefore be to combine carefully selected paired and unpaired individuals, considering genetic diversity, body condition, age structure, sex ratio, and behavioural information where available. While some established pairs may not remain bonded after translocation, moving some paired individuals may still be appropriate, as these birds have already formed successful partnerships and may be well placed to establish new bonds and continue contributing reproductively after release. Likewise, unpaired birds may provide useful founders when their lack of a partner reflects external or demographic constraints, such as skewed sex

ratios, limited mate availability, or strong competition. In these cases, they may still have high reproductive value if released into a population where such constraints are relaxed. Importantly, any removal of birds should also consider the potential consequences for source populations, including disruption of existing breeding pairs, loss of reproductive adults, altered local sex ratios, and reduced future reproductive output.

Translocations that mix individuals from different source populations can increase population size and growth rates, in part because the introduction of new genetic material can enhance genetic diversity and reduce the effects of inbreeding depression, thereby increasing the chance of genetic rescue (Ingvarsson, 2001; Frankham, 2015; Bell et al., 2019; Undin et al., 2021a; Undin and Castro, 2022). Consistent with this expectation, kiwi on Ponui Island show elevated genetic diversity at both genome-wide SNP markers (Undin et al., 2021a) and MHC IIβ exon 2 (Chapters 3 and 4), despite originating from a small founder group. These results demonstrate that hybrid translocation can help preserve genetic diversity—and crucially, functional immune variation—even in recently established populations. However, caution is warranted as mixing differentiated populations can also result in outbreeding depression and reduced offspring fitness (Allendorf et al., 2001; Edmands, 2007; Thavornkanlapachai et al., 2019). Such contrasting scenarios leave conservation managers with difficult choices when selecting source populations for translocations, as these decisions can strongly influence both immediate translocation success and long-term population persistence (Thavornkanlapachai et al., 2019). While kiwi on Ponui Island indicates that mixing divergent populations can reinforce genetic variation, the growing use of translocation in kiwi conservation (Jahn et al., 2022) highlights the need for further studies to inform when and how multiple source populations should be incorporated into translocation planning.

Adjustments in mate choice following translocation, particularly shifts in assortative mating, are important to consider as they can alter pairing dynamics and influence both reproductive outcomes and genetic structure (Bradley et al., 2014; Engler et al., 2019; Undin et al., 2021b). This is especially pertinent when translocating individuals from multiple source populations, which may differ in reproductive behaviour or genetic background (Bradley et al., 2014; Undin and Castro, 2022). Despite the central role often attributed to MHC in mate choice (Mays & Hill, 2004; Setchell & Huchard, 2010), we

found no evidence that MHC IIβ exon 2 influenced mating patterns or pair-bond stability in kiwi on Ponui Island (Chapter 4). Likewise, mate choice based on morphological traits does not appear to operate in this species (Bain, 2018; Undin et al., 2021a; Chapter 5). Interestingly, genome-wide SNP data suggest that disassortative mating does occur in kiwi on Ponui Island (Undin et al., 2021a), a pattern that may help maintain genetic diversity and reduce extinction risk through genetic rescue (Undin et al., 2021a; Undin and Castro, 2022). While we found no evidence of assortative mating for the MHC region or the phenotypic traits examined, further research is clearly needed to investigate the generality of this apparent pattern. Nevertheless, mating decisions depend not only on preferences for specific traits but also on the availability of potential mates expressing them (Crespi, 1989; Wang et al., 2019; Höbel et al., 2022; Chapter 5). This has important implications for translocations, as opportunities for mate choice may be substantially constrained in small and isolated kiwi populations (Undin et al., 2021a).

Collectively, the findings of this thesis suggest that kiwi reproductive associations emerge through the interaction of behavioural flexibility, ecological context, and genetic background within a translocated population. While evidence for direct mate choice based on MHC compatibility or morphological similarity was limited, behavioural factors such as incubation investment and pair stability appeared more strongly associated with reproductive dynamics. Together, these results suggest that post-translocation mating systems may be shaped less by strict genetic or phenotypic matching and more by the interaction between demographic structure, behavioural compatibility, and opportunities for social association.

Data limitations, challenges, and opportunities

Kiwi are the most translocated bird species in Aotearoa New Zealand (Cromarty & Alderson, 2013; Miskelly & Powlesland, 2013), and with translocation projects continuing to expand (Jahn et al., 2022), identifying the factors that distinguish successful from unsuccessful outcomes is essential for improving future efforts. Although kiwi reproductive behaviour holds clear relevance for conservation planning, the breeding systems and mating strategies have only recently been proposed as important considerations for conservation management (Undin and Castro, 2022). This is partly

because foundational knowledge of reproductive behaviour remains limited for most kiwi species, constraining our ability to incorporate it effectively into management strategies. Kiwi are cryptic and nocturnal birds that nest in subterranean or tree cavities and abandon incubation when disturbed (Taylor et al., 2014), which poses challenges for studying their mating behaviour. These challenges are particularly pronounced in studies of breeding systems and mating preferences, which require the identification of breeding units and data collection from mated individuals. This process demands intensive fieldwork, including repeated tracking of individuals across multiple reproductive seasons to establish pair or group membership, as well as direct nest inspections to confirm reproductive activity.

Recent advances in miniaturisation of electronic devices have enabled the use of animal-borne loggers, which provide a valuable source of data by recording information directly from individual animals (Wilmers et al., 2015). Among these are Chick Timer™ transmitters (wildtech.co.nz/Kiwi.aspx), which are equipped with algorithms that register and interpret activity patterns in kiwi and have been crucial for overcoming key challenges in studying their behaviour (Taylor et al., 2014; Colbourne et al., 2020; Undin and Castro, 2022). However, employing such devices entails repeated handling, which poses questions for data reliability due to possible post-handling behavioural changes (De Rosa, 2021). While kiwi on Ponui Island have been monitored using Chick Timer™ transmitters since 2010, which has enabled consistent long-term tracking and improved reproductive monitoring, the number of tracked pairs is still small to meet the sample size requirements of robust statistical analyses. Expanding monitoring on Ponui Island is therefore essential, but equally important is the collection of comparable long-term behavioural data from other NI brown kiwi populations. Such data would help clarify variation in incubation effort, the influence of environmental conditions on reproductive investment, and the demographic contexts that facilitate alternative breeding systems and mating strategies across the species' range. Obtaining comparable data from natural populations is urgently needed, as such information can provide a baseline for evaluating whether patterns observed in translocated populations are typical of the species or influenced by the translocation process.

Reliable density estimates are essential for effective conservation planning and management and are urgently needed for managed kiwi populations (including source

populations), whether they are presumed to occur at low or high densities (see De Rosa, 2021). Addressing this limitation is crucial for examining how breeding systems and mating strategies shift with population density, social context and ongoing habitat change (Undin and Castro, 2022). Such data are also important because population size is often linked to genetic diversity (Frankham, 1996) and can therefore assist in identifying suitable source populations for future translocations, although confirmation using genome-wide data remains necessary. Likewise, individual- and population-level genomic data can be used to assess effective population size, admixture, breeding behaviour, and mate choice over time, and to evaluate the effectiveness of conservation interventions (Semenov et al., 2017; Thavornkanlapachai et al., 2019; Galla et al., 2020; Undin et al., 2021a; Undin and Castro, 2022). Deepening our understanding of how breeding systems and mating preferences contribute to reproductive success will ultimately strengthen conservation strategies—particularly translocations—by ensuring that the fundamental processes underpinning population viability are properly integrated into management decisions.

Concluding remarks

This study has provided important insights into how breeding behaviour in NI brown kiwi (or kiwi as a genus, for that matter) can inform translocation planning and management, reinforcing the need to embed reproductive ecology into species recovery frameworks (e.g., Undin et al., 2021a; Undin and Castro, 2022). As discussed above, translocations can introduce kiwi to novel selective contexts, where shifts in resource distribution, demographic structure, and social environments may reshape reproductive tactics. Because these efforts often involve removing individuals from wild populations (Jahn et al., 2022), the breeding behaviour of source populations may also be affected, further complicating the already challenging task of predicting translocation outcomes. The breeding system and mate preferences are flexible aspects of breeding behaviour that are particularly sensitive to environmental and demographic conditions (Emlen & Oring, 1977; Quader, 2005; Carrete et al., 2006; Undin & Castro, 2022), with the potential to shape individual reproductive success, genetic admixture, population growth, and ultimately population viability (Anthony and Blumstein, 2000; Quader, 2005; Bradley et

al., 2014; Koenig et al., 2019; Undin and Castro, 2022). This is particularly relevant for conservation programmes involving small founder groups or mixed-source releases, where post-release mate availability and partner redistribution can influence both reproductive output and genetic structure.

Given the scale and pace of anthropogenic habitat alteration, destruction, and fragmentation, shifts in breeding systems and mating preferences are increasingly likely (Caro and Sherman, 2011; Candolin and Wong, 2016; Buchholz et al., 2019; Undin and Castro, 2022), reinforcing the importance of incorporating behavioural dimensions into conservation planning. Advancing our understanding of the drivers and conditions that shape breeding systems and mating strategies presents a valuable opportunity to enhance conservation management and support the recovery of threatened species (Griesser and Suzuki, 2016; Koenig and Dickinson, 2016; Griesser et al., 2017; Koenig, 2017; Klug, 2018; Undin and Castro, 2022). Kiwi on Ponui Island offer a rare example indicating that the translocation of a small number of individuals from divergent source populations into a novel habitat—despite the presence of pest mammals—can result in successful population growth. This outcome appears to be supported by behavioural flexibility in breeding, which may have facilitated lineage admixture and increased genetic diversity (see also Undin et al., 2021a; Undin and Castro, 2022). While further research is needed to clarify the factors underlying the success of kiwi on Ponui Island, this population offers a valuable system for advancing understanding of breeding behaviour and its integration into conservation management (Undin and Castro, 2022). Such insight might be critical to ensuring that future translocations support the long-term viability of kiwi diversity across Aotearoa New Zealand. Broadly, this thesis highlights the importance of integrating behavioural ecology, reproductive biology, and conservation genetics when managing translocated populations, particularly in long-lived species with complex social systems and constrained mate availability.

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