Evolutionary consequences and fitness correlates of extra-pair mating in the tūī, *Prosthemadera novaeseelandiae*

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Ecology

at Massey University, Auckland,

New Zealand

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2014

Frontispiece

“Me he korokoro tūī”

“How eloquent he is; he has the throat of a Tūī”

Maori proverb

Abstract

Animals of many different taxa exhibit sexual dimorphism in phenotypic traits that have evolved through male-male competition and/or female mate choice. Many bird species also engage in extra-pair mating that can exert significant selection pressures on male secondary sexual characters. Studies examining the evolution of sexual dimorphism in birds have demonstrated that the degree of plumage dimorphism is positively related to rates of extra-pair paternity (EPP) among species. In contrast, it is generally argued that sexual size dimorphism (SSD) is not related to EPP but instead is associated with social polygamy. Thus understanding the adaptive function of extra-pair mating may shed light on the evolution of male sexually selected traits. It is argued that females increase offspring fitness by maximising the benefits they receive from males through extra-pair mating. Females are hypothesised to gain direct benefits such as parental care or territory quality through social mate choice, as well as indirect benefits such as compatible genes or good genes benefits through extra-pair mate choice. However, despite much research, the exact adaptive function of female genetic polyandry is still debated.

This thesis investigates the influence of sexual selection on the evolution of reproductive behaviours and male secondary sexual characters. We examine correlates and fitness consequences of female within-pair and extra-pair mate choice in the New Zealand tūī, *Prosthemadera novaeseelandiae*. Tūī are unusual for a socially monogamous passerine in that they exhibit extreme SSD. They also possess unique ornamental white throat feather plumes which are larger in males, but of which the functional significance is unknown. We discover that tūī possess one of the highest rates of EPP of any socially monogamous bird: EPP occurred in 72% of all broods and 57% of all offspring were extra-pair. Our results show that tūī exhibit a level of SSD normally associated with strong polygamy, with males being 50% heavier than females. In addition, male body size and ornament size are strong predictors of paternity success. Thus, contrary to current opinion, these findings demonstrate that EPP can be causal in the evolution of SSD. Furthermore, this study provides needed evidence that selective pressures, acting via EPP, can lead to the evolution of male ornaments.

Females paired with large social males gained direct benefits that increased offspring survival. Parental ability was not related to male size suggesting that large males may instead provide higher quality territories. Two traits correlating with male extra-pair mating success also predicted offspring fitness: extra-pair young and the offspring of genetically dissimilar sires possessed faster growth rates, and females mated to highly ornamented males overproduced sons to gain offspring with greater reproductive potential. These findings suggest that females gain indirect benefits from extra-pair mate choice, and support both the Fisher-Zahavi good genes and compatible genes hypotheses as functional benefits of female genetic polyandry.

Examination of tūī parental provisioning rates revealed that males provided significantly less parental care than females. Male ornament size was inversely related to paternal effort, providing further support that ornamentation indicates indirect rather than direct benefits in tūī. In addition, males with lower paternity of

their broods provided more care than males gaining full paternity. When considered together with female choice for highly ornamented extra-pair males, these findings suggest the existence of alternative male reproductive strategies in which males trade-off investment in offspring with mating effort in response to their residual reproductive potential.

Tūī are unusual in that they possess several attributes commonly associated with social polygyny, such as extreme SSD, multiple male traits, and reduced paternal care. Although the selection pressures leading to the evolution of ornamentation and SSD in tūī remain to be clearly resolved, the relationship between these two traits is likely complex. We argue that these traits may have evolved through male-male competition but are reinforced by female choice for dominant males. This study substantially furthers our knowledge of tūī mating behaviours and makes a significant contribution towards understanding the functional significance of male traits and EPP. Finally, our findings have implications for sexual selection theory pertaining to the evolution of SSD.

Acknowledgements

The biggest thanks go to my supervisors who have each taught me so much during this time. I feel very lucky to have no less than three supervisors that have each contributed different sets of skills towards this thesis. First and foremost I would like to thank the endless dedication of my supervisor Weihong Ji. Thank you for giving me the opportunity to do my PhD at Massey. You have always had such faith in me, which has spurred me on when things were not going quite to plan. You have been a mentor as well as a supervisor. Your words of encouragement and the scientific discussions we have had have been a sounding board for many ideas that I hope we can build on in the future. Many thanks to my secondary supervisor Dianne Gleeson and Landcare Research, without whom this PhD would never have happened. Dianne was one of the first people to give me a job in New Zealand and it has all gone from there. Thank you for organising all the research funding for the genetics work, I am very grateful and feel very lucky that I had never had to worry about running out of money. Well, that is, until I ran out of money! Thank you for all your support with the genetics analysis, it has been great to have someone so knowledgeable onboard to guide me. Last but definitely not least, many thanks to the supreme king of framework, my third supervisor Jim Dale. Your extensive breadth of knowledge and critical input has been invaluable and has helped to guide this thesis to where it is today. You always found time to respond to my many requests for advice and comments and they were always very insightful.

I must sincerely acknowledge Landcare Research who provided the funding for genetic analysis for this project and have been involved in my career in New

Zealand back in 2005. In particular, Gary Houliston and Frank Molinia went out of their way to make sure I had what I needed after Dianne Gleeson moved to Australia. This is an incredible company made possible by incredible people, and it has been great to be a part of it.

Fieldwork for this project was conducted at Tawharanui Regional Park with permission from Auckland Council and the Department of Conservation. In addition, a huge thank you is needed to Tim Lovegrove and Matt Maitland at the Auckland Council for their financial and logistic support for this project. I am incredibly grateful to you both for funding the fieldwork, and allowing my volunteers and I to stay at Tawharanui for almost 5 months a year. I would not have been able to achieve half of what I have without your support. A big thank you also to Moe and Colin at Tawharanui for your support with my fieldwork. You made my time up at the “vol hole” a fun experience and I never got tired of seeing your cheeky faces at the end of a long day. Moe, I hope the “bo” finds its’ “at”.

No PhD would be complete without extensive, sometimes fatiguing, statistical analysis. I am indebted to Beatrix Jones, David Aguirre, and Adam Smith for help with statistical analysis. Some of you may have driven me to tears of frustration (you know who you are!) but it was worth it in the end. Thanks also to Dianne Brunton for giving me the opportunity to be part of the Ecology and Conservation group and your advice on my project.

Lab work can be sometimes tedious, but never a dull day was had with Ana Ramón-Laca, Diana Prada, Robyn Howitt, Shelley Myers, Julia Allwood, and Tom Winstanley. My Landcare girls (sorry Tom!) provided so much help and support and always had time to work through a solution to a problem with me. Thank you for providing epic lunchtime feijoa-eating competitions accompanied by good banter. I am also grateful for the advice of Duckchul Park who always has the answer for any genetic problem you can possibly think of, and to Shaun Forgie for making me laugh with his hilarious attempts at conducting lab work and our HK BBQ sessions with Quentin Paynter. May that place remain open, cheap, and with amazingly hot sauce forever.

A huge thank you to the many volunteers I had in the field, Fatima Torrico (Fats), Helen Cadwaller, Damien Menard, Ulla Kail, Jordi Segars, Mariska Kraaij, and Mithuna Sothieson. Particularly to Sam Hill who had to spend a month alone with me on the Chatham Islands, you kept me sane. I feel very lucky to have had such great volunteers and without whom I would not have been able to accomplish so much. A big thanks to Kevin Parker, Luis Ortiz Catedral, Michael Anderson, Mark Delaney, Marleen Baling, and David Gudex-Cross, both for their help in the field and for collecting samples for me. I am grateful to Barry Lett, for allowing me to catch so many tui on his property and for turning a blind eye to the Tourettes which seemed to grip me whenever a tui managed to get a good hold of me.

Merci beaucoup à « Crazy Christophe », t'es complètement fou mais ça m'a fait plaisir de partager un bureau avec toi. Encore merci pour ton aide sur le terrain et pour tes corrections sur ma thèse. C'est à ton tour maintenant ! J'espère que je pourrais aussi t'aider quand tu reviendras.

To all my friends and colleagues at Massey and around the world, from New Caledonia to the UK, thank you so much for your support during this project. A special mention goes Marc Oremus for starting this whole thing and having faith in me, to Sara, Jez, and Loosie for being the best flatmates and being so supportive, to ma poulette, Anne-Sophie Boyer, for her support and encouragement, to Megan Young for the surf trips that gave me perspective, to Hannah Wannah Jones for weekends in Raglan and mutual rants about PhD issues, and to my girls in Europe who I have missed so much: Sophie Hodder, Lucy Nicholson, Harriet Sandliands, Sarah Ellis, Lucy Abrahams, Charlie Palmer, Mathilda Evans, Sara Peterman, and Heather Davies. I know I may not have been in as much contact with many of you as I should have but I promise that will now change!

Finally my long-suffering family deserve much credit and my gratitude for putting up with, and supporting, a permanent student. Thanks particularly to my auntie Caro for your encouragement and to Granny S for being so amazing at 95 years old and showing no signs of slowing down! To my soon-to-be family, the "Van clan"; Paula and Jeff, Carly and Scott, Jason, and Margaret and Eddy, thank you for all your support you have given me over the past years. I feel very lucky to have a second family so close, while mine is so far away. To my amazing Dylan, I honestly do not think I could have done this without you by my side. You have got me through endless meltdowns and your smile at the end of the day is all that I have needed to make the world seem right again. I can't wait for the surf trips and the new adventures. Love you newt. And last but not least, I dedicate this thesis to my parents. You started this whole thing by getting me into nature at a very young age, showing me the small things, and taking me on hikes armed with a bumblebee survey kit (the joys of having a science teacher as a mother!). You have nurtured and supported me without question my whole life, and I feel very lucky to have been guided by your immense knowledge and wisdom. Thank you for everything.

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General Introduction

Female tūī, *Prosthemadera novaeseelandiae*, with pollen on her head from feeding on flax, *Phormium tenax*.

1. General Introduction

1.1 Variation in extra-pair paternity

As recently as the 1980's, it was widely assumed that over 90% of all bird species were monogamous (Lack 1968). However, the study of mating systems has been revolutionised by the advent of molecular techniques, such as the polymerase chain reaction, in assigning parentage. This led to the discovery that the broods of many previously assumed monogamous species contained offspring fathered by more than one male (Gowaty and Karlin 1984; Birkhead and Møller 1998), and provided evidence that females engage in extra-pair matings with males outside of the observed social pair-bond (Møller 1988). This discovery led to a proliferation of studies re-examining mating systems in many bird species (reviewed in Griffith et al. 2002). Consequently a distinction between genetic and social mating systems is now recognised, and extra-pair matings are now known to occur in the majority of avian species, and in as much as 86% of all passerines (Griffith et al. 2002).

Rates of extra-pair paternity (EPP) vary considerably between species (Fig. 1.1). The average rate of EPP within socially monogamous species is 11% of all offspring (Griffith et al. 2002) but ranges from 0% of offspring in the New Zealand robin, *Petroica australis* (Ardern et al. 1997), to the highest rate of EPP found to date in the cooperatively breeding superb fairy wren, *Malurus cyaneus*, where 76% of offspring could be attributed to extra-pair matings (Mulder et al. 1994). Variation in rates of EPP also exists both within and among populations of the same species, suggesting a role of ecological constraints in the evolution of EPP (e.g. Bouwman et al. 2006; Schmoll 2011). For example, the rate of EPP has been shown annual variation between 7% and 33% in the bluethroat**,** *Luscinia svecica* (Johnsen and Lifjeld 2003), while rates varied between 0% and 50% between different populations of willow warblers, *Phylloscopus trochilus* (Gyllensten et al. 1990; Bjornstad and Lifjeld 1997). Such variation between populations may at least partially be explained by differences in breeding densities (e.g. Griffith et al. 1999; Canal et al. 2012; Mayer and Pasinelli 2013), although many other ecological explanations have been proposed (reviewed in Neudorf 2004; Schmoll 2011).

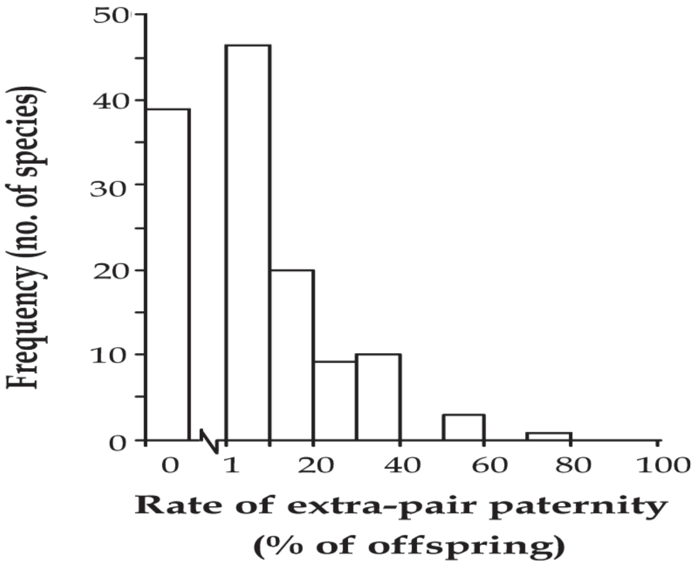

Figure 1.1: Rates of extra-pair paternity among passerines with different mating systems (from Griffith et al. 2002). The average rate among socially monogamous birds is 11%. The highest rate of 76% has been found in the cooperatively breeding superb fairy wren**,** *Malurus cyaneus* (Mulder et al. 1994).

EPP can only evolve as a function of the change in social or ecological interactions between the female, the social male, and the extra-pair male (Lifjeld et al. 1994). Yet we currently lack knowledge of whether extra-pair matings are male or female driven (reviewed in Eliassen and Kokko 2008). There is little contention that the opportunity to gain additional matings is likely to be adaptive for males. Indeed in species such as the wandering albatross, *Diomedea exulans* (Jouventin et al. 2007), extra-pair matings appear to be forced upon females by males. In others, males seek out extra-pair matings that may, or may not be accepted by females (Wagner 1998). Females may accept extra-pair copulations as the costs of accepting are lower than the potential costs associated with resisting (Arnqvist and Kirkpatrick 2005). Evidence is accumulating that females actively solicit extra-pair matings (Kempenaers et al. 1992; Sheldon 1994; Double and Cockburn 2000). EPP is often argued to be a female strategy to evade the mate choice restrictions imposed by social monogamy (Foerster et al. 2003). However, the specific consequences of EPP for female fitness, which is primarily limited by fecundity, are less obvious (reviewed in Forstmeier et al. 2014). Apart from a few notable exceptions where females gain access to food resources (e.g. Gray 1997), females obtain no apparent direct benefits from extra-pair males. Consequently, much research has been conducted on the possible indirect benefits of female extra-pair mating. Multiple mating may increase offspring fitness by simply increasing sperm competition so that only the fittest sperm fertilize eggs (Madsen et al. 1992; Evans et al. 2003). Alternatively, extra-pair mating may be a form of fertility insurance to guard against social mate infertility (Wetton and Parkin 1991; Sheldon 1994; Vedder et al. 2011). As it shall be seen, EPP can also create significant selective pressure on male traits and reproductive behaviour. However, despite decades of research into the adaptive function of EPP, findings are equivocal and no consensus has been reached (Wan et al. 2013). Westneat and Sherman (2003) asserted that, in order to further our knowledge of the evolutionary significance of EPP, future research should examine within-population variation in rates of EPP in association with individual traits across a wide range of species. Therefore, this thesis aims to address these deficiencies in knowledge using a species of honeyeater, the New Zealand tūī, *Prosthemadera novaeseelandiae*, that is new to the EPP literature.

1.2 The origin of sexual selection

Anisogamy, the differential investment in gametes, is generally assumed to explain the origin of sexual selection. Anisogamy inherently limits the number of gametes that a female is able to produce, while the mass-production of sperm creates the competition for mating upon which sexual selection is founded (reviewed in Lessells et al. 2009). The primary outcomes of anisogamy were identified by Bateman (1948) who asserted that a male's reproductive success positively increases with the number of matings he can achieve. Therefore, in order to increase fitness, males should endeavour to maximise their reproductive success by seeking additional matings. In contrast, he further demonstrated that a female's ability to produce offspring is limited by fecundity. As a direct consequence, females are commonly the choosy sex and may invest heavily in selecting mates that maximise offspring fitness (Emlen and Oring 1977).

This fundamental difference between males and females underlies the basis of sexual selection. Hence, sexual selection can ultimately be defined as competition over mates (Andersson 1994). Sexual selection can thusly be partitioned into competition between males for mating access (intrasexual selection) or female choice for high quality males that maximise fitness (intersexual selection). Both models of sexual selection can lead to the evolution of male phenotypic traits that increase a male’s mating opportunities. Darwin (1871) was the first to recognise and attribute male characters that do not function to increase survival to the processes of intra- and intersexual selection. Later, Trivers’ (1972) seminal paper built on the observations of Darwin, and this work forms the basis of sexual selection theory today. Understanding the adaptive processes of male-male competition and female choice is central to understanding the evolution male traits.

1.3 Sexual selection and the evolution of male displays

A main aim of sexual selection theory is to explain the evolution of secondary sexual characters that exert no apparent benefit on survival (Darwin 1871). Sexual selection of a trait can be ascribed to differences in reproductive success caused by competition over mates (Andersson 1994). As a consequence, both female choice and male-male competition can lead to the evolution of male traits that advertise male quality. Male-male competition can create selective pressures on male traits due to its ability to exclude the losers from mating opportunities, and therefore increase the variance in male mating success. Male-male competition has been documented to drive the evolution of male traits such as ornaments and colouration (e.g. Ligon et al. 1990; Pryke and Andersson 2003; Santos et al. 2011) that are used as “badges of status” and indicate dominance. Such traits are purported to be kept honest through continual testing during male-male competition (Berglund et al. 1996; Qvarnström and Forsgren 1998). More commonly, traits such as body size or weapons that assist a male’s fighting ability and dominance over other males are typically assumed to evolve through male-male competition (Berglund et al. 1996).

Female choice for high quality sires may select for the evolution of costly (and therefore condition-dependent) male traits that advertise the genetic benefits for offspring that can be gained by mating with him (Grafen 1990; Iwasa and Pomiankowski 1994, 1999). However, there is mixed empirical support for female preference for male traits (Akçay and Roughgarden 2007).

Male-male competition and female choice are often mutually reinforcing (Bradbury and Davies 1987; Berglund et al. 1996; Qvarnström and Forsgren 1998; Wong and Candolin 2005). Indeed, there is empirical evidence that male traits driven by both male-male competition and female choice impart benefits for offspring survival (Norris 1993; Petrie 1994; Sheldon et al. 1997; Andersson et al. 2002). However, the strength and direction of intra- and intersexual selection interactions may also vary (Bradbury and Davies 1987; Qvarnström and Forsgren 1998; Wong and Candolin 2005; Hunt et al. 2009). For example, male traits that have evolved through male-male competition do not always provide fitness benefits for females (reviewed in Qvarnström and Forsgren 1998; Arnqvist and Rowe 2005). For example in three-spined sticklebacks, *Gasterosteus aculeatus* (Sargent 1985), sand gobies, *Pomatoschistus minutes* (Forsgren 1997), and collared flycatchers, *Ficedula albicollis* (Qvarnström 1997), dominant males incur a fitness cost for females as a result of trade-offs between investment in male-male competition and parental care. Thus females should not always prefer dominant males (e.g. Moore et al. 2003). In such instances intra- and intersexual selection may be antagonistic, and the evolution of the trait will depend on the outcome of balancing selection (Moore and Moore 1999). Such mechanisms have been invoked to explain the persistent variation in individual trait display (Moore and Moore 1999). Alternatively, competitively superior males may override female preference so that male mating success does not correlate with female preference (e.g. Petersson et al. 1999). Similarly, promiscuous females may be constrained from engaging in extra-pair mating by superior mate guarding by dominant social males (e.g. Komdeur et al. 1999; Chuang-Dobbs et al. 2001; Wilson and Swaddle 2013). Thus, understanding of the potential interaction between male-male competition and female choice in the evolution of male traits is still lacking (Wong and Candolin 2005; Hunt et al. 2009). The evolution and adaptive significance of male advertisement forms a central part of this thesis. In Chapter 3 I address whether selection pressures are acting on male traits and discuss the potential for these to have arisen from intra- or intersexual selection. In Chapters 4 and 6 I examine the influence of male sexually selected traits on offspring fitness to examine the possible pathways that have led to the evolution of these traits.

1.3.1 The evolution of multiple male traits

Males of many species exhibit multiple traits that function as signals of quality or dominance in male displays. Two main hypotheses have been proposed to explain the adaptive significance of informative multiple traits in male display (Møller and Pomiankowski 1993; Johnstone 1996). First, the “multiple messages” hypothesis states that these displays represent different cues for different aspects of male quality. Different traits may be used to gain advantages in male-male competition and female choice. For example in the polygynous red-collared widowbird, *Euplectes ardens*, females prefer males with long tails, while males with larger carotenoid feather patches are more competitive in male contests and hence were more likely to hold territories (Andersson et al. 2002). In water boatmen, *Sigara falleni*, females select males with a larger body and leg size, but body mass influenced mating success through male-male competition (Candolin 2005). Alternatively, females may make mating decisions based on traits indicating multiple aspects of male quality (Møller et al. 1998; Rowe 1999; McGraw and Hill 2000; Blows et al. 2003; Candolin 2003; Coleman et al. 2004; Hebets and Papaj 2005; McGlothlin et al. 2005). For example, female three-spined sticklebacks, *Gasterosteus aculeatus*, prefer both redder males for indirect benefits, and large males for parental quality (Kraak et al. 1999). Few studies, however, have identified different male traits associated with within-pair and extra-pair mate choice (Yezerinac and Weatherhead 1997; Lehtonen et al. 2009).

Second, the “back-up” hypothesis asserts that multiple cues act as reinforcement for the same trait to provide an increased accuracy of assessment (Møller and Pomiankowski 1993; Johnstone 1996). For example, both the size of a black pigment patch and the rate of display in male tawny dragons, *Ctenophorus decresii*, indicate aggression levels, and therefore function of badges of status in male-male competition (Osborne 2005). In fence lizards, *Sceloporus undulatus*, females use a combination of head size, throat patch size, and body condition to make mate choice decisions (Swierk et al. 2012). Back-up signals appear to be rarer than multiple traits advertising multiple messages (Candolin 2003).

The evolution of multiple traits is thought to be stable only when the costs of choosiness are not significantly increased by additional assessment (Iwasa and

Pomiankowski 1994). Much research focus has been given to investigating different traits that influence mating success, and yet relatively little attention has been focussed on the fitness consequences of multiple trait display (Candolin 2003). As discussed below, little is known of the relative importance of direct and indirect benefits in driving the evolution of male sexually selected traits, yet their influence on multiple male traits is even less clear (Candolin 2003; Andersson and Simmons 2006). I therefore address this issue in this thesis using a species that exhibits multiple secondary sexual characters and the possibility to gain both direct and indirect benefits via social and extra-pair mate choice. Findings from each chapter are applied in Chapter 7 to discuss the functional significance of multiple male traits in this species.

1.3.2 The role of mating systems in the evolution of sexual dimorphism

Sexual dimorphism is thought to be mediated through sexual selection in relation to the mating system and parental care of a species (Andersson 1994). For example, it is posited that the high levels of sexual dimorphism observed in polygamous species are the result of the high variation in male mating success produced by intense male-male competition and female choice. Sexual dimorphism may manifest as differences in plumage, such as colouration or the moderation of feathers into ornaments, or as differences in structural size or mass i.e. sexual size dimorphism (SSD). Extreme examples are found in polygynous lekking species such as peacocks and birds of paradise, where it is purported that female choice has driven the evolution of these male displays (Petrie 1994). In these systems males contribute little to offspring development apart from sperm, instead trading-off investment in elaborate plumage to increase mating opportunities.

In contrast, the advantage of size in male-male competition is thought to be the primary driver of SSD (Darwin 1871). Consistent with this hypothesis, SSD is common in male-male competitive systems such as resource-defence polygyny, in which large males dominate in competition for territories, and hence ultimately attract more mates (e.g. Searcy and Yasukawa 1983). Comparative studies investigating the association of SSD and social mating systems have further asserted that polygyny favours the evolution of SSD (Webster 1992; Owens and Hartley 1998; Dunn et al. 2001). Indeed, there are relatively few examples of substantial SSD in socially monogamous passerines (Payne 1984). The polygynous brown songlark, *Cinclorhamphus cruralis*, exhibits the highest rate of SSD currently known, in which males are 76% heavier than females (Magrath et al. 2003). The polygynous red-winged blackbird also exhibits extreme SSD with males 50% heavier than females (Searcy and Yasukawa 1983). In comparison, the highest rate of SSD currently known among socially monogamous passerines is in the Australian yellow wattlebird, *Anthochaera paradoxa*, where males are 37% heavier than females (Milledge 2001). The yellow wattlebird, whose genetic mating system in unknown, is also the largest of the honeyeaters (*Meliphagidae*).

Socially monogamous birds are generally characterised by less extreme sexual dimorphism than polygamous species, as energy is instead invested in parental care (Andersson 1994). Payne (1984) thus argued that sexual selection is stronger in polygamous than monogamous species. However, in apparent conflict with this hypothesis many socially monogamous species do exhibit sexual dimorphism, particularly in plumage (Møller 1986). The discovery of EPP provided the pathway to create the necessary high variance in male reproductive success (e.g. Vedder et al. 2011), and provoked a proliferation of studies investigating the influence of female extra-pair mate choice on the evolution of male traits. As a consequence, associations between male mating success and the expression of male secondary sexual characters have been reported, and EPP is now considered to be the principle driver of plumage dimorphism (Møller and Birkhead 1994; Owens and Hartley 1998; but see Dunn et al. 2001). In contrast, these studies found no association between the degree of SSD and EPP (Owens and Hartley 1998; Dunn et al. 2001). However, there has been no study of the influence of EPP on SSD in a socially monogamous species with both a high rate of EPP and SSD, where a correlation would be most expected. I address this issue in the tūī, a species exhibiting both extreme SSD and EPP rates.

1.4 Adaptive significance of mate choice

Kokko et al. (2006) eloquently defined mate choice as “the outcome of the inherent propensity of an individual to mate more readily with certain phenotypes of the opposite sex, and the extent to which an individual engages in mate sampling before deciding to mate”. Females are commonly the choosy sex and may invest greatly in selecting mates that optimise offspring fitness (Darwin 1871; Andersson 1994; Kokko et al. 2003). Two models of potential fitness benefits have been proposed to explain the adaptive significance of female mate preferences (Kirkpatrick and Ryan 1991).

1.4.1 Direct benefits

Direct benefits may accrue to females when mate choice is based on the quality of the immediate benefit to themselves. The "good parent" hypothesis predicts that direct benefits are signalled by sexually selected traits that indicate any male quality that increases female survival chances, fertility, or fecundity (Haywood 1989; Hoelzer 1989; Iwasa and Pomiankowski 1999). These advantages may take the form of access to food resources, protection from predators, courtship feeding, insurance against mate infertility, or parental care (reviewed in Andersson 1994). For example, an early study by Hill (1990) demonstrated that female house finches, *Carpodacus mexicanus*, preferred colourful males that provide relatively more parental care than dull males. In this species, bright colouration is associated with dietary carotenoid intake and thus is an honest indicator of male condition. Other positive correlations between parental ability and colouration have been found in brown boobies, *Sula leucogaster* (Montoya and Torres 2014), northern cardinals**,** *Cardinalis cardinalis* (Linville et al. 1998), European kestrels, *Falco tinnunculus* (Palokangas et al. 1994), and American kestrels, *Falco sparverius* (Wiehn 1997), while other studies have shown a correlation between song quality and male parental ability (Catchpole 1986; Welling et al. 1997; Buchanan and Catchpole 2000)**.**

In addition to parental care, territory quality is likely to have a significant direct effect on offspring fitness (reviewed in Andersson 1994). Females may either choose males based on traits that indicate a male's dominance, or on territory quality directly (Alatalo et al. 1986; Calsbeek and Sinervo 2002). Correlations between male phenotypic characteristics and territory quality have been demonstrated in the great reed warbler, *Acrocephalus arundinaceus*, where territory quality correlates with male song quality (Forstmeier and Leisler 2004), and in three-spine sticklebacks where large males of a superior competitive ability gain higher quality territories (Candolin and Voigt 2001). Traits that have evolved through male-male competition are more likely to provide females with an honest indicator of potential direct benefits. Yet, although it is argued that female choice should often be correlated with dominance (but see Qvarnström and Forsgren 1998; Wong and Candolin 2005 for reviews), studies investigating the fitness consequences of female choice for dominant males are lacking (Qvarnström and Forsgren 1998; Hunt et al. 2009).

However, selection may not always favour the advertisement of direct benefits (Kokko 1998). For example, if ornament size instead advertises male attractiveness or his genetic quality, smaller ornamented males with a reduced mating success may instead compensate by increasing investment in parental care (e.g. Burley 1986; Sanz 2001; Jawar and Breitwisch 2004). Thus, studies investigating the correlation between male advertisement and parental ability are equivocal.

1.4.2 Indirect benefits

Recent focus has turned to the more contentious genetic benefits that females may gain from mate choice. Species with EPP provide a good test of indirect benefits as in many species females gain no apparent direct benefits from extra-pair mates (but see Burke et al. 1989). Indirect benefits occur when linkage disequilibrium exists between female mate preference genes and genes influencing fitness, and consequently preferred males provide genetic benefits that either increase offspring survival (Fisher 1915) or mating success (Fisher 1958). Various functional hypotheses have been proposed to explain female mate choice for indirect benefits (reviewed in Griffith et al. 2002; Griffith and Immler 2009), however the following two hypotheses are typically considered to offer the greatest support as adaptive explanations for female multiple mating.

1.4.2.1 The "good genes" hypothesis

The "good genes" hypothesis assumes that there is additive genetic variance in fitness between males and that female preference is based on male sexually selected characters that are genetically correlated with total fitness (Kirkpatrick 1996). Males should be selected to visually advertise their genetic quality, and females should prefer to mate with males possessing the most exaggerated phenotype. Zahavi (1975) asserted that, in order to maintain honesty of the signal, sexually selected characters should be costly to produce, i.e. a "handicap", such that only high-quality males can endure these costs. There has been much focus on this hypothesis in recent years, and it has received substantial support with studies finding positive correlations between paternity success and the expression of sexually selected male traits such as body size (e.g. Kempenaers et al. 1992, 1997; Canal et al. 2011), ornamentation (e.g. Petrie 1994; Sheldon and Ellegren 1999; Thusius et al. 2001), colouration (e.g. Johnsen et al. 2001; Estep et al. 2005), and song diversity (e.g. Hasselquist et al. 1996; Poesel et al. 2006). However, support is equivocal (Akçay and Roughgarden 2007; Forstmeier et al. 2014).

Assortative mating from generation to generation, i.e. females preferring large ornaments produce offspring that also prefer large ornaments as offspring inherit preference genes as well as fitness genes, can lead to rapid positive directional selection on the trait, termed the “Fisherian runaway” (Fisher 1930). This process may evolve in conflict with adaptation through natural selection, and the trait then becomes a disadvantage. For example, males with conspicuous plumage or ornaments may become more obvious to predators. The expression of the trait is then dependent on balancing selection between the cost of carrying the trait and the gain in reproductive success it offers. Although both good genes and Fisherian runaway mechanisms arise through linkage disequilibrium between genes for female preference and high fitness at other loci (Cameron et al. 2003; but see Maklakov and Arnqvist 2009), in contrast to good genes, increased offspring viability is not a necessary outcome of Fisherian runaway mating. However, fitness comprises both survival and reproductive success, and instead, females mated to attractive males should overproduce sons that increase fitness through increased reproductive success in the next generation i.e. the “sexy son” hypothesis (Weatherhead and Robertson 1979; Burley 1981). However, this theory has been disputed and theoretical models predict that an evolutionary equilibrium may only be reached when female preference is associated with males that maximise fecundity (Kirkpatrick 1985). In contrast, others have suggested that female choice for sexy sons benefits may be stable when there is a cost associated with the expression of the trait (Zahavi 1975; Eshel et al. 2000), and it has been argued that good genes and sexy son benefits should not be distinguished as they are both justifiable by the same indirect benefits model (Kokko et al. 2002; Chandler et al. 2013). However, the topic is still widely debated (Cameron et al. 2003; Kokko et al.

2006; Chandler et al. 2013) and therefore it is currently unresolved whether this mechanism exists in nature. To add to this debate, a basic prediction of the sexy son hypothesis, that females should overproduce sons when mated to attractive males, is tested in this study.

1.4.2.2 The "compatible genes" hypothesis

Indirect benefits may also be non-additive and mediated through the combination of male and female genotypes. Thus contrary to the good genes hypothesis, not all males will provide equal fitness benefits to all females (Tregenza and Wedell 2000; Puurtinen et al. 2005). The "compatible genes" hypothesis asserts that female attain indirect benefits by mating with genetically compatible individuals that increase offspring viability through heterozygote overdominance or avoidance of the deleterious effects of inbreeding (Zeh and Zeh 1996, 1997; Jennions 1997; Tregenza and Wedell 2000). Inbreeding depression is a common consequence of intragenomic conflict caused by increased identity by descent and lowered functional genome-wide heterozygosity (Coulson et al. 1998). However, most tests of the compatible genes hypothesis have focussed on multi-locus heterozygosity (MLH) at neutral markers as a proxy of functional heterozygosity. MLH has been shown to not accurately reflect functional heterozygosity in some circumstances (e.g. Sauermann et al. 2001; Hansson et al. 2004; Westerdahl et al. 2005). Nevertheless, positive associations between MLH and male reproductive success (Foerster et al. 2003; Seddon et al. 2004), offspring survival (Hansson et al. 2001; Foerster et al. 2003), as well as sexually selected characters such as plumage ornamentation (Foerster et al. 2003), and song structure (Seddon et al. 2004) or repertoire size (Marshall et al. 2003) have been demonstrated. Furthermore, there is increasing empirical evidence for females to use genetic dissimilarity as a criterion for mate choice (e.g. Kempenaers et al. 1999; Foerster et al. 2003; Kawano et al. 2009). However, the role of female choice in the compatible genes theory is contested, and it has been argued that apparent female choice for genetically dissimilar mates and the subsequent enhanced fitness may result from post-copulatory differential fertilisation success of sperm by genetically dissimilar males (Tregenza and Wedell 2000; Colegrave et al. 2002; Griffith and Immler 2009).

1.4.2.3 Good genes and compatible genes are not mutually exclusive

Good genes and compatible genes benefits may be in balance in the population or may cycle depending on the cost-benefit relationship (Colegrave et al. 2002; Neff and Pitcher 2005). For example, Foerster et al. (2003) found evidence of both types of benefits occurring simultaneously in a population of blue tits, *Cyanistes caeruleus*. Temporal plasticity in female mate choice has been demonstrated in mice (Roberts and Gosling 2003) and house finches, *Carpodacus mexicanus* (Oh and Badyaev 2006) in which female preference shifted between good genes and compatible genes depending on the variability of the selected traits in the population. Furthermore, recent evidence suggests that these mechanisms may not be mutually exclusive concepts, as correlations between male heterozygosity and the expression of sexually selected traits have been demonstrated, i.e. the good-genes-as-heterozygosity hypothesis (Marshall et al. 2003; Reid et al. 2005; Ilmonen et al. 2009). Nevertheless, few studies have investigated the relative strengths and importance of each benefit, thus is it still unresolved whether females employ both criteria in mate choice. Thus a greater knowledge of the evolution of ornamental traits and their function in relation to genetic compatibility is needed (Mays and Hill 2004). In this study I test these hypotheses in a species which possesses the potential to gain significant indirect benefits from mate choice.

1.4.3 Relative importance of direct versus indirect benefits

Among lekking species, where males typically contribute only sperm, female mate choice is attributed to genetic benefits (Kokko et al. 2003; Mead and Arnold 2004). Mate choice may become more complex in monogamous species with a resource-based mating system where females may stand to gain either direct or indirect benefits (Westneat et al. 1990). Alternatively, females may obtain both types of benefits from the same mate. For example, females may mate with genetically compatible extra-pair males who also assist in nest defence (Gray 1997), or female preference for a male trait that provides direct benefits may therefore also provide genetic benefits if the trait is heritable (Cordero and Eberhard 2003). For example, superior males of high genetic quality often also win superior territories (Montgomerie and Thornhill 1989; Alatalo et al. 1991). Indeed, direct benefits are likely to be important for female reproductive success (Price et al. 1993; Iwasa and Pomiankowski 1999) and have been overlooked in recent studies (Kotiaho and Puurtinen 2007). When direct and indirect benefits are dissociated, females may also face a trade-off between the two (Calsbeek and Sinervo 2002; Oneal et al.

2007). Thus it is important to consider both models in studies investigating the evolution of mate choice (Maklakov and Arnqvist 2009).

Few studies have examined direct and indirect benefits simultaneously (Kokko et al. 2003; but see Jones et al. 1998; Iyengar and Eisner 1999; Calsbeek and Sinervo 2002; Kojima et al. 2009) thus it is difficult to assess the generality and importance to female fitness of each model. The strength of each type of selection will be proportional to the covariance between fitness and the trait (Kokko et al. 2006). However, the relative fitness magnitudes of each model are still debated (Kokko et al. 2003; Hunt et al. 2004; Kotiaho and Puurtinen 2007; Hettyey et al. 2010) and have been, at least in part, hampered by the difficulty in empirically quantifying absolute fitness (Hosken and Tregenza 2005). It has been proposed that female choice for indirect benefits asserts a relatively weak influence on selection in comparison to direct benefits (Kirkpatrick 1996; Kirkpatrick and Barton 1997; Arnqvist and Kirkpatrick 2005; Arnqvist and Rowe 2005; Orteiza et al. 2005; Akçay and Roughgarden 2007; Maklakov and Arnqvist 2009; Lebigre et al. 2012). However other studies have shown that indirect selection on fitness can be of almost equal importance than direct selection (Møller and Alatalo 1999; Møller and Jennions 2001), or even outweigh the associated costs of lost direct benefits (Head et al. 2005). Thus there is a need to study species exhibiting both forms of selection in order to determine the relative importance of direct and indirect selection, as well as their interactions, on mate choice (Maklakov and Arnqvist 2009). In this study, the determination of potential direct or indirect pathways, and their contributions to offspring fitness are therefore a central theme in this thesis.

1.5 The role of EPP in the evolution of alternative reproductive strategies

Resource allocation in parental care is constrained by fitness trade-offs occurring between current and future reproduction (Stearns 1992). For example, investment in offspring may divert resources away from investment in survival or mating opportunities (Alonzo-Alvarez and Velando 2012). As a consequence parents must weigh up the costs and benefits to overall fitness that each reproductive event entails. Reproductive strategies become more complex in systems with bi-parental care, as sexual conflict may arise in the amount of care that each parent is willing to invest (Parker et al. 2002; Johnstone and Hinde 2006; Lessells 2006).

Bateman's principle predicts that males should endeavour to maximise reproductive success by seeking additional matings, and hence social polygyny would appear to be the optimal male reproductive strategy. Yet in contrast, 90% of all bird species are socially monogamous (Clutton-Brock 1991). Monogamy is hence purported to have originated from the necessity of bi-parental care in raising offspring (Lack 1968), which is adaptive for males when the fitness benefits of investing in offspring outweigh the costs of reduced mating opportunities (Emlen and Oring 1977; Székely and Cuthill 1999). Conversely, the optimisation of offspring fitness should play a major role in female reproductive strategy. Females may achieve this either through mate choice (see above), or via investment in offspring.

Differential allocation occurs when reproductive investment is influenced by mate attractiveness (Sheldon 2000). This hypothesis predicts that females mated to high quality males should invest relatively more in these offspring in order to gain more attractive offspring (Burley 1986). As a consequence, males are predicted to provide relatively less care and instead invest in future reproductive success (Burley 1986). There is substantial empirical support for this hypothesis (e.g. de Lope and Moller 1993; Petrie and Williams 1993; Kolm 2001; Limbourg et al. 2004; Bonato et al. 2009; McFarlane et al. 2010; Robart 2012).

Negative associations between male ornamentation and paternal effort may also arise due to evolutionary trade-offs between current investment in offspring and either investment in male-male competition or mating effort (Magrath and Komdeur 2003). Under this hypothesis high quality males are predicted to provide less parental care because the fitness benefits from the alternative strategy outweigh those acquired through parental investment. However, such mechanisms may often be more complex than theory predicts. For example, high quality males may experience less of a trade-off as they may have more resources to invest in both behaviours (Magrath and Komdeur 2003). In addition, although male-male competition is likely to be costly and thus representative of a trade-off (Pryke and Griffith 2009), it has been argued that acquiring additional matings requires little effort on the part of the male (Stiver and Alonzo 2009), and thus a trade-off may not always occur.

In contrast, alternative theoretical models predict a positive relationship between male advertisement and paternal care, when male advertisement is an honest indicator of direct benefits (i.e. the good parent hypothesis, see above). Thus it is not easy to predict the relationship between the expression of male traits and parental investment. However, the benefit to males of each strategy depends on the marginal fitness gains from multiple mating. Increasing marginal gains are predicted in systems where genetic quality has a significant influence on offspring fitness, and thus male advertisement should be negatively related to paternal care in species with extra-pair mating (Kokko 1998).

Sexual selection may favour the existence of multiple alternative male reproductive strategies within populations depending on a male's residual reproductive value (Gross 1996; Badyaev and Hill 2002; Kelly and Alonzo 2009). However, the benefit of certain reproductive strategies is complicated in species engaging in EPP by a reduced average certainty of paternity. Socially monogamous species are characterised by generally high rates of paternal care (Clutton-Brock 1991), thus raising the question of how a lowered average paternity of a brood should affect male parental investment. As paternity decreases, the benefit to males of providing care decreases (Fig. 1.2). Trivers (1972) stated that a male should be selected to reduce investment in offspring that are not his own. Indeed, socially monogamous species exhibit a large variance in rates of paternal care between species (Clutton-Brock 1991), and negative interspecies correlations found between the rate of EPP and paternal effort in comparative studies of socially monogamous birds suggest that reduced paternal care may be an adaptive response to a male's greater uncertainty of paternity (Møller and Birkhead 1993; Møller and Cuervo 2000; Sheldon 2002; Matysiokova and Remes 2013).

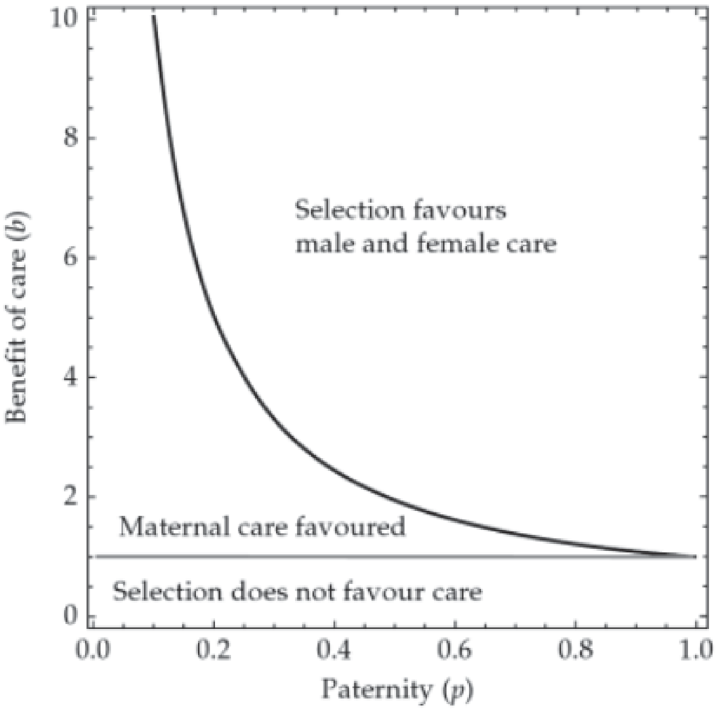

Figure 1.2: Visual representation of Queller's (1997) relationship between paternity, the fitness benefits of parental care, and the outcomes of selection on parental care, from Alonzo & Klug (2012). Lines show the boundaries at which selection favours changes in male or female parental care. Overall multiple paternity reduces the benefits to males of providing care. Note, however, males are still predicted to care even at low levels of paternity (<0.2) if the benefits of care are high.

The relationship between mating patterns and parental care is likely to be complex and may be influenced by multiple factors such as sexual conflict, male quality, paternity, as well as the probability of future reproductive events (Westneat and Sherman 1993; Freeman-Gallant 1997; Neff and Sherman 2002; Alonzo 2012). For example, a recent meta-analysis found that, in order to avoid the adverse dilemma of reducing care to potentially related offspring, a reduction in paternal investment is predicted only when EPP is frequent in the population, and when care is costly to a male's future reproductive success (Griffin et al. 2013). Furthermore, there may be an inverse relationship between paternity and paternal effort if cuckolded males are of low quality but actually are better parents in compensation (Eliassen and Kokko 2008). These aspects make it difficult to draw definite conclusions about the causality of detected links between male quality and reproductive strategies. This thesis attempts to contribute further knowledge of these systems in a species which is characterised by both a high variation in male parental effort and high rates of infidelity, thus potentially providing an important addition to the parental care and paternity debate. In Chapter 5 I draw on conclusions from other chapters to address the question of whether males exhibit alternative reproductive strategies that influence their parental investment in relation to their quality and parentage.

1.6 Life histories of the honeyeaters (*Meliphagidae*)

The *Meliphagidae* honeyeaters are a large and diverse family of passerines consisting of over 182 species that are found in a wide variety of habitats (Ford and Paton 1976). They are a predominantly tropical family originating in the Austro-Papuan region (Ford and Paton 1976) but are found as far as New Caledonia and New Zealand. Honeyeaters are primarily nectivorous and are equipped with a brush-tipped tongue, but are also opportunistic feeders of insects and fruit (Driskell and Christidis 2004).

Honeyeaters are aggressive and will readily attack or chase away conspecifics or other bird species trying to gain access to food resources (McFarland 1986b;

Franklin et al. 1989). Dominance hierarchies are common among the Australian honeyeaters, where large species have been observed to dominate smaller species (McFarland 1986b). This aggression may be partly a result of the sporadic and unpredictable nature of nectar sources (Armstrong 1991). In Australia, these food resources typically attract flocks composed of different honeyeater species competing for access (McFarland 1986b). The larger species require greater energy supplies and depend more heavily on nectar, and thus have more to gain through aggressive encounters (McFarland 1986b).

Honeyeaters are typically multi-brooded and clutch sizes are small, with modes of two-egg clutches among many species (Ford and Tremont 2000). However larger three and occasionally four-egg clutches are found among the single-brooded friarbirds due to their shorter breeding season (Ford and Tremont 2000). Most socially monogamous honeyeaters exhibit bi-parental care. There is little information on levels of parental investment in socially monogamous honeyeaters. However, females typically built the nest and incubate the eggs (Oliver 1998). Male parental care is typically confined to nest defence and provisioning of nestlings (but see Noske 1998), and males appear to contribute less towards offspring provisioning than females.

The Meliphagidae are under-represented in the mating systems literature. They exhibit various different social mating systems (Ford et al. 1988), although most research has focussed, perhaps disproportionately, on species characterised by a cooperative breeding strategy (Clarke 1997) such as obligate cooperative breeders such as the noisy miner, *Manorina melanocephala*, and bell miner, *Manorina melanophrys*, or facultative cooperative breeders such as the helmeted honeyeater, *Lichenostomus melanops cassidix*, (Franklin et al. 1995). Many other species are socially monogamous (e.g. the New Holland honeyeater, *Phylidonyris novaehollandiae* (Paton 1985; McFarland 1986a), rufous-banded honeyeater, *Conopophila albogularis* (Noske 1998), and regent honeyeater, *Xanthomyza Phrygia* (Oliver 1998)).

There have been only three published studies investigating the genetic mating systems of honeyeaters, of which two were conducted on cooperative breeders. In both the noisy and bell miners, EPP was found to be virtually non-existent (Poldmaa et al. 1995; Conrad et al. 1998). It is purported that their complex social structure, in which only the dominant male and female breed assisted by highly related helpers, precludes the opportunity to gain genetic benefits from extra-pair matings (Poldmaa et al. 1995).

There has only been one published study to date investigating the rates of EPP in socially monogamous honeyeaters. Ewen et al. (2008) studied the genetic mating systems of the Australian yellow-faced, *Lichenostomus chrysops,* and crescent, *Phylidonyris pyrrhopterus*, honeyeaters. They found very high rates of EPP of 44 % and 58 % of all offspring studied in the yellow-faced and crescent honeyeater respectively. However, these estimates are based on very small sample sizes of only 18 and 19 offspring studied in the yellow-faced and crescent honeyeater respectively, and thus some caution is needed in interpreting these results.

Of the 182 species of honeyeaters, only two are found in New Zealand; the bellbird, *Anthornis melanura*, and the tūī, *Prosthemadera novaeseelandiae*. Both are endemic and a recent phylogeny of the honeyeaters identified them as sister taxa and placed an estimate of their divergence time at 2.9 Mya (Driskell et al. 2007). Thus meliphagids have been present in New Zealand since at least the Pliocene era (Driskell et al. 2007). The breeding biology of bellbirds and tūī are similar, both being socially monogamous with female only incubation and bi-parental care. In bellbirds, males were found to contribute significantly less to nestling provisioning than females, and spent more time on territory defence (Anderson and Craig 2003). Bellbirds typically lay clutches of three to four eggs (Anderson and Craig 2003), which is also typical of tūī, although two, and occasionally five, egg clutches have been observed in tūī (Wells unpublished data).

A study by Cope (2007) investigated rates of EPP in the bellbird using southern-blotting and found unusually high rates of EPP, with 81% of all offspring studied being extra-pair young. However the accuracy of the methodology used in this study has been questioned (D.H. Brunton *pers comm*). Nevertheless, together with the study of Ewen et al. (2008), this finding suggests a high rate of EPP may exist among the honeyeaters.

1.7 Study species and study site

To date, most studies on New Zealand's avifauna have focussed on critically endangered species. Other more common, yet endemic, species have received much less attention. The tūī is one such species. The tūī is primarily distributed in areas of native bush throughout the mainland and is an important pollinator of many New Zealand endemic plants (Robertson et al. 1999). Two subspecies are currently recognised based on morphology, *P.n. novaeseelandiae*, the mainland tūī, and *P.n. chathamensis*, the endangered Chatham Island tūī (Oliver 1955). Although classified as widespread, its distribution is non-continuous in the South Island, being virtually absent to the west of the Southern Alps. Despite its patchy South Island distribution, tūī are good dispersers, and are known to travel distances up to 30 km in search of food sources (Bergquist 1989).

There is a need to establish sound baseline data on the tūī. Although a widespread and iconic endemic species in New Zealand, very little is known about their life histories. With the exception of their inclusion in multi-species accounts documenting the importance of honeyeaters as pollinators of endemic plants, the published literature on tūī can be summed up into a recent study on variation in tūī vocalisations (Hill et al. 2013) and a few papers published over 20 years ago on foraging ecology (Craig et al. 1981, 1985; Bergquist 1985a, b) and dominance hierarchies (Craig 1984; Stewart and Craig 1985; Bergquist and Craig 1988).

Like other honeyeaters, tūī are highly aggressive and territorial, both on breeding territories, and at food resources throughout the year (Stewart and Craig 1985). Aggression is projected towards subordinate species such as the bellbird, as well as conspecifics (Craig 1984; Stewart and Craig 1985). They possess a highly complex song (Hill et al. 2013) that is used in resolution of dominance hierarchies and in mate attraction (Bergquist and Craig 1988; Bergquist 1989). Dominance between the honeyeaters appears to be size-related (Craig 1985; McFarland 1986b) and tūī dominate over the smaller bellbird at food resources (Craig 1985). Thus it is highly likely that, as in many other species (Qvarnström and Forsgren 1998), large males are more dominant and gain access to the best territories and food resources. In addition, access to nectar resources is likely to be important to tūī breeding as females paired to dominant individuals have also been found to have a higher annual reproductive success (Bergquist and Craig 1988).

Tūī are socially monogamous, forming pair-bonds that vary in duration from one breeding attempt to multiple years (Wells, unpublished data). However, there has been no study of tūī genetic mating systems. As is typical of honeyeaters (Oliver 1998), female tūī incubate the eggs, but they exhibit bi-parental care. However, the sex-specific contributions to parental care are unknown.

The tūī provides an interesting model for studies of sexual selection as they possess some unique behaviours and secondary sexual characteristics which have not been examined in detail. For example, male tūī exhibit intense male-male competition and are considerably larger than females; traits that are commonly associated with polygyny. Indeed, the most highly sexually size dimorphic socially monogamous passerine currently known is the yellow wattlebird honeyeater (see above). However, the degree of SSD in tūī compared with other socially monogamous birds, and the adaptive significance of this SSD has not been investigated. In addition, both male and female tūī possess an unusual feather ornament that consists of modified white throat feathers that form two groups of plumes (Fig. 1.3). These plumes appear to be larger in males, although this has not been quantified, and the adaptive function of the plumes is unknown. Indeed, there has been little study on the function of white ornaments (Santos et al. 2011). Achromatic traits may be used as badges of status in male contests (Mennill et al. 2003; Santos et al. 2011) however other studies indicate they are condition-dependent (McGlothlin et al. 2007; Gladbach et al. 2011). The expression of achromatic plumage has been associated with conspicuous visual behavioural displays (Galván 2008; Guindre-Parker et al. 2013). Indeed, the throat plumes appear to play a role in both male-male competition and courtship in tūī where they are prominently displayed during song (Wells, *pers obs*). During these displays, males will also puff out their feathers to appear larger (Fig. 1.3), thus further suggesting a role of both ornamentation and size in sexual selection in tūī.

Figure 1.3: Male tūī displays. Clockwise from top right: typical male display posture during territorial and courtship song. Note the puffed out feathers to appear larger and prominent white feather plumes; male displaying to an interested female; male-male competitive displays during a song contest on a flame tree. The male on the right was one of the resident territory holders on the flame tree for the whole duration of this study, and in this photo is shown with his feathers puffed out.

This thesis is based on the study of a wild population of tūī at Tawharanui Regional Park (36°22'S, 174°50'E) on the North Island of New Zealand. This study was conducted over four breeding seasons (October to February) between 2009 and 2012. Introduced pest mammals have been eradicated from inside the park and a predator-proof fence has been constructed to prevent re-incursions. In addition, a tightly controlled buffer area is maintained outside the fence. Tawharanui is a multi-use park, consisting of a mixture of pastureland and native broadleaf-podocarp forest patches. I concentrated my research effort on two main areas of the park, although I also sampled nests occurring near these areas. Ecology bush is a large remnant forest patch located inside the predator-proof fence (Fig. 1.4). Jones Bay is located outside the fence in the buffer zone and consists of a small patch of forest surrounded by farmland. The Jones Bay site was particularly important for this study as its relatively non-continuous distribution allowed us to sample a significant proportion of the resident population. This site is also particularly interesting as it contains a large number of nectar resources, such as flame trees, *Erythrina sykesii*, New Zealand flax, *Phormium tenax*, and pohutakawa, *Metrosideros excelsa*, that are important dietary components for tūī, and consequently, competition for territories with access to these resources is fierce.

Figure 1.4: Tawharanui Regional Park, the study site for this thesis, with the locations of the two main locations highlighted by yellow dots: Jones Bay to the west, and Ecology Bush to the east. The thick red line across the peninsula denotes the location of the predator-proof fence. Dark green colouration represents native forest. Pastureland is indicated by light green colouration.

1.8 Thesis structure and objectives

The main aims of this thesis are to firstly determine the genetic mating system in a little studied New Zealand passerine, the tūī, *Prosthemadera novaeseelandiae*. There is a paucity of data on the genetic mating systems of the honeyeaters, thus this thesis will make a significant contribution to furthering our knowledge of honeyeater life histories. Secondly, I aim to advance the knowledge of the adaptive function of female genetic polyandry, and the role of sexual selection in the evolution of male secondary sexual characters. Although there has been much research conducted on sexual selection and the adaptive significance of extra-pair paternity over the past two decades, the hypotheses are still contentious and no consensus has been reached. The tūī is particularly suitable for assessing these hypotheses as they possess some intriguing sexually dimorphic characteristics and behaviours that are not normally associated with monogamy. Thus the tūī may provide us with valuable information of the evolution and maintenance of mixed reproductive strategies. I achieve this by examining the role of male phenotypes in male mating success, and the fitness consequences to females of mating with males possessing certain genotypic and phenotypic traits.

This thesis comprises four research chapters (Chapters 3 to 6) with an introductory (Chapter 1), methods (Chapter 2) and concluding (Chapter 7) chapter. This thesis is concerned with the subject of sexual selection and fitness consequences of female mate choice and thus some of the theories and conclusions explored in these chapters invariably overlap and resulted in some unavoidable repetition. For this reason each chapter should be considered with respect to the preceding and following chapters. Every effort has been made to limit duplication where appropriate, and therefore a chapter describing the genetic methodology has been included.

Chapter 1 provides a general overview to the theories explored in this thesis. A background to extra-pair paternity is given and current theories that have been proposed to explain the adaptive significance of extra-pair paternity are detailed. A background to general sexual selection theory and its role in the evolution of male sexually selected traits is presented. I also summarise the current published literature on the life histories of the study species and the *Meliphagidae* family.

Chapter 2 details the methodology used to develop and amplify microsatellite markers for the tūī that are used in every chapter of this thesis. This chapter was published as a paper: S. J. Wells, W. Ji, S. M. Baillie, and D. Gleeson (2013).

Characterisation and cross-amplification of fourteen microsatellite loci for the New Zealand endemic tūī (*Meliphagidae*), *Prosthemadera novaeseelandiae*. *Conservation Genetic Resources* 5: 113–116.

Chapter 3 examines the rate of extra-pair paternity and SSD in a wild population of tūī at Tawharanui Regional Park. I first review the current evidence for selection pressures, acting via extra-pair paternity, on the evolution of sexual size dimorphism in birds. I then investigate this in the tūī by assigning genetic parentage to offspring, and examining male within-pair and extra-pair paternity success in relation to male phenotypic and genotypic characteristics in a population where a significant proportion of the candidate males were sampled. This chapter has been published as a paper: Wells S.J., Ji W., Dale J., Jones M. B., and Gleeson D. (2015) Male size predicts extra-pair paternity in a socially monogamous bird with extreme sexual size dimorphism. *Behavioral Ecology* 26 (1): 200-206

Chapter 4 assesses the importance of direct and indirect benefits in female mate choice in the tūī. I draw on the outcomes of male mating success in the previous chapter and investigate the benefits that these males may provide for offspring survival and offspring reproductive value (measured as offspring sex) as two common proxies of offspring fitness. I determine whether these males provide direct or indirect benefits by disassociating the benefits provided by social and genetic fathers. This chapter is currently being submitted to the journal *Animal Behaviour*.

Chapter 5 investigates whether EPP has led to the selection for alternative male reproductive strategies in the tūī. I examine this by determining whether males of differing phenotypic quality invest differentially in parental effort. As parental care has not been studied in tūī, I firstly quantify two measures of male and female provisioning as proxies for relative parental effort. I then apply these rates to test various sexual selection hypotheses that predict the parental effort of male and female parents in response to male quality and reduced average certainty of paternity as a consequence of EPP.

Chapter 6 provides insight into nestling growth rates in tūī and examines offspring growth, as an important proxy of fitness, in relation to female mate choice. First, nestling growth rates and size at hatching are quantified and the hypothesis of sex-specific growth rates due to SSD in tūī is investigated. Second, I determine the effect of male and female provisioning rates on offspring growth. Third, I examine whether females gain direct or indirect benefits from mate choice that influence offspring growth and size at hatching by comparing traits of the social and genetic fathers and within-pair and extra-pair maternal half-siblings.

Chapter 7 concludes by summarising findings from each research chapter and integrating them into a general framework. I consider these findings in relation to current sexual selection hypotheses and discuss my conclusions on the adaptive significance of male secondary sexual characters and extra-pair paternity in tūī. I outline the novel contribution that this thesis has contributed to these theories and suggest priorities for future research.

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2

Characterisation and cross-amplification of fourteen microsatellite loci for the endemic New Zealand tūī (*Meliphagidae*), *Prosthemadera novaeseelandiae*

The impressive iridescent plumage of a male tūī.

2. Characterisation and cross-amplification of fourteen microsatellite loci for the endemic New Zealand tūī, *Prosthemadera novaeseelandiae*

2.1 Abstract

I describe eight novel microsatellite loci for the tūī, *Prosthemadera novaeseelandiae,* and the cross-amplification of six polymorphic loci developed for the bellbird (*Anthornis melanura*). Fifty tūī samples from Tawharanui Regional Park, New Zealand, were genotyped using the 14 loci described. Numbers of alleles ranged from 3 to 16. Observed and expected heterozygosities ranged between 0.180-0.940 and 0.237-0.878, respectively. There was no evidence of linkage disequilibrium. One locus (Ame-22) significantly deviated from Hardy-Weinberg Equilibrium. This study highlights the benefits of long-repeat microsatellites in minimising scoring errors. These are the first microsatellites to be developed for the tūī and also provide potential markers for studying other honeyeaters throughout the Austro-Papuan region.

2.2 Introduction

The tūī, *Prosthemadera novaeseelandiae*, is one of only two endemic honeyeaters (*Meliphagidae*) in New Zealand. Despite being one of the largest honeyeaters and distributed across a wide latitudinal range from the sub-tropical Kermadec, to the sub-Antarctic Auckland Islands, there is a lack of knowledge of their important ecological aspects such as phylogeography, population genetics, mating and social systems. Since tūī are a widespread forest passerine, they can assist in our understanding of the effects that human-induced fragmentation, off-shore island isolation, and rapid urbanization in New Zealand have had on the population dynamics of common and less abundant species alike (Gaston 2010).

2.3 Methods

Blood samples were collected from 50 tūī at Tawharanui Regional Park in the North Island and stored in Seutin's buffer (Seutin et al. 1991). Genomic DNA was extracted using the QIAxtractor™ (QIAGEN). One sample of ~5ng genomic DNA was sequenced using the Roche/454 GS FLX System at the University of Otago High-Throughput Sequencing Unit according to Margulies et al. (2005). 14,000 reads were analysed with MSATCOMMANDER™ (Fairclough 2008) to detect microsatellite repeat arrays. Sixty eight di- to hexa-nucleotide loci were identified and PRIMER3 (Rozen and Skaletsky 2000) was used to detect reproducible loci and design primers. 18 loci were consequently tested for polymorphism using a M13(-21) tail attached to a fluorescent FAM label (Schuelke 2000). Amplification was performed in 15 μL reactions with 1x PCR buffer, 2mM $MgCl_2$, 0.2 mM dNTP, 0.4 μM M13-tagged forward primer, 0.2 μM untagged forward primer, 0.4 μM reverse primer, and 1.0 U FastStart Taq DNA Polymerase (Roche Diagnostics). Amplification conditions were 95°C for 4 minutes, followed by 8 cycles of 94°C for

15 s, 59°C minus 0.5°C every cycle for 30 s, and 72°C for 20 s, followed by 94°C for 15 s, 51°C for 30 s, and 72°C for 20 s for 4 cycles, then 94°C for 15 s, 49°C for 30 s, and 72°C for 20 s for 26 cycles, with a final extension of 72°C for 20 minutes. Products were resolved on an ABI-PRISM 3130xl sequencer using the GENESCAN™ 500-ROX™ size standard and scored using GENEMAPPER™ v4.0. Eight loci proved polymorphic. 12 microsatellite loci previously developed for the bellbird, *Anthornis melanura* (Paterson et al. 2010), were also screened. Eight proved sufficiently polymorphic and six were chosen for further study.

The 14 loci (Table 2.1) were initially amplified in two multiplexes using 5'-attached fluorophores (Applied Biosystems) on each forward primer. However, due to insufficient amplification, four loci were subsequently amplified separately in multiplexes 3 and 4 and added to the original multiplexes of 1 and 2 respectively, before genotyping (Table 2.1). Multiplexes were 10 µL in volume with 1x PCR buffer with 2mM $MgCl_2$, 0.2 mM dNTP, and 0.9 U FastStart Taq DNA Polymerase. Primer volumes are detailed in Table 2.1. PCR cycling parameters were: 95°C for 4 minutes, followed by 94°C for 30 s, 58°C for 45 s, and 72°C for 45 s for 38 cycles, with a final extension of 72°C for 40 minutes. Products were resolved on an ABI-PRISM 3130xl sequencer using the GENESCAN™ 500-LIZ™ size standard and scored manually using GENEMAPPER™ v4.0.

Ten per cent of the total samples were re-genotyped in order to quantify scoring error rates (Hoffman and Amos 2005). Error rates were also calculated using known mother-offspring pairs (N = 156) and testing for mismatches. The number of alleles, expected and observed heterozygosities and the Polymorphic Information Content of each loci (Table 2.1) were calculated using CERVUS v3.0.3

(Marshall et al. 1998). MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004) was used to test for genotyping errors. Evidence for linkage disequilibrium and deviations from Hardy-Weinburg Equilibrium (HWE) was assessed using GENEPOP v4.0.10 (Rousset 2008).

Table 2.1: Characterisation of 14 microsatellite loci for the tūī (*Prosthemadera novaeseelandiae*)

Locus	5' Fluorescent label	Primer sequence (5' - 3')	Primer (pmol)	Repeat motif	Multiplex	Ta	Allele size range	N	Na	H_O	H_E	PIC
Pnov02	PET	F: ACGAGTGCGTTTATTGGCTG R: TACATGTGGTCCAGTGCCC	5	(CT)6	3	58	184-194	50	4	0.380	0.633	0.553
Pnov03	6-FAM	F: CTCCCACTGCTCCCATCTC R: CTCTGGCTCTCTCCAGCAC	2	(AGC)12	1	58	134-200	50	16	0.720	0.803	0.773
Pnov04	VIC	F: AAGCCTCCGAGAGCGAC R: GGTTCCTCCGCTAGCACC	8	(AGG)7	1	58	158-173	50	5	0.720	0.675	0.606
Pnov07	NED	F: ACGAGTGCGTTGGGACAAG R: CCTGAAAGAAATACACACATACACAC	6	(GT)6	2	58	112-130	50	5	0.620	0.710	0.663
Pnov10	NED	F: TGCAGGATTTCTTGCTGCTG R: GTCCTGACGTGGGGTAATTAAG	1	(GT)6	2	58	167-173	50	3	0.180	0.237	0.221
Pnov20	6-FAM	F: ACGAGTGCGTGAAGCAAAG R: ATCCACTCCCATACGTCCC	8	(CCATT)11	2	58	179-214	50	8	0.780	0.801	0.762
Pnov23	PET	F: GAGTGCGTAAACTGTGAGCC R: ACTCTCATCTTCTCACGGCAG	8	(ATTTT)6	4	58	154-189	50	14	0.860	0.864	0.842
Pnov25	PET	F: ATAGGGGTGTTTGTCGGGG R: ACTCAACCCAACCAATTCAAC	8	(GGGTT)8	3	58	204-354	50	12	0.760	0.813	0.781
Ame-9[a]	NED	F: TTGCTATCCCCAGCCCTGAC R: GCAAGGCAGAGCCAAACAGG	1	(AATTGG)6	2	58	183-249	50	11	0.840	0.805	0.776

Ame-10[a]	6-FAM	F: GGCTGATGGTGGGTGCCTTA R: CCGGAGGGGACAACGAAAAT	1	(AAAC)5	2	58	158-166	50	3	0.400	0.479	0.379
Ame-14[a]	NED	F: CCCCAACAAAACAAGCACAAA R: TTCGGCTGATGGTAAGGGCTA	1	(ATTTC)7	1	58	120-175	50	12	0.940	0.878	0.857
Ame-20[a]	PET	F: ACGAAGGGTTTTCCCAGTCT R: CACTGCACCTCATTTTGCAT	2	(AAAC)6	4	58	210-222	50	3(6)[§]	0.340	0.296	0.267
Ame-22[a]	VIC	F: TGACAACATCCAGGGTCACA R: CAGGGTTGTTCTCCCTGTGT	1	(CCATT)12	2	58	145-175	50	7	0.340	0.686	0.625
Ame25[a]	NED	F: TCTCTTTCCTTAAACCAACCAACC R: CCCTCTTCTGCAACAGCCTCA	5	(ACAA)9	1	58	190-210	50	6	0.740	0.796	0.754

Ta annealing temperature; N number of samples; Na number of alleles; H_O observed heterozygosity; H_E expected heterozygosity; PIC Polymorphic Information Content. [a] Paterson et al. (2010); [§] alleles can be counted as 6 or 3 if indel is considered, or not, respectively.

2.4 Results

No loci were in linkage disequilibrium. One locus (Ame-22) significantly deviated from HWE after sequential Bonferroni correction (Rice 1989) due to an excess of homozygotes. Ame-22 and Pnov02 showed signs of potential null alleles as identified by MICROCHECKER. This may have resulted from weighted sampling (30 of 50 samples) from an area of potentially related groups of birds on breeding territories possessing alleles identical by descent from a common ancestor. This can be resolved by further study of site philopatry in the tūī (Hedrick 2009).

Three loci had indels in one or both allele copies in some individuals. These were reproducible, and were found to be inherited when mother-offspring pairs were compared. When sequenced, the Ame-20 indel was found to result from the occasional presence of a 1 bp insertion in the flanking sequence of the repeat. This indel could be found in all three alleles and was visualised as a 1 bp shift on the microsatellite profile. This could be used to provide further resolution for parentage analyses. Pnov23 has a deletion of two T's in some copies of the repeat causing alleles not to be consistently 5 bp apart, as evidenced by clean peaks without stuttering. Similarly, Ame-14 has an allele in-between two repeat lengths caused by a TC deletion in one of the repeat units.

The mean error rate across all loci based on differing allele scoring between repeated genotypes was 1.6%. Mother-offspring mismatches produced the same error rate to that identified by repeat genotyping (1.6%). These error rates were predominantly a result of allelic drop-out of some of the longer Pnov03, Pnov25, and Ame-9 alleles, and therefore in all further analyses, all apparent homozygotes at any loci were re-genotyped separately to minimise scoring errors. An error rate of 1.6% was subsequently input as an error rate for paternity analyses to allow for potential low frequency null alleles.

As is common among longer tandem repeat microsatellites (Bacher and Schumm 1998), no loci exhibited stuttering, resulting in profiles that were easily read with minimal chance for scoring errors. This study shows that longer tandem repeats can be highly polymorphic, and with increasingly commonly reported genotyping errors found in microsatellite studies (Dewoody et al. 2006), researchers should consider these repeats more frequently for genetic studies.

In summary, this study reports the first microsatellites developed for the tūī, as well as the cross-amplification of loci designed for another honeyeater, the bellbird. These markers will be useful in studies of population genetics, and behavioural aspects such as mating systems, of the New Zealand honeyeaters and potentially honeyeaters of the Austro-Papuan region.

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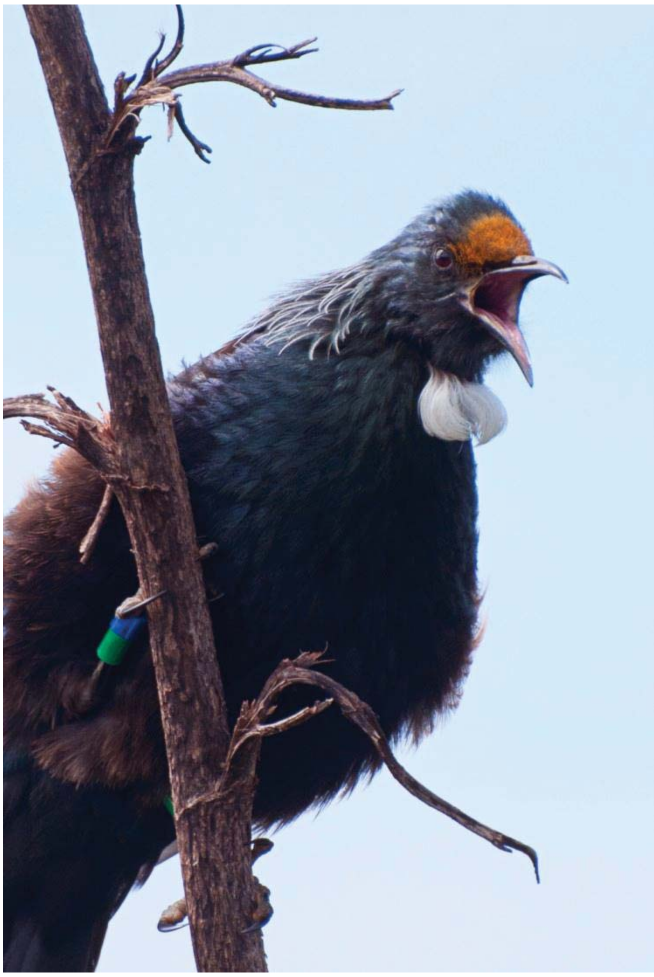
3

Male size predicts extra-pair paternity in a socially monogamous passerine with extreme sexual size dimorphism



Male tūī engaged in territorial song display

3. Male size predicts extra-pair paternity in a socially monogamous passerine with extreme sexual size dimorphism

3.1 Abstract

Extra-pair paternity (EPP) is purported to be an important contributor to the evolution of plumage dimorphism, and yet relatively few studies have demonstrated that EPP creates selection pressures on male traits. The New Zealand tūī, *Prosthemadera novaeseelandiae*, is a socially monogamous passerine exhibiting extreme sexual size dimorphism (SSD) and sexual plumage dimorphism. Although SSD is typically associated with polygyny rather than EPP, nothing is known about the genetic mating system of tūī. I examine whether EPP has contributed to the evolution of SSD in body size and ornament size in the tūī. I discover one of the highest rates of EPP currently known, with extra-pair young occurring in 72% of broods and accounting for 57% of all offspring. Both male body size and ornament size were strong correlates to EPP, with within-pair paternity success positively related to both traits. These results contrast with patterns observed in the majority of socially monogamous species exhibiting extra-pair mating. Male ornament size, but not body size, was a significant predictor of EPP success, suggesting different fitness benefits may be gained from within-pair and extra-pair males. Although these patterns may have arisen through either

male-male competition or female choice, these two mechanisms are unlikely to be mutually exclusive in tūī. I also found support for the genetic compatibility hypothesis as extra-pair sires were less genetically related to females than their within-pair counterparts. This study provides needed evidence that EPP can both create selection pressures on male traits, and contribute to the evolution of SSD.

3.2 Introduction

Sexual selection has been recognised as a significant contributor to the evolution of sexual dimorphism (Andersson 1994; Gontard-Danek and Møller 1999). Sexual dimorphism may manifest as differences in plumage, such as colouration or ornaments, or differences in body size i.e. sexual size dimorphism (SSD). Variation in levels of sexual dimorphism between species are thought to be a consequence of differences in mating system and parental care (reviewed in Andersson 1994). Since the opportunity for sexual selection increases with the variance in reproductive success (Arnold and Wade 1984), polygynous species typically exhibit greater sexual dimorphism than monogamous species (Darwin 1871; Dunn et al. 2001).

However many socially monogamous species are also sexually dimorphic (Møller 1986). Extra-pair paternity (EPP) is purported to increase the variance in male reproductive success, and hence the opportunity for sexual selection (Gibbs et al. 1990; Kempenaers et al. 1992; Yezerinac et al. 1995; Vedder et al. 2011). Comparative studies have demonstrated that plumage dimorphism and rates of

EPP are positively correlated (Møller and Birkhead 1994; Owens and Hartley 1998). Consequently, EPP has been put forward as the principle protagonist in the evolution of many secondary sexual characters in socially monogamous species (e.g. Smith et al. 1991; Hasselquist et al. 1996; Sundberg and Dixon 1996; Hoi and Hoi-Leitner 1997; Kempenaers et al. 1997; Walker et al. 2014) .

In contrast, comparative analyses assert that SSD has evolved in association with polygamous mating systems, but not with rates of EPP (Owens and Hartley 1998; Dunn et al. 2001). Owens and Hartley (1998) thus declared that SSD and plumage dimorphism had arisen through different evolutionary mechanisms. It is argued that plumage dimorphism has evolved through intersexual selection (female choice), while SSD is driven by intrasexual selection (male-male competition) for access to females or territories. However, the evolutionary origin of sexual dimorphism is still widely debated, and other studies have indicated that social mating system may be a better predictor of both types of dimorphism (Dunn et al. 2001).

The expression of secondary sexual characters is thought to be costly and therefore positively related to an individual's fitness. As a consequence, male-male competition or female choice can create selection pressures that favour the evolution of male traits that advertise either direct benefits such as territory size or parental ability (Kirkpatrick and Ryan 1991), or indirect benefits such as "good genes" (reviewed in Mays and Hill 2004). In addition, females are also posited to gain non-additive indirect benefits that are not typically signaled by male traits. Such "compatible genes" benefits are obtained by mating with genetically dissimilar males that increase offspring viability (reviewed in Kempenaers 2007;

Griffith and Immler 2009). However, despite decades of research into the role of EPP in sexual selection, the fitness advantages for females are still debated (reviewed in Griffith et al. 2002; Akçay and Roughgarden 2007; Forstmeier et al. 2014), and few studies have shown that EPP creates selection pressures on male traits within species.

Although large body size is often favoured in species with intense male-male competition (Mitani et al. 1996; Dunn et al. 2001; Lindenfors et al. 2003; Raihani et al. 2006; Hunt et al. 2009) such as polygynous species (Clutton-Brock and Harvey 1977; Owens and Hartley 1998), body size is typically related to dominance and therefore may also impart benefits for females (Qvarnström and Forsgren 1998). Thus, of all male sexually selected traits, body size is purported to be under the greatest selection pressure from both intra- and intersexual selection (Hunt et al. 2009).

If SSD is driven only by social mating system and not by EPP, reproductive success and body size should be positively correlated among sexually size dimorphic species and unrelated among socially monogamous species. Table 3.1 summarizes the relationship between male size and gains in either within-pair or extra-pair paternity success across 42 studies of 28 bird species. Some studies, such as those on the red-winged blackbird, *Agelaius phoeniceus,* in which males are 50% heavier than females (Searcy and Yasukawa 1983), support the hypothesis that selection on body size in polygynous species has led to the evolution of SSD. In this species large males gain larger harems and subsequently enjoy greater reproductive success (Weatherhead and Boag 1995). Although studies of some socially monogamous birds have reported significant associations between male size and paternity success (Table 3.1; see also Akçay and Roughgarden (2007) for a meta-analysis finding a positive correlation), there is a paucity of information in species exhibiting both high rates of EPP and significant SSD, where a positive association may be most expected. Additionally, although body size is generally heritable in birds (e.g. Alatalo and Lundberg 1986) it is challenging to separate the effects of good genes from direct benefits that large males may bestow (Alatalo et al. 1986). Large males may instead gain higher reproductive success through their increased capacity to guard their mate (Møller 1987), obtain extra-pair copulations by force (Burg and Croxall 2006), or intrude onto other males' territories (Hutchinson and Griffith 2008). In summary, the influence of EPP on selection for SSD is unresolved, especially among socially monogamous species.

In this study, I examine the effects of EPP on the SSD of multiple male traits in the tūī, *Prosthemadera novaeseelandiae*, an endemic New Zealand honeyeater (*Meliphagidae*). The *Meliphagidae* are a large family of Austro-Papuan origin (Driskell and Christidis 2004) exhibiting a variety of mating systems (Clarke 1997). This family is under-represented in the EPP literature, with only one study (Ewen et al. 2008) examining the genetic mating systems of socially monogamous honeyeaters. Although this study is based on <20 nestlings, Ewen et al. (2008) found very high EPP rates; 44% and 58% of all offspring in the yellow-faced, *Lichenostomus chrysops* and crescent, *Phylidonyris pyrrhopterus*, honeyeaters respectively.

Very little is known of tūī life histories, one of the largest members of the *Meliphagidae*. Tūī exhibit dominance hierarchies and males are aggressive and highly territorial (Craig 1985), with a complex and highly diverse song (Hill et al.

2013). Tūī are particularly suited to studies of sexual selection, as although the sexes are monomorphic in their iridescent plumage, they are highly sexually size dimorphic; an unusual trait among monogamous passerines. Both sexes also possess characteristic modified white throat feathers, forming two ornamental plumes. Although the adaptive significance of the plumes is unknown, they are larger in males. There has been little research on the function of white ornaments (Santos et al. 2011), although they likely function as either badges of status in male contests (Mennill et al. 2003; Santos et al. 2011) or condition-dependent signals (McGlothlin et al. 2007; Gladbach et al. 2011). Achromatic plumage has also been associated with complex behavioural displays (Galván 2008; Guindre-Parker et al. 2013). In support of these findings, male tūī prominently display their plumes during male contests. Groups of two or more males will face each other, puff out their feathers to look larger, and warble their throat plumes whilst singing. These displays are common around resource-rich territories at the beginning of the breeding season, and provide anecdotal evidence that both body size and ornament size may be advantageous in male-male competition. However, females often join the circle of males to watch these contests, and males also adopt the same pose during courtship. Thus these displays may have important outcomes for both male-male competition and female choice.

In this study I provide the first measure of rates of EPP in tūī, and examine male mating success in relation to correlates of male phenotypic and/or genetic quality. Specifically, I hypothesize that SSD and ornamentation in tūī are driven by sexual selection operating through EPP, and therefore males with the greatest expression of these traits should exhibit a reproductive advantage. To assess this I investigate these traits firstly in relation to a male's within-pair paternity success, and secondly, using pairwise comparisons of within-pair males (WPMs) and extra-pair males (EPMs) to examine traits predicting EPP success. Finally, I consider the importance of these findings in relation to current theories surrounding the evolution of SSD.

Table 3.1: Correlations of male body size and mass traits to within-pair (WP) and extra-pair (EP) paternity success in birds. The degree of sexual size dimorphism (SSD) is given for each species.

Species	MS	%SSD	SSD	%EPP	Trait	P	WP or EP	Reference
Tyrannus tyrannus	SM	1.9-2.9	0^	47	Tarsus	NS	WP	Dolan et al. 2007
					Mass	NS	WP	
					Tarsus	+	EP	
Acanthiza pusilla	SM	14.0	1	6.2	Body size	+	WP	Green et al. 2002
Rhipidura albiscapa	SM	-1.1	0	55	Body size	NS	EP	Hoffman et al. 2010
Parus major	SM	7.0	1	14	Tarsus	+	WP	Blakey 1994
				8	Tarsus	NS^+	WP	Krokene et al. 1998
					Mass	NS^+	WP	
				8.5	Tarsus	NS	WP	Strohbach et al. 1998
					Tarsus	NS^-	EP	
				6.5	Tarsus	NS^-	EP	Van Oers et al. 2008
					Mass	NS^-	EP	
				17.2	Tarsus	NS^-	EP	Kawano et al. 2009

Species	MS	%SSD	SSD	%EPP	Trait	P	WP or EP	Reference
Delichon urbica	SM	-1.5	0	19	Tarsus	NS^+	$WP+EP^\#$	Whittingham & Lifjeld 1995
					Mass	+	$WP+EP^\#$	
Hirundo rustica	SM	-6.1	-1	28	Tarsus	NS^-	WP	Moller & Tegelstrom 1997
					Mass	NS^+	WP	
				15.6	Tarsus	NS^+	WP	Kojima et al. 2009
					Wing	NS^+	WP	
					Tarsus	NS^+	EP	
Progne subis	SM	1.5	0	23.5	Mass	NS^+	WP	Wagner et al. 1996
Setophaga caerulescens	SM	-3.1	0	23.5	Tarsus	NS^-	EP	Webster et al. 2001
					Mass	NS^-	EP	
Emberiza citrinella	SM	-0.4	0	37.0	Tarsus	NS^+	EP	Sundberg & Dixon 1996
Emberiza schoeniclus	SM*	10.1	1	55.0	Tarsus	NS^-	WP	Bouwman et al. 2007
					Mass	NS^+	WP	
					Tarsus	NS^+	EP	

					Mass	NS^{+}	EP	
Geothlypis trichas	SM	2.1	0	20.0	Mass	NS^{+}	EP	Garvin et al. 2006
Setophaga petechia	SM	4.2	0	33.1	Body size	+	WP	Yezerinac & Weatherhead 1997
					Body size	NS^{+}	EP	
Setophaga citrina	SM	0-8.2	1	26.7	Tarsus	NS^{+}	WP	Stutchbury et al. 1997
					Mass	NS^{-}	WP	
					Tarsus	NS^{0}	EP	
					Mass	NS^{0}	EP	
Passer domesticus	SM	0.7	0	11	Tarsus	NS^{+}	WP	Stewart et al. 2006
					Tarsus	NS^{-}	WP	Veiga & Boto 2000
					Mass	NS^{-}	WP	
					Tarsus	NS^{-}	EP	
Cygnus atratus	SM	22.9	2	15.1	Tarsus	NS	EP	Kraaijeveld et al. 2004
Sialia mexicana	SM*	-8.1	-1^	20	Tarsus	NS^{+}	EP	Dickinson 2001
Ficedula albicollis	SM*	3.2	0^	15	Body Size	NS^{-}	WP	Sheldon & Ellegren 1999

Species	MS	%SSD	SSD	%EPP	Trait	P	WP or EP	Reference
Ficedula albicollis	SM*	3.2	0^	15	Body Size	NS^{-}	EP	Sheldon & Ellegren 1999
Oenanthe oenanthe	SM*	-1.3	0	11	Tarsus	NS^{+}	EP	Currie et al. 1998
					Mass	NS^{-}	EP	
Cyanistes caeruleus	SM*	6.1	1	15.7	Tarsus	+	EP	Foerster et al. 2003
				15	Tarsus	NS	WP	Delhey et al. 2003
					Mass	NS	WP	
					Tarsus	NS	EP	
					Mass	NS	EP	
				NG†	Tarsus	NS	WP	Poesel et al. 2006
					Tarsus	NS	EP	
				11	Tarsus	+	WP	Kempenaers et al. 1992
				11	Tarsus	+	WP	Kempenaers et al. 1997
					Tarsus	+	EP	
				16.1-25.4	Tarsus	NS^{+}	WP	Charmantier et al. 2004
					Mass	NS^{+}	WP	

					Tarsus	NS^{-}	EP	
					Mass	NS	EP	
				7	Tarsus	NS^{+}	WP	Krokene et al. 1998
					Mass	NS^{+}	WP	
Phylloscopus trochilus	SM*	1.0-6.3	0	33	Tarsus	NS^{+}	WP	Bjornstad & Lifjeld 1997
					Mass	+	WP	
Locustella luscinioides	SM*	0.6	0	4.1	Tarsus	-	WP	Neto et al. 2010
Charadrius alexandrinus	SM*	2.1	0$^{\$}$	3.9	Tarsus	NS	WP	Küpper et al. 2004
					Mass	NS	WP	
Passerculus sandwichensis	PG	5.6 -1.9	1	33.7	Mass	NS^{-}	WP	Freeman-Gallant et al. 2006
					Mass	NS	EP	
Ficedula hypoleuca	PG	-3.9	0	11	Tarsus	NS	WP	Ratti et al. 1995
					Mass	NS	WP	
				20	Tarsus	NS^{-}	WP	Canal et al. 2011
					Mass	NS^{-}	WP	

Species	MS	%SSD	SSD	%EPP	Trait	P	WP or EP	Reference
Ficedula hypoleuca	PG	-3.9	0	11	Tarsus	+	EP	Canal et al. 2011
					Mass	NS^{+}	EP	
				4.4	Tarsus	NS^{+}	WP	Lehtonen et al. 2009
					Tarsus	NS^{-}	EP	
Tachycineta bicolor	PG	8.8	1	38	Mass	NS	WP	Lifjeld et al. 1993
				53	Tarsus	NS^{+}	WP	Dunn et al. 1994
					Mass	NS^{+}	WP	
					Tarsus	NS	EP	
					Mass	NS	EP	
				51.1	Tarsus	NS^{+}	WP	Kempenaers et al. 1999
					Mass	NS^{+}	WP	
					Tarsus	NS^{-}	EP	
					Mass	NS^{+}	EP	
				52	Tarsus	NS	WP+EP$^{\#}$	Kempenaers et al. 2001
					Mass	NS	WP+EP$^{\#}$	

					Tarsus	NS^{+}	EP	
					Mass	+	EP	
Phylloscopus fuscatus	PG	5.9	1	45	Body size	NS^{-}	EP	Forstmeier 2002
					Mass	NS^{-}	EP	
Euplectes orix	PG	16.6	2	17.6	Tarsus	NS	WP	Friedl & Klump 2002
					Mass	NS	WP	
					Tarsus	+	EP	
					Mass	NS^{+}	EP	
Agelaius phoeniceus	PG	54	5	25.6	Body Size	+	WP	Weatherhead & Boag 1995
					Body size	NS^{+}	EP	
					Body size	NS^{+}	WP	Westneat 2006
					Body size	NS	EP	
Sturnus vulgaris	PG	3.8-7.1	1	8.7	Tarsus	NS^{+}	WP	Smith & von Schantz 1993
Acrocephalus arundinaceus	PG	8.0	1	3.0	Tarsus	NS	EP	Hasselquist et al. 1996
Acrocephalus schoenobaenus	SM	13.3	1	8.0	Mass	NS^{-}	EP	Marshall et al. 2007
Cracticus tibicen	CO*	5.5	1	44	Tarsus	NS^{-}	WP	Durrant & Hughes 2006

Species	MS	%SSD	SSD	%EPP	Trait	P	WP or EP	Reference
					Mass	NS^{+}	WP	
Malurus splendens	CO	10.4	1	65	Tarsus	NS	WP	Tarvin et al. 2005
					Mass	NS	WP	
					Tarsus	NS	EP	
					Mass	NS	EP	

%SSD, percentage difference of male body mass in relation to female body mass. SSD: -1, females 5– 15% heavier; 0, less than 5% difference between sexes; 1, males 5 - 15% heavier; 2, males 15 – 25% heavier; 3, males 25 – 35% heavier; 4, males 35 – 45% heavier; 5, males 45 – 55% heavier. MS, social mating system: SM, socially monogamous; PG, polygynous; CO, cooperative breeder. %EPP, percentage of extra-pair offspring. P, significance of test: + significant positive correlation; NS^{+}, non-significant positive correlation; NS^{-}, non-significant negative correlation; NS^{0}, zero correlation; NS, non-significant but no obvious direction of correlation given. This table was compiled using Google Scholar and the Web of Science databases searching for the keywords “body size”, “tarsus”, “mass”, “weight”, and “extra-pair”. *with some polygamy, ^Males have larger wing and tail lengths, $^{\$}$Morphology varies between populations, $^{\#}$Total reproductive success, †NG, data not given.

3.3 Methods

3.3.1 Field methods

Between 2009 and 2012, 390 tūī were caught in mist-nets from a wild population at Tawharanui Regional Park (36°22'S, 174°50'E) in New Zealand which supports approximately 1,000 breeding pairs of tūī (Wells, *unpublished data*). Large proportions of the subpopulations inhabiting two areas of the park separated by 2.5km were caught: Jones Bay, which is comprised of small fragments of native bush separated by farmland; and Ecology Bush, a larger native broadleaf-podocarp forest remnant. Due to the distance between these two areas, and the restricted range of tūī during the breeding season, these areas were considered discrete. I searched for nests during the breeding seasons (Oct-Feb) of 2009-2012 at these sites, and mist-nets were used to catch breeding pairs. Once captured, birds were color-banded, bled, and body mass and tarsus, head-bill, wing, and tail length were recorded. For a subset of the individuals the length of the white throat plumes were measured with calipers from the base of the feathers to the tip in its natural curved shape, and the number of feathers in each plume were recorded. Sex was assigned by a combination of field observations; male territorial song, presence of a brood patch, breeding behavior; or by tarsus length, which has no overlap between the sexes in tūī (female mean: 42.2mm ± 1.14, range: 39.9-44.3mm, N = 46; male mean: 47.0mm ± 1.04, range: 44.4-49.0mm, N = 96) as determined by molecular sexing following Fridolfsson & Ellegren (1999). Tūī lay 2 to 4 eggs per clutch and fledge up to two broods per season. Blood samples were collected from parents and nestlings of 56 nests; 11 nests in 2009, 26 in 2010, 15 in 2011, and 4 in 2012. In three nests I managed to sample only one of the two parents; two females and one male.

3.3.2 Paternity analysis

To investigate paternity, 14 microsatellites were developed for the tūī (Wells et al. 2013). Two markers (Pnov02 and Ame-22) were removed from further analyses due to high occurrence of null alleles in the population. The combined exclusion probability calculated in CERVUS v.3.0.3 (Marshall et al. 1998) for the remaining 12 markers was >0.999. Parentage analyses were conducted in CERVUS and verified using COLONY v.2.0.2.2 (Jones and Wang 2010), and NEWPATXL (Worthington Wilmer et al. 1999). Parentage analyses in all programs were conducted for the two sites independently, as well as jointly, which allowed a more precise estimate of proportion of sampled candidate males at each site to be entered. However, this had no effect on parentage assignment, as EPMs that were assigned from different areas during the joint analysis had low support with consistently one or more mismatches. Furthermore, the three programs drew almost identical conclusions, and therefore only results from the joint CERVUS analysis are discussed further. All males sampled in the two areas during the study period were included as candidate fathers in the analysis. Parameters used in simulations and to assess confidence of paternity were estimated from field data and were: 5 candidate males per offspring, 0.6 proportion of the candidate parents sampled, and a genotyping error of 0.01 to account for loci with known low frequencies of null alleles. All males with a positive LOD score for each nestling were identified, and the most likely father was determined using the LOD score. WPMs were discounted as being the true genetic father if they (a) mismatched at one or more loci within the mother-offspring dyad that could not be accounted for by the presence of a null allele or (b) mismatched at three or more loci where null alleles could be present, but were unlikely due to their low frequencies, and if also (c) the WPM did not receive a positive LOD score. EPMs were assigned to a nestling only if they mismatched at zero loci within the mother-offspring dyad.

3.3.3 Statistical analysis

3.3.3.1 SSD in tūī

Data on the mass of 100 males and 46 females caught at Tawharanui between 2009 and 2012 were collated to quantify the degree of SSD in tūī. Records included were limited to measurements taken by the two main observers (S.J.W. and W.J.) to reduce measurement bias. Birds were molecularly sexed according to Fridolfsson and Ellegren (1999). In all analyses, tarsus length was used as a measure of body size as it thought to be the most accurate measurement of body size (Senar and Pascual 1997). Mass was not used because, due to seasonal and yearly fluctuations in mass, the different timings of sampling would confound comparison between males. The mean number of feathers in the two throat plumes possessed by an individual was used in further analyses.

3.3.3.2 Variation in within-pair paternity success

I used generalized linear mixed models (GLMMs) with binomial error distributions and logit link functions to test two measures of within-pair paternity success; the proportion of EPP in a nest with brood size as the binomial denominator, and the probability (0 or 1) of EPP occurring in a nest. To evaluate if females seek good genes from social mate choice I tested whether predictors of male quality (tarsus length, heterozygosity, plume length, and number of plumes) were related to male within-pair paternity success. Three estimates of male heterozygosity were calculated using Rhh (Alho et al. 2010); standardized heterozygosity, internal relatedness (IR), and homozygosity by loci (HL). All three measures showed high multicollinearity (VIF>20), and therefore only IR was included in the models (Amos et al. 2001). However, all models were also tested using HL, which did not change the model outcomes. To determine instead if genetically dissimilar males gained more paternity, I included a predictor of social-pair genetic relatedness. Different relatedness estimates may perform better on different datasets (van de Casteele et al. 2001). Therefore the most suitable relatedness metric was assessed using COANCESTRY v.1.0.1.1 (Wang 2011). COANCESTRY analyses a simulated data based on the user’s actual data and produces correlation coefficients among seven different relatedness estimators and the simulated data. The estimator with the highest correlation coefficient is most suitable estimator for the data (Wang 2011). Based on results of this analysis, relatedness estimates calculated from TrioML, a maximum likelihood estimator (Wang 2007), were included in the models. Clutch number (first or second clutch of the year), was included to test for differences in EPP due to timing of the breeding season, and female tarsus length was included to test for differences due to female size or quality. Male and female identities were used as crossed random effects to account for the non-independence of multiple broods from the same individuals (Pinheiro and Bates 2000).

I estimated the opportunity for sexual selection (I_S) by calculating the standardized variance in apparent and realized male within-pair fertilization and reproductive (number of within-pair young fledged) success (Arnold and Wade 1984). I do not provide an estimate of male total reproductive success because this dataset comes from a large wild population where I was unable to sample all nests in the given areas, and because sampling effort varied between years. Taken together with the high rates of EPP in this population, any estimate of total reproductive success would be inaccurate.

3.3.3.3 Comparison of WPMs and EPMs

To compare traits of analogous WPMs and EPMs I used GLMMs with a binomial error distribution and logit link function to examine the probability of a male being an EPM as the response variable. I included possible indicators of male phenotypic or genetic quality (tarsus length, plume number and length, and heterozygosity) as well as pair genetic relatedness, as predictors to determine if these traits predicted male EPP success and therefore potentially advertise indirect benefits to females. To examine if extra-pair success is correlated between females, I included a predictor specifying the number of different females for which each male had sired extra-pair offspring. As some nests had more than one EPM, and to account for some males being chosen as EPMs by different females, female identity was used as a random effect.

There was no significant difference in the mean proportion of EPP in a brood between years ($p = 0.2$), therefore years were pooled for each analysis. No collinearity was evident in predictors (all $VIF<5$, $r \leq 0.6$), therefore the initial model contained all relevant predictors, with the exception of plume length and number which were run in separate models due to the reduced sample size for these variables. Stepwise selection was then employed, using hypothesis testing approaches, to reduce the model down to the final model including only significant predictors (Zuur et al. 2009). Stepwise selection may increase the risk of Type 1 errors (Forstmeier and Schielzeth 2011). Therefore I conducted a full model likelihood ratio test against the null model (Forstmeier and Schielzeth 2011). All full models were significant (all $p \leq 0.01$) and thus I am confident that stepwise selection did not increase the likelihood of Type 1 errors in our models. Final models were validated using the Hosmer-Lemeshow goodness-of-fit test using the maximum number of data partitions allowed by the data. Deviations from expected event rates, and therefore a poor model fit, are given by significant χ^2 values (Hosmer and Lemeshow 2004). All models gave non-significant results (proportion of EPP, $g = 10$, χ^2: 3.717, $p = 0.882$; probability of EPP, $g = 10$, χ^2: 7.738, $p = 0.459$; comparison of WPMs and EPMs, $g = 8$, χ^2: 9.395, $p = 0.153$) and therefore fit the data well. All models were run using the lme4 package (Bates 2010) in R v.2.15.1 (R Development Core Team 2011).

3.4 Results

3.4.1 Paternity analysis

Our ability to detect social polygyny is this population is limited by the subsample of the population studied. Nevertheless, after extensive observations of the social pairs in this study there was no evidence of any polygynous pairings, suggesting that, as has been previously documented (Stewart 1980) tui are socially monogamous. There were, however, three instances of mate-swapping occurring between breeding attempts. Extra-pair young were present in 72% of all nests studied (41 of 57) and accounted for 55% of all offspring studied (90 of 163). When broods from the same females are averaged to give independent results, 72% of all independent nests (24.5 of 34) contained EPP, and 57% of all offspring (55.5 of 97.8) were sired by EPMs. The mean proportion of extra-pair young among EPP nests was 0.75 (SD 0.25; 95% CIs: 0.671, 0.829, N = 41 nests). EPMs could be identified for 55.5% (50 of 90) of the total extra-pair young, in 25 of the 41 nests, with 16 of these being from the Jones Bay area. Of the 25 nests, 13 were from different females, with the remaining 12 being either a repeated social pair or the same female with a different WPM. In clutches with two or more extra-pair young and in which EPMs could be identified, 29% (7 of 23) contained offspring from two EPMs. In total, 16 individual EPMs were identified for 42 of the 90 extra-pair young (47%). Four of these, all from Jones Bay, fathered offspring from 2 different females, and two fathered offspring from three different females. Of all the extra-pair matings for which the EPM could be identified, 75% (n = 21) were with males either holding territories within two territories of the female, or seen within two territories away of the female's territory. Seven additional matings were with EPMs known from the same area, but whose territory was unidentified. EPP resulted in a standardized actual variance in male within-pair fertilization success 16 times higher than over that which would result from genetic monogamy (apparent: I_s = 0.05, mean = 3.0, variance = 0.47; realized: I_s = 0.81, mean = 1.29, variance = 1.34) and a standardized realized variance in male within-pair reproductive success 10 times higher than apparent variance in reproductive success (apparent: I_s = 0.09, mean = 2.55, variance = 0.62; realized: I_s = 0.96, mean = 1.09, variance = 1.14).

3.4.2 Data analysis

3.4.2.1 SSD in tūī

Males were on average 50% heavier than females (males 129g, females 86g; Fig. 3.1), which to our knowledge is the highest level of SSD in a socially monogamous passerine (among 578 socially monogamous passerines for which I had information on both social mating system and level of SSD (data from Dale et al. 2007), tūī were found to be the top ranked most sexually size dimorphic). Males also possessed on average 19.5% longer and 46.2% more plume feathers than females (mean plume length, female: 13.4mm SD 2.1, male: 16.0mm SD 1.7; mean plume number, female: 12.3 SD 3.3, male: 18.0 SD 3.8). There was a significant correlation between a male's tarsus length and the number of plume feathers he possessed (Pearson’s r = 0.22, p = 0.04) but not to plume length (r = 0.015, p = 0.9). Neither plume length (r = -0.004, p = 0.9), or number of plumes (r = 0.155, p = 0.3) were significantly correlated with tarsus length in females.

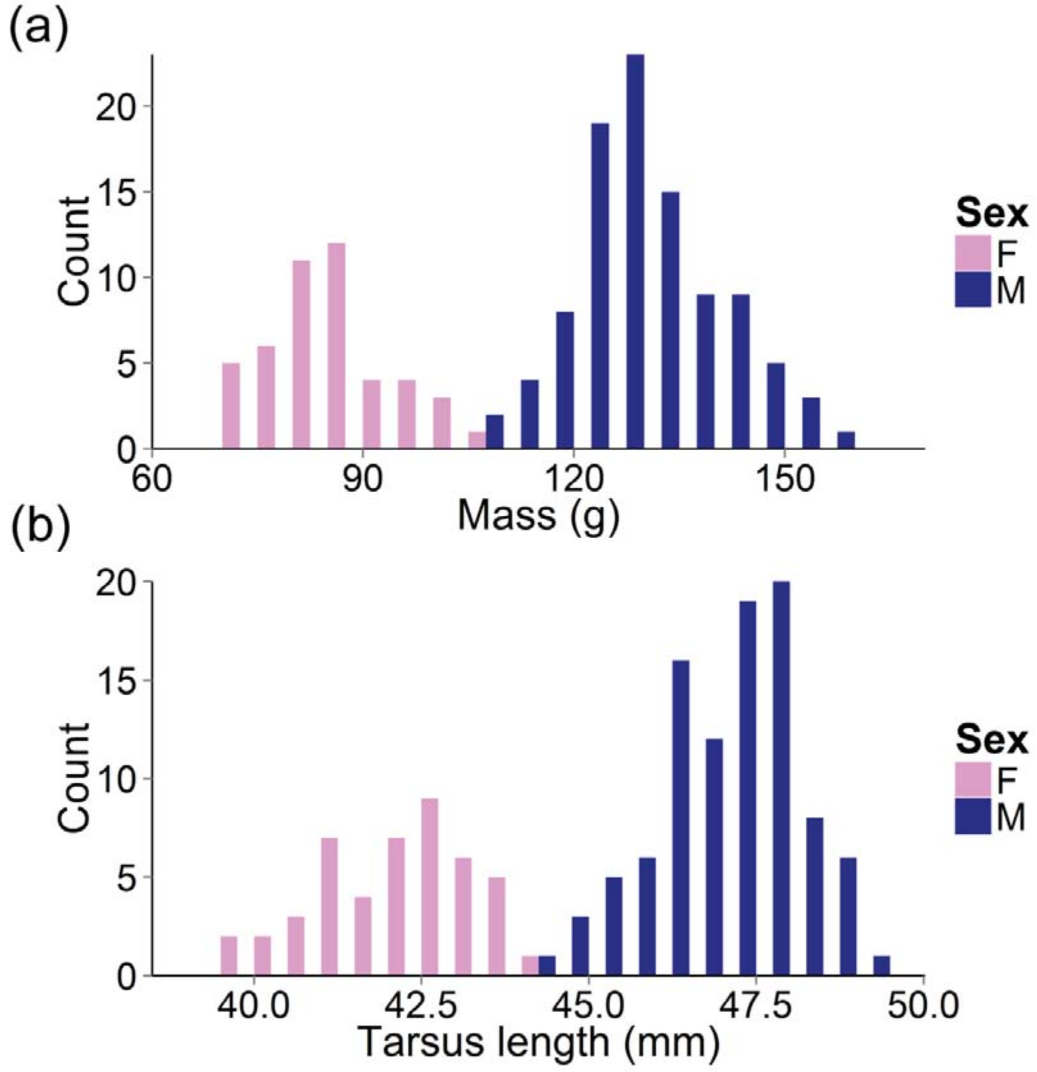


Figure 3.1: Frequency histograms showing the degree of SSD in tūī in (a) body mass in grams in 5g bin widths and (b) tarsus length in millimeters in 0.5mm bin widths. Data from 46 female (pink) and 98 male (blue) molecularly sexed adults.

3.4.2.2 Variation in within-pair paternity success

The probability and the proportion of EPP in a brood were significantly inversely related to WPM tarsus length, as well as the length and number of plume feathers he possessed (Fig. 3.2; Table 3.2). The proportion of EPP was also significantly higher in second broods of the season (Fig. 3.2), although clutch number had no effect on the probability of EPP occurring. Male heterozygosity was unrelated to within-pair paternity success. Neither female tarsus length, nor plume length and number were significantly related to either the probability or proportion of EPP (Table 3.2). Genetic similarity of the social-pair did not significantly predict male within-pair success (Table 3.2).

Proportion of EPP				Probability of EPP			
Predictor	β ± SE	LRT χ^2	P	Predictor	β ± SE	LRT χ^2	P
Relatedness	-1.321 ± 2.222	0.324	0.569	Male IR	0.013 ± 2.441	0.001	0.996
Male IR	-1.348 ± 1.485	0.774	0.379	Relatedness	2.299 ± 3.589	0.429	0.513
Female tarsus length	-0.509 ± 0.483	1.029	0.310	Clutch number	0.870 ± 0.872	0.929	0.335
Clutch number	**0.968 ± 0.473**	**3.865**	**0.049**	Female tarsus length	-1.329 ± 0.813	3.163	0.075
Male tarsus length	**-0.906 ± 0.303**	**7.787**	**0.005**	**Male tarsus length**	**-0.916 ± 0.435**	4.778	**0.029**
Male plume length	0.553 ± 0.375	2.351	0.125	Male plume length	0.049 ± 0.663	0.005	0.942
Male plume number	**-0.564 ± 0.221**	**9.073**	**0.003***	**Male plume number**	**-0.914 ± 0.525**	**6.747**	**0.009***
Female plume number	0.051 ± 0.150	0.114	0.735	Female plume length	0.041 ± 0.212	0.038	0.845
Female plume length	0.042 ± 0.110	0.146	0.702	Female plume number	0.237 ± 0.302	0.682	0.409

Table 3.2: Effect of social parent and nest characteristics on the probability and proportion of EPP in a nest. Horizontal lines delimit separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a χ^2 test between nested GLMMs while accounting for all other predictors remaining in the model. Coefficients in the final model are listed at the bottom of the table in bold. *This dataset contained an outlier with a large, although not unusual, number of plume feathers. However, results were robust to its removal (proportion of EPP: $\beta = -0.552 \pm 0.225$, $\chi^2 = 6.331$, $p = 0.012$; probability of EPP: $\beta = -0.912 \pm 0.529$, $\chi^2 = 4.450$, $p = 0.034$).

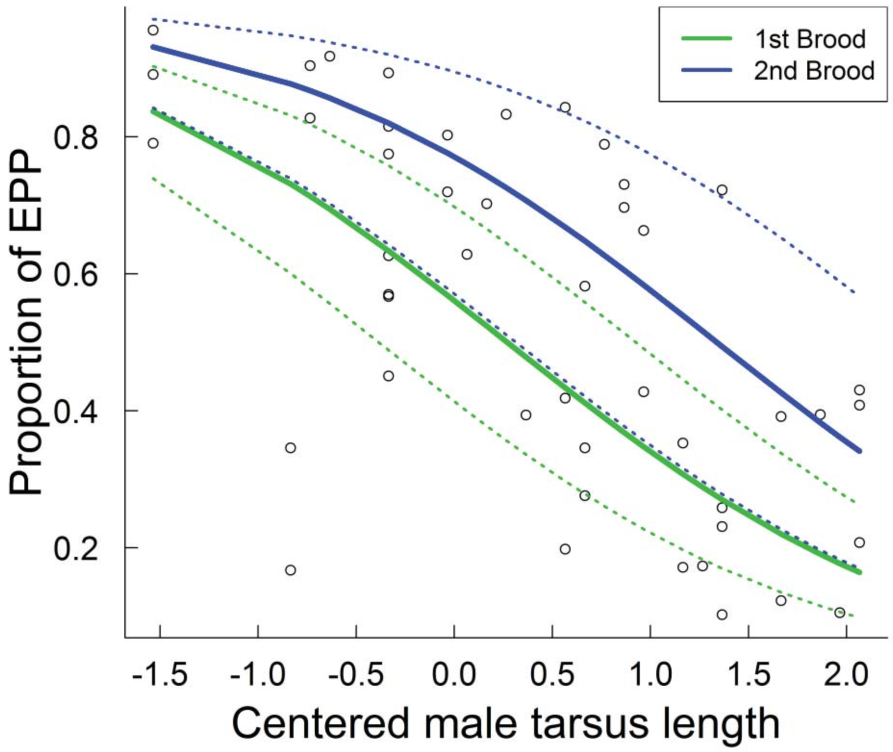


Figure 3.2: Predicted probabilities and fitted values from a GLMM with binomial error distribution showing the mean relationship between the proportion of EPP in a nest and WPM mean-centered tarsus length for first (n = 30) and second (n = 26) broods of the season as shown by unbroken lines. 95% confidence intervals for each brood are shown by dashed lines. Tarsus length was mean-centered in the GLMM to avoid collinearity with the model intercept relating to the proportion of EPP when male tarsus length is equal to zero.

3.4.2.3 Comparison of WPMs and EPMs

Males were more likely to be an EPM of a female if they were also an EPM for other females (Table 3.3), suggesting that certain males are consistently successful at gaining extra-pair matings. Despite the small sample size (n = 6 comparisons), EPMs had significantly more and longer plume feathers than their WPM counterparts (Table 3.3). There was no significant difference in IR between EPMs and WPMs. However, EPMs were also significantly less related to the female than the WPMs they cuckolded (n = 28, Table 3.3; Fig. 3.3).

Predictor	β ± SE	LRT χ^2	P
Male tarsus length	0.585 ± 0.506	1.444	0.230
IR	-3.655 ± 2.418	2.514	0.113
Relatedness	**-5.543 ± 2.420**	**7.751**	**0.005**
EPM for another nest	**2.719 ± 0.944**	**12.735**	**0.0004**
Male plume number	**0.776 ± 0.442**	**4.353**	**0.037**
Male plume length	**2.047 ± 1.245**	**5.191**	**0.023**

Table 3.3: Effects of WPM and EPM characteristics on the likelihood of being an EPM. Horizontal lines delimit separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a χ^2 test between nested GLMMs while accounting for all other predictors remaining in the model. Coefficients in the final model are listed at the bottom of the table in bold.

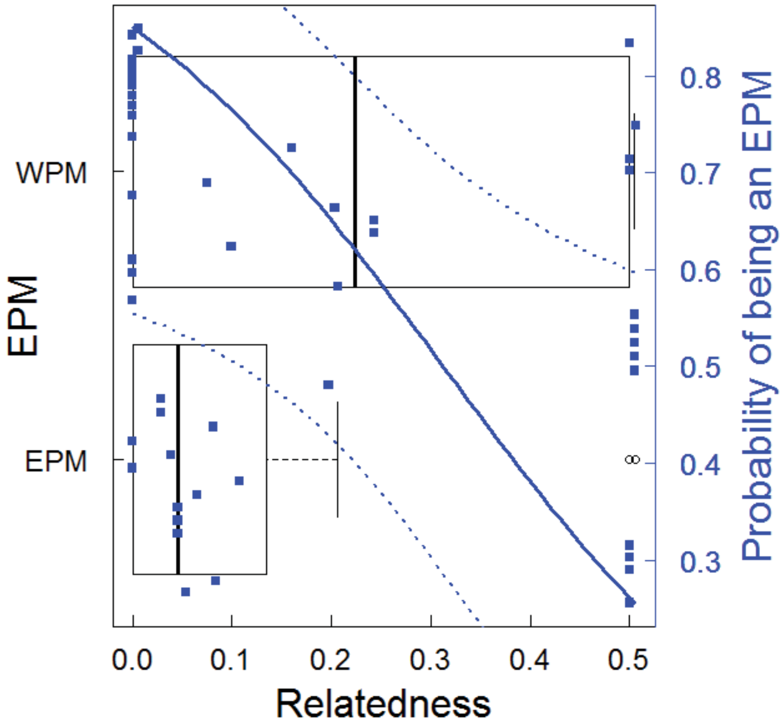


Figure 3.3: Predicted probabilities and fitted values (in blue) from a GLMM with binomial error distribution showing the mean relationship between the likelihood of a male being an EPM as a function of his genetic relatedness to the female. Data are from pairwise comparisons of corresponding WPMs and EPMs (N = 28 comparisons). Also shown are the raw data boxplots showing the difference in genetic relatedness between analogous WPMs and EPMs (in black).

3.5 Discussion

The rate of EPP in tūī in this study (57%) is one of the highest currently found in any socially monogamous bird. Male tūī are aggressively territorial which may provide females with the opportunity to engage in frequent extra-pair copulations, as males are too distracted to accurately monitor female behavior, and therefore faithfulness (Kokko 1999). Tūī exhibit levels of SSD normally associated with strong polygamy. SSD was a significant correlate to EPP, with large males, and males with large ornaments, achieving greater paternity success. This study provides needed evidence of EPP creating selection pressures on male sexual traits. Moreover, comparative studies have previously asserted that SSD is a consequence of polygyny, but not EPP (Owens and Hartley 1998; Dunn et al. 2001). Yet in this study, I demonstrate that EPP can generate selection pressures on male size, and that this selection has contributed to the evolution of one of the highest levels of SSD known among socially monogamous birds.

It is unclear whether the success of large and highly-ornamented males arises from female choice or male-male competition. For example, in this study large males won a greater paternity of their broods, and therefore may simply be more aggressive in defending their paternity (Møller 1987; Komdeur 2001). The three EPCs witnessed in this study arose from active solicitation by females during forays into the EPMs territory. Therefore, in tūī, the ability of males to satisfactorily guard their mate may be important. This is further supported by higher rates of EPP in second clutches when, during their second fertile period, females turn over the duty of provisioning first brood fledglings to males. During this period, males may be subject to trade-offs between parental care and mate-guarding, allowing females to pursue more extra-pair copulations.

However, it is not yet fully understood how intra- and intersexual selection interplay (reviewed in Qvarnström and Forsgren 1998; Wong and Candolin 2005; Hunt et al. 2009) , and the two processes are unlikely to be mutually exclusive. Berglund et al. (1996) posited that traits originating through male-male competition may be intensified by female choice for dominant males (e.g. Passos et al. 2013) . Our results are consistent with this possibility. Female observation of male territorial song contests, in which size appears an advantageous trait, further supports this hypothesis. These male displays provide the ideal arena for females to assess male dominance and quality prior to choosing a mate; a behavior common to polygamous species. Although copulations have not been observed as an outcome of these male aggregations, this behavior is reminiscent of the “hidden lek” hypothesis (Wagner 1998), in which subordinate males parasitize the attractiveness of dominants during male displays and steal matings from soliciting females (Cockburn et al. 2009). Even if female choice for large WPMs is assumed, it is unclear whether choice is based on size, or rather on size-associated traits, particularly if large males also win higher quality territories. Alatalo et al. (1986) separated the confounding factors of good genes and direct benefits in social mate choice, and found that female pied flycatchers, *Ficedula hypoleuca,* made mating decisions based on territory quality, not male phenotypic characteristics *per se*. Indeed, in tūī, WPM but not EPM body size is positively related to offspring survival (Chapter 4). This suggests that large males may indeed confer direct benefits such as territory quality and, as these are likely attained only through social pairings, is additionally supported by EPMs not being significantly larger than WPMs.

EPP is purported to be responsible for the evolution of plumage dimorphism (Møller and Birkhead 1994). Yet few studies have identified the ornamental traits responsible for male EPP success (reviewed in Akçay and Roughgarden 2007). In our study, male plume size was positively related to both within-pair and extra-pair paternity success. If ornamentation in tūī signals additive good genes benefits, female choice of EPMs should be synonymous (Neff and Pitcher 2005). Our finding that EPMs typically gained EPP from multiple females supports this hypothesis. However, I cannot unequivocally rule out that EPMs are simply more competitive, or exhibit less of a trade-off between parental care and mating effort, than unsuccessful males. Male body size, in contrast, was unrelated to EPP success. These findings suggest that females gain different benefits from within-pair and extra-pair mate choice. Differential within-pair and extra-pair success of male traits in has been demonstrated in yellow warblers, *Setophaga petechia*, in which male size and within-pair success were positively related, and yet females preferred more ornamented EPMs (Yezerinac and Weatherhead 1997). Lehtonen et al (2009) also found that different male traits influenced within-pair and extra-pair success in pied flycatchers. A comparable pattern to this study was found in dark-eyed juncos, *Junco hyemalis*, in which body size was only advantageous in male-male competition, while a large condition-dependent white tail patch was favored in both female choice and male-male competition (McGlothlin et al. 2005). The authors concluded that correlational selection, driven by the interaction of intrasexual competition and intersexual choice on tail white and body size, resulted in the evolution of honest signaling of the ornament (LeBas et al. 2003). Our findings are consistent with this hypothesis. Plumes varied in condition, becoming discoloured or broken in some individuals and therefore, although unlikely to be costly to produce, the ability to maintain them may be an indicator of male quality which is kept honest through continual testing during territorial disputes (Berglund et al. 1996). Male body size and ornament size are positively related, and possible correlational selection on these traits may arise from the superior ability of large male tūī to defend territories or food resources, and therefore gain condition. Female preference for ornamented males may then lead to the reinforcement of these traits. Thus male body size and ornamentation may be subject to different selective pressures and may reflect separate, yet correlated, aspects of male quality (Møller and Pomiankowski 1993; Johnstone 1996; Coleman et al. 2004; McGlothlin et al. 2005; Ibáñez et al. 2013) . Further studies on the influence of intra- and intersexual selection on male traits should be conducted to assess this hypothesis.

EPMs were also less related to the female than WPMs in this study, which may be an adaptive response to a generally high average relatedness in the population (seven social pairs were either first or second degree relatives). These results suggest females could simultaneously optimize fitness via both good genes and compatible genes, although examination of female fitness benefits from EPP is needed to confirm this. Adaptive female behavioral plasticity for multiple genetic benefits in response to male availability has been frequently suggested (Colegrave et al. 2002; Mays and Hill 2004; Neff and Pitcher 2005; Puurtinen et al. 2005), yet only empirically demonstrated in a small number of studies (Roberts and Gosling 2003; Fossøy et al. 2008). Specifically, evidence for female choice for both good genes and compatible genes has been found in blue tits, *Cyanistes caeruleus* (Foerster et al. 2003), while temporal plasticity in mate choice was demonstrated by Oh and Badyaev (2006) who found that female house finches**,** *Carpodacus mexicanus*, chose males based on plumage coloration early in the breeding season, but chose genetically dissimilar males towards the end. However, in tūī, the likelihood of cuckoldry did not increase with the genetic similarity of the social-pair, suggesting that female tūī cannot accurately assess relatedness. This begs the question why genetically dissimilar males were successful EPMs. This pattern could arise if EPMs are more exploratory and therefore disperse further (Van Oers et al. 2008). Alternatively, sperm competition, in which sperm of more compatible males are more likely to fertilize an egg (Tregenza and Wedell 2000), may also result in apparent female preference for less-related sires.

In conclusion, I found very high rates of EPP in tūī that were correlated with extreme SSD. This study provides evidence that EPP can create selection pressures on male traits, and can therefore be an important contributor towards the evolution of both plumage dimorphism and SSD. The effects of inter- and intrasexual selection on SSD may be additive, and I propose that the SSD seen in tūī is maintained through male-male competition, but also intensified by female choice. Our results further suggest that multiple male display traits in tūī may represent different aspects of male quality. In addition, the greater EPP success in genetically dissimilar, as well as highly ornamented, males suggests that the opportunity to obtain multiple genetic benefits from extra-pair mating exists. The throat plumes possessed by tūī are a unique type of ornamentation with no obvious comparisons in other avian taxa. This study suggests that plumes may be used as both badges of status in male-male competition, and honest indicators of male quality in female choice. Although robust conclusions of the function of male plumes cannot yet be drawn, the positive relationship seen between both a male's within-pair and extra-pair success and ornamentation incites further study.

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4 Seeking the best of both worlds: the effects of direct and indirect benefits on offspring survival and sex ratios in a socially monogamous passerine

Juvenile tūī feeding on *Coprosma robusta* berries. Tūī only acquire their throat plumes about 6 weeks after fledging.

4. Seeking the best of both worlds: the effects of direct and indirect benefits on offspring survival and sex ratios in a socially monogamous passerine

4.1 Abstract

Females can maximise offspring fitness by 1) seeking direct or indirect benefits in mate choice that increase either offspring viability or attractiveness or 2) overproducing sons of a high reproductive value when mated to attractive males. The relative importance of direct versus genetic benefits remains unresolved, and their fitness advantages can be difficult to disentangle. Female mate choice for different fitness benefits has been proposed in the tūī, *Prosthemadera novaeseelandiae*, where females may gain direct benefits in the form of territory quality from large within-pair males (WPMs), but also potential indirect benefits from highly ornamented or genetically dissimilar extra-pair sires. In this study, I dissociated the fitness advantages of female within-pair and extra-pair mate choice by examining the effects of WPM and genetic sire traits on offspring survival and sex ratios. I argue that when direct benefits predominate, offspring fitness should be related to qualities of the WPM. In contrast, if indirect benefits are of primary importance, offspring fitness should be associated with traits of the genetic sire.

Consistent with direct benefits received from large males, offspring survival was positively related to WPM body size, regardless of the offspring's extra-pair status. In addition, offspring of highly ornamented and genetically dissimilar genetic sires were more likely to be male. To our knowledge, this is the first study in a socially monogamous bird to show that females use multiple male traits to simultaneously optimise different components of offspring fitness through within-pair and extra-pair mate choice, and emphasises the importance of considering both direct and indirect benefits in studies of sexual selection.

4.2 Introduction

Two suites of potential fitness benefits have been proposed to explain the adaptive significance of female mate preferences (Kirkpatrick and Ryan 1991). First, direct benefits occur when females choose mates based on the quality of the immediate benefit to themselves (e.g. territory quality or parental care). Direct benefits are likely to be important consideration in female mate choice and may be signalled by male traits such as song diversity or body size or condition (reviewed in Andersson 1994). Second, indirect benefits occur when preferred males provide genetic benefits that increase offspring survival (Fisher 1915) or mating success (Fisher 1958). Females actively engaging in extra-pair mating are often argued to be maximizing indirect benefits through either "compatible genes" or "good genes" benefits (reviewed in Neff and Pitcher 2005). The compatible genes hypothesis argues that females choose genetically compatible partners to avoid the deleterious effects of inbreeding, or to increase heterosis (Zeh and Zeh 1996, 1997; Jennions 1997; Tregenza and Wedell 2000). This hypothesis has gained support from evidence of its positive effect on offspring viability in some studies (Kempenaers et al. 1999; Johnsen et al. 2000; Foerster et al. 2003; Masters et al. 2003; Brouwer et al. 2011; but see Mays et al. 2008). In contrast, the Fisher-Zahavi "good genes" hypothesis posits that females choose mates based on a trait indicative of male additive genetic quality, such as song complexity or ornamentation (Kokko et al. 2003). Such benefits may either increase offspring viability (e.g. Hasselquist et al. 1996; Sheldon et al. 1997; Møller and Alatalo 1999; Foerster et al. 2003; Dunn et al. 2013), or offspring attractiveness and hence reproductive value (e.g. Gwinner and Schwabl 2005; Head et al. 2005; Prokop et al. 2012) . Theoretical research predicts that under certain social or environmental conditions, females should display plasticity in mate choice for different genetic benefits in response to the relative fitness advantages of each strategy (Colegrave et al. 2002; Mays and Hill 2004; Neff and Pitcher 2005; Puurtinen et al. 2005). There are currently few studies, however, that have demonstrated this empirically (Foerster et al. 2003; Roberts and Gosling 2003; Schmoll et al. 2005; Oh and Badyaev 2006).

The relative magnitudes of direct and indirect benefits are debated. Some studies dispute the relevance of indirect benefits in female mate choice (Kirkpatrick and Barton 1997; Cameron et al. 2003; Arnqvist and Rowe 2005; Akçay and Roughgarden 2007). Yet, other studies suggest that indirect fitness benefits can be of comparable importance to direct benefits (Moller and Alatalo 1999; Moller and Jennions 2001), and can outweigh the costs of potential losses in direct benefits, such as reduced paternal care (Weatherhead & Robertson 1979, Head et al. 2005). However, direct and indirect benefits are not necessarily mutually exclusive (Kirkpatrick 1996) and females are generally expected to adopt behavioural strategies that optimise both kinds of fitness returns simultaneously (reviewed in Wong and Candolin 2005). For example, if a costly male trait reflects both physical condition and genetic quality, then it could honestly signal direct and indirect benefits to females (Berglund et al. 1996; Bart and Earnst 1999; e.g. Montoya and Torres 2014). Alternatively, females may utilise multiple traits in their mate choice decisions as a means to maximize each type of benefit individually (Møller and Pomiankowski 1993; Johnstone 1996) although there is little empirical evidence of this. More research is required in order to resolve the relative importance of indirect versus direct benefits.

When variance in male reproductive success is high, sexual selection can lead to high fitness differentials between the production of sons and daughters. Under these circumstances, both indirect and direct benefit models predict that females should allocate more resources to the sex with the greatest reproductive value (Trivers and Willard 1973). Under indirect benefits models, females mating with more attractive males should overproduce sons, on the assumption that these sons will inherit traits associated with attractiveness and sire more offspring in the next generation (Weatherhead and Robertson 1979; Burley 1981; Fawcett et al. 2007). Similarly, under direct benefits models, sex allocation may be adaptive if direct benefits increase offspring attractiveness (e.g. Grafen 1988; McGraw et al. 2005). Furthermore, females may manipulate offspring sex ratios if superior rearing environments can support more offspring of the more vulnerable or costly sex

(Bradbury and Blakey 1998; Nager et al. 1999; Kalmbach et al. 2001; Clout et al. 2002; Hasselquist and Kempenaers 2002; Pryke and Rollins 2012; Bowers et al. 2013). This is of particular relevance in sexually size dimorphic species (Wiebe and Bortolotti 1992; Sheldon et al. 1998; Sheldon and West 2004) where faster growth rates and higher energy demands in the larger sex are common (Clutton-Brock et al. 1985; Anderson et al. 1993).

Indeed, there is mounting evidence that females of some avian species possess substantial control over offspring sex (Ellegren et al. 1996; Heinsohn et al. 1997; Komdeur et al. 2002; Griffith et al. 2003; Dreiss et al. 2006; Pryke and Griffith 2009). In birds, females are the heterogametic sex, potentially enabling them to manipulate chromosomal sex determination (Pike and Petrie 2003). However support appears species-specific (Saino et al. 1999; Grindstaff et al. 2001; Leech et al. 2001; Westneat et al. 2002; Rosivall et al. 2004; Kingma et al. 2011), and the proximate maternal mechanisms involved remain unclear (Hasselquist and Kempenaers 2002; Alonso-Alvarez 2006; Navara 2013; Henderson et al. 2014).

Direct and indirect benefits can be difficult to disentangle. Additionally, empirical evidence for sex ratio adjustment in response to male quality is equivocal. This has been partially attributed to the analysis of sex ratios in relation to traits of within-pair males (WPMs), rather than those of genetic sires (Komdeur 2012). In this study I disassociate the effects of direct versus indirect benefits by elucidating whether two proxies of offspring fitness, survival and reproductive potential (here measured by offspring sex), are related to male traits predicting within-pair and extra-pair paternity success. If direct benefits are more important to offspring fitness, offspring survival and/or reproductive value should be associated with indicators of WPM quality. If indirect benefits are more important, offspring fitness should be related to attributes possessed by the genetic sire (Komdeur 2012).

I use the New Zealand tūī, *Prosthemadera novaeseelandiae* (*Meliphagidae*) to examine these hypotheses. The tūī provides an excellent model to test this, because among socially monogamous bird species, tūī are extreme in terms of both high rates of EPP (57% of all offspring) and high degree of sexual size dimorphism (SSD; males are 50% heavier than females, Chapter 3). The high EPP rate provides the opportunity for females to gain both indirect and direct benefits from mate choice. In addition, the high variance in male reproductive success (Chapter 3) suggests the presence of potential high fitness differentials between sons and daughters required for the evolution of sex ratio adjustment (Komdeur et al. 2002; Whittingham and Dunn 2005). Indeed, previous work suggests that females seek multiple benefits from mate choice. Females sought extra-pair mates that were either more genetically dissimilar, or that possessed a larger white throat feather ornament, than her social mate (Chapter 3). In contrast male body size may instead be associated with direct benefits because although large males enjoyed greater within-pair paternity success, these males did not win more extra-pair matings. Alternatively, large males may be simply better able to defend their paternity (Møller 1987; Komdeur 2001).

Our study makes four specific predictions. Females paired to dominant male tūī gain access to nectar resources and consequently have a higher reproductive success (Bergquist and Craig 1988). As dominance is thought to be size-related in tūī (Craig 1985), I firstly hypothesise that females gain direct benefits from large WPMs, and therefore the offspring of large WPMs should have a higher probability of survival. Second, due to the high SSD in tūī, I predict that females paired to large dominant males will produce more sons. This prediction assumes that sons are more energetically expensive to rear and females can afford these increased costs when mated to larger males. If alternatively, large males do not provide direct benefits, but possess superior paternity defence mechanisms, WPM body size should be unrelated to offspring survival or sex. Third, female choice for large-plumed males (Chapter 3) suggests that male ornamentation indicates attractiveness, and I therefore predict that under sex allocation theory offspring sired by highly ornamented males should be more likely to be male. Fourth, given the extra-pair mating advantage of genetically dissimilar males in this population, I assume that these males provide indirect benefits for offspring fitness, and predict that offspring sired by genetically dissimilar males should have higher survival rates due to the higher viability of outbred individuals. These predictions aim to determine whether females obtain different benefits from within-pair and extra-pair mate choice.

4.3 Methods

4.3.1 Field methods

I caught 390 wild tūi using mist-nets at Tawharanui Regional Park, in the North Island of New Zealand, between 2009 and 2012. For all birds, body mass, tarsus, head-bill, wing, and tail length were recorded, and they were colour-banded and blood-sampled. For a subset of the individuals, the natural curved length of the white throat plumes, and the mean number of feathers in each plume were recorded as measures of ornamental plume size.

Nests were located during breeding seasons (October to February). Blood samples were collected from five days old nestlings (N =162) of 56 nests. Thirty eight nests were from unique social-pairs and 18 were multiple broods from either a repeated male or female. Of these nestlings, 116 were followed through to fledging to determine nestling mortality rates resulting from starvation (these nestlings were from 37 nests, including 30 nests from different social pairs and 7 from repeat individuals). Any broods in which mortality from predation occurred were excluded, as it was unknown whether they would have survived to fledging.

4.3.2 Genetic analysis

Twelve microsatellite loci were employed as in Wells et al. (2013) to determine the paternity of all nestlings (see Chapter 3 for paternity details). I estimated genetic fathers and nestling heterozygosity using a measure of internal relatedness (IR: Amos et al. 2001). The genetic relatedness of social and genetic pairs was determined using TrioML, a maximum likelihood estimator (Wang 2007) using the software COANCESTRY (Wang 2011; see Chapter 3 for details on calculations of these variables). Molecular sexing was performed by amplification of an intron of the avian CHD1 gene using the 2550F and 2718R primers (Fridolfsson and Ellegren 1999). These primers are able to accurately sex non-ratite birds because the CHD1 intron occurs in different lengths on the W and Z chromosomes on non-ratite birds. Amplification of this intron produces products of different sizes and number according to sex: males being the homogametic sex in birds produce a single DNA fragment during gel electrophoresis which corresponds to the amplification of two copies of the CHD1Z intron; while females, being the heterogametic sex, produce products of two different sizes corresponding to amplification of the W and Z linked CHD1 introns. I performed PCR amplification of the CHD1 intron following the methodology of Fridolfsson and Ellegren (1999) and visualised the products on a 2% agarose gel using an ethidium bromide stain. Five samples were sequenced and amplification of the CHD1 intron was confirmed using NCBI BLAST (Altschul et al. 1990). The product sizes produced for male and female tui were consistent for all samples, with the CHD1Z intron visualised as around 600 base pairs on the gel, while the product size for the CHD1W intron was around 400 base pairs, allowing for easy distinction between males and females.

4.3.3 Statistical analysis

4.3.3.1 Fledging success

If direct benefits are important to female reproductive success, I reasoned that nestling fledging success should be dependent on WPM characteristics. Therefore our first model tested the influence of predictors of the social parents' quality on offspring survival to fledging. These predictors included WPM and female tarsus length, WPM plume length and number, female body condition index (BCI), and social-pair genetic relatedness. For females that were sampled in the same year as her brood, female BCI was calculated from the residuals of a regression of body mass on tarsus length (Reist 1985). To adjust female BCI for the time of year, a polynomial regression of BCI residuals on the sampling month was plotted, and the residuals used as predictors in statistical analyses. I used generalised linear mixed models (GLMMs) with a binomial error structure and a logit link function to investigate the probability of pre-fledging brood mortality as a binary response variable (0 = no mortality, 1 = one or more nestling mortality). To account for variability due to the rearing environment, I included the following additional predictors: overall brood genetic diversity as a function of the overall variance in genetic distance between siblings (Cohas et al. 2007), brood sex ratio (the proportion of male offspring), clutch number (first or second brood of the season), and a binary factor variable specifying the presence of EPP in the nest. Finally a clutch size offset was included as an unmodelled regression parameter to account for potentially higher mortality of larger broods.

If indirect benefits are more important to offspring survival, *individual* nestling fledging success should be irrespective of WPM characteristics, but should instead be related to traits of the genetic father. Therefore in our second model, the effects of WPM and genetic father characteristics on the probability of an individual nestling surviving to fledging were compared in a GLMM with a binomial error and logit link. I included both social and genetic male traits that may signal genetic quality, such as tarsus lengths, plume lengths and numbers, and heterozygosity. To determine if genetic compatibility effects are instead of greater importance to offspring survival, I also included predictors of nestling IR and social-pair and genetic-pair relatedness.

Random intercepts for maternal identity (model 1), and brood identity nested within maternal identity (model 2) were used to account for non-independence of observations from the same female, and from offspring within a brood, respectively (Pinheiro and Bates 2000). However, the variance in random effects for both models was zero, and thus the models were re-fitted as GLMs.

4.3.3.2 Offspring sex ratios

To examine the factors affecting offspring primary brood sex ratios, I used GLMMs with a binomial error structure and a logit link function to investigate the response variable of the proportion of males in a brood with the brood size as the binomial denominator. A random effect for female identity was initially included in all models to account for the presence of multiple broods from the same female. However, because the variance in the random intercepts was zero for all models, the models were re-analysed as GLMs. Although all nestlings in a brood were sampled from the majority of the nests studied and therefore represent actual primary sex ratios, in 12 out of the 56 broods (42 of 162 nestlings) there was a chance that incomplete broods were sampled due to partial mortality before blood sampling. However, because complete broods do not represent primary sex ratios more accurately than incomplete broods, both can be included in sex ratio studies (Fiala 1980; Krackow and Neuhauser 2008). To determine if the inclusion of these broods affected our conclusions, all models were fitted with and without incomplete broods included and the results compared.

Our first model was built to determine if social parent quality or the rearing environment predict offspring sex ratios. I therefore fitted predictors of social parent traits (WPM plume length and number, social-pair genetic relatedness, WPM and female tarsus lengths, and female BCI) and the rearing environment (brood number, clutch size, and a factor variable for whether EPP was present in the nest).

Our second model was used to test the hypothesis that females should overproduce sons when mated to attractive genetic sires. Twenty two out of 56 broods were of mixed paternity due to either two EPMs siring different offspring in the same brood, or offspring sired from both the WPM and EPM being present. Therefore, in order to test the influence of genetic father traits on the brood sex ratio I took the mean of the traits from all genetic fathers known from each brood. Where I only had data from one of two genetic fathers of a brood, I included this data. I specified predictors of the genetic fathers' traits (tarsus length, plume length and number), and the genetic relatedness of the genetic pair.

In both of the above models, I included random effects for maternal identity and brood identity nested within maternal identity. As there was no difference in fledging success (GLMM: $\chi^2 = 1.13$, $p = 0.2$), or offspring sex ratios (GLMM: $\chi^2 = 4.84$, $p = 0.2$) among years, years were pooled in all analyses. All variables were tested for collinearity using variance inflation factors (VIFs) and correlation coefficients prior to inclusions in models. Tarsus length and plume predictors were mean-centred (Zuur et al. 2009). For each approach, the initial model contained all predictors, and then backwards stepwise selection was employed (using hypothesis testing approaches) to reduce the model down to the final model with only significant predictors (Zuur et al. 2009). Stepwise regression may increase the likelihood of making type 1 errors (Forstmeier and Schielzeth 2011). However, as all final significant predictors were also significant in the full model, type 1 errors are unlikely to have affected our results (Forstmeier and Schielzeth 2011). Male plume variables were fitted in separate models in all analyses due to the reduced sample size for these variables (offspring survival: N = 41 nestlings from 20 broods, offspring sex: N = 60 nestlings from 28 broods). Additionally, I also fitted separate models to test female condition because of a smaller sample size of broods in which females were sampled in the same year as her brood (offspring survival: N = 65 nestlings from 21 broods, offspring sex: N = 83 nestlings from 28 broods). All models were analysed using the lme4 package (Bates 2010) in R v.2.15.1 (R Development Core Team 2011) and validated using the Hosmer-Lemeshow goodness-of-fit test (Hosmer and Lemeshow 2004).

4.4 Results

4.4.1 Genetic relatedness between pairs

The mean genetic relatedness of social parents in this study was 0.14 SD 0.18 while the mean genetic relatedness of genetic parents was 0.10 SD 0.15. Parents of 10 of the 37 broods (6 independent pairs) studied in this population were either first or second degree relatives. Three of the four pairs for which I had information on their pairings from multiple years, maintained pair-bonds for multiple years, and two of these maintained pair-bonds across all four years in this study.

4.4.2 Statistical analysis

4.4.2.1 Fledging success

Broods with WPMs with longer tarsi were significantly less likely to experience nestling mortality than WPMs with shorter tarsi (Table 4.1; Fig. 4.1a). This is suggestive of larger WPMs having a direct positive effect on offspring survival (see below). The probability of all nestlings fledging increased as the proportion of male nestlings in a brood decreased (Table 4.1; Fig. 4.1b). Females with long tarsi or in good condition were no more likely to fledge all their chicks than females with short tarsi or in poor condition. Broods with EPP had no more likelihood of fledging all nestlings than nests without, and nest overall genetic diversity did not influence fledging success. Social-pair genetic relatedness did not influence the probability of mortality (Table 4.1).

Predictor	β ± SE	LRT χ^2	P
Nest genetic diversity	0.193 ± 0.355	0.301	0.584
Female tarsus length	-0.525 ± 1.095	0.237	0.627
Social relatedness	2.621 ± 3.481	0.578	0.447
EPP present	1.364 ± 1.044	1.783	0.182
Clutch number	-1.152 ± 0.903	1.718	0.190
Social male tarsus length	**0.986 ± 0.447**	**5.750**	**0.016**
BSR	**-4.101 ± 1.519**	**9.690**	**0.002**
Female BCI	0.235 ± 0.281	0.787	0.375
Male plume number	0.0177 ± 0.306	0.003	0.954
Male plume length	-0.866 ± 0.799	1.367	0.242

Table 4.1: Effect of social parents and nest characteristics on the probability of all chicks in a nest surviving to fledging. Horizontal lines delimit separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a χ^2 test between nested GLMMs while accounting for all other predictors remaining in the model. Coefficients in the final models are listed in bold.

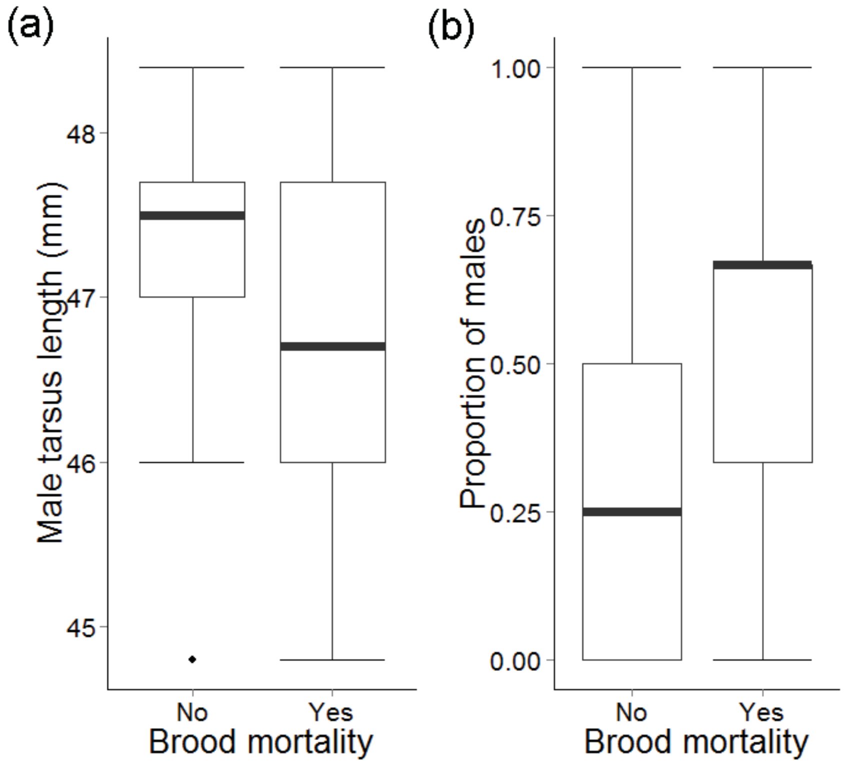

Figure 4.1: Tukey boxplots showing differences in (a) WPM mean-centred tarsus length and (b) the proportion of male nestlings between broods experiencing nestling mortality and those in which all nestlings fledged (N = 37).

The above indication that direct benefits associated with large WPMs are responsible for the increased offspring survival was supported when traits of the WPMs and genetic sires of individual offspring were compared. Although no genetic sire trait influenced the likelihood of fledging (Table 4.2), WPM tarsus length was positively related to an individual offspring's probability of survival to fledging (Fig. 4.2; Table 4.2), regardless of the offspring's extra-pair status. Surprisingly, I found that the likelihood of fledging was also higher in nestlings whose social parents were more genetically similar (Fig. 4.2; Table 4.2), but was unrelated to genetic similarity of the genetic parents.

All predictors had low collinearity ($r \leq 0.6$, VIF < 5) except for social and genetic male tarsus which showed slight collinearity ($r = 0.8$, VIF = 6). However, I continued with these predictors tested in the same model, as I reasoned this was the single best way to test whether an effect on the response was due to traits of the WPM or genetic male. Therefore, to verify the effect of genetic male tarsus without the possibility of collinearity with WPM tarsus influencing the result, I tested the significance of genetic male tarsus by itself in the model. The result was not significant ($\chi^2 = 0.009$, $p = 0.9$), and so I felt confident that the removal of genetic male tarsus in the stepwise selection process was not an artefact of collinearity.

Predictor	β ± SE	LRT χ^2	P
Genetic pair relatedness	-1.793 ± 4.089	0.191	0.662
Nestling IR	-1.594 ± 1.953	0.667	0.414
Social father IR	2.414 ± 2.703	0.819	0.366
Genetic father IR	-1.810 ± 2.053	0.831	0.362
Genetic father tarsus length	-1.169 ± 0.723	2.755	0.110
Social pair relatedness	**5.597 ± 2.413**	**7.565**	**0.006**
Social father tarsus length	**0.827 ± 0.293**	**8.617**	**0.003**
Social plume length	-0.420 ± 0.644	0.460	0.498
Social plume number	0.153 ± 0.273	0.382	0.536
Genetic plume length	-0.103 ± 0.299	0.123	0.726
Genetic plume number	0.069 ± 0.158	0.208	0.649

Table 4.2: Effect of social and genetic fathers' characteristics on the probability of nestling mortality from a binomial GLMM with a logit link function. Significant results are indicated in bold. Horizontal lines delimit separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a χ^2 test between nested GLMMs while accounting for all other predictors remaining in the model. Coefficients in the final models are listed in bold.

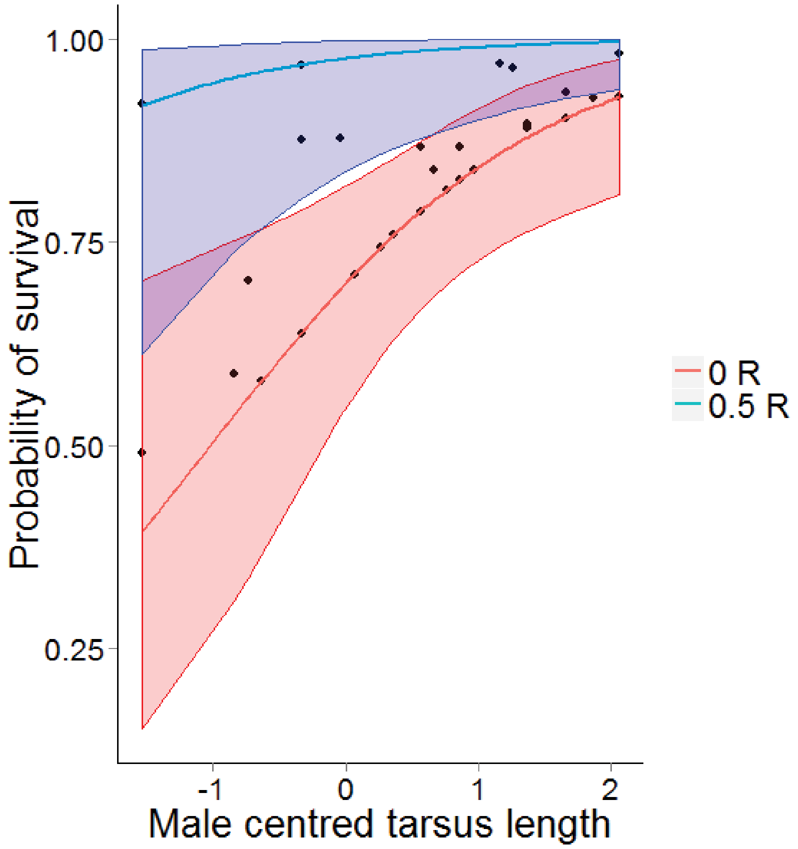

Figure 4.2: GLMM predicted probabilities and fitted values with 95% confidence intervals showing the relationship between an individual nestling's probability of survival and WPM mean-centred tarsus length at social-pair genetic relatedness values of 0 (red) and 0.5 (blue) (N = 116).

4.4.2.2 Offspring sex ratios

There was no evidence for an effect of direct benefits on offspring sex ratios, because no traits of the social parents influenced the number of sons produced (Table 4.3). In addition, broods with EPP were no more likely to produce sons than broods without EPP, and offspring sex was also unrelated to clutch size (Table 4.3). Excluding potentially incomplete broods had no effect on the model outcome.

Predictor	β ± SE	LRT χ^2	P
Social male tarsus length	0.008 ± 0.200	0.001	0.970
Clutch size	-0.033 ± 0.278	0.014	0.905
Clutch number	0.219 ± 0.335	0.429	0.513
Social relatedness	-0.812 ± 0.955	0.732	0.392
EPP present	-0.508 ± 0.391	1.699	0.192
Female tarsus length	-0.263 ± 0.212	1.580	0.209
Female BCI	0.096 ± 0.063	2.450	0.118
Male plume length	0.187 ± 0.333	0.317	0.573
Male plume number	0.175 ± 0.148	1.600	0.206

Table 4.3: Effect of social parent qualities on the proportion of sons in a brood for the full dataset. Horizontal lines delimit separate models. P values are based on the result of a likelihood ratio test (LRT) between nested GLMs while accounting for all other predictors in the model.

However, females produced significantly more sons when the true sire possessed a large number of plume feathers (Table 4.4; Fig. 4.3) and when the genetic parents were genetically dissimilar (Table 4.4). These predictors remained significant when potentially incomplete broods were excluded from the analysis (plume number: GLM $\beta = 0.331 \pm 0.165$, $\chi^2 = 4.507$, $p = 0.034$, genetic relatedness: GLM $\beta = -3.651 \pm 2.012$, $\chi^2 = 3.740$, $p = 0.050$). The tarsus length of the genetic father did not significantly predict offspring sex ratios (Table 4.4).

Predictor	β ± SE	LRT χ^2	P
Tarsus length	0.223 ± 0.167	1.797	0.180
Relatedness	**-3.089 ± 1.594**	**4.042**	**0.044**
Plume length	-0.222 ± 0.189	1.429	0.232
Plume number	**0.312 ± 0.121**	**9.204**	**0.002**

Table 4.4: Effect of genetic father traits on the proportion of sons in a brood using the full dataset. Horizontal lines delimit separate models. P values are based on the result of a likelihood ratio test (LRT) between nested GLMs while accounting for all other predictors in the model. Significant predictors are highlighted in bold.

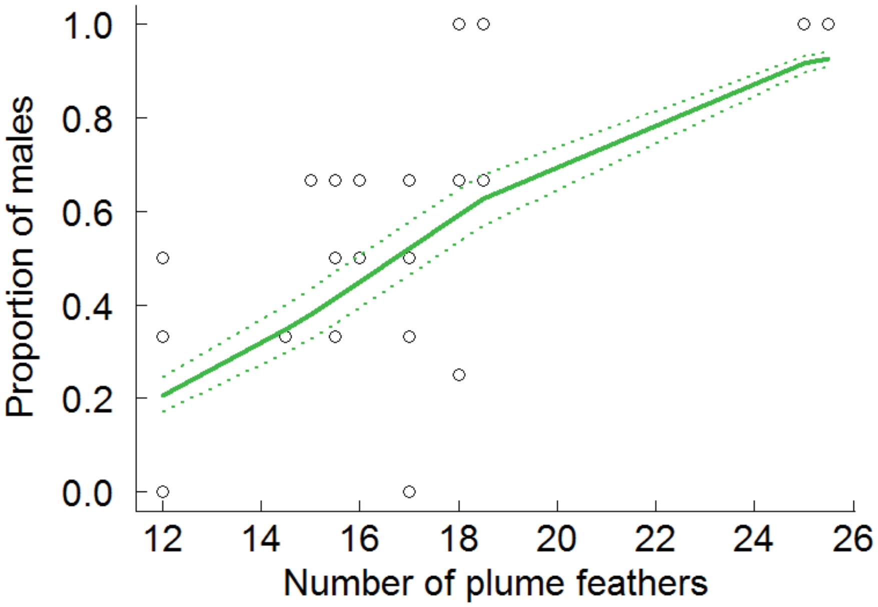

Figure 4.3: Effect of the number of plume feathers possessed by genetic sires on the proportion of male nestlings in a brood from a GLM with binomial error distribution. The figure shows raw data points from the full dataset (N = 29 broods) and model predicted probabilities (solid line) with 95% confidence intervals (dashed lines).

4.5 Discussion

In this study, direct benefits were supported by the finding that offspring survival was positively related to the body size of the WPM, regardless of whether they were within-pair or extra-pair offspring. In addition, indirect benefits were also supported, as females mated to highly ornamented, as well as genetically dissimilar genetic sires, overproduced male offspring. To our knowledge, this is the first study to provide evidence of female simultaneous use of multiple male traits to optimise different aspects of offspring fitness via the differential effects of direct and indirect benefits.

Offspring in broods of large WPMs were more likely to survive to fledging, whereas the body size of the genetic sire had no effect. This suggests that direct benefits from being paired to large WPMs, rather than overall genetic quality, are of greater importance to offspring survival. Such direct benefits could include higher territory quality and/or better paternal care, which can have profound effects on offspring survival rates (Clutton-Brock 1991). However, I argue that this is unlikely to be due to parental care, as provisioning rates are unrelated to WPM body size in tūī (Chapter 5). Previous studies on tūī have suggested that dominance hierarchies are size-related (Craig 1985), and demonstrated that winners of male-male competition gain territories containing important food resources, and consequently fledge more young (Bergquist and Craig 1988). I therefore argue that the direct benefits provided by large males are likely to be related to dominance and their ability to win better territories through male-male competition (reviewed in Qvarnström and Forsgren 1998). Regardless of the mechanism, our study supports the hypothesis that the benefits that large males offer are not genetic, and explains why extra-pair males are not significantly larger than WPMs in this population (Chapter 3).

Among species with male-biased SSD, sons are asserted to be more costly to rear than daughters, as a consequence of their faster growth rates and higher energy demands that result in higher mortality if the required increased investment cannot be met by parents (Clutton-Brock et al. 1985). Our finding that brood mortality is positively related to the proportion of males in a brood is consistent with this hypothesis. If sons are more energetically expensive to rear, and large WPMs provide direct benefits, one may also expect the broods of large WPMs to contain more sons (Kölliker et al. 1999; Yamaguchi et al. 2004; Abroe et al. 2007). This was not the case in our study. Indeed, no WPM traits influenced offspring sex, thus it would appear that the fitness consequences of direct benefits is limited to offspring survival rather than future reproductive potential. Surprisingly, I found no evidence for the effect of female body size or condition on either fitness measure. This contrasts with findings in other species (e.g. Wiebe and Bortolotti 1992; Nager et al. 1999; Alonso-Alvarez and Velando 2003; Warner et al. 2007) and suggests that male quality, rather than female quality, is more important to offspring fitness in tūī. This result is nevertheless consistent with the expectation that male quality plays a larger role in species like the tūī where there is evidence of intense sexual selection (Trivers 1972).

Contrary to expectations, nestling mortality was significantly lower in social-pairs of a greater genetic similarity. Given the consistent pairings of related individuals across years in this population, I argue that inbreeding in tūī is unlikely to be random (reviewed in Szulkin et al. 2009). However, exactly what benefits, if any, being paired to a relative might provide is unclear (reviewed in Kokko and Ots 2006). One explanation is that related WPMs may be less likely to reduce parental care or divorce unfaithful females as, under Hamilton's (1964) rule, they still share genes with extra-pair young. Alternatively, the costs of avoiding inbreeding may be too high (Foerster et al. 2003; Kokko and Ots 2006) especially if local males, using acquired local knowledge, are more likely than immigrants to procure the best territories. A detailed study of the levels of philopatry and inbreeding depression in this population is needed to examine these patterns further.

Although survival was not greater in offspring of genetically dissimilar biological sires (contrary to compatible genes theory), these offspring were significantly more likely to be male. In our study, females also overproduced sons by large-plumed genetic sires. Interestingly, both genetic relatedness and male ornament size are traits by which females appear to select extra-pair partners (Chapter 3). The congruence in traits predicting both offspring sex ratios and male extra-pair success is similar to findings in collared flycatchers, *Ficedula albicollis*, (Ellegren et al. 1996), blue tits, *Cyanistes caeruleus*, (Sheldon et al. 1999) and peafowl, *Pavo cristatus* (Chandler et al. 2013) where females manipulated primary sex ratios in response to the attractiveness of their mate. Our findings add to a growing body of evidence in birds that females can facultatively manipulate primary sex ratios in response to mate quality, and suggest that this mechanism is more likely to operate under indirect rather than direct selection. However, it remains to be determined whether females commonly possess such a finite control over offspring sex to be able to bias the sex of individual offspring without biasing the sex of other offspring within a clutch (Komdeur 2012). Indeed, only four studies have demonstrated this to date (Kempenaers et al. 1997; Schwarzova et al. 2008; Johnson et al. 2009; Du and Lu 2010).

The overproduction of sons is not a common prediction of compatible genes theory. Recent studies have found that heterozygosity can be correlated to good genes indicators such as song complexity or ornamentation i.e. the good-genes-as-heterozygosity hypothesis (e.g. Marshall et al. 2003; Reid et al. 2005; Ilmonen et al. 2009). As genetically dissimilar pairs will have more heterozygous offspring (e.g. Chapter 6, Tregenza and Wedell 2000), it is possible that producing heterozygous sons may also increase offspring reproductive potential. This hypothesis is particularly pertinent to our study population in which inbreeding in some social pairs is substantial, and therefore producing outbred sons may generate significant fitness advantages. Further research is required to test if male heterozygosity and ornamentation are positively correlated in this population.

In conclusion, previous research on patterns of extra-pair mating in this population of tūī argued that larger males likely provide direct benefits only, and proposed that females optimise both direct and indirect benefits in mate choice (Chapter 3). The results reported here confirm these hypotheses. There is mounting evidence of female choice for multiple male traits (reviewed in Candolin 2003). However, although female choice for multiple male traits that collectively act to increase one aspect of offspring fitness has been demonstrated (e.g.

Lancaster et al. 2009), this study is the first to demonstrate female choice for multiple male sexually selected traits that influence disparate aspects of offspring fitness. This was achieved through the optimisation of direct and indirect benefits: direct benefits had a significant effect on offspring survival, while indirect benefits instead predicted an offspring's reproductive potential. Females paired to large WPMs refrain from engaging in extra-pair copulations (Chapter 3). Thus it seems likely that females not mated to large social partners compensate for lost direct benefits by producing offspring with a higher reproductive potential through extra-pair matings. However, it is unknown whether such indirect benefits can fully compensate for the loss of direct benefits. Theoretical work predicts that selection on indirect benefits is relatively weak compared to selection on direct preferences (Kirkpatrick 1996; Kirkpatrick and Barton 1997). Yet empirical studies present contrary evidence (e.g. Head et al. 2005). Considering the intense male-male competition for territories and selection pressure indicated by the extreme SSD in tūī, I argue that indirect benefits are unlikely to completely compensate for direct benefits provided by territory quality. However, our data is limited to the nestling phase and further work quantifying the relative contributions of indirect versus direct benefits to female fitness post-independence is required. Nevertheless, these results highlight the importance of considering both direct and indirect benefits simultaneously in studies of sexual selection. To our knowledge, this is the first study to provide evidence of female mate choice for multiple male traits advertising different fitness aspects through direct and indirect benefits.

4.6 References

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5 Attractiveness influences male reproductive strategies in the tūī, *Prosthemadera novaeseelandiae*

Female tūī feeding a brood of four hungry nestlings

5. Attractiveness influences male reproductive strategies in the tūī, *Prosthemadera novaeseelandiae*

5.1 Abstract

Males of many species exhibit alternative reproductive strategies depending on their future mating opportunities. Depending on the fitness advantages of these strategies, male sexually selected traits may signal either parental ability or genetic benefits. These strategies predict opposing levels of paternal effort in response to the expression of the trait. However, the cost and benefits of alternative reproductive strategies may be complex when the certainty of parentage is reduced by extra-pair paternity (EPP). Tūī, *Prosthemadera novaeseelandiae*, are particularly suited to the study of alternative reproductive strategies as sexual size dimorphism (SSD) and frequent EPP are typically associated with reduced male care and a high variance in male mating success. Parental care has not been studied in tūī, therefore I first compared male and female provisioning rates in response to offspring age and environmental variables. In order to examine whether selection favours alternative male reproductive strategies in the tūī, I then assess the relationship between male parental effort and the expression of two sexually selected traits known to reflect male quality and influence paternity. Parental effort decreased with offspring age but showed annual variation in response to fluctuations in a major food resource. Contrary to expectations in species with SSD, parental effort was not greater in male-biased broods, although male effort did increase with clutch size. Despite a population-level reduction in male care suggestive of an adaptive response to reduced paternity assurance, cuckolded males provided significantly more care than males gaining full paternity of their broods. While male body size did not influence parental care, male ornament size was inversely related to paternal effort, supporting the hypothesis that ornamentation signals attractiveness rather than parental ability. Females mated to highly ornamented males did not increase their own parental effort in compensation as predicted by the differential allocation hypothesis. Our findings thus support the hypothesis that attractive males trade-off parental effort with mating effort, and I argue that unattractive males compensate for a reduced reproductive potential by increasing investment in current offspring. This study suggests the existence of alternative male reproductive strategies in which males adjust parental investment in response to their residual reproductive value.

5.2 Introduction

Males of many species possess ornaments or colourations that are driven by male-male competition and/or female choice (reviewed in Andersson 1994). However, evidence is divided whether male advertisement should signal direct benefits such as parental ability (reviewed in Kokko 1998). Parental care involves a trade-off between current and future reproductive success (Clutton-Brock 1991), and consequently males may exhibit plasticity in reproductive investment depending on their quality and residual reproductive value (e.g. Smith 1995; Badyaev and Hill 2002; Harris and Uller 2009). Indeed, studies investigating the direction of the relationship between paternal investment and male advertisement are equivocal and tend to be species and context-specific. Three hypotheses have been proposed to explain this contention.

First, the good parent hypothesis asserts that male advertisement signals a male's parenting ability (Heywood 1989; Hoelzer 1989; Andersson 1994; Wolf et al. 1997). Investment in sexually selected characters may incur costs that result in life-history trade-offs with other aspects of viability or reproduction. Consequently it is purported that the trait is an honest indicator of a male's parental ability, as only high quality males can bear the cost of the trait (Zahavi 1975; Grafen 1990). Although this hypothesis has received some support (Hill 1991; Linville et al. 1998; Buchanan and Catchpole 2000; Keyser and Hill 2000; Massaro et al. 2003; Siefferman and Hill 2003; Krebs et al. 2004; Germain et al. 2010), a comparative analysis revealed that ornamentation was only positively correlated to male care in species with low rates of extra-pair paternity (EPP; Møller and Thornhill 1998). Consequently, this hypothesis is presumed to predominate when direct benefits are of primary importance to reproduction (Kokko 1998; Kelly and Alonzo 2009).

Second, the trade-off hypothesis predicts a negative correlation between male advertisement and paternal effort (Magrath and Komdeur 2003). Bateman's (1948) principle asserts that males should maximise fitness by seeking additional matings. Male mating success is positively correlated with the expression of secondary sexual characters in many species (reviewed in Griffith et al. 2002).

Therefore, when the fitness benefits from multiple matings outweigh those acquired through parental investment, a negative correlation between male advertisement and paternal effort is expected (Magrath and Komdeur 2003). Despite receiving some support (Studd and Robertson 1985; Sanz 2001; Mitchell et al. 2007), others have challenged the hypothesis that investment in mating effort is costly (Stiver and Alonzo 2009). However, trade-offs may also occur with male-male competition rather than mating effort *per se*, and such trade-offs are indeed likely to be costly (e.g. Pryke and Griffith 2009). Evidence for trade-offs with male-male competition derives from studies in which experimentally enhanced attractiveness led to an increase in male-male competition at the expense of parental effort (Qvarnström and Forsgren 1998; Sanz 2001; Mazuc et al. 2003). This relationship may be at least partly mediated by testosterone levels, which is an indicator of dominance (Ketterson et al. 1992) and is hence often negatively correlated to paternal care (Hegner and Wingfield 1987; Mazuc et al. 2003; Schwagmeyer et al. 2005; McGlothlin et al. 2007; but see van Duyse et al 2000, Qvarnström et al. 2000).

Third, the differential allocation hypothesis argues that when ornamentation indicates indirect benefits, highly ornamented males should provide less parental care because their mates' increase parental investment to compensate for their attractive partner (Burley 1986; Kokko 1998). The male is then free to invest in his own survival, and the costs to female fitness of the additional investment are offset by siring offspring of a higher reproductive value (Burley 1986). This hypothesis has received much support (e.g. Burley 1988; Petrie & Williams 1993; de Lope & Møller 1994; Qvarnström 1997; Badyaev and Hill 2002; Mahr et al. 2012;

Limbourg et al. 2013; but see Bluhm & Gowaty 2004, Gowaty et al. 2007) and should persist when indirect benefits are of principal importance to offspring fitness (Kokko 1998).

Overall, the most advantageous reproductive strategy will depend on the magnitude of fitness benefits that can be gained from multiple mating (Kokko 1998). However, 86% of all passerines engage in EPP (Griffith et al. 2002), and therefore the benefits of each strategy may also be contingent on an individual male's share of paternity. Males should be selected to reduce paternal effort in response to a lowered paternity (Trivers 1972; Queller 1997; Matysioková and Remeš 2013). Indeed, there is evidence that males of some species facultatively adjust parental effort in response to realised or perceived paternity (Burke et al. 1989; Dixon et al. 1994; Freeman-Gallant 1997; Møller and Tegelstrom 1997; Sheldon et al. 1997; Lifjeld et al. 1998; Sheldon and Ellegren 1998; Neff and Gross 2001; Osorio-Beristain and Drummond 2001). However, other studies are equivocal (Whittingham et al. 1993; Whittingham and Lifjeld 1995; Kempenaers et al. 1998; MacDougall-Shackleton and Robertson 1998; Svensson et al. 1998; Kamel and Grosberg 2012; Hoi et al. 2013), and a recent meta-analysis demonstrated that in order to avoid the adverse dilemma of reducing care to potentially related offspring, a reduction in paternal investment is predicted only when EPP is frequent in the population (Griffin et al. 2013). Furthermore, the relationship is expected to be complex. For example, poor quality males with low paternity are predicted to have fewer future mating opportunities and should thus increase parental investment (Burley 1986; Kokko 1998). Consequently, there is no clear consensus on how paternity will influence male reproductive strategies.

Understanding the causality of differential investment in parental care in relation to male traits is essential before a prediction can be made about the magnitude of sexual selection exerted on alternative reproductive strategies. However, there are many competing predictions, and empirical studies have resulted in disparate conclusions (reviewed in Horváthová et al. 2012). In this study I investigate alternative reproductive strategies in the tūī, *Prosthemadera novaeseelandiae*, by examining how EPP and multiple sexually selected male traits influence parental effort. Tūī exhibit bi-parental care, but also extreme sexual size dimorphism (SSD; males are 50% heavier than females) and male-male competition (Chapter 3). Tūī are an interesting model for this study as these traits are commonly associated with social polygyny and reduced paternal care. They also possess SSD in an ornamental throat feather plume (Chapter 3). Both male body size and plume size are sexually selected traits that correlate positively with paternity and there is evidence that these traits indicate different fitness benefits. Female reproductive success is positively correlated to social male body size suggesting that male body size signals direct benefits (Chapter 4). Yet tūī also exhibit very high rates of EPP (57% of all offspring studied), and thus there is the potential for females to gain appreciable indirect benefits from extra-pair mate choice (Chapter 3). Female preference for large-plumed extra-pair sires, and overproduction of sons when mated to these males, is consistent with the hypothesis that ornamentation in tūī signals attractiveness (Burley 1981).

Parental care in tūī has not been studied in detail. Therefore, I first examine differences in male and female provisioning rates (a proxy of parental effort) in response to offspring age and rearing conditions such as clutch size, clutch number (first or second of the season), and brood sex ratio (BSR). I predict that 1) male provisioning rates will be significantly lower than females because of known characteristics of tūī commonly associated with reduced paternal care such as high rates of EPP, intense territoriality, and extreme SSD; 2) because of the high degree of SSD in tūī, sons are expected to have higher energy demands and faster growth rates (Chapter 6; Anderson et al. 1993), and I predict that parental provisioning should increase with the proportion of male offspring in a brood.

I then investigate male and female contributions towards parental care as a function of EPP and male quality. Specifically, I aim to determine whether male body size and ornamentation advertise parental ability, or whether these traits indicate different fitness benefits that have evolved from alternative male reproductive strategies. I thus make three further predictions: 3) I predict that large dominant males should invest less in parental care than small males due to a trade-off with investment in male-male competition. This prediction is derived from previous anecdotal evidence that males with significant nectar resources on their territory make fewer feeding visits to nestlings due to more time spent defending the territory (Stewart and Craig 1985); 4) based on the differential allocation hypothesis, I predict that male ornamentation advertises indirect benefits and due to the predicted reduced parental effort of large-plumed males, females should allocate more resources to offspring when paired with attractive males; finally, 5) because males are expected to reduce investment in unrelated offspring, I predict that males that lose paternity of their brood should provide less parental care than males gaining full paternity.

5.3 Methods

5.3.1 Data collection

Nestlings and parents from 26 nests were colour-banded at Tawharanui Regional Park in the North Island of New Zealand during the 2010 and 2011 breeding seasons. Blood samples and morphological measurements (tarsus, head-bill, wing and tail lengths) were taken, and the number and length of plume feathers were also recorded for a subset of the male parents (N = 9 fathers of 11 nests). I conducted nest observations to determine parental provisioning rates by sitting within sight of the nest and observing with binoculars. Care was taken to place the observer far enough away from the nest in order not to affect parental visitation rates. A suitable location was chosen during the first observation. If an adverse reaction to observer presence occurred (alarm calling or hesitation to go to the nest), the observer moved a few metres further away. This process was repeated until no reaction was observed. Data from this observation period was not included in the analysis, and the final location was then used for the rest of the study. For each observation I recorded two estimates of parental provisioning: the number of visits by each parent to the nest, and the length of time spent on the nest by each parent. Visit lengths exclude time spent by females brooding chicks, and only includes offspring feeding and nest maintenance, responsibilities which both parents undertake. Nest observations were predominantly two hours long and were conducted 2 or 3 times a week throughout the nestling phase and, where it was possible to follow fledglings, through to independence. Some observations differed in length due to unfavourable weather conditions.

5.3.2 Paternity analysis and molecular sexing

All chicks were sexed using molecular markers following the method of Fridolfsson and Ellegren (1999). Microsatellites were developed for the tūī (Wells et al. 2013; Chapter 2) and paternity analyses were conducted in CERVUS (see Chapter 3 for details of the methodology used to determine parentage).

5.3.3 Data analysis

Mixed effects models are appropriate for this study as they are able to efficiently model unbalanced repeated measures, where measurements were not taken on the same day for each grouping (Bates 2010). Linear and generalised linear mixed effects models based on Bayesian Markov Chain Monte Carlo (MCMC) inference were used to analyse parental provisioning rates and nest visitation rates in the package MCMCglmm (Hadfield 2010) in R v.2.15.1 (R Development Core Team 2011). I examined two response variables. First, I fitted a Gaussian distribution to the provisioning rate, calculated as the time spent on the nest per observation period in seconds. Second, I fitted a Poisson distribution to the nest visitation rate measured as the number of visits to the nest during the observation period.

For each model I fitted a baseline model investigating the effect of nestling age on the response variable. Preliminary plots identified non-linear trends with nestling age, particularly for females (Fig. 5.1), and therefore quadratic terms of nestling age were included in each model. Observation length was included as a covariate to account for unequal observation periods and year was included as a categorical predictor to account for significant annual variation.

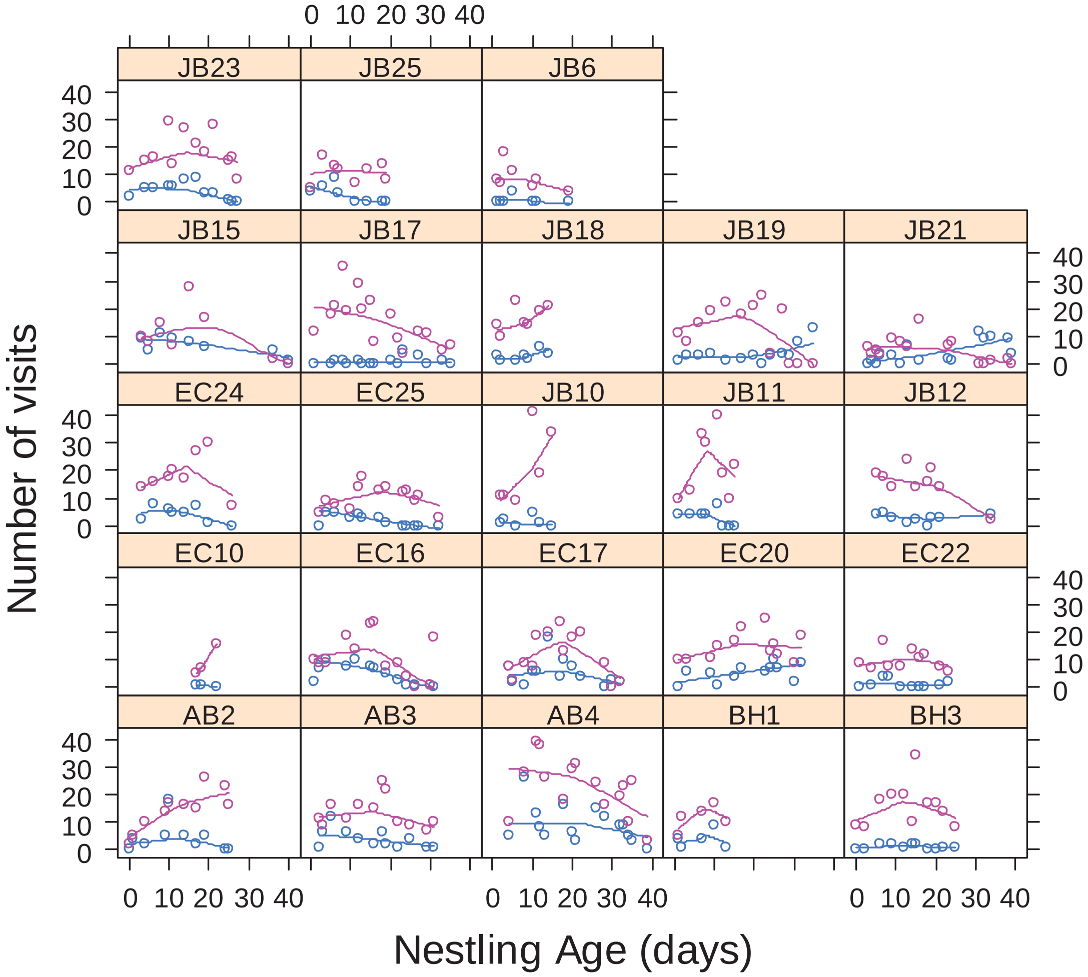

Figure 5.1: Male (blue) and female (pink) raw data showing nest visitation rates with nestling age (N = 23 nests). A loess (local regression) smoother (span = 1) with has been added to show the general trend. Three nests were removed for which a smoother could not be drawn as males provided no visits throughout the observation period.

This model was then used as a baseline to test predictors of parental quality and the rearing environment. To prevent fitting an over-parameterised model, I tested the significance of each additional predictor by adding them separately to the baseline model. The same predictors were tested for each response variable. These predictors included environmental variables such as clutch size, clutch number (first or second clutch of the season), and the brood sex ratio (BSR). Interactions of clutch number with the nestling age were included to test the significance of the field observation that males tend to provide less care towards the end of the second brood as they abandon the territory before the female. Parental traits such as male and female tarsus length, male throat plume length and plume number were tested in each model. Continuous predictors of male and female tarsus length and male plume length were mean-centred to prevent collinearity with the intercept (Zuur et al. 2009).

Each model included a random intercept for female identity to account for non-independent multiple observations from the same parent, and linear and quadratic random slopes for each female to allow for variance in the rate of provisioning with offspring age. A fully specified variance-covariance matrix was included. I specified parameter-expanded prior distributions for the random effects with prior variances corresponding to observed variation for each response, and inverse-Wishart prior distributions for the residual error terms (Gelman and Hill 2007). Models were run with for 500,000 iterations with a burnin of 100,000 iterations with samples recorded every 400 iterations. This resulted in 1000 MCMC samples which were used to calculate posterior means and 95 % credible intervals for the parameters. Significance of the parameters is indicated by the pMCMC values corresponding to the probability that the posterior distribution does not differ significantly from zero. Models were inspected for convergence using effective sample sizes and inspection of trace plots of the posterior distributions of the parameters. Posterior predictive checks according to Hadfield (2010) were conducted to determine whether the applied model was a good fit of the data (Appendix 2).

5.4 Results

5.4.1 Provisioning rates

Table 5.1 and Figure 5.2 show the posterior mean values of the time spent on the nest for female and male parents across nestling age and broods 1 and 2. The larger random intercept for females is indicative of greater variation among different females, than there is among males. Males spent significantly less time on the nest per observation period than females, as indicated by the non-overlapping credible intervals for male and female intercepts. Both parents showed a significant curvilinear decline in time spent on the nest with nestling age. This decline was less steep for males. Mean provisioning rates were significantly higher for both parents in 2010 than in 2011 (Table 5.1).

Clutch size had a significant effect on males, but not on females, with males providing more provisioning in larger broods (Table 5.1). However, there was no difference in either male or female provisioning rates depending on the BSR. Neither parent’s provisioning rate differed between first or second clutches. Male provisioning rates did not significantly decline as expected at the end of the second brood (Table 5.1). Male and female body size were unrelated to both their own and the other parent’s provisioning rate (Table 5.1). Female provisioning did not differ between broods with or without extra-pair young. However, cuckolded males had significantly higher rates of provisioning than non-cuckolded males (Table 5.1). Females paired to large-plumed males did not attend the nest more than females paired to small-plumed males. However, large-plumed males had significantly lower provisioning rates than small-plumed males (Table 5.1).

Predictor	Female				Male			
	Mean	Low 95%	Upp 95%	PMCMC	Mean	Low 95%	Upp 95%	pMCMC
Observation time	**937.1**	**0.004**	**0.014**	**<0.001**				
Intercept	**184.9**	**107.0**	**251.4**	**<0.001**	-11.75	-50.83	24.34	0.562
Age	**-2386**	**-2950**	**-1824**	**<0.001**	**1804**	**1313**	**2345**	**<0.001**
$(\text{Age})^2$	**654.4**	**220.9**	**1107**	**0.004**	**-458.9**	**-942.6**	**-3.752**	**0.050**
Year (2011)	**-87.12**	**-133.3**	**-39.13**	**<0.001**	**-20.22**	**-39.46**	**-2.370**	**0.034**
Random intercept	13306	4017.0	24549		683.7	108.6	1477.8	
Random slope	-33462	-105500	19084		0.001	0.0002	0.002	
Male tarsus	-21.19	-66.41	19.57	0.310	-1.946	-13.27	9.882	0.768
Female tarsus	-0.212	-6.641	1.957	0.310	-6.005	-17.04	6.502	0.302
Male plume number	-0.199	-64.15	54.38	0.978	-0.382	-9.196	8.791	0.858
Male plume length	5.854	-29.77	32.81	0.666	**-25.03**	**-54.10**	**0.858**	**0.048**
Clutch size	180.9	-179.6	599.0	0.390	**26.80**	**14.58**	**39.53**	**<0.001**
BSR	18.66	-16.14	53.64	0.296	7.716	-24.84	39.48	0.610
Clutch number	9.839	-110.1	108.5	0.864	5.973	-23.78	34.30	0.674
Age : Clutch number (2)	15.69	-72.58	111.4	0.782	538.6	-420.8	1513.0	0.268

$(\text{Age})^2$: Clutch number (2)	**-1132**	**-2065**	**-229.0**	**0.026**	131.1	-855.0	980.7	0.754
EPP (Yes)	40.55	-32.04	115.9	0.284	**27.66**	**4.335**	**55.65**	**0.036**

Table 5.1: Posterior means and lower and upper 95% higher posterior density credibility intervals for all predictors and random-effect variance parameters (in italics) for the effect of the rearing environment and parental characteristics on female and male time spent on the nest. The horizontal line denotes the partition of the baseline model (above the line) to which each predictor was tested. Observation time was not interacted by trait and was included just to account for variation in observation time length. The probability that a fixed-effect estimate does not differ from zero is provided by the pMCMC values. Fixed effects with pMCMC < 0.05 are highlighted in bold. For categorical predictors, the level estimated, relative to the baseline intercept level, is provided in parentheses.

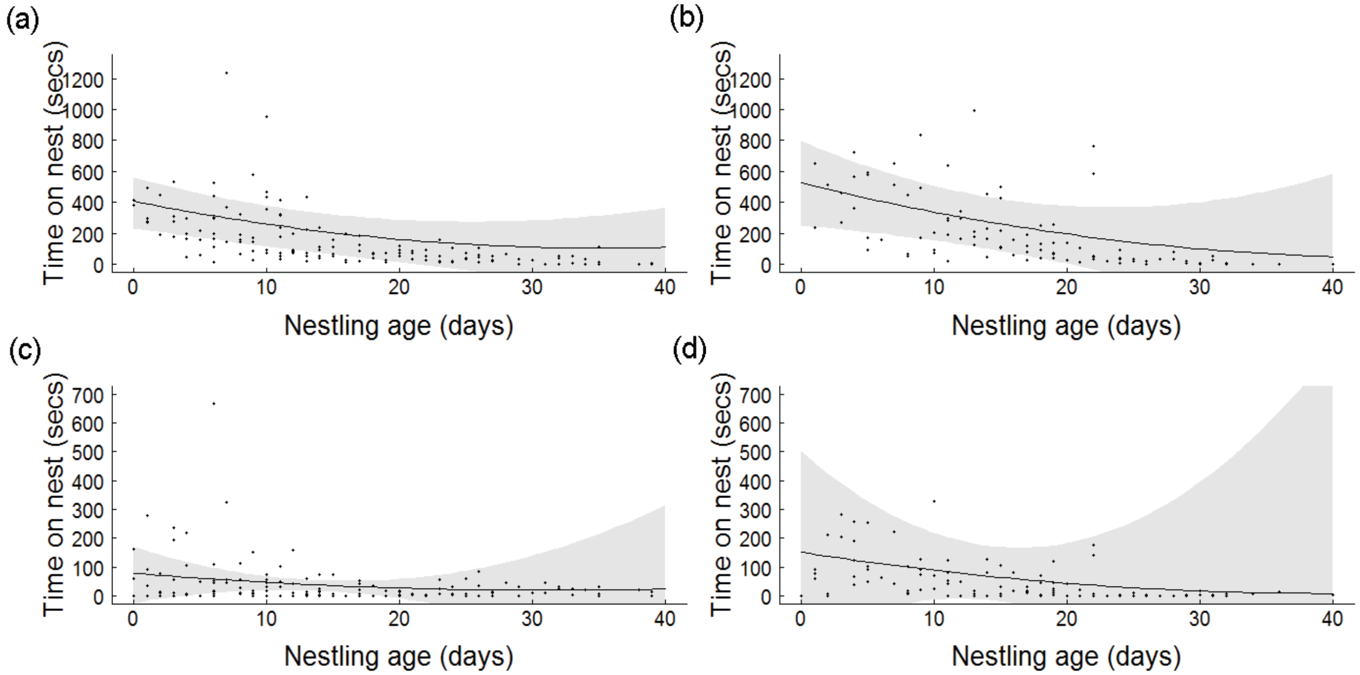

Figure 5.2: Posterior predicted mean effects (solid lines) with 95 % credible intervals (shaded areas) of the predicted time spent on the nest during each observation period (N = 26 broods). Plots are for (a) female provisioning for first broods of the season, (b) female provisioning for second broods of the season, (c) male provisioning for first broods of the season, (d) male provisioning for second broods of the season. Data has been averaged by year. Points are raw data values for each parent for each brood.

5.4.2 Number of visits

Table 5.2 and Figure 5.3 show the posterior mean values of parental nest visitation rates with offspring age in broods 1 and 2. Males visited the nest significantly less than females. In contrast to the steady decline in provisioning rate, the number of visits made by each parents initially increases after hatching, reaching a maximum number of visits of around three logged visits (20 visits) per observation period around day 18 for females, and around one to two logged visits (three to seven visits) per observation period around day 12 for males (Fig. 5.3). Male, but not female, visitation rates were significantly lower in 2011 than 2010. While overall visitation rates were similar between first and second broods of the season, males made significantly less visits towards the end the second brood than at the end of the first brood (Table 5.2, Fig. 5.3). Similarly to the provisioning rate, there was a greater variation in the number of visits among females than among males (Table 5.2).

The number of visits made by parents was unrelated to the BSR. Male visitation rate was, however, positively related to clutch size (Table 5.2). Neither male nor female visitation rate was related to male body size. There was an almost significant ($p = 0.062$) trend for visitation rates to be lower in nests of large females (Table 5.2). Although female visitation rate was unrelated to male plume size, there was a trend ($p = 0.078$) for large-plumed males to make less visits to the nest than small-plumed males. Cuckolded males made significantly more visits to the nest than males that gained full paternity of their nests (Table 5.2).

Predictor	Female				Male			
	Mean	Low 95%	Upp 95%	PMCMC	Mean	Low 95%	Upp 95%	pMCMC
Observation time	**0.0002**	**0.0001**	**0.0002**	**<0.001**				
Intercept	**1.266**	**0.762**	**1.744**	**<0.001**	-0.334	-1.079	0.449	0.372
Age	-5.977	-9.616	-3.028	**<0.001**	-4.632	-15.87	7.006	0.426
$(\text{Age})^2$	**-9.290**	**-12.70**	**-6.313**	**<0.001**	2.328	-5.308	10.75	0.560
Year (2011)	-0.112	-0.280	0.067	0.217	**-0.637**	**-1.087**	**-0.235**	**0.004**
Random intercept	0.071	0.009	0.147		0.524	0.138	0.999	
Random slope	0.0005	0.0001	0.0009		0.0005	0.0001	0.0009	
Male tarsus	0.097	-0.076	0.256	0.226	0.028	-0.458	0.470	0.824
Female tarsus	-0.185	-0.376	0.0005	0.062	-0.318	-0.937	0.300	0.282
Male plume number	0.030	-0.068	0.145	0.552	0.063	-0.134	0.295	0.534
Male plume length	-0.120	-0.515	0.320	0.552	-0.599	-1.354	0.085	0.078
Clutch size	0.119	-0.043	0.307	0.164	**0.562**	**0.230**	**0.862**	**<0.001**
BSR	-0.199	-0.704	0.274	0.432	-0.221	-1.330	0.077	0.710
Clutch number (2)	-0.145	-0.597	0.313	0.512	0.316	-0.490	1.071	0.426
Age : clutch number (2)	0.462	-5.892	7.379	0.916	**-14.53**	**-25.75**	**-1.208**	**0.024**

$(Age)^2$: clutch number (2)	-2.519	-8.524	3.303	0.410	-2.156	-12.33	8.209	0.684
EPP (Yes)	-0.024	-0.336	0.340	0.906	**0.846**	**0.167**	**1.452**	**0.008**

Table 5.2: Posterior means and lower and upper 95% higher posterior density credibility intervals for all predictors and random-effect variance parameters (in italics) for the effect of the rearing environment and parental characteristics on female and male nest visitation rate. The horizontal line denotes the partition of the baseline model (above the line) to which each predictor was tested. Observation time was not interacted by trait and was included just to account for variation in observation time length. The probability that a fixed-effect estimate does not differ from zero is provided by the pMCMC values. Fixed effects with pMCMC < 0.05 are highlighted in bold. For categorical predictors, the level estimated, relative to the baseline intercept level, is provided in parentheses.

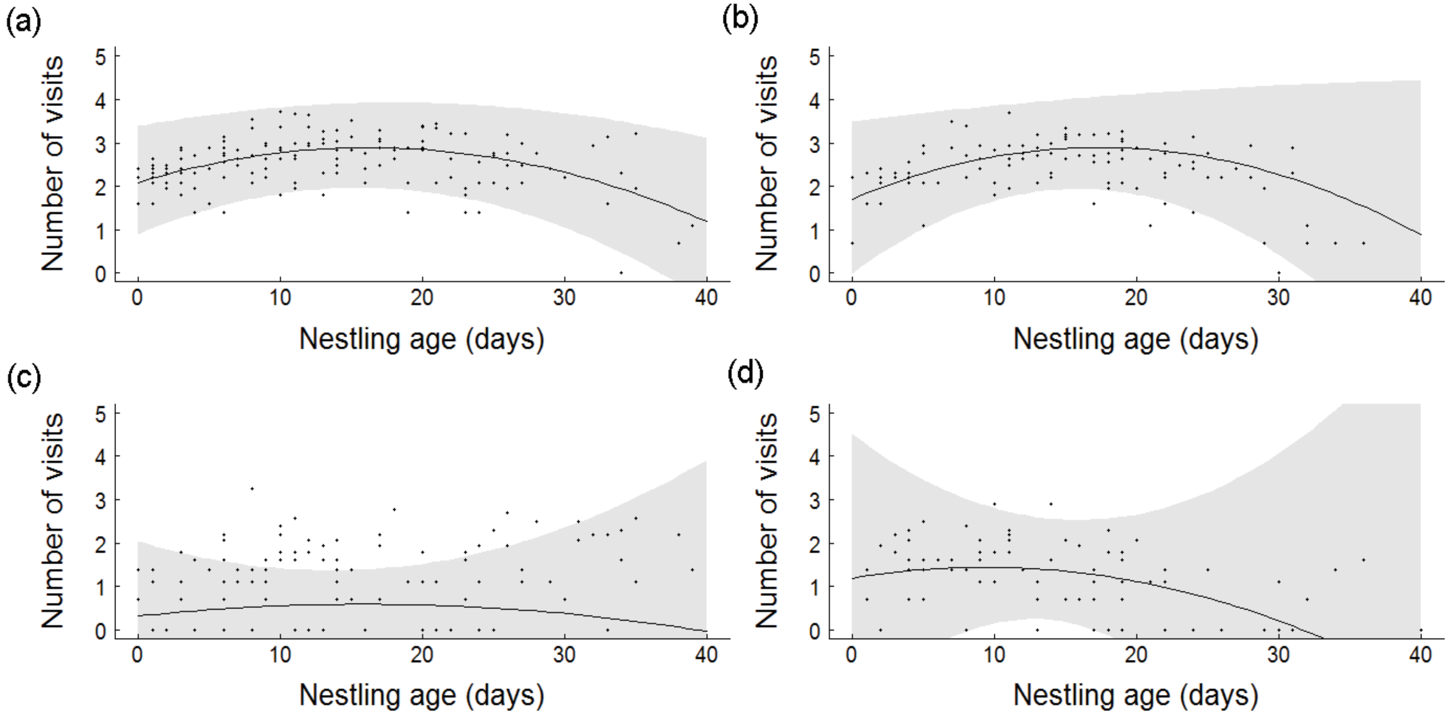

Figure 5.3: Posterior predicted mean effects (solid lines) with 95 % credible intervals (shaded areas) of the predicted number of visits to the nest during each observation period (N = 26 broods). Number of visits is shown on the log scale for comparison with model posterior parameter estimates in Table 2. Plots are for (a) female provisioning for first broods of the season, (b) female provisioning for second broods of the season, (c) male provisioning for first broods of the season, (d) male provisioning for second broods of the season. Data has been averaged by year. Points are raw data values for each parent for each brood.

5.5 Discussion

5.5.1 Male and female parental effort in tūī

Female parental effort was consistently greater than males, and showed a significant decline in provisioning rate with offspring age. In contrast, male parental effort, while lower, was more consistent across the nestling period, and showed a less steep decline than females with offspring age. A decrease in parental effort with offspring age is contrary to findings in other studies on passerines (Breitwisch et al. 1986; Haggerty 1992; Goodbred and Holmes 1996; LaBarbera et al. 2011) where provisioning increases across the nestling period. However, our data includes post-fledging parental care, where diminishing parental effort is expected. In addition, the provisioning rate in this study included nest maintenance (removing of faeces and cleaning), which is most intensive at the beginning of the nestling stage and likely contributes to the decline in provisioning rate. Consistent with this decrease in time spent on nest maintenance and a corresponding increase in time spent feeding nestlings with nestling age, visitation rates of both parents increase until around days 18 to 20. This asymptote in visitation rate corresponds to just before fledging (mean fledging age = 21.5 days). After this period, visitation rates, as well as provisioning rates steadily decline. Similar reductions in provisioning at the end of the nestling period have been found in other birds (e.g. pigeon guillemots, *Cepphus columba* (Emms and Verbeek 1991)), and may be a parental strategy to initiate fledging (Ydenberg 1989).

5.5.2 Effect of environmental variables on parental effort

Both male and female parental effort was greater in 2010 than 2011. 2010 was a prolific flax, *Phormium tenax*, flowering year (Wells, *pers obs*); the primary food resource for adult tūī during the breeding season (Stewart and Craig 1985). Additional food for offspring would have been available in 2010 as nectar is also given to older nestlings. This additional food source would also supply females with greater energy resources to allocate to parental investment. This suggests that female parental effort may be condition or resource-dependent, and hence access to food resources may be an important consideration in female mate choice.

Owing to the difficulty in following mobile fledglings, there is less data available for the post-fledging period, leading to larger confidence intervals in the amount of parental effort. Contrary to the observation that males often desert the territory after the second brood has fledged, male provisioning rate was not significantly lower at the end of the second brood. However, males did show significantly lower visitation rates during this period. I propose that this discrepancy may be caused by male provisioning rates already being very low at the end of the broods (males often spent only a few seconds feeding fledglings), and combined with a low amount of data for this period, may have led to the decrease not being significant. Early territory desertion has also been reported in the polygynous great reed warbler, *Acrocephalus arundinaceus* (Ezaki 1988). However, unlike great reed warblers, male tūī only desert the territory after there are likely to be no more mating opportunities, and thus this behaviour is unlikely to incur costs associated with a reduction in reproductive success. Indeed, deserting male great reed warblers commenced moulting earlier, settled on territories earlier the following season, and consequently were more likely to be polygynous (Urano 1992). Therefore, territory desertion in tūī may have benefits for male condition that enable them to increase mating success in the following season. Females did not increase parental effort to compensate for loss of male provisioning, suggesting that male care may not be essential at this stage.

Maternal provisioning was independent of the majority of environmental variables in this study, indicative of an intensive and essential level of parental effort. Although large females made less visits to the nest than small females, they did not spend less time on the nest, suggesting that large females may be able to bring larger, or a greater number of prey items per visit. Foraging efficiency and food loads are positively correlated to parental body size and experience in European shags (e.g. Daunt et al. 2007). Large females may therefore be more experienced, or in better condition and consequently be more proficient foragers than inexperienced small females. Contrary to our second hypothesis, neither parent increased parental effort in broods with a higher proportion of sons. This result is surprising given the extreme SSD in tūī which commonly leads to higher energy demands of the larger sex (Anderson et al. 1993; Chapter 6). However, our conclusions are limited because I cannot discount the possibility that parents with male-biased broods bring more food biomass per visit. Nevertheless, this finding is consistent with the greater mortality observed in male-biased broods (Chapter 4). In contrast, male, but not female, parental effort did increase with clutch size. Westneat et al. (2011) found a similar pattern in house sparrows, *Passer* *domesticus*. It is posited that males should increase care in large broods because the potential gains to reproductive success are greater (Westneat 1988; Manica 2002). Thus males may trade-off male care with investment in mating effort or survival, depending on the relative value of the brood (Magrath and Komdeur 2003). Alternatively, males of large broods may be better parents in general (e.g. Gustafsson and Sutherland 1988), or they may invest only the minimum required to achieve reproductive success (Rytkönen et al. 1993; Itzkowitz et al. 2001; Markman et al. 2002). Data across broods of different clutch sizes from the same parents are required to distinguish between these hypotheses.

5.5.3 Parental effort in relation to parental traits

Our results support the hypothesis that ornamentation in tūī indicates attractiveness rather than parental ability, as male parental effort was inversely related to the size of a sexually selected male ornament. The differential allocation hypothesis predicts that females should increase parental effort when mated to attractive males (Burley 1986). I found no evidence to support this hypothesis. Yet, the finding that females did not increase provisioning to offspring of more attractive males does not necessarily refute the differential allocation hypothesis. Reproductive investment may occur via pathways other than provisioning, for example via maternal differential investment in eggs (e.g. McFarlane et al. 2010; Giraudeau et al. 2011). As a relevant example, it has been demonstrated in tūī that the offspring of more attractive males are more likely to be male (Chapter 4). If sons are more costly to rear than daughters, as suggested by the higher SSD and pre-fledging mortality of sons in tūī (Chapters 3 and 4), then female manipulation of BSRs to overproduce sons would exemplify a case of differential allocation (Sheldon 2000).

Highly ornamented male tūī have a higher extra-pair mating success in this population (Chapter 3). Thus our findings are more consistent with the trade-off hypothesis in which highly ornamented males trade-off investment in parental care with extra-pair mating effort (Magrath and Komdeur 2003). Similar findings have been demonstrated in yellow warblers, *Setophaga petechia* (Studd and Robertson 1985), bluethroats, *Luscinia svecica* (Johnsen et al. 1998), and common yellowthroats, *Geothlypis trichas* (Mitchell et al. 2007). In order for such a trade-off to be adaptive, the benefits of multiple matings should exceed the costs of the reduced parental investment. Male ornamentation tended to be inversely related to offspring growth in tūī (Chapter 6) suggesting that reduced care by ornamented males may incur a fitness cost. However, the offspring of highly ornamented males were also smaller at hatching and so it is unclear if this relationship is a result of reduced male care, as this may also be a consequence of a greater proportion of sons in a brood (Chapter 4, see also below). On the other hand, highly ornamented males are equally as likely to fledge all their young as lesser ornamented males (Chapter 4), thus there do not appear to be fitness consequences for within-pair reproductive success. Concurrent female choice for the same extra-pair males suggests that these males are likely to obtain significantly higher extra-pair reproductive success (Chapter 3). Therefore it remains to be determined whether the reduced growth of offspring of highly ornamented males has any long-term fitness consequences for males that sufficiently counteract fitness gains through extra-pair matings.

I cannot definitively discount the possibility that the correlation between ornamentation and parental effort results from a trade-off between parental care and male-male competition, as has been found in other passerines (e.g. collared flycatcher, *Ficedula albicollis* (Qvarnström 1997); pied flycatcher, *Ficedula hypoleuca* (Sanz 2001); Gouldian finches, *Erythrura gouldiae* (Pryke and Griffith 2009)). Although the significance of plume ornaments in male-male competition remains to be determined, they appear to be displayed during male contests, and may function as badges of status (Chapter 3). As a consequence, more ornamented males may possess higher quality territories and consequently expend more effort in defending them (Qvarnström and Forsgren 1998).

Indeed, I also predicted that large males would exhibit a trade-off between parental care and male-male competition. Yet I found no significant difference in parental effort between large and small males. However, the amount of care provided by dominant males may vary depending on the net fitness gain from reducing care. For example, dominant males may be in better condition and consequently have more energy resources to contribute to both male-male competition and parental care (e.g. Qvarnström et al. 2000; Magrath and Komdeur 2003). Although it is purported that large male tūī are more dominant (Craig 1985), the relative effort expended by large males on male-male competition was not part of this study. Further studies that quantify male investment in mating effort and male-male competition in relation to phenotype are required to clarify that trade-offs are occurring.

5.5.4 Effect of extra-pair paternity on male parental effort

Contrary to the hypothesis that males should reduce care in response to lower paternity (Trivers 1972; Queller 1997), cuckolded males actually provided more parental care than males with full paternity of their brood. This is surprising given that it should be adaptive to adjust parental effort in response to paternity when 1) frequency of cuckoldry is high (Griffin et al. 2013) and 2) there is a high probability of future reproduction (Dickinson 2003); both of which are relevant to tūī, who are long-lived and survive until at least six years old (Wells *unpublished data*). However, theoretical models predict that males should be selected to adjust care only if parentage can be readily assessed and fluctuates across breeding attempts (Houston and McNamara 2002). This study therefore supports findings in other passerines that males in many species are unable to accurately assess parentage (e.g. tree swallows, *Tachycineta bicolor* (Lifjeld et al. 1993); red-winged blackbirds, *Agelaius phoeniceus* (Westneat et al. 1995); purple martins, *Progne subis* (Wagner et al. 1996); , western bluebirds, *Sialia mexicana* (Dickinson 2003)). Kin recognition is rare in birds (Kempenaers and Sheldon 1996), and it is posited that males instead equate paternity with female behaviour during her fertile period (e.g. Davies et al. 1992; Sheldon et al. 1997), which may in some instances not be a very accurate determinant of paternity. Indeed, male adjustment of parental effort may only occur in species where there are strong indicators of lost paternity. For example, male hihi, *Notiomystis cincta*, adjusted provisioning according to the number of witnessed extra-pair copulations on his social female (Ewen and Armstrong 2000). In this species extra-pair copulations are forced, and commonly occur in front of the social male (Ewen 1998), thus providing the male with an accurate gauge of parentage. Furthermore, anecdotal, yet intriguing evidence in tūī suggests that females may deceive males in their perceived paternity. For example, one consistently cuckolded male that provided the lowest amount of care with one female, provided significantly more care when paired with a new female that arrived mid-season (she ejected his previous female from the territory) even though he lost the same amount of paternity. I see two possible explanations for this behaviour. Either the male provided more care to "impress" the new female with his parental ability, or the female was able to convince the cuckolded male that he had a high paternity (Shellman-Reeve and Reeve 2000) by engaging in extra-pair copulations prior to her arrival in the area. Indeed, I was unable to identify the extra-pair male which suggests that he was an outsider, and supports the latter explanation.

The correlation between male parental effort and paternity in the opposite direction predicted by theoretical models suggests that the relationship is complex and may depend on additional factors such as sexual conflict, male quality, future reproductive opportunities, and the cost of providing care (Dunn and Cockburn 1996; Freeman-Gallant 1996; Neff and Sherman 2002; Westneat and Stewart 2003; Griffith 2007; Benowitz et al. 2013). For example, an inverse relationship between paternity and paternal effort may arise if cuckolded males are of low quality, but are better parents in compensation (Houston and McNamara 2002; Alonzo and Klug 2012). Cuckolded males are smaller or less ornamented in this population (Chapter 3). I therefore argue that this is a likely strategy in tūī.

I did, however, find support for the hypothesis that EPP has coevolved with reduced levels of paternal care (Møller and Birkhead 1993; Møller and Cuervo 2000; Matysioková and Remeš 2013), as males provided consistently less parental care than females in this study. The correlation between population-level rates of EPP and male care is purported to arise through an adaptive reduction in male care in response to either a diminished average certainty of paternity (Trivers 1972; Queller 1997), or a reduced dependence on male care to successfully rear offspring (Mulder et al. 1994; Gowaty 1996; Møller 2000). The lack of evidence for a positive correlation between a male's paternity of his brood and his parental effort at first contests the former hypothesis. However, a facultative change in male behaviour is not an essential prerequisite for the evolution of a population-wide association (Sheldon 2002). For example, when chances of cuckoldry are high, a mutation selecting for reduced paternal care would be adaptive regardless of paternity. Furthermore, while this study investigates parental care in relation to paternity across individuals, comparisons among broods of the same individuals would be a more accurate test of this hypothesis (Lessells 1994). Therefore I cannot currently distinguish between these two competing hypotheses.

If males do not adjust care in accordance with paternity, promiscuous females are not penalised for their actions, and consequently only small benefits acquired from EPP are sufficient to lead to a reduced average paternity over evolutionary time (Slatyer et al. 2011). However theoretical models predict that monogamy with very high rates of EPP (> 50%) can be an evolutionary stable strategy only if 1) males are unable to reliably assess parentage, 2) the benefits of EPP are not high, and 3) females cannot fully compensate for a reduction in male care (Kokko 1999). If one of these is violated, male care will decrease over generations, leading to eventual polygyny and female only care; the so-called “tragedy of the commons” (Kokko 1999; Kokko and Jennions 2012). While there is evidence of the first prediction in tūī, the second and third remain unknown. It is therefore currently unclear whether the high rates of EPP in tūī are an evolutionary stable strategy, or represent a transition towards social polygyny. However, tūī do share traits with polygynous species such as intense male-male competition and high SSD. The territorial polygyny model asserts that social polygyny will evolve whenever inequities in territory quality are great enough that female reproductive success would be greater with the polygynous male despite reduced paternal care (Verner and Willson 1966; Orians 1969). Although there is no evidence of socially polygynous pairings in tūī, mid-season mate-swapping and competition between females for males does occur, particularly in territories associated with abundant nectar resources (Wells *unpublished data*). Thus the potential may exist for polygyny to evolve.

In conclusion, this study provides the first valuable insight into the parental allocation of resources to reproduction in tūī. However, experimental studies that control for maternal effects, and studies based on variation in parental care, paternity, and male quality *within*, rather than *between*, individuals are needed (Sheldon 2002). This study also highlights the importance of considering different aspects of parental effort, as the same variables did not necessarily influence both estimates of provisioning. For example, if parental effort was solely measured by the nest visitation rate, one would have incorrectly drawn the conclusion that large females provide less care than small females. Male body size was unrelated to parental effort, suggesting that the direct benefits provided by large males may instead be related to territory quality. This study supports the hypothesis that ornamentation signals attractiveness in species with high rates of EPP where indirect benefits and the chance to gain additional matings are significant. Our findings support the trade-off hypothesis in which attractive males trade-off parental effort with mating effort. However, I am unable to definitively rule out differential allocation arising from maternal effects. This study further suggests that alternative reproductive strategies may have evolved through sexual selection in tūī, with less attractive males compensating for reduced paternity success by increasing investment in parental care to obtain breeding opportunities. Such condition-dependent strategies have been found in other socially monogamous birds such as house finches, *Carpodacus mexicanus* (Badyaev and Hill 2002). Further study on male investment in mating effort should be conducted to confirm that trade-offs are occurring.

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6 Evidence of a genetic compatibility benefit from extra-pair mating in the tūī, *Prosthemadera novaeseelandiae*

Tūī chick at around five days old

6. Evidence of a genetic compatibility benefit from extra-pair mating in the tūī, *Prosthemadera novaeseelandiae*

6.1 Abstract

Despite much focussed research, there remains no clear consensus on the fitness benefits of female mate choice for sexually selected male characters. Previous studies in tūī, *Prosthemadera novaeseelandiae*, have shown that two male sexually selected traits, body size and ornament size, may reveal discrete direct and indirect potential benefits for offspring survival and reproductive value, respectively. However, tūī also frequently engage in extra-pair mating with genetically dissimilar, as well as highly ornamented, males. Here I investigate the effect of female mate choice for these traits on two proxies of offspring fitness; size at hatching and growth rates. To limit potentially confounding effects of the rearing environment, I also compare within-pair and extra-pair maternal half-siblings, and in addition examine the potential effects of annual variability, clutch size, and brood sex ratios on sex-specific offspring growth. Offspring growth was greater in years of higher food abundance. Large clutches and male-biased brood sex ratios were detrimental to male hatching size, indicating that males may be more vulnerable to competitive rearing conditions, as predicted by the extreme sexual size dimorphism in tūī. Contrary to evidence of a positive effect of male size

on offspring survival, I found no influence of male body size on offspring growth. This finding is, however, consistent with a study that found no difference in offspring provisioning rates between large and small males. Male body size may instead indicate territory quality that enables females to maximise reproductive success. Ornamentation in tūī did not signal direct or good genes benefits for offspring. To the contrary, offspring growth was unexpectedly inversely related to male ornament size. Our results suggest that females may incur a fitness cost by pairing with an attractive social male which may be a result of attractive males trading-off parental effort with extra-pair mating effort. In contrast, extra-pair offspring tended to be larger than their within-pair maternal half-siblings, and offspring growth was negatively correlated to parental genetic relatedness, suggesting a genetic compatibility benefit. Together with previous findings in tūī that demonstrated female preference for genetically dissimilar extra-pair males, our results support the hypothesis that female extra-pair mating is a functional adaptation to increase female fitness.

6.2 Introduction

Mate choice is considered to be an adaptation that increases offspring fitness (Trivers 1972). Selection should therefore favour the evolution of male traits advertising the fitness benefits that females can gain by mating with them (reviewed in Andersson 1994). Females are argued to receive direct benefits that enhance either her ability to provide for offspring, or that directly increase offspring fitness (Kirkpatrick and Ryan 1991). For example, large dominant males may possess high quality territories, or males in good condition may possess greater energy reserves to provision nestlings (reviewed in Qvarnström and Forsgren 1998). Indeed, direct benefits and rearing conditions are known to have a strong influence on offspring growth and survival (reviewed in Lindstrom 1999). In monogamous species with extra-pair paternity (EPP), female mate choice is complicated by the opportunity to select different social and genetic partners for different potential fitness benefits (Neff and Pitcher 2005). Whether females obtain indirect genetic benefits from extra-pair mating and what forms these benefits take are still contentious (reviewed in Griffith et al. 2002; Westneat and Stewart 2003; Akçay and Roughgarden 2007; Forstmeier et al. 2014). However, the "good genes" and "compatible genes" hypotheses for indirect benefits have been widely accepted in explanation for female genetic polyandry (but see Akçay and Roughgarden 2007).

Firstly, the "good genes" hypothesis posits that females choose males based on phenotypic traits indicating genetic quality that either increase offspring survival or future mating success (Hamilton and Zuk 1982; Kirkpatrick and Ryan 1991; Andersson 1994; Kokko et al. 2002; 2003). Numerous studies have demonstrated female preferences for male advertisements such as song quality (e.g. Hasselquist et al. 1996; Poesel et al. 2006; Woodgate et al. 2011) or plumage traits (Møller 1988; Thusius et al. 2001; Estep et al. 2005; Guindre-Parker et al. 2013), and notable studies have also demonstrated a link between male advertisement and offspring fitness (Norris 1993; Hasselquist et al. 1996; Sheldon et al. 1997; Foerster et al. 2003; Chargé et al. 2010; Dunn et al. 2013).

Secondly, the “compatible genes” hypothesis predicts that females should allocate paternity to genetically compatible males that increase offspring viability through heterozygote overdominance or avoidance of the deleterious effects of inbreeding (Zeh and Zeh 1996; Jennions 1997; Zeh and Zeh 1997; Tregenza and Wedell 2000). Inbreeding depression is a consequence of intragenomic conflict caused by increased shared genetic similarity by common descent and lowered functional genome-wide heterozygosity (Coulson et al. 1998). As a consequence, inbreeding can have detrimental effects on offspring birth weights, growth, and survival (Keller and Waller 2002). This hypothesis is emerging as an important consideration in female choice (Blomqvist et al. 2002; Masters et al. 2003; Richardson et al. 2005; Cohas et al. 2006; Dowling and Mulder 2006; Freeman-Gallant et al. 2006; Rubenstein 2007; Thiel et al. 2013; but see Mays et al. 2008), and recent studies have revealed positive associations between female choice for dissimilar mates and offspring heterozygosity and/or fitness (e.g. Foerster et al. 2003; Reid et al. 2005; Tarvin et al. 2005; Oh and Badyaev 2006; Cohas et al. 2007; Suter et al. 2007; Fossøy et al. 2008; Brouwer et al. 2010).

Despite the multitude of studies investigating female extra-pair mate choice (reviewed in Griffith et al. 2002; Neff and Pitcher 2005), fewer studies have demonstrated the critical connection between female genetic polyandry and offspring fitness (Sheldon et al. 1997; Johnsen et al. 2000; Seddon et al. 2004; Freeman-Gallant et al. 2006; Garvin et al. 2006; Fossøy et al. 2008). This may, in part, be due to the influence of confounding factors, such as maternal effects (e.g. Magrath et al. 2009; Tschirren et al. 2012), or the environment (reviewed in Lindstrom 1999; Schmoll 2011) on offspring fitness.

In this study I investigate whether male traits that correlate with mating success confer either direct or indirect benefits for offspring fitness in the tūī, *Prosthemadera novaeseelandiae*. The tūī provides a good model for this study as they exhibit a very high rate of EPP, and this permits the direct comparison of within-pair young (WPY) and extra-pair young (EPY) maternal half-siblings that controls to some degree confounding effects due to variation in rearing environments (Sheldon et al. 1997; Griffith et al. 2002).

Previous studies in tūī have demonstrated that male body size predicts within-pair paternity success (Chapter 3). I hypothesise that females paired to large males remain faithful to prevent the loss of direct benefits such as territory quality, through divorce or reduction in parental care (Chapter 3). In support of the direct benefits hypothesis, female reproductive success is positively related to social male body size (Chapter 4). In contrast, females prefer either highly ornamented or genetically dissimilar extra-pair sires (Chapter 3). Females also overproduced sons when mated to these males, suggesting female adjustment of offspring sex to gain offspring of a higher reproductive potential (Chapter 4, Weatherhead and Robertson 1979; Burley 1981). Thus it is argued that female tūī exhibit a complex reproductive strategy in order to maximise offspring fitness via multiple pathways (Chapter 4).

Here, I investigate offspring size at hatching and pre-fledging growth rates as two proxies of offspring fitness. These variables are common predictors of probability of survival to recruitment age (Magrath 1991; Schwagmeyer and Mock 2008) or future fecundity (Haywood and Perrins 1992). I make nine specific predictions in relation to these indicators of offspring fitness. Nestling growth has not been studied in tūī, therefore I firstly 1) investigate sex-specific growth rates to determine whether the high degree of sexual size dimorphism (SSD) in tūī (Chapter 3) necessitates faster growth rates in male nestlings (Anderson et al. 1993), and 2) determine whether the common positive correlation between parental provisioning rates and offspring growth rates is present in tūī. Environmental conditions are likely to have an effect on offspring growth. I take advantage of significant annual variations in a major food resource at the onset of breeding season to determine how food availability and the rearing environment influence offspring growth. I predict that 3) growth rates should decrease with clutch size, and 4) I predict that the greater competition for resources in male-biased broods will adversely affect offspring growth. This prediction is based on a study of parental provisioning rates in tūī which indicated that parents do not increase parental care to male-biased broods (Chapter 6).

I then examine the consequences of female within-pair and extra-pair mate choice on offspring fitness. I predict that 5) offspring of large social males should experience enhanced growth rates due to their ability to dominate access to food resources (Craig 1985; Qvarnström and Forsgren 1998). I further predict that females gain indirect benefits from extra-pair mating, and thus 6) EPY should be fitter, and therefore larger, than their WPY half-siblings in mixed-paternity broods. Specifically, I predict 7) if ornamentation signals good genes benefits, the offspring of genetic fathers with large plume ornaments should experience faster growth rates. Alternatively, 8) if genetic compatibility is of primary importance to offspring growth, nestlings with less-related genetic parents, and 9) who are more heterozygous, should have faster growth rates. Finally I test a prediction of the compatible genes hypothesis that 10) offspring heterozygosity should be inversely related to the genetic relatedness of the true parents.

6.3 Methods

Between 2009 and 2012, 390 tūī were colour-banded and sampled at Tawharanui Regional Park as specified in Chapter 3 in order to obtain data on a significant proportion of the possible candidate extra-pair males in the study areas. To investigate nestling growth for this study, 20 tūī nests of known hatching date were studied during the breeding seasons (October to February) of 2010 and 2011. Parents of each nest were caught using mist-nets. Blood samples were taken, birds were colour-banded, and standard morphological measurements (mass, tarsus length, head-bill, wing and tail length) were recorded. Measurements of male plume size (mean number of plume feathers in each plume and natural curved plume length) were collected for a subset of the males (N = 11). At three stages of the nestling phase; day 0-1, day 4-6, and day 9-11, nestlings from the 20 broods (N = 57) were weighed and standard morphological measurements were taken. For eight nestlings from three nests, measurements from days 15-16 were also available. Sampling was conducted at the same time of day to reduce variation in diurnal nestling mass. Nestlings were ringed with elastic cotton bands on the first measurement to allow future identification. On the second measurement they were provisionally banded with a single plastic colour band until they could be permanently banded on the third measurement. Blood samples for genetic sexing (see Fridolfsson and Ellegren 1999 for methodology) and paternity analysis were taken at day 5 (see Chapter 3 for details on parentage analyses). Nine out of 57 nestlings died during the observation period. In order to explore the factors affecting offspring growth rather than pre-fledging mortality, which is detailed in Chapter 4, data from fledged nestlings only was used in this study (N = 48).

6.3.1 Data analysis

Linear mixed effect models (LMMs) were employed to test the nine predictions in the seven analyses listed below (Table 6.1). For all analyses except 2 and 7, I analysed two proxies of offspring fitness; size at hatching and growth rates. To investigate each of these fitness traits I used the response variables of nestling mass, and nestling tarsus length as a measure of structural size (Senar and Pascual 1997), and fitted both responses to each measure of offspring fitness. For analyses 1, 3, 5, and 6, which test multiple predictors, the included predictors are listed in Table 6.1 (Chapter 3 details how genetic relatedness and measures of heterozygosity were calculated). For all tarsus and mass at hatching models in analyses 3 to 6, I also included the offspring age predictors from analysis 1 to account for nestling age (Table 6.2).

Prediction number	Prediction	Analysis number
1	Male offspring have faster growth rates than female offspring	1
2	Growth rates increase with parental provisioning	2
3	Growth rates should decrease with clutch size	3
4	Growth rates decrease with increasing proportion of sons in a brood	3
5	Large males produce offspring with faster growth	3
6	EPY should experience faster growth than WPY	4
7	Large-plumed genetic fathers should sire offspring with enhanced growth	5
8	Genetically dissimilar genetic fathers should produce offspring with faster growth rates	5
9	Heterozygous offspring should have faster growth rates	6
10	Offspring heterozygosity should increase with increasing parental genetic dissimilarity	7

Table 6.1: List of predictions of the factors affecting offspring growth and the corresponding analysis that tests each prediction

For analysis 1, and all models examining hatching size, I suppressed a random intercept for each nestling due to the low variation in hatching size, and over-parameterisation identified by convergence errors when a random intercept was specified. I included linear random slopes for each female identity, and for each nestling identity nested within female identity. These account for both multiple unbalanced repeated measures from each nestling, as well as non-independence of nestlings from the same female (Pinheiro and Bates 2000). To calculate a measure of individual offspring growth rates for use as the second response variable, I extracted an individual nestling's residual growth rate from the random slopes fitted in analysis 1. A random intercept for female identity was included for all growth rate models.

For all analyses, the variables of social and genetic male plume size were tested in separate models due to the reduced sample size for this dataset. Continuous predictors of tarsus lengths and plume length and number were mean-centred (Zuur et al. 2009). Stepwise selection using information-theoretic and hypothesis testing approaches, was employed to reduce the model down to the final model including only significant predictors (Zuur et al. 2009). Variance structures were applied where necessary to correct for heteroskedasticity of residuals (Zuur et al. 2009), and final optimum models were verified by the inspection of residual plots (Pinheiro and Bates 2000; Appendix 1). All models were fitted in R v.2.12.2 (R Development Core Team 2011) using the packages lme4 and nlme (Bates 2010).

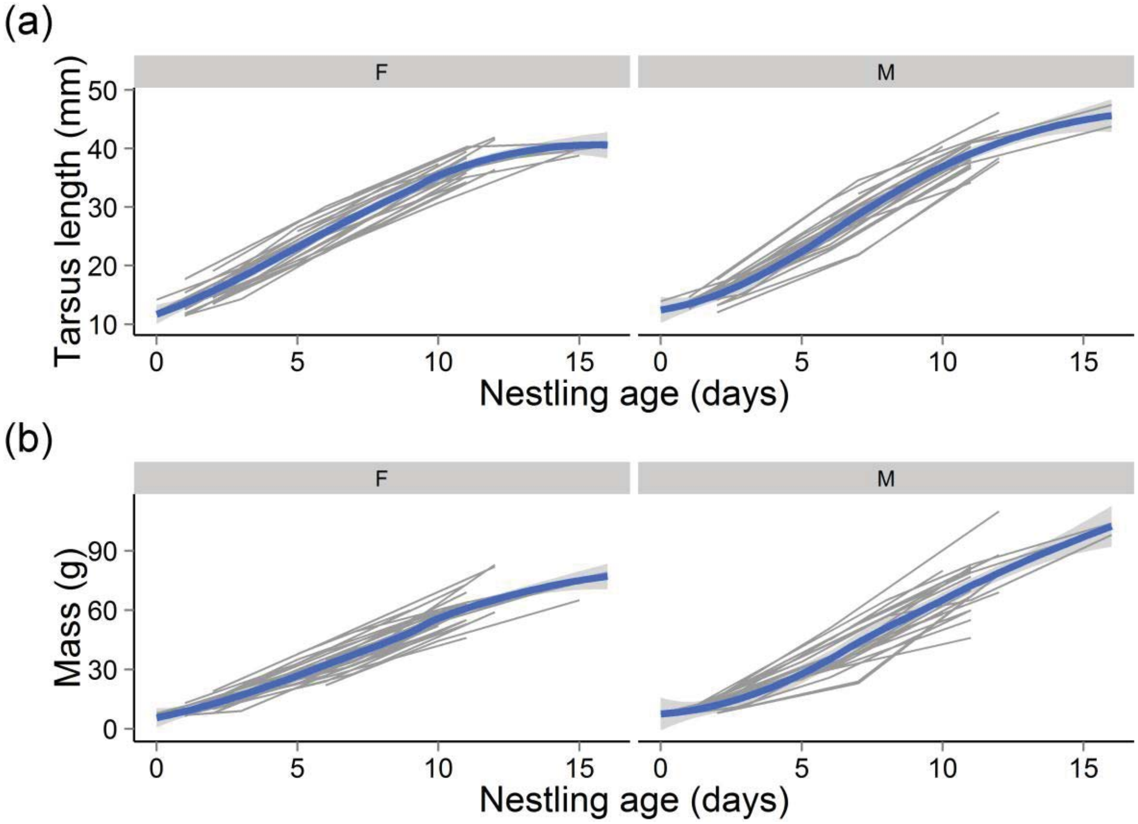

Figure 6.1: Growth rates of individual nestlings in (a) tarsus length in mm and (b) mass in grams with nestling age for female (left plots, N = 26) and male (right plots, N = 22) nestlings. A locally weighted regression (lowess) line (in blue) with 95 % confidence interval shading has been added to show the overall trend with age.

6.3.1.1 Nestling growth rates

To investigate nestling growth rates I fitted predictors of nestling age to the response variables of nestling tarsus and mass. Due to non-linear trends observed in data exploration (Fig. 6.1), I fitted third degree polynomials for nestling age rather than the linear term alone (Table 6.2). To answer prediction 1, I fitted sex-specific nestling growth rates by including separate interactions of offspring sex to each nestling age predictor. I tested the significance of the sex-specific growth rates by refitting the model without the sex interactions and conducting a likelihood ratio test.

6.3.1.2 Effect of parental provisioning rates on offspring growth rates

Prediction 2 assumes that there should be a positive correlation between parental provisioning rates and offspring growth rates. To test this, analysis 2 investigates the relationship between mean nest growth rates, and mean male and female provisioning rates. In order to obtain mean nest growth rates and mean male and female provisioning rates, I refitted the model in analysis 1 with a random slope for each nest, and refitted the provisioning rate model in Chapter 5 with a random intercept for each nest. I then extracted the random slope coefficients from the growth rate model as a measure of mean nest residual growth rate to use as our response variable. For the provisioning rate predictors, I extracted the random intercept coefficients of male and female residual mean provisioning rates from each nest of the provisioning rate model. To account for multiple broods from the same parents, random intercepts for female identity and male identity were fitted for female and male provisioning models respectively.

Analysis 1	Analysis 3	Analysis 5	Analysis 6
Sex	Social male tarsus length	Genetic male tarsus length	Nestling IR
Age	Female tarsus length	Genetic male IR	(Nestling IR)^2
Age^2	Social-pair R	Genetic male mean d^2	Nestling mean d^2
Age^3	Year	Genetic-pair R	(Nestling mean d^2)^2
Sex:Age	BSR	Genetic male plume length	
Sex:(Age)^2	Sex : BSR	Genetic male plume number	
Sex:(Age)^3	Clutch size		
	Sex : Clutch size		
	Social male plume length		
	Social male plume number		

Table 6.2: Predictors tested in analyses 1 to 4 for each of the response variables of tarsus length and mass at hatching and tarsus length and mass growth rates. Tarsus and mass at hatching models in analyses 2 to 4 also included predictors in analysis 1 to account for nestling age. Male plume variables and nestling heterozygosity variables were tested in separate models. BSR: brood sex ratio, IR: internal relatedness, R: genetic relatedness.

6.3.1.3 *Effect of social parent and rearing environment on offspring growth*

In order to explore predictions 3 to 5, analysis 3 examines how nestling growth is affected by environmental fluctuations and nest conditions, and whether direct benefits provided by characteristics of the social parents are important to offspring growth. To achieve these aims I included the predictors listed in Table 6.2. To determine if SSD in tūī has led to differential sex-specific influences of the rearing environment on growth, an offspring sex interaction was included for clutch size and brood sex ratio (BSR) predictors.

6.3.1.4 Comparison of WPY and EPY

Prediction 6 argues that, if females gain genetic benefits from extra-pair mating, EPY should be fitter than WPY in mixed paternity broods. To test this, analysis 4 examines whether EPY grow faster or are larger at hatching than their WPY maternal half-siblings. I achieve this by specifying a factor variable indicating the nestling's extra-pair status (0 = WPY, 1 = EPY). However, almost half (5 of 13) of the broods with EPY contained 100% EPP, therefore leaving few mixed paternity broods to test this hypothesis. Therefore, I also conducted this analysis using data from all broods containing EPY (including the nests with 100% EPP).

6.3.1.5 Effect of genetic father and parental genetic relatedness on offspring growth

Only offspring for which I knew the identity of the true genetic father (N = 41) were included in analysis 5. Predictions 7 and 8 were tested by examining whether traits of the genetic parents (i.e. the effect of indirect benefits) rather than traits of the social parents, are more important to tūī nestling growth. Specifically, I tested the influence of predictors specifying phenotypic and genetic traits of the genetic father, as well as parental genetic relatedness (Table 6.2) on offspring hatching size and growth rates.

6.3.1.6 Effect of offspring heterozygosity on offspring growth

Heterozygosity is predicted to positively influence offspring vigour, and therefore growth. Analysis 6 therefore tests prediction 9 by investigating the effect of two measures of offspring heterozygosity on growth: internal relatedness (IR; Amos et al. 2001) and mean d^2 (Coulson et al. 1998). However, the influence of heterozygosity on fitness may be non-linear due to potential outbreeding depression (Kempenaers 2007) and therefore greatest at intermediate values. I therefore included quadratic functions of offspring heterozygosity as predictors. I fitted a separate model for the two measures of nestling heterozygosity. I initially included these predictors in analysis 3, however, the quadratic functions led to convergence errors due to over-parameterisation. Nestling heterozygosity can also be considered a function of the relatedness of the genetic parents and could therefore be included in analysis 5. However, due to the smaller dataset of known genetic parents I fitted these predictors separately in analysis 6 in order to use all available data.

6.3.1.7 Effect of parental genetic relatedness on offspring heterozygosity

Compatible genes theory asserts that parental genetic relatedness should be inversely related to offspring heterozygosity (prediction 10). Therefore, in analysis 7 I test for this predicted correlation using nestling IR or mean d^2 as response variables, and genetic relatedness of the true parents as the predictor. To use the greatest amount of data possible for this test I used the larger dataset of sampled nestlings from Chapter 3 for which I knew the identity of both the genetic parents (N = 118). A random intercept for female identity was included.

6.4 Results

6.4.1 Nestling growth rates

The sex-specific interactions for nestling tarsus growth rates were highly significant ($\chi^2 = 19.57$, $p < 0.001$). Hatching tarsus lengths did not differ significantly between males and female nestlings, being around 12mm for both sexes. However, male and female nestlings diverge in their development with age (Table 6.3; Fig. 6.2). Both males and females showed a linear phase of growth in tarsus of about 2mm per day between days 0 to 7 (Fig. 6.2a). While the growth rate of female nestlings begins to slow around day 7, male growth rate maintains linearity until the inflection point around day 9. The quadratic functions of nestling age emphasise the sharply decelerating growth rates towards the end of the measurement period (Table 6.3; Fig. 6.2). The model predicted a slight negative growth rate trend after day 15, however this is most likely a result of the lack of data and subsequent wide confidence intervals at this age (Fig. 6.2a). Although this study does not cover the full nestling period, as nestlings fledge around 21 days old, a similar trend of tapering growth may be expected for the last 5 days of the nestling phase. By day 16, both males and females have reached 90% of their adult tarsus length (female mean: 42.2mm; male mean: 47.0mm, see Chapter 3) and the SSD in tūī is apparent.

As found for tarsus growth rates, the sex-specific interactions for nestling mass growth rates were highly significant ($\chi^2 = 21.77$, $p < 0.001$). The average nestling mass at hatching was 7g and was not significantly different between the sexes.

Nestling mass showed a similar trend to tarsus of logistic growth, however there was a greater sex-specific difference in growth rates for mass than for tarsus (Fig. 6.2b). Both males and females experienced an initial almost exponential growth phase in which mass increased by about 3g per day until about day 5. The quadratic functions of nestling age were highly significant (Table 6.3) and after day 5 male and female growth rates start to diverge, with males reaching the inflection point at around day 11 compared to around day 9 for females (Fig. 6.2b). After this period, growth rates begin to asymptote, but less severely than for tarsus. By day 16 females have achieved around 81% of the mean adult female weight (86g, see Chapter 3), while males have only reached around 74% of the mean adult male weight (129g). As for structural size, the SSD in mass can be observed by the end of the observation period.

Predictor	Nestling tarsus length			Nestling mass		
	β ± SE	LRT χ^2	P	β ± SE	LRT χ^2	P
Intercept	**11.980 ± 0.629**	**0.628**	**<0.001**	**6.977 ± 0.700**	**0.700**	**<0.001**
Sex	0.312 ± 0.923		0.738	1.081 ± 1.156		0.357
Age	**1.324 ± 0.377**		**<0.001**	**1.175 ± 0.708**		**0.101**
(Age)^2	**0.223 ± 0.057**		**<0.001**	**0.652 ± 0.134**		**<0.001**
(Age)^3	**-0.012 ± 0.002**		**<0.001**	**-0.029 ± 0.007**		**<0.001**
Sex : Age	-0.449 ± 0.555	0.687	0.407	-0.754 ± 1.099	0.970	0.325
Sex : (Age)^2	0.093 ± 0.084	1.296	0.255	0.296 ± 0.218	1.938	0.164
Sex : (Age)^3	-0.003 ± 0.004	0.744	0.388	-0.011 ± 0.012	0.502	0.479

Table 6.3: Output from LMMs of nestling tarsus and mass growth rates showing the mean coefficients ($\boldsymbol{\beta}$) with standard errors (SE). Likelihood ratio tests (LRT) scores are not available for some predictors as their effects could not be separated from their higher-level interactions by a χ^2 test. P values (P) for these coefficients instead come from Wald tests. Significant predictors are highlighted in bold.

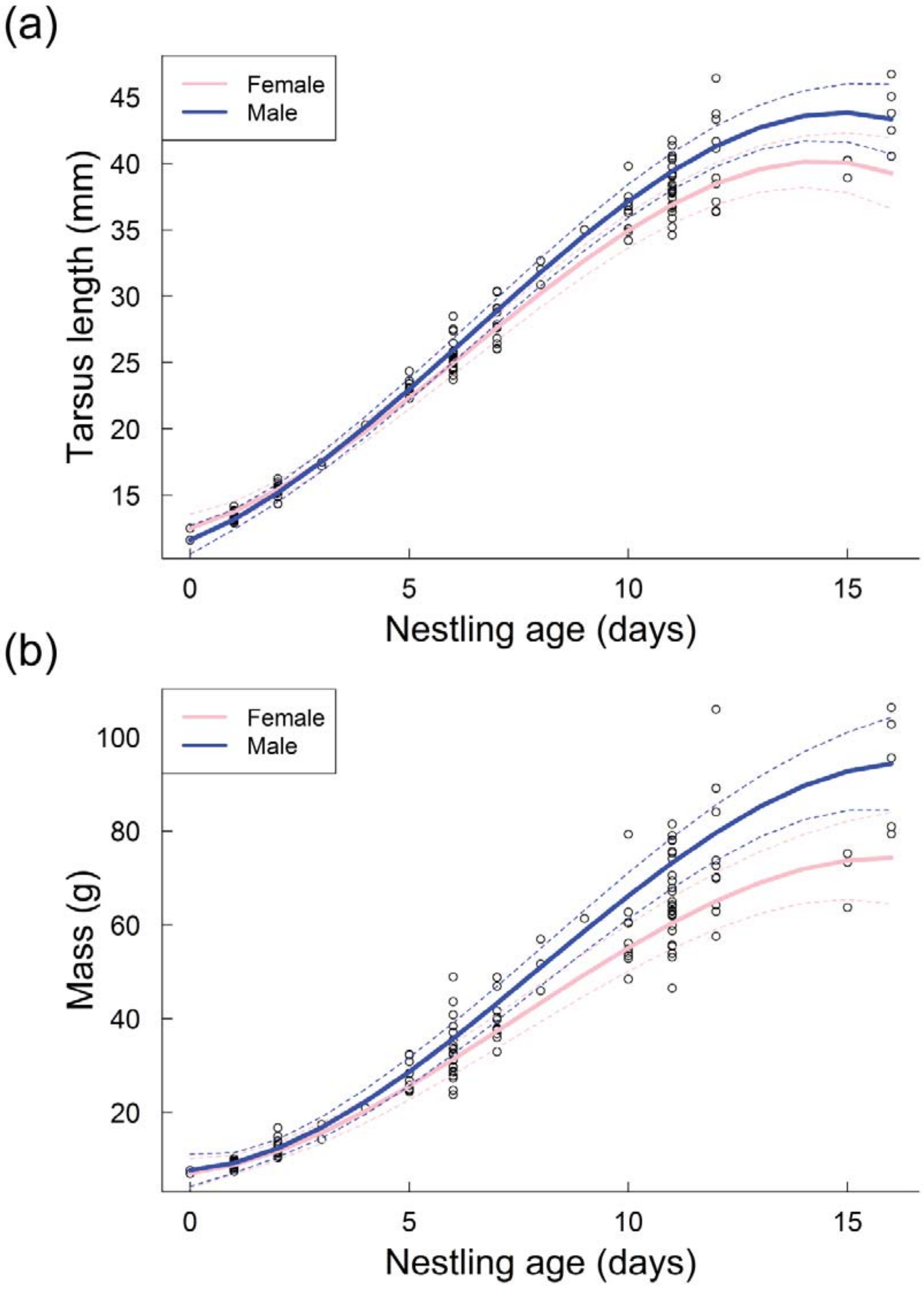

Figure 6.2: LMM fitted values (points) and mean pre-fledging growth rates (solid lines) with 95% confidence intervals (dashed lines) in a) tarsus length in mm and b) mass in grams for male (blue, N = 22) and female (pink, N = 26) nestlings.

6.4.2 Effect of parental provisioning rates on offspring growth rates

Female provisioning rates were unrelated to nestling growth in either tarsus length (β = -0.023 ± 0.042, χ^2 = 0.015, p = 0.591) or mass (β = 0.039 ± 0.336, χ^2 = 0.015, p = 0.903; Fig. 6.3a). There was a trend for male provisioning rate to be positively related to growth rates in mass (β = 0.115 ± 0.081, χ^2 = 1.968, p = 0.161; Fig. 6.3b) but not tarsus length (β = 0.014 ± 0.020, χ^2 = 0.528, p = 0.468).

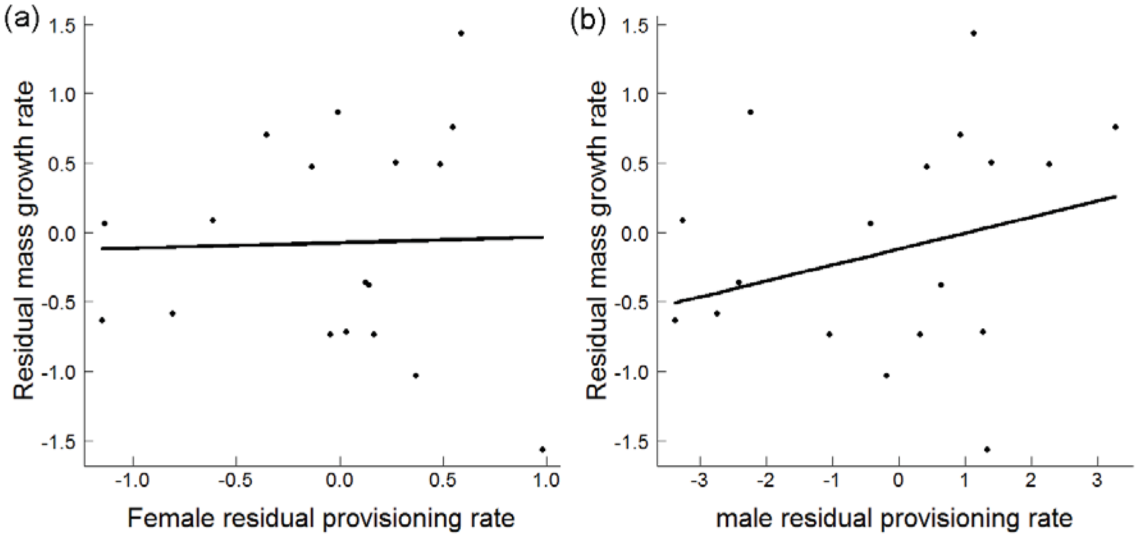

Figure 6.3: Correlation between mean (a) male and (b) female nest provisioning rates and mean mass growth rates of nestlings from the same female. Growth rates are residual mean rates for a female from the random slopes of an LMM. Mean provisioning rates were taken from the random intercept of an LMM investigating male and female time spent on the nest from Chapter 5. Although both results were non-significant there was a positive trend with male provisioning rate depicted by the regression line.

6.4.3 Effect of social parent and rearing environment on nestling growth

Tarsus and mass showed similar responses to most predictors, indicating that these measurements, as expected, are highly correlated. An effect of year on hatching size and mass as well as growth rates was found, being in all respects greater in 2010 than 2011 (Tables 6.4 and 6.5). Female hatching size and mass were unrelated to the BSR, although males were smaller than females in tarsus length at hatching (with also a non-significant trend for mass at hatching) in broods with a higher proportion of males (Table 6.4). Similarly, while female hatching size was unaffected by clutch size, large clutches had a significantly negative effect on the hatching size of male nestlings (Table 6.4).

Social male tarsus length was unrelated to any measures of nestling size or mass (Tables 6.4 and 6.5). Social male plume size was significantly negatively related to size and mass at hatching, with the same trend seen in nestling growth rates, although this fell below significance (Tables 6.4 and 6.5).

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
Female tarsus length	0.176 ± 0.530	0.106	0.744	Female tarsus length	-0.973 ± 1.748	0.302	0.583
Social-pair R	-0.698 ± 1.169	0.355	0.551	Sex : Clutch size	-1.586 ± 1.631	0.891	0.345
WPM tarsus length	-0.198 ± 0.195	1.003	0.317	Clutch size	0.989 ± 1.003	0.958	0.328
Clutch size	**0.470 ± 0.351**			Social-pair R	-5.636 ± 3.655	2.329	0.127
BSR	**-0.420 ± 1.025**			WPM tarsus length	-0.822 ± 0.600	1.747	0.186
Sex : BSR	**-2.949 ± 1.386**	**4.802**	**0.028**	Sex : BSR	-7.681 ± 4.545	2.803	0.094
Sex : Clutch size	**-1.123 ± 0.441**	**6.703**	**0.010**	BSR	-2.385 ± 2.373	0.932	0.334
Year	**-2.384 ± 0.373**	**38.051**	**<0.001**	**Year**	**-2.217 ± 0.613**	**12.857**	**<0.001**
WPM plume number	**-0.295 ± 0.069**	**12.476**	**<0.001**	**WPM plume number**	**-0.563 ± 0.223**	**4.254**	**0.039**
WPM plume length	**-1.580 ± 0.208**	**36.520**	**<0.001**	**WPM plume length**	**-1.418 ± 0.547**	**8.060**	**0.005**

Table 6.4: Effects of social parent and nest traits on nestling tarsus length and mass at hatching. The horizontal line between predictors delimits the separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a χ^2 test between nested LMMs while accounting for all other predictors remaining in the model. LRT scores are not available for some predictors as their effects could not be separated from their higher-level interactions by a χ^2 test. Coefficients in the final model are highlighted in bold. BSR: brood sex ratio, IR: internal relatedness, R: genetic relatedness, WPM: Within-pair male.

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
WPM tarsus length	-0.0005 ± 0.006	0.007	0.934	Social-pair R	-0.063 ± 0.252	0.063	0.802
Sex : BSR	-0.010 ± 0.036	0.083	0.774	Female tarsus length	-0.041 ± 0.052	0.201	0.654
Social-pair R	-0.010 ± 0.024	0.167	0.683	WPM tarsus length	-0.029 ± 0.042	0.461	0.497
Female tarsus length	0.005 ± 0.009	0.266	0.606	Sex : Clutch size	-0.108 ± 0.127	0.715	0.398
BSR	-0.028 ± 0.013	1.620	0.203	Clutch size	-0.005 ± 0.065	0.005	0.943
Sex : Clutch size	-0.024 ± 0.013	3.240	0.072	Sex : BSR	-0.326 ± 0.346	0.874	0.350
Sex	-0.003 ± 0.009	0.085	0.770	Sex	-0.001 0.101	0.0001	0.992
Clutch size	0.006 ± 0.007	0.688	0.407	BSR	0.006 ± 0.145	0.001	0.969
Year	**-0.023 ± 0.009**	**6.188**	**0.013**	Year	-0.144 ± 0.085	2.858	0.091
Male plume number	-0.007 ± 0.005	1.470	0.225	Male plume number	-0.019 ± 0.017	0.525	0.469
Male plume length	-0.003 ± 0.002	3.211	0.073	Male plume length	-0.068 ± 0.052	1.833	0.176

Table 6.5: Effects of social parent and nest traits on nestling tarsus length and mass growth rates. The horizontal line between predictors delimits separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a LRT χ^2 test between nested LMMs while accounting for all other predictors remaining in the model. Coefficients in the final model are highlighted in bold. BSR: brood sex ratio, IR: internal relatedness, R: genetic relatedness, WPM: Within-pair male.

6.4.4 Comparison of EPY and WPY

EPY had significantly larger mass ($\beta = 1.681 \pm 0.746$, $\chi^2 = 5.241$, $p = 0.022$) and tarsus ($\beta = 0.776 \pm 0.394$, $\chi^2 = 4.152$, $p = 0.042$) at hatching than WPY. EPY also possessed significantly faster growth rates in mass ($\beta = 0.210 \pm 0.100$, $\chi^2 = 4.349$, $p = 0.037$), and tended to have faster growth rates in tarsus ($\beta = 0.020 \pm 0.011$, $\chi^2 = 2.700$, $p = 0.100$), than WPY. Within mixed-paternity nests, EPY also tended to have a larger mass at hatching and faster growth in mass than WPY (Fig. 6.4b). However, due to 5 out of the 13 nests containing 100% extra-pair paternity and consequently being removed from this analysis, this test probably lacked power and results consequently fell below significance (hatching: $\beta = 1.857 \pm 1.242$, $\chi^2 = 2.254$, $p = 0.133$; growth rate: $\beta = 0.146 \pm 0.110$, $\chi^2 = 1.857$, $p = 0.173$). However, comparisons of tarsus length between corresponding EPY and WPY were not significant (hatching: $\beta = 0.293 \pm 0.444$, $\chi^2 = 0.489$, $p = 0.484$; growth rate: $\beta = 0.008 \pm 0.014$, $\chi^2 = 0.326$, $p = 0.568$) although a similar trend to mass was evident (Fig. 6.4a).

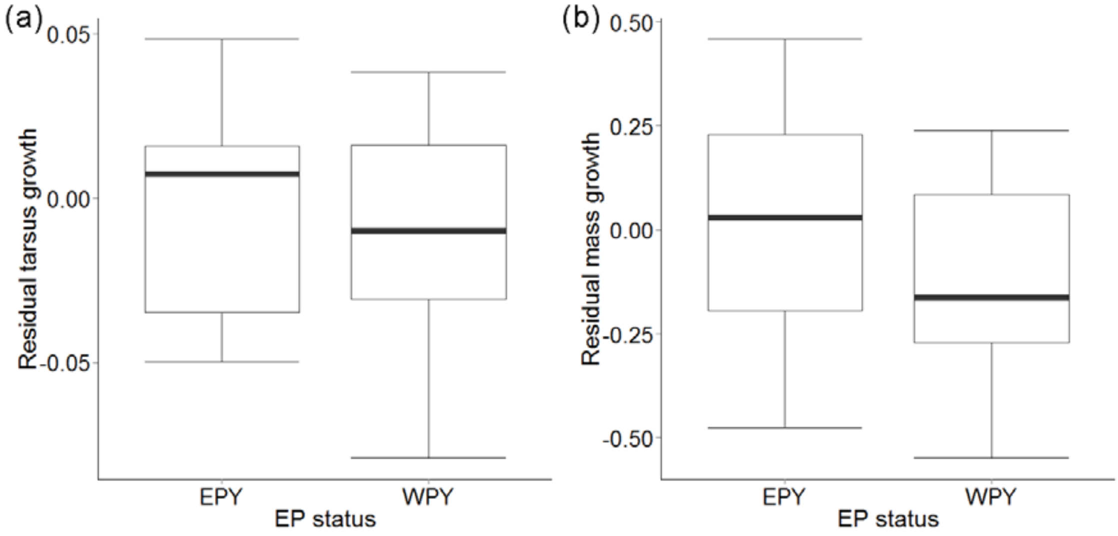

Figure 6.4: Difference in residual growth rates in a) tarsus length and b) mass between EPY and WPY maternal half-siblings in mixed paternity broods (N = 24 nestlings in 8 brood comparisons). Growth rates were extracted from the random slopes of LMMs modelling nestling growth with time (analysis 1).

6.4.5 Effect of genetic father and parental genetic relatedness on nestling growth

Less-related genetic parents produced offspring that were significantly larger at hatching in both size and mass (Table 6.6), and that exhibited almost significantly faster growth rates (Table 6.7). Neither measure of genetic father heterozygosity influenced nestling size or growth, although there was a trend for nestlings of males with higher mean d^2 values to grow faster in mass (Table 6.7). The tarsus length of the genetic father was unrelated to either fitness measure of nestling growth (Tables 6.6 and 6.7). However, the number of plumes possessed by the genetic father was significantly inversely related to both size and mass at hatching, although it was unrelated to growth rates (Tables 6.6 and 6.7).

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
Male IR	0.379 ± 1.336	0.080	0.777	Male mean d^2	-0.002 ± 0.163	0.0001	0.993
Male mean d^2	0.023 ± 0.046	0.240	0.624	Male tarsus length	0.112 ± 0.560	0.040	0.841
Male tarsus length	0.145 ± 0.179	0.652	0.420	Male IR	1.168 ± 3.938	0.088	0.767
Genetic pair R	**-3.917 ± 1.328**	**8.868**	**0.003**	**Genetic pair R**	**-6.580 ± 2.360**	**7.603**	**0.006**
Male plume length	-0.453 ± 0.222	3.193	0.074	Male plume length	0.065 ± 0.736	0.007	0.934
Male plume number	**-0.431 ± 0.121**	**10.538**	**0.001**	**Male plume number**	**-0.409 ± 0.193**	**4.212**	**0.040**

Table 6.6: Effects of genetic parent traits on nestling tarsus length and mass at hatching. The horizontal line between predictors delimits the separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a LRT χ^2 test between nested LMMs while accounting for all other predictors remaining in the model. Coefficients in the final model are highlighted in bold. IR: internal relatedness, R: genetic relatedness.

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
Male IR	0.020 ± 0.033	0.361	0.548	Male tarsus length	-0.010 ± 0.039	0.071	0.791
Male tarsus length	0.003 ± 0.004	0.390	0.532	Male IR	0.283 ± 0.280	1.008	0.315
Male mean d^2	0.001 ± 0.001	1.126	0.289	Male mean d^2	0.019 ± 0.011	2.837	0.092
Genetic pair R	-0.065 ± 0.035	3.454	0.063	Genetic pair R	-0.571 ± 0.313	3.351	0.067
Male plume length	0.001 ± 0.005	0.015	0.902	Male plume number	-0.013 ± 0.024	0.272	0.602
Male plume number	-0.003 ± 0.002	1.968	0.161	Male plume length	-0.037 ± 0.048	0.648	0.421

Table 6.7: Effects of genetic parent traits on nestling tarsus length and mass growth rates. The horizontal line between predictors delimits the separate models for the plume variables. Predictors are listed in the order of removal from the model based on the result of a LRT χ^2 test between nested LMMs while accounting for all other predictors remaining in the model. Coefficients in the final model are highlighted in bold. IR: internal relatedness, R: genetic relatedness.

6.4.6 Effect of nestling heterozygosity on offspring growth

There was no evidence of a non-linear effect of nestling heterozygosity on offspring hatching size, or growth rates (Tables 6.8 and 6.9). Neither of the linear predictors of mean d^2 or IR significantly predicted nestling size or mass at hatching (Table 6.8), or nestling growth rates (Table 6.9), although there was a non-significant trend for hatching tarsus length to be greater for nestlings with higher mean d^2 values (Table 6.8).

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
IR	-0.205 ± 0.983	0.043	0.836	IR	-1.904 ± 2.963	0.405	0.525
$(IR)^2$	3.032 ± 3.276	0.851	0.356	$(IR)^2$	-0.492 ± 9.788	0.003	0.960
Mean d^2	0.042 ± 0.024	2.946	0.089	Mean d^2	0.063 ± 0.075	0.684	0.408
(Mean $d^2)^2$	0.0003 ± 0.002	0.016	0.900	(Mean $d^2)^2$	0.006 ± 0.007	0.710	0.400

Table 6.8: Effects of nestling heterozygosity predictors on nestling tarsus length and mass at hatching. IR (internal relatedness) and mean d^2 predictors were tested in separate models. Non-significant quadratic terms were removed to then test significance of the linear terms. Significance was assessed using LRT χ^2 tests.

Predictor	Nestling tarsus length			Predictor	Nestling mass		
	β ± SE	LRT χ^2	P		β ± SE	LRT χ^2	P
IR	-0.003 ± 0.028	0.014	0.905	IR	-0.305 ± 0.245	1.524	0.217
$(IR)^2$	0.031 ± 0.091	0.115	0.734	$(IR)^2$	0.005 ± 0.814	<0.001	0.995
Mean d^2	0.0008 ± 0.0007	2.256	0.133	Mean d^2	0.009 ± 0.006	1.704	0.192
(Mean $d^2)^2$	0.00001± 0.0001	0.054	0.816	(Mean $d^2)^2$	0.0003 ± 0.001	0.280	0.597

Table 6.9: Effects of nestling heterozygosity predictors on nestling tarsus length and mass growth rates. IR (internal relatedness) and mean d^2 predictors were tested in separate models. Non-significant quadratic terms were removed to then test significance of the linear terms. Significance was assessed using LRT χ^2 tests.

6.4.7 Effect of parental genetic relatedness on offspring growth

The LMM testing the effect of parental genetic relatedness on nestling heterozygosity showed that nestling IR was highly significant and positively correlated with the genetic relatedness of the biological parents ($\beta = 0.609 \pm 0.112$, $\chi^2 = 25.212$, $p < 0.001$; Fig. 6.5) but not to mean d^2, although a trend in the predicted direction was observed ($\beta = -3.859 \pm 4.057$, $\chi^2 = 0.804$, $p = 0.370$).

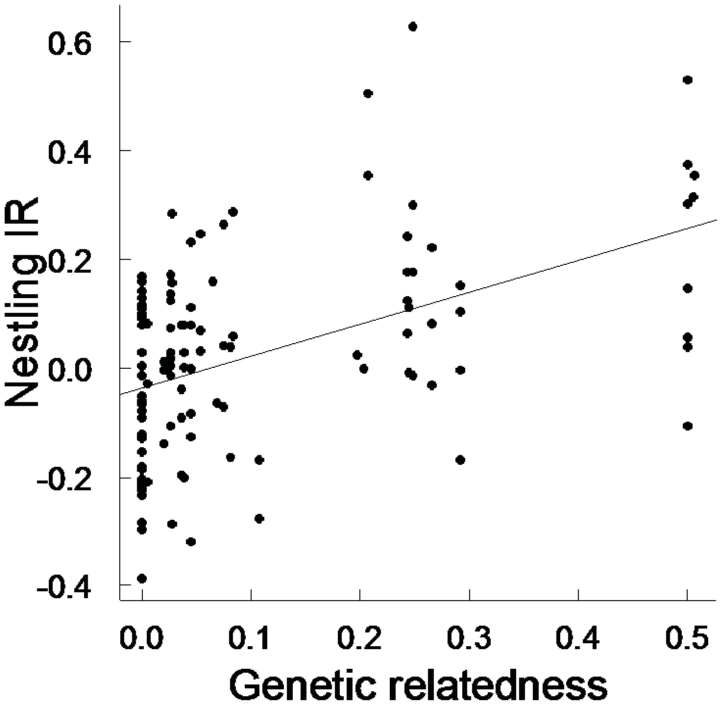

Figure 6.5: Effect of parental genetic relatedness (X axis) on a measure of offspring homozygosity (IR, Y axis) with regression line (N = 118). Points show the actual data.

6.5 Discussion

6.5.1 Nestling growth rates

There was no sex-specific difference in hatching size in this tūī population. This finding is common in birds, even among the most sexually size dimorphic species (Teather 1989; Burke 1992). As predicted in species exhibiting SSD (Anderson et al. 1993), female nestlings had significantly lower asymptotic mass and size than males. Nevertheless, males exhibited a slower growth rate relative to their eventual size than females. This was particularly apparent for mass, in which females reach 81% of their total mass by day 16 while males only achieve 74%. This slower relative growth rate in the larger sex has been found previously in sexually size dimorphic species (Richter 1983). In contrast, both sexes achieved almost full adult tarsus length by the end of the observation period. The importance of the ability to fly in foraging and avoiding predation to post-fledging survival is likely to have contributed to the greater focus on structural size over mass during development. The earlier development in size over mass in both sexes is a frequent occurrence in birds with clutch sizes greater than one, in which full development of the skeletal system is crucial to a nestling's survival prospects due to dramatic reductions in parental investment to the nest once the first nestling (usually the largest) has fledged (Nilsson and Svensson 1996).

6.5.2 Direct benefits and rearing conditions on offspring growth

There was significant annual variation in offspring growth, which was greater in all respects in 2010 than in 2011. 2010 was a prolific flax, *Phormium tenax*, flowering season, which is an important nectar source for tūī. This increased food availability most likely enhanced parental condition and therefore allowed greater energy expenditure on parental care. This conclusion is consistent with our finding of higher parental provisioning rates in 2010 than 2011 (Chapter 5). I found some support for the predicted adverse effect of male-biased broods and large clutches on offspring growth, although these conditions only affected the hatching size of sons. Smith and Fretwell (1974) showed that there is an optimal amount of resources to be invested in each offspring during oogenesis and that all else being equal, selection should favour the production of more offspring. The larger size of sons may make them more vulnerable to fluctuations in these optimal investments. Males did not, however, have significantly slower growth rates under these conditions, suggesting that any disadvantages in hatching size may be somewhat compensated by post-hatching ontogenetic growth (e.g. Schwabl 1996). Nevertheless, there was still a trend for males in larger clutches to have slower growth rates, indicating that this compensation may not be complete (Schwabl 1996).

Offspring growth rates in this study were not significantly correlated to parental provisioning rates. Female provisioning rate in particular had no apparent influence on either growth in tarsus or mass. This result is likely due to the smaller variance in mean provisioning rates between females than between males (Fig.

6.3). Indeed, males differed greatly in their mean provisioning rates and there was a trend for growth rates in mass to be greater in nests where males provided relatively more care. Consequently, as has been demonstrated for many birds with bi-parental care (Lyon et al. 1987; Reynolds and Székely 1997), male parental ability may make a significant contribution to offspring fitness, although more data is need to confirm this in tūī.

Male body size is positively related to offspring survival in tūī (Chapter 4). Therefore, under the assumption that large males defend higher quality territories (Craig 1985, Bergquist & Craig 1988) and therefore provide direct benefits for offspring fitness, I predicted that the offspring of large social males would exhibit faster growth rates. Our findings, however, did not support this hypothesis as offspring growth was unrelated to social male body size. As our study included only offspring that survived to fledging, different traits may have potentially influenced offspring growth and survival. Similarly, Suter et al. (2009) found that direct benefits in the form of male provisioning rates influenced offspring survival but not growth in reed buntings, *Emberiza schoeniclus.* These findings are consistent with Smith and Fretwell's (1974) hypothesis that selection favours the production of more offspring over fewer offspring of a higher quality. Thus high parental quality does not always predict high offspring quality (Klug et al. 2012). Alternatively, such a result may occur if large males are also closely related to their female (see below), counteracting the direct benefits of male size. Both natal philopatry and genetic relatedness appear high in this population (mean R = 0.08), and perhaps only large dominant males are successful in winning high quality territories in their natal area. However there was no evidence of a positive trend between male body size and relatedness in this study (results not shown).

6.5.3 Indirect benefits on offspring growth

Inbreeding can have detrimental effects on offspring birth weights and growth (Keller and Waller 2002), and studies of socially monogamous birds have demonstrated positive associations between genetic dissimilarity of extra-pair mates and offspring growth (Suter et al. 2007) or survival (Foerster et al. 2003; Oh and Badyaev 2006). These studies highlight the potential indirect fitness benefits that can be gained from compatible genes mating. Previous findings in tūī have shown that genetically dissimilar males gain more extra-pair matings (Chapter 3). In the current study, offspring of genetically dissimilar parents were larger at hatching and exhibited faster growth rates, providing further support for compatible genes effects. There was also a trend for EPY to have faster growth in mass than their WPY half-siblings. The weak association of this trend may be due to two factors. Firstly, this analysis lacked power due to the small sample size remaining after removal of nests with 100% EPP. Secondly, although the comparison of half-siblings controls for environmental effects that may otherwise influence growth (Sheldon et al. 1997; Griffith et al. 2002), other confounding factors such as maternal effects may obscure any significant result (Tschirren et al. 2012). For example, females may differentially allocate more resources to superior offspring during ontogeny (Schwabl 1993, 1996; Williams 1994; Lipar et al. 1999; Royle et al. 2001, 2003; Gilbert et al. 2006), or bias the laying order so that these offspring hatch earlier and gain a competitive advantage (Johnson et al. 2009;

Magrath et al. 2009; Ferree et al. 2010). Other possible mechanisms include non-random fusion of gametes due to post-copulatory sperm competition in which the sperm of genetically dissimilar males are more likely to fertilise an egg (Olsson et al. 1996; Wedekind et al. 1996; Marshall et al. 2003; Griffith and Immler 2009).

If compatible genes enhance offspring fitness, then offspring of genetically dissimilar parents should possess greater genome-wide heterozygosity (Wright 1921; Brown 1997). I found a highly significant positive relationship between genetic dissimilarity of the true parents and nestling heterozygosity. Yet I found no evidence of either linear or non-linear effects of nestling heterozygosity on growth. I see two possible reasons for this to occur. Firstly, multi-locus heterozygosity (MLH) may not be a reliable indicator of inbreeding or functional heterozygosity. Microsatellite loci also tend to be chosen based on their polymorphism, and therefore may not accurately reflect genome-wide heterozygosity. Indeed, several studies have reported no correlation between heterozygosity at MHC loci and MLH based on microsatellite loci (Sauermann et al. 2001; Hansson et al. 2004; Westerdahl et al. 2005). Moreover, there is evidence in some species that the effect of genetic compatibility on offspring fitness may be independent of heterozygosity. For example, immunocompetence is commonly related to both genetic compatibility and heterozygosity (e.g. Johnsen et al. 2000; reviewed in Neff and Pitcher 2005). However, Fossøy et al. (2008) found that although EPY from genetically dissimilar matings were more heterozygous and more immunocompetent than WPY in bluethroats, *Luscinia svecica*, these two traits were unrelated. They therefore concluded that females gained multiple genetic benefits from compatible males. Furthermore, inbreeding between first or second order relatives was relatively common in this tūī population, thus the effect of genetic relatedness on fitness is likely to be detectable in this population. In contrast, inbred offspring will still possess modest MLH due to stochastic processes during gamete formation, such as the random fusion of gametes, recombination, and mutation. This factor, in addition to the smaller sample size used to estimate heterozygosity (two alleles in heterozygosity versus four alleles in genetic relatedness) suggests that the relationship between offspring MLH and fitness may be less robust than that between parental relatedness and fitness (Fossøy et al. 2008). However, until the exact mechanisms underlying the association of these different fitness aspects are resolved, how heterozygosity does, or does not, influence fitness will remain unclear.

I found no support for good genes benefits on offspring growth in this study. To the contrary, I found evidence of an adverse effect of a sexually selected ornament on offspring growth. However, the plume dataset is significantly smaller than the full dataset, and males of 13 out of the 28 nestlings were the genetic as well as the social father. Therefore it is difficult to disentangle whether these findings are attributed to direct or indirect effects. Nevertheless, several alternative explanations may have contributed to this result.

Firstly, this discrepancy may arise because the genetic benefits of ornamentation may manifest via pathways other than ontogenetic growth. For example, fitness benefits may become apparent after recruitment and consequently require extensive studies spanning the reproductive lifetime of an individual. For example, studies on dark-eyed juncos, *Junco hyemalis* (Gerlach et al. 2012), and song sparrows, *Melospiza melodia* (Sardell et al. 2012), demonstrated that EPY were more likely to recruit and had a higher reproductive success than WPY. In support of this hypothesis, neither social nor genetic father ornament size influences offspring survival to fledging (Chapter 4). Instead, offspring of highly ornamented genetic sires are more likely to be male. Together, these results suggest that ornamentation in tūī signals attractiveness and increased reproductive success in the next generation (Fisher 1930), rather than viability. However, this hypothesis does not justify a significant negative relationship between ornamentation and fitness.

Negative correlations may result from complex life-history trade-offs between ornament size and growth. For example, trade-offs in energy expenditure between ornament size and development may occur during ontogeny (Kotiaho 2001). This, however, is unlikely in tūī as ornament development occurs post-independence. Instead, trade-offs may arise due to antagonistic effects on different aspects of fitness at the genic level. Antagonistic pleiotropy may result if the gene for the expression of ornament size also causes the expression of phenotypic traits that are detrimental, as well as beneficial, to offspring fitness (Brooks 2000). For example, Barber et al. (2001) found that the offspring of more ornamented three-spined sticklebacks, *Gasterosteus aculeatus*, grew slower than offspring of dull males, although they possessed a superior immune response to disease. Similar trade-offs with growth have also been found in birds (Soler et al. 2003; Brommer 2004; Garvin et al. 2006).

Similar consequences may ensue if the gene for ornament size is in linkage disequilibrium with a gene causing detrimental fitness effects (Brooks 2000). However this requires that the gene for ornament size is located on the nonrecombining male chromosome where deleterious alleles can accumulate in conjunction with the ornament trait, i.e. genetic hitchhiking. Consequently, this scenario is unlikely in tūī, as not only do both sexes possess plume ornaments, and therefore the gene should be located on a chromosome possessed by both sexes, but males are the homogametic sex in birds and therefore recombination is not restricted.

On the other hand, the findings that extra-pair males possessed larger ornaments than within-pair males in this population (Chapter 3), and that EPY had faster growth rates than WPY in this study, suggest that this negative association may not have a genetic basis. This is further substantiated by the greater significance of the correlation for social fathers than genetic fathers. Attractive social males may trade-off investment in parental care with alternative reproductive strategies such as investment in territorial defence or seeking additional matings (Magrath and Komdeur 2003). This hypothesis is consistent with a study of parental provisioning in tūī (Chapter 5) in which highly ornamented males provided less care than lesser ornamented males. As an alternative explanation, because the offspring of large-plumed sires are more likely to be male (Chapter 4), the smaller hatching size of offspring of large-plumed social males may be attributed to these nests possessing a male-biased BSR; an effect which reduced the hatching size of male nestlings in this study. However the robustness of this conclusion is limited by the sample size and the multiple covariances occurring between traits. Therefore experimental studies should be employed to partition the possible interactions that may have contributed to these findings.

In summary, this study provides the first investigation of nestling growth rates in tūī. The high degree of SSD in tūī has resulted in sex-specific differences in growth rates in mass, although relative growth rates in structural size were similar. There were also adverse effects of male-biased brood sex ratios on the growth of male nestlings, which can be attributed to their higher energy demands and faster growth rates. I suggest that the negative correlation between male ornament size and offspring growth is likely to be a result of attractive males trading-off parental effort with mating effort. Studies investigating male allocation of resources to parental care and mating effort are needed to confirm this hypothesis. In contrast, our results indicate that female tūī obtain compatible genes benefits from extra-pair mating. However, I cannot discount the possibility that the offspring size advantages attributed to genetic dissimilarity and extra-pair paternity in this study are due to maternal effects. Experimental cross-fostering, as well as long-term empirical, studies are needed to tease apart the exact mechanisms underlying the observed findings, and to examine not just proxies of offspring fitness, but lifetime reproductive success. Nevertheless, regardless of the mechanism, our findings suggest that female genetic polyandry in tūī may be adaptive, and that compatible genes may provide an alternative pathway to direct benefits through which females can enhance offspring viability.

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7

General discussion

Male tūī puffing out his feathers in a territorial display whilst defending a food resource. Note the blue tree fushia, *Fuchsia excorticate*, pollen on the bill.

7. General discussion

7.1 Overall summary

In this thesis I provide the first investigation into the genetic mating system of tūī. The aims of this thesis were to examine the influence of sexual selection, acting via extra-pair paternity (EPP), on the evolution of male secondary sexual characters and male reproductive behaviours. I also investigate the adaptive function of female genetic polyandry. In chapter 1, the basic theoretical concepts involved in sexual selection and the evolution of male sexually selected traits are described. Chapter 2 describes the development of genetic markers for the tūī that are used to answer questions relating to paternity and inbreeding throughout this thesis. Chapter 3 explores the contribution of EPP to the evolution of sexually selected male traits in tūī. I show that contrary to the findings presented in other studies, EPP can be a significant contributor towards the evolution of sexual size dimorphism. I also provide evidence of sexual selection, acting via EPP, favouring ornamental traits. Here, I further propose that females may gain multiple genetic benefits from extra-pair matings. In Chapters 4 and 6 I build on these findings by examining the mechanistic pathways linking male phenotype to fitness in female mate choice. I demonstrate that multiple male sexually selected traits are not mutually reinforcing signals, and that male display traits in tūī have evolved to differentially advertise direct and indirect benefits to females. In addition, I provide support for both good genes and compatible genes hypotheses as adaptive functions of female genetic polyandry. Multiple male traits signalling different aspects of male quality suggests the possible existence of alternative male reproductive strategies. In Chapter 5 I provide further support for the evolution of male alternative reproductive strategies, and show how they may play an important role in mediating the trade-off between parental care and mating effort in the tūī. In the current chapter, I draw together conclusions from previous chapters to discuss how these have advanced our knowledge of the adaptive function of female genetic polyandry, and the role of sexual selection in the evolution of male secondary sexual characters.

7.2 Implications for sexual selection theory

7.2.1 The evolution of multiple male traits

EPP is known to play a large role in sexual selection and the evolution of male traits. Most studies have generally focussed on one particular trait, and yet many animals display multiple traits used in either male-male competition or female choice (Andersson et al. 2002). Multifaceted functional male displays may either advertise different aspects of male quality (i.e. the "multiple messages" hypothesis), or act as "back-up" signals to reinforce the honesty of the same signal (Møller and Pomiankowski 1993; Johnstone 1996). For example, multiple messages were responsible for female preference for both red males and large males in three-spined sticklebacks, *Gasterosteus aculeatus* (Kraak et al. 1999). Tūī represent a good model for testing these hypotheses as they are highly sexually size dimorphic in both body size and ornament size, and these two traits are displayed during both male-male competition and courtship. In addition, tūī exhibit both a high degree of male-male competition and EPP which presents the opportunity for significant selection pressures to be acting on these traits.

Comparative analyses assert that EPP plays no selective role the evolution of sexual size dimorphism (SSD), which is instead thought to have evolved in association with social polygamy (Owens and Hartley 1998; Dunn et al. 2001). To test this hypothesis, in Chapter 3 I examined the influence of body size and ornament size on male mating success in the tūī. I found one of the highest rates of EPP (57% of all offspring studied were extra-pair), as well as one of the highest levels of SSD among socially monogamous birds. Both body size and ornament size were strong correlates to the rate of EPP. Few studies to date have provided evidence of selection pressures, acting via EPP, on male traits, and in particular on an ornament. By examining a species exhibiting both extreme SSD and EPP I demonstrate that, in contrast to current sexual selection theory, EPP can contribute to the evolution of SSD.

I then demonstrated in Chapters 4 and 6 that body size and ornament size provided direct and indirect benefits respectively, and that these influenced disparate aspects of offspring fitness. These findings support the multiple messages hypothesis. To our knowledge, this is the first study to show that females simultaneously optimise different components of offspring fitness through multiple male traits used in within-pair and extra-pair mate choice.

It remains to be determined whether male-male competition or female choice has led to the evolution of these traits in tūī. Although the relationship may be complex, I argue that these two forms of selection are unlikely to be mutually exclusive. While SSD in tūī has probably originated through male-male competition that allows large males to dominate access to food resources and territories, it seems likely that SSD has also been intensified by female choice for direct benefits (Berglund et al. 1996). It remains to be determined whether females actually *prefer* males of a large body size, or whether they instead select males based on territory quality (e.g. Alatalo et al. 1986; Calsbeek and Sinervo 2002). While disassociating the effects of territory quality and male size on female choice presents significant challenges in a wild population, further studies should first aim to clarify the influence of male body size and plume size on the outcomes of male-male competition and territory quality.

Although I am unable to unequivocally identify the mechanisms that have led to the evolution of these traits, I propose some hypotheses to be investigated in future studies. Female choice for large ornaments is likely, yet it remains to be tested whether these traits indicate attractiveness or good genes. Cameron et al. (2003) argue that male traits rarely conform to either sexy sons or good genes benefits, as most traits are condition-dependent (e.g. Andersson 1986; Price et al. 1993; Johnstone 1995; Rowe and Houle 1996; Griffith et al. 1999), i.e. the Fisher-Zahavi model (Eshel et al. 2000; Kokko et al. 2003). Indeed, tūī plume feathers appear to be condition-dependent. Yet, their apparent use in male-male competition also suggests they are used as badges of status. Similarly, white plumage traits in dark-eyed juncos, *Junco hyemalis*, and snow buntings, *Plectrophenax nivalis*, are displayed both during male contests and courtship, and signal both male dominance and condition (Balph 1979; McGlothlin et al. 2007; Guindre-Parker et al. 2013). These two functions are not mutually exclusive, as badges of status indicate dominance, but dominance is also likely to be condition-dependent. Hence, while white ornaments are typically inexpensive to produce, maintaining them may be an indicator of male quality. I argue that plume size is kept honest through continual testing during male territorial contests, and female preference for ornamented males leads to the reinforcement of this trait. I further suggest that both plume size (i.e. plume feather length and number) and condition-dependent plume quality may be important in female choice.

It is, however, unknown whether plume size is also heritable. The positive correlation between male body size and plume size suggests possible linkage disequilibrium between the two traits. However, this relationship may also arise through correlational selection. For example, large dominant males winning access to food resources are likely to gain condition, and consequently high quality ornaments. Therefore, body size and ornament quality in tūī may represent long-term and current male quality respectively (Candolin and Reynolds 2001; Candolin 2003), and thus to their advertisement of different fitness benefits. Consistent with the hypothesis of correlational selection, the results presented in this thesis suggest that large, highly ornamented males obtain a higher overall mating success due to their combined success in within-pair and extra-pair paternity than males possessing only one of the two traits (McGlothlin et al. 2005).

7.2.2 Fitness consequences of female mate choice

There is considerable debate on the adaptive significance of female mate choice. In particular the importance of direct versus indirect benefits is unresolved (Calsbeek and Sinervo 2002; Kotiaho and Puurtinen 2007; Maklakov and Arnqvist 2009). The relative magnitudes of these benefits may depend on the frequency of EPP and hence the opportunity to gain indirect benefits. The good genes and compatible genes hypotheses provide the most consistent explanations for female genetic polyandry (Petrie and Kempenaers 1998; Griffith et al. 2002; Mays and Hill 2004; Neff and Pitcher 2005), and yet the existence of these mechanisms are still questioned (Arnqvist and Rowe 2005; Akçay and Roughgarden 2007; Forstmeier et al. 2014). In Chapters 4 to 6 I examined the fitness consequences of female within-pair and extra-pair mate choice for males of differing phenotypic quality. Our findings suggest that females are able to optimise offspring fitness by obtaining different benefits from within-pair and extra-pair mate choice. Females paired to large males remained faithful and gained direct benefits that increased reproductive success. I argue that due to the significant competition for nectar resources in tūī, direct benefits that increase offspring survival are likely to play a significant role in female fitness, and hence mate choice. In contrast, females engaging in extra-pair mating obtained indirect benefits for offspring. In addition, I also found evidence for a commonly predicted, yet rarely documented hypothesis of female choice for different extra-pair males providing multiple genetic benefits. Female preference for highly ornamented males and subsequent overproduction of sons suggests that these males may provide Fisher-Zahavi good genes that increase offspring reproductive value. In contrast females mating with genetically dissimilar males produced offspring with faster growth rates, indicative of a genetic compatibility benefit. Few studies to date have shown the existence of multiple genetic benefits as a consequence of extra-pair mating. However, further studies are needed that investigate the mating success of offspring of attractive males, to determine if ornament size is indeed heritable. This study points to exciting avenues for future research, as it remains to be tested whether females show temporal plasticity in mate choice in response to social and environmental conditions (Oh and Badyaev 2006), or whether these form alternative female reproductive strategies (Foerster et al. 2003).

This study raises the question of whether females actually seek genetically dissimilar sires. Indeed, there is little empirical evidence to suggest that females can accurately assess relatedness (Kempenaers 2007). I argue that females may not choose genetically dissimilar extra-pair males *per se* and this relationship may be indirectly driven by the existence of highly related social pairs in which post-copulatory processes of sperm selection may account for apparent female preference for genetically dissimilar males (reviewed in Griffith and Immler 2009).

Female overproduction of sons by large-plumed males suggests that ornamentation in tūī indicates attractiveness rather than good genes *per se* (Weatherhead and Robertson 1979; Burley 1981). Indeed, I found no support for ornamentation benefiting offspring viability. Similar findings were reported in a recent meta-analysis, in which evidence for Fisherian “sexy sons” benefits, but not intrinsic good genes benefits, was found across the 55 species studied (Prokop et al. 2012). Female choice for genetic benefits from attractive extra-pair sires is straightforward. However, highly ornamented social males provided less parental care (Chapter 5) and offspring growth was consequently slower (Chapter 6). Therefore, the higher within-pair paternity won by highly ornamented social males (Chapter 3) raises the question of whether female choice for sexy son benefits can sufficiently compensate for these direct costs associated with pairing with attractive males. Theoretical research argues that indirect benefits are likely to exert relatively weak selection on female mate choice (Kirkpatrick 1985; Cameron et al. 2003; Arnqvist and Rowe 2005; Akçay and Roughgarden 2007). Cameron et al. (2003) assert that the sexy son hypothesis can never be a stable reproductive strategy as costly female preference cannot be solely maintained by the benefits provided by sexy sons. However, the magnitude of sexy son benefits may vary depending on the relative importance of mating success to total male fitness (Kokko et al. 2006). Certainly, the high rates of EPP and female preference for the same extra-pair sires in tūī (Chapter 3) suggest that highly ornamented sons may gain a significant mating advantage. However, it is unclear how reduced ontogenic growth may affect the expression of the adult ornament if ornamentation is condition-dependent (Rowe and Houle 1996).

Furthermore, it cannot be definitively ruled out that a viability benefit to ornamentation may occur post-fledging. Indeed, investment in offspring of a higher reproductive value does not necessarily discount good genes, as elements of the sexy son process will always occur when there are mating preferences (Cameron et al. 2003; Kokko et al. 2006; Mays et al. 2008). For example, if ornamentation is heritable and reflects dominance, it could be adaptive for females to produce sons by highly ornamented males. However, theoretical debate on the stability of the sexy son hypothesis and it’s exclusivity in relation to good genes benefits are still ongoing (Cordero and Eberhard 2003; Kokko et al. 2006; Mays et al. 2008; Lee et al. 2012; see also above). Thus, it remains to be tested whether ornamentation signals attractiveness alone, or another viability benefit not measured by this study.

This work has shed light on the adaptive function of female genetic polyandry and is one of the few studies to find evidence of female optimisation of fitness from multiple disparate genetic benefits from extra-pair mating. These findings also emphasise the current debate over the magnitude of direct and indirect benefits in female mate choice. From this study it is unclear whether indirect benefits gained from extra-pair mating are of comparable significance to the benefits for reproductive success obtained from large social males. The intense male-male competition for territories, the high rate of EPP, and the demonstrated selection pressures from these behaviours on SSD in tūī, suggest that the magnitude of selection on female choice is likely to be significant for both models. Future empirical work encompassing offspring lifetime reproductive success and experimental studies that can tease out potential confounding factors is required to quantify the magnitude of different fitness benefits of female mate choice.

7.2.3 Alternative male reproductive strategies

Under certain social or environmental conditions, selection may favour plasticity in male reproductive investment that enables them to maximise fitness (Shuster and Wade 1991; Badyaev and Hill 2002; Harris and Uller 2009). As a consequence, the optimum reproductive strategy for an individual may depend on innate characteristics of the male, such as quality or age. I tested the existence of male reproductive strategies in Chapter 5 by investigating how a male's mating success, and hence his reproductive potential, influenced investment in parental care. I found support for the hypothesis that attractive males with a high reproductive potential trade-off investment in parental care with mating effort, as ornamentation was inversely related to paternal effort (Magrath and Komdeur 2003). These findings further support the hypothesis that ornamentation in tūī signals male attractiveness and indirect benefits rather than parental ability. In addition, cuckolded unattractive males with a low mating success increased parental effort, suggesting that it may be adaptive for these males to compensate by investing in current offspring of which he may have sired a small proportion, at the cost of also investing in unrelated offspring (Houston and McNamara 2002; Alonzo and Klug 2012). Nevertheless, the balance between the costs and benefits of this strategy also depend on the population frequency of EPP and consistency of cuckoldry among broods (Griffin et al. 2013). Yet, there are limitations associated with this study. For example, the correlational aspect of this research highlights potential complications that may arise from confounding factors. This is particularly relevant to determination of the influence of paternity on male reproductive strategies, which should be tested within, rather than across, individuals. Furthermore, parental allocation of time to mating effort was not investigated in this thesis, therefore further research that quantifies these investments is needed to clarify that trade-offs are indeed occurring. In addition, further study is needed to determine if ornamentation influences the outcomes of male-male competition, and thus whether it may also provide direct benefits such as territory quality.

7.3 Implications for tūī behavioural ecology

Little literature has been previously published on tūī, and prior to this study knowledge of tūī mating behaviours was limited. This work therefore contributes vital baseline knowledge of mating behaviours of one of New Zealand's most iconic, yet overlooked species.

The Meliphagidae exhibit intense male-male competition for access to limited and ephemeral nectar resources. Tūī are one of the largest honeyeaters and therefore because of their higher energy demands, are likely to experience even greater competition for food resources. These characteristics are likely to have played a role in the evolution of honeyeater mating systems. For example, this study supports the hypothesis that the rate of EPP should be positively correlated with the variation in territory quality (Petrie and Kempenaers 1998). Although I found no evidence of females obtaining access to food resources from extra-pair males, this possibility should be investigated further. Nevertheless, the aggregation of individuals around food resources may promote EPP by driving male-male competition and offering frequent opportunities for female simultaneous comparison of mates that facilitate female choice (Cohas and Allainé 2009). Socially dominant males will obtain the best resources, and consequently provide considerable advantages for female reproductive success. Therefore, dominance is likely to have a significant impact on fitness, and contribute to the disparity in male reproductive success.

This study highlights the importance that EPP can play in sexual selection and the evolution of reproductive strategies. The high rates of EPP observed in tūī created a high variance in male mating success and intense sexual selection. Such increased sexual selection can be costly to survival and can be detrimental to population viability. For example, a high variance in male reproductive success can decrease the effective population size and consequently increase inbreeding (Nunney 1993). In addition, species exhibiting either male-male competition or EPP are generally characterised by reduced male parental care or nest defence. As a consequence, these species may experience reduced reproductive success as a result of decreased offspring survival through either starvation or predation (e.g. Weatherhead et al. 1994). Moreover, it has been demonstrated that sexually size dimorphic species (that experience strong sexual selection) experience higher mortality rates due to trade-offs with male-male competition (Promislow et al. 1992). As sexual selection and natural selection often favour contrasting phenotypes, sexual selection in such species may override the effect of natural selection, such that the species is not optimally adapted to respond to environmental pressures (McLain et al. 1995). These effects may render sexually size dimorphic species more vulnerable to stochastic environmental events (Sorci et al. 1998). Despite the high dispersal ability of tūī (Bergquist 1985), competition for territories in this study also likely contributed towards the unexpected level of inbreeding. This inbreeding may further increase the variance in male reproductive success due to the reduced survivorship and competitive ability of inbred individuals, and hence exacerbate the effects of inbreeding (e.g. Meagher et al. 2000).

Although tūī are not endangered, habitat loss due to deforestation is nevertheless occurring (Ewers et al. 2006), and has the potential to create reproductively isolated populations. In these populations habitat availability, and hence competition for resources, is likely to be even greater and thus may exacerbate sexual selection. This is particularly relevant for isolated island populations such as the endangered Chatham Island tūī, *P.n. chathamensis*, where inbreeding may erode population genetic diversity and increase the risk of extinction (Frankham 2005). The Chatham Island tūī has suffered population declines and local extirpations (Dilks 2004), and translocations have been conducted to re-establish new populations. This thesis contributes valuable information that can be used in translocations. For example, knowledge of mating patterns and levels of inbreeding in both the source and destination populations can help to establish over how wide a geographic distance to obtain founders and how many of each sex to source in order to obtain a good sample of the gene pool and maintain population genetic diversity (Frankham et al. 2002; Sigg et al. 2005). Knowledge about tūī behaviour can therefore have important implications for tūī conservation management (Sutherland 1998).

7.4 Directions for future research

This study forms the first investigation into tūī mating systems, and thus there is considerable research remaining to be done that I cannot possibly hope to cover here. Therefore, I focus on the most pressing issues highlighted by this study. First, there is a need to determine whether, as proposed in this thesis, male-male competition and female choice have exerted mutually reinforcing selection pressures on male body size and ornament size. Most importantly, the influence of body size and ornament size on dominance during male contest competition should be quantified. Experimental manipulation should be employed to assess the importance of plume quality, in addition to plume size, on male contest and mating success. The outcomes of male contests should then be examined in relation to territory quality and access to nectar resources to clarify exactly what direct benefits socially dominant male tūī provide. While access to nectar resources is likely to be a significant contributor to direct benefits, the availability of suitable nesting sites should also be assessed.

The adaptive function of female ornamentation in tūī should also be further explored. In contrast to males, I found no evidence of a correlation between female ornament size and body size, nor to the likelihood of EPP, suggesting that female plume size may not be under selection. However, our dataset for the latter is small. Female ornamentation may either be driven by correlated effects of sexual selection on males, or be the product of female competition for mates (reviewed in Amundsen 2000). The competition for territories suggests that there should be significant competition between females for males. Indeed, in this study I observed mid-season mate-swapping on nectar-rich territories that coincided with the arrival of new females in the area. I recommend future exploration of potential mechanistic pathways linking female ornamentation with fitness. Insight into selection acting on female ornamentation will assist in clarification of the adaptive function of ornamentation in tūī, and the potential role of female competition in the evolution of tūī mating behaviours.

Considering the evidence found in this study of inbreeding occurring in a non-reproductively isolated population, further study should be conducted into the potential adaptive significance of inbreeding. Nectar resources are likely to play a significant role in the causality of inbreeding, as average genetic relatedness was higher in the nectar-rich area of Jones Bay than Ecology Bush (Wells *unpublished data*). I did not find evidence of female inbreeding avoidance through increased frequency of extra-pair copulations in this study, although extra-pair males were less-related. This suggests that inbreeding avoidance may be costly for females. For example, are related social males more likely to gain access to the best territories? Perhaps philopatric males use local knowledge to gain territorial access? Natal philopatry appears to be high, with both male and female first year birds returning to their natal sites to breed. However, a formal study of philopatry is required in order to assess whether this has significantly influenced population substructure.

Finally, while fitness proxies such as offspring survival and growth can provide us with important information on the factors influencing some aspects of fitness, offspring lifetime reproductive success is a vital component of total fitness. This work established the beginnings of a long-term study of this population, and thus I was unable to include any measures of offspring lifetime reproductive success. However, ongoing research into this population should aim to compare the recruitment and lifetime reproductive success of within-pair and extra-pair half-siblings sired by males of different quality. Such knowledge will shed light on the ultimate fitness advantages provided by male sexually selected characters and female genetic polyandry.

7.5 References

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Appendices

Male tui feeding on Kowhai, *Sophora microphylla*

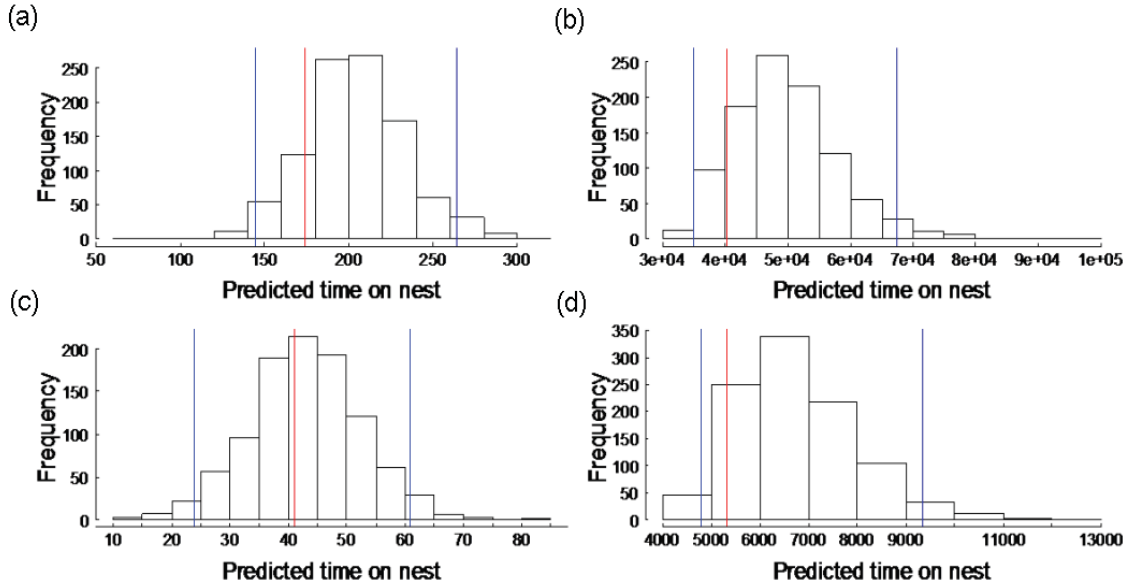

Figure 1: Histogram of the posterior predictive distribution generated under a Gaussian distribution to check the model fit for the provisioning rate (time spent on the nest in seconds) data for a) female mean provisioning rate, b) female variance in provisioning rate, c) male mean provisioning rate, and d) male variance in provisioning rate. Blue lines show the 95% credible intervals for the model predictions and the red line shows the mean parental provisioning rate. The red line should lie within the blue lines if the model is a reliable representation of the data.

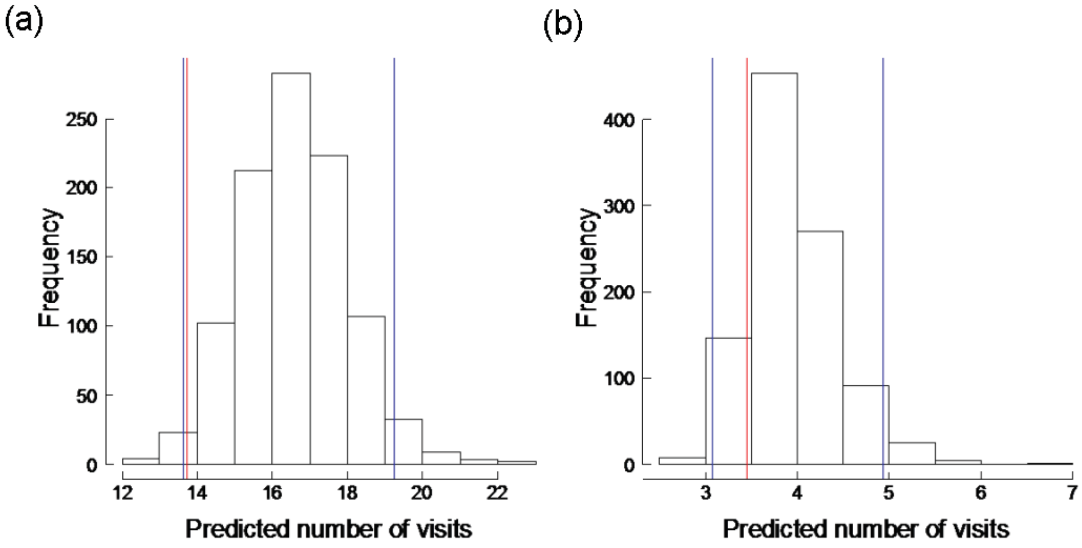

Figure 2: Histogram of the posterior predictive distribution generated under a Poisson distribution to check the model fit for the nest visitation rate (number of visits) data for a) female mean provisioning rate, b) male mean provisioning rate. Blue lines show the 95% credible intervals for the model predictions and the red line shows the mean parental provisioning rate. The red line should lie within the blue lines if the model is a reliable representation of the data.

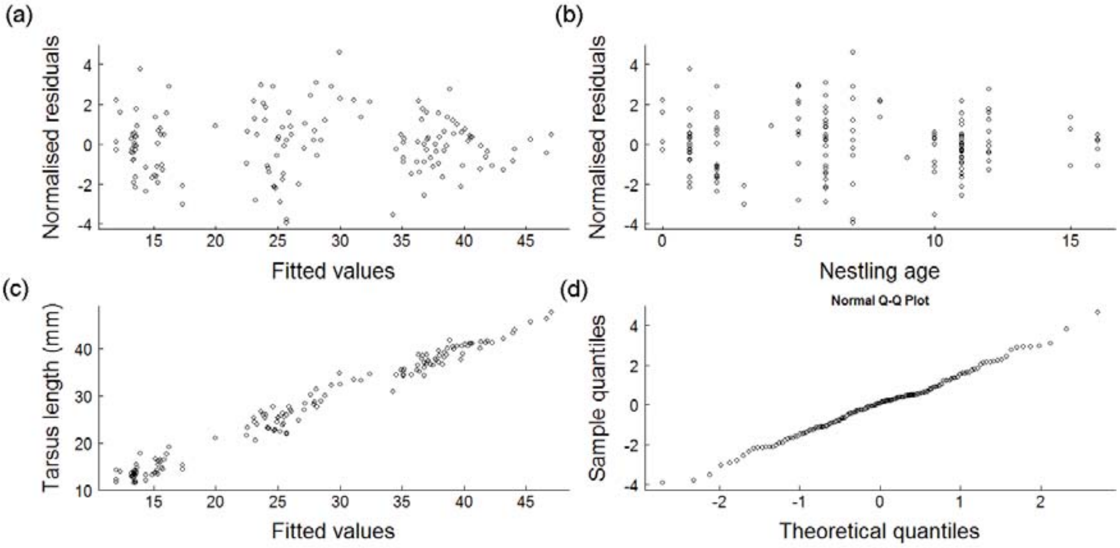

Figure 1: Residual plots showing graphical diagnostics of the nestling tarsus length growth rate model. a) model fitted values on model normalised residuals to check for violations of homogeneity, b) nestling age predictor (days) on model residuals, c) model fitted values on the tarsus length response variable (mm) to check the model fit, and d) quantile-quantile plot to assess normality of the model residuals, showing theoretical expected values on quantiles of the dataset. Plots a) and b) should show no trends, and plots c) and d) should equate to roughly a straight line if the model assumptions are not violated.

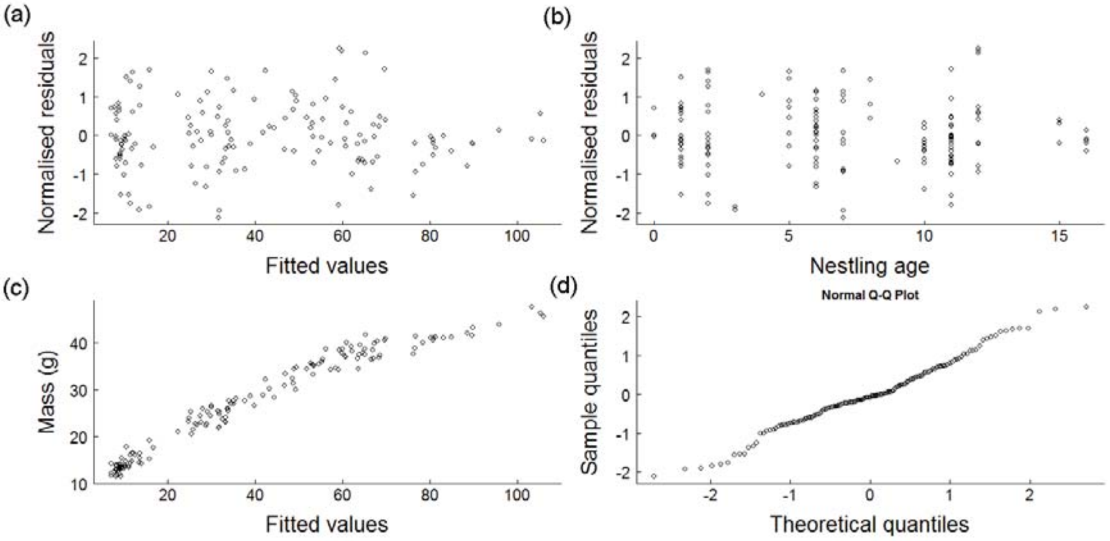

Figure 2: Residual plots showing graphical diagnostics of the nestling mass growth rate model. a) model fitted values on model normalised residuals to check for violations of homogeneity, b) nestling age predictor (days) on model residuals, c) model fitted values on the tarsus length response variable (mm) to check the model fit, and d) quantile-quantile plot to assess normality of the model residuals, showing theoretical expected values on quantiles of the dataset. Plots a) and b) should show no trends, and plots c) and d) should equate to roughly a straight line if the model assumptions are not violated.