THE EVOLUTIONARY BASIS OF MORPHOLOGICAL AND BEHAVIOURAL VARIATION IN THE NEW HOLLAND HONEYEATER (*PHYLIDONYRIS NOVAEHOLLANDIAE*)

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SUMMARY

This thesis discusses the current field of evolutionary biology and examines patterns and processes of divergence in the morphology and behaviour of a key model species, the New Holland Honeyeater (*Phylidonyris novaehollandiae*). More specifically, the cause of phenotypic divergence between island and mainland populations, and populations exposed to different climatic conditions, is investigated in *P. novaehollandiae*. Island-mainland comparisons showed that island birds were larger than mainland birds in tarsus (2.5%) and bill length (3.7%), had a wider foraging niche (mostly due to greater insect consumption), and foraged more from the bark and air (sallying). Island birds also had longer foraging times than mainland birds, which may be evidence for reduced resource availability. This evidence, and evidence from the literature, suggests that a paucity of resources on Kangaroo Island has most likely driven niche expansion, facilitated by the absence of some bird species on the island. Larger body size in island birds appears to be a response to local conditions on the island and may be driven by natural selection or population-scale phenotypic plasticity. Comparisons across a climatic cline showed that variation in all morphological traits in males and two of four morphological traits in females correlated with variation in rainfall. Additive genetic variation exceeded that of neutral genetic variation for all morphologic traits, indicating a strong signal of selection -- the observed environmental correlation suggests an environmental driver. These observations are consistent with the hypothesis that, in drier climates in South Australia, reduced and unpredictable nectar availability drives natural selection for increased aerial insect foraging (and maybe dispersal) efficiency. The lack of correlation found for some female traits was most likely explained by female biased-dispersal weakening the signal of the

selective source. The findings of this research add to a body of research that aims to understand and predict the evolutionary response of organisms under a changing climate.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for the award of any other degree or diploma in any university; and that to the best of my knowledge, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

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At the beginning of my PhD journey I couldn't have begun to imagine the challenges that lay ahead; but at the end of four years I've finally emerged on the other side, having overcome each one of those challenges. Now that the journey is almost over I look back and it's difficult to fathom just how far I've progressed both personally and professionally; my understanding and general outlook on the world has changed at a rate unequalled except possibly by my early years on earth. But at the same time I have to pause and look ahead to others that have completed a similar journey and have gone on to make the most of that experience, such as my supervisors and other leaders in the field. I then realise that this is only the tip of the iceberg if I want to enjoy a distinguished career in science -- but now I am prepared. This journey would not have been possible without the help and support of many people, to whom I must extend my gratitude.

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CHAPTER 1: GENERAL INTRODUCTION

Evolution

Evolution, the change in allele frequencies within a population, is a key principle in biology; it is an observable phenomenon, an outcome, a process, and a theory (discussed in Price 2008). Essentially, evolution is the result of interactions between a number of natural mechanisms that include genetic mutation, gene transfer (with inheritance), and death. The various outcomes of these interactions can be observed at the scale of the population, and these outcomes are classified according to their effect on allele frequencies. Here I introduce the natural mechanisms fundamental to evolution, before explaining in further detail the evolutionary forces that can be observed as a result of their interactions.

The first of the natural mechanisms that I introduce is genetic mutation (Hereafter referred to as mutation). Mutation describes irreversible change in an individual's genetic code, occuring at the scale of the molecule. Mutations typically arise through errors during DNA replication and may take the form of substitutions, insertions, deletions, inversions, or duplications of nucleotides in a DNA sequence (Lynch & Conery 2000, Zhang 2003, Zhang et al. 2003). Mutation is unique among the natural mechanisms that contribute to evolution in that it is the sole mechanism by which genetic variation (i.e., allelic variants) is introduced.

The second natural mechanism is gene transfer. Gene transfer describes the exchange of genes (alleles) between individuals, with a potential for inheritance (i.e., incorporation into the germline). A number of mechanisms exist by which genes can transfer between

individuals, and these can be divided into two general categories; (1) vertical gene transfer, and (2) horizontal gene transfer (Lawrence 2005, Keeling & Palmer 2008). Vertical gene transfer describes gene transfer through descent (e.g., via self-replication or sexual recombination), while horizontal gene transfer describes gene transfer across individuals, from one individual into another (potentially across taxa; e.g., Richards et al. 2009). Gene transfer contributes to evolution by shuffling existing allelic variants between individuals; it can operate at the scale of the individual, or any scale higher.

The third mechanism, death, is a relatively self-explanatory mechanism that operates at the scale of the individual. Death contributes to evolution by removing allelic variants from within a population. Death may result from a number of mechanisms including violence, aging, disease, and nutritional stress.

It is important to remember that the mechanisms described above are not mutually exclusive; they influence each other in a number of complex ways. The ultimate outcome of these interactions is a change in allele frequencies at the scale of the population; the phenomenon classified as evolution. The various outcomes of evolution reflect the net action of the natural mechanisms described above, and these actions can be considered the driving forces of evolution (evolutionary forces). The evolutionary forces are effectively classified according to the direction of change in allele frequencies in relation to the environment and other populations; they include genetic drift, natural selection (which includes sexual selection and other sub-variants), and gene flow.

Genetic drift and natural (and sexual) selection are considered creative forces in evolution, as they commonly generate variation between populations. Genetic drift is the random

change in allele frequencies due to finite population size (Slatkin 1987) -- effectively sampling error. Distinct from this is natural selection, the change in allele frequencies with a tendency toward beneficial traits (Slatkin 1987). Unlike genetic drift, natural selection can lead to variation between populations even when there is high gene flow, provided the relative fitness of alleles varies between populations (reviewed in Coyne & Orr 2004).

Natural selection and genetic drift can adequately explain all evolutionary change occurring *within* a population. However, populations are not reproductively isolated entities (discussed in Waples 2006), and genetic exchange is likely to occur between them (it may even occur between species; e.g., hybridisation). A third evolutionary force, gene flow, describes the exchange of genes between populations (Slatkin 1985, Slatkin 1987, Beebee and Rowe 2004). Gene flow often acts as a constraining force to evolution by inhibiting the establishment and maintenance of local genetic differences (Slatkin 1987, Beebee and Rowe 2004, Morjan and Rieseberg 2004); however, under certain circumstances gene flow can act as a creative force (e.g., Wright's Shifting Balance Theory; Wright 1943).

Natural Selection & Adaptation

Natural selection operates at an *individual* scale through the effect of an individual's phenotype on its fitness, and has an affect at a *population* scale by altering the phenotypic distribution *within* a population (Brodle, 1995) – generally, fitness of an individual is a measure of its relative reproductive success (Fisher 1930, Lande 1982), and is an intrinsic balance between survival and reproduction. Under natural selection, three modes of change are possible; these modes are characterised by the distribution of allele frequencies within a population at time *b*, relative to the distribution at time *a*. These modes include

directional selection, stabilising selection, and disruptive selection. Directional selection is effectively a shift in the mean phenotype of a population in a single direction. Stabilising selection describes an increase in frequency of the mean phenotype, resulting in a less heterogeneous population. Disruptive selection describes a divergence in the mean phenotype of a population, resulting in a more heterogeneous population. The mode of selection is important in the course of evolution; stabilising selection can be considered a conservative force in evolution, while disruptive and directional selection can be considered diversifying forces (important in relation to speciation).

Despite the mode of selection, in the absence of other forces natural selection should theoretically lead to local adaptation. Organisms that best suit their environment are more likely to survive and reproduce, increasing the frequency of their alleles in the population and leading to local optimisation of phenotypic traits for local conditions (Mayr 1963, Boag & Grant 1981, Schluter & Grant 1984, Benkman & Lindholm 1991, Benkman 2003). Local adaptation across a heterogeneous environment can lead to the establishment of phenotypic variation between populations -- a process known as adaptive divergence (Schluter & Nagel 1995; Schluter 1996; Schluter, 2000; Burger et al. 2006). Instances of local adaptation and adaptive divergence provide a paradigm for testing evolutionary hypotheses about specific trait-environment interactions. One system that illustrates this is Darwin's finches, one of the most well documented cases of adaptive radiation (Boag & Grant 1981; Gosler 1986; Schluter 2002; Blondel et al. 2006; Grant & Grant 2006; Hendry et al. 2008). Widespread spatial and temporal variability in resource availability on the Galapagos archipelago has driven adaptive divergence in behaviour and morphology in a group of finches, resulting in large-scale speciation and widespread subspecific variation within this group (Boag & Grant 1981, Grant & Grant 1997, 2006). Ultimately, it has been

the miriad of unique environments found both within and between islands that has driven the radiation of Darwin's finches, facilitated by a significant paucity of predator and competitor species on the archipelago. It is these characteristics that have made the Galapagos archipelago a hot-spot for species diversification – not only in finches – and subsequently a popular target for the study of speciation, and evolution in general. Further contributing to its usefulness as a model system for the study of evolution, the Galapagos archipelago is volcanic, meaning that species colonisation and subsequent diversification from their mainland counterparts is easily traced back in evolutionary history. Unfortunately, the characteristics that make this system such an ideal model are rarely found in nature and, as a result, the mechanisms that drive evolution in this system may not reflect the mechanisms of evolution that are perhaps more commonly encountered elsewhere.

Ecotypic rules

The effectiveness and ubiquity of adaptation is exemplified by the observation of broad-scale patterns of adaptation (essentially convergent evolution) to geographically related phenomena, otherwise known as ecotypic rules. Ecotypic rules describe general trends in morphology and related traits across geographic clines. The generality of such trends and their causal mechanisms have become a key focus of ecological and evolutionary biology as they provide insight into the evolutionary processes driving local adaptation and regional diversification. Some of the most robust trends include latitudinal variation in body size (Bergman 1847) and latitudinal variation in appendage size (Allen 1877). These two trends arise as the result of a common physiological driving mechanism – optimisation of temperature regulation – coupled with stable, long-term climatic conditions influenced by geography. Organisms with larger bodies and shorter appendages

have smaller surface-area-to-volume ratios and conserve heat more effectively than organisms with smaller bodies and longer appendages (Hamilton 1961, James 1970). As temperature generally decreases with increasing latitude, a relationship between large body size and high latitude can be observed. Temperature also tends to decrease with increasing altitude; subsequently, altitudinal variants of Bergman's and Allen's rules have also been identified (Rensch 1938). Despite having a single unifying cause, ecotypic rules may also be influenced by other factors such as resource availability (Rosenzweig 1968, Olson et al. 2009) and species richness (Brown & Nicoletto 1991, Cardillo 2002, Olson et al. 2009); which has fuelled much debate about the generality of the mechanisms that drive these rules.

Island rule

Another ecotypic rule that has received much attention concerns broad-scale patterns of adaptation on islands. Islands are important models for the study of evolution as newly formed islands act as a blank slate for colonisation and subsequent diversification (Losos & Ricklefs 2009), which in most cases can be traced back to the island's origins. In addition to this, islands present novel environments that drive local adaptive responses (Grant 1998, Losos & Ricklefs 2009). The broad-scale and consistent characteristics of islands, such as depauperate species diversity, are responsible for a clear trend in island evolution. Island evolution is characterised by a general tendency for size increase in smaller species and size decrease in larger species, within taxa. This pattern, coined the 'island rule' by Van Valen (1973), was first described by Foster (1964, 1965), when he proposed that certain mammalian orders evolve smaller body size on islands, while others display the opposite pattern; and some taxa show no discernable trend. Lomolino (1985) modified this pattern to make it size-based rather than taxon-dependent, although it still

applied only to mammals. The island rule was later described in birds (Clegg & Owens 2002, Lomolino 2005) and other taxa (Boback & Guyer 2003), and has since been generalised to a variety of taxa (Lomolino 2005). While the island rule explains a general pattern of body-size evolution on islands, the underlying processes are complex and may result from a number of different mechanisms. Mechanisms that drive the island rule include decreased interspecific competition (Grant 1965), thermoregulation (Brown & Maurer 1986), and dominance over resources in high-density populations (Kikkawa 1980). The relative influence of these mechanisms is determined by the characteristics of the islands (such as size and habitat diversity) and biology of the species in question (such as dispersal ability and position in the food chain) (MacArthur & Wilson 1967, Lack 1976, Williamson 1981, Clegg & Owens 2002, Meiri et al. 2008). The mechanisms that drive the island rule may also drive other, less general patterns of island evolution; for example, decreased interspecific competition has been found to drive reduced dispersal and expansion of ecological niche (Grant 1965, MacArthur & Wilson 1967, Williamson 1981, Mathys & Lockwood 2009). Similarly, decreased predator diversity has been found to drive expansion of ecological niche (Lomolino 2005).

Climate change

The ecotypic rules highlight the powerful influence that climate can have on the microevolution of species. Change in climate can therefore have serious consequences for the ecology and ethology of species. The climate on earth is continually changing, and it has been doing so since the formation of the earth; the majority of evidence for this changing climate is found in the geological strata. Climate change is driven by external and internal forces (outside and inside the climate system, respectively). External elements are an area for current concern as they are generally harder to predict (as they are less

understood) than internal forces, and have relatively drastic and long-lasting effects on the climate system (i.e. they affect the internal forces). External forces include volcanic activity, solar variations, forest fires, changes in earth's orbit, and, more recently, anthropogenic activity (Hegerl et al. 2010). Populations can respond to conditions of changing climate in either one of three ways; adapt, disperse, or perish (Davis & Shaw 2001; Hannah et al. 2008; Raxworthy et al. 2008). There is evidence that historical climate change, without the impact of anthropogenic activities, has been responsible for changes in the phenology and distribution of many extant plant and animal species worldwide (reviewed in Chambers et al. 2005 and Parmesan 2006), and is accountable for the mass extinction event at the end of the Permian (Benton & Twitchett 2003). Current climate change is driven by these factors, plus anthropogenic activity. Burning of fossil fuels and the subsequent release of carbondioxide (CO_2) into the atmosphere is one of the largest contributers to anthopogenic climate change. In just a few hundred years humans (*Homo sapiens*) have released massive amounts of CO_2 into the atmosphere, CO_2 that had taken millions of years for plants and algae to sequester. Analyses of climate change have shown that recent rates of climate change are accelerated on historic values, presumably due to the added impact of anthropogenic activities (Allen et al. 2000; IPCC 2007; Rosenzweig et al. 2008). Studies show that the rate of climate change may have a more critical effect on populations than its magnitude and duration (Davis et al. 2005), and recent changes in climate have driven important ecological changes in both plants and animals; including shifts in spring events (e.g. leaf unfolding, blooming time, migration, time of reproduction), species distributions, and community structure (reviewed in Rosenzweig et al. 2008). Given the increased rate of climate change, and its effect on ecological communities, it is important to develop a further understanding of its current and potential biological impact (Visser 2008).

An Afterword on Sexual Selection

Sexual selection operates in addition to natural selection; it deals specifically with selection that arises through differences in the average success of individuals obtaining a mate or mates (Grant & Grant 2008). Sexual selection was originally theorised to explain selection for conspicuous traits that, considering environmental factors alone (i.e., not including intrasexual competition), should reduce individual fitness (Darwin 1871). Darwin proposed two main mechanisms of sexual selection that have since been confirmed as operating in natural populations: male-male (or female-female) competition, and mate choice (Hunt et al. 2009). Male-male competition may include contests for mating privileges, sperm competition, or physiological adaptations such as earlier development or traits that allow earlier or prolonged access to mates, or prolonged virility; mate choice may include choice of a mate based on behavioural or ornamental displays, or coercion by threat or force (reviewed in Andersson & Iwasa 1996, and Clutton-Brock 2007). The outcome of many of these mechanisms may oppose mechanisms of natural selection, and, in natural populations, sexual selection and natural selection are often found to counteract each other (Brennan 2010).

The Model – The New Holland Honeyeater (Phylidonyris novaehollandiae)

In this thesis, I use the New Holland Honeyeater (*Phylidonyris novaehollandiae*) as a model to investigate key concepts of evolutionary biology. *Phylidonyris novaehollandiae* is a small Australian endemic passerine that is present in large numbers in areas of south-eastern and south-western Australia. *Phylidonyris novaehollandiae* is mostly restricted to habitats with a dense shrub layer, such as woodlands, heathlands, heathy woodlands, and mallee scrub (Recher 1971, Paton et al. 2002); but is also common in suburban parks and

gardens. *Phylidonyris novaehollandiae* appears to be a socially monogamous bird, having a tendency to mate for life, and with no observed sign of co-operative breeding (McFarland 1985); but these observations are yet to be examined genetically. Breeding in *P. novaehollandiae* occurs in autumn and spring (McFarland 1986, Paton 1985); however, breeding activity is generally higher during the spring following the wet season (Rooke 1979, Paton 1985, McFarland 1985, Armstrong & Pyke 1991). During breeding, the sexes partition roles, with males spending more time defending their territory and nest from predators and resource competitors (both inter- and intra-specific), while females spend more time nesting, including nest construction, incubation, and a majority of the nestling care (McFarland 1985, Clarke & Clarke 1999). Females forage closer to the nesting site than males and use more nectar resources (McFarland 1985). Outside of the breeding season, *P. novaehollandiae* may continue to maintain territories used for breeding, either in mating pairs or as individuals, or they may disperse (McFarland 2002). However, movement records (n=11,260) suggest that long distance dispersal is not common, with 99.1% of birds moving <10 km from location of banding (Higgins & Peter 2002). The relatively contained movement of *P. novaehollandiae*, coupled with its specialist diet, makes it an ideal model for the investigation of adaptive responses to environmental variation.

Aims

Sex of an individual is an important detail required in many biological studies. A number of sex determination methods exist for birds; some are more reliable than others. Without a standard method for sex determination, the possibility of error in sex determination remains unneccesarily large. Chapter 3 aims to test the reliability of methods that incorporate morphological measurements for sex determination, using genetic sex as a

standard of comparison and using the New Holland Honeyeater (*P. novaehollandiae*) as a model.

The isolated and often replicated nature of island biogeography makes islands ideal systems for ecological and evolutionary research. The 'island rule', a general pattern of body-size evolution observed on islands, is a keystone of island evolution research. Recent research has revealed that processes driving the 'island rule' are complex and may result from a number of different mechanisms, such as a paucity of resources, interspecific competition, and predation (MacArthur & Wilson 1967, reviewed in Lomolino 2005). Chapter 4 aims to investigate variation in morphology and foraging behaviour of a specialist forager – *P. novaehollandiae* – between island and mainland to test the mechanisms of island evolution. It is expected that under conditions of reduced competition on the island, ecological release will be favoured and a shift toward a more generalist diet will be observed. A shift in morphology toward traits that allow a more generalist diet should also be observed.

Climate change has been implicated in a number of mass extinction events and is responsible for changes in the phenology and distribution of many extant plant and animal species worldwide. The recent increase in the rate of climate change as a direct influence of anthropogenic activity has raised concern for the conservation of global biota. Species can respond to a changing climate in one of three ways: through dispersal, adaptation, or extinction. Extinction is no doubt preceded by strong selection; monitoring the adaptive response of species for variation in the rate of change, that may be influenced by climate change, can provide early indications of the effects of climate change. Chapter 5 aims to investigate differences in morphological and genetic variation across a climatic cline to

test the potential for climate driven adaptive responses in *P. novaehollandiae* -- a specialist forager that has its main food source directly influenced by climate. Under natural selection (evidence of an adaptive response), it is expected that morhpology will correlate with climate such that morphology will match predictions of local foraging requirements that are dependent on climate.

Organisation of the thesis

This thesis is organised as a series of manuscripts that have been published in international peer-reviewed journals (Chapters 2 and 4), or that will be submitted for publication in peer-reviewed journals (Chapters 3 and 5), with me as the first author in all cases. In all cases, I was responsible for writing the manuscript and received supervision in regards to field techniques, molecular techniques, and critical comments in regards to the manuscript content. Each manuscript is a separate chapter and formatted consistently for the purpose of the thesis. The thesis focuses on the role of evolutionary forces in behavioural and morphological divergence across heterogeneous environments; in particular across an island-mainland comparison and a climatic cline. The thesis order follows a logical progression: Chapter 1 describes the current field of evolutionary biology, paying particular attention to island biogeography and the role of the environmental in evolution, and introduces relevant background information and the aims of this thesis; Chapter 2 describes a methodological component, of which the findings are critical to the development of Chapter 5 -- the crowning chapter of this thesis; Chapter 3 is largely a methodological chapter that contributes to later chapters, but it also contains data that is discussed in detail within an ecological and evolutionary framework; Chapters 4 and 5 are data rich and investigate patterns of morphological and behavioural variation that have been identified in the model species, *P. novaehollandiae*. They also go on to examine the

subsequent evolutionary processes responsible for these patterns; Chapter 4 is concerned with differences between island and mainland evolution and ecology whereas Chapter 5 focusses on the influence of climate; Chapter 6 ties together the findings of each chapter and suggests possible directions for future research that may fill gaps in understanding left by this thesis.

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CHAPTER 2: NEW HOLLAND HONEYEATER (*PHYLIDONYRIS NOVAEHOLLANDIAE*) MICROSATELLITES: ISOLATION AND CHARACTERISATION OF 15 NOVEL MARKERS USING AN ENRICHMENT METHOD

FOREWORD

This chapter is purely methodological in nature; it describes the isolation and characterisation of microsatellite loci for the New Holland Honeyeater (*Phylidonyris novaehollandiae*). Microsatellites are short DNA sequences repeated in succession. The short DNA sequences (motifs) of microsatellites may range from two (di-motif) to six (deca-motif) bases in length; longer sequences may occur but are classified as minisatellites or satellites. Due to the evolutionary properties (i.e., the mode of mutation) of microsatellites, shorter motifs and shorter motif repeats are more common (discussed in Ellegren 2004). Microsatellites are ubiquitous among eukaryotes, and are typically relatively neutral with respect to selection and highly polymorphic; these properties make microsatellite loci ideal for use as genetic markers (discussed in Parker et al. 1998). Since the first documented use of microsatellite loci as genetic markers in natural populations (Schlotterer et al. 1991, Ellegren 1992), microsatellites have become a fundamental tool in many studies of ecology and evolution. The microsatellites described in this chapter play a pivotal role in this thesis; they are used in chapter 5 to provide information on population genetic structure, sex-specific dispersal patterns, and quantitative genetic variance.

ABSTRACT

The New Holland Honeyeater (*Phylidonyris novaehollandiae*) plays a significant role in the pollination and sustainability of Australia's endemic flora, and hence fauna. Despite this key ecosystem function, knowledge of *P. novaehollandiae* life-history traits and population dynamics remain poorly understood. I describe the development of primers amplifying 22 *P. novaehollandiae* microsatellite loci. Fifteen of the loci were found to be polymorphic, with observed heterozygosity between 0.500 and 1.000, and from four to 17 alleles per locus in adult birds from the study site.

MAIN BODY

The New Holland Honeyeater (*Phylidonyris novaehollandiae*) is an Australian endemic passerine, and one of the first bird species described from Australia. *Phylidonyris novaehollandiae* is a pollinator of many endemic plant groups (Driskell & Christidis 2004); hence, the loss of this species heralds the decline of intact ecosystems. A recent decline in woodland bird species as a result of habitat fragmentation (Paton et al. 2004) emphasizes the importance of monitoring population dynamics of key biota. Despite the significance of *P. novaehollandiae* for both ecosystem function and sustainability, research into its population dynamics has been limited, partly due to the lack of suitable molecular genetic markers. Therefore, I developed microsatellite markers to investigate breeding strategies, population structure, and dispersal dynamics in *P. novaehollandiae*.

I used a modified version of the enrichment technique of Gardner et al. (2008) to isolate microsatellites. DNA from one *P. novaehollandiae* individual was digested with RsaI (New England Biolabs; NEB) and BstUI (NEB), separately. Digested DNA was ligated with linkers (S475 and S476) and amplified. Only RsaI-cut fragment amplicons yielded

suitable products and were used in size selection, where fragments of between 350 bp and 1200 bp were selected and purified. Purified DNA was co-enriched for AC with AG; and AAC with AAAG microsatellite-containing fragments using a biotinylated enrichment procedure. Enriched fragments were recovered, cleaned, and amplified by polymerase chain reaction (PCR). Only enrichment of di-motif repeats was successful. Amplified enriched di-motif products were ligated into a StrataClone Vector (Stratagene) and transformed into competent Escherichia coli cells. Insert-containing clones were screened for microsatellites using a PCR method (Gardner et al. 1999) where vector primers (M13pUCF and M13pUCR) were used with simple sequence repeat-specific primers (AC and AG).

A total of 288 insert-positive clones were screened with 204 (72.2%) identified as containing microsatellites. Of these, 131 (63%) were amplified with universal vector primers (M13pUCF and M13pUCR) and sequenced. Ninety-six (73.3%) of the inserts contained microsatellites of suitable length and flanking regions. Primers for 48 (50%) loci were designed using the programs Primer 3 version 0.4.0 (Rozen & Skaletsky 2000) and AutoDimer version 1 (Vallone & Butler 2004). These primers were trialled with DNA from eight unrelated *P. novaehollandiae* samples (South Australian Museum: ABTC 2500, 2503, 2536, 2546, 2560, 2582, 2599, 2713). Amplifications (15 μL) were performed in 1× Eppendorf Hotmaster buffer (containing 1.5 mm MgCl 2), 0.8 mm total dNTPs, 0.1 μm labelled dUTPs, 400 nm each primer, 0.034 U/μL Eppendorf Hotmaster Taq polymerase, and 10–50 ng DNA. Cycling parameters were: initial denaturation at 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 55 °C for 60 s, 70 °C for 60 s; and final extension at 70 °C for 15 min. Primers for bell miner (*Manorina melanophrys*) loci BMC2 and BMC4 were tested for amplification using published conditions (Painter et al. 1997).

Polymorphism was tested by polyacrylamide gel electrophoresis (Gel-Scan 2000, Corbett Research).

Twenty-two (46%) loci (Table 2.1) were consistently amplifiable, polymorphic and did not amplify nonspecific amplicons. Two genotyping multiplexes were designed which included 12 and 8 of the loci, respectively, with amplicons ranging from 90 to 520 bp and from 120 to 410 bp. Two loci (Pn17 and Pn20) were not analysed further due to overlapping allele sizes with the multiplexes. The forward primer of each multiplexed locus was 5′-labelled with a fluorescent dye (Table 2.1) for detection by a 3730 capillary DNA Analyser (Applied Biosystems).

The 18 loci were amplified in a sample of 30 *P. novaehollandiae* from Newland Head Conservation Park. Amplification conditions were as previously but included adjustments to primer concentration, annealing temperature and cycle repeat numbers (Table 2.1). Pooled products were separated on a 3730 DNA Analyser, and sized and scored using the GeneMapper 3.7 software (Applied Biosystems). Locus Pn21 was discarded as it appeared to be inherited in a non-Mendelian fashion. Pn24 was also discarded, due to inconsistent amplification. Genotype data for the remaining loci were used to test for deviations from Hardy–Weinberg equilibrium using the software packages GENEPOP version 3.4 (Raymond & Rousset 1995) and GENALEX version 6 (Peakall & Smouse 2006). After applying Bonferroni corrections, loci Pn16 and Pn18 showed a consensus of significant heterozygote deficiency across both programs and were therefore considered to significantly deviate from Hardy–Weinberg equilibrium. MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) was used to test null allele frequencies for all loci. Pn16 and Pn18 showed homozygote excess, probably due to null alleles ($P < 0.05$). Pn16 was

subsequently discarded. However, the deviation in Pn18 was accounted for by probable sex linkage: an expanded analysis (n = 90) showed all females (n = 45) were homozygous while when only males were considered, Pn18 was in Hardy–Weinberg equilibrium (P = 0.076). No significant linkage was detected among loci (GENEPOP version 3.4). The number of alleles per locus ranged from four to 17 (average 10.39) and expected heterozygosity ranged from 0.387 to 0.943 (CERVUS version 3.0.3; Marshall et al. 1998).

The availability of these 17 informative loci for *P. novaehollandiae* will provide useful markers for analyses of population structure and mating systems.

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Table 2.1. Characterization of 18 microsatellite loci in the passerine *Phylidonyris novaehollandiae*. Fluorescent primer labels: P, PET; F, 6-FAM; V, VIC; N, NED. Primer concentration [Primer] in μM, final annealing temperature in °C (T_A); number of cycle repeats (N_R), number of individuals successfully genotyped from a possible 30 individuals (N_I), number of alleles (N_A), allele size range in base pairs, and the observed (H_O) and expected (H_E) heterozygosities are listed for each locus. A range in T_A indicates a touchdown PCR starting at the highest temperature and decreasing by 1 °C each cycle repeat to the lowest temperature.

Primer specifications			Reaction conditions						Heterozygosity		
Locus	Repeat motif in clone	Primer sequence (5'-3')	[Primer]	T_A	N_R	N_I	N_A	Size range	H_O	H_E	GenBank Accession
Pn1	$(TG)_{14}$	F: ^{P}CCACATCGTAGAAGGAAAGACC	0.2	60	34	30	11	288-312	0.733	0.869	FJ154859
		R: CACCATACCTCCTTTGCATTCCTCC									
Pn2	$(AC)_{15}$	F: ^{F}GGCTCTTGAGAGGACAAGAAA	0.3	60-55	40	30	12	91-119	0.833	0.858	FJ154860
		R: CTCATCCCTCTTCTCTGGAATG									
Pn3	$(TG)_{17}$	F: ^{V}AGTTTTTGTGGTGGGAGCAG	0.2	65-60	34	30	14	228-262	0.833	0.879	FJ154861
		R: GGTGCAAACTCAGACACAGAAG									
Pn4	$(AC)_{11}$	F: ^{V}GGGACTAGAGATTACCAGAGGGAC	0.2	60	34	30	8	288-302	0.800	0.771	FJ154862
		R: GGTGTTAGCCTCCGCATTAG									
Pn5	$(AC)_{8}$	F: ^{F}CTGTCCTTTCATCACTTTCATC	0.3	60	38	30	4	238-244	0.600	0.620	FJ154863
		R: CAGGTTTGTTTTCAGCAGCA									
Pn7	$(TG)_{8}$…$(TA)_{6}$	F: ^{F}GAGATAGAAACAACTACCAG	0.45	60	38	22	6	153-163	0.818	0.717	FJ154864
		R: ACCCCAGGACAAGCCAAAG									
Pn8	$(GT)_{10}$	F: ^{F}GCTGCTGTGCAATGAGCTG	0.2	65-60	38	24	8	506-524	0.500	0.636	FJ154858
		R: GAGAACAGGTTTGCCCCCAAG									

A dot in the repeat motif represents a single non-motif base; † loci showing significant heterozygote deficiency.

Table 2.1. continued.

Primer specifications			Reaction conditions						Heterozygosity		
Locus	Repeat motif in clone	Primer sequence (5'-3')	[Primer]	T_A	N_R	N_I	N_A	Size range	H_O	H_E	GenBank Accession
Pn11	$(AG)_8$...$(GT)_{3..}(GT)_8$	F: ^{V}AGAGAGAGAGAGAGTGCGTGTG	0.3	65-60	38	23	17	157-187	1.000	0.943	FJ154865
		R: AGGCAAGGAAGAAGGGATTT									
Pn12	$(TG)_{10}$	F: ^{P}ACTGCTTGAGGAGGGATGTG	0.45	65-60	34	29	7	232-244	0.621	0.651	FJ154866
		R: TGCCAGTCCGTTGGGAAATAC									
Pn13	$(CA)_{15}$	F: ^{F}AAAGAGGGAGTGGTGGTATG	0.3	60	38	28	12	316-336	0.893	0.905	FJ154867
		R: AAACGGCCAGTCAGATGTGTAG									
Pn15	$(AC)_{15}$	F: ^{F}AGAAGAGCCTCCAGACCACA	0.2	65-60	38	29	14	182-214	0.897	0.917	FJ154868
		R: TTGGGAAAGTCTCAACTGGC									
Pn16†	$(TG)_{10}$...$(TG)_{13}$	F: ^{N}CAGGGCTACGCAATCTATGAA	0.35	65-60	38	30	14	162-206	0.700	0.869	FJ154869
		R: CATATACATGAACACACACACACAC									
Pn18	$(GT)_{16}$	F: VCCTCCCATTAGATCACCTCC	0.2	60	34	30	11	382-413	0.500	0.858	FJ154871
		R: CAAAGCCAAAGGAAACCCCAAAGG									
Pn22	$(GT)_{15}$	F: ^{N}AAAGCAGGAACGCATCATCT	0.45	65-60	40	30	13	270-294	0.867	0.912	FJ154873
		R: CACATCAGGATCAGAAGTCC									
Pn23	$(CT)_{23}$	F: ^{F}AAAGTCTGACACTGCCTCTCC	0.2	65-60	40	30	10	142-164	0.867	0.845	FJ154874
		R: TGGGGAACTGAACTCATCCT									
Pn25	$(TG)_{15}$	F: ^{V}TTATTGTCAGGGCACATGGA	0.2	65-60	38	30	10	135-155	0.700	0.834	FJ154876
		R: AAAAACCGCGCGCACACAC									
BMC2	$(CA)_{19}$	see Painter *et al*. 1997	0.2	65-60	34	25	6	161-175	0.360	0.387	AF005375
BMC4	$(CA)_{23}$	see Painter *et al*. 1997	0.3	60	34	30	10	170-188	0.800	0.780	AF005377

A dot in the repeat motif represents a single non-motif base; † loci showing significant heterozygote deficiency.

CHAPTER 3: SEX DETERMINATION BY MORPHOLOGY IN THE NEW HOLLAND HONEYEATER (*PHYLIDONYRIS NOVAEHOLLANDIAE*): CONTRASTING TWO POPULAR TECHNIQUES ACROSS REGIONS

ABSTRACT

Sex determination of individuals is often required for ecological and behavioural studies but is difficult to carry out in the field for species that are only slightly dimorphic. To address this issue, researchers may use a variety of methods that rely solely on morphological measurements for sex determination. There are two main groups of morphologic methods; (1) based on discriminant analysis, and (2) based on resolving mixed-modal distributions. Here, I use one method from each of the two groups to sex the slightly dimorphic New Holland Honeyeater (*Phylidonyris novaehollandiae*) in South Australia, and I compare results of the two methods in relation to a genetic standard. I found that performance of both methods was comparable, but varied between populations. I also found regional differences in the best discriminating variables for morphological sex determination. This regional variation in performance of methods indicates that a single method for morphological sex determination cannot be applied across regions, even within species; furthermore, average morphological trait values should be reviewed across years given the possible role of selection or drift to influence phenotype.

INTRODUCTION

Sex determination of individuals is often required for ecological and behavioural studies where sex ratio, paternity, and parental care are commonly investigated. For sexually

dimorphic species, visual inspection of individuals is sufficient for determining sex. However, many bird species lack consistently observable sexual dichromatism or dimorphism. In such cases, sex may be determined non-invasively using behavioural cues (Baeyens 1981), or by the presence of a brood patch on incubating females or a protruding cloaca in males. However, these approaches are usually only possible with sexually active individuals during the breeding season.

The difficulties of sex determination for species with only slight sexual dimorphism may be overcome by examining morphological measurements. Two techniques that can be used to determine sex based solely on morphological measurements are cited above all others: (1) more traditional methods based on resolving mixed-modal distributions (Disney 1966, Rogers et al. 1986, Rogers & Rogers 1995, Higgins & Peter 2002, Twedt 2004, Morgan 2005), and (2) a more recently emerging method that utilises discriminant analysis (Phillips & Furness 1997, Bavoux et al. 2006, Kesler et al. 2006, Alarcos et al. 2007, Hermosell et al. 2007, Jakubas & Wojczulanis 2007, Svagelj & Quintana 2007, Kochert & McKinley 2008, Pitzer et al. 2008). Discriminant analysis explores the predictive ability of a number of user-defined independent variables on a single categorical dependent variable. The best linear combination of traits is used to calculate the discriminant function. The probability that an individual with given measurements will belong to either sex can be calculated from the discriminant function. On the other hand, for the mixed modal method, researchers identify the single best predictor trait, and the mean and standard deviation for each sex for this trait are used to construct normal density curves. The probability that an individual with a given measurement will belong to either sex can be calculated from these curves. This information is used to develop upper and lower cut-off values for which any individual having a

measurement above or below can be assigned a sex with a given margin of uncertainty chosen by the user.

The New Holland Honeyeater (*Phylidonyris novaehollandiae*) is a common Australian passerine that plays a key role in ecosystem function (Ford & Paton 1977, Ford et al. 1979, Paton 1981, 1982, Driskell & Christidis 2004). Sex determination has been an ongoing challenge in *P. novaehollandiae* (Disney 1966, Griffiths 1968, Rogers et al. 1986, Pyke & Armstrong 1993, Rogers & Rogers 1995). Disney (1966) reported a morphological sex difference in *P. novaehollandiae* and proposed that measurement of the extreme wingspan could discriminate between the sexes. However, taking this measurement in live, wild birds is difficult and can trigger undesired stress response in small birds; hence, this measurement is seldom taken. A more common measurement that has been used for sex determination in birds, including honeyeaters, is the bill-head length measurement (Rooke 1976). Many highly regarded bird manuals report use of bill-head length with the mixed modal method for sex discrimination (Rogers et al. 1986, Schodde & Mason 2000, Higgins & Peter 2002); however, reliable criteria for *P. novaehollandiae* have not been published.

One factor impeding the development of morphologic methods for sex determination has been the need for a large sample size to accurately represent the overall study population (Rogers & Rogers 1995). The development of primers for genetic sexing in birds (Griffiths et al. 1998, Kahn et al. 1998, Jensen et al. 2003) enables a reliable method for sex determination that can be easily applied to large sample sizes. This approach allows examination of morphological sex variation at a large scale. In *P. novaehollandiae*, further impediment in development of methods arises through regional variation in body size (Latham 1790, Mathews 1918-27, Salomonsen 1966, Pyke & Armstrong 1993, Higgins & Peter 2002). My

study area spans two geographically distinct regions, Fleurieu Peninsula and Kangaroo Island, for which previous body size variation has been observed (Mathews 1918-27). In this study on *P. novaehollandiae* in South Australia, I (1) genetically sex individual birds for which I have morphological data, (2) investigate the extent of sexual size dimorphism for all measured morphological traits in Kangaroo Island and Fleurieu Peninsula, (3) develop criteria for sex determination by the two morphologic methods (mixed-modal and discriminant analysis), and (4) compare the predictive power of both morphologic methods.

METHODS

I mist-netted birds across sites over a five-year period between 2004 and 2008 (see also Oorebeek & Kleindorfer 2008a, b, Oorebeek et al. 2009, Oorebeek & Kleindorfer 2009, Chapman et al. 2009). To minimise sampling bias across the sampling period, banding trips to each site were carried out annually during the same months (May, June, September). I caught and measured a total of 417 adult birds. Each bird was banded with an aluminium identification band, measured for morphological characteristics, and sampled for blood that was stored on FTA paper for DNA analysis (see Kleindorfer et al. 2006, Chapter 2).

Morphologic data were collected at seven sites within South Australia: (1) Newland Head Conservation Park, (2) Scott Conservation Park, (3) Cox Scrub Conservation Park, (4) Sandy Creek Conservation Park, (5) Scott Creek Conservation Park, (6) Flinders Chase National Park, and (7) Pelican Lagoon Conservation Park (Figure 1). The sites span two geographically distinct regions; (1) Fleurieu Peninsula (FP), and (2) Kangaroo Island (KI). Dominant flora at each site is described in Rix (1976), Ford & Paton (1977, 1982), Westphal et al. (2003), Kleindorfer et al. (2006), Schlotfeldt & Kleindorfer (2006), McGuire & Kleindorfer (2007), Galligan & Kleindorfer (2008), and Chapter 4.

I determined sex of each bird by genetics using the polymerase chain reaction (PCR) method of Kahn et al. (1998) and conditions outlined by Jensen et al. (2003). Briefly, each PCR contained 1x Taq polymerase buffer, 4 mM $MgCl_2$, 0.8 mM dNTPs, 0.16μM each primer, and 0.02 U/μL Taq polymerase (AmpliTaq Gold). The temperature profile included an initial denaturation at 94°C for 9 min; followed by 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s; and a final extension of 72°C for 6 min. All 417 birds had sex successfully assigned using this method. I assumed 100% accuracy of sex assignments; however, there is likely to be a small degree of error associated with this method (~ 0.5%) due to contaminating agents entering the PCR (Daniel et al. 2007). While there are methods available to minimise and essentially eradicate this error, they are costly and time consuming, and a 0.5% error is not likely to have a significant effect on the results. Therefore, I opted against using methods to alleviate this small degree of error. Template DNA for use in PCR was extracted from FTA using method #4 of the protocols outlined by Smith and Burgoyne (2004). Briefly, I took a small disc (1 mm^2) of blood-soaked FTA paper and submerged it in 500 mL lysis buffer solution (100mM Tris [free base], 0.1% sodium dodecyl sulphate [SDS]). After a thirty minute wash, I removed the supernatant and submerged the FTA disc in 500 mL DNAzol (guanidinium thiocyanate). After a ten minute wash, I removed the supernatant and washed the disc once in 95% ethanol, and twice in reverse osmosis water. Finally, I submerged the disc in 80 μL reverse osmosis water and incubated at 90°C for 5 minutes, to release the DNA from the disc.

I examined 8 morphological traits that have been shown to correlate with body size in birds: (1) bill-head length; (2) bill length from the tip of the bill to the base of the bill, where the feathers begin (bill-feathers length); (3) bill length from the tip of the bill to the anterior

extreme of the nostril (bill-nostril length); (4) bill depth, measured at the base of the bill (bill depth); (5) bill width at the base of the bill (bill width); (6) tarsometatarsus length (tarsus); (7) length of the flattened wing (wing); and (8) Mass (Rising & Somers 1989, Piersma & Davidson 1991, Senar & Pascual 1997). To reduce measurement error, all morphologic measurements were made by Sonia Kleindorfer (SK) and Margot Oorebeek (MO) (banding permit ABBBS 2601). Bill measurements of recaptured, remeasured birds (n=30) were used in t-tests with original measurements to analyse measurement error. Bill-head length showed the lowest measurement error (0.35%), while bill length nostril and bill length feathers showed higher error (1.9% and 3.4%, respectively). I therefore conservatively estimate that measurement error for any given trait was < 5%.

To determine the extent of sexual dimorphism in *P. novaehollandiae* and the influence of region, I carried out MANOVA using the 8 morphological traits as independent variables and sex, as identified by genetics, as a fixed factor, as well as region (SPSS 14.0; SPSS Inc., Chicago, IL). The MANOVA showed a significant effect of sex ($F = 52.510$; $P < 0.001$; Wilk's Lambda = 0.491; Partial $ETA^2 = 0.509$) and region ($F = 17.589$; $P < 0.001$; Wilk's Lambda = 0.743; Partial $ETA^2 = 0.257$) on morphological variation, and no significant interaction effect between sex and region. This result suggests that, in this system, regions should be considered separately for the development of morphologic methods of sex determination; and this is how I proceeded.

To identify traits that showed significant variation between sexes in each region I carried out analysis of variance (ANOVA) across regions for each morphological variable using sex as a fixed factor. I applied Holm corrections (Holm 1979, Aickin & Gensler 1996) to ANOVA

results to adjust for multiple comparisons that address a common null hypothesis (Rice 1989). I also calculated descriptive statistics across regions for each morphological trait for each sex.

I calculated means, standard deviation, and Mahalanobis distance across regions for each morphological variable. Mahalanobis distance provides the difference between two means as the number of standard deviations different from zero. Therefore, the trait showing the largest Mahalanobis distance will have the greatest power for discrimination between sexes. The mean and standard deviation for each sex and trait that showed the largest Mahalanobis distance were used to construct a graph of normal densities. I assumed a normal distribution of measurements for each sex, and sex ratios equivalent to those of the sample population. The probability that a bird with any given measurement will be a given sex is obtained by dividing the ordinate of that sex at that measurement by the sum of the ordinates for both sexes.

To develop discriminant functions (d) for sex determination for each region I applied stepwise discriminant analysis to morphological variables. In the discriminant analysis, genetic sex was used as the dependent variable and the morphological measurements showing significant variation between regions by MANOVA were used as covariates.

From the discriminant function the probability (P) that a bird with a given morphology is male can be estimated as

$$P_{male} = e^{d} / 1 - e^{d}$$

where d is the discriminant function and e is the base of the natural logarithms. The probability of being female is

$$P_{female} = 1 - P_{male}$$

Based on these equations, I calculated the accuracy of assignments made using a minimum probability of correct sexing of 90%.

RESULTS

Table 3.1 shows results of the ANOVA carried out for each trait using sex as the fixed factor. All morphological measurements differed significantly between sexes. Examination of the descriptive statistics (Table 3.2) shows that all traits were larger in males than females. The trait that explained the largest variation in the model for Fleurieu Peninsula was bill-head length (Part $ETA^2 = 0.494$), and for Kangaroo Island was wing length (Part $ETA^2 = 0.661$).

Table 3.2 shows sample size, mean, and standard deviation for morphological traits for each sex across regions. Two traits (bill-head length, wing length) were above the 90% level (Mahalanobis distance > 1.645) for Fleurieu Peninsula, while three traits (bill-head length, wing length, mass) were above the 90% level for Kangaroo Island. The trait that showed the least overlap between sexes for Fleurieu Peninsula was bill-head length, and for Kangaroo Island was wing length (see Figures 3.1 and 3.2).

Table 3.3 shows the percentage of the sample population correctly sexed, unsexed, and incorrectly sexed for each region applying criteria developed using the mixed-modal method at a minimum probability of correct sexing of 90%.

Table 3.4 shows the discriminant functions for each region, and the percentage of the sample population correctly sexed, unsexed, and incorrectly sexed for each region applying criteria developed using these discriminant functions at a minimum probability of correct sexing of 90%.

DISCUSSION

I found variation between sexes in *P. novaehollandiae* for all morphological variables in both the island and mainland regions in South Australia, with males as the larger sex. Male biased size dimorphism is common in birds (Bjorklund 1990, Ellrich et al. 2010) and has previously been observed in *P. novaehollandiae* (Disney 1966, Rooke 1976, Paton & Collins 1989). Size dimorphism is thought to be related to sexual selection and the reproductive role of each sex (Fairbairn 1997, Owens & Hartley 1998), although proximate causes are also possible (Potti & Merino 1997). *Phylidonyris novaehollandiae* exhibit sex specific breeding behaviour; male *P. novaehollandiae* maintain and defend territories during the breeding season, and larger males are more successful at territory defence (McFarland 1985, Clarke & Clarke 1999). Males that maintain a territory produce more offspring than males that do not maintain a territory. This mating strategy appears to be a likely candidate for the mechanism driving selection for larger males.

I found size variation in birds between Kangaroo Island and Fleurieu Peninsula, consistent with findings by Mathews (1918-27) and Rogers et al. (1986). Kangaroo Island birds were larger for all measured traits with the exception of bill depth and width, which did not differ between regions, and body mass, for which Kangaroo Island birds had lower mass. This regional morphological variation may represent local adaptation through natural selection as a result of regional environmental variation (see Chapters 4, 5, & 6), given that island resources are generally depauperate compared with the mainland (Robinson-Wolrath & Owens 2003, Lomolino 2005).

The morphological trait that showed the most variation between the sexes was different in each region. In Fleurieu Peninsula, bill-head length was most different between the sexes, whereas on Kangaroo Island, wing length was most different between the sexes. These two traits are commonly cited in the literature as being useful for discriminating sex, probably due to the low intrinsic error of these measurements relative to other common measurements. Discriminant analysis for each region estimated that a linear combination of traits was a better predictor of sex than any single trait on its own. The linear combination of traits for Fleurieu Peninsula included bill-head length, bill-feather length, bill depth, and wing length, and for Kangaroo Island included bill-head length, wing length, and mass.

Comparing the two morphologic methods directly, at a minimum correct sexing of 90%, the mixed-modal method correctly sexed a larger proportion of the population than the discriminant analysis method for the Fleurieu Peninsula sample. However, almost twice as many samples were incorrectly sexed using the mixed-modal method. For the Kangaroo Island sample, the discriminant analysis method correctly sexed a larger proportion of the population than the mixed-modal method. These results showed that discriminant analysis and the mixed-modal method are comparable methods for sex determination in *P. novaehollandiae* in South Australia. Their performance will depend on the morphological characteristics of the population under examination.

I found regional variation in the traits that best discriminate sex, which indicates variation in sexual size dimorphism between Kangaroo Island and Fleurieu Peninsula. Although regional variation in morphologies has been previously observed (Latham 1790, Mathews 1918-27, Salomonsen 1966, Pyke & Armstrong 1993, Higgins & Peter 2002), it has not been interpreted in relation to sexual size dimorphism. Avian studies over the last decade have

provided evidence that sexual size dimorphism correlates with mating strategy and parental care (Fairbairn 1997, Benito & González-Solís 2007, Dale et al. 2007). The regional variation in size dimorphism that I observed in *P. novaehollandiae* may indicate region-specific selection pressures for parental care or foraging competition. However, this remains to be examined (but see Chapter 6).

The extensive regional variation in morphologies observed in *P. novaehollandiae*, both in this study and previous studies, suggests that protocols for morphologic-based sex determination developed for a particular site may not be suitable for landscape-level sex determination. A suitable method and suitable measurements must be determined for any given area and time span given neutral and adaptive changes in morphology (Price & Grant 1984, Schluter & Smith 1986, Reimchen 1995). Even within an area and time span, I was not able to accurately assign sex to the entire sample population based solely on morphological measurements. Morphologic methods for assigning sex are intrinsically error prone because there is a trade-off between the accuracy of sex determination and the percentage of the population that can be sexed (Twedt 2004). Before implementing morphologic methods for sex allocation, the implications of this unreliability should be seriously considered in relation to the desired application and the degree of tolerance that this application allows.

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(Flinders University) and were approved by the Animal Welfare Committee of Flinders University (permit E190, E203).

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Table 3.1. Results of the ANOVA testing the effect of sex on variation in morphology for *P. novaehollandiae* across regions in South Australia. The regions are Fleurieu Peninsula and Kangaroo Island. Sample size (N), Degrees of freedom (df), F-values (F), Partial ETA^2 (Part ETA^2), P-values, and corresponding Holmed P-values are shown. Bold indicates significant values.

	Fleurieu Peninsula						Kangaroo Island					
Trait	N	df	F	Part ETA^2	P	P_H	N	df	F	Part ETA^2	P	P_H
Bill-head length	336	1	**326.66**	**0.494**	**<0.001**	**<0.008**	79	1	**113.32**	**0.592**	**<0.001**	**<0.008**
Bill length feather	336	1	**15.77**	**0.045**	**<0.001**	**<0.007**	79	1	**24.40**	**0.238**	**<0.001**	**<0.007**
Bill length nostril	336	1	**86.51**	**0.205**	**<0.001**	**<0.006**	79	1	**23.08**	**0.228**	**<0.001**	**<0.006**
Bill depth	336	1	**102.99**	**0.235**	**<0.001**	**<0.005**	79	1	**27.36**	**0.260**	**<0.001**	**<0.005**
Bill width	336	1	**58.45**	**0.149**	**<0.001**	**<0.004**	79	1	**18.76**	**0.194**	**<0.001**	**<0.004**
Tarsus	336	1	**83.82**	**0.200**	**<0.001**	**<0.003**	79	1	**19.19**	**0.197**	**<0.001**	**<0.003**
Wing	336	1	**215.96**	**0.392**	**<0.001**	**<0.002**	79	1	**151.87**	**0.661**	**<0.001**	**<0.002**
Mass	336	1	**125.84**	**0.273**	**<0.001**	**<0.001**	79	1	**85.64**	**0.523**	**<0.001**	**<0.001**

Table 3.2. Descriptive statistics describing the extent of sexual dimorphism in *P. novaehollandiae* across regions of South Australia. The regions are Fleurieu Peninsula (FP) and Kangaroo Island (KI). Shown are mean values and standard deviation (SD) for males and females per trait for each region, as well as the common standard deviation (Common SD) and Mahalanobis distance (Mahal dist). The largest value for Mahalanobis distance is in bold.

Region	Trait	Male			Female			
		N	Mean	SD	N	Mean	SD	Mahal dist
FP	Bill-head length	208	42.0	0.99	129	40.0	0.98	**2.028**
	Bill length feather	208	23.7	1.86	129	22.9	1.71	0.444
	Bill length nostril	208	10.6	0.65	129	9.9	0.54	1.151
	Bill depth	208	5.2	0.27	129	4.9	0.26	1.128
	Bill width	208	5.2	0.34	129	4.9	0.34	0.882
	Tarsus	208	23.3	0.89	129	22.4	0.72	1.091
	Wing	208	78.6	2.83	129	74.0	2.75	1.643
	Mass	208	21.8	1.76	129	19.6	1.76	1.25
KI	Bill-head length	46	42.7	1.11	34	40.3	0.79	2.464
	Bill length feather	46	24.7	1.52	34	23.1	1.27	1.132
	Bill length nostril	46	10.9	0.70	34	10.2	0.58	1.079
	Bill depth	46	5.2	0.31	34	4.9	0.28	1.010
	Bill width	46	5.2	0.35	34	4.9	0.32	0.890
	Tarsus	46	24.0	0.97	34	23.0	0.90	1.064
	Wing	46	79.7	2.43	34	73.4	2.08	**2.762**
	Mass	46	20.8	1.32	34	18.3	1.05	2.075

Table 3.3. The potential accuracy of sex assignments using the mixed-modal method across regions in South Australia. Cut-off values and percentage of the population correctly sexed, unsexed, and incorrectly sexed with 90% confidence using the mixed-modal method are shown for each region. See Table 3.2 for a list of the mean±sd per sex.

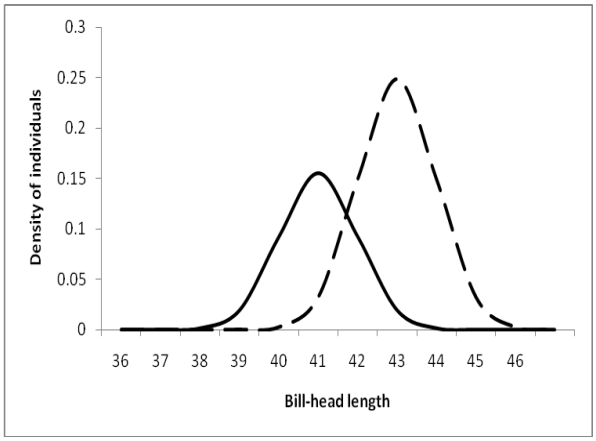
Region	Variable	Cut-off values	% Correctly sexed	% Unsexed	% Incorrectly sexed
Fleurieu Peninsula	Bill-head length	Female ≤39.6 mm, Male ≥41.0 mm	70.6	21.4	8.0
Kangaroo Island	Wing length	Female ≤74.5 mm, Male ≥77.9 mm	76.2	22.5	1.3

Table 3.4. The potential accuracy of sex assignments using the discriminant analysis method across regions in South Australia. Multivariate discriminant functions, and percentage of the population correctly sexed, unsexed, and incorrectly sexed with 90% confidence using these functions are shown for each region.

Region	Discriminant Function	% Correctly sexed	% unsexed	% Incorrectly sexed
Fleurieu Peninsula	D = 0.787(BHL) - 0.127(BFL) + 0.675(BD) + 0.139(wing) - 43.585	65.9	29.4	4.7
Kangaroo Island	D = 0.542(BHL) + 0.243(wing) + 0.236(mass) - 45.956	88.7	8.8	2.5

Figure 3.1. Normal density curves based on morphology for each sex. Solid line indicates females. Dashed line indicates males.

a) Density curves for bill-head length for Fleurieu Peninsula.



b) Density curves for wing length for Kangaroo Island.

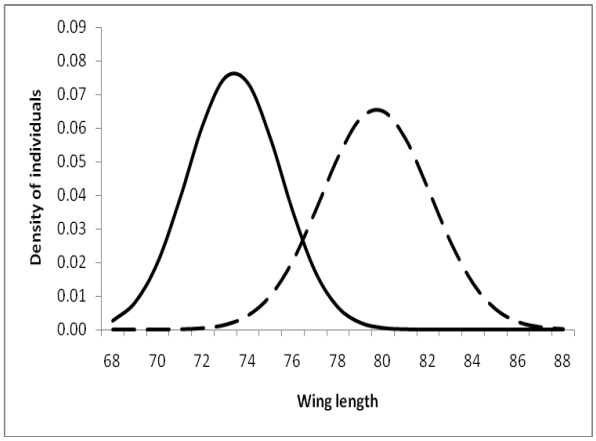
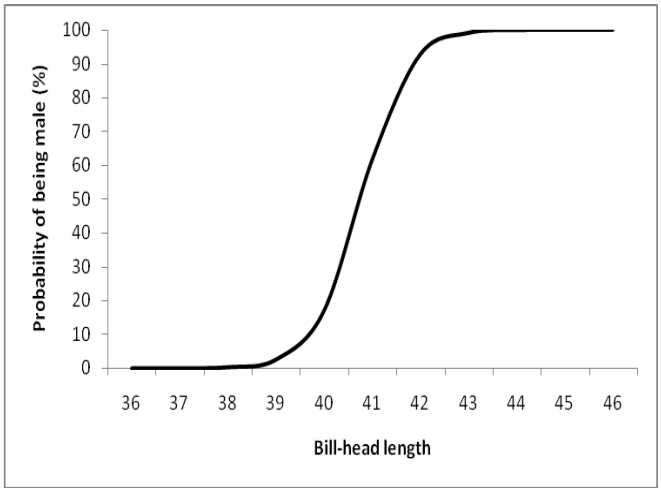
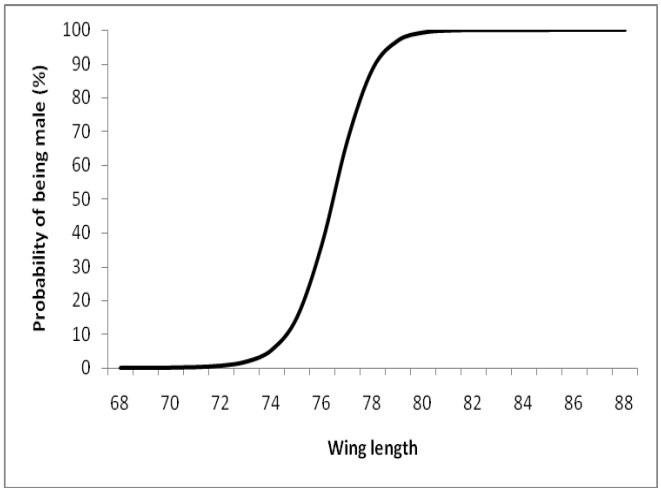


Figure 3.2. Probability curves that an individual with a given measurement for a given trait will be male.

a) Probability curve based on bill-head length for Fleurieu Peninsula.



b) Probability curve based on wing length for Kangaroo Island.



CHAPTER 4: DIVERGENCE IN THE NEW HOLLAND HONEYEATER (*PHYLIDONYRIS NOVAEHOLLANDIAE*): EVIDENCE FROM MORPHOLOGY AND FEEDING BEHAVIOUR

ABSTRACT

Studies of island versus mainland populations have provided insights into processes that shape adaptive divergence (e.g., through selection in allopatric populations), as well as identifying patterns of morphological change. In this study, I compare the morphology and foraging behaviour in New Holland Honeyeaters (*Phylidonyris novaehollandiae*) between Kangaroo Island and the South Australian mainland. I (1) provide descriptive information on morphological traits across populations to test previously described differences; (2) test the prediction that island birds will have a wider foraging niche, different use of foraging substrate, and different foraging techniques, and (3) test for evidence of reduced prey diversity on islands as evidenced by longer foraging time. The results provided support for the predictions. Island birds were larger than the mainland birds in tarsus (2.5%) and bill length (3.7%), had a wider foraging niche (mostly due to greater insect consumption), and foraged more from the bark and air (sallying). Consistent with other studies of island biogeography, at least one common mainland predator and several bird species are absent from Kangaroo Island, which may facilitate niche expansion in the island birds. A wider niche may also be favoured by lower prey diversity on islands: in this study, island birds had longer foraging times than mainland birds, which may be evidence for lower total prey density.

INTRODUCTION

Island populations have played a key role in understanding processes and patterns of allopatric divergence due to their geographic isolation, combined with typical ecological and environmental characteristics shared by islands (Williamson 1981, Grant 1998, Whittaker 1998, Schluter 2000). Comparisons between morphological traits of island and mainland fauna reveal consistent patterns, which include the 'island rule' (reviewed in Lomolino 2005, Lomolino et al. 2006). According to the island rule, small organisms tend to become larger (towards gigantism) on islands and large organisms tend to become smaller (towards dwarfism) on islands (Foster 1964, Van Valen 1973, Lomolino 1985). This emergent pattern, as with other ecotypic patterns, results from predictable differences in selective pressures among island species, with a tendency for convergence toward optimal phenotypes. Clearly, different processes acting both within and across populations or taxa could shape this pattern. Common explanations for changes in morphology and ecology of island forms include a paucity of resources, interspecific competition, and predation (MacArthur & Wilson 1967, reviewed in Lomolino 2005). In birds, larger bill size is thought to confer the advantage of a wider ecological niche and a more generalist diet, which may be facilitated by fewer interspecific competitors and also foraging in predator-free zones (e.g. ground foraging in the absence of specific predators) (Grant 1965, Keast 1968a, Abbott 1974a, 1977). Occupation of a wider ecological niche may be necessitated if island prey density is lower than mainland prey density, and may subsequently be maintained by reduced interspecific competition (Svanbäck & Bolnick 2007).

Honeyeaters (Meliphagidae) constitute the largest family of birds within Australia, containing some 70 species (Ford et al. 1979). They are predominantly nectar-feeders and are known to pollinate over 600 plant species (Keighery 1982), including many Australian endemics such as species of the genera *Banksia*, *Dryandra*, *Hakea*, *Melaleuca*, *Xanthorrhoea* and *Eucalyptus* (Driskell & Christidis, 2004). Honeyeaters partition resources according to phenotype, with long-billed honeyeaters (e.g. *Phylidonyris* spp.) spending more time foraging for nectar than insects compared to short-billed honeyeaters (Ford & Paton 1976, Ford & Paton 1977a, b, Ford 1979, Recher 1981). Larger honeyeater species are also somewhat territorial and tend to dominate the defence of food resources over smaller species (e.g. Keast 1968a, Ford & Paton 1976, 1982, Ford 1979, Pyke 1980, Paton 1986).

The New Holland Honeyeater (*Phylidonyris novaehollandiae*) is arguably one of the most abundant species of honeyeater. In 1906, Campbell noted morphological differences between the Kangaroo Island form in South Australia (which had a longer bill) compared with that of south western Victoria. This difference in bill length was the justification for classification of two subspecies: *P. n. campbelli* on Kangaroo Island and *P. n. novaehollandiae* on the mainland (Mathews 1918–1927). Later studies also found variation in morphology between mainland and Kangaroo Island birds, including longer bill and wing length, and lower body mass in birds on Kangaroo Island (Keast 1968a, Ford 1976).

Kangaroo Island is a relatively large (~4 500 km^2) continental island located approximately 14 km from mainland South Australia; it was last connected to the mainland around 8 900 years ago due to lower global sea levels (Abbott 1974a, b, Belperio & Flint 1999, Paton et al. 2002). Kangaroo Island displays characteristics typical of a large continental island, differing ecologically from the adjacent mainland, having fewer animal species (Keast 1968a, Abbott 1974b). Several notable avian families are missing from Kangaroo Island, including the trunk and bark-feeding treecreepers (Climacteridae), sittellas (Neosittidae), and shrike-tits (Pachycephalidae) (Abbott 1974b, Paton et al. 2002). In addition to this, Kangaroo Island hosts fewer species of honeyeater compared to the mainland; it has 10 resident honeyeater species (Paton et al. 2002) compared with 12 species in the Mount Lofty Ranges on the mainland (Paton & Ford 1977); of these, six are considered subspecies of the mainland forms (Davies et al. 2002, Higgins & Peter 2002, Paton et al. 2002). These characteristics make Kangaroo Island ideal for the investigation of island evolution.

In this study, I compare the morphology and foraging behaviour of *P. novaehollandiae* on Kangaroo Island with the adjacent South Australian mainland form. This study differs from others that have compared mainland and kangaroo island honeyeaters as I am concerned with processes responsible for patterns of variation, and have significantly larger sample sizes for foraging ecology and morphology. I provide descriptive information on morphological traits across populations to test for variation. It is common for islands to be characterised by a depauperate fauna community, including fewer prey species, fewer predators, and fewer interspecific competitors (MacArthur 1965). Here I test for indirect evidence of

reduced food availability as an explanation for a wider foraging niche in island birds. Based on ecomorphology (Leisler & Winkler 1985, 1991), I predict that island birds will (1) differ in use of foraging substrate and foraging technique (in the absence of bark-feeding birds) than mainland birds and (2) will have a wider foraging niche and longer search times for food on the island. I also predict that (3) mainland birds will have longer search times and a wider foraging niche during the low rainfall compared with the high rainfall season, when prey items are also presumably less abundant.

METHODS

Study species

The New Holland Honeyeater (*Phylidonyris novaehollandiae*) is a small passerine (~20g) endemic to Australia. It has a wide distribution throughout coastal regions of south east Australia, Tasmania, and south west Australia (see Higgins et al. 2001). Five subspecies are currently recognised, in Western Australia, mainland south-eastern Australia, Kangaroo Island, Bass Strait islands, and Tasmania, respectively (Schodde & Mason 1999). Within its South Australian range, *P. novaehollandiae* is among the most abundant passeriformes (Oorebeek & Kleindorfer 2009). The sexes exhibit no marked dichromatism or dimorphism, although males tend to be slightly larger on average (Rooke 1976, Chpater 3). *Phylidonyris novaehollandiae* appear to be socially monogamous during the spring and autumnal breeding periods, with only the female incubating the eggs but both parents feeding the young (Paton 1985). They are mostly nectarivorous but will frequently supplement their carbohydrate requirements with manna, honey dew, and lerp, and their protein requirements with insects (Paton 1979, 1982).

Study sites

In 2004, 2005, 2006, and 2007, I collected data on morphology and foraging behaviour from two locations in South Australia: (1) Kangaroo Island (island), and (2) Mount Lofty Ranges region (mainland) (Table 4.1). I sampled from two island sites: (1) Flinders Chase National Park (FCNP), and (2) Pelican Lagoon Conservation Park (PLCP); and three mainland sites: (1) Newland Head Conservation Park (NHCP), (2) Scott Conservation Park (SCP), and (3) Sandy Creek Conservation Park (SAC).

All sites were dominated by *Eucalyptus* spp. FCNP, PLCP and NHCP were primarily mallee heath land (Ford & Paton 1982, Kleindorfer et al. 2006a, Schlotfeldt & Kleindorfer 2006), with large areas of scrubland at NHCP (Kleindorfer et al. 2006b). SCP was dominated by *Eucalyptus* woodland; SAC had two distinct vegetation types, comprising *Callitris* woodland and *Eucalyptus* woodland (Rix 1976, Ford & Paton 1977b, Westphal et al. 2003, Schlotfeldt & Kleindorfer 2006, Kleindorfer et al. 2006b, Galligan & Kleindorfer 2008).

Morphology

All morphologic measurements were made by Sonia Kleindorfer (SK) and Margot Oorebeek (MO) to reduce measurement error (banding permit ABBBS 2601); SK and MO banded birds at both sites, and their measurements were comparable (see Chapter 3 Methods). Mist-nets were used to capture *P. novaehollandiae*. Each bird had blood taken for genetic analysis using previously described methods (Kleindorfer et al. 2006b, Chapter 2) and was subsequently ringed with a unique aluminium reference

band. A total of 372 birds were banded and measured; 291 at mainland sites, and 81 birds at island sites (see Table 4.1). For this study, I analysed 11 morphological traits: (1) bill length to the back of the head (bill-head length) (mm), (2) bill length to the base of feathers at the skull (bill–feathers length) (mm), (3) bill length from the anterior nostril opening (bill–nostril length; mm), (4) bill depth at base (bill depth; mm), (5) bill width at base (bill width; mm), (6) tarsometatarsal length (tarsus length; mm), (7) length of the flattened wing (wing; mm), (8) tail length (tail; mm), (9) mass (g), (10) foot span with claws (mm), and (11) foot span without claws (mm). Foot and claw measures were made using an indentation of the foot in plasticine.

A genetic-based method was used to determine bird sex (Kahn et al. 1998). DNA was extracted from FTA using method #4 of the protocols outlined by Smith and Burgoyne (2004) (see Chapter 3).

Foraging behaviour

Between March and November in 2005 and 2006, I collected opportunistic data on foraging behaviour at all study sites. On the island, I observed birds for 44 days across the sites. On the mainland, I observed birds for 48 days across the sites.

For all foraging observations, I recorded only the first feeding observations (the first food item ingested) per encountered bird per day to avoid statistical non-independence of the data (discussed in Kleindorfer et al. 2006a). *Phylidonyris novaehollandiae* is a visually conspicuous bird, even when sitting still, and it is unlikely that my observations were biased by conspicuous behaviour.

For each foraging event, I recorded the following information: (1) Foraging technique: sally (pursuit of aerial prey), probe (insertion of bill into the substrate), glean (prey removal from a foliage surface), pick (prey removal from a non foliage surface), and bite (part of the food item ingested) (adapted from Ford 1989); (2) Foraging substrate: air, flower, green leaves, live bark, dead bark, cone, and ground; and (3) Genus of plant that was used as a foraging substrate, for example: *Acacia, Allocasuarina, Amyema, Astroloma, Banksia, Callistemon, Callitris, Correa, Eucalyptus, Hakea, Leucopogon, Melaleuca,* and *Xanthorrhoea*. For a subset of foraging events (N=294), I also recorded the time (sec) from first observation until the time that the bird was observed to feed. To do this, I used a stop watch; observations < 3 s in duration (often at flowers or fruits) were not monitored with a stop watch. I analysed the foraging time (sec) in relation to location (island, mainland) and rainfall period (low, high) using ANOVA.

Niche breadth

Niche breadth was calculated for the three foraging categories (technique, substrate, plant genus) using the following Shannon-Wiener formula for (1) location (island, mainland), (2) season (low rainfall, high rainfall), and (3) for the entire year (seasons combined):

$$H' = - \sum (p_i \ln p_i),$$

where p_i is the proportion of observations within each foraging category *i* (Werner & Sherry 1987, Antos & Bennett 2006, Schlotfeldt & Kleindorfer 2006). Because H' does not take into account the number of behaviour states within each foraging category, I calculated J', which provides a measure of niche breadth on a 0 - 1 scale:

$$J' = H'/\ln n$$

where *n* is the number of behaviour states within each foraging category *i* (Krebbs 1999, Antos & Bennett 2006). A simple measure of niche breadth was also calculated according to Antos and Bennett (2006): I tallied the number of behaviour states that were used in more than five per cent of observations. This resulted in a ratio of behavioural states within foraging categories: a larger number denotes a wider niche breadth (Antos & Bennett 2006).

Seasonal effects

Phylidonyris novaehollandiae mostly feed on flower nectar (Paton 1982), which is reliant on rainfall (Law et al. 2000). Using monthly rainfall data from Bureau of Meteorology rainfall stations, measurements from up to two of the closest stations to each site for each month (Jan-Dec) in 2005 and 2006 were averaged over the island and mainland. Based on this data, two annual rainfall seasons were identified: May to October (hereafter 'high rainfall') and November to April (hereafter 'low rainfall'). Ford and Paton (1977b) identified a comparable seasonal shift in rainfall for their study of ten honeyeater species in the Mount Lofty Ranges: April to September and October to March.

RESULTS

Morphology

A comparison of the mean values for 11 morphological variables between locations for each sex is presented in Table 4.2. In males, bill-head length and bill-feathers length were significantly larger in island birds compared with mainland birds. In females, prior to Holm corrections, only bill-nostril length was significantly different across locations, but not after Holm corrections. This may be a result of Holm corrections being over-conservative (Aickin & Gensler, 1996). Tarsus length was significantly larger in island birds for both sexes and island birds had significantly lower mass than mainland birds.

Foraging technique

During both the high and low rainfall periods, foraging technique differed significantly between locations (Likelihood Ratio: $P < 0.001$, d.f. = 4; and Likelihood Ratio = $P < 0.001$, d.f. = 3, respectively) (Fig. 1). Island birds were observed sallying and gleaning more frequently than mainland birds while mainland birds were observed probing more frequently than island birds.

Foraging substrate

Foraging substrate differed significantly between locations during the high rainfall (Likelihood Ratio: $P < 0.001$, d.f. = 6) (Fig. 2*a*) and low rainfall period (Likelihood Ratio: $P < 0.001$, d.f. = 4) (Fig. 2*b*). Both rainfall periods showed a similar pattern:

island birds foraged more in the air and on live bark (and green leaves during the high rainfall period only) whereas mainland birds foraged more on flowers.

Plant genera used for foraging

There was a significant difference across locations in the use of plant genera for foraging during the high rainfall (Likelihood Ratio: $P < 0.001$, d.f. = 12) (Fig. 3*a*) and low rainfall periods (Likelihood Ratio: $P < 0.001$, d.f. = 9) (Fig. 3*b*). During the high rainfall period, most foraging was observed on *Eucalyptus* plants. During the low rainfall period, mainland birds foraged more on *Eucalyptus* than island birds (Fig. 3*b*). In contrast, island birds foraged on *Correa* and *Melaleuca*, which was not observed on the mainland (although this was observed in previous years). Mainland birds also foraged more frequently on *Astroloma* and *Banksia* than island birds during the high rainfall period. During the low rainfall period, foraging on *Correa* was only observed on the island (the most frequent plant genus visited during this period, followed by *Eucalyptus*) and foraging on *Amyema* was only observed on the mainland (the second most frequent plant genus after *Eucalyptus* for this period).

Niche breadth

In both seasons, island birds showed greater diversity in resource use, as indicated by consistently higher J' values (Table 4.3). The only exception to this trend was in the use of plant genera in the wet season. When data were pooled, island birds showed greater diversity in all three categories.

Foraging time

Foraging time (seconds) for each rainfall period (low, high) was significantly longer for island (36.1 ± 5.3; 17.1 ± 3.3) than mainland (6.7 ± 0.6; 4.2 ± 0.5) birds (ANOVA: location: $F = 71.2$, $P < 0.001$; rainfall period: $F = 10.8$, $P < 0.001$; interaction term: $F = 18.34$, $P < 0.001$; corrected total = 294). Figure 4.4 shows the results for foraging time (seconds) for the foraging techniques "glean" and "pick". As above, I found longer foraging time in island birds, and a non-significant trend for longer foraging time during the period of low versus high rainfall (ANOVA: location: $F = 25.9$, $P < 0.001$; rainfall: $F = 3.4$, $P = 0.07$; interaction term: $F = 10.0$, $P = 0.002$; corrected total = 102).

DISCUSSION

This study found that island birds generally had longer bills and tarsi, but not longer wings, than mainland birds. Island birds also had lower body mass. In addition to morphological differences, island birds occupied a wider foraging niche, and foraged significantly more on bark as well as in the air (sallying). Most prey items from bark feeding and sallying were insects. This study found indirect evidence for reduced prey availability on the island as an explanation for occupation of a wider foraging niche. Across the year, island birds had longer foraging times compared with mainland birds. On the island and mainland, foraging time was longer during the low rainfall versus high rainfall period, which I suspect was related to lower total prey density (this was not measured) during the low rainfall period (Schlotfeldt 2010). Thus, the results of this study suggest that reduced prey availability on the island may be an explanation for a wider foraging niche, as explained by optimal foraging theory (Stephens & Krebs 1986).

Previous studies have also found comparable morphological differences between Kangaroo Island and mainland forms of *P. novaehollandiae*, namely longer bills and lower body mass (e.g. Keast 1968a, Ford 1976). Body mass and morphology affect foraging movements: lower body mass limits energy storage but reduces metabolic rate and increases aerial efficiency (see review in Collins & Paton 1989, Pyke 1980, Forstmeier & Keßler 2001). I observed more aerial insect foraging in island than mainland birds; therefore, lower mass in island birds may be an adaptation to their more insectivorous diet, allowing greater aerial insect foraging efficiency. Although body mass is prone to diurnal and seasonal variation linked to fluctuations in food availability, parasite prevalence, and other ecological factors that can act at a regional scale (Creswell 1998, Oorebeek & Kleindorfer 2008); even when controlling for season, island birds had significantly lower body mass.

Bill length is a highly heritable quantitative trait (Keller et al. 2001); a large proportion of the phenotypic variation in this trait is explained by additive genetic variation. Bill wear does not have a significant effect on phenotypic variation (Sulloway & Kleindorfer *in review*, Myers et al. comparison of recapture measurements *unpubl. data*). Bill length is known to be strongly shaped by selection pressure from feeding ecology (Rakotomanana 1998, Blondel 1999, Schluter 2000, Christensen & Kleindorfer 2007, Christensen & Kleindorfer 2009). Therefore, it is hypothesized that food source shapes selection pressure for bill length in *P. novaehollandiae*. Ford (1976) suggested that the longer bills found on Kangaroo Island birds were the result of directional selection. In particular, tubular flowers (such

as island *Correa*, which had longer corollas than the mainland form) appeared to be more important nectar sources for the island birds than open-cupped Eucalypt flowers. Indeed, during the low rainfall season when nectar resources are scarce, *Correa* was the most important food source on the island, while Eucalyptus was most important on the mainland (Fig. 3). However, insects appeared to be more important than nectar in the diet of island birds throughout the year. These observations suggest an alternative to Ford's hypothesis; that insect foraging influences selection on bill length.

Tarsus length and mass were the only traits to significantly differ between island and mainland birds in both sexes. Tarsus, like bill length, has been shown to be a highly heritable trait in birds (Grant 1983, Smith 1993, Keller et al. 2001), and due to its role in foraging, tarsus is often a key target of selection (Blondel et al. 1999, Székely et al. 2004, Thessing & Ekman 1994). Long tarsi have been shown to favour gleaning behavior during foraging (Fitzpatrick 1985) and also increase spring, allowing quick sallying from the ground (Sherry 1982); while short tarsi promote balance and stability in arboreal birds (Fitzpatrick 1985, Keast & Recher 1997). Island birds had longer tarsi, which should favour sallying and gleaning.

Island birds were observed to sally and glean more than mainland birds (84.3% vs 35.5%), while mainland birds probed their beaks more into flowers. Gleaning from foliage surfaces may indicate consumption of alternative carbohydrates such as manna, honeydew, and lerp (Paton 1982), so I cannot make any conclusions about the diet derived from gleaning, which I suspect included a significant proportion of insects. However, based on sallying behaviour, which was always for insects, it is

apparent that island birds most likely consumed more insects than mainland birds. Other studies have found that *P. novaehollandiae* forage equally for nectar and insects (e.g. Keast & Condon 1968, Ford 1976), whereas in this study, island birds appeared to have much lower nectar consumption (perhaps due to drought conditions). The foraging time (seconds) until a food item was ingested was significantly longer in island than mainland birds, and was longer within each location comparing the low and high rainfall period. Combined, these findings suggest a continuous production of nectar-producing flowers on the mainland during the low rainfall season, and lower food availability (or less accessible prey) on the island across the year. This paucity of island resources seems to be responsible for a shift in morphology and behaviour toward a more insectivorous diet. A similar study (Schlotfeldt & Kleindorfer 2006) on morphology and foraging behaviour in the Superb Fairy-wren, *Malurus cyaneus*, found comparable results: island birds had a wider niche breadth and were more generalist foragers than mainland birds. Niche expansion may be a response to increased intraspecific competition (Svanbäck & Bolnick 2007), which in this case is likely to reflect limited resource availability (not measured).

My morphological comparisons showed significant variation between males in bill length at both locations that was not observed in females, although a trend for larger bills in island females was observed. Sexual size dimorphism is thought to reflect the adaptation of individual sexes to their different reproductive roles, possibly to reduce competition when cooperating to rear offspring (Fairbairn 1997). Given that bill length is strongly influenced by the selection pressures of feeding ecology, these data suggest a possible shift in the feeding and foraging ecology of island males but not

females, and furthermore a shift in patterns of intraspecific habitat use. It is possible that reduced food availability on the island has resulted in exaggerated sex-specific foraging differences not found on the mainland, as a mechanism to minimise intraspecific competition (Selander 1966, Shine 1989; but see Chapters 5 and 6). This sex difference warrants further investigation.

Island birds showed overall greater diversity in resource use compared with mainland birds (Table 4.3), and higher diversity during the dry versus wet season. The increase in resource use in island birds matches a trend observed for generalist foragers (Christensen & Kleindorfer 2009). It is unclear if my observations of a wider niche breadth in island birds are the result of many individual foraging specialists or a population of generalist foragers, as is often assumed (see Werner & Sherry 1987; Bolnick et al. 2003, Scott et al. 2003). Irrespective of the underlying structure of individual specialist or generalists, a wider niche breadth would be favoured by the absence of some bird species on the island. Notable families of birds that are missing from the island avifauna are the trunk and branch feeding specialists such as the sittellas, shrike-tits, and treecreepers. Loss of these families on Tasmania has been implicated in ecological and morphological differentiation of at least three honeyeater species (Keast 1970).

The Kangaroo Island form of *P. novaehollandiae* is a recognised subspecies of the mainland form. Here I showed that island birds were morphologically different from mainland birds, and also had different foraging behaviour. The island birds appear to be geographically separate from the mainland birds; based on mark-recapture data

from long-term banding studies (e.g. Paton et al. 2002, Kleindorfer et al. unpublished data) there is at present little evidence for frequent dispersal (despite being highly mobile birds). However, current examination of gene flow between the two locations suggests that it may be relatively high (Chapman et al. 2009, Chapter 5). Data from the present study were insufficient to explicitly test the underlying processes for the observed morphology patterns, but the results support the argument for adaptive divergence in foraging ecology and morphology under conditions of reduced interspecific competition and reduced food availability on the island.

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Table 4.1. An overview of study sites and sample sizes. Shown are the names of the study sites (site), the abbreviation for each study site (abbr.; used throughout the text), regional location (i.e. island, mainland; location), GPS coordinates, area of the park in hectares (area), and sample sizes for morphology and foraging data.

Site	Abbr.	Location	GPS	Area (ha)	Morphology	Foraging
Flinders Chase National Park	FCNP	Island	35˚56´S 136˚44´E	32828	34	257
Pelican Lagoon Conservation Park	PLCP	Island	35˚48´S 137˚47´E	[a]407	47	91
Newland Head Conservation Park	NHCP	Mainland	35˚36´S 138˚31´E	1036	151	193
Scott Conservation Park	SCP	Mainland	35˚24´S 138˚44´E	210	54	308
Sandy Creek Conservation Park	SAC	Mainland	35˚36´S 138˚51´E	143	85	270

Table 4.2. Mean ± se values of 11 morphological traits for *P. novaehollandiae* sampled at island and mainland sites. The sample size was 372 individuals: 47 males and 34 females for the island; 175 males and 116 females for the mainland. T-test P-values (P) and corresponding Holmed P-values (P_H) for each trait are listed. Bold indicates significant values.

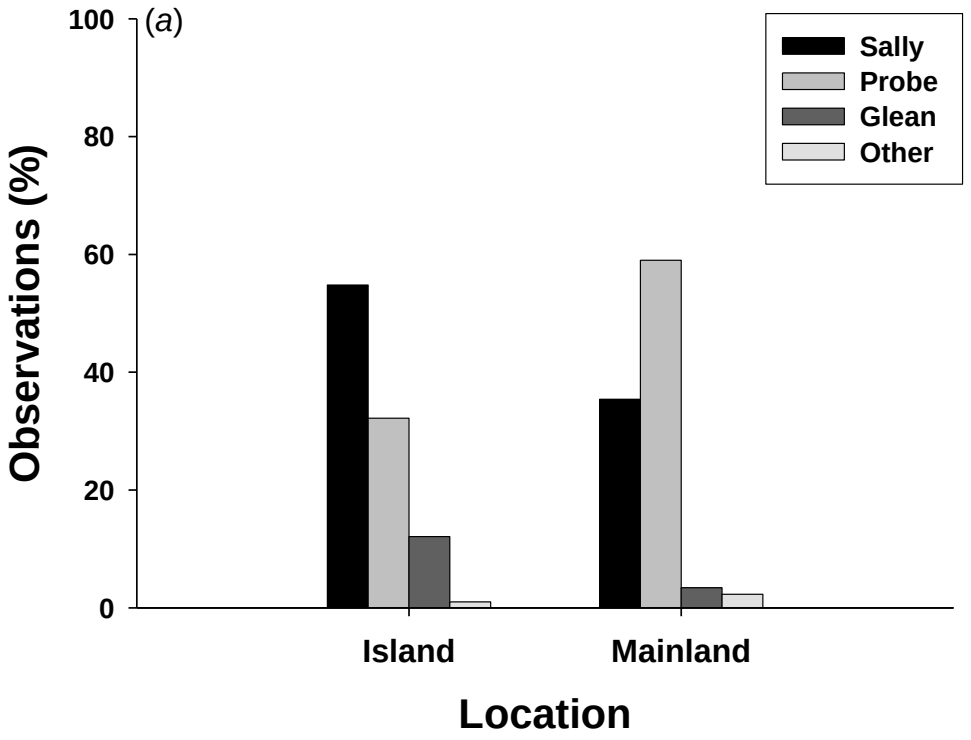
Trait	Male				Female			
	Island (mm)	Mainland (mm)	P	P_H	Island (mm)	Mainland (mm)	P	P_H
Bill length head	**42.73±0.16**	**42.09±0.08**	**0.001**	**0.009**	40.32±0.14	40.03±0.1	0.088	0.616
Bill length feathers	**24.73±0. 23**	**23.66±0.17**	**<0.001**	**0.011**	23.09±0.22	23.01±0.19	0.825	0.825
Bill length nostril	**10.93±0.1**	**10.61±0.06**	**0.009**	**0.063**	10.38±0.18	9.95±0.06	0.033	0.297
Bill depth	5.21±0.05	5.19±0.02	0.696	2.784	4.86±0.05	4.9±0.03	0.515`	1.545
Bill width	5.24±0.05	5.22±0.03	0.815	1.63	4.9±0.06	4.97±0.04	0.337	1.348
Tarsus	**23.89±0.14**	**23.34±0.07**	**0.001**	**0.008**	**23.01±0.16**	**22.44±0.07**	**0.001**	**0.010**
Wing	79.78±0.35	79.10±0.2	0.1	0.6	73.35±0.36	74.14±0.27	0.304	1.52
Tail	82.22±0.55	81.98±0.33	0.713	2.139	76.38±0.73	75.44±0.54	0.084	0.672
Mass	**20.71±0.21**	**21.96±0.15**	**<0.001**	**0.010**	**18.23±0.19**	**19.63±0.17**	**<0.001**	**0.011**
Footspan with claws	33.84±0.59	33.49±0.23	0.523	2.615	31.52±0.58	32.75±0.25	0.093	0.558
Footspan without claws	25.57±0.45	25.56±0. 2	0.985	0.985	24.43±0.6	24.8±0.32	0.592	1.184

Table 4.3. Niche breadth within and between seasons (high rainfall, low rainfall) and locations (island, mainland). Niche breadth was calculated using Shannon's formula and was adjusted to a range of 0-1, (J'). 'Entire year' refers to both seasons (high rainfall, low rainfall) combined.

Foraging Variable	Niche Breadth								
	High rainfall			Low rainfall			Entire year		
	Island	Mainland	*difference*	Island	Mainland	*difference*	Island	Mainland	*difference*
Technique	0.718	0.550	0.168	0.793	0.547	0.246	0.759	0.557	0.202
Substrate	0.625	0.598	0.027	0.864	0.55	0.314	0.701	0.494	0.207
Plant Genus	0.631	0.501	0.130	0.575	0.656	-0.081	0.611	0.537	0.074

Figure 4.1. Foraging technique (% of observations per category) is shown for each location and time period. The category 'Other' includes the foraging techniques bite and pick.

a) High rainfall (May to October)



b) Low rainfall (November to April)

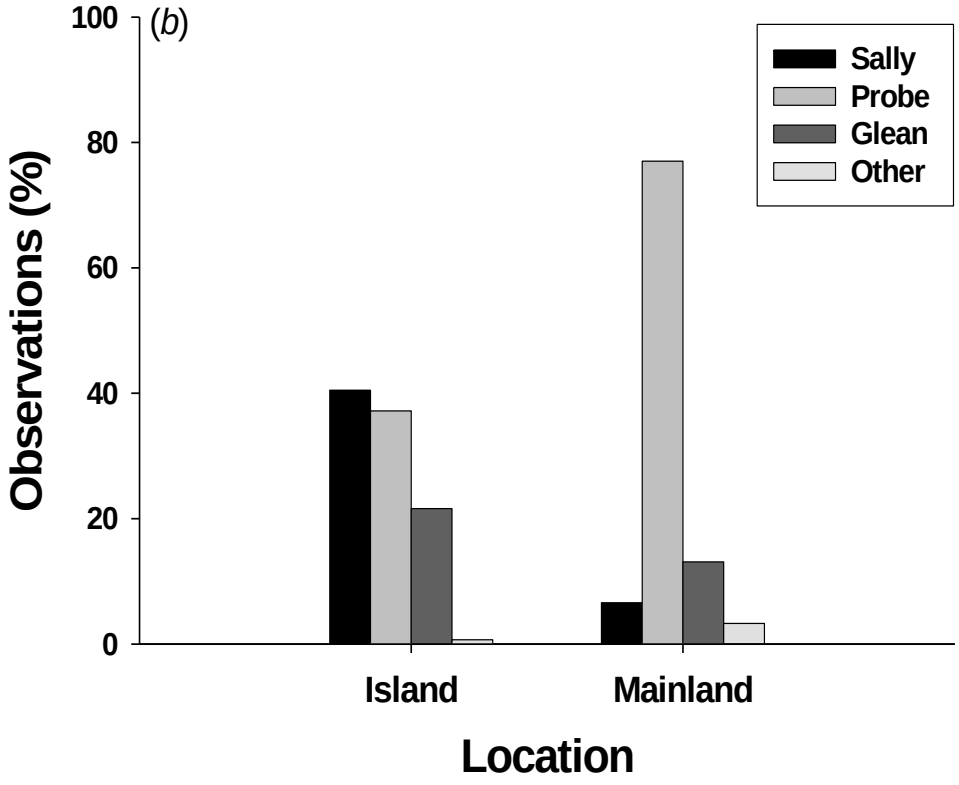
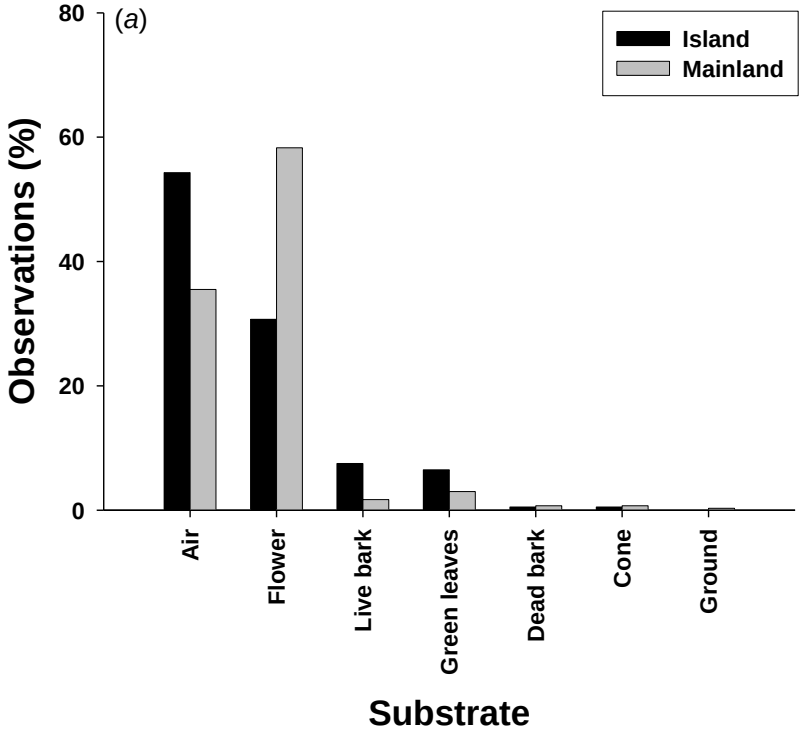


Figure 4.2. The foraging substrate (% of observations per category) is shown for each location and time period.

a) High rainfall (May to October)



b) Low rainfall (November to April)

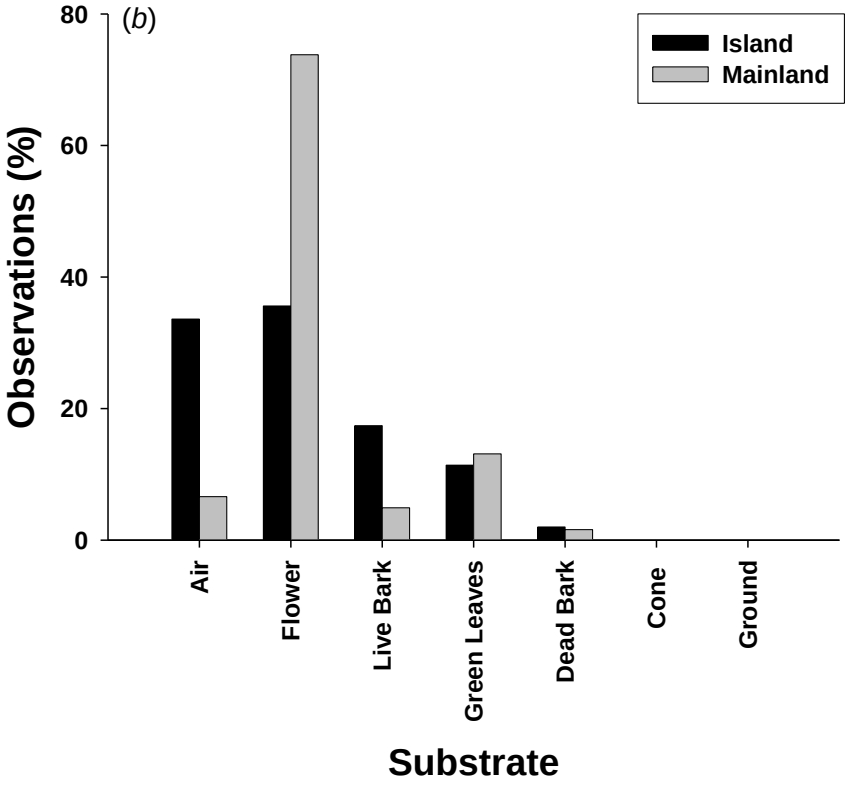
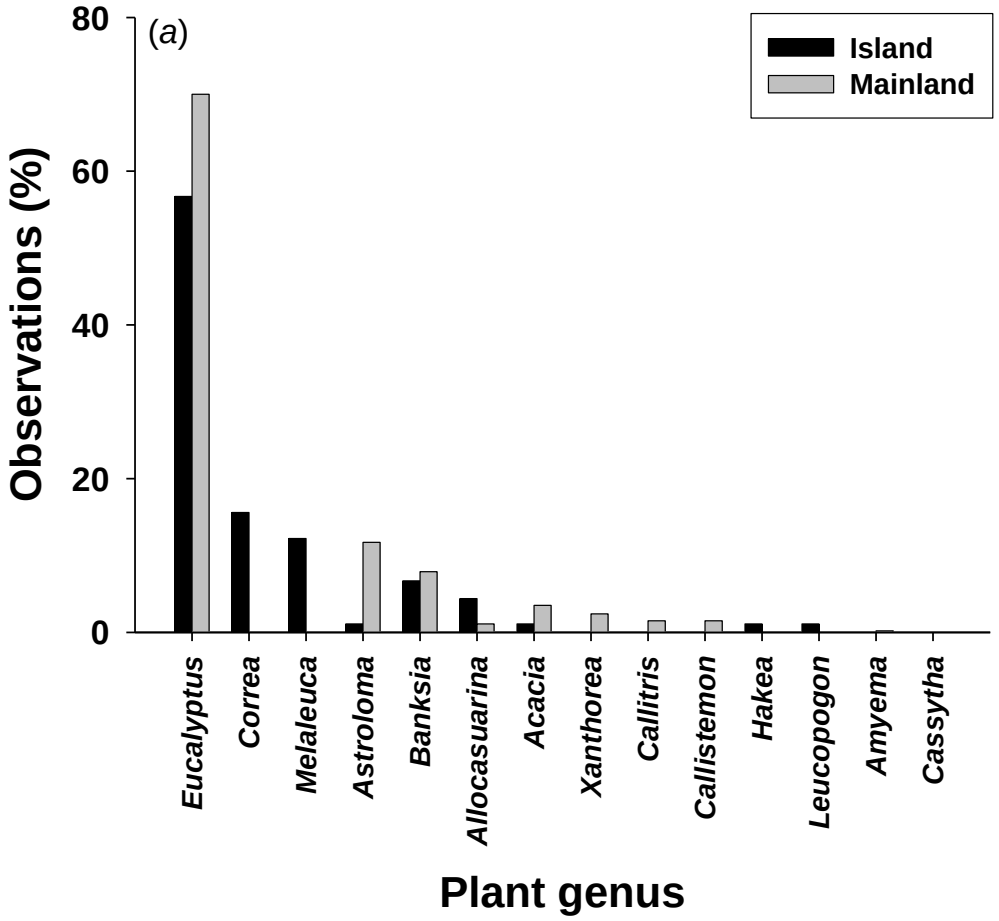


Figure 4.3. The plant genus (% of observations per genus) is shown for each location and time period.

a) High rainfall (May to October)



b) Low rainfall (November to April)

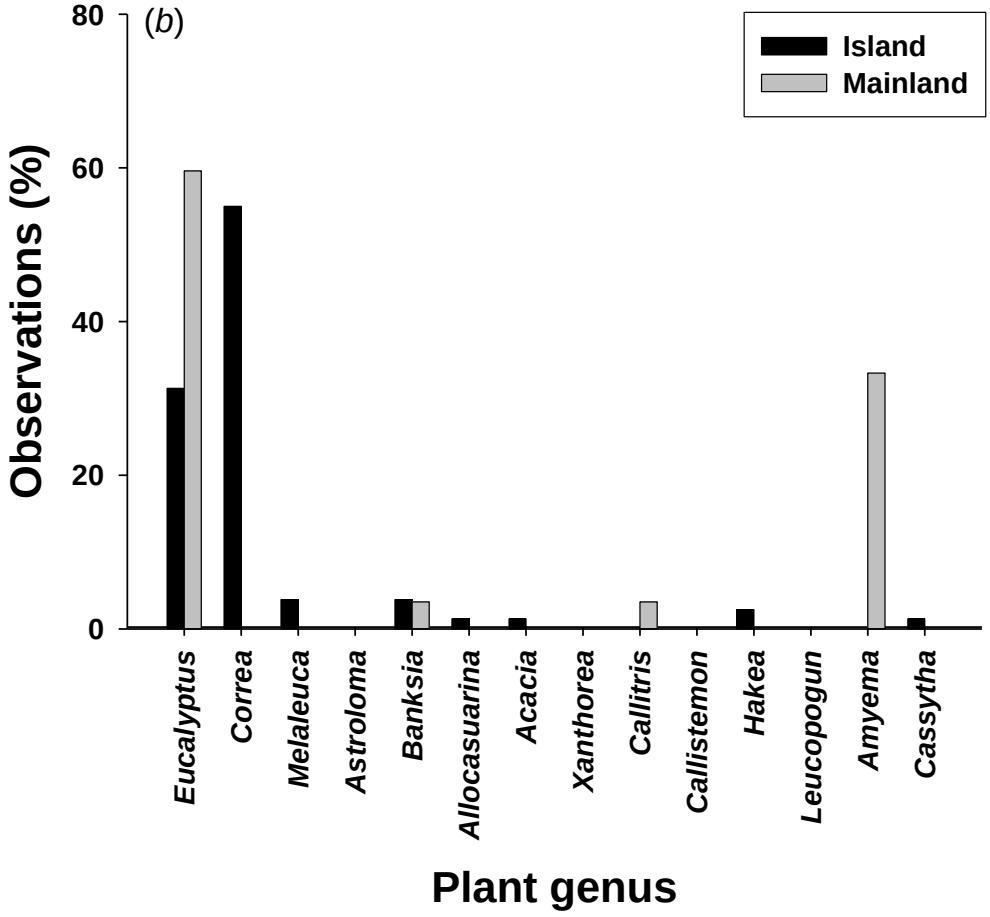
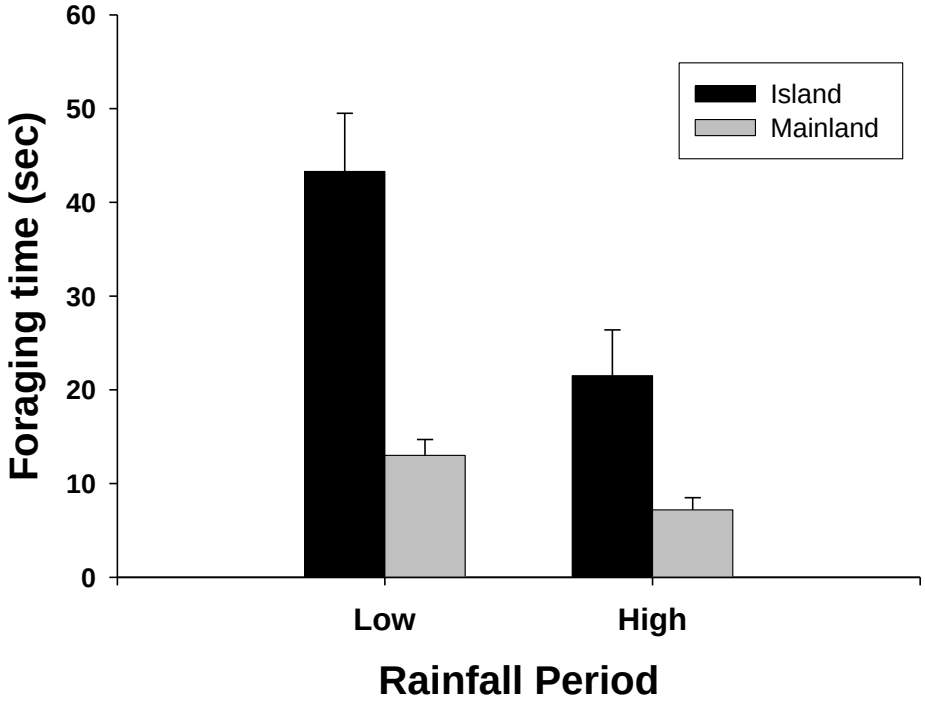


Figure 4.4. The mean foraging time in seconds is shown for each location and time period.



CHAPTER 5: CLIMATE DRIVES DIVERGENT NATURAL SELECTION IN THE NEW HOLLAND HONEYEATER (*PHYLIDONYRIS NOVAEHOLLANDIAE*: PASSERIFORMES: MELIPHAGIDAE) IN SOUTHERN AUSTRALIA.

ABSTRACT

Recent acceleration in climate change will test the evolutionary potential of populations. Identifying environmentally driven changes in traits that serve an ecological function is essential for predicting evolutionary outcomes of climate change. I examined, across a climatically variable landscape, variation in quantitative traits of a passerine bird, the New Holland Honeyeater (*Phylidonyris novaehollandiae*), which has strong reliance on rainfall-dependent foraging resources such as nectar and insects. Morphological traits with ecological function (bill dimensions, tarsus length, and body size) varied significantly across sites in South Australia. I compared neutral genetic differentiation (F_{ST}) with additive genetic variance (Q_{ST}) to test the relative roles of genetic drift and natural selection for the observed patterns of morphological variation. In all circumstances, Q_{ST} exceeded F_{ST} in both sexes indicating a predominant role of divergent natural selection on morphology. I tested the potential for rainfall to drive divergent natural selection in ecological traits by examining correlations between morphological variation and rainfall. In males, all morphological traits correlated with rainfall. In females, only two of four traits (bill base circumference and body size) correlated with rainfall. I was able to conclude that, at a landscape scale, rainfall drove divergent natural selection in morphology in *P. novaehollandiae*, but female biased-dispersal has most likely weakened the signal of the selective source in females, explaining the observed sex difference.

INTRODUCTION

Climate change has a worldwide impact upon biodiversity and is considered responsible for rapid changes in the phenology and distribution of many plant and animal species (Avise & Walker 1998, Root et al. 2003, Thomas et al. 2004, reviewed in Parmesan 2006). Population responses to conditions of changing climate can have either one of three outcomes; adaptation, dispersal, or extinction (Davis & Shaw 2001, Hannah et al. 2008, Raxworthy et al. 2008). Given the acceleration of climate change through anthropogenic activity, research now focuses on the adaptive capacity of organisms to respond to rapid climate change (Allen et al. 2000, IPCC 2007, Rosenzweig et al. 2008) to help understand the current and likely future consequences of climate change (Visser 2008). One way to monitor animal response to climate change is to measure the strength of selection and rate of evolution of functionally important traits in the context of environmental variation. Identifying environmentally driven changes in traits that serve an ecological function will increase our theoretical understanding of the timeframe for adaptive response to climate and will inform the conservation management of species with key ecosystem functions. Birds are an ideal model system to examine selection because many facets of their foraging ecology and survival can be predicted from specific morphological traits (Bowman 1961, Lederer 1975, Fitzpatrick 1985, Grant & Grant 1989a, Blomqvist et al. 1997, Keast & Recher 1997, Forstmeier & Keßler 2001). Here, I investigate the role of selection and genetic drift on divergence in functional morphological traits in a key pollinator species, the New Holland Honeyeater (*Phylidonyris novaehollandiae*), across an environmentally variable landscape in southern South Australia.

The majority of ornithological studies have been conducted in the Northern Hemisphere where avian life history strategies differ discernibly from those in the Southern Hemisphere (see Martin 1996). Long distance migration is a common trait shared by Northern

Hemisphere birds that is likely to confound investigation of a species' adaptive response to local environmental change as it creates two strong and potentially contrasting periods of selective pressure per year (that is, from non-breeding and breeding sites). In contrast, most Australian songbirds are opportunistically nomadic, remaining in or near breeding territories and tracking local resources as they become available (Collins et al. 1984, Higgins & Peter 2002). I use *P. novaehollandiae* to examine the role of local environmental conditions on variation in morphology for two main reasons: (1) *P. novaehollandiae* follows local nectar resources as they become available, indicating strong reliance on food resources that are influenced by environmental conditions (McFarland 2002); and (2) movement records (n=11,260) suggest that long distance dispersal is not common, with 99.1% of birds moving < 10 km from location of banding (Higgins & Peter 2002), suggesting that populations endure local environmental conditions year-round. This relatively contained movement, coupled with a specialist diet, makes *P. novaehollandiae* an ideal model for the investigation of adaptive responses to environmental variation.

The diet of *P. novaehollandiae* has two major components: nectar, and aerial insects (Recher 1977, Ford & Paton 1977, Paton 1982). Nectar is the main source of energy for *P. novaehollandiae*, while insects provide a rich source of protein (Paton 1982, Ford & Paton 1982). *Phylidonyris novaehollandiae* spend the majority of their time foraging for nectar, and are most likely to be limited by their ability to meet their energy requirements from nectar than by their ability to meet their protein requirements (Paton 1982). The abundance of inflorescences, and subsequently nectar availability, is directly influenced by rainfall: periods of high rainfall lead to increased inflorescence abundance, and wetter climates experience higher inflorescence abundances (Porter 1978, Wooller et al. 1998, Keaser et al. 2008). South Australia is characterised by spatially variable rainfall and experiences arid, semi-arid, and

dry sub-humid climates (Australian Bureau of Meteorology; http://www.bom.gov.au/index.shtml), allowing for comparison between sites experiencing high- and low-rainfall. Rainfall can also be variable and unpredictable, especially in South Australia, and the system is sometimes referred to as having unpredictable 'boom and bust' conditions, namely high- or low-rainfall periods respectively (Kingsford et al. 1999, Jenkins et al. 2005, Robin et al. 2009).

Evidence for an increasingly insectivorous diet under conditions of reduced food availability, including divergence of key functional morphological traits and foraging behaviour, has previously been observed in *P. novaehollandiae* (Chapter 4). Therefore, under conditions of lower rainfall I expect reduced nectar availability to drive niche expansion, via natural selection, toward an increasingly insectivorous diet. This niche expansion should be reflected by changes in morphological traits that have a key role in feeding and foraging ecology, including bill shape, tarsometatarsal length, and body size. Shorter, deeper bills provide increased crushing force and allow handling of larger prey items (Bowman 1961; Lederer, 1975; Grant & Grant 1989). Typically, more insectivorous honeyeaters have shorter, less slender bills than nectarivorous honeyeaters (Lederer 1975, Wooller 1984, Wooller 1988). Longer tarsi improve the efficiency of gleaning behaviour during foraging (Fitzpatrick 1985) and increase spring, allowing quicker sallying from a perch (Sherry 1982). Gleaning and sallying are typical insect foraging behaviours, and an insectivorous honeyeater is expected to benefit from having a longer tarsus. Smaller body size increases aerodynamic efficiency and aerial agility (Székely et al. 2004, Raihani et al. 2006) allowing more efficient aerial foraging of insects. Therefore, under conditions of lower rainfall, I expect to observe a shift towards shorter, less slender bills, longer tarsi, and smaller body sizes.

In this study I used morphological, rainfall, and molecular genetic data to test for evidence of divergent natural selection. I (1) examined variation in morphological traits of *P. novaehollandiae* across a spatially variable landscape in South Australia; (2) examined the spatial genetic structure of *P. novaehollandiae*; (3) examined spatial variation in rainfall intensity; and (4) used comparative analyses to detect signals of selection from rainfall on morphology.

METHODS

Study species and sites

The New Holland Honeyeater, (*Phylidonyris novaehollandiae*), is a common species of small passerine that occurs in coastal regions of south-eastern and south-western Australia. Breeding in *P. novaehollandiae* occurs in autumn and spring (McFarland 1986, Paton 1985a), although breeding activity is generally higher during the spring following the wet season (Rooke 1979, Paton 1985a, McFarland 1985, Armstrong & Pyke 1991, Lambert et al. 2006). During breeding, the sexes partition roles, with males spending more time defending their territory and nest, while females spend more time nesting, including nest construction, incubation, and providing the majority of nestling feeding (McFarland 1985, Clarke & Clarke 1999). Females forage closer to the nesting site than males and use more nectar resources (McFarland 1985). Outside of the breeding season, *P. novaehollandiae* may continue to maintain territories used for breeding, either in mating pairs or as individuals, or they may disperse (McFarland 2002), though these behaviours have not been well studied; and never using genetic markers.

I studied *P. novaehollandiae* at seven sites in southern South Australia comprising one island and three mainland peninsulas: (1) Flinders Chase National Park (Kangaroo Island); (2)

Pelican Lagoon Conservation Park (Kangaroo Island); (3) Sandy Creek (Fleurieu Peninsula); (4) Cox Scrub/Scott Conservation Parks (Fleurieu Peninsula), (5) Newland Head Conservation Park (Fleurieu Peninsula); (6) Innes National Park (Yorke Peninsula); and (7) Port Lincoln (Eyre Peninsula) (Fig. 1). Vegetation at each site, excluding Port Lincoln, is described in Rix (1976), Ford and Paton (1977), Ford and Paton (1982), Westphal et al. (2003), Kleindorfer et al. (2006b), Schlotfeldt and Kleindorfer (2006), Galligan and Kleindorfer (2008), Oorebeek et al. (2009), and Chapter 4. Vegetation at Port Lincoln comprises mallee. Coastal White Mallee *Eucalyptus diversifolia* and Yorrell *Eucalyptus gracilis* are dominant tree species found throughout thickly vegetated areas. *Eucalyptus diversifolia* is accompanied often by understorey species including Wallowa *Acacia calamifolia*, Coastal Velvet-bush *Lasiopetalum discolor*, Felted Wallaby Bush *Beyeria lechnaultii*, Dwarf Hop Bush *Dodonaea humilis*, and the Shiny Ground Berry *Acrotriche patula* (South Australian Department of Environment and Heritage 2009).

Sample collection

I used only adult birds in this study, aged by morphology (Disney 1966, Higgins & Peter 2002). I mist-netted a total of 670 birds between 2004 and 2009 at the seven sites. I sampled over five days at each site twice per year: once during the wet season (June to November; breeding) and once during the dry season (December to May; non-breeding). Sample size per site, year, and season is shown in Appendix 5.A. Each bird was banded with an aluminium identification band, measured for morphological characteristics, and sampled for blood that was stored on FTA paper for DNA analysis (see Kleindorfer et al. 2006b).

Sex determination

A subset of birds (N = 575) was sexed using the molecular genetic method of Kahn et al. (1998), using conditions outlined in Jensen et al. (2003). DNA was extracted from FTA using method #4 of the protocols outlined by Smith and Burgoyne (2004) (see Chapter 3). The remaining birds (N = 95) had sex assigned using a univariate morphological sexing method. Morphological data from the genetically sexed individuals were used to calculate sexing criterion using the SHEBA sexing programs (Rogers 1995a and 1995b). Sexing criteria were calculated for a minimum probability of correct sexing at 90% and were calibrated regionally to account for regional variation in morphology (Ellrich et al. 2010, Chapter 3). SHEBA estimated the percentage of correctly sexed birds in the population to be 97.2%, so combined with the genetically sexed subset I expect an accuracy of approximately 99.6% for all sex assignments in my data set, assuming 100% accuracy of the molecular genetic method (but see Chapter 3 Method).

Morphology

At the time of banding, I measured six morphological traits; (1) bill length from the tip of the bill to the back of the head (bill-head length); (2) bill length from the tip of the bill to the anterior extreme of the nostril (bill-nostril length); (3) bill depth measured at the base of the bill (bill depth), (4) bill width measured at the base of the bill (bill width); (5) tarsometatarsal length (tarsus length); and (6) body mass (mass). Bill-head length, bill-nostril length, bill depth, bill width, and tarsus length were measured to the nearest tenth of a millimetre using callipers. Mass was measured to the nearest tenth of a gram using scientific scales. All measurements were made by SK (N = 514) and SM (N = 156), who measured birds in all seven study sites. Variation in measurement between researchers for all traits was not significant (t-test; $P > 0.05$), and was less than the variation across sites reported in Appendix 5.B. A second test of measurement error tested morphological variation across sites for each

researcher separately, which mirrored the findings for both researchers combined (Appendix 5.C). Multivariate analysis of variance (MANOVA) with sex as a fixed factor, as well as site, year, and season, revealed a significant effect of sex on morphological variation ($F = 105.95$; $P < 0.001$; Wilk's Lambda = 0.48; Partial $ETA^2 = 0.52$). Examination of trait means for each sex indicated that males had larger measurements for all traits, consistent with previous observations of sexual dimorphism in *P. novaehollandiae* (Disney 1966, Rogers et al. 1986, Higgins & Peter 2002, Chapter 3). Interaction effects with sex showed that variation between sexes did not differ significantly between sites, but did differ significantly across years ($F = 2.30$; $P < 0.001$; Wilk's Lambda = 0.90; Partial $ETA^2 = 0.03$) and seasons ($F = 2.84$; $P = 0.010$; Wilk's Lambda = 0.97; Partial $ETA^2 = 0.03$). Variation between sexes also differed significantly between sites across years ($F = 2.19$; $P < 0.001$; Wilk's Lambda = 0.84; Partial $ETA^2 = 0.03$) and between seasons across years ($F = 2.16$; $P = 0.012$; Wilk's Lambda = 0.95; Partial $ETA^2 = 0.01$), but not between sites across seasons. Due to this variability in morphology between sexes, I separated the sexes for all further analyses of morphology.

A number of the measured traits are expected to be correlated, and I have prior expectations regarding these correlations. The shape of the bill is determined by the interaction of length and base circumference (width and depth). The development of these bill dimensions is largely decoupled, allowing them to be differentially expressed (Abzhanov et al. 2004, Abzhanov et al. 2006, Abzhanov et al. 2007). Therefore, measurements of bill length should be relatively uncorrelated with measurements of bill base circumference. However, measurements taken within each bill dimension should be highly correlated. Bill length measurements should be highly correlated, as each measurement contains a fraction of the other measurement. Bill-head length may also be correlated with body size, as it contains a measure of skeletal head size that is correlated with body size. Bill depth and bill width

measurements should also be highly correlated, considering that their development is tightly linked. Tarsus length should be uncorrelated with other traits, but in some circumstances may correlate with body size (Rising & Somers 1989, Piersma & Davidson 1991, Senar & Pascual 1997). Body mass in birds is commonly correlated with current energy stores and body size (Rising & Somers 1989, Piersma & Davidson 1991, Senar & Pascua, 1997). I carried out a confirmatory factor analysis that included all six measured traits to test these hypothesised relationships; I predict the data set will fit five factors; bill length (high loading for bill-head length and bill-nostril length), bill base circumference (high loading for bill depth and bill width), tarsus length (high loading for tarsus length), mass (high loading for mass), and body size (high loading for any of bill-head length, mass, and tarsus length). I used Varimax rotation to align components most closely with observed variables to facilitate more informative factor analyses. Examination of the correlation matrix showed that it supported my hypothesised relationships in both sexes; it also indicated that mass and bill-head length, but not tarsus, were highly correlated (correlation value > 0.3), presumably due to the shared component of body size. The model indicated a good fit to the data for males (KMO value = 0.62; chi square = 547.76; $P < 0.001$) and females (KMO value = 0.68; chi square = 311.22; $P < 0.001$). In males, factor 1 explained 27.61% of the variation in the model and showed strong loading (> 0.5) of bill depth (0.908) and bill width (0.890); factor 2 explained 20.44% of the variation in the model and showed strong loading of bill-nostril length (0.962) and bill-head length (0.518); factor 3 explained 18.34% of variation in the model and showed strong loading of mass (0.958); factor 4 explained 16.76% of variation in the model and showed strong loading of tarsus length (0.973); factor 5 explained 11.74% of variation in the model and showed strong loading of bill-head length (0.706). In females, factor 1 explained 27.06% of the variation in the model and showed strong loading (> 0.5) of bill depth (0.895) and bill width (0.868); factor 2 explained 17.32% of the variation in the model and showed strong

loading of bill-nostril length (0.946); factor 3 explained 17.04% of variation in the model and showed strong loading of mass (0.956); factor 4 explained 17.00% of variation in the model and showed strong loading of tarsus length (0.973); factor 5 explained 15.82% of variation in the model and showed strong loading of bill-head length (0.706). Based on biological reasoning and the loadings observed in each factor for both sexes, I use these factors to represent morphological traits for all subsequent analyses: factor 1 represents bill base circumference, factor 2 represents bill length, factor 3 represents mass, factor 4 represents tarsus length, and factor 5 represents body size. As mass represents *in situ* energy stores, it is likely to be affected by a number of external parameters such as variation in food consumption, activity, ambient temperature, and condition of health. The majority of this variation is plastic, without an evolutionary basis, and any variation in mass that is not plastic is most likely related to a residual component of body size. Therefore, I removed the mass factor from all analyses testing the evolutionary basis of variation. The full loading of measured traits across the five factors for both sexes can be seen in Appendix 5.D.

Our sample sites vary in composition of ecological variables, and I expect this variation to influence the optimal morphology of key ecological traits. To determine if *P. novaehollandiae* showed variation in morphology between sites I performed a MANOVA (SPSS 17.0; SPSS Inc., Chicago, IL) using the four morphology factors as independent variables and site as a fixed factor. To control for annual and seasonal variation in morphology I included year and season (wet, dry) as fixed factors in the MANOVA. Analysis of variance was also carried out for each fixed factor and their interaction effects. I applied Holm corrections (Holm 1979; Aickin & Gensler 1996) to ANOVA results to adjust for multiple comparisons that address a common null hypothesis (Rice 1989).

Molecular genetic analysis

I genotyped 330 individuals at 10 microsatellite loci: *Pn2, Pn3, Pn4, Pn5, Pn8, Pn13, Pn15, Pn22, Pn23, Pn25* (Chapter 2). Polymerase chain reaction conditions were as outlined in Chapter 2. Prior to performing analyses I tested the suitability of my data for analysis with F- and R- statistics (Hardy et al. 2003). The results of these tests showed that all of the 10 loci were better analysed by F-statistics. The number of alleles (N_A), expected and observed heterozygosities (H_E, H_O), and the inbreeding co-efficient (F_{IS}) were calculated for each locus by site using GENEPOP v4 (Raymond & Rousset 1995) (Appendix 5.E). The same values were also calculated globally for each locus using the program GENEPOP v4 (Appendix 5.F).

I carried out tests of linkage disequilibrium for each locus by site using GENEPOP v4. After Bonferroni correction (Rice 1989), significant departure from linkage disequilibrium was detected for 13 locus pairs across the sites ($P < 0.01$), although 12 of these pairs were found to depart from linkage disequilibrium at only one site. I followed recommendations by Kaeuffer et al. (2007) and estimated the correlation co-efficient (r_{LD}; Black & Krafsur 1985) for these locus pairs using Linkdos software (Garnier-gere & Dillman 1992) (http://genepop.curtin.edu.au/linkdos.html). The r_{LD} for each locus pair was < 0.55 ($P < 0.05$), indicating a distance of greater than 3 cM between loci (Kaeuffer et al. 2007), which is sufficient distance that any linkage effect does not bias clustering analyses (Pritchard and Wen 2004); therefore I retained all loci for further analyses. I tested for Hardy-Weinberg equilibrium within each site using GENEPOP v4. After Bonferroni correction (Rice 1989), two loci, *Pn15* and *Pn5*, differed significantly from Hardy-Weinberg equilibrium at one and two sites respectively, both showing heterozygote deficiency ($P < 0.01$). I investigated the effect of these loci on my analyses by comparing results obtained with and without them. Results were consistent in all cases and I concluded that the observed departures from Hardy-

Weinberg equilibrium for these loci were most likely not enough to significantly bias results. Therefore, keeping these violations in mind I retained all loci for further analyses, assuming the extra information in these loci outweighs any potential biases they may add.

Sex-biased dispersal

In birds with a resource defence mating system (Greenwood 1980, Clarke et al. 1997), such as *P. novaehollandiae* (McFarland 1985; Pyke et al. 1989), there is a tendency for male-biased philopatry, where males remain in or near their natal territory. Sex-biased dispersal can contribute to sexual variation in patterns of spatial (and temporal) neutral genetic differentiation and phenology. I tested sex-biased dispersal using the program FSTAT v2.9.3.2 (Goudet 1995; Goudet 2001).

Goudet et al. (2002) recommend two tests with overlapping ranges of effectiveness for examining sex-biased dispersal -- a test that examines sexual variation in the variance of assignment index (vAIc), and a test that examines sexual variation in the proportion of neutral genetic variation between samples (F_{ST}). The vAIc test is ineffective at detecting biased dispersal when dispersal frequencies are high, but performs better than the F_{ST} test when dispersal frequency is low. These tests are one-sided, based on the principle that genotypes of the more philopatric sex will be more similar (relative to the dispersing sex) in the population in which they were sampled. I tested the hypothesis that, in *P. novaehollandiae*, males are the more philopatric sex.

The tests showed that male vAIc was not significantly greater than female vAIc within sample populations (P=0.081), but male F_{ST} was significantly lower than female F_{ST} within sample populations (P=0.038). These results support my hypothesis of male-biased

philopatry. It is most likely that dispersal frequency is too high for the vAIc test to detect bias. Comparing my results to those of exhaustive sampling by Goudet et al. (2002), I can estimate the dispersal rate in *P. novaehollandiae* to be approximately > 20% in females. Given the sex-specific effect that this biased dispersal may have on distributions of morphology and genotypes, I consider each sex separately in all further analyses.

Isolation by distance

Evidence from mark-recapture data of *P. novaehollandiae* indicates a limited capacity for dispersal (c.a. 110 km max.) that spans a shorter distance than the distances between some study sites (Paton et al. 2004). Additionally, museum vouchers and sight records (fig. 1), as well as a general survey of the land, suggest that there are no obvious geographical or environmental discontinuities between the study sites that are likely to disrupt dispersal. With limited dispersal and no significant barriers to gene flow between the study sites, I expect to observe a pattern of isolation by distance. I tested the contribution of isolation by distance to genetic differentiation. When dispersal is unrestricted in two dimensions, a positive regression slope of $F_{ST}/(1-F_{ST})$ on log of distance is expected (Rousset 1997). I used the program SPAGEDi v1.2 (Hardy & Vekemans 2002) to calculate pairwise $F_{ST}/(1-F_{ST})$ for each sex. I calculated the shortest distance across land between two points (km); I assumed that dispersal between Kangaroo Island and mainland South Australia only occurs at the narrowest point across Backstairs Passage (a distance of ~14 km). Log of distance was subsequently computed from these distances. I evaluated the correlation between log of distance and pairwise $F_{ST}/(1-F_{ST})$ for each sex using $1x10^7$ randomisations. I accounted for non-independence of distance correlations inherent with matrix data by using Mantel tests in the software program zt (Bonnet & Van de Peer 2002).

Genetic population structure analysis

To determine population structure using multi-locus genotype data, I used a model-based clustering method based on a Bayesian model in the program STRUCTURE v2.3.2 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). I used STRUCTURE because it is a standard reference for Bayesian inference of population structure, most likely due to the variety of extensively tested modelling options available to the user. All Bayesian models are based on assumptions of two types: (1) a prior distribution for unknown quantities (such as clustering and allele frequencies); and (2) a likelihood function relating these unknown parameters to the observed genotypes. In STRUCTURE, the unknown parameters are inferred through Markov Chain Monte Carlo (MCMC) computation. The standard STRUCTURE model makes no assumptions about prior clustering, and therefore the assumed prior for clustering is uniform (but see Hubisz et al. 2009). For data sets when differentiation is low, Hubisz et al. (2009) suggest using the LOCPRIOR model, which differs from the standard admixture model by incorporating site information into the inference. More specifically, the LOCPRIOR model with admixture uses a modified prior distribution for clustering that allows the distribution of cluster assignments to vary by site. This prior is such that, when site information is uninformative, the LOCPRIOR model behaves like the standard model; but when site information is informative, the LOCPRIOR model will depart from the standard model (Hubisz et al. 2009).

In the STRUCTURE model the number of clusters, K, is a fixed parameter, set by the user. Basically, the model works by probabilistically assigning individuals to a population (or populations, in the case of admixture) in a way that minimises departure from Hardy-Weinberg equilibrium at each locus while conforming to the set value of K. The model assumes that loci *within* populations are in Hardy-Weinberg equilibrium and linkage

equilibrium. The procedure to find the number of clusters, K, which best fit the data consists in running multiple MCMC replicates for varying values of K and inferring the most likely value for K from the approximation of their posterior probabilities. Based on mark-recapture data of *P. novaehollandiae* (Paton et al. 2004), I expect structure to be relatively weak. Therefore, I used the admixture model which is recommended for closely related populations. This model allows each individual to have partial ancestry in each population and accordingly clusters admixed individuals into two or more populations indicated by their genotypes. For the same reason, I used the option that takes into account the likelihood that allele frequencies are correlated across populations. Pritchard et al. (2000) suggest that chains should converge within $1x10^4$ and $1x10^5$ MCMC iterations, so I investigated convergence and mixing properties of chains by running three replicates of $1x10^5$ MCMC iterations in length for each value of K, ranging from 1-8 (K = 8 is the maximum number of populations that my data set can be expected to reliably detect within the constraints of sample size, and I am making no a priori assumptions about the number of populations that best fit the data). Sensitivity of the data to alternative hyper-parameter priors was also tested by running further MCMC chains using different priors. All chains converged with mixing within $3x10^4$ MCMC iterations; therefore, I chose a relatively conservative burn-in of $5x10^4$ MCMC iterations, which I fixed for all further runs. Runs using different hyper-parameters showed consistent results, but I reverted to the default priors (mean = 0.01, standard deviation = 0.05, lambda = 1) for all further runs as they make the algorithm more sensitive to subtle structure (Falush et al. 2003). I explored the data for consistency between replicate runs by running multiple MCMC replicates for a range of longer and shorter chains, for a range of K. Because longer runs are time consuming, I ran these exploratory chains for only three values of K (K = 1, K = 4, and K = 8) spread across my range of possible K (K = 1-8). The results indicated that a chain length of $1x10^5$ MCMC iterations was most appropriate. Using the optimised burn-in

length ($5x10^4$ iterations) and MCMC length ($1x10^5$ iterations), I ran 10 MCMC replicates for K = 1-8. Although there is some debate about the best method for inferring clusters (Evanno et al. 2005), my data appeared to best fit the method published in the original structure paper -- I have a relatively large data set, I expect a small number of discrete clusters, and I expect homogeneous patterns of dispersal between clusters -- which involves comparing mean log likelihoods penalized by one-half of their variance (Pritchard et al. 2000).

Rainfall

Our sample sites vary in distance from coast, latitude, altitude, and other variables that influence rainfall intensity. Therefore, I expect rainfall intensity to vary between sites. To represent rainfall intensity at each site, I used the measure of mean monthly rainfall calculated across the study period. Rainfall data were obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au/index.shtml) using the nearest meteorological station to each site (minimum distance = 0 km; maximum distance = 51 km; average distance = 17 km): Port Lincoln: North Shields meteorological station; Innes NP: Warooka meteorological station; Flinders Chase NP: Cape Borda meteorological station; Pelican Lagoon CP: Cape Willoughby meteorological station; Newland Head CP: Parawa meteorological station; Cox and Scott CPs: Kuitpo Forest Reserve meteorological station; and Sandy Creek: Rosedale meteorological station. To determine if rainfall intensity varied among sites, I used a univariate ANOVA (SPSS 17.0; SPSS Inc., Chicago, IL) with rainfall as the dependent variable and year, season, and site as the fixed factors.

Morphology-rainfall correlation

I expected to observe habitat-phenotype correlations, such that dominant phenotypes will reflect requirements imposed by the habitat. I used the four morphology factors to represent

the phenotypic variables and I used mean monthly rainfall at each site (which I refer to as rainfall intensity) to represent the habitat variable. I expected a biologically meaningful correlation between rainfall intensity and morphology. According to the role of each morphological trait in foraging and the expected effect of rainfall intensity on food availability (addressed in the introduction), I expected bill length and body size to be positively correlated with rainfall ($r > 0$); and I expected bill base circumference and tarsus length to be negatively correlated with rainfall ($r < 0$). To test these correlations, I carried out regression analyses using the four morphology factors as the dependent variables and rainfall intensity (mean monthly rainfall) as the independent variable. I applied Holm corrections (Holm 1979; Aickin & Gensler 1996) to adjust for multiple comparisons that address a common null hypothesis (Rice 1989).

Phenotypic and genetic differentiation (P_{ST}, Pseudo-Q_{ST}, and F_{ST})

Phenotypic variation between populations can arise from genetic drift alone, or coupled with any combination of natural selection, habitat selection, or phenotypic plasticity. Spitze (1993) developed a measure of genetic differentiation at quantitative traits, the quantitative genetic differentiation coefficient (Q_{ST}):

$$QST = \frac{V_{A}between}{V_{A}between + 2V_{A}within} \qquad [1]$$

where $V_{A_{between}}$ and $V_{A_{within}}$ are the additive genetic variances between and within populations. Generally speaking, phenotypic variances, weighted by heritability values, can be used as a substitute for the additive genetic variances, such that:

$$QST = \frac{h2between\ V_{P}between}{h2between\ V_{P}between + h2within\ 2V_{P}within} \qquad [2]$$

The quantitative genetic differentiation coefficient is analogous to the neutral genetic differentiation coefficient, F_{ST}. The Q_{ST} of a trait, or suite of traits, can be compared to F_{ST} to give an indication of the dominant force driving population divergence. If Q_{ST} significantly

exceeds F_{ST}, there is evidence to suggest that the trait has diverged more than expected by genetic drift alone (but see O'Hara & Merilä 2005, Whitlock 2008). However, calculating Q_{ST} requires quantifying the additive genetic components of variance for traits within and among populations, which can only be achieved with intricate common-garden experiments. In cases where quantitative genetic designs are not practical, P_{ST} -- the phenotypic analogue of Q_{ST} -- may be used (Leinonen et al. 2006, Raeymaekers et al. 2007, Gay et al. 2009). For P_{ST}, additive genetic variance is replaced by phenotypic variance, such that:

$$PST = \frac{V_B between}{V_B between + 2V_B within} \qquad [3]$$

Assuming the contribution of environmental and non-additive genetic variation to phenotypic variation is low, P_{ST} will closely resemble Q_{ST} and can be used as a valid substitute. However, it is rare to find this scenario in nature and it should definitely not be assumed (Puyol et al. 2008). With that said, precautions can be taken to avoid artefacts due to strong environmental effects or non-additive genetic variance when the additive genetic components of variance, or heritability, are unknown. Examining how Q_{ST} (equation 2) behaves given a range of between- and within- heritability values provides estimates that take into account the uncertainty of heritability, known as 'pseudo-Q_{ST}'. I allowed the between population additive genetic proportions of difference (h^2 between) to vary from 0.05 to 1; 0.05 is a conservatively low estimate of between population heritability, based on heritabilities reported in the literature (Boag & Grant 1978, Smith & Zach 1978, Wiens & Rotenberry 1980, Boag 1983, Grant 1983, Noordwijk et al. 1988, Wiggins 1989, Potti & Merino 1994, Hõrak & Tammaru 1996, Keller et al. 2001). I set the within population additive genetic proportions of difference (h^2 within) at 0.25 and 1. I calculated the morphological variances among populations by performing a one-way analysis of variance (ANOVA) with population as the dependent variable on each morphology factor for each sex. I used the mean square estimates

to calculate the between- ($V_{P_{between}}$) and within- ($V_{P_{within}}$) population variance. I calculated P_{ST} and pseudo-Q_{ST} between all pairwise comparisons for each morphology factor for each sex. Conventional methods for estimating variance in Q_{ST} estimates consider a global Q_{ST} and necessitate a large number of populations -- two conditions not met by my data. Therefore, as a conservative measure, I calculated the standard deviation of pseudo-Q_{ST} estimates across pairwise comparisons and used them as upper (for estimates using $h^2_{within} = 0.25$) and lower (for estimates using $h^2_{within} = 1$) confidence intervals. I estimated global neutral genetic differentiation between sites -- F_{ST} -- according to Weir & Cockerham (1984), using the program SPAGEDi v1.2 (Hardy & Vekemans 2002). To estimate the variance in F_{ST}, I estimated 95% confidence intervals for F_{ST} by jack-knifing over loci.

RESULTS

Morphology

In males, multivariate analysis of variance (MANOVA) with site as a fixed factor, as well as year, and season, revealed a significant effect of site ($F = 4.13$; $P < 0.001$; Wilk's Lambda = 0.75; Partial $ETA^2 = 0.07$), but not year or season, on morphological variation. A significant interaction effect of site and year ($F = 2.15$; $P < 0.001$; Wilk's Lambda = 0.80; Partial $ETA^2 = 0.06$) on morphological variation was observed, indicating variation in morphology between sites across years.

Table 5.1 shows results of the ANOVAs testing the effect of dependent variables found to have a significant effect on morphological variation (MANOVA) on each morphology variable in males. The ANOVAs indicated significant variation in all morphological factors between sites. A significant interaction effect of site and year was only observed for bill length and bill base circumference.

In females, MANOVA revealed a significant effect of site ($F = 3.02$; $P < 0.001$; Wilk's Lambda = 0.71; Partial $ETA^2 = 0.08$), and season ($F = 5.82$; $P < 0.001$; Wilk's Lambda = 0.90; Partial $ETA^2 = 0.10$), on morphological variation. A significant interaction effect of site and year ($F = 2.48$; $P < 0.001$; Wilk's Lambda = 0.69; Partial $ETA^2 = 0.09$) and season and year ($F = 5.19$; $P < 0.001$; Wilk's Lambda = 0.82; Partial $ETA^2 = 0.09$), on morphological variation was observed, indicating variation in morphology between sites across years, and between seasons across years.

Table 5.2 shows results of the ANOVAs testing the effect of dependent variables found to have a significant effect on morphological variation (MANOVA) on each morphology variable in females. The ANOVAs indicated a significant effect of site on variation in bill length and bill base circumference. A significant effect of season on bill length and bill base circumference was also observed. The following interaction effects were significant: site and year on bill length and body size; and season and year on bill length and bill base circumference.

Molecular Genetic analysis

Of 3300 data points (genotypes for 330 individuals at 10 loci), there were 192 (5.8%) missing values. The number of alleles per locus ranged from 6 to 18 (mean 13.5), expected heterozygosity ranged from 0.672 to 0.913 (mean 0.827), and allelic richness ranged from 4.450 to 13.719 (mean 10.152).

Isolation by distance

Figure 5.2 shows a scatter plot of pairwise $F_{ST}/(1-F_{ST})$ correlated with log of distance for each sex. Mantel tests examining the correlation between pairwise $F_{ST}/(1-F_{ST})$ and log of pairwise distance showed significant positive correlations in both males (r=0.77, P=0.033) and females (r=0.75, P=0.024).

Genetic population structure analysis

Estimates of the logarithm of probability of the data averaged over the 10 MCMC replicates for each value of K were maximal for K = 1. Mean logarithm of probability of the data for all values of K are shown in Appendix 5.G. As population differentiation appears to be low for these data, I also implemented the LOCPRIOR model. Applying the LOCPRIOR model to the data, and using the admixture model, allowing for correlated allele frequencies, I investigated convergence and mixing properties of chains, and sensitivity of the data to alternative priors for the hyper-parameters as previously stated in the Methods for the standard model. All chains converged with mixing within $2x10^4$ MCMC iterations; therefore, I chose a relatively conservative burn-in of $5x10^4$ MCMC iterations, which I fixed for all further runs. As stated in the Methods, I reverted to the default priors (mean = 0.01, standard deviation = 0.05, lambda = 1) for all runs. I explored the data for consistency between replicate runs as previously stated in the Methods for the standard model. The results indicated that a chain length of $1x10^5$ MCMC iterations was most appropriate. Using the optimised burn-in length ($5x10^4$ iterations) and MCMC length ($1x10^5$ iterations), I ran 10 MCMC replicates for K = 1-8. Averaged over the 10 MCMC replicates for each value of K, the logarithm of probability of the data was maximal for K = 3; different to the estimate of K = 1 made using the standard model. Mean logarithm of probability of the data for all values of K are shown in Appendix 5.G. I examined the estimated cluster membership for each individual of the MCMC replicate having the highest logarithm of probability of the data for K = 3, and assigned individuals to

the cluster for which the membership was the highest (Appendix 5.H). One cluster had 256 individuals assigned to it, another had 36 individuals assigned to it, and the final cluster had 34 individuals assigned to it. The three clusters generally represented spatial groups, with individuals from (1) Kangaroo Island and Fleurieu Peninsula (KI/FP), (2) Innes National Park (INP), and (3) Port Lincoln (PL), respectively. Five individuals did not conform to this pattern of spatial clustering: three individuals from Flinders Chase National Park were assigned to the PL cluster (mean membership to PL cluster = 0.674), one individual from Innes National Park was assigned to the KI/FP cluster (membership to KI/FP cluster = 0.648), and one individual from Scott/Cox Conservation Parks was assigned to the PL cluster (membership to PL cluster = 0.528). Despite these exceptions, the LOCPRIOR model was successful in delimiting structure that is biologically meaningful. Three facts support the findings of the LOCPRIOR model over those of the standard model: (1) a number of individuals from spatially distinct sites were clustered together; (2) there is a strong indication of shared ancestry between the clusters, which is expected with high gene flow; and (3) the clusters make biological sense by conforming to patterns of geographic separation. Therefore, population estimates based on the LOCPRIOR model should most accurately represent the true populations. I believe the most likely reason for the standard structure model failing to detect structure was due to the strong signal of isolation by distance, which can swamp clustering estimates (Pritchard & Wen 2003).

I carried out standard molecular genetic analysis for microsatellite loci within the three clusters identified by the STRUCTURE program. The number of alleles (N_A), expected and observed heterozygosities (H_E, H_O), and the inbreeding co-efficient (F_{IS}) were calculated for each locus by cluster using GENEPOP v4 (Appendix 5.I). I carried out tests of linkage disequilibrium for each locus by cluster; after Bonferroni correction, significant departure

from linkage disequilibrium was detected for 1 locus pair in one population ($P < 0.01$). The estimated correlation co-efficient (r_{LD}) for this locus pair, calculated using LINKDOS, indicated that the loci were separated by a distance of greater than 3 cM ($r_{LD} < 0.55$; $P < 0.05$). I tested for Hardy-Weinberg equilibrium within each population; after Bonferroni correction, two loci, *Pn4* and *Pn5*, differed significantly from Hardy-Weinberg equilibrium in one population (KI/FP), both showing heterozygote deficiency ($P < 0.01$).

Rainfall

Table 5.3 shows results of the ANOVA examining the effect of year, season, and site on variation in rainfall. The ANOVA showed a significant effect of season and site on rainfall, and a significant interaction effect between season and year on rainfall.

Morphology-rainfall correlation

Table 5.4 shows the regression coefficient (r) and one-tailed significance for regressions examining the correlation between morphology factors and rainfall intensity (mean monthly rainfall) for both sexes. For males, bill length and body size showed a significant positive correlation with rainfall intensity, while tarsus length and bill base circumference showed a significant negative correlation. For females, body size showed a significant positive correlation with rainfall intensity, while bill base circumference showed a significant negative correlation.

Phenotypic and genetic differentiation (P_{ST}, pseudo-Q_{ST}, and F_{ST})

In males, genetic differentiation (F_{ST}) among regions ranged from 0.2% to 4.7%. Ten of 21 pairwise comparisons showed significant deviation from 0 (Appendix 5.J). In females, genetic differentiation among regions ranged from 0% to 3.2%. Three pairwise comparisons

showed negative F_{ST} (effectively 0) and one showed an F_{ST} of 0. Seven pairwise comparisons showed significant deviation from 0 (Appendix 5.J). A geographic pattern was observed for F_{ST} estimates in males and females: all significant pairwise comparisons included either Port Lincoln or Innes National Park. Differentiation of all tested morphological factors in both males and females exceeded neutral genetic marker differentiation, even assuming very low values of additive genetic variance (Figure 5.3).

DISCUSSION

I found evidence for variation in morphological traits with ecological function across populations of *P. novaehollandiae* in both males and females in South Australia. The strong and almost exclusive effect of site on variation for all male morphological factors suggests that site-related phenomena, such as prevailing ecological conditions, were the driving force of divergence. These findings are in line with the prediction that divergent natural selection between sites will be strong. Alternatively or in concert, these patterns may also arise via genetic drift (Slatkin 1987), habitat selection (discussed in Stamps et al. 2005, and Stamps 2009), or phenotypic plasticity (discussed in Scheiner 1993). In light of these possible alternative factors, I must consider my evidence from genetic and environmental data to gain further insight into the mechanistic basis for divergence. Having said this, variation between sites across years was observed for bill length and bill base circumference. If genetic drift were driving divergence in these traits, the divergence should be uniform between sites across years (unless there is a gene flow bias between populations), and a pattern such as this is not likely to develop. Therefore, genetic drift does not appear to be driving the observed patterns in this case, but evidence from genetic data is required to be sure.

I found sex differences in the magnitude of variation across sites and seasons. In females, I found both a weaker effect of site on morphological variation (only bill length and bill base circumference showed significant variation between sites) and a stronger effect of season on morphological variation than in males. Both of these findings are consistent with the observation of higher dispersal in females. Dispersal is energetically demanding (Van Vuren & Armitage 1994, Merilä 1997) and therefore is likely to act selectively on traits that benefit energy acquisition (i.e. foraging) or enable energy efficiency in dispersal. In birds with male-biased philopatry, such as what I observed in *P. novaehollandiae*, female-biased post-natal dispersal is expected (Greenwood 1980). Dispersal frequency should be seasonally bimodal -- minimal during the breeding season when birds aggregate for mating, and therefore maximal during the non-breeding season. Therefore, selection driven by dispersal should be seasonal, and stronger in females than males. However, in an absence of direct evidence that dispersal has a selective cost in the study species, more evidence is required to test the hypothesis that natural selection is driving morphological divergence.

The fact that all male traits, but only some female traits, showed variation between sites indicates a potential for asymmetry between the sexes in mechanisms that affect the spatial pattern of morphological distribution that I found. The significant interaction effects of sex with year, season, site and year, and season and year, on morphological variation between the sexes observed in the MANOVA with sex as the fixed factor supports the idea of sexual asymmetry in mechanisms affecting the spatial morphological distribution. However, further examination of these interaction effects by ANOVA (correcting for multiple comparisons) showed that the effect of sex with season, and season and year, was not significant for any morphological variable. The effect of sex with year was only significant for mass, and the effect of sex with site and year was only significant for bill depth. Therefore, the case for

asymmetry in the processes driving morphological divergence between males and females is only supported for mass and bill depth; and even then, the support is weak. Mass may have a large component of variance influenced by the environment; and the fact that bill width did not show the same pattern of variation as bill depth, despite a strong correlation in the development of bill depth and width, weakens the case for bill depth. However, if such asymmetry between the sexes were to exist, it may arise from the sex-specific roles identified in *P. novaehollandiae* during breeding such as uniparental incubation and territory defence (Recher 1977, McFarland 1985, Clarke et al. 1997, Arnold et al. 2007, Arlt & Pärt 2008), and sex-specific foraging ecology (Shine 1989, Pasinalli 2000, Thaxter et al. 2009, Weimerskirch et al. 2009, Herrel et al. 2010). Results of ANOVAs carried out for significant interaction effects in the MANOVA with sex as a fixed factor can be seen in Appendix 5.K.

The pattern of isolation by distance (IBD) observed in both sexes was particularly strong ($r \geq 0.75$), which I expected with an absence of physical barriers coupled with the limited dispersal capacity of *P. novaehollandiae*. A signal of IBD confirms that gene flow between populations is possible but restricted (Slatkin 1993, Rousset 1997, Hutchinson & Templeton 1999). The geographic pattern that I found for pairwise estimates of genetic differentiation (F_{ST}) is consistent with the observed pattern of IBD. Under IBD, allele frequencies are expected to vary gradually with distance, and genotypes from the most remote sites are expected to be most differentiated. Accordingly, I found that pairwise comparisons including either Port Lincoln or Innes National Park, the two most remote sites, were the only sites with significant F_{ST}, which is also reflected in the number and composition of clusters found by STRUCTURE using the more powerful LOCPRIOR model The contrasting finding from the original STRUCTURE model and the LOCPRIOR model likely reflect insensitivity of STRUCTURE

to an underlying population model of IBD with inadequate spatial sampling of populations in a "stepping stone" distribution (Pritchard et al. 2010).

Based on the expected ecological function of quantitative traits I predicted that, across a heterogeneous environment, the additive genetic component of variation of quantitative traits would exceed neutral genetic differentiation; therefore, I sought to compare these measures. However, my study lacked information on genetic variance of quantitative traits (Q_{ST}), so I used phenotypic variation (P_{ST}) as a substitute. When comparing neutral genetic variation (F_{ST}) with phenotypic variation (P_{ST}), a number of assumptions must be considered (see Whitlock 2008). It is pertinent that estimates of neutral genetic variation conform to the expectations of neutral divergence. Analyses carried out on microsatellite data adhering to the genetic clusters identified by STRUCTURE confirm neutrality for the majority of loci, showing no significant deviation from Hardy-Weinberg equilibrium and linkage disequilibrium. However, the loci *Pn4* and *Pn5* showed significant heterozygote deficit, which was identified as most likely the result of null alleles. In the presence of null alleles, F_{ST} and genetic distances tend to be overestimated (Chapuis & Etoup 2007). Null alleles are most likely to be observed in (1) populations with large effective population sizes; (2) loci with an unusually high mutation rate in the flanking regions; and (3) loci that have diverged from the population from which the clone was sampled (Chapuis & Etoup 2007). I do not expect microsatellites to have diverged from the sample, as the sample originated within the study area, and within the last 50 years. It is also unlikely that null alleles are caused by high mutation rates in the flanking regions, as one would expect deviations to occur in all populations; instead, we see deviation in only the population with the largest population size. These facts are parsimonious with null alleles resulting from the effect of large effective population size. Regardless, presence of null alleles should not affect my conclusion as Q_{ST} estimates

exceeded F_{ST} in every comparison. To test the effect of the loci *Pn4* and *Pn5* on my F_{ST} estimates I re-calculated F_{ST} using a data set with these loci removed -- the subsequent F_{ST} estimates (male = 0.016; female = 0.008) were lower than the original estimates (male = 0.018; female = 0.013), as predicted.

Similarly for P_{ST}, estimates should accurately reflect the partitioning of genetic variance. Unfortunately, P_{ST} assumes a relatively small effect of environment and non-additive genetic variance (Saether et al. 2007) that confounds the effects of plasticity with additive genetic variance. This can lead to overestimation of P_{ST} if a large component of phenotypic variance is explained by plastic expression. To avoid this, I examined how P_{ST} estimates varied with the additive genetic proportion of differences between and within populations, giving pseudo-Q_{ST} values. In males and females, the value of Q_{ST} exceeded that of F_{ST} for all values of additive genetic proportion of differences between and within populations, for all traits. Because the traits I examined have been found in other birds to be highly heritable (Boag & Grant 1978, Smith & Zach 1978, Wiens & Rotenberry 1980, Boag 1983, Grant 1983, Noordwijk et al. 1988, Wiggins 1989, Potti & Merino 1994, Hõrak & Tammaru 1996, Keller et al. 2001), and because I tested comparatively low between- and within-population heritability values using the pseudo-Q_{ST} method, I am confident that the effect of environment and non-additive genetic variance has been accounted for. Subsequently, the large values observed for Q_{ST}, relative to F_{ST}, for all morphological variables in males and females indicates a predominant role of selection in the divergence of morphological traits. However, my study does not control for spatial variation that may arise by dispersal (ie habitat selection), and this apparent signal of divergent natural selection may instead be a signal of habitat selection.

All sites showed seasonal and annual variation in rainfall. Further investigation of the data revealed that seasonal variation was explained by the predictable wet and dry seasons that are characteristic of southern South Australia. Rainfall averaged 56.3 mm/month during the wet season and 31.3 mm/month during the dry season. The significant annual variation in rainfall was explained by an early wet season in 2007, when the months of January and April received anomalously high rainfall. Most importantly, however, mean monthly rainfall varied across sites (as did *P. novaehollandiae* morphology), which I expected based on the geographic dissimilarities apparent between the sites.

I predicted that, under conditions of lower rainfall, reduced nectar availability would drive niche expansion toward a more generalist diet (i.e. increased proportion of insects). Based on ecological function, I expect that natural selection will shape morphology toward shorter, less slender bills, longer tarsi, and smaller body sizes under conditions of lower rainfall. This should be reflected by positive correlations between bill length and rainfall, and body size and rainfall; and negative correlations between bill base circumference and rainfall, and tarsus length and rainfall. In males, all morphological traits correlated with rainfall as predicted if diet, influenced by rainfall, were driving divergent natural selection. In females, bill base circumference and body size correlated with rainfall as we would predict if rainfall were driving divergent natural selection.

The hypothesis that habitat selection is driving morphological divergence is not parsimonious with the observed morphology-rainfall correlations, as habitat selection should result in an environmental correlation with only a single trait, or multiple traits with linked expression or co-dependent function. In addition, under habitat selection, in a system with female-biased dispersal such as what I observed in *P. novaehollandiae*, variation between sites should be

greater in females than in males; however, the opposite is observed. Likewise, the hypothesis of phenotypic plasticity is not parsimonious with these correlations for a similar reason. Under phenotypic plasticity, a strong correlation with environment is not expected in such a large number of traits – except if plasticity is adaptive (Via et al. 1996, Charmantier et al. 2008). However, adaptive plasticity requires a relatively unchanging environment to develop (Via et al. 1996). To the contrary, the study area is characterised by 'boom and bust' conditions (Kingsford et al. 1999, Jenkins et al. 2005, Robin et al. 2009). Furthermore, the low values of genetic differentiation indicate high rates of dispersal which suggests a large amount of movement between different habitat types. Having rejected the hypotheses that habitat selection, phenotypic plasticity, or genetic drift are responsible for the observed divergence across habitats for all morphologic traits, and considering the evidence in favour of selection – additive genetic variance that exceeds neutral genetic variance, and in most cases a significant correlation with rainfall – it is safe to assert that selection must be the driving force of spatial variation in the traits investigated. For all morphological traits in males, and bill base circumference and body size in females, correlations with rainfall were as predicted if, under conditions of lower rainfall, reduced nectar availability drives niche expansion toward an increasingly insectivorous diet.

The strong signal of natural selection found for bill length and tarsus length in females indicated by pseudo-Q_{ST} and not explained by an effect of rainfall has a number of possible explanations. Density-dependent sexual selection (Kokko & Rankin 2006) and density-dependent natural selection (Spottiswoode 2007) are both candidate processes as they can be relatively free from environmental influence. However, density-dependent sexual selection is not a likely explanation because in birds with a territory defence mating system where males maintain breeding territories, such as in *P. novaehollandiae* (Recher 1977, McFarland 1985),

mate choice is typically by females, and sexual selection should be largely focussed on males. Density-dependent natural selection is a more likely explanation: female *P. novaehollandiae* are the sole incubators (Recher 1977, McFarland 1985) and could therefore experience higher predation risk, as predation risk has been shown to be directly proportional to time spent incubating in another passerine (Kleindorfer & Hoi 1997); and predation risk is known to be density-dependent (Ferrière et al. 2004). In terms of selection for the observed morphology: under predation we may expect selection to favour longer tarsi as longer tarsi increase spring (Sherry 1982), potentially enabling quicker escape. It is not clear that density-dependent natural selection, especially that influenced by predation, could influence bill length in females, and I conclude that density-dependent selection on bill length is not likely. A more parsimonious hypothesis is that rainfall drives selection in all four morphological traits in females, but female dispersal dilutes the signal of correlation between morphology and rainfall; this assumes that the strength of selection is weaker for bill length and tarsus length. This hypothesis is supported for bill length, by the observation of significant seasonal variation in bill length. Analysis of the patterns of bill length distribution across wet and dry seasons shows a correlation with rainfall that is predicted if rainfall is driving divergent natural selection; with larger bills, on average, observed in wet seasons. Observations for tarsus length are also compatible with this hypothesis. This hypothesis is further supported by the observation of female-biased dispersal and male-biased sex ratio (Paton 1985b) in *P. novaehollandiae*.

Based on the combined evidence of my data, I conclude that rainfall has shaped divergent natural selection in bill length and slenderness, tarsus length, and body size across a climatically varied landscape in South Australia. Nectar is an important resource for *P. novaehollandiae* (Recher 1977, Ford & Paton 1977) and is their primary source of energy

(Paton 1982, Ford & Paton 1982). Under conditions of reduced nectar availability, *P. novaehollandiae* increase their foraging niche (Myers et al. 2010). During low rainfall conditions, birds in our study had (1) a shorter and wider bill, (2) longer tarsi, and (3) smaller body size. While speculative, these traits could be considered adaptive under low rainfall conditions for the following reasons. A shorter and wider bill favours increased crushing force and prey size handling ability, thereby potentially increasing the range of prey sizes as well as crushing force for hard prey characteristic of drought conditions (Bowman 1961; Lederer, 1975; Grant & Grant 1989). Longer tarsi extend an individual's reach during gleaning (defined as the removal of prey from a surface) and allow quicker sallying from a perch (Fitzpatrick 1985, Sherry 1982). Finally, smaller body size increases aerodynamic efficiency and aerial agility (Székely et al. 2004, Raihani et al. 2006).

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Table 5.1. Results of ANOVAs testing the effect of dependent variables on variation in morphology carried out for variables found to have a significant effect on morphological variation (MANOVA) in males. Degrees of freedom (df), F-values (F), partial ETA^2, P-values, and corresponding Holmed P-values are shown. Bold indicates significant values.

Trait	Fixed Factor	df	F	Partial ETA^2	P	P_H
Bill length	site	6	**3.535**	**0.061**	**0.002**	**0.004**
	site*year	9	**2.469**	**0.063**	**0.010**	**0.029**
Bill base circumference	site	6	**4.292**	**0.073**	**0.000**	**0.001**
	site*year	9	**2.891**	**0.073**	**0.003**	**0.011**
Tarsus length	site	6	**3.543**	**0.061**	**0.002**	**0.002**
	site*year	9	1.561	0.041	0.126	0.251
Body size	site	6	**4.180**	**0.071**	**0.000**	**0.001**
	site*year	9	0.892	0.024	0.533	0.533

Table 5.2. Results of ANOVAs testing the effect of dependent variables on variation in morphology carried out for variables found to have a significant effect on morphological variation (MANOVA) in females. Degrees of freedom (df), F-values (F), partial ETA^2, P-values (P), and corresponding Holmed P-values are shown. Bold indicates significant values.

Trait	Fixed Factor	df	F	Partial ETA^2	P	P_H
Bill length	site	6	**3.215**	**0.086**	**0.005**	**0.015**
	season	1	**10.421**	**0.048**	**0.001**	**0.006**
	site*year	8	**5.279**	**0.171**	**< 0.001**	**< 0.001**
	season*year	2	**14.017**	**0.120**	**< 0.001**	**< 0.001**
Bill base circumference	site	6	**5.621**	**0.141**	**< 0.001**	**< 0.001**
	season	1	**9.634**	**0.045**	**0.002**	**0.007**
	site*year	8	0.792	0.030	0.610	1.221
	season*year	2	**5.169**	**0.048**	**0.006**	**0.019**
Tarsus length	site	6	1.854	0.051	0.090	0.181
	season	1	0.081	0.000	0.776	0.776
	site*year	8	0.374	0.014	0.933	0.933
	season*year	2	0.047	0.000	0.954	0.954
Body size	site	6	1.006	0.029	0.423	0.423
	season	1	1.292	0.006	0.257	0.514
	site*year	8	**3.770**	**0.128**	**< 0.001**	**0.001**
	season*year	2	1.301	0.013	0.274	0.549

Table 5.3. Results of ANOVA test of between site, year, and season effects on variation in rainfall. F, Partial ETA2, and P-values are shown. Bold indicates significant values.

Fixed factor	df	F	Partial ETA^2	P
Site	6	**3.964**	**0.094**	**<0.001**
Year	5	**2.251**	**0.047**	**0.050**
Season	1	**41.972**	**0.154**	**<0.001**
Site*year	11	0.363	0.017	0.969
Site*season	6	0.381	0.010	0.891
Season*year	5	**11.452**	**0.199**	**<0.001**

Table 5.4. Results of regressions between morphology and rainfall. The regression coefficient (r), two-tailed significance (P), and corresponding Holmed P-values (P_H) are shown. Bold indicates significant values.

Sex	Trait	r	P	P_H
Males	Bill length	**0.112**	**0.012**	**0.012**
	Bill base circumference	**- 0.203**	**< 0.001**	**< 0.004**
	Tarsus length	**- 0.213**	**< 0.001**	**< 0.003**
	Body size	**0.276**	**< 0.001**	**< 0.002**
Females	Bill length	0.059	0.305	0.610
	Bill base circumference	**- 0.160**	**0.006**	**0.018**
	Tarsus length	- 0.050	0.381	0.381
	Body size	**0.354**	**< 0.001**	**< 0.004**

Figure 5.1. Maps showing (a) Australia with study area inset; (b) New Holland Honeyeater distribution for South Australia based on museum vouchers and sight records from the South Australian Department for the Environment and Heritage; and (c) study sites in South Australia.

a)

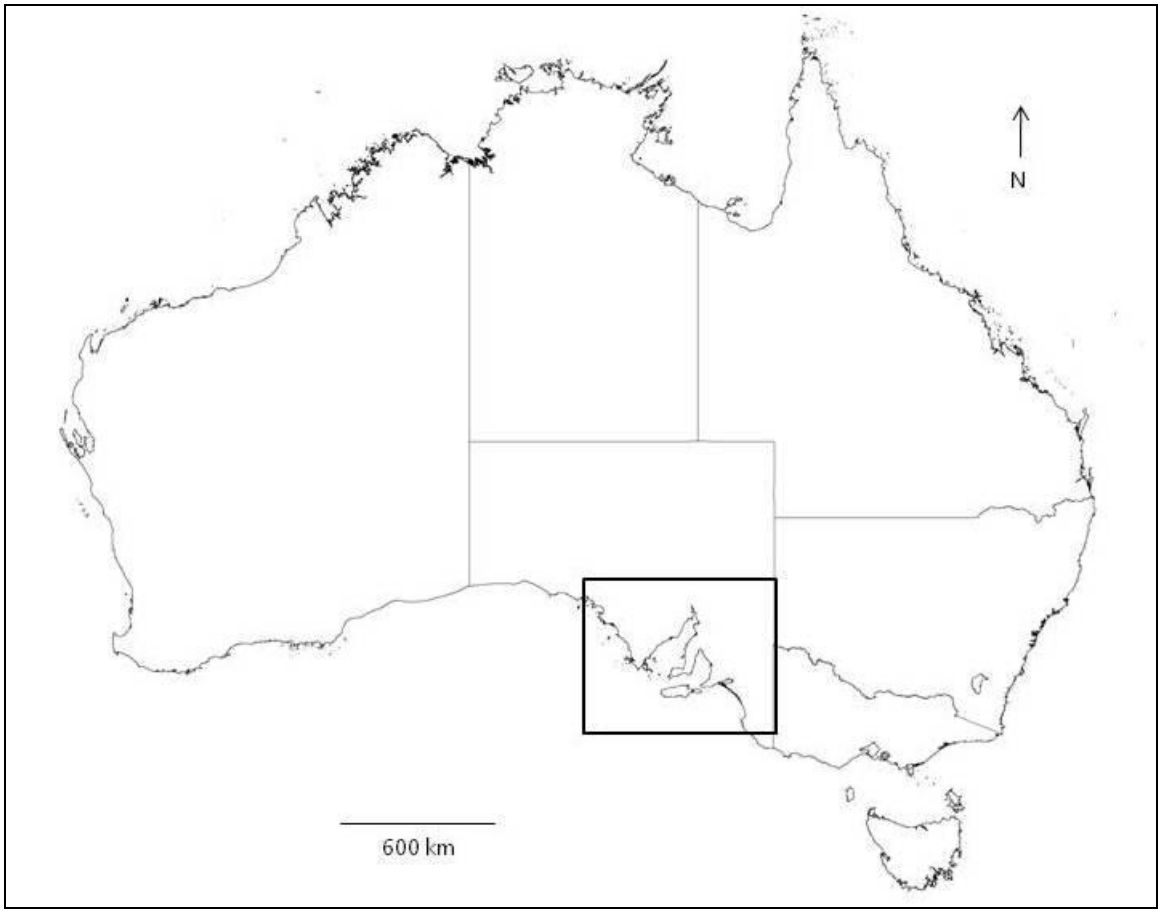
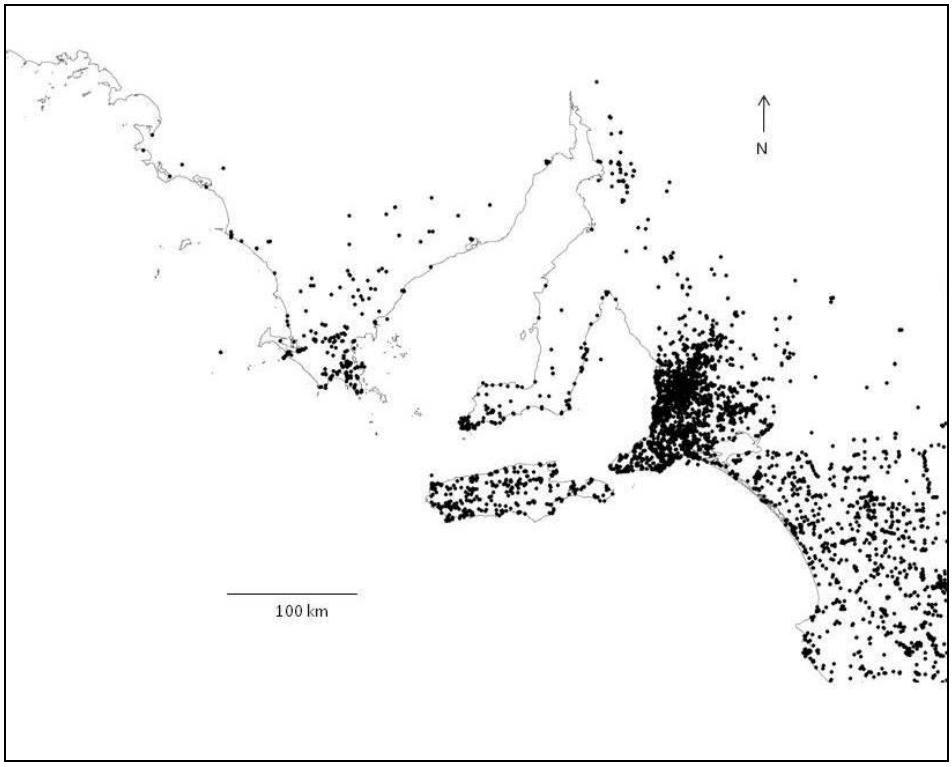


Figure 5.1. continued.

b)



c)

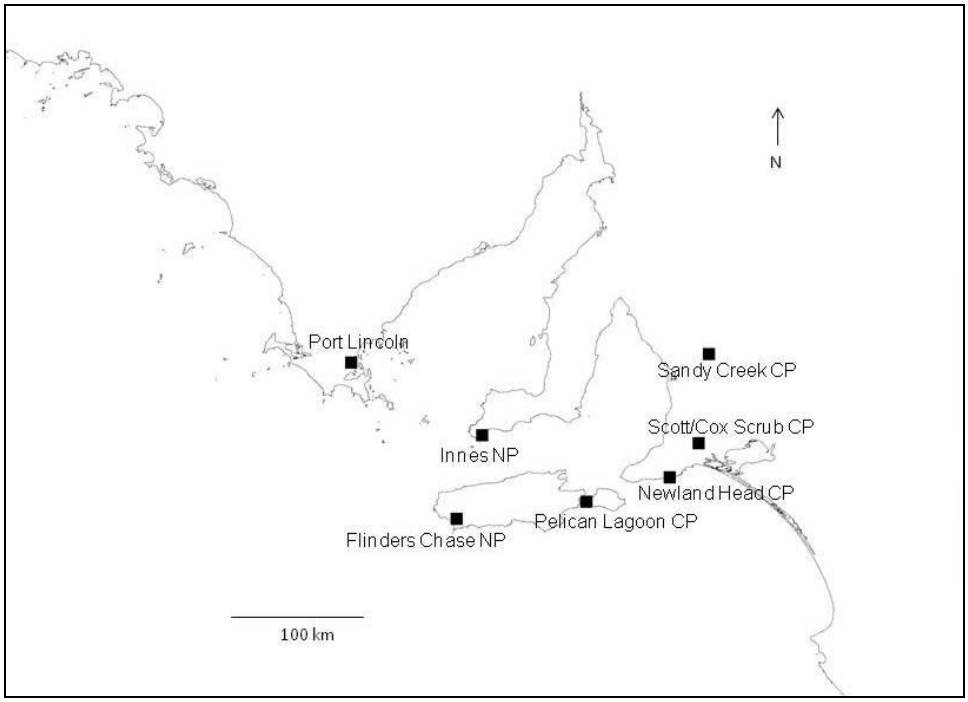
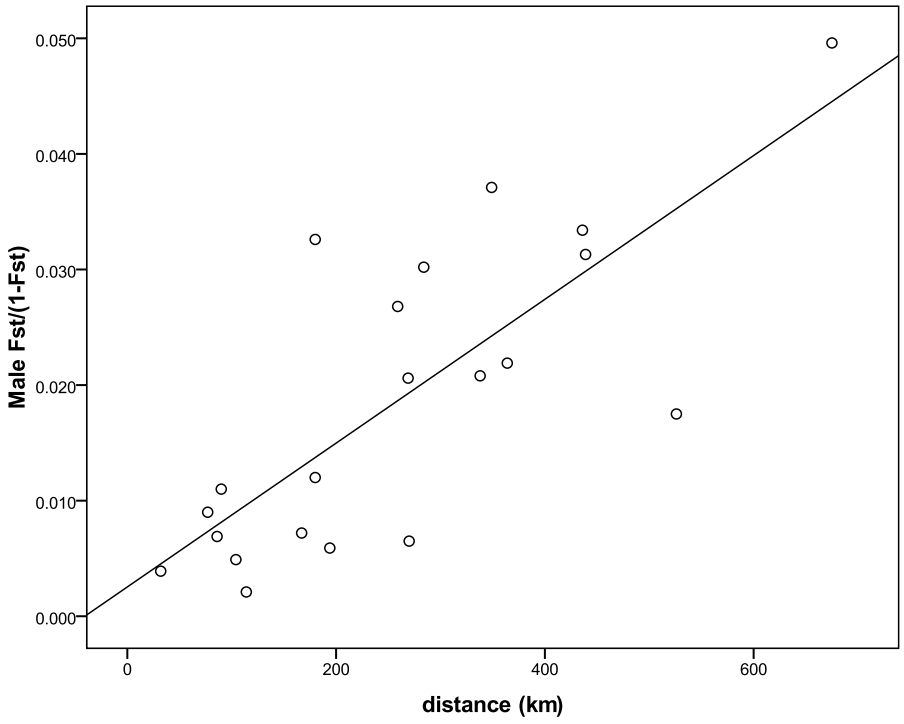


Figure 5.2. Isolation by distance analysis.

a) males (r = 0.773)



b) females (r = 0.748)

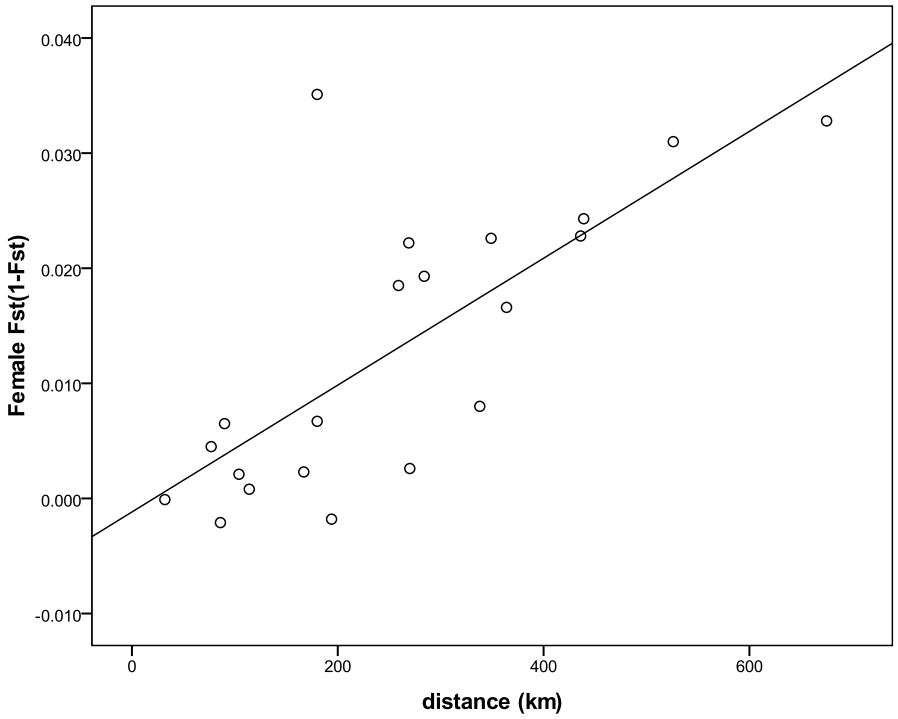
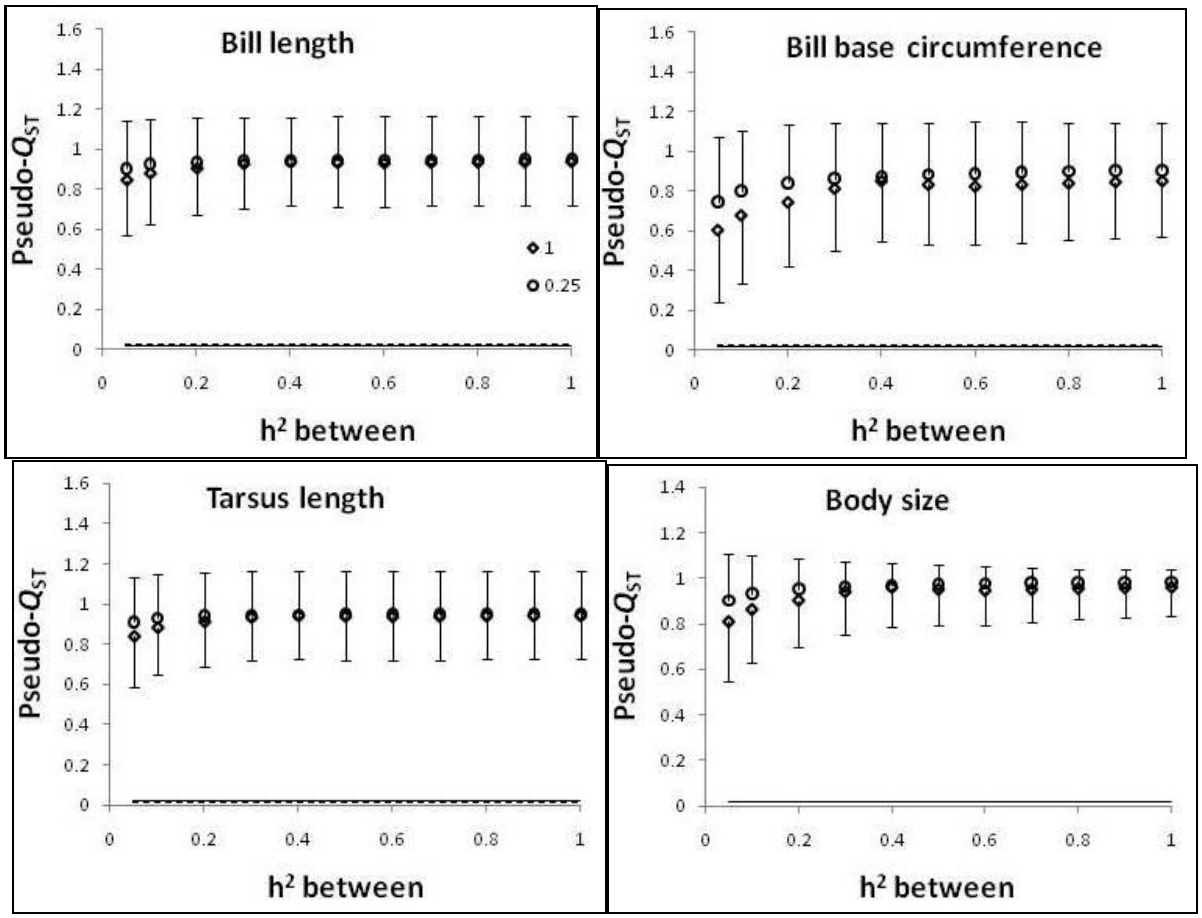
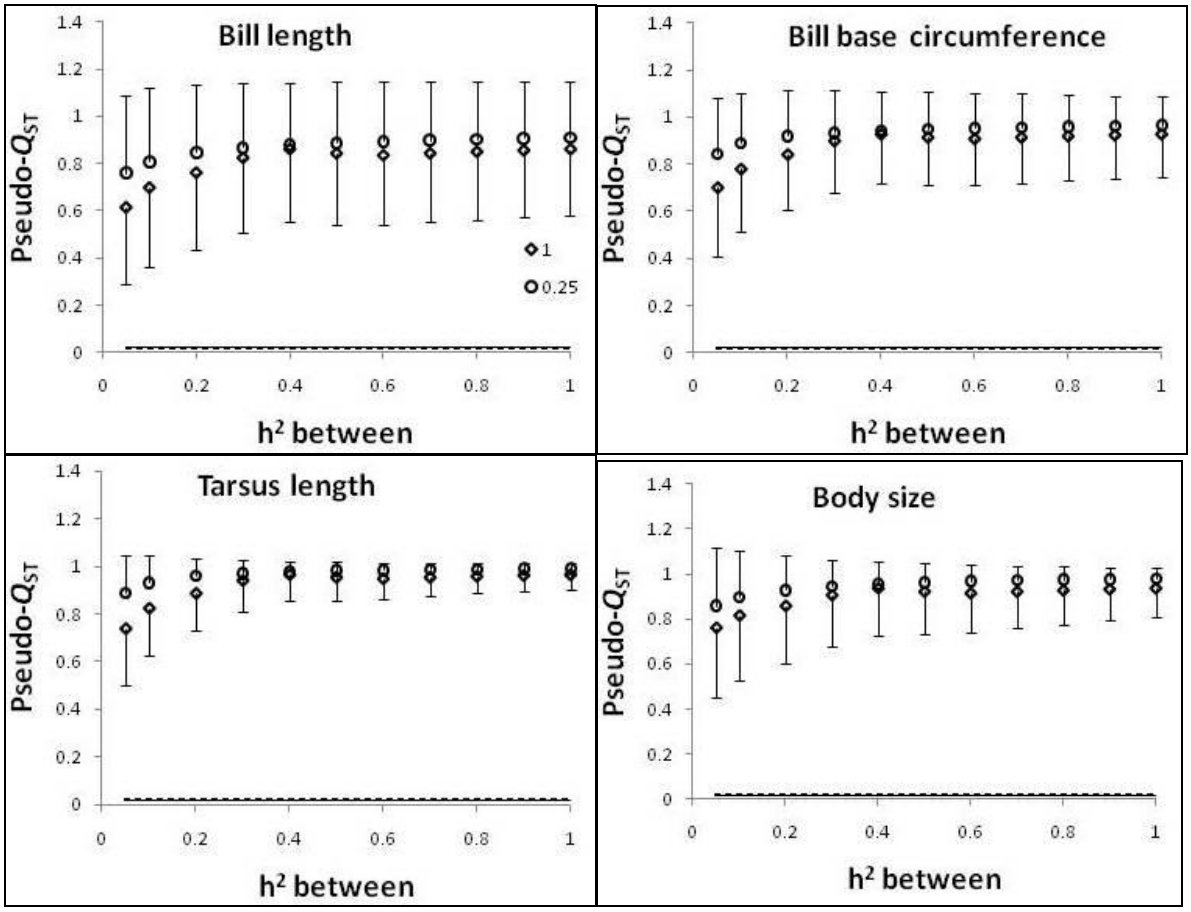


Figure 5.3. Comparison of pseudo-QST and FST for each trait for (a) males and (b) females. Variation of pseudo-QST with the additive genetic proportion of differences between- (x-axis) and within- (two different values: 1; 0.25) sites is represented by the markers ±sd. Estimate of neutral genetic differentiation (FST) is represented by the black line ±sd (dashed lines).

a)



b)


Bill length
Bill base circumference
Tarsus length
Body size
Pseudo-Q_{ST}
h² between
1
0.25
0
0.2
0.4
0.6
0.8
1
1.2
1.4

Appendix 5.A. Sample size (*N*) per site, year, and season for morphological data.

Site	Year	Season	*N*
Sandy Creek	2004	Dry	30
		Wet	0
	2005	Dry	21
		Wet	0
	2006	Dry	0
		Wet	38
	2007	Dry	14
		Wet	2
	2008	Dry	0
		Wet	1
	2009	Dry	0
		Wet	0
Scott/Cox Conservation Parks	2004	Dry	15
		Wet	0
	2005	Dry	12
		Wet	0
	2006	Dry	31
		Wet	14
	2007	Dry	0
		Wet	0
	2008	Dry	23
		Wet	0
	2009	Dry	0
		Wet	0
Newland Head Conservation Park	2004	Dry	0
		Wet	20
	2005	Dry	0
		Wet	94
	2006	Dry	14
		Wet	35
	2007	Dry	4
		Wet	0
	2008	Dry	0
		Wet	0
	2009	Dry	0
		Wet	0

Appendix 5.A. continued.

Site	Year	Season	*N*
Flinders Chase National Park	2004	Dry	0
		Wet	0
	2005	Dry	0
		Wet	6
	2006	Dry	18
		Wet	0
	2007	Dry	1
		Wet	0
	2008	Dry	0
		Wet	10
	2009	Dry	0
		Wet	0
Pelican Lagoon Conservation Park	2004	Dry	7
		Wet	0
	2005	Dry	10
		Wet	9
	2006	Dry	5
		Wet	9
	2007	Dry	9
		Wet	22
	2008	Dry	0
		Wet	2
	2009	Dry	0
		Wet	0
Innes National Park	2004	Dry	0
		Wet	0
	2005	Dry	0
		Wet	0
	2006	Dry	6
		Wet	0
	2007	Dry	0
		Wet	0
	2008	Dry	35
		Wet	19
	2009	Dry	0
		Wet	0

Appendix 5.A. continued.

Site	Year	Season	*N*
Port Lincoln	2004	Dry	0
		Wet	0
	2005	Dry	0
		Wet	0
	2006	Dry	0
		Wet	0
	2007	Dry	0
		Wet	0
	2008	Dry	0
		Wet	64
	2009	Dry	70
		Wet	0

Appendix 5.B. The extent of variation for each morphological trait across sites in *P. novaehollandiae*. Shown are mean values and standard deviation (SD) for males and females per trait for each site, as well as the common standard deviation (Common SD).

Site	Trait	Male			Female		
		N	Mean	SD	N	Mean	SD
Sandy Creek Conservation Park	Bill-head length	70	42.44	0.80	36	39.69	0.86
	Bill-nostril length	70	10.94	0.55	36	9.91	0.60
	Bill depth	70	5.32	0.28	36	5.02	0.26
	Bill width	70	5.38	0.37	36	5.07	0.38
	Tarsus	70	23.56	0.88	36	22.52	0.77
	Mass	70	22.29	1.89	36	19.73	1.85
Cox Scrub/Scott Conservation Parks	Bill-head length	60	41.73	1.20	35	40.00	1.23
	Bill-nostril length	60	10.44	0.63	35	9.87	0.71
	Bill depth	60	5.11	0.31	35	4.85	0.33
	Bill width	60	5.20	0.40	35	4.90	0.38
	Tarsus	60	22.96	0.94	35	22.39	0.82
	Mass	60	20.95	1.37	35	19.90	1.92
Newland Head Conservation Park	Bill-head length	93	42.20	0.94	74	39.88	0.99
	Bill-nostril length	93	10.50	0.73	74	9.92	0.47
	Bill depth	93	5.13	0.24	74	4.83	0.26
	Bill width	93	5.15	0.31	74	4.88	0.34
	Tarsus	93	23.28	0.79	74	22.35	0.69
	Mass	93	22.16	1.82	74	19.51	1.78
Flinders Chase National Park	Bill-head length	18	43.30	1.09	17	40.55	0.78
	Bill-nostril length	18	11.13	0.54	17	10.31	0.46
	Bill depth	18	5.29	0.29	17	4.95	0.28
	Bill width	18	5.27	0.37	17	4.99	0.23
	Tarsus	18	24.46	0.90	17	23.48	0.96
	Mass	18	20.96	0.92	17	17.70	0.91
Pelican Lagoon Conservation Park	Bill-head length	38	42.50	0.94	35	39.68	0.76
	Bill-nostril length	38	11.04	0.92	35	10.07	0.80
	Bill depth	38	5.12	0.29	35	4.73	0.27
	Bill width	38	5.22	0.38	35	4.79	0.38
	Tarsus	38	23.73	0.83	35	22.63	0.60
	Mass	38	21.16	1.84	35	19.10	1.40

Appendix 5.B. continued.

Site	Trait	Male			Female		
		N	Mean	SD	N	Mean	SD
Innes National Park	Bill-head length	35	39.95	1.34	25	38.34	0.91
	Bill-nostril length	35	9.59	0.68	25	9.48	0.64
	Bill depth	35	5.27	0.35	25	4.96	0.29
	Bill width	35	5.28	0.62	25	4.93	0.31
	Tarsus	35	23.52	0.87	25	22.07	0.81
	Mass	35	20.10	1.80	25	18.40	1.10
Port Lincoln	Bill-head length	91	41.03	0.94	43	38.55	1.13
	Bill-nostril length	91	10.24	0.51	43	9.59	0.62
	Bill depth	91	5.31	0.35	43	4.86	0.28
	Bill width	91	5.29	0.41	43	4.85	0.32
	Tarsus	91	23.57	0.91	43	22.40	1.02
	Mass	91	20.20	1.10	43	18.00	1.45

Appendix 5.C. Assessing individual researcher measurement error on morphological measurements by comparison of variation across sites by sex for each researcher (SK, SM) separately, and combined. Independent samples t-tests were carried out only between the two sites with the fewest samples (Port Lincoln and Innes National Park), as sampling error should be maximal at these sites by probability. The sample sizes (*N*; Innes National Park, Port Lincoln) and two-tailed significance assuming equal variances (P) are shown. Bold indicates significant values (< 0.05).

	males						females					
	SK		SM		combined		SK		SM		combined	
	N	P	*N*	P	*N*	P	*N*	P	*N*	P	*N*	P
Bill-head length	14, 42	**0.000**	21, 49	**0.002**	35, 91	**0.000**	13, 17	0.336	11, 26	0.814	24, 43	0.439
Bill-nostril length	14, 42	**0.001**	21, 49	**0.000**	35, 91	**0.000**	14, 17	0.238	11, 26	0.880	25, 43	0.467
Bill depth	14, 42	0.602	21, 49	0.766	35, 91	0.603	14, 17	0.286	11, 26	0.366	25, 43	0.161
Bill width	14, 42	0.114	21, 49	0.386	35, 91	0.972	14, 17	0.455	11, 25	0.526	25, 42	0.329
Tarsus length	14, 42	0.076	21, 49	0.397	35, 91	0.802	14, 17	0.294	11, 26	0.393	25, 43	0.172
mass	10, 40	0.111	18, 47	0.490	28, 87	0.728	11, 16	0.177	10, 26	0.570	21, 42	0.307

Appendix 5.D. Matrix of factor loadings after Varimax rotation for confirmatory factor analysis using six morphological measurements and predicting five factors in (a) males and (b) females. Bold indicates strong loadings (> 0.5).

a)

	Factors				
	1	2	3	4	5
Bill-head length	0.046	**0.518**	0.371	0.083	**0.706**
Bill-nostril length	0.050	**0.962**	0.103	0.079	0.159
Bill depth	**0.908**	0.093	0.146	0.108	-0.226
Bill width	**0.890**	-0.015	-0.024	0.141	0.322
Tarsus length	0.171	0.084	0.118	**0.973**	0.049
mass	0.084	0.127	**0.958**	0.119	0.153

b)

	Factors				
	1	2	3	4	5
Bill-head length	.150	.303	.223	.158	**.879**
Bill-nostril length	.060	**.946**	.075	.128	.246
Bill depth	**.895**	-.057	.039	.008	.249
Bill width	**.868**	.166	.214	.163	-.059
Tarsus length	.111	.122	.074	**.973**	.128
mass	.173	.075	**.956**	.076	.185

Appendix 5.E. Allelic variability of ten *P. novaehollandiae* microsatellite loci at seven sites calculated using the program GENEPOP. Bold indicates loci that depart significantly from Hardy-Weinberg equilibrium.

		N	N_A	H_E	H_O	F_{IS}
Newland Head Conservation Park						
	Pn2	91	15	0.852	0.802	0.0590
	Pn3	89	18	0.880	0.876	0.0037
	Pn4	88	8	0.758	0.784	-0.0344
	Pn5	81	5	0.664	0.691	-0.0408
	Pn8	74	9	0.669	0.554	0.1722
	Pn13	80	12	0.881	0.913	-0.0363
	Pn15	88	17	0.912	0.932	-0.0219
	Pn22	69	17	0.900	0.870	0.0342
	Pn23	91	14	0.681	0.728	-0.0690
	Pn25	91	13	0.864	0.813	0.0594
Pelican Lagoon Conservation Park						
	Pn2	33	12	0.843	0.818	0.0303
	Pn3	33	12	0.903	0.848	0.0608
	Pn4	32	8	0.785	0.813	-0.0353
	Pn5	**34**	**4**	**0.644**	**0.500**	**0.2262**
	Pn8	34	6	0.639	0.588	0.0808
	Pn13	34	11	0.892	0.971	-0.0895
	Pn15	34	17	0.882	0.765	0.1346
	Pn22	23	10	0.880	0.696	0.2134
	Pn23	34	8	0.795	0.735	0.0756
	Pn25	33	9	0.848	0.788	0.0725
Sandy Creek Conservation Park						
	Pn2	34	12	0.866	0.941	-0.0881
	Pn3	34	16	0.890	0.706	0.2092
	Pn4	34	7	0.777	0.588	0.2461
	Pn5	**32**	**5**	**0.721**	**0.531**	**0.2665**
	Pn8	32	8	0.682	0.750	-0.1014
	Pn13	32	12	0.887	0.781	0.1213
	Pn15	34	15	0.922	0.912	0.0111
	Pn22	24	8	0.862	0.750	0.1321
	Pn23	34	10	0.828	0.735	0.1139
	Pn25	34	9	0.856	0.765	0.1076

Appendix 5.E. continued.

		N	N_A	H_E	H_O	F_{IS}
Scott/Cox Conservation Parks						
	Pn2	66	16	0.867	0.815	0.0595
	Pn3	67	17	0.902	0.864	0.0429
	Pn4	56	7	0.733	0.716	0.0228
	Pn5	66	6	0.665	0.500	0.2495
	Pn8	55	8	0.715	0.742	-0.0383
	Pn13	68	13	0.904	0.891	0.0145
	Pn15	65	16	0.912	0.882	0.0324
	Pn22	55	16	0.902	0.800	0.1136
	Pn23	66	14	0.849	0.894	-0.0528
	Pn25	67	11	0.856	0.821	0.0408
Flinders Chase National Park						
	Pn2	31	9	0.832	0.968	-0.1666
	Pn3	32	15	0.920	0.875	0.0493
	Pn4	33	7	0.804	0.667	0.1732
	Pn5	30	4	0.693	0.567	0.1851
	Pn8	34	8	0.721	0.706	0.0210
	Pn13	29	11	0.898	0.931	-0.0370
	Pn15	**34**	**16**	**0.900**	**0.794**	**0.1191**
	Pn22	30	11	0.882	0.867	0.0182
	Pn23	34	11	0.836	0.794	0.0511
	Pn25	34	10	0.856	0.882	-0.0307
Innes National Park						
	Pn2	33	11	0.847	0.788	0.0704
	Pn3	36	12	0.880	0.861	0.0212
	Pn4	38	8	0.798	0.816	-0.0223
	Pn5	37	4	0.649	0.595	0.0844
	Pn8	35	8	0.680	0.600	0.1191
	Pn13	34	9	0.804	0.853	-0.0616
	Pn15	38	15	0.851	0.816	0.0422
	Pn22	33	10	0.845	0.788	0.0683
	Pn23	36	9	0.791	0.889	-0.1262
	Pn25	38	10	0.855	0.816	0.0466

Appendix 5.E. continued.

		N	N_A	H_E	H_O	F_{IS}
Port Lincoln						
	Pn2	34	8	0.783	0.676	0.1380
	Pn3	34	12	0.881	0.971	-0.1039
	Pn4	33	6	0.785	0.818	-0.0435
	Pn5	33	4	0.546	0.576	-0.0546
	Pn8	34	6	0.686	0.676	0.0136
	Pn13	29	12	0.814	0.759	0.0688
	Pn15	34	15	0.903	0.912	-0.0104
	Pn22	34	10	0.825	0.824	0.0022
	Pn23	33	8	0.779	0.818	-0.0505
	Pn25	33	9	0.864	0.909	-0.0532

N, sample size; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding co-efficient.

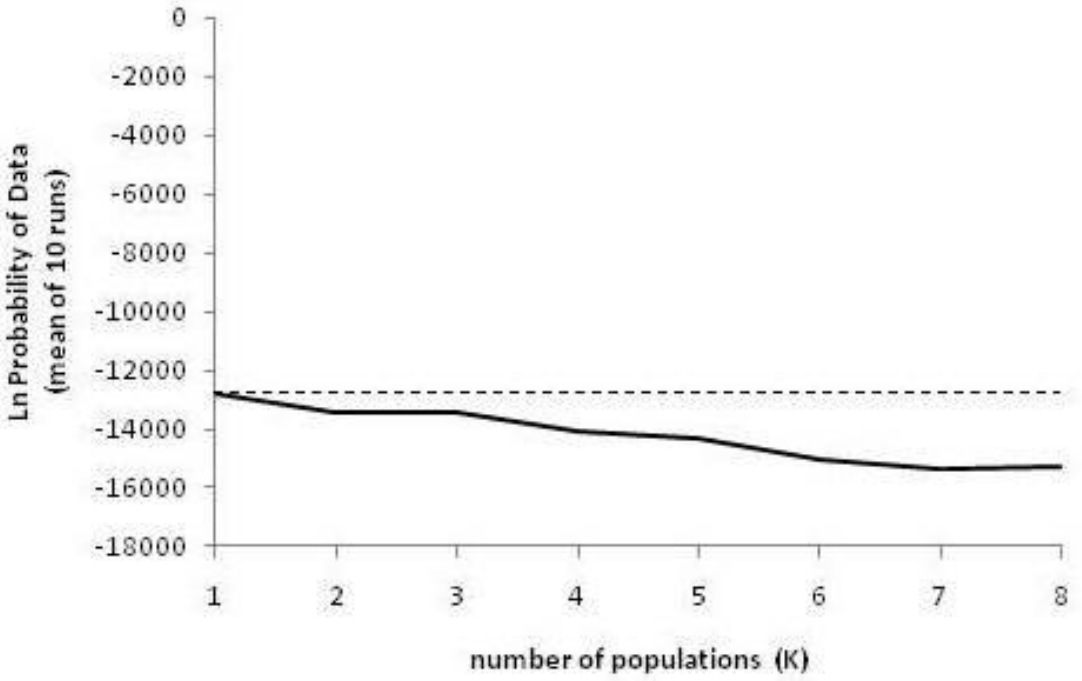
Appendix 5.F. Global allelic variability of ten *P. novaehollandiae* microsatellite loci at seven sites calculated using the programs CERVUS and GENEPOP. Bold indicates loci that depart significantly from Hardy-Weinberg equilibrium.

Locus	N	N_A	H_O	H_E	P	F_{IS}	A_T
Pn2	318	16	0.827	0.851	0.840	0.0282	11.079
Pn3	322	18	0.860	0.898	0.128	0.0417	13.719
Pn4	323	8	0.749	0.777	<0.000	0.0354	7.104
Pn5	301	6	0.578	0.672	<0.000	0.1399	4.450
Pn8	307	10	0.655	0.699	0.089	0.0640	7.522
Pn13	291	15	0.880	0.886	0.606	0.0071	11.534
Pn15	328	18	0.872	0.913	0.020	0.0445	14.831
Pn22	266	17	0.812	0.888	0.041	0.0860	11.653
Pn23	325	14	0.837	0.820	0.457	-0.0208	9.484
Pn25	327	13	0.823	0.864	0.789	0.0475	10.145

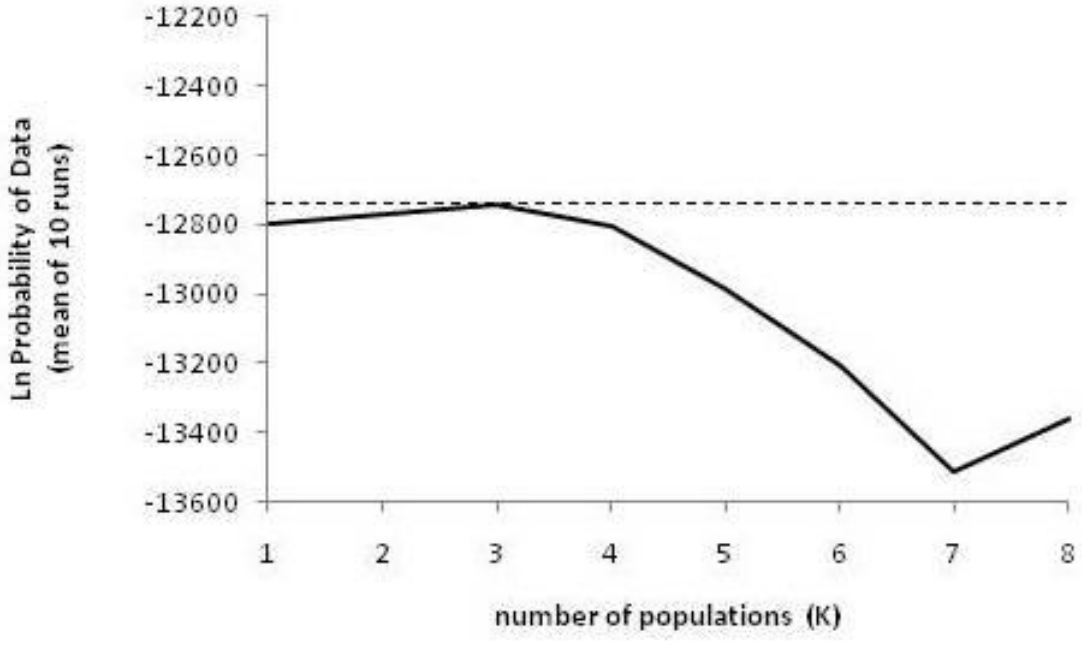
N, sample size; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; P, P-value for tests of Hardy-Weinberg equilibrium; F_{IS}, inbreeding co-efficient; A_T, allelic richness.

Appendix 5.G. Results of STRUCTURE analyses. Shown are the mean estimated logarithm of probability of the data for 10 runs, for K = 1-8 using (a) the standard admixture model and (b) the LOCPRIOR model. Models incorporated the correlated allele frequencies model.

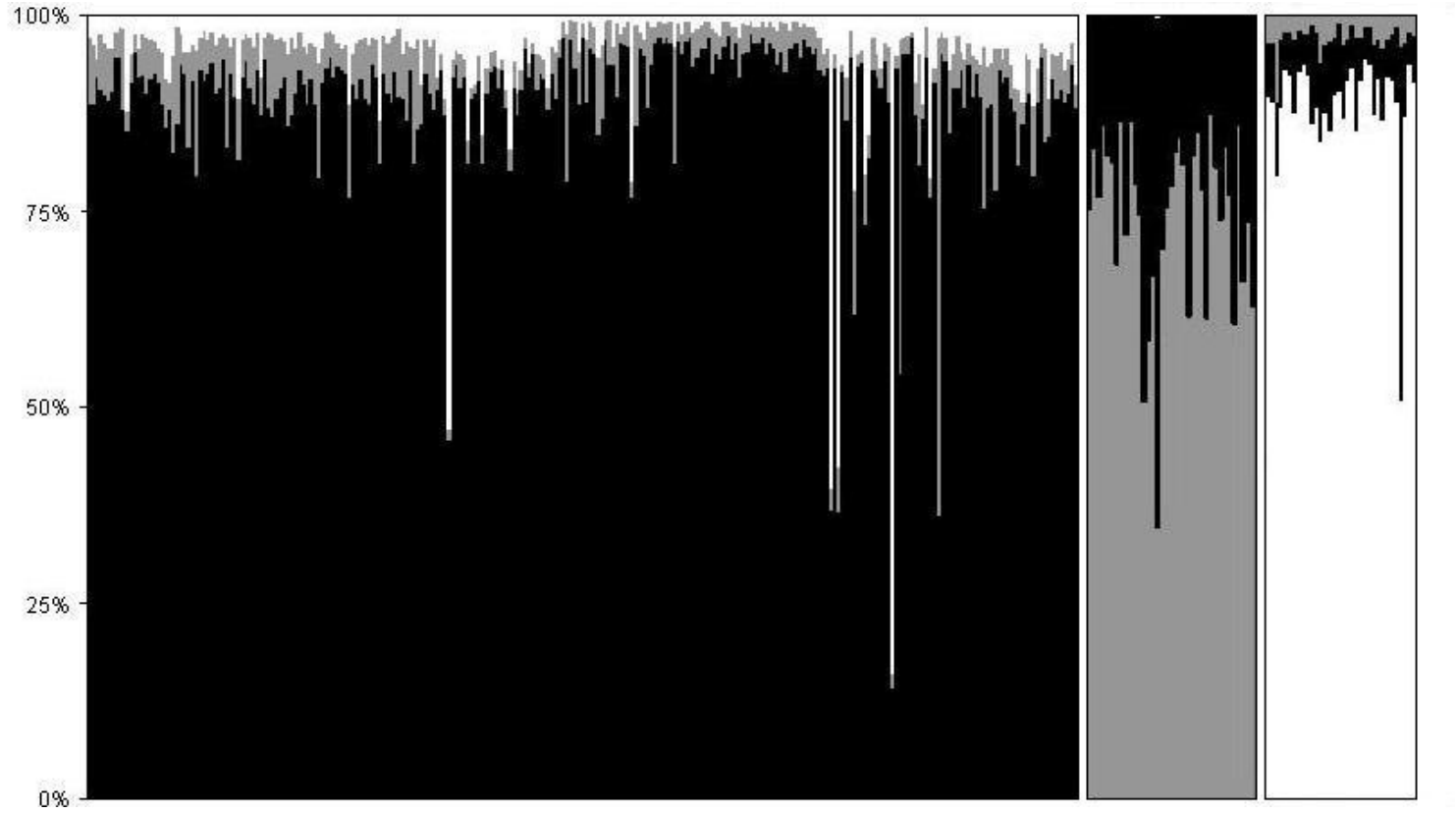
a)



b)



Appendix 5.H. Percentage membership of individuals in each cluster as estimated by the LOCPRIOR model. Box 1 shows percentage membership of individuals sampled from Kangaroo Island and Fleurieu Peninsula (KI/FP); box 2 shows percentage membership of individuals sampled from Innes National Park (INP); box 3 shows percentage membership of individuals sampled from Port Lincoln (PL). Black columns represent percentage membership in the KI/FP cluster; grey columns represent percentage membership in the INP cluster; white columns represent percentage membership in the PL cluster.



Appendix 5.I. Allelic variability of ten *P. novaehollandiae* microsatellite loci within the three populations identified by the STRUCTURE program, calculated using the program GENEPOP. Bold indicates loci that depart significantly from Hardy-Weinberg equilibrium.

		N	N_A	H_E	H_O	F_{IS}
Kangaroo Island/Fleurieu Peninsula						
	Pn2	249	16	0.854	0.847	0.008
	Pn3	250	18	0.897	0.848	0.055
	Pn4	**250**	**8**	**0.767**	**0.728**	**0.052**
	Pn5	**229**	**6**	**0.684**	**0.576**	**0.158**
	Pn8	236	10	0.688	0.657	0.045
	Pn13	226	15	0.893	0.898	- 0.005
	Pn15	254	18	0.914	0.874	0.044
	Pn22	197	17	0.894	0.812	0.092
	Pn23	254	14	0.826	0.831	- 0.005
	Pn25	254	13	0.859	0.811	0.056
Innes National Park						
	Pn2	33	16	0.852	0.818	0.040
	Pn3	36	17	0.871	0.833	0.043
	Pn4	36	8	0.778	0.806	- 0.036
	Pn5	35	6	0.663	0.600	0.097
	Pn8	34	8	0.659	0.618	0.064
	Pn13	32	14	0.826	0.875	- 0.061
	Pn15	36	17	0.860	0.806	0.064
	Pn22	31	15	0.825	0.806	0.023
	Pn23	34	11	0.792	0.882	- 0.116
	Pn25	36	12	0.854	0.806	0.058
Port Lincoln						
	Pn2	36	14	0.793	0.694	0.126
	Pn3	36	17	0.885	0.972	- 0.101
	Pn4	37	7	0.795	0.838	- 0.054
	Pn5	37	5	0.568	0.568	0.001
	Pn8	37	9	0.702	0.676	0.037
	Pn13	33	14	0.832	0.758	0.091
	Pn15	38	18	0.900	0.921	- 0.024
	Pn22	38	15	0.837	0.816	0.026
	Pn23	37	10	0.778	0.838	- 0.078
	Pn25	37	13	0.867	0.919	- 0.060

N, sample size; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding co-efficient.

Appendix 5.J. Variation in F_{ST} for pairwise comparisons of sites for males (top matrix) and females (bottom matrix). P-values were obtained after 21,000 permutations and significant deviations above 0 (P<0.002381 after adjustment for multiple tests) are in bold.

		Males						
	Site	SAC	Scott/Cox	NHCP	FCNP	PL	Innes	Lincoln
Females	SAC		0.007	0.002	0.007	0.012	**0.020**	**0.032**
	Scott/Cox	-0.002		0.004	0.006	0.005	**0.020**	**0.026**
	NHCP	0.000	-0.000		0.007	0.009	**0.021**	**0.029**
	FCNP	0.003	-0.002	0.002		0.011	**0.017**	**0.030**
	PL	0.007	0.002	0.004	0.006		**0.032**	0.036
	Innes	0.022	0.008	**0.016**	**0.030**	**0.022**		**0.047**
	Lincoln	**0.034**	0.018	**0.019**	0.024	**0.022**	**0.032**	

Appendix 5.K. Results of ANOVAs testing the effect of interactions between sex and other fixed factors on variation in morphology carried out for interactions found to have a significant effect on morphological variation (MANOVA). Degrees of freedom (df), F-values (F), partial ETA^2, P-values, and corresponding Holmed P-values are shown. Bold indicates significant values.

Trait	Fixed Factor	df	F	Partial ETA^2	P	P_H
Bill-head length	sex*site	4	1.782	0.013	0.131	0.524
	sex*season	1	0.407	0.001	0.524	0.524
	sex*site*year	7	2.314	0.029	0.025	0.099
	sex*season*year	2	0.166	0.001	0.847	0.847
Bill-nostril length	sex*site	4	2.518	0.019	0.040	0.202
	sex*season	1	3.372	0.006	0.067	0.267
	sex*site*year	7	1.824	0.023	0.080	0.161
	sex*season*year	2	4.138	0.015	0.016	0.099
Bill depth	sex*site	4	0.367	0.003	0.832	0.832
	sex*season	1	4.928	0.009	0.027	0.161
	sex*site*year	7	**3.492**	**0.044**	**0.001**	**0.007**
	sex*season*year	2	2.156	0.008	0.117	0.467
Bill width	sex*site	4	1.010	0.008	0.402	0.803
	sex*season	1	1.308	0.002	0.253	0.760
	sex*site*year	7	1.868	0.024	0.073	0.218
	sex*season*year	2	3.565	0.013	0.029	0.145
Tarsus length	sex*site	4	1.099	0.008	0.356	1.068
	sex*season	1	0.532	0.001	0.466	0.932
	sex*site*year	7	0.361	0.005	0.925	0.925
	sex*season*year	2	0.168	0.001	0.845	1.691
Mass	sex*site	4	**7.238**	**0.051**	**0.000**	**0.000**
	sex*season	1	3.094	0.006	0.079	0.396
	sex*site*year	7	2.411	0.031	0.019	0.097
	sex*season*year	2	0.791	0.003	0.454	1.362

CHAPTER 6: GENERAL DISCUSSION

MAIN FINDINGS

For large datasets, the application of morphology-based sexing methods has the potential to alleviate the time, cost, and effort otherwise involved in sexing by other methods. In Chapter 3, I found morphological variation between sexually mature male and female (as identified by genetic methods) New Holland Honeyeaters (*Phylidonyris novaehollandiae*) in South Australia. However, this variation was found to be insufficient for reliable, accurate sex discrimination based on morphology. The relatively poor performance of morphologic methods was due to the intrinsic error involved with morphologic-based sexing (i.e., uncertainty in the region of overlap between sexes and unavoidable misidentifications). Additionally, the finding of regional and temporal variation in morphology suggests that application of sexing criteria should be restricted exclusively to samples from within the time and location from which the criteria were developed (Van de Pol et al. 2009, Chapter 4). Therefore, when accurate sex discrimination is important, an alternate, reliable method, such as anatomical or genetic identification, should be favoured above morphologic methods; at the very least, if a morphological method is used, an alternate reliable method should be applied for sexing individuals in the unavoidable overlap. This study has demonstrated, using South Australian *P. novaehollandiae*, the limitations of morphological sexing methods. These data and evidence from the literature (e.g., Van de Pol et al. 2009) suggest that these limitations most likely apply to the majority of sexually monomorphic bird species.

The sexual size dimorphism observed in *P. novaehollandiae* in Chapter 3 was most likely explained by sexual selection toward larger males, as a consequence of mating strategy.

Logically, an opposing force to this selection must exist, and, considering the small degree of size dimorphism in *P. novaehollandiae*, this force should be strong. This suggests that sexual selection toward larger males is counteracted by selection toward smaller body size by at least one other selective force; sexual selection (in males) and/or natural selection (in both sexes). A similar balance of selective forces governing sexual size dimorphism has been found in shorebirds, where sexual selection for size increase was counteracted by sexual selection for male agility (Székely et al. 2004). A separate study, investigating mechanisms driving Rensch's rule (a tendency for sexual size dimorphism to be more pronounced in larger species; Rensch 1950), found a strong positive correlation between the strength of positive allometry and aerial display agility, where subfamilies of birds with more agile displays demonstrated stronger positive allometry (Dale et al. 2007). This broad-scale correlation between degree of allometry and aerial display agility suggests that sexual selection for agile displays, as observed by Székely et al. (2004), is a common mechanism that counteracts sexual selection for larger males. However, sexual selection on aerial display agility is not likely in *P. novaehollandiae*; the flight component of their flight songs (which are carried out to defend territories) serves primarily to increase height for more efficient projection of song (Clarke & Clarke 1999), and they generally do not contain difficult manoeuvres (Rooke 1979). It is more likely that, in *P. novaehollandiae*, smaller body size is driven by natural selection for more efficient aerial insect foraging. This provides an alternative to sexual selection on aerial display agility balancing sexual selection for larger body size, as proposed by the sexual selection hypothesis of size allometry (Smith 1977, Abouheif & Fairbairn 1997, Fairbairn 1997); this is of particular significance for species that do not demonstrate sexual selection on aerial display agility.

In Chapter 4, I showed that island birds had longer tarsi and bills, but lower body mass than mainland birds. Bill-head length (the measure used in Chpater 4 to represent bill length) and body mass are both reflective of body size in *P. novaehollandia*; however bill-head length was shown to be most informative of body size (Chapter 5). This may indicate larger body size in island birds relative to mainland birds, which conforms to the island rule; although this is speculative without a proper measure of body size. Increased body size on islands may be an adaptation resulting from decreased interspecific competition leading to ecological release (Grant 1965), optimisation of body size for thermoregulation (Brown & Maurer 1986), or increased intraspecific competition selecting for dominance over resources (Kikkawa 1980). Birds on Kangaroo Island had a broader ecological niche than mainland birds, which is parsimonious with the ecological release hypothesis. Niche expansion on Kangaroo Island has most likely been driven by a paucity of food resources (evidenced by longer search times) and the absence of some bird species on Kangaroo Island, in particular the trunk- and branch-feeding specialists. However, an alternative to the ecological release hypothesis is the influence of niche expansion and change in diet on physical development (ie phenotypic plasticity). Kangaroo Island birds had lower nectar consumption and higher insect consumption than mainland birds. A lower carbohydrate (nectar) and higher protein (insects) diet is likely to increase structural growth and overall body size, and decrease mass relative to body size. The observed high rate of gene flow between island and mainland (Chapter 5), which is likely to counteract selection, suggests phenotypic plasiticity may be the most likely mechanism to explain the observed changes in island birds; however, natural selection can also lead to population divergence with high gene flow (reviewed in Coyne & Orr 2004).

In Chapter 5, I found that in sites with drier climates birds had shorter bills and smaller body sizes than birds in sites with wetter climates. Furthermore, in sites experiencing drier climates

male birds had wider bills and longer tarsi. These observations match expectations if natural selection is driving size distributions in these traits. The absence of a significant correlation of bill length and tarsus length with rainfall in females could be explained by reduced selection pressure on these traits relative to bill width and body size in females, coupled with female-biased dispersal. Nectar is an important resource of *P. novaehollandiae* (Recher 1977, Ford & Paton 1977) and is its primary source of energy (Paton 1982, Ford & Paton 1982). Nectar availability is positively influenced by rainfall (Porter 1978, Wooller et al. 1998, Keasar et al. 2008), and it is reasonable to assume that nectar resources are less available in drier climates, provided similar flora. Under conditions of reduced nectar availability, *P. novaehollandiae* must find an alternate source of energy, which primarily includes insects (Paton 1982). Under conditions of high insect demand; a shorter, less slender bill allows increased crushing force and size handling ability (Bowman 1961, Lederer 1975, Grant & Grant 1989, Grant & Grant 1996); longer tarsi improve efficiency of gleaning behaviour and allows quicker sallying from perches (Fitzpatrick 1985, Sherry 1982); and smaller body size increases aerodynamic efficiency and aerial agility (Székely et al. 2004, Raihani et al. 2006). I conclude that the most parsimonious explanation for my data is that rainfall drives divergent natural selection in bill length and slenderness, tarsus length, and body size of *P. novaehollandiae* across a climatically varied landscape in South Australia. These findings demonstrate that, under conditions of reduced food availability, we can expect populations to evolve toward traits that favour a more generalist diet. In many cases, food availability is dependent on climate. Therefore, under a changing climate the strength of selection can be monitored to provide valuable information about the stability of a population.

The direction of change in traits of birds in drier climates observed in chapter 5 mirrors change in traits of Kangaroo Island birds relative to mainland birds observed in chapter 4. If

the Kangaroo Island sites experience a drier climate than do the mainland sites, then the evidence would suggest that natural selection, not phenotypic plasticity, is driving divergence in the Kangaroo Island-mainland system; this would also add support to the hypothesis that conditions of reduced food availability on the island drive adaptive divergence in foraging ecology and morphology (Chapter 4). I investigated rainfall intensity between mainland and Kangarro Island (using methods used in chapter 5) and found evidence for a wetter climate in mainland sites (Australian Bureau of Meteorology; http://www.bom.gov.au/index.shtml), which supports the hypothesis of natural selection. This means that, while an absence of interspecific competitors on Kangaroo Island has allowed ecological release, niche expansion in island birds has most likely been driven by reduced nectar availability, as influenced by rainfall, leading to greater intraspecific competition (Svanbäck & Bolnick 2007). This scenario provides an alternative to the classic ecological release hypothesis. Under the ecological release hypothesis, where niche expansion preceeds a change in diet (Grant 1965), the only logical mechanism to drive niche expansion is population density (i.e. intraspecific competition; see model in Einum et al. 2008). Under the hypothesis proposed here, variation in climate, and subsequently available resources, drives niche expansion. This study is among the first to demonstrate the role of climate as a mechanism that influences island evolution; it provides an alternative to the classic ecological release hypothesis and gives insight into the potential influence of climate on island evolution, which has, until now, been largely overlooked.

PERSPECTIVES FOR FUTURE RESEARCH

Chapter 3 describes sexual size dimorphism in the New Holland Honeyeater (*Phylidonyris novaehollandiae*), with males as the larger sex. Based on my combined data and evidence from the literature I proposed that this dimorphism is driven by sexual selection toward larger

males – the consequence of a territory defence mating system – counteracted by natural selection for smaller body size in both sexes. This hypothesis can contribute to the theoretical understanding of Rensch's rule of allometry for sexual size dimorphism; it provides a mechanism that helps explain the observation of Rensch's rule in bird species that do not demonstrate sexual selection on aerial display agility (see Chapter 6 'Main findings' above). Future research may choose to focus on testing this hypothesis, using more direct evidence than was available in this study. An experimental design to test this hypothesis may include investigation of the correlation between male body size and breeding territory size, or available territory resources, and male body size and clutch size. These tests will give an indication of (1) territory quality relative to body size, and (2) reproductive success relative to body size; the mating system hypothesis would predict a positive correlation between body size, territory quality, and reproductive success. Other research may test the role of sexual (aerial display agility) and natural (efficiency of aerial insect foraging) selection in constraining body size. This may include investigation of the correlation between male body size and agility of aerial display and foraging success.

One of the key findings of this study, addressed in Chapter 4, was of adaptive divergence in foraging ecology and morphology. Based on the evidence, I proposed two possible explanations; (1) ecological release and (2) developmental plasticity. Chapter 5 provided data that suggested ecological release, driven by depauperate resources influenced by rainfall, was responsible; however my data are not conclusive. Currently, no studies have demonstrated that developmental pasticity influences patterns of island evolution – despite plasticity being implemented in population responses to changing environmental conditions (Charmantier et al. 2008). A sensible line of approach to test these hypotheses would be to investigate the role of plasticity in morphological divergence between Kangaroo Island and mainland Australia.

This may be examined relatively easily using a ‘common garden’ experiment, where individuals from different populations are reared and maintained under common conditions (Rasner et al. 2004, Yeh 2004). Furthermore, both the ecological release hypothesis and the developmental plasticity hypothesis assume that reduced nectar availability on Kangaroo Island has driven niche expansion and subsequent variation in diet. However, this assumption was based on indirect evidence from foraging behaviour. A direct quantification of nectar availability, and possibly of *P. novaehollandiae* diet, across the study area would most likely benefit this study.

The second major finding of this study was addressed in Chapter 5; birds in drier climates had shorter, wider bills, longer tarsi, and smaller body sizes. I proposed that reduced nectar availability under dry conditions drives niche expansion and subsequent selection on traits that support a more insectivorous diet. However, much of the evidence presented in this study is indirect and relies on a number of assumptions. For instance, firstly I assume, based on evidence from the literature (Bowman 1961; Lederer, 1975; Grant & Grant 1989, Fitzpatrick 1985, Sherry 1982, Székely et al. 2004, Raihani et al. 2006), that the morphological traits I investigated influence foraging success in *P. novaehollandiae*; however these ecological relationships have not been tested in *P. novaehollandiae* (but see Chapter 3). A direct quantification of foraging niche, foraging success, and diet relative to morphology would improve this study. Secondly, I assume, based on indirect evidence from rainfall data, that reduced nectar availability in drier climates (Porter 1978, Wooller et al. 1998, Keaser et al. 2008) has driven niche expansion and subsequent selection on morphological traits. A direct quantification of nectar availability across the study area can test this assumption. Finally, a degree of uncertainty remains about assumptions of heritability of morphological traits, despite taking all possible precautions to ensure the assumptions were valid (see Chapter 5).

An estimate of heritabilities of morphological traits in *P. novaehollandiae* would remove the need for such assumptions. This could be achieved through a pedigree analysis (eg. along the lines of Blondel et al. 1999, Forstmeier 2005, Rønning et al. 2007). Future research with a more direct approach and better developed, more specific hypotheses, should be able to resolve greater detail from this system and contribute a wealth of knowledge to the field of evolution; in particular, to the subjects of sexual dimorphism, island biogeography, and climate driven adaptation.

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