Local adaptation in the wedge-tailed shearwater (*Puffinus pacificus*).

Thesis submitted by

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“I may not have gone where I intended to go, but I think I have ended up where I needed to be.”
Douglas Adams
(1952 - 2001)

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Peck, D. R., Smithers, B. V., Krockenberger, A. K. & Congdon, B. C. (2004) Sea-surface temperature constrains wedge-tailed shearwater foraging success within breeding seasons *Marine Ecology Progress Series* 281, 259-266.

Peck, D. R. & Congdon, B. C. (2005) Colony-specific foraging behaviour and co-ordinated divergence of chick development in the wedge-tailed shearwater *Puffinus pacificus Marine Ecology Progress Series* 299, 289-296.

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Peck, D. R. & Congdon, B. C. (2006) Sex-specific chick provisioning and diving behaviour in the wedge-tailed shearwater *Journal of Avian Biology* 37, 1-7.

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SUMMARY

Models of speciation that involve adaptation to local environmental conditions rather than physical barriers have rarely been examined in vertebrate taxa. Seabirds offer a unique opportunity to test such models because they have the potential to disperse widely. This means that large-scale geographical barriers to gene flow are less likely. In addition, breeding colonies are constrained to forage locally, thus promoting the optimisation of life history and fitness-related morphological traits among colonies. Ultimately, these changes may lead to a reduction in gene flow and genetic divergence may ensue: the first step towards speciation. To explore adaptive models of speciation, the role of local foraging conditions in promoting molecular, morphological (including physiological) and behavioural divergence among breeding colonies of the wedge-tailed shearwater (*Puffinus pacificus*) was examined.

To this end levels of variation in morphology and neutral genetic markers were measured among four spatially disjunct breeding colonies located in Australian waters (Rottnest Island, Raine Island, Heron Island and Lord Howe Island). In addition, data on foraging behaviour, chick developmental patterns and sensitivity to background environmental conditions (sea surface temperature) were obtained from two of these colonies representing climatic and oceanographic extremes for this species: the sub-tropical 'reef' colony (Heron Island) and Lord Howe Island, a temperate 'oceanic' colony.

Results from the foraging behaviour and chick developmental component of this research suggests that wedge-tailed shearwaters are sensitive to fluctuations in sea surface temperature, and consistently use different foraging strategies during the chick rearing period in accordance with where they breed. Specifically, at Heron Island, birds use a 'dual-foraging' strategy involving alternative 'short' and 'long' trips. This strategy is consistent with adults self-provisioning from distant locations and chick-provisioning from near colony locations. However, at Lord Howe Island, a dual foraging strategy was not observed, suggestive of a more productive environment.

As an indirect result of divergent oceanographic regimes, chick developmental patterns between the two locations also differed; chicks at Lord Howe Island grow faster than those at Heron. However, when the differences in meal mass per night were accounted for, Heron Island chicks were consistently heavier than those at Lord Howe Island.

Overall, the foraging and chick development data suggest that; (1) chick developmental patterns and foraging behaviour are coordinated in wedge-tailed shearwaters, (2) the foraging environment experienced by wedge-tailed shearwaters at Heron Island is less productive than at Lord Howe Island and (3) chicks at Heron Island appear to 'store' mass as an adaptation to consistently poor provisioning rates (driven by poor foraging conditions experienced by adults). The chick developmental pattern is likely to be driven by an obligate rather than a facultative mechanism because it is doubtful chicks can react to changing provisioning rates over the (small) period of time that the response took place.

Within the general patterns of foraging, sex-specific differences were also evident. Females spent more time at sea resulting in a lower provisioning rate compared to males. The average maximum dive depth also differed according to sex, with males diving consistently deeper than females. The most parsimonious explanation for the differences is that competition has lead to niche partitioning at the foraging grounds, although direct evidence will be required to substantiate this hypothesis. Subtle differences in the extent of sex-specific foraging between Heron Island and Lord Howe Island could promote a barrier to gene flow via. reinforcement if inter-colony pairings result in lower provisioning to chicks. Again, further evidence will be required to test this idea.

Morphological analyses highlighted significant variation within (sex-specific) and among breeding colonies. A canonical discriminant functions analysis was conducted using four traits: wing, tarsus, culmen and tail. Discriminant function 1 (CV1) explained 57.46 % of the variation among groups and was correlated most strongly with tarsus (a measure of skeletal size) followed by tail. CV2 explained a further 38.30 % and was strongly correlated with culmen. In general, birds from Rottnest

Island are significantly larger in overall body size compared to east-coast colonies, however Raine Island birds have significantly longer culmens than elsewhere.
Within colonies males are subtly larger than females, but relative to overall size, only bill morphology was significantly larger. A novel form of mate choice (disassortative) based on bill width was also observed at both Heron and Lord Howe Islands. Patterns of morphological variation and pair formation do not fit with those expected if environmental conditions alone (i.e. plasticity) are responsible. Instead, the results suggest that morphological diversity is more likely to involve selection.

Finally, levels of gene flow were gauged and compared to morphological variation to determine if gene flow constrains morphological divergence among colonies. Three intron and three microsatellite loci were used. Gene flow estimates differed according to the type of marker. Introns suggest substantial inter-colony movement whereas microsatellites imply that gene flow is restricted. The different estimates reflect differences in the mutation rates of the two markers. Consequently, introns (evolving more slowly) likely reveal historical connections during the Pleistocene, with microsatellites representing more contemporary patterns. A lack of congruence between the amount of morphological and genetic differentiation suggests that genetic drift alone can not explain all of the observed morphological diversity in wedge-tailed shearwaters.

Taken together, the results from this study clearly suggest that oceanographic/environmental regimes have an important function in the development and maintenance of seabird diversity and can substantially influence the direction of micro-evolutionary change. This has important implications from a management perspective, as some colonies will need to be considered independently. Future work should focus on assessing the role of selection in causing the observed patterns by evaluating the relationship of behavioural, morphological and physiological (chick development) traits to fitness in alternative habitats.

CHAPTER 1

1.0 INTRODUCTION

The majority of biologists believe that speciation is a continuous process in which genetic variation gradually becomes segregated between populations (Turelli et al. 2001). However, the exact route to speciation remains unclear. Darwin (1859) believed that speciation was a direct result of natural selection and therefore driven by ecological conditions. Subsequent studies used Darwin's proposition as the central tenet upon which to elaborate and expand models and theories regarding speciation (Futuyma 1998).

However, the mid 1900s saw a shift in focus away from natural selection as the principal means of speciation/diversity, towards the idea that genetic drift in conjunction with geographical separation caused the majority of observed diversity (i.e. allopatric speciation) (Dobzhansky 1937; Mayr 1959; Mayr 1963; Futuyma 1998). It was thought that populations of organisms with dispersal capabilities that promoted inter-population movement could not diverge genetically and/or morphologically because of the homogenising effects of gene flow (Mayr 1959; Maynard Smith 1966).

Gene flow, and the subsequent recombination of locally adapted genotypes, is still thought to be the major hurdle that needs to be overcome by proponents of non-allopatric speciation (Bush 1975; Via 2001; Kirkpatrick and Ravigne 2002). However, within the last 15 years, an ever-increasing body of work (encompassing both empirical and modelling studies) has shown that this 'antagonism' between selection and recombination can be reduced or even completely negated, and that genetic divergence (the first step towards speciation) may occur in a non-allopatric manner across a wide variety of taxa (Via 2001; Dres and Mallet 2002, Doebeli et al. 2005).

1.1 Non-allopatric speciation

Mathematical models based on standard allopatric models of speciation are tenable, and many empirical studies have illustrated genetic divergence resulting from a combination of allopatric processes (eg. vicariance) and random genetic drift (Futuyma 1998; Grant, Grant et al. 2000). However, models of speciation based on non-allopatric processes have proven harder to illustrate empirically because of the difficulty involved in separating underlying allopatric signatures (usually historic in nature) from recent non-allopatric mechanisms (Endler 1982; Berlocher 1998; Barraclough and Nee 2001). Despite this, modelling suggests that non-allopatric speciation is plausible, and that natural selection is principally involved (Kirkpatrick and Ravigne 2002). This work has highlighted conditions that are predicted to favour non-allopatric divergence. These conditions provide a context in which non-allopatric divergence may take place.

1.1.1 Conditions that favour non-allopatric speciation

Firstly, the 'ability' to make a habitat/resource use 'shift' appears to be integral for non-allopatric divergence to occur (Bush 1975; Via 2001). With this ability, individuals and populations can radiate into new habitats, and adapt to a new set of resources. This is known as adaptive radiation (Turner 1994; Schluter 1996; Futuyma 1998). The most well known examples are morphological changes entrained by trophic niche shifts in Rift Lake cichlids (Turner 1994; Kornfield and Smith 2000) and Galapagos Island finches (Grant and Boag 1981; Grant and Grant 1996). In both cases, variation in locally adapted trophic morphologies provides evidence that the ability to make a resource 'shift' exists.

Another condition thought to favour non-allopatric divergence and speciation is when individuals within populations choose mates according to one or more locally adapted morphological traits (assortative mating). This means that as populations adapt to local habitats, a barrier to gene flow arises because mating is increasingly restricted to individuals within each population (Bush 1975). For this to occur, either co-inheritance (linkage disequilibrium) of the genes for both the adaptive traits and for the behaviour to mate assortatively is required (refer Via 2001, Dres and Mallet 2002 for a reviews), or pleiotropy exists (eg. the gene for the adaptive trait also controls the behaviour to mate assortatively).

Finally, it is proposed that for non-allopatric divergence to occur, an 'intermediate' habitat/resource is not present (Kondrashov and Mina 1986; Rice 1987). This scenario limits gene flow between populations by causing selection against hybrid individuals (reinforcement) (Bush 1975; Bush 1994). Examples of this scenario exist in phytophagous insect species where two host-plant species represent distinct resources with no intermediaries (Bush 1994; Feder 1998; Dres and Mallet 2002).

1.1.2 Models of non-allopatric of speciation

Numerous models have been developed in order to understand non-allopatric divergence and speciation (see Rundle and Nosil 2005). In their simplest form, non-allopatric models of speciation have concentrated on showing how reinforcement can produce significant genetic divergence and by extension, speciation (Fisher 1930; Dobzhansky 1940; Mayr 1963). These models are commonly referred to as 'divergence with gene flow' (DWGF) models of speciation and often rely on the condition (outlined above) that population specific assortative mating is linked with morphological trait/s that are under ecologically driven divergent selection (Maynard Smith 1966; Dickinson and Antonovics 1973; Felsenstein 1981).

However, it is unlikely that non-allopatric speciation can proceed to completion in this way because recombination during meiosis breaks up evolving gene complexes, severing the connection between the gene/s for population specific assortative mating and those for the traits under divergent selection. Therefore, an unrealistically close association is required between these two genes (Felsenstein 1981; Sanderson 1989; Rice and Hostert 1993; Bush 1994; Kirkpatrick 2000).

1.1.3 Bushs' Model

In 1994 Guy Bush suggested a variation on these original models that appeared to make the antagonism between gene flow, recombination and selection less likely and therefore increase the feasibility of non-allopatric models of speciation (Bush 1994).
Bush (1994) proposed that the behaviour to stay within the confines of a preferred habitat or location (i.e. habitat fidelity) was crucial, and could theoretically create genetic divergence when population specific assortative mating occurs as a 'by-product' of this behaviour. This is because the genetic association between selected traits and mate choice does not rely exclusively on the formation of linkage disequilibrium

between unlinked genes. Thus, recombination is negated because selection would act directly on habitat choice, thereby directly producing assortative mating (Rice 1984, 1987, Bush 1994). Bush and others have provided empirical support from host specific insects that this mechanism can create significant genetic divergence among putative host 'races' (Hawthorne, Via et al.). However, the model has yet to be applied to taxa that have behavioural traits analogous to host specificity, such as species that exhibit natal philopatry.

Natal philopatry is the tendency for an individual to return to the place of birth in order to breed. This behaviour is analogous to host specificity in insects because individuals feed and breed in the local area, thus encouraging local adaptation. Moreover, population specific assortative mating occurs as a by-product of natal philopatry thereby reinforcing local adaptations and acting to further isolate populations.

1.1.4 Empirical evidence for non-allopatric divergence

The majority of empirical work on taxa where dispersal ability and/or behavioural characteristics predict non-allopatric divergence in the way described by Bush (1994) has been with host specific insect taxa (Table 1). Compared to invertebrates, empirical studies that examine the possibility of non-allopatric speciation in vertebrates are rare (Table 1). Nonetheless, one group (stream/lake fish) have been of considerable use (Turner 1994).

Empirical evidence suggests that populations or 'flocks' of cichlids confined to lakes in Africa have adaptively diverged in accordance with differential habitat/trophic niche use. A similar situation has been highlighted in populations of sticklebacks from glacial lakes (Schluter 1996). Numerous studies have examined the role of selection during the diversification of these lake fish taxa (see Turner 1994).

To summarise this literature, divergence under non-allopatric conditions in lake fish are thought to involve one or (likely) a combination of the following selective scenarios; (1) changes in morphology (ecologically driven) that are directly linked to assortative mating when the trait under selection is also the trait used to assortatively mate (Hendry, Wenburg et al. 2000), (2) ecologically selected changes in a life history trait that reinforces the assortative mating trait (Schliewen, Rassmann et al. 2001), (3)

habitat fidelity and brood site choice (Markert, Arnegard et al. 1999; Danley, Markert et al. 2000; Schliewen, Rassmann et al. 2001),(4) reinforcement of reproductive isolation (Rundle and Schluter 1998), and (5) sexual selection (Schliewen, Tautz et al. 1994; Seehausen, van Alphen et al. 1997; Wilson, Noack-Kunnmann et al. 2000). Evidently, mechanisms of non-allopatric speciation in lake fish are complex and case specific, indicating that the relatively simple model proposed by Bush is unlikely to be of primary importance in this group (Kornfield and Smith 2000).

Vertebrate taxa in which DWGF models might also be appropriate are marine vertebrates (other than fish), such as cetaceans, turtles, and seabirds. This is because natal philopatry is a common behavioural trait in these taxa. Moreover, population level morphological, behavioural and/or genetic divergence has been evidenced in some cases, despite a lack of obvious physical barriers to dispersal, suggesting that natural selection may be involved (Table 1).

1.2 Divergence in pelagic seabirds

Pelagic seabirds represent an ideal group with which to examine the plausibility of DWGF models of speciation. Seabirds are highly mobile and so gene flow between breeding populations is not impeded by large-scale physical barriers and therefore has the potential to be high. This trait also means that populations are widely distributed and likely to be associated with different resource bases thereby providing an opportunity for adaptive processes isolate populations. In addition, the prevalence of natal philopatry means that population specific assortative mating (central to Bushs' DWGF model) will occur as a by-product.

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1.3 Thesis structure and aims

I aimed to evaluate the applicability of DWGF models of speciation to pelagic seabirds by examining the possibility of local adaptation in the wedge-tailed shearwater (*Puffinus pacificus*). To this end, a number of traits were measured within and between colonies including morphology, foraging behaviour, and chick growth/developmental characteristics. Gene flow between/among populations was also gauged to determine the effect different levels had on morphological variation. Foraging behaviour and chick developmental patterns were quantified primarily at two colonies that are constrained to

use predictably different resource bases during the breeding period; Heron Island (Great Barrier Reef) and Lord Howe Island (south-western Pacific Ocean).

This thesis is divided into 6 chapters. The general methods are described in Chapter 2, where information regarding sampling design, locations, laboratory methods and genetic analyses are given. Chapter 3 focuses on documenting and comparing local oceanographic conditions, foraging behaviour and chick developmental patterns between Heron Island and Lord Howe Island. Chapter 4 examines sex-specific foraging patterns within Heron Island and Lord Howe Island in order to assess the possibility that intra-colony processes can promote isolation. Chapters 5 (morphology) and 6 (gene flow) document both intra and inter-colony morphological and genetic divergence, with a general discussion on the overall implications of this study included in Chapter 6.

Table 1. Empirical studies of taxa where dispersal ability and/or behavioural characteristics make non-allopatric divergence possible.

Taxa	Findings	Reference
Birds		
Little greenbul *(Andropadus virens*)	habitat dependent morphological differentiation	Smith et al. (1997)
Spectacled eider (*Somateria fisheri*)	genetic structuring among populations	Scribner et al. (2001)
Seabirds		Burg & Croxall (2001)
Brown booby (*Sula leucogaster*)	genetic structuring among populations	Steeves et al. (2003)
Procellariiforme Seabirds		
Cory's shearwater (C*alonectris diomedea ssp.*)	genetic structuring among populations	Rabouam et al.(2000)
	morphological differentiation	Granadeiro (1993)
Black-browed albatross (*Thalassarche melanophris*)	genetic structuring correlated with foraging grounds	Burg & Croxall (2001), Waugh et al.(1999)
Mammals		
Humpback whale (*Megaptera novaeangliae*)	significant genetic structuring within ocean basin	Baker et al. (1998)
Killer whale (*Orcinus orca*)	morphological and genetic structuring within ocean basin	Hoelzel & Dover (1991),Ford et al. (1998)
Bottlenose dolphin (*Tursiops spp.*)	morphological and genetic structuring within ocean basin	Wang et al. (1999)
Pilot whales (*Globicephala melas*)	genetic srtucturing within ocean basis	Fullard et al. (2000)
Reptiles		
Green turtle (*Chelonia mydas*)	significant genetic structuring within ocean basin	Encalada et al. (1996)
Invertebrates		
Butterfly (*Heliconius spp.*)	morphological differentiation	Jiggins et al. (2001)
	selection against hybrids	
Pea aphid (*Acyrthosiphon pisum*)	significant genetic structuring between host populations	Via (1999)
	selection against hybrids	Via (2000)
Apple maggot fly (*Rhagoletis pomonella*)	significant genetic structuring between host populations	Feder et al. (1988)(1990)
		Prokopy (1988), Feder (1994)
Larch budmoth (*Zeiraphera diniana*)	significant genetic structuring between host populations	Emelianov et al.(1995)
Fruit fly (*Drosophila serrata/birchii*)	morphological differentiation	
	selection against hybrids	
Fish		
Sockeye salmon (*Oncorhynchus nerka*)	significant genetic structuring between 'ecotypes'	Higgie et al. (2000)
White fish (*Coregonus clupeaformis*)	significant genetic structuring between 'ecotypes'	Lu & Bernatchez (1999)
	morphological differentiation	
Cichlid (*Tilapia spp.*)	significant genetic structuring between 'ecotypes'	Schliewen et al. (2001)
	morphological differentiation	

CHAPTER 2

2.0 GENERAL METHODS

2.1 Study locations and experimental design

Details regarding the experimental design for the behavioural component of this project (foraging behaviour, mating systems) are found in the relevant chapters. Nonetheless, a generalised template for sample/data collection was used. In order to examine the possibility of ecological based mechanisms to cause genetic divergence, two colonies were chosen that represented predictably different environments in which wedge-tailed shearwaters (*Puffinus pacificus*) forage during the chick-rearing period. These were Heron Island (representative of a sub-tropical environment) and Lord Howe Island (a more temperate locale). In addition to these two colonies, blood samples for genetic analyses were obtained from two further colonies (Fig. 1), and a limited number from Hawaii (Oahu). Table 2 shows the data that was obtained for each of these colonies. These locations were chosen so that data could be collected from colonies that experienced the maximum potential for differences in foraging/ environmental conditions, as well as according to distance and the existence of large-scale geographical barriers. This was so that differences in measured traits could be assessed in light of potential causes. For example, by sampling the Rottnest Island colony, it was hoped that the relative importance of large-scale geographical barriers (in this case the Australian continent) in causing any observable genetic or morphological divergence could be assessed.

2.2 Study species

The wedge-tailed shearwater is a pelagic, tube-nosed, burrow nesting seabird (Procellariidae) that breeds on islands primarily in tropical locales but also in more temperate locations. Breeding colonies are distributed right across the Indo-Pacific ocean basin. In Australian Pacific waters, breeding colonies occur from southern New South Wales to the tip of Cape York Peninsula including coral cays of the Great Barrier Reef (GBR)(Marchant and Higgins 1990). The small amount of banding data that is available suggests that adults are able to disperse widely and travel vast distances when not breeding. However, during the breeding season, they are constrained by the need to forage locally to provision chicks. Adults rear a single chick over a 60 - 70 day nestling period.

2.3 Laboratory Methods and Materials

2.3.1 Sample Collection

Blood samples for genetic analyses were obtained from eighty-nine wedge-tailed shearwaters at five locations across the indo-pacific (Table 2, Fig. 1). All samples came from breeding birds except those from Hawaii (Table 2). Samples from Hawaii came from dead birds that either died of natural causes (beach strandings), or were killed by cars. Samples from breeding birds were collected during the breeding period (December – March). Details of sample times and numbers are included in Table 2. Samples were collected from each member of a pair. Individual pairs constituted replicates used for the foraging behaviour study (Chapter 3). Birds were caught by hand at night after returning to feed chicks. Blood from all living birds was extracted from the main tarsal vein. Approximately 25-50 µ1 of blood was taken from each individual and stored in 100 µ1 of Queen's Lysis buffer (Tris, NaCl, EDTA, Sarkusyl). The birds were then released adjacent to where they were caught.

Table 2. Data collected during the course of this project.

Location	Lat./Long.	Tissue samples	Morphology (Individuals)	Foraging Behaviour (Year)	Chick Growth (Year)
Heron Is.	23° 26' S, 151° 51' E	21	235	2003, 2005	2003, 2005
Raine Is.	12° 00' S, 144° 00' E	20	38	2002	_
Lord Howe Is.	31° 33' S, 159° 05' E	20	72	2004	2004
Rottnest Is.	32° 00' S, 115° 30' E	20	50	_	_
Hawaii (Oahu)	21° 18' N, 157° 52' W	8	_	_	_

2.3.2 DNA Extraction

Total genomic DNA was extracted using the DNeasy® Tissue Kit (Qiagen Pty Ltd). Initially, recommended protocols were used; however, protocols were optimized to improve efficiency and DNA yield. As both blood and tissue from feathers were used as the initial starting material, optimal protocols differed. For blood samples, approximately 45 µl of blood/Queen's Lysis was used. Samples were digested at 70ºC with the recommended concentration of proteinase K for a minimum of 4 hrs. For feathers, 5 – 10 mg of feather root (with attached blood/skin) was ground, using a micro-pestle, in a 1.5 µ1 microfuge tube. Samples were digested at 70ºC with the recommended concentration of proteinase K for a minimum of 5 hrs.

The quality of DNA obtained using this extraction procedure was checked by loading five microlitres (µl) of resuspended DNA along with 5 µl of x 1 TA buffer and 2 µl of loading dye (Bromophenol Blue) onto a 2 % agarose gel for electrophoresis. Gels included 0.005µl of ethidium bromide (EtBr)/ml agarose so that DNA could be visualised. Gels were run in x1 TA buffer at 45 MA for approximately 30 minutes. DNA was detected using ultraviolet light (GelDoc 1000 image system, BIORAD). To gauge the average amount of DNA recovered from the above protocols, a Hoefer® DyNa Quant® Fluorometer was used in accordance with the protocols provided.

2.3.3 Population Genetic Markers and Polymerase Chain Reaction (PCR)

A number of considerations should be made before screening populations for genetic variation and structure. The most important of these is the choice of genetic marker. Population genetic markers can be dominantly inherited or codominantly inherited. The former are multilocus markers (eg. RAPDs, AFLPs), that are technically convenient, but possess a number of weaknesses. The major concern with dominant multilocus markers is that a proportion of the observed variation can be non-heritable. This means that patterns of genetic structure among populations can only have meaning relative to other populations in the same study. Moreover, allele frequencies cannot be calculated, which severely restricts both the power and scope of the genetic analyses and therefore the interpretations that can be made from the data (Sunnucks 2000). Codominant markers however enable allele frequencies and, consequently, gene genealogies/phylogenies to be calculated. This allows for analyses that can infer past

population processes, and can separate past demographic processes from current ones. This is an important consideration when attempting to determine the relative importance of past vs. present barriers to gene flow (Peck and Congdon 2004).

It is also appropriate to consider using more than one codominant marker when attempting to determine if genetic structuring exists. This has two main advantages. Firstly, it can reduce the effect of random sampling regimes that can cause different patterns in markers that have similar histories (Palumbi and Baker 1994). Secondly, using more than one codominant marker will improve confidence that observed patterns are real. This is particularly relevant when using analyses that measure the amount and direction of gene flow (such as coalescent analysis) (Edwards and Beerli 2001).

2.3.3.1 Mitochondrial control region

Mitochondrial DNA (mtDNA) control region (or 'D' Loop) gene genealogies have been used to construct intra-specific phylogenies for numerous animal populations (Avise et al. 1987). mtDNA is maternally inherited and thus non-recombining. This characteristic means that the effective population size of a group of individual haplotypes is ¼ that of bi-parentally inherited markers. Moreover, mtDNA has a high net mutation rate respective to nuclear DNA, resulting in greater resolution at the mtDNA locus than at nuclear loci (Birkey et al. 1989). For these reasons, the mtDNA control region is extremely useful for studies relating to population divergence.

In birds, Part I of the mtDNA control region has been shown to be the most variable (Wenink et al. 1994), although other regions have been shown to be useful in elucidating barriers to gene flow in seabirds (Peck and Congdon 2004). The polymerase chain reaction (PCR) primers that were used initially in this study were those described by Desjarins and Morais (1990);

TS437R 5′-GGGTTGCTGATTTCACGTGA-3′,
CH16746L 5′-ACCCCAAGGACTACGGCTTGAA-3′.

This primer pair targeted Part 1 of the control region. These primers have been used successfully on sooty terns (*Sterna fuscata*) by Avise et al. (1994), and produced a PCR product of approximately 400 base positions (bp) in length. However, this product was unrecognisable according to the published mtDNA sequences of Avise et al. as well as; (1) The chicken mtDNA control region sequence published by Desjarins and Morais (1990) and (2) two shorebird mtDNA control region sequences (a turnstone and a dunlin)(Wenink et al. 1994). All four of these sequences were originally cloned from purified mtDNA and so were not likely to be nuclear copies. A search of Genbank also failed to produce a match to the sequence obtained using the above-mentioned primers.

Because sampled sequences could not be reconciled with the published sequences, and amplification of the non-targeted product could not be avoided using extensive optimisation procedures, further PCR analysis using primers that targeted different sections of Part I of the mitochondrial control region were conducted (Table 3). Unfortunately, the same problem was encountered. Recently, a number of workers have encountered similar problems in seabird studies using the mtDNA control region (Friesen pers. comm. 2003). Non-targeted products have been identified as nuclear copies, or in some cases, duplicate control regions that are evolving in concert (Friesen pers. comm. 2003, Peck and Congdon 2004, Abbott and Double 2005). As a result of these problems, a different suite of molecular markers had to be used.

2.3.3.2 Microsatellites

Microsatellites (short repeated base position motifs) are used routinely to examine population genetic structure and to infer gene flow among populations. Microsatellites are highly variable and codominant, making them ideal for this purpose. Microsatellite primers are presently unavailable for the wedge-tailed shearwater. Consequently, a number of previously published primers designed to amplify loci in seabirds, as well as in more distantly related taxa were trialled (Table 3).

Table 3. Primer trials for wedge-tailed shearwaters.

Locus	Primer Sequence (5'-3')	Result	Reference
Dc5	F: AGGAGGGAAACTTCTCCCAG R: AGCAGGGAGTGACTTGAGGAG	Monomorphic	Burg (1999)
Dc9	F: CGTGGTATATAGCTTATGGGCA R: GAGATTGTACTCCTGGGGCA	NP	Burg (1999)
Dc16	F: TTTTCCAAAGAGATGGCACCA R: GACAGCAGAGGTGGGTCTGT	NP	Burg (1999)
Dc20	F: GGATTGCTGTGGTTTTGCTT R: AACATGACACAGGAGAGTGGC	Monomorphic	Burg (1999)
Dc27	F: CACCCATTTTTGCAGTTCAC R: TCCCCTTGCTTGTTGATTATG	NP	Burg (1999)
De11	F: CCTGGAAAAGGCCCTTATATTC R: CACCGAGTACCATCATTCCC	Monomorphic	Burg (1999)
De35	F: CAAACCTGAAACCTTCCAAAAC R: CCCCCTGTTTCTACTCTGGTC	Monomorphic	Burg (1999)
HrU2	F: CATCAAGAGAGGGATGGAAAGAGG R: GAAAAGATTATTTTTCTTTCTCCC	Monomorphic	Primmer et al. (1995)
Paequ3	F: TGTGGGTGCAGTAGAGCA R: CAATAAGAAGATCAGCAGAACAGAC	Polymorphic	Techow & O'Ryan (2004)
Paequ7	F: TGCAGACCTGACTTTCACAGCTC R: CCTCCAAACATCCAGCCATC	Polymorphic	Techow & O'Ryan (2004)
Paequ8	F: TATTCTGAGACTTGCGTTATCC R: GTGATCCATTAGTTGATGTCTACTG	Monomorphic	Techow & O'Ryan (2004)
Paequ13	F: GACCTGCAGCAATAGCACGAC R: TGCCTTCATCAGAATCCTCCTG	Polymorphic	Techow & O'Ryan (2004)
Gapd	F: ACCTTTAATGCGGGTGCTGGCATTGC R: CATCAAGTCCACAACACGGTTGCTGTA	Polymorphic	Friesen et al. (1997)
Lamin	F: CCAAGAAGCAGCTGCAGGATGAGATGC R: CTGCCGCCCGTTGTCGATCTCCACCAG	Polymorphic	Friesen et al. (1997)
Aldolase	F: ATCATCAAAGAAAAAGGCATGGTGGTGGG R: AGCACCATCTTTCTTGTACTGGGCACAGCG	Polymorphic	Friesen et al. (1997)

Ultimately, three loci were used based on levels of polymorphism and the ability of the published primers to amplify the loci (Table 3). Primers for these loci were originally developed for white-chinned petrels (*Procellaria aequinoctialis*) (Techow and O'Ryan 2004). PCR reactions were carried out in a 10 µl reaction volume containing 10x PCR Buffer (200mM Tris-HCL (pH 8.4), 500mM KCl); 2.5mM $MgCl_2$; 5pmol of fluorescently labelled forward primer; 5pmol of unlabelled reverse primer; 0.1 mM of each dATP, dTTP, dCTP, dGTP and 1 unit of *Taq* polymerase (Life Technologies). The only exception was locus Paequ3 for which 1.5 mM $MgCl_2$ was used. Loci were amplified using a 'step-down' annealing procedure: one cycle for 120s at 94ºC, 30s at 57ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 56 ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 55 ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 53 ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 51 ºC, 45s at 72 ºC, 10s at 94ºC and one final cycle of two minutes at 72ºC. Again, the only exception to this protocol was locus Paequ3 for which a step-down annealing procedure of: one cycle for 120s at 94ºC, 30s at 61ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 60 ºC, 45s at 72 ºC, 10s at

94ºC; five cycles for 30s at 58 ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 57 ºC, 45s at 72 ºC, 10s at 94ºC; five cycles for 30s at 55 ºC, 45s at 72 ºC, 10s at 94ºC and one final cycle of two minutes at 72ºC was used.

At the completion of the PCR, 1 µl of the PCR reaction was mixed with 2 µl of 0.3 mg/ml of bromophenol blue/100% formamide. Samples were heat denatured for three minutes and 1 µl was loaded onto a 6% denaturing acrylamide gel. Gels were run using the Gel-Scan 2000 (Corbett Research).

2.3.3.3 Introns

The use of nuclear introns in population genetic studies is becoming increasingly common. This is because introns have a number of benefits. Firstly, intron loci present genetic variation that is representative of the entire genome (Friesen 2000). This is in contrast to single gene markers (such as mtDNA) that may not necessarily be typical of genetic variation across the genome. In addition, universal intron primers have been developed that anneal to exons of highly conserved nuclear genes, thus avoiding the time and cost of developing species-specific primers. Finally, introns have potentially high rates of sequence evolution and have been shown to be highly variable within species (Palumbi and Baker 1994).

In the current study, three introns were screened for sequence variation (Table 3). The primers for these loci were developed by Friesen et al. (1997), and have subsequently been shown to work effectively in a range of vertebrate taxa (Friesen et al. 1997). PCR reactions were carried out in a 10 µl reaction volume containing 10x PCR Buffer (200mM Tris-HCL (pH 8.4), 500mM KCl); 4.0mM $MgCl_2$; 5pmol of each primer; 0.1 mM of each dATP, dTTP, dCTP, dGTP and 1 unit of *Taq* polymerase (Life Technologies). The only exception was Gapdh for which 2.5 mM $MgCl_2$ was used. Loci were amplified using a 'step-down' annealing procedure: five cycles for 90s at 94ºC, 30s at 94ºC, 30s at 69ºC, 90s at 72ºC; five cycles for 30s at 94ºC, 30s at 68ºC, 90s at 72ºC; ten cycles for 30s at 94ºC, 30s at 65ºC, 90s at 72ºC; fifteen cycles for 30s at 94ºC, 30s at 64ºC, 90s at 72ºC; and one final cycle of seven minutes at 72ºC. Again, the only exception to this protocol was locus Gapd for which a step-down annealing procedure of: five cycles for 90s at 94ºC, 30s at 94ºC, 30s at 70ºC, 90s at 72ºC; five cycles for 30s at 94ºC, 30s at 68ºC, 90s at 72ºC; ten cycles for 30s at 94ºC, 30s at 65ºC, 90s at 72ºC;

fifteen cycles for 30s at 94ºC, 30s at 64ºC, 90s at 72ºC; and one final cycle of seven minutes at 72ºC was used.

At the completion of the PCR, 5 µl of the PCR reaction was subjected to electrophoresis through a 2% agarose gel using the same protocols as those used for checking DNA extraction quantity and quality. To ascertain the size of the PCR product 0.1 µg of 50bp DNA ladder (Invitrogen) was run on each gel for comparison. PCR reactions were then purified using the UltraClean™ PCR purification kit (MOBIO Inc.) using protocols supplied by the manufacturer. Purified products were eluted into 50 µl of ultraviolet treated water. Quantification for sequencing reactions was achieved in the same manner as for quantification for genomic DNA extractions (i.e. using the Hoefer® DyNa Quant® Fluorometer).

2.3.3.3.1 Intron sequencing

PCR products were cycle sequenced using two protocols. Initially the Big Dye ™ primer incorporation method was used (Applied BIOSYSTEMS). Reactions were carried out in 0.2 ml thin-walled PCR tubes. Each reaction consisted of 4 µl of x5 CSA buffer, 0.5 µl of 10mM forward primer and approximately 20 ng of template DNA. Ultra clean water (UV treated) was added to make a total volume of 20 µl. Initially, three individuals were sequenced in the reverse direction also so that the 3' end of the PCR product could be compared with the published intron sequence of both the marbled murrelet (seabird) and the chicken (Friesen et al. 1997). This allowed confirmation that the product was the targeted intron. Cycling conditions for the sequencing reactions were as follows; initial denaturing step of 96 ºC for 10s, annealing step of 50 ºC for 5s, extension step of 60 ºC for four minutes. After 35 cycles, a final extension step of 60 ºC for seven minutes was completed.

Sequencing products were purified using protocols supplied by the Automated DNA sequencing facility (University of N.S.W., Sydney), and then submitted to the same facility where they were run on a vertical electrophoresis acrylamide gel, in a Perkin-Elmer ABI Prism™ 377 DNA sequencer. The sequence of each sample is determined by a laser beam that reads the frequency of dye labelled nucleotides. This information is transformed into electropherograms (using ABI Prism 377 Sequencing Analysis Software), that allows for the visual inspection of individual sequences.

Sequences were aligned using the sequence alignment program ProSeq v 2.91 (Filatov 2002), and checked visually for reading errors and nucleotide base changes among individuals. Any base positions that could not be reconciled were scored as 'N' and subsequently not included in the analysis.

2.3.3.3.2 Intron allele assignment

Sequencing intron loci results in chromatograms of two overlapping alleles. This is because introns are bi-parentally inherited nuclear markers. Therefore, in some instances, sequenced individuals would show base position changes at more than one base position (> 1 bp heterozygotes), thus making allele assignment difficult.

In these instances, the program PHASE was used to assign unknown heterozygotes using a Bayesian approach (Stephens et al. 2001, 2003). Statistical analyses (i.e. AMOVAs) were carried out on both the dataset without unknown heterozygotes (reduced sample size) and with unknown heterozygotes assigned using PHASE.

2.3.4 Molecular sexing

Molecular sexing of wedge-tailed shearwaters was carried out using a modification of protocols developed by Fridolfsson and Ellegren (1999). This technique utilises the presence of the CHD-gene on the female sex-linked chromosome and a non sex-linked chromosome. A PCR with the primers

2550F (5'-GTTACTGATTCGTCTACGAGA-3') and
2718R (5'-ATTGAAATGATCCAGTGCTTG-3'),

results in two different sized bands in females and one band in males when the PCR product is run out with 2 µl of loading dye (Bromophenol Blue) on a 2% agarose gel at 33 MA for ~30 minutes. PCR reactions were carried out in 25 µl volumes, consisting of: 10 – 20 ng of DNA; 10x PCR Buffer (200mM Tris-HCL (pH 8.4), 500mM KCl); 2.0mM $MgCl_2$; 5pmol of each primer; 0.15 mM of each dATP, dTTP, dCTP, dGTP and 1 unit of *Taq* polymerase (Life Technologies). PCR cycle conditions were as follows: one cycle for 90s at 94ºC; thirty cycles for 45s at 50ºC, 30s at 72ºC, 30s at 94ºC; one cycle for 60s at 50ºC, 5 minutes at 72ºC. Known pairs were examined together on a gel

and for every pair, both a male and a female individual were identified, thus confirming the robustness of the technique.

2.3.5 Statistical Analysis

2.3.5.1 Tests of assumptions

Methods of estimating population genetic structure and/or gene flow from molecular data make a number of assumptions. The most important are; (1) that effective population sizes have remained stable over long periods of time (i.e. that populations have not undergone recent expansions), (2) that sampled genes are neutral to selection (Beerli 1998), (3) that migration rates between populations are equal, (4) that loci are unlinked (linkage disequilibrium), and (5) that recombination doesn't occur within loci (i.e. that alleles have independent evolutionary histories).

These assumptions maybe unrealistic, and therefore represent significant limitations regarding results and subsequent conclusions of these analyses. However, recent advances, especially in the use of coalescent theory, enable some of these assumptions to be evaluated independently (Beerli 1998).

Mismatch distributions describe the amount of genetic difference between pairs of sequences in a sample (Slatkin and Hudson 1991, Rogers and Harpending 1992). These distributions can be informative about the demographic history of a sample of sequences (Rogers and Harpending 1992, Harpending 1993). In a stable population, the expected mismatch distribution conforms to a geometric distribution, while in a population that is undergoing rapid expansion, a unimodal distribution is expected (see eg. Harpending 1993). Thus, a mismatch analysis enables examination of the assumption that population sizes have remained stable over long periods (assumption 1 above).

A range of other neutrality statistics were also examined in order to detect traces of past population growth, decline or stability in the sequences (assumption 1). Fu's F*s* tests specifically for population growth (Fu 1997) and distinguishes excesses of low frequency alleles in an expanding population as compared to the number expected in one that is static (Fu 1997, Federov and Stensev 2001). In addition, Fu and Li's F* and D* statistics were calculated so that a comparison could be made with Fu's F*s*.

Population growth or range expansion can be distinguished from the effects of background selection by the pattern of significance of F*s*, F* and D* (Fu 1997). A range expansion is indicated when F*s* is significant and F* and D* are not, whereas the reverse indicates selection (Fu 1997, Joseph et al. 2002).

Tajima's D test is useful as a measure of neutrality (assumption 2 above) in stable populations at mutation-drift equilibrium (Kimura 1980, Tajima 1989):

$$D = (\pi - \theta_b) / \sqrt{(\pi - \theta_b)}$$

The basis of this test is that under neutrality, the number of nucleotide differences between sequences from a random sample (π) should be equal to the number of differences between the segregating (polymorphic) sites only (θ). Departures of Tajima's D from zero can be due to selection, but they can also be due to a number of other factors. Population expansions can cause significant negative departures of Tajima's D from zero (Tajima 1989a.b, Aris-Brosou and Excoffier 1996), and non-significant values of Tajima's D may result from a combination of population expansion and mutation rate heterogeneity, and so do not necessarily support neutrality. Tajima's D was calculated for combined intron data after randomising the order in which alleles were recorded for each individual to avoid establishing pseudo-linkages across loci (Congdon et al. 2000).

In addition to the statistical procedures outlined above, tests for deviations from Hardy-Weinberg frequencies were conducted. Significant deviations from Hardy-Weinberg equilibrium can provide evidence for selection, non-random mating, population structure, small population size, mutation and/or the presence of null alleles. Thus, in combination with the statistical tests outlined above, comparisons of expected allele frequencies vs. observed can be extremely useful. Allele frequency deviations from Hardy-Weinberg equilibrium were tested using an exact probability test (Haldane 1954), and a Markov chain (Guo and Thompson 1992) using the software program TFPGA (Miller 1997). Statistical tests and mismatch distributions were calculated using both Arlequin (Schneider et al. 1997) and DnaSP 3.51 (Rozas and Rozas 1999) to

ensure consistency of results. Hardy-Weinberg deviations were examined using TFPGA (Miller 1997).

Estimates of F_{ST} may be misleading when it is assumed that the exchange of individuals between sub-populations is equal ((3) above) and/or if population sizes differ (Beerli and Felsenstein 1998). Because this assumption may not be biologically realistic for wedge-tailed shearwater populations sampled in this study, gene flow was also estimated using a maximum-likelihood method based on coalescent theory that allows for asymmetrical exchange of individuals between different sized populations (Beerli and Felsenstein 1998). In addition, this maximum-likelihood method is less sensitive to small sample sizes, and can still give accurate results with only a few loci (Beerli and Felsenstein 1998). The direction and magnitude of gene flow was estimated using the computer program MIGRATE (Beerli 2002).

For all analyses the default settings of MIGRATE were used. Introns and microsatellites were analysed independently. The sequence model was used for the intron loci and the Brownian microsatellite model was used for the microsatellites. Heating was activated and four temperatures used; 1.0, 1.5, 2.5, and 6. This method allows for exploration of a very large 'data space' thereby improving confidence interval estimation (Beerli 2002). MIGRATE was run a minimum of four times for each marker set to ensure consistency of results. For each run the random number seed was changed.

Finally, linkage disequilibrium among loci (assumption 4) was analysed using a Markov chain for individual populations, or a Fisher exact test for all populations (using Arlequin), and lack of recombination (assumption 5) was tested by calculating recombination rates using the MIGRATE software (Beerli 2002). Where appropriate, statistical tests were conducted with sequential Bonferroni adjustments in order to control for Type I errors resulting from multiple comparisons from single sampling sites (Rice 1989).

2.3.5.2 Population genetic structure

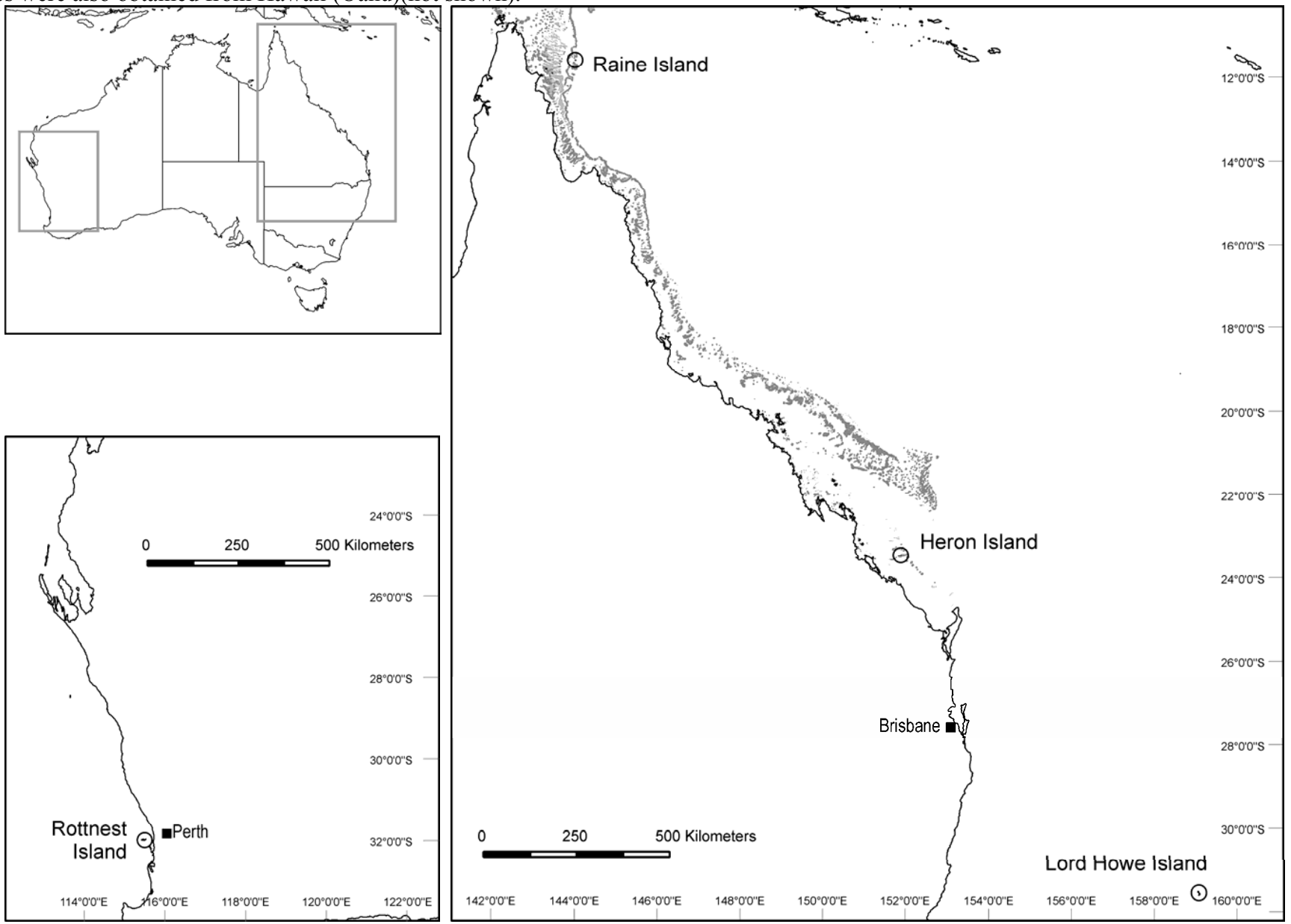
In order to examine spatial patterns of population structure in microsatellite data, Wright's F_{ST} (Wright 1965) was calculated using an analysis of variance on genotype

frequencies (using Arlequin). Population structure using intron loci was examined using both allele frequency and sequenced based methodologies. ϕ^{ST}, an analogue of Wright's F_{ST} that incorporates information on sequence divergence among haplotypes (Excoffier 1992, Michalakis and Excoffier 1996), was calculated for sequence data using analysis of molecular variance (AMOVA). Intron sequence information from each individual was input into AMOVA analyses as two 'haplotypes'. To avoid establishing pseudo-linkages across loci, the order in which haplotypes were recorded in software package spreadsheets was randomized. In the first instance, all samples/individuals were classified as a single group so that variation among sampling sites, and ϕ^{ST} could be calculated. The significance of estimates of ϕ^{ST} was determined using a Markov chain analysis (Raymond and Rousset 1995) with 5000 permutations (Schneider et al. 1997).

2.3.5.3 Isolation by distance

Isolation by distance was examined using the mean of immigration and emigration between populations calculated with MIGRATE. Geographic distances between sites were calculated using Distance Finder (http://www.indo.com/distance [2001]). Pair-wise geographic distances ranged from 1148 km to 4099 km. Associations between gene flow and geographic distance were examined with a Mantel (1967) test. Mantel tests use random permutations of matrix combinations to test if correlations between distance matrices are greater than expected by chance (Sokal and Rohlf 1995). Mantel tests thus allow for the lack of independence in regression points, and so are not violating the major assumption of OLS regression. Mantel tests were conducted in GenAlEx (Version 5.04)(Peakall and Smouse 2002) and also IBD (Bohonak 2002) to ensure consistency of results.

Fig. 1. Map showing the location of wedge-tailed shearwater breeding colonies sampled in this study. A small number of samples for genetic analyses were also obtained from Hawaii (Oahu)(not shown).



CHAPTER 3

3.0 INTRODUCTION

Increasingly, studies of population-specific foraging behaviour suggest that selection on life-history traits associated with foraging in different background environments can lead to local adaptation and promote genetic divergence between populations (Foster 1999, McLaughlin 2001, Remeš and Martin 2002). Despite this, empirical studies examining spatial and temporal variation in foraging behaviour at the population level are rare (Smith and Skúlason 1996, Foster 1999). This is the case in seabirds, where a limited number of comparative studies exist (e.g. Waugh et al. 2000, Hamer et al. 2001, Falk et al. 2002, Tremblay and Cherel 2003). The relative paucity of studies belies the fact that seabirds are good model taxa for examining local adaptive processes (Chapter 1).

Where colony-specific resources differ, substantially different foraging strategies may be favoured at alternative locations, each maximising lifetime reproductive output relative to background resource availability (Endler 1977, Suryan et al. 2000, Vos and Hemerik 2002). Entrained by population-specific foraging behaviour, physiological characteristics such as chick developmental patterns may also diverge and become population-specific (Arendt 1997). If inter-colony matings then produce chicks with developmental patterns unsuited to either foraging environment, character displacement may serve to further promote population differentiation and eventually even speciation (see Endler 1977).

Foraging behaviour in many Procellariiform seabird species varies according to local resource availability, which in turn may be linked to local oceanographic parameters such as productive upwelling zones and sea surface temperature (SST) (eg. Granadeiro et al. 1998, Catard et al. 2000, Waugh et al. 2000). SST has been shown to influence wedge-tailed shearwater breeding success over seasonal time scales (Smithers et al.

2003). However it is possible that day-to-day fluctuations may also impact on prey availability/accessibility and therefore indirectly affect provisioning rates and chick development. If SST influences prey accessibility differently at alternative locations, then this will also promote local adaptation and enhance character displacement.

When local resources are poor Procellariiforms use a specialized bimodal foraging strategy that alternates multiple short foraging trips (1-4 days duration) in near-colony waters for chick provisioning, with longer self-provisioning trips (>5 days duration) to highly productive areas located at-distance from breeding colonies (Weimerskirch 1998, Weimerskirch and Cherel 1998, Congdon et al. 2005). However, at colonies where near-colony resources are sufficient, they provision using a unimodal strategy based on local productivity only (Granadeiro et al. 1998, Waugh et al. 2000). The wedge-tailed shearwater is one such species. The population at Heron Island, Australia uses a bimodal strategy (Congdon et al. 2005) while the population at Tern Island-Hawaii adopts a unimodal strategy (Baduini 2002). Currently, it is not known if differences between colonies are colony-specific (obligate) or vary according to temporal changes in resource availability (facultative). Nor is it known if chick development at each location reflects differences in provisioning rates associated with these two strategies.

This chapter has three aims;

(1) To examine the sensitivity of wedge-tailed shearwater foraging success to sea surface temperature fluctuations at Heron Island and Lord Howe Island.

(2) To assess background productivity levels at Heron Island - GBR, and Lord Howe Island - south western Pacific Ocean.

(3) To document foraging behaviour at these two locations, and examine the relationship between chick developmental patterns and adult foraging behaviour at both locations.

This was done to determine if wedge-tailed shearwaters respond to oceanographic parameters such as SST in the same way at both locations and to assess the potential for

co-ordinated local adaptation between adult foraging behaviour and chick development to restrict gene flow and promote population divergence in this species.

3.1 METHODS

3.1.1 Study sites

Foraging and provisioning behaviour data was primarily collected at Heron Island (2003) and Lord Howe Island (2004) during the chick-rearing period (February/March). However, limited data on foraging behaviour was also obtained at Heron Island in 2005 and during the prospecting/incubating period at Raine Island during December 2002. Since the methodology differed at Raine Island, data collection and statistical analyses for this location are treated separately. Further details on study locations can be found in Chapter2.

3.1.2 Sensitivity to SST

ANCOVA was used to gauge the sensitivity of wedge-tailed shearwaters to SST at Heron and Lord Howe Islands. Relative daily mass change was used as the response variable, SST as the co-variate and island as the factor. Relative daily mass change was calculated as chick mass change over each 24 h period (i.e. the difference between 2 consecutive 16:00 h masses) divided by the chick mass at the start of the 24 h period. These were then averaged for all chicks for each day of the study period. Sea surface temperature (SST) values were obtained from NASA satellite-derived images (http://poet.jpl.nasa.gov/), and tested *a priori* for autocorrelation.

3.1.3 Primary productivity: Heron Island vs. Lord Howe Island

To compare background levels of productivity between Heron Island and Lord Howe Island across multiple years, sea-surface chlorophyll concentrations were obtained from SeaWiFS Local Area Coverage (LAC) ocean colour data (accessed 21/03/05; http://reason.gsfc.nasa.gov/OPS/Giovanni/ocean.seawifs.shtml) (as per Murtugudde et al. 1999, Weimerskirch et al. 2005). Data consisted of monthly mean chlorophyll concentrations for 50 km^2 of ocean surrounding each island, from 1998 to 2004, at the scale of 0.1 º. Chlorophyll data from each island in each year were tested for homogeneity of variance using Levene's F tests. Different levels of variance were consistently observed between locations but not among years at each location (see results). As a consequence, between-year comparisons were undertaken separately at

each location using monthly samples in a repeated measures ANOVA, while between locations comparisons were performed using monthly samples in a paired t-test that adjusted for unequal variance among samples (Welch's t-test).

3.1.4 Foraging behaviour and chick growth: Heron Is. vs. Lord Howe Is.

For both Heron and Lord Howe Island, work was carried out during the first month of the chick-rearing period. A total of twenty experimental nests were monitored at each location. At Heron Island, monitoring was conducted in 2003 and 2005, however the 2005 season was truncated resulting in only ~12 days of data compared to ~28 days in 2003. During monitoring, burrow entrances were fitted with a one-way trapdoor of clear Perspex. After an adult had entered and chick feeding was complete, as indicated by chicks no longer begging, the visiting adult was captured, weighed (Persola spring balance ± 0.25), identified and released. Adult provisioning rates and chick mass were monitored at each nest daily. Chicks were weighed twice a day at 09:00 and 16:00 hours using an electronic balance (± 0.1g). Between these times no adult visits or chick feeding were observed. At each burrow, both adults were banded for individual recognition and attendance was monitored continuously from 17:00 to 05:00 hours daily. Five control nests were also established at each location where chick growth was monitored but adults experienced no trapping or handling. As nest monitoring was continuous, no adults were left trapped for more than ten minutes. After each capture traps were reset so that subsequent visits by the same or other adults could be detected. Chick weights were obtained immediately following any known adult visit to a nest. At each nest chick skeletal growth measurements (tarsus) were obtained at four-day intervals using dial callipers (± 0.1 mm).

To avoid pseudoreplication, mean values for each parent were used as replicates in analyses of foraging behaviour at each colony. Measures of foraging behaviour obtained per individual included; provisioning rate (meals/night), meal mass (g), meal mass/night (g/night), and foraging trip length (days). In addition, to examine adult foraging efficiency, the mean proportionate daily mass change per individual using the following equation was calculated: Proportionate mass loss = (AM – DM)/DM, where AM and DM is arrival mass and departure mass in grams (González-Solís 2000). Post-hoc analyses were conducted using Tukey's HSD.

Adult foraging patterns were examined using a frequency distribution of the mean time spent (as a proportion of days) on foraging trips of different length for each individual. Kruskal-Wallis ANOVA and post-hoc pair-wise Mann-Whitney tests among trip-length categories (with Bonferroni correction) were used to test for bimodality and colony-specific patterns (as per Congdon et al. 2005). All proportions were arcsine transformed prior to analysis.

Mass increase in Procellariiform chicks can be described accurately by the logistic equation: $W = A/1 + e^{-K\ (T-Tm)}$, where W is chick weight at time T and A is the asymptotic value of the curve (see Ricketts and Prince 1984, Congdon 1990). Data from the first 20-30 days post-hatching provides an accurate estimate of growth parameters over the entire pre-fledging period for seabirds having this pattern of development (Congdon 1990). Growth data from individual chicks were fitted to this equation using least squares regression. To facilitate inter-colony comparisons the asymptotic value (A) for all chicks regardless of colony was standardized to 347.68g. This is the average mass (S.E. = 5.14, n = 32) of wedge-tailed shearwaters at fledging across a range of colonies (Petit et al. 1984, Marchant and Higgins 1990, Carter et al. 1996, Peck et al. unpub. data). A standard *t*-test was used to test for differences in the mean growth rate constant (K) between breeding colonies using chicks as replicates.

The growth of seabird chicks has previously been shown to vary among and within populations in response to spatial and temporal differences in the total quantity of food provided by adults (Cairns 1987, Huin et al. 2000, Tremblay and Cherel 2003). Therefore, to look for underlying difference in developmental rates beyond those attributable to differences in the total quantity of food provided, chick growth at both Heron and Lord Howe Islands was compared relative to per gram of food received by individual chicks. To this end, an ANCOVA with colony location as a factor, meal mass per day as the covariate, and mass change per day (i.e. the difference between two consecutive 16:00 hr weightings) as the response variable was conducted. To account for the potential influence of chick developmental stage on mass changes, the change in mass per day was standardised relative to total chick mass for each individual. A similar analysis was used to examine differences in patterns of tarsus growth between the two colonies with the exception that tarsus length was measured at four rather than one day intervals.

All statistical analyses were conducted using JMP ver. 4.0.2 (SAS Institute Inc.). The normality of each set of measurements was tested using the Shapiro-Wilk W test. Data were either log or square root transformed prior to analysis if deviations from normality were detected.

3.1.5 Data collection and statistical analyses: Raine Is.

Adult incubation characteristics were monitored at Raine Island from 29/01/2001 – 10/12/2002. Each day a small number of burrows were examined for signs of occupancy. When encountered, incubating birds were banded and weighed. Marked burrows were then monitored once daily between 18:00 hrs and 04:00 hrs for the presence of an adult. Any adult present was identified and re-weighed. However, any single incubating adult was only weighed a total of three times. This was to avoid the possibility of nest desertion due to the weighing procedure. Minimum incubation change over period (in days) was obtained from these data, as were rates of food assimilation for incubating birds. Food assimilation rate was calculated as the mean decrease in weight per consecutive 24-hour period.

Each night for a minimum period of ~5 hours between 20:00 and 05:00 all adults encountered on the surface within a ~20m by ~150m study area were caught by hand or with a small net and banded. Adult foraging trip duration was defined as the time elapsed between successive recaptures of the same adult. There is no certainty that this period reflects the actual time spent foraging by an individual adult, as a small number of individuals may have returned elsewhere in the colony between encounters. However, since a comprehensive sample of a random subsection of the colony was obtained, analyses assume that the temporal distribution of recaptures within the study plot reflects the general pattern of attendance at the colony. In addition, due to the level of site philopatry observed in this and similar shearwater species (Wooller et al. 1992), the number of banded birds that may have returned to other areas of the colony would be low. To check this assumption a systematic survey in adjoining areas of the colony around the study site was undertaken each night and on two occasions extensive surveys outside the study site were conducted. No banded birds were found during any of these surveys.

Changes in adult body mass whilst foraging, relative to the weight loss observed in incubating birds (see above) were used to determine the minimum level of adult self-provisioning (minimum assimilated food). A histogram of foraging trip duration was used to examine the foraging pattern and analysed in the same way as described above for Heron and Lord Howe Islands. An ANCOVA was used to establish if food assimilation rates changed with trip duration.

3.2 RESULTS

3.2.1 Sensitivity to SST

A priori autocorrelation analysis indicated an effect at the scale of 1 day. To account for this the degrees of freedom were reduced during significance testing. Relative chick mass change was negatively correlated with SST at both locations ($F_{1,41}$ = 5.31, p = 0.02, r^2 = 0.11) and shows zero or negative growth at around 28°C (Fig. 2). There was also an effect of island on mass change ($F_{1,41}$ = 5.94, p = 0.01), however the slopes of the regression lines for each island were not significantly different from each other ($F_{1,41}$ = 138, p = 0.24).

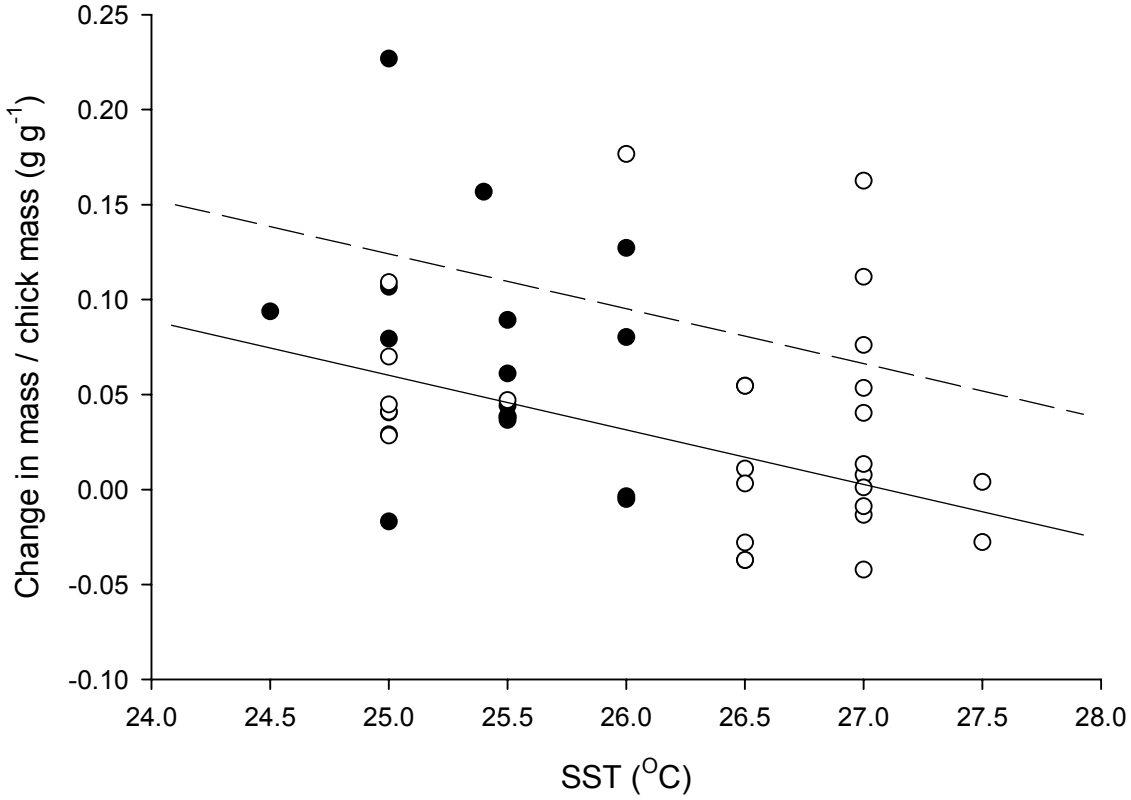


Fig. 2. Relative change in wedge-tailed shearwater chick mass (g) in relation to sea surface temperature (SST ^{o}C) from Heron Island (○) and Lord Howe Island (●). The solid line represents the regression line for Heron Island chicks and the dashed line that for Lord Howe Island chicks.

3.2.2 Primary productivity: Heron Is. vs. Lord Howe Is.

Primary productivity as measured by chlorophyll concentration in surrounding waters was not significantly different among the seven years at either location (Fig. 3, Heron, $F_{6, 83} = 1.69$, p = 0.13; Lord Howe, $F_{6, 83} = 1.88$, p = 0.09). Mean monthly chlorophyll concentration was significantly more variable (Levene's F test; $F_{1, 174} = 57.17$, p = 0.0001) and consistently higher (Welch pairwise t-test; $t_{165} = 4.31$, p = 0.001) at Lord Howe Island (mean = 0.19 mg chlorophyll m^{-3}, ± 0.007, n = 84) than at Heron Island (mean = 0.15 mg chlorophyll m^{-3}, ± 0.007, n = 84) (Fig. 3).

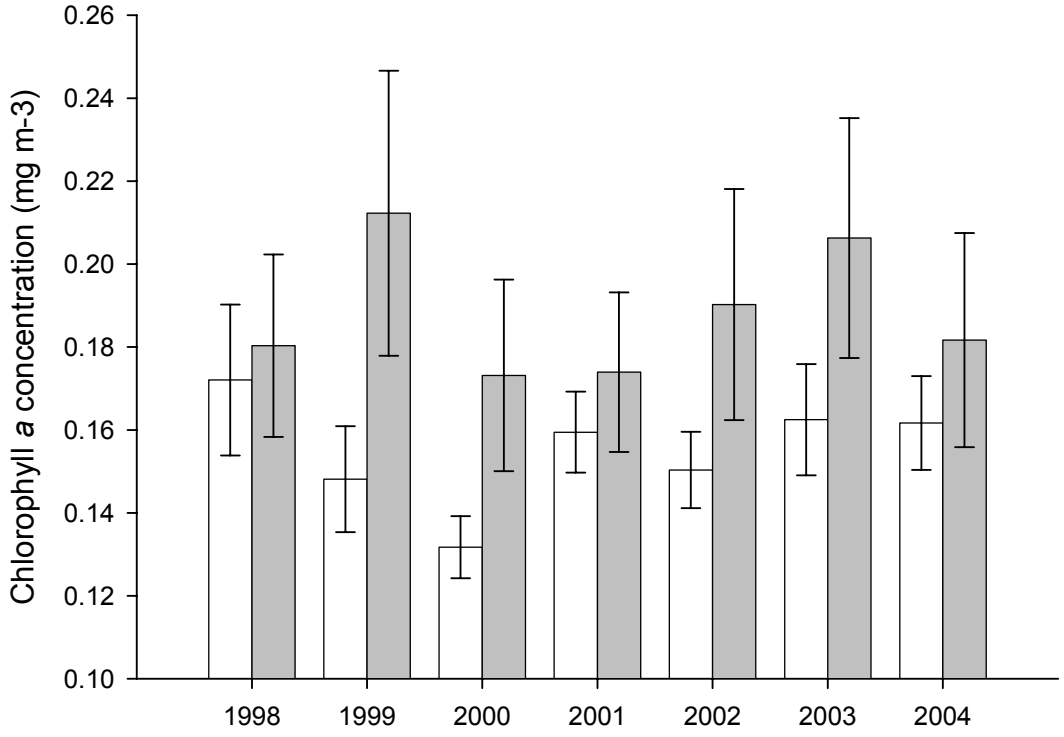


Fig. 3. Variation among years (mean ± SE) in chlorophyll *a* concentration at Heron Island, southern Great Barrier Reef (unshaded) and Lord Howe Island, south-west Pacific Ocean (shaded).

3.2.3 Foraging behaviour: Heron Is. vs. Lord Howe Is.

The majority of adult foraging trips lasted 1-3 days at both study sites. However, mean trip length was significantly longer at Heron Island in 2003 than at Heron in 2005 or Lord Howe Island (Table 4). At Heron Island in 2003, significantly less time was spent on 4 day trips than on either 1-2 day trips or trips of > 5 days (Fig. 4a, Kruskal-Wallis $\chi^2_{11} = 183.71$, $p < 0.001$). This pattern clearly identifies the use of a bimodal foraging strategy at Heron Island with short trips (ST) ≤ 4 days and long trips (LT) of ≥ 5 days. At Lord Howe Island, the proportion of time spent on foraging trips of different length decreased with trip length. Consequently a unimodal foraging strategy was observed (Fig. 4b).

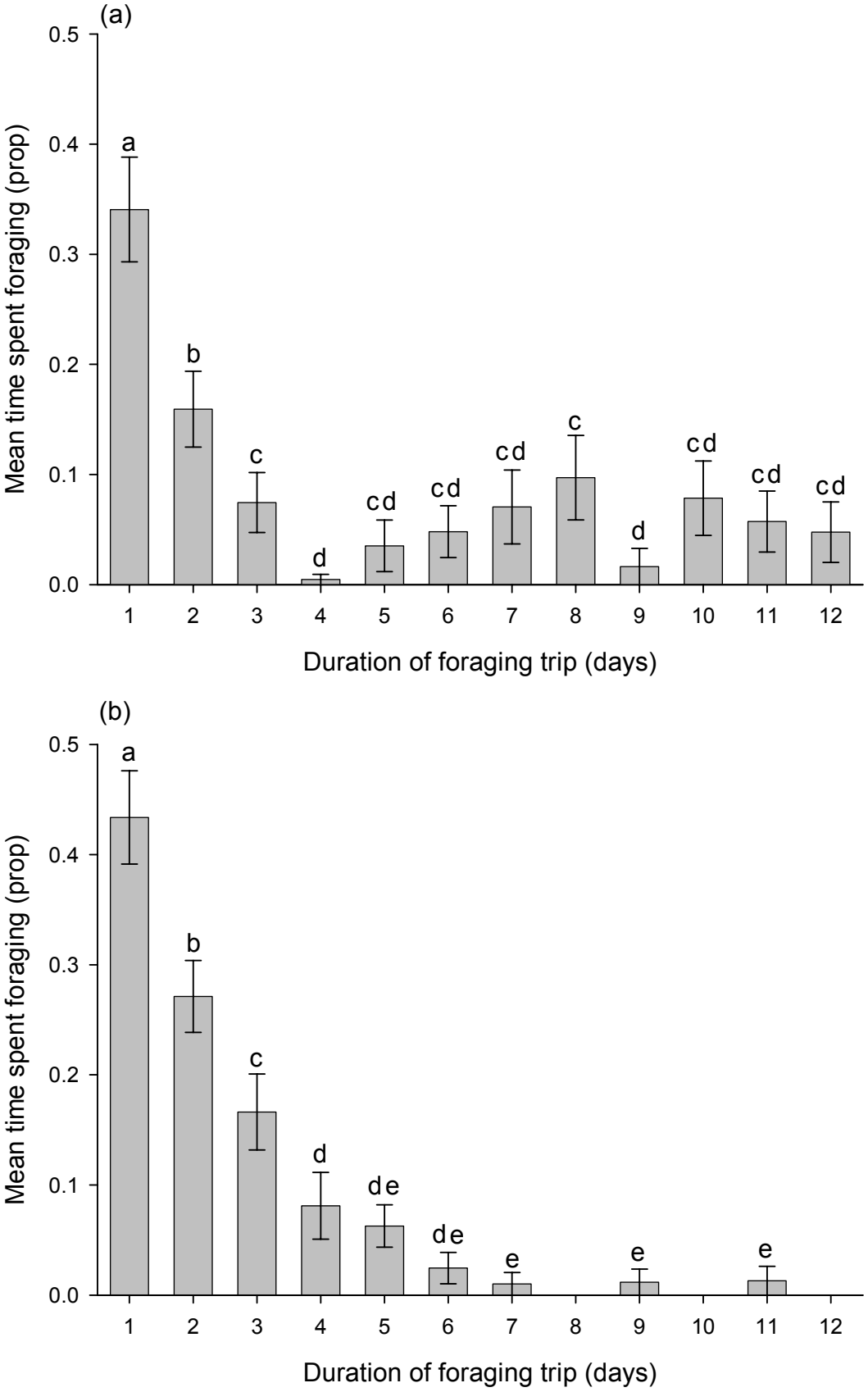


Fig. 4. Mean (± 2 SE) proportion of time spent on foraging trips of different lengths by individual wedge-tailed shearwaters at (a) Heron Island and (b) Lord Howe Island. Different letters indicate means that are significantly different.

The bi-modal foraging pattern seen at Heron Island in 2003 is similar to that observed at the same colony in 2001 (Congdon et al. 2005). As with 2001, in 2003, the second modal interval occurred at foraging trips of eight days duration (Fig. 4a). The pattern of mass loss during short trips (≤ 4 days) and mass gain during long trips (≤ 5 days) observed for Heron Island in 2003 was also consistent with the pattern seen during the 2001 breeding season, and with that seen in all Procellariids that use a dual foraging strategy (e.g. Granadeiro et al. 1998, Weimerskirch 1998). The pattern was not observed at Heron in 2005 because data was only gathered over a 12-day period thereby severely restricting the possibility of observing long trips.

Mean meal mass fed to chicks at Heron Island was not different to that at Lord Howe Island (Table 4). However, the mean meal mass delivered at Heron Island 2003 was significantly more variable than at Lord Howe (Levene's F test; $F_{1, 78} = 3.68$, p = 0.05). After adjusting for unequal variances, mean meal mass delivered at each colony was still not significantly different (Welch t-test; $t_{72} = 1.04$, p = 0.30).
Meal mass delivered to chicks after ST also did not differ significantly between Heron and Lord Howe Islands (Heron mean = 31.99 g, ± 2.12, n = 38, Lord Howe mean = 34.48 g, ± 1.72, n = 40; $t_{76} = -0.914$, p = 0.36). However, chicks at Lord Howe Island were fed on average twice as much per night than those at Heron Island in 2003 (Table 4). This was because chicks at Lord Howe had a higher probability of being fed by both parents each night (Table 4). At Heron in 2005, meal mass and feed probability was also higher than Heron 2003 (Table 4). This is due to the fact that an adult 'change-over' between short and long trips occurred during this time resulting in an increased number of feeds over the truncated study period.

Regardless of foraging trip length, adults at Heron Island 2003 and Lord Howe Island gained similar amounts of mass whilst foraging relative to their mass at the beginning of each foraging trip (Table 4). However, a significant difference was observed between Heron Island 2005 and Lord Howe Island (Table 4), a result of only obtaining short trip data (see following). Foraging efficiency of adults at Heron Island 2003 was significantly lower during trips of 4 days or less than during foraging trips of 5 days or greater ($t_{23} = 5.70$, $p < 0.001$). In fact, adults at Heron Island gained weight during LT and lost weight during ST (Mean foraging efficiency LT = 0.07, ± 0.01, n = 24, Mean

foraging efficiency ST = -0.04, ± 0.006, n = 24), while adults at Lord Howe maintained a relatively constant mass during the provisioning period.

Mean values of K (the growth rate constant) for mass change were significantly different between the two locations. Chicks at Lord Howe Island had a higher mean K value and thus faster growth to fledging mass than those at Heron Island (K = 0.09 ± 0.004 and 0.03 ± 0.02 respectively; t_{35} = -3.70, p < 0.001). Change in chick mass at both locations was positively correlated with the amount of food received (Fig. 5a, $F_{1, 526}$ = 90.80, p < 0.0001, adjusted r^2 = 0.17). The ANCOVA revealed a significant effect of island/location on mean mass change (Fig. 5a, $F_{1, 497}$ = 7.37, p = 0.007) but no difference among chicks or between the slopes of the regression lines for each island (Fig. 5a, $F_{27, 497}$ = 1.13, p = 0.29 and $F_{1, 1}$ = 1.50, p = 0.22 respectively). Therefore, at Heron Island chick mass increase for a given amount of food was consistently higher than at Lord Howe Island. Chicks at Heron Island added approximately 0.03 grams (per gram of chick) more per gram of food delivered than those at Lord Howe Island (Fig. 5a).

Change in tarsus for chicks at both locations was also positively correlated with the amount of food received (Fig. 5b, $F_{1, 165}$ = 25.93, p < 0.0001, adjusted r^2 = 0.17). ANCOVA revealed a significant effect of island/location on mean mass change (Fig 5b, $F_{1, 137}$ = 4.13, p = 0.04) but no difference among chicks or between the slopes of the regression lines for each island (Fig. 5b, $F_{26, 137}$ = 0.69, p = 0.85 and $F_{1, 1}$ = 0.14, p = 0.70 respectively). Therefore, at Heron Island tarsus growth for a given amount of food was consistently lower than at Lord Howe Island. At Lord Howe Island chicks required approximately half the amount of food for the equivalent amount of tarsal growth (Fig. 5b).

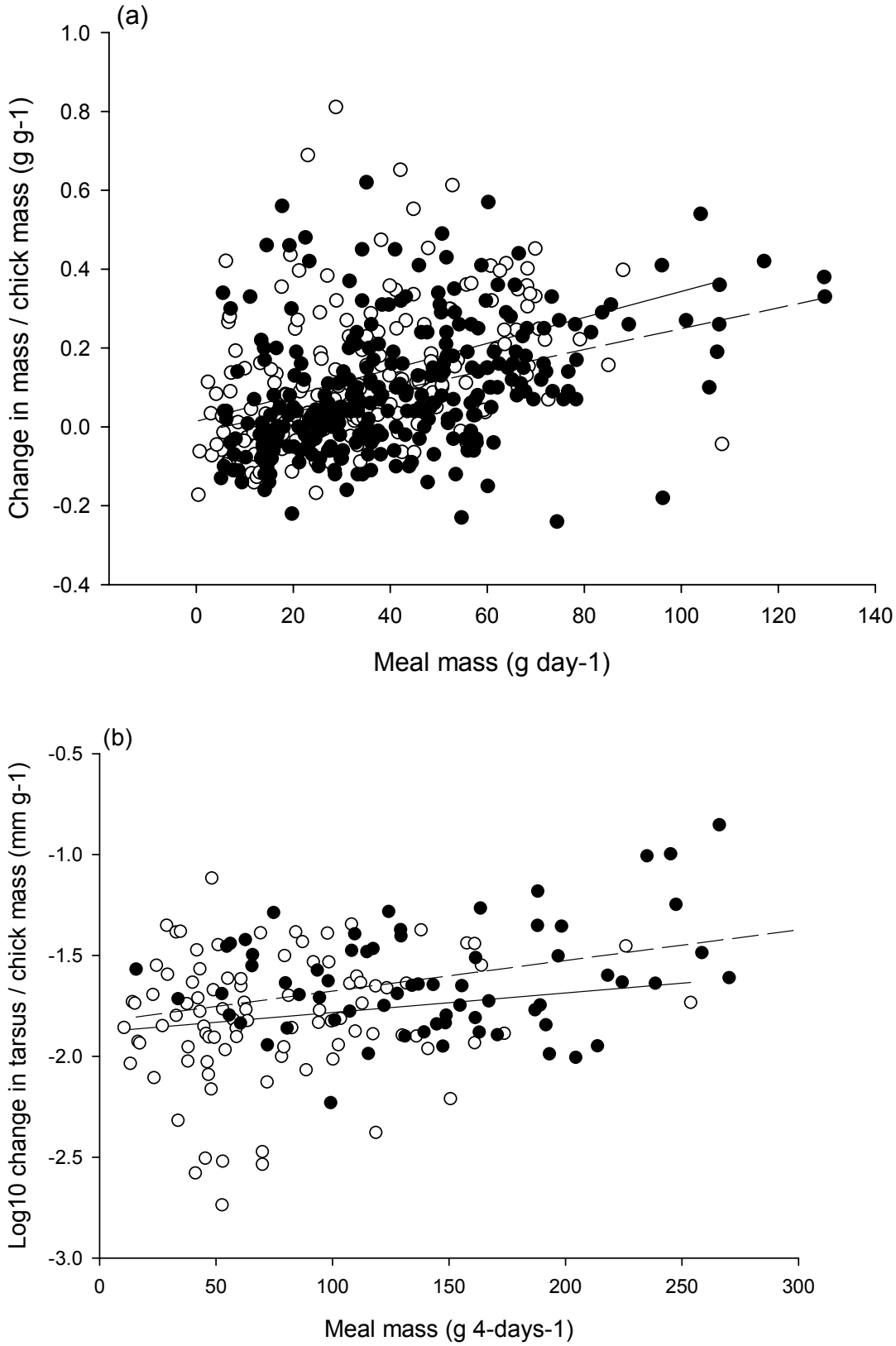


Fig. 5. Relative change in wedge-tailed shearwater chick mass (g) in relation to meal mass (g) (a) and relative change in chick tarsus (mm) in relation to meal mass (g) (b) from Heron Island (○) and Lord Howe Island (●). The solid line represents the regression line for Heron Island chicks and the dashed line that for Lord Howe Island chicks.

3.2.4 Foraging behaviour during incubation: Raine Is.

Daily burrow inspections showed that individual adults incubated continuously for a minimum of 3-4 days. Because of time constraints the full incubation changeover period for a single adult was not observed. Incubating birds did not feed, and lost body mass at a rate of 0.7 to 0.9g/hour or a mean of 18.24g/day (SE=0.052, n=10). Based on maximum (457g) and minimum (312g) adult weights observed this rate of loss gives a predicted maximum incubation changeover period of 6-8 days for an average adult.

Analysis of capture/recapture data from banded adults is consistent with a dual foraging pattern (Fig. 6). Adults were only recaptured after either short 1-3 day trips or long 6-8 day trips (Fig. 6).

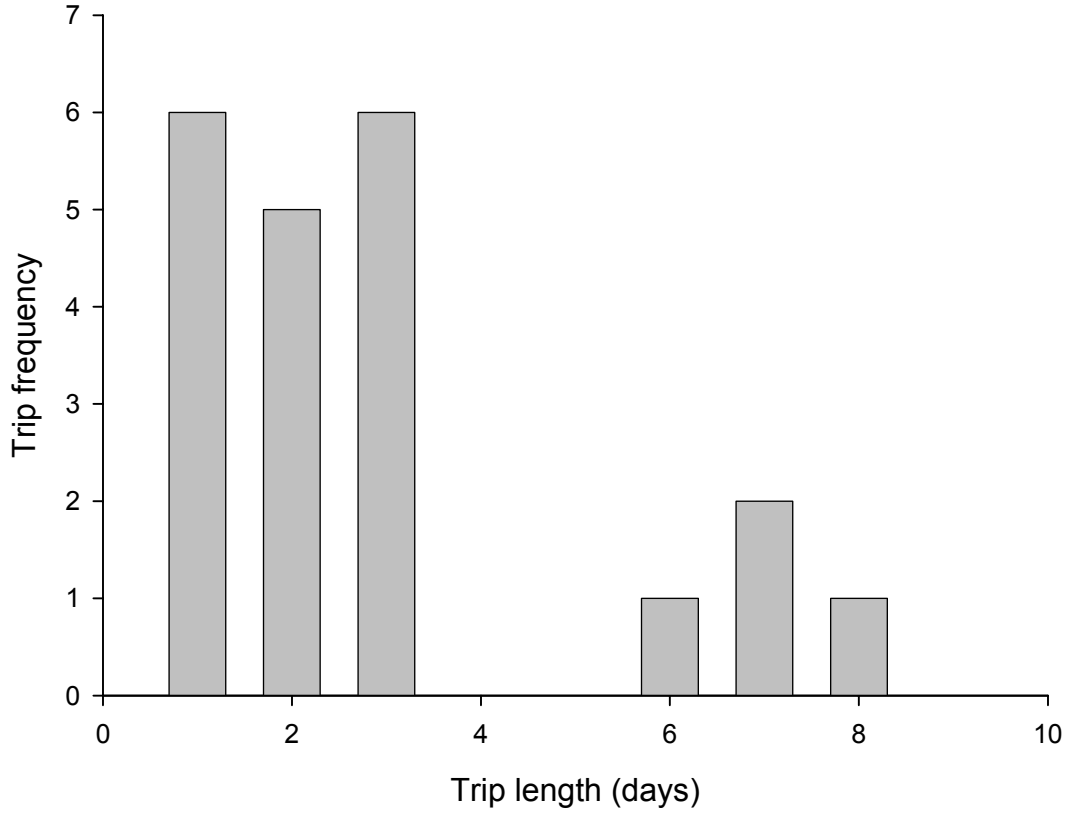


Fig. 6. Foraging trip length frequency distribution of wedge-tailed shearwaters during the incubation/burrow prospecting period at Raine Island 2002.

Minimum food assimilated was significantly associated with trip length (Fig. 7; ANCOVA, $F_{3,17}$= 83.28; r^2 = 0.94; P = < 0.0001) but was not dependant on trip type (i.e. 'short' or 'long'), (Fig 2, $F_{1,1}$ = 0.0493; P = 0.8269). Food assimilation rates of ~20g/day were observed for both short and long trips (Fig. 7). This result combined with the mass loss of ~19g/day in incubating birds indicates that 6-8 day trips were not the result of birds having spent 1-3 days incubating before undertaking a 1-3 day trip. If so, the rate of assimilation over 6-8 day trips would on average be only half that observed during 1-3 day trips. Therefore, the bimodal foraging pattern observed (Fig. 6) does not result from birds having spent 1-3 days incubating before undertaking a 1-3 day trip.

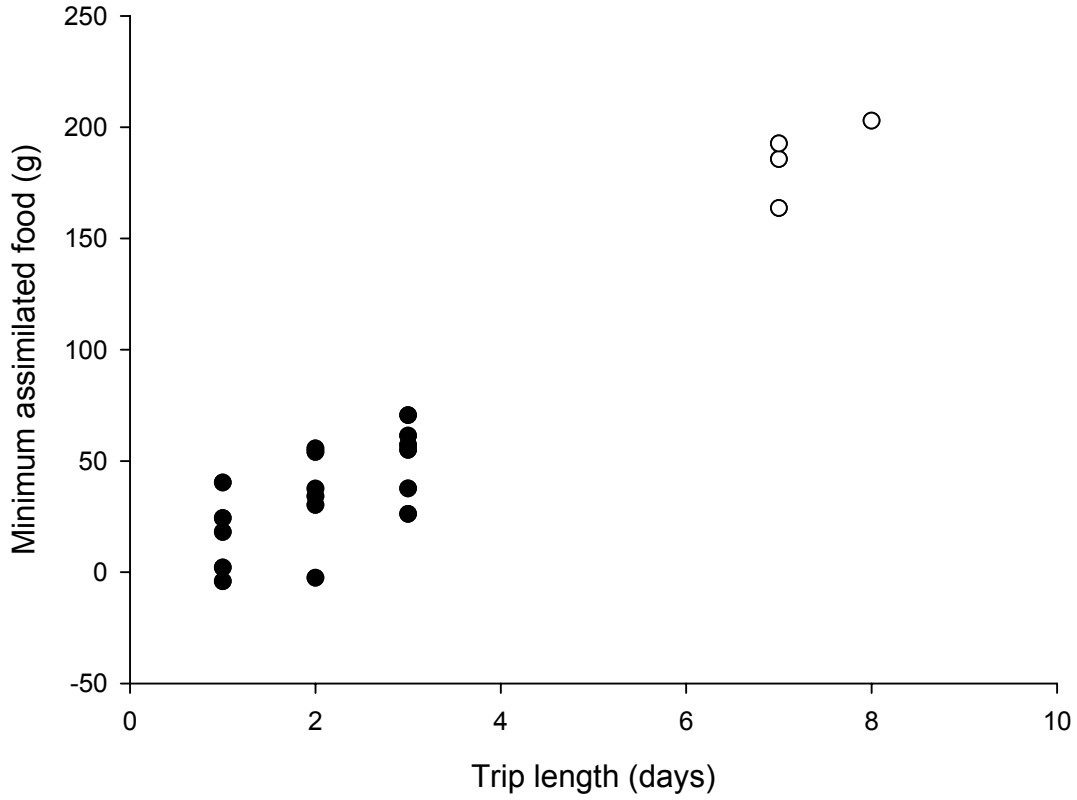


Fig. 7. Minimum assimilate food (g) in relation to foraging trip length (days) for wedge-tailed shearwaters at Raine Island during the prospecting period (2001/2002).

3.3 DISCUSSION

3.3.1 SST sensitivity and Primary productivity

Relative chick mass change was related to SST on a daily basis at both locations (Fig. 2). Therefore, small-scale daily fluctuations in SST significantly impact chick growth of wedge-tailed shearwaters both at Heron Island and at Lord Howe Island. A significant effect of island indicated that this effect was greater per degree of temperature increase at Lord Howe Island compared to Heron Island (Fig. 2).

Annual or decadal variation in SST have often been correlated with fluctuations in seabird reproductive success (Francis et al. 1998) and have generally been framed in the context of large-scale (both spatial and temporal) atmospheric processes such as ENSO (in the Pacific Ocean) or NAO (in the North Atlantic Ocean) (Cushing 1990, Ainley et al. 1995, Ramos et al. 2002, Durant et al. 2003). However, fluctuating chick mass was observed on a daily basis (Fig. 2) indicating that other factors were operating within the system over much shorter temporal-scales. This is evidence that wedge-tailed shearwaters are very sensitive to increases of SST, probably via. the effect that these temperature changes have on prey availability and/or accessibility.

Moreover, it would appear that wedge-tailed shearwaters are more sensitive at Lord Howe Island as evidences by the significant island effect in the ANCOVA (refer results, Fig. 2). This means that SST will impact chick development differently at different locations and thus illustrates; (1) divergent foraging environments at the two locations, (2) a link between these differing environments and chick development (3), a role for adaptive responses by wedge-tailed shearwaters to local oceanographic conditions and (4) local environmental differences that could help to promote reinforcement/character displacement between these two populations.

Other oceanographic parameters also suggest divergent foraging environments ((1) above) between Heron Island and Lord Howe Island. Mean chlorophyll concentrations at Heron Island were consistently lower than those at Lord Howe Island and were similar to other regions of known low productivity in the tropical Pacific (Murtugudde et al. 1999) and Indian Oceans (Weimerskirch et al. 2005). Moreover, chlorophyll concentrations at both locations remained relatively stable and divergent across the seven years for which data were available (Fig. 3). This suggests that the foraging

environments experienced at each location were consistent across the two years of the study and that the colony-specific differences observed likely reflect general long-term patterns of resource availability at each location.

3.3.2 Divergent foraging strategies

Birds at Lord Howe Island used a unimodal strategy as described previously for wedge-tailed shearwaters in Hawaii (Baduini 2002). Both in Hawaii and elsewhere, unimodal foraging patterns have been attributed to birds provisioning using only near-colony areas of high productivity (eg. Granadeiro et al. 1998, Baduini 2002). The results suggest that Lord Howe Island adults may also forage exclusively using productive near-colony locations. This contrasts with the bimodal strategy observed at Heron Island both in 2001 (Congdon et al. 2005) and 2003 (presented here). Comparisons with other Procellariiforms (Weimerskirch 1998, Weimerskirch and Cherel 1998) imply that at Heron Island wedge-tailed shearwaters adopt a bimodal strategy to supplement food input to chicks beyond levels possible using only resource-poor near-colony waters. .

Therefore, combining results from both oceanographic/productivity and foraging analyses suggests that prey availability at Lord Howe Island differs substantially and consistently from that at Heron Island and that adult foraging strategies at each location are adjusted accordingly. Previously, temporal and spatial variation in food availability or productivity between locations has been shown to drive population-specific foraging behaviour in a number of seabird species (eg. Hamer et al. 2001, Falk et al. 2002, Tremblay and Cherel 2003). The results suggest that the same is true for wedge-tailed shearwaters.

3.3.3 Chick growth and local adaptation

Chick physiology and growth at the two study locations are significantly divergent in a number of ways. Firstly, overall growth in body weight, (as measured by K), was significantly higher at Lord Howe Island than at Heron Island. This was not surprising given that Lord Howe Island chicks were provisioned approximately twice the amount of food per unit time and that seabird growth is sensitive to provisioning rates (Pettit et al. 1984, Cairns 1987). It also reinforces the suggestion that resource availability was lower at Heron Island. By itself, this result implies that overall growth rates at the two locations respond to different levels of provisioning by parents (Ricklefs 1973, 1979).

However, when comparing developmental changes per gram of food received, growth patterns for chicks at the two locations also differed significantly. Chicks at Heron Island showed substantially less skeletal development per gram of food obtained than those at Lord Howe, but gained significantly larger amounts of body mass, with the pattern reversed at Lord Howe Island. This has not been seen previously in seabirds. Such growth patterns imply that chicks at Heron Island may 'store' mass at the expense of skeletal growth; possibly as an adaptation to lower and less predictable long-term provisioning rates (Hulsman and Smith 1988, Congdon 1990, Schaffner 1990, Hamer et al.2000). The differences observed must reflect either physiological or facultative season-specific responses at each colony, or colony-specific adaptations resulting from divergent selection. Based on available evidence, as outlined below, colony-specific adaptation is more likely.

Firstly, chicks at Heron Island do not show suppressed overall development (per gram of food) with decreased provisioning. This has been the standard within or between season physiological response observed in many Procellariiformes and other seabirds when provisioning rates and/or food availability declines (e.g. Ricklefs et al. 1987, Hamer et al. 1998, Takahashi et al. 1999, Weimerskirch and Lys 2000).

Alternatively, the relative increase in mass gains at Heron Island (per gram of food) may reflect a physiological response to differences in the nutritional content of prey at each location. For example the results may be explained by higher lipid content in prey at Heron Island compared to Lord Howe Island. Unfortunately data on prey energy content at each colony needed to test this hypothesis directly, but again the results contrast with expectations from the literature. In general, the energy density of forage-fishes is positively correlated with lipid content (Van Pelt et al. 1997) implying that high-lipid-content prey is also high-quality prey. In seabirds, high-energy, high-lipid-content diets have been shown to increase body mass gains (Litzow 2002, Dahdul and Horn 2003), fat reserves, and rates of wing development, without effecting tarsus or culmen growth (Dahdul and Horn 2003). This suggests that a high-lipid diet at Heron Island could produce the increase in relative mass gain observed at this location, but not the differences in relative tarsus growth between colonies.

Thirdly, the findings do not agree with expectations of the only previously documented facultative response of seabird chicks to reduced provisioning rates (Congdon 1990). This model suggests that in pelagic foraging seabirds under low predation pressure, mass should be preferentially maintained at the expense of other types of growth during periods of increased food stress (Congdon 1990, Ashton and Armstrong 2002). Under this model, in similarly adapted populations, equivalent mass storage at both locations and retarded skeletal growth only at Heron Island is expected. This was not the case.

It is possible that the findings reflect an as yet undocumented facultative response. If so, then chick developmental physiology at each location must be responding to a season-specific assessment of background food availability that has occurred in the very early stages of chick development; well within the 26 day study period. The previously documented variability in day-to-day provisioning rates in both wedge-tailed shearwaters (Baduini 2002, Peck et al. 2004) and other pelagic foraging seabirds (Ricklefs et al. 1985, Congdon 1990, Warham 1990, Catard et al. 2000) make such a season-specific assessment theoretically difficult in the time available. Therefore, while a facultative mechanism cannot be discounted entirely, such a response would highlight a novel and significant physiological ability not previously documented in other taxa.

3.3.4 Foraging behaviour: Raine Is.

The bimodal foraging pattern observed on Raine Island of 1-3 day 'short' trips and 6-8 day 'long' trips is surprisingly similar to the pattern observed for Heron Island wedge-tailed shearwaters during chick rearing (Figs. 4a, 6). Dual foraging in Procellariids has only previously been observed during chick rearing and is thought to be associated with adults recovering body mass at highly productive foraging areas at distance from breeding colonies (Weimerskirch and Cherel 1998; Klomp and Schultz 2000; Congdon 2005). The results from Raine Island indicate that this pattern may exist during the courting/incubation period as well (Fig. 6).

However, that assimilation rates were consistent across both short (1-3 day) and long (6-8 day) trips suggests that Raine Island birds were not accessing zones of higher relative productivity during long trips. It is more likely that the results represent two 'groups' of birds using the same near colony resource; one remaining at-sea to recover mass following incubation change-over while the other, comprising non-mated or

courting birds, returns at shorter intervals to prospect for burrows or mates. As the sample size for both 'long' and 'short' trip categories are small, all analyses must be interpreted with caution. Further sampling is required to clarify these results.

In summary, wedge-tailed shearwaters breeding at Heron and at Lord Howe Islands have divergent foraging strategies consistent with a resource-poor local foraging environment at Heron Island, and a relatively rich foraging environment at Lord Howe Island. Some of the variation in resource availability may be attributed to the differential impact of SST on prey availability at the two locations. Concomitant divergence occurs in; (1) the way chicks respond (indirectly through adult provisioning) to local oceanographic conditions such as SST and (2), chick development at the two locations. Per ^{o}C increase in SST, chicks at Heron Island lose less mass than chicks at Lord Howe Island. However, per gram of food delivered, chicks at Heron Island exhibit a marked increase in body mass development over skeletal growth, relative to chicks at Lord Howe.

Based on previously observed facultative responses to changes in food availability and theoretical expectations on the rate at which season-specific assessments of background food availability can occur, the growth patterns observed are most likely colony-specific physiological adaptations to differences in long-term provisioning rates at each location. If so, then results demonstrate co-ordinated environmentally determined divergent coevolution of chick and adult life-history parameters across these two locations, and thus have important implications for models of population divergence and speciation in seabirds.

Raine Island findings are preliminary, and therefore need to be interpreted with caution. Nonetheless the data suggest that forage resources and consequently the foraging behaviour of wedge-tailed shearwaters at this location may be unique. Further work at Raine Island is required to substantiate these findings.

Table 4. Foraging and provisioning parameters of wedge-tailed shearwaters breeding at two locations in eastern Australian waters (mean ± SE). Significant pairwise comparisons are indicated.

	Length of foraging trip (days)	Meal mass (g)	Meal mass (g night $^{-1}$)	Probability of feed (meals night $^{-1}$)	Adult mass change (g g mass $^{-1}$)
Heron Is.(2003)	2.69 ± 0.22	32.65 ± 1.80	7.40 ± 0.69	0.21 ± 0.01	-0.002 ± 0.005
Heron Is.(2005)	1.37 ± 0.09	37.91 ± 2.12	12.81 ± 1.19	0.35 ± 0.02	-0.02 ± 0.006
Lord Howe Is. (2004)	1.75 ± 0.09	35.01 ± 1.34	14.43 ± 1.13	0.37 ± 0.02	-0.001 ± 0.005
p	< 0.001	0.12	< 0.001	< 0.001	0.03
Tukey's HSD	(LH/H2003),(H2005/H2003)	n.s.	(LH,H2003),(H2005,H2003)	(LH/H2003),(H2005/H2003)	(LH/H2005)

CHAPTER 4

4.0 INTRODUCTION

In theory, offspring survival can be enhanced if sex-specific foraging patterns by adults interact in such a way that they maximise provisioning relative to the background environment. This could promote a barrier to gene flow (via. reinforcement) when adults pair with non-local individuals because offspring provisioning and survival would decrease. By highlighting the mechanisms that underpin any observed sex-specific patterns of foraging behaviour within populations using divergent resource bases, significant insights into the potential for sex-specific foraging patterns to enhance barriers to gene flow between populations can be obtained.

Empirical studies have consistently documented sex-specific foraging strategies in sexually size-dimorphic birds in general (e.g. Peters and Grubb 1983, Gosler 1987, Hogstad 1991, Aho et al. 1997, Pasinelli 2000) and seabirds in particular (eg. Weimerskirch et al. 1997, Gonzalez-Solis et al. 2000, Phillips et al. 2004, Congdon and Preker 2004). As a result, the development of sex-specific foraging behaviour in seabirds is generally attributed to size-related mechanisms (Gonzalez-Solis et al. 2000, Kato et al. 2000, Phillips et al. 2004).

Based on these models, sex-specific differences in foraging strategy are not expected in sexually monomorphic seabirds. However, recent empirical work demonstrates that sex-specific differences exist in at least two monomorphic seabird species (Gray and Hamer 2001, Lewis et al. 2002). Because of this, the general applicability of size-related models of foraging behaviour divergence appears less certain (Lewis et al. 2002). Mechanisms underlying sex-specific foraging behaviour in monomorphic taxa are unknown. Currently four potential explanations have been proposed.

Firstly, if one sex is in poorer condition at the beginning of the chick-rearing period this could lead to preferential self-provisioning by that sex, resulting in measurable

differences in foraging trip lengths and/or provisioning rates (Weimerskirch 1998, Gray and Hamer 2001). The initial poor condition of one sex could result from costs incurred during egg production, in the case of females, or simply that one sex assumes a greater responsibility during incubation (Martin 1987, Hatch 1990, Lewis et al. 2002). An extension to this hypothesis is that differences arise because nutrient requirements differ between the sexes (Carey 1996, Lewis et al. 2002). For example, females may require calcium at the start of the chick-rearing period in order to replenish that lost during eggshell formation (Carey 1996, Graveland and Drent 1997, Lewis et al. 2002).

A second explanation is that nest attendance times differ between sexes (Woo et al. 1999). For example, if one sex forages at night and the other during the day, differences in foraging and provisioning patterns may arise because different at-sea foraging conditions are experienced at these two times (Wilson et al. 1993, Woo et al. 1999, Kato et al. 2000, Hays 2003). While plausible for some species, this hypothesis cannot explain sex-specific foraging divergence in seabird taxa that show no differences in nest attendance times (eg. Gray and Hamer 2001).

A third possibility is that one sex is more sensitive to chick condition and adjusts foraging effort accordingly (i.e. differences in provisioning rules between the sexes) (Quillfeldt et al. 2004). In Manx shearwaters (*Puffinus puffinus*), Quillfeldt et al. (2004) have shown that chick begging intensity and chick condition are strongly negatively correlated and that only females use this cue to decrease provisioning rates when chicks are in good condition. The generality of this novel result is yet to be tested in other species.

Finally, differences in foraging behaviour could evolve if, despite being monomorphic, one sex is less efficient at foraging (Lewis et al. 2002). The less efficient sex could then be out-competed and forced to forage in a less productive spatial or temporal niche as a way of reducing inter-sexual competition (Hunter 1983, Gray and Hamer 2001, Gonzalez-Solis et al. 2000). The unpredictability of the marine environment means that competition within and between sexes for forage resources is likely to be strong (Ashmole 1971, Lewis et al. 2001). Therefore, inter-sexual competition of this type could theoretically result in niche partitioning and promote foraging behaviour divergence between sexes (Selander 1966). However, at present

no proximal mechanism has been proposed for why one sex would consistently out-compete the other in a monomorphic species.

Sex-specific foraging behaviour has been documented twice in a monomorphic species of the genus *Puffinus,* the Manx shearwater (*P. puffinus)* (Gray and Hamer 2001, Quillfeldt et al. 2004). To test the generality of these findings, further elucidate mechanisms driving sex-specific foraging behaviour in monomorphic seabirds and evaluate the possibility that differences in the extent or direction of sex-specific foraging behaviour can promote local adaptation and isolation, chick provisioning and adult dive behaviour was simultaneously compared in the wedge-tailed shearwaters at both Heron and Lord Howe Islands.
This chapter has three aims;

(1) To determine if sex-specific foraging behaviour exists in this species and if it differs between populations.

(2) To identify any coordinated divergence in at-sea foraging behaviour between the sexes and chick provisioning rates.

(3) To determine the mechanism/s underpinning any observed patterns and evaluate the likelihood that differences in sex-specific foraging between colonies could lead to a reduction in gene flow via. reinforcement.

4.1 METHODS

Details on study sites are given in Chapter 2. Methods relating to measuring foraging behaviour and chick provisioning are described in section 3.1.4. Adult birds were sexed at the conclusion of the field season using protocols described in section 2.3.4.

Estimating dive capability is a good way to highlight links between sex-specific morphology and sex-specific foraging behaviour. To this end, maximum depth gauges (MDG's) were attached to all 40 adults at least once during the duration of the study. Depth gauges have been used extensively in the past to examine dive capabilities of seabirds (Burger and Wilson 1988, Mougin and Mougin 2000, Burger 2001). Depth gauges were made of PVC tubing of 0.8 mm internal diameter (Tygon) and were 120 mm long. The internal surface was lined with icing sugar, heat sealed at one end and

the whole length except the heat-sealed portion (L_s) measured to the nearest 0.5 mm. Tubes were attached to the basal portion of the central tail feather shaft using waterproof tape (Tesa). The open end of the gauge was pointed towards the end of the tail so that water entered only when birds were fully submerged.

Gauges were attached to adult birds after chick feeding, and recovered at the next feeding visit. The length of the undissolved icing sugar was measured to the nearest 0.5 mm (L_d). Maximum dive-depth (D_{Max}) was calculated from the following equation (Burger and Wilson 1988):

$$D_{Max} = 10.08 (L_s/L_d - 1)$$

It has been shown that calculating maximum dive capability in seabirds using this method is very reliable (see Burger and Wilson 1988, Hedd et al. 1997, Zavalaga and Jahncke 1997). Before deploying MDGs their accuracy was tested and an error of < 2 % in calculations involving depths of up to fifteen metres was observed (Peck *unpub. data*). This error rate was similar to that recorded by Burger and Wilson (1988).

4.1.2 Statistical analysis

All statistical analyses were conducted using JMP ver. 4.0.2 (SAS Institute Inc.). The normality of each set of measurements was tested using the Shapiro-Wilk *W* test. Data were log-transformed prior to analysis if deviations from normality were detected.

To avoid pseudoreplication, the mean foraging behaviour values obtained for each parent from the same nest were compared in paired t tests. Measures of foraging behaviour obtained and details on how foraging patterns were analysed are described in section 3.1.4. Paired t-tests using parents from the same nests were also used to test for differences in maximum dive-depths between the sexes.

In order to calculate chick body condition on a specific day, chick body mass at 16:00 hrs was regressed against the tarsus measurement obtained on the same day (i.e. every fourth day of the study period). The residuals of this regression were then divided by the predicted values to generate a measure of chick body condition (relative to size) prior to feeding on that day (Hamer and Hill 1993). The relationship between this measure of chick condition and the meal size delivered by adults of each sex was

examined by a two-factor ANCOVA, with chick and sex as factors, and meal size as the covariate (~ three - six meal masses/chick).

Chick condition was calculated by using chick body mass at 09:00 hrs on the day after tarsus measurements were made. This is the relative condition of the chick at the start of an adult foraging trip (Quillfeldt et al. 2004). Spearman rank order correlation was used to examine the effect that this had on the length of subsequent foraging trips for each sex.

4.2 RESULTS

4.2.1 Sex-specific foraging patterns and dive-depths

Mean trip length differed significantly between the sexes at Lord Howe Island, with males making significantly shorter trips than females (Table 5). This pattern is similar to that seen in other members of the same genus (Gray and Hamer 2001). However, this pattern was not consistent between islands, with no difference in mean foraging trip length between the sexes at Heron Island in any year (Table 5).

At Lord Howe Island, the mean maximum dive-depth regardless of trip length was 4.98 m (±0.33) with a range of 1.97 – 11.72 m. Mean dive-depth differed significantly according to sex (Table 5)(Fig. 8). Males dived significantly deeper than females (Fig. 8). Although the trend was similar at Heron Island, the observed difference was not significant (Table 5)(Fig. 8).

Table 5. Differences in sex-specific patterns of foraging and provisioning behaviour in wedge-tailed shearwaters breeding at Heron and Lord Howe Islands.

	Sex	Trip length (days)	MDG (m)	Meal mass (g)	Meals (night^{-1})	Provisioning rate (g night-1)	Arrival time (hrs:mins)	Adult foraging efficiency (g day^{-1})
Heron 2003	Male	3.05 ± 0.36	_	33.20 ± 3.60	0.21 ± 0.02	6.73 ± 0.82	6:30 ± 0:27	0.005 ± 0.008
	Female	2.47 ± 0.32	_	36.28 ± 3.50	0.22 ± 0.02	8.04 ± 1.11	5:19 ± 0:26	-0.0004 ± 0.01
d.f.		16	_	14	14	14	15	14
t		1.1	_	-0.71	-0.23	-1.05	1.68	0.39
p		0.28	_	0.48	0.81	0.31	0.11	0.7
Heron 2005	Male	1.46 ± 0.13	3.52 ± 0.54	36.70 ± 2.67	0.44 ± 0.04	16.12 ± 2.18	5:04 ± 0:20	-0.01 ± 0.01
	Female	1.37 ± 0.22	2.93 ± 0.43	39.05 ± 4.28	0.27 ± 0.03	10.87 ± 1.66	5:15 ± 0:20	-0.04 ± 0.01
d.f.		12	8	15	13	15	18	10
t		0.39	0.74	-0.46	2.7	1.8	1.22	1.47
p		0.69	0.47	0.64	**0.01**	0.09	0.23	0.17
Lord Howe 2004	Male	1.61 ± 0.11	5.43 ± 0.44	34.27 ± 1.54	0.44 ± 0.035	16.37 ± 1.80	5:40 ± 01:05	-0.003 ± 0.006
	Female	1.97 ± 0.13	4.14 ± 0.44	35.75 ± 2.23	0.32 ± 0.027	12.37 ± 1.30	5:30 ± 00:21	0.001 ± 0.008
d.f.		19	19	19	19	19	19	16
t		-2.07	-2.13	-0.59	2.6	1.84	-0.93	-0.04
p		**0.04**	**0.04**	0.56	**0.01**	0.08	0.36	0.69

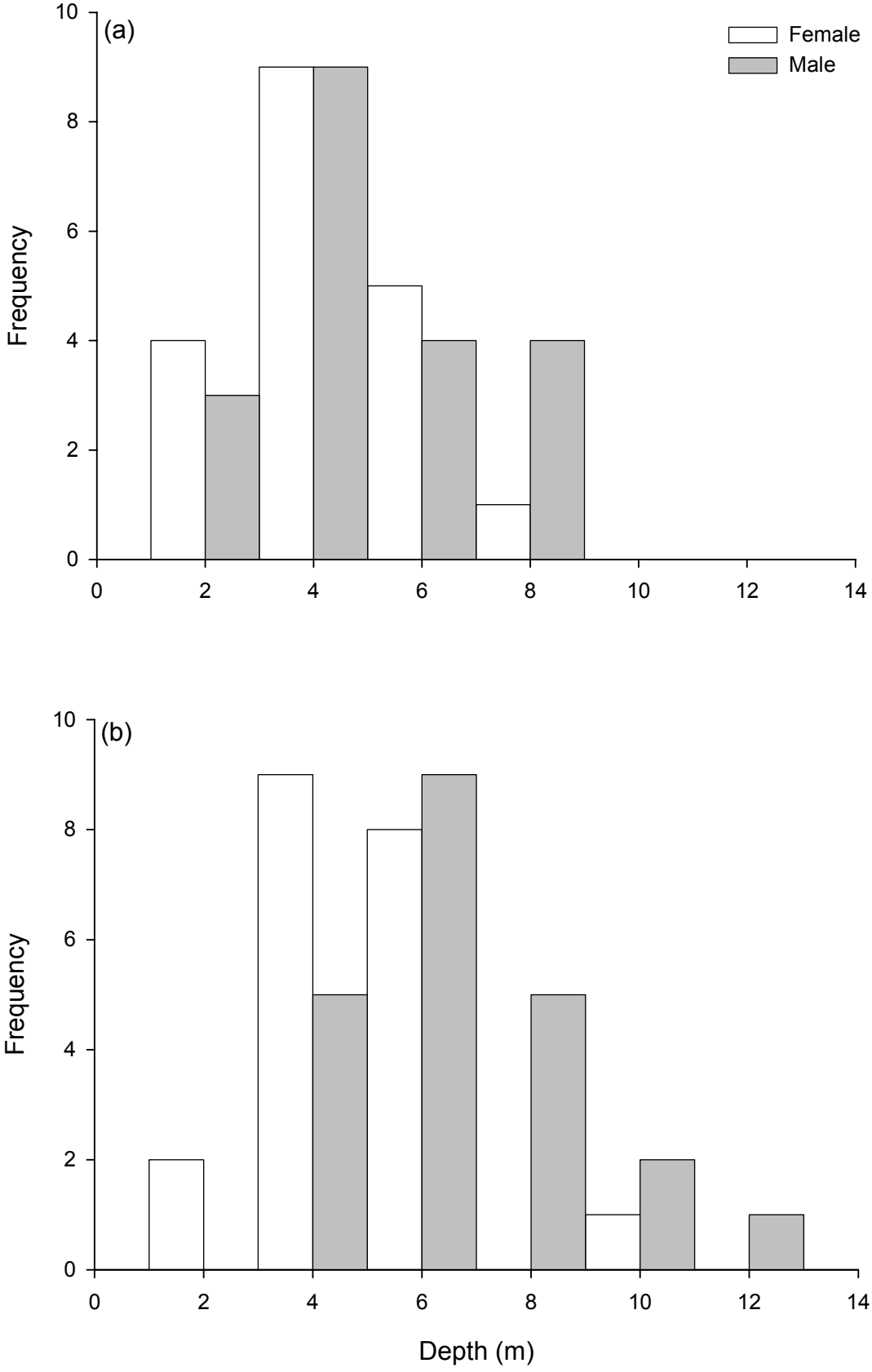


Fig. 8. Maximum dive depths during foraging bouts of male and female wedge-tailed shearwaters during the chick-rearing period at Heron Island during 2005 (a) and at Lord Howe Island during 2004 (b).

4.2.2 Sex-specific food provisioning, nest attendance and foraging efficiency

Throughout the study, only a small proportion (<5%) of chicks were fed more than once per night. When this occurred meal values were summed and the 'nightly' meal mass values were used in subsequent analyses. For the arrival time analysis, the first visit time was used.

Overall, mean meal mass fed to chicks by wedge-tailed shearwaters breeding at Lord Howe Island did not differ according to sex (Table 5). The same was true of breeding birds at Heron Island (Table 5). However, males had a significantly higher probability of feeding chicks than females at Heron Island in 2005 and at Lord Howe Island (Table 5). This led to a tendency for males to provision chicks more per night than females at Heron Island in 2005 and Lord Howe Island (Table 5). This difference was very close to being statistically significant at both locations (Table 5). There was no evidence that the probability of feeding chicks, or provisioning rate was greater for males at Heron Island during 2003 (Table 5).

There was no significant difference in arrival times measured as hours elapsed since 16:00 hours at any location or during any year (Table 5). Foraging efficiency of adult wedge-tailed shearwaters was measured as the proportionate daily mass gained by adults whilst foraging (González-Solís 2000, Chapter 3, section 3.1.4). Foraging efficiency for all trip lengths combined did not differ significantly between the sexes at any location or during any year at Heron Island (Table 5).

Sex-specific differences in incubation behaviour could mean that one sex temporarily loses more weight during the incubation period, and would therefore need to replenish lost reserves during the chick-rearing period (Martin 1987). This could lead to sex-specific foraging patterns. To examine sex-specific differences in adult body mass immediately after the incubation period, masses of both parents from eight nests at Lord Howe Island (from the sample of twenty) that had hatched a chick within the previous twelve hours were compared. No significant difference between the sexes in adult mass was observed ($t_7 = -0.28$, $p = 0.787$).

Quillfeldt et al. (2004) proposed that sex-specific foraging behaviour might result from one sex adjusting provisioning/foraging behaviour according to chick condition. No evidence of this was found in wedge-tailed shearwaters. Meal mass delivered decreased significantly with improved chick body condition prior to feeding at Lord Howe Island but not elsewhere (Table 6). In fact, at Heron in 2005 meal mass was close to being significantly positively related to chick condition prior to feeding (Table 6). There was also an effect of chick at Lord Howe Island and at Heron Island in 2003 (Table 6). However, no difference in provisioning patterns between adult sexes was observed at any location or year (Table 6) and no significant higher order interactions were observed.

In addition, the length of foraging trips made by males or females was not related to the body condition of chicks post-feeding at any location or year, although interestingly, a negative correlation was nearly significant at Lord Howe Island where males tended to make shorter trips when chicks were in poor condition (Spearman rank order correlation: $r_{36} = -0.32$, $p = 0.06$).

Table 6. Results from two-factor ANCOVA examining the impact of chick condition on adult foraging/provisioning at Heron Island (two years) and Lord Howe Island.

		Meal mass	Chick	Sex
Heron 2003	d.f.	1,28	15,28	1,28
	r^2	-0.008	_	_
	F	0.11	1.9	0.27
	p	0.74	0.06	0.6
Heron 2005	d.f.	1,26	16,26	1,26
	r^2	0.02	_	_
	F	3.32	0.9	1.08
	p	0.07	0.57	0.3
Lord Howe	d.f.	1,60	20,60	1,60
	r^2	-0.008	_	_
	F	5.8	2.57	0.02
	p	**0.01**	**0.002**	0.87

4.3 DISCUSSION

Within the general pattern of foraging behaviour observed for wedge-tailed, shearwaters (Chapter 3), sex-specific patterns were evident (Figure 1a,b, Table 5). This was especially true at Lord Howe Island, where females spent a greater proportion of time on long (four and five day) foraging trips compared to males, resulting in a significantly longer mean trip length for females (Table 5). As a result, chick-feeding frequency was higher in males than in females over the study period at that location (Table 5). Sex-specific patterns of dive behaviour in wedge-tailed shearwaters breeding at both Lord Howe Island and Heron Island were observed (Fig. 8). Males dived deeper than females despite negligible differences in overall body size (Chapter 5). Differences in diving behaviour have only been observed twice before in a monomorphic species (Woo et al.1999, Lewis et al. 2002). The findings further support Lewis et al's (2002) suggestion that relative size may not necessarily be the driving force behind sexual differences in seabird foraging behaviour.

Theoretically, the differences in female provisioning and foraging behaviour (fewer meals/night, greater mean trip length) could result from females staying longer at foraging grounds and self-provisioning more in order to regain body reserves (condition) lost during the egg production, and/or incubation periods (Weimerskirch 1998, Gray and Hamer 2001, Martin 1987, Hatch 1990). The results do not support this model. If body condition in females is consistently poorer during the initial stages of chick rearing, significant sex-specific differences in adult mass immediately after hatching is expected. This was not the case. In addition, females did not self-provision more than males as meal sizes fed to chicks and mass gain whilst foraging did not differ significantly between the sexes.

Female birds lose nutrients (such as calcium) as a result of egg production, and may need to regain them once incubation is complete (Carey 1996, Graveland and Drent 1997). If females require prey with specific nutrient components, and these prey differ in their vertical distribution, then differences between sexes in maximum dive depths may be evident (Lewis et al. 2002). Detailed information on sex-specific patterns of prey selection do not exist for this or other shearwaters. Until these data are available, this possibility cannot be completely discounted. However it seems unlikely that this is the case given that prey used by shearwaters at Lord Howe Island is predominantly of one type (squid - Peck *unpub. data*), and that evidence from other seabird species

suggests that nutrients transferred from adult tissues to eggs is small compared to adult body reserves (Grau 1982, Harrison et al. 1983).

Different patterns of nest attendance could result in each sex foraging at different times and under different environmental conditions (i.e. light levels and/or prey types and availability) (Wilson et al. 1993, Woo et al. 1999, Jones et al. 2002). This could potentially affect characteristics such as maximum dive-depth and provisioning rate (Wilson et al. 1993, Woo et al. 1999). However, this is unlikely to be influencing wedge-tailed shearwater foraging patterns at Lord Howe Island as nest attendance patterns (mean arrival time in hours after 16:00 hours), did not differ between the sexes.

Another possible explanation for the results is sex-specific differences in provisioning rules, where one sex mediates foraging behaviour according to chick body condition (Quillfeldt et al. 2004). The reasons for differences in provisioning rules are unclear, however it could be that offspring care is more costly in one sex than the other, and that care would therefore need to be allocated more precisely in that sex (Quillfeldt et al. 2004). There was evidence that chick body condition prior to feeding influenced the amount of food delivered (Table 6). This result fits with previous findings (Congdon et al. 2005). However, there was no difference in provisioning patterns according to adult sex. There was also no relationship between chick condition at the beginning of adult foraging trips, and subsequent trip lengths by either males or females. Therefore, decision rule differences cannot explain the divergent foraging behaviour observed between male and female shearwaters during the study period/s. However, the possibility that decision rule differences may be important in other seasons having different overall patterns of resource availability cannot be discounted (Quillfeldt and Masello 2004). Data from subsequent years is required to fully explore this possibility.

Finally, sex-specific foraging behaviour could evolve if, despite being monomorphic, one sex is less efficient at foraging (Lewis et al. 2002). This could theoretically lead to the less efficient sex being out-competed and result in niche-divergence as a way of reducing inter-sexual competition (Hunter 1983, Gray and Hamer 2001, Gonzalez-Solis et al. 2000). The observed pattern could be explained by this scenario if females are being out-competed by males and as a consequence take longer to accumulate

equivalent food loads at the same foraging grounds, or undertake longer trips to more distant foraging grounds to avoid competition (Hunter 1983, Gray and Hamer 2001, Gonzalez-Solis et al. 2000). Furthermore, the fact that males also dive deeper during foraging bouts indicates that males may be better able to, or are more prepared to, pursue prey than females. Wedge-tailed shearwaters breeding in the Seychelles (Indian Ocean) have been recorded diving to depths of over sixty metres with a mean of fourteen metres (Burger 2001). A maximum dive depth of over fifteen metres was never recorded (Fig. 8). The fact that this species has the capacity to dive much deeper than observed, indicates that females have the ability to dive to the same depth as males during foraging bouts, but that at Lord Howe they either choose not to (different prey selection) or are out-competed at deeper depths by males. To date, no proximate mechanism has been proposed to explain how inter-sexual competition in a monomorphic seabird could precipitate divergent foraging behaviour. One possibility is that prey-capture and handling efficiency (a function of numerous morphological traits including wing and culmen shape) may allow one sex to out-compete the other (González-Solís 2000, Ruxton et al. 2001).

4.3.1 Population differences in sex-specific foraging/provisioning behaviour

A barrier to gene flow (via. reinforcement) could arise if sex-specific foraging patterns maximise provisioning relative to the background environment. By comparing patterns of sex-specific foraging and provisioning between Heron and Lord Howe Islands, one aim of this Chapter was to explore this possibility.

Overall, sex-specific foraging and provisioning patterns observed in wedge-tailed shearwaters breeding at Heron and Lord Howe Islands were congruent, except that at Lord Howe Island, females made significantly longer foraging trips than males, and dived to significantly shallower depths than males. No differences were observed between the sexes in these two parameters at Heron Island. The difference in trip length between the sexes at Lord Howe but not at Heron Island may be confounded by the fact that birds use a dual foraging strategy at the later location (Chapter 3). Interspersing long with short foraging trips would lead to greater variation in mean trip length and therefore make it less likely that a difference is detected. However, differences in maximum dive depth at Lord Howe and not Heron may be because of stronger inter-sexual competition at the former, and therefore may serve to subtly promote reinforcement between the two localities. This interpretation is necessarily

tentative at this stage but nonetheless; the results suggest that a more detailed study into this intriguing possibility is warranted.

Overall, the results show that sex-specific provisioning and dive behaviour exists in wedge-tailed shearwaters breeding at Lord Howe Island. Simultaneous divergence in both of these aspects of foraging behaviour has not been documented before in a monomorphic seabird. The results support Lewis et al's (2002) assertion that relative size may not always explain sex-specific foraging patterns in seabirds. Results suggest that replenishment of body condition by females after the incubation period is unlikely to explain the observed patterns. Sex-specific patterns of nest attendance and decision rule differences are also unlikely explanations. Therefore, by a process of elimination inter-sexual competition at local foraging grounds is the most parsimonious explanation for the sex-specific behaviour observed. However, without further direct evidence of competition this conclusion must remain tentative. This interpretation is consistent with that observed for dimorphic species (Weimerskirch et al. 1993, González-Solís 2000, Phillips et al 2004), and suggests that inter-sexual competition at local foraging grounds may be more general than previously thought. Moreover, subtle differences in the pattern of sex-specific foraging between the two colonies may serve to promote genetic divergence via. reinforcement.

Based on these findings, further critical investigation into sex-specific niche partitioning and into the interaction between sex-specific foraging and background environmental conditions is warranted in order to fully explore adaptive explanations for the observed phenomena.

CHAPTER 5

5.0 INTRODUCTION

Documenting and comparing patterns of morphological divergence among populations can often be an informative step towards understanding the origin of biological diversity (Shine 1989, Edwards and Knot 1995, Smith et al. 2001, Gonzalez-Solis 2004). This approach may be informative in relation to non-allopatric mechanisms of speciation if populations are distributed across shifting resource bases or habitat types, and gene flow between populations is not impeded by physical barriers (Smith et al. 2001). Intriguing results have been achieved using this approach (Smith et al. 2001, Miller-Butterworth 2003).

Smith et al. (1997) showed that greater morphological divergence exists between geographically close ecotone and forest populations of the little greenbul (*Andropadus virens*) than between distant populations occupying similar habitats. This was despite considerable gene flow among the study populations (Smith et al. 1997). With high gene flow, and morphological divergence of functional traits (in this case wing, tarsus and bill measurements) between ecotone/rainforest populations, selection was posited as the most likely mechanistic explanation (Smith et al. 1997).

However, morphological differentiation at the population level need not involve selection. Morphology may simply be responding to environmental fluctuations over short time periods (phenotypic plasticity) (West-Eberhard 1989, Barbraud et al. 1999). If this is the case, morphological variation is not genetically inherited. For example, Rhymer (1992) found that body size divergence among populations of mallard (*Anas platyrhynchos*) was mostly caused by fluctuations in environmental conditions during the growth period rather than genetic inheritance.

Morphological divergence may also result from genetic drift (Wright 1931). This can occur if morphological variation is heritable, there is little or no gene flow among

populations and effective population size is small (Wright 1931). In this instance morphological characteristics will be idiosyncratic to each population, and there should be concordance between morphological and neutral genetic markers among populations.

Along with natural selection and drift, the trajectories of morphological variation within and between populations may be subject to sexual selection (Selander 1966). Differences between/among populations in mating systems, sex-related niche partitioning (Chapter 4) or non-adaptive (inherited or culturally determined) sensory bias may influence the variation observed (Shine 1989, Barbraud and Jouventin 1998, Karubian and Swaddle 2001, Pearson et al. 2002).

Seabird populations offer an interesting scenario for studying the mechanisms that underlie geographic variation in morphological and other characters (Chapter 1). It is therefore surprising, that existing information on the degree of morphological divergence among seabird colonies relates to species that have breeding populations that experience similar background environmental conditions (eg. Barbraud and Jouventin 1998, Waugh et al. 1999, Bretagnolle et al.2000). To date, no study has examined morphological differences in a species that breeds across an environmentally heterogeneous landscape with breeding populations that experience predictably different environmental conditions.

By examining morphometric differences within and between populations of the wedge-tailed shearwater (*Puffinus pacificus*) breeding in different locations, this chapter has three aims.

(1) To document the amount of morphological divergence among colonies in Australian waters and evaluate the relative importance of a genetic process (i.e. selection, drift) verus a plastic response in maintaining any observed patterns.

(2) To quantify sexual dimorphism and the degree of assortative mating among colonies in order to assess the importance of sexual selection in creating/maintaining any observed intra- or inter-population divergence in morphology.

(3) To estimate the heritability (h^2) of tarsus length as a proxy for body size at Lord Howe Island and thereby explore the possibility that divergence in this trait (and by extension overall size) is genetically driven.

5.1 METHODS

5.1.1 Study sites

Four populations were sampled representing tropical (Raine Island), sub-tropical (Heron Island) and temperate (Lord Howe Island, Rottnest Island) locales and are separated by varying degrees of geographical distance (Fig. 1). This sampling regime allowed comparisons to be made between populations located at different latitudes and consequently experience different environmental conditions/selection regimes. Further details on study locations can be found in Chapter2.

5.1.2 Data collection

Four morphological characteristics were measured across all populations using Vernier callipers (±0.1 mm): culmen length (CL), wing length (WL), tarsus length (TAR) and tail length (TL). In addition, bill depth (BD) and bill width (BW) were measured at Rottenest, Heron and Lord Howe Islands (refer to Table 2, Chapter 2 for specific sample sizes). Culmen measurements were taken from the start of culmen exposure to the tip, and tarsus from the 'notch' in the intertarsal joint to the 'nub' created by the bent over foot. Wing length was measured to the nearest 0.5 mm using a steel stopped ruler as the length from the carpal joint to the tip of the longest feather and tail length as the distance from the base of the two central tail feathers to the tip of the longest tail feather. Bill depth was measured from the start of the exposed culmen to the ventral edge of the lower mandible. Bill width was also measured at the exposed culmen. These are standard measurements and are defined in the ABBS banding manual (Lowe1989).

It is unknown if subtle differences in morphometric traits exist between the sexes of wedge-tailed shearwaters, because sex identification in the field is difficult. In order to examine this possibility, and the effect that sexual dimorphism may have on the level of divergence between colonies, individual birds were sexed using the molecular technique outlined in Chapter 2.

5.1.3 Statistical analysis

All statistical analyses were conducted using JMP ver. 4.0.2 (SAS Institute Inc.). The normality of each measurement was tested using the Shapiro-Wilk W test. If the data did not fit this assumption, they were transformed using the appropriate method in each case.

Two-factor ANOVAs were used to examine sexual and geographic variation in morphology. To compare the relative proportions of birds from each population, residual scores from general linear regressions using tarsus as the independent variable and each trait as the dependant were calculated. This gave the extent (and direction) to which an individual deviated in a particular trait from that expected for a bird of that size. Tarsus was used as an index of body size rather than body mass because body mass is likely to vary according to environmental conditions (Rising and Somers 1989,Cuervo and MØller 2001,Bull et al. 2004). Although results from these analyses are presented based on residual scores (to facilitate interpretation), statistical comparisons were conducted using the more robust approach of ANCOVA, where the independent variable (tarsus length) was used as the covariate (Sokal and Rohlf 1995, Pearson et al. 2002). Results are shown as ± 1 standard error (SE) unless otherwise stated. All single tests are two-tailed and significance was set to $p<0.05$.

Multivariate analyses were also conducted in order to further distinguish differences between populations and sexes. Discriminant Functions Analysis (DFA) is a multivariate technique that combines variables in order to maximise group separation (Quinn and Keough 2002). At the population level, a DFA was performed using tarsus, tail, wing and culmen length in order to detect the best discrimination between groups (populations). Bill depth and width were not used in the DFA because these values were not available for Raine Island. The standardised DFA coefficients were calculated to highlight which variables contributed the most to each discriminant function. Eigenvalues were calculated so that the percentage of overall variation explained by each function could be assessed. Combined characters were analysed using a multivariate analysis of variance to determine if groupings differed significantly. In order to measure the relative distinctiveness of populations, the percentage of observations correctly classified into their *a priori* groupings was also calculated. A DFA was also conducted to detect sexual dimorphism. In this case, all five traits (tarsus, wing, tail, culmen length, bill-depth and bill-width) were used.

The coefficient of variation (CV) was calculated in order to compare levels of variation within and between morphological measurements, and between the sexes. Levene's *F* test was used to gauge levels of significance for comparisons of the CV. The degree of sexual dimorphism (SD) ach trait in adult wedge-tailed shearwaters within each population was quantified using the equation SD = Log (Male/Female) (Greenwood 2003).

Possible assortative mating based on morphological traits was explored using univariate linear regressions. If regressions were significant, population differences in patterns were tested using ANCOVA with the male trait as the co-variate.

Narrow sense heritability (h^2) is a commonly used measure representing the proportion of genetic variance that can be transmitted to the next generation. Estimates for tarsus were calculated from fledglings at Lord Howe Island in 2004 using offspring/mid-parent regressions (Falconer 1989, Larsson et al. 1997). It was not possible to calculate h^2 for other traits or at other locations as this data was not available.

5.2 RESULTS

5.2.1 Sexual and geographic variation in morphology

Two-factor ANOVAs revealed that the populations studied differ in all traits except bill depth (Table 7, Fig. 9). The longest tarsi were found at Rottnest Island and the smallest at Lord Howe Island (Fig. 9a), indicating that these populations are largest and smallest in skeletal size respectively. Wing size is greatest at Heron Island and smallest at Rottnest Island (Fig. 9b). Rottnest Island birds also have the smallest tail measurements (Fig. 9c). In terms of culmen length, Raine Island birds have the longest culmens, with Lord Howe Island birds having the shortest (Fig. 9d). Finally, Rottnest Island birds were found to have substantially narrower bills than birds from all other colonies sampled (Fig. 10b). In addition, significant differences between the sexes in all traits exist (except tail), with males being the larger sex in all cases (Table 7, Fig. 9a-d, Fig. 10a,b). Interestingly, females had close to significantly larger tails than males, with a p value of 0.09 (Table 7, Fig. 9c).

For all morphological measurements the coefficient of variation was between 1 % and 6 % (Table 8). Levene's *F* test indicated that this did not differ significantly between

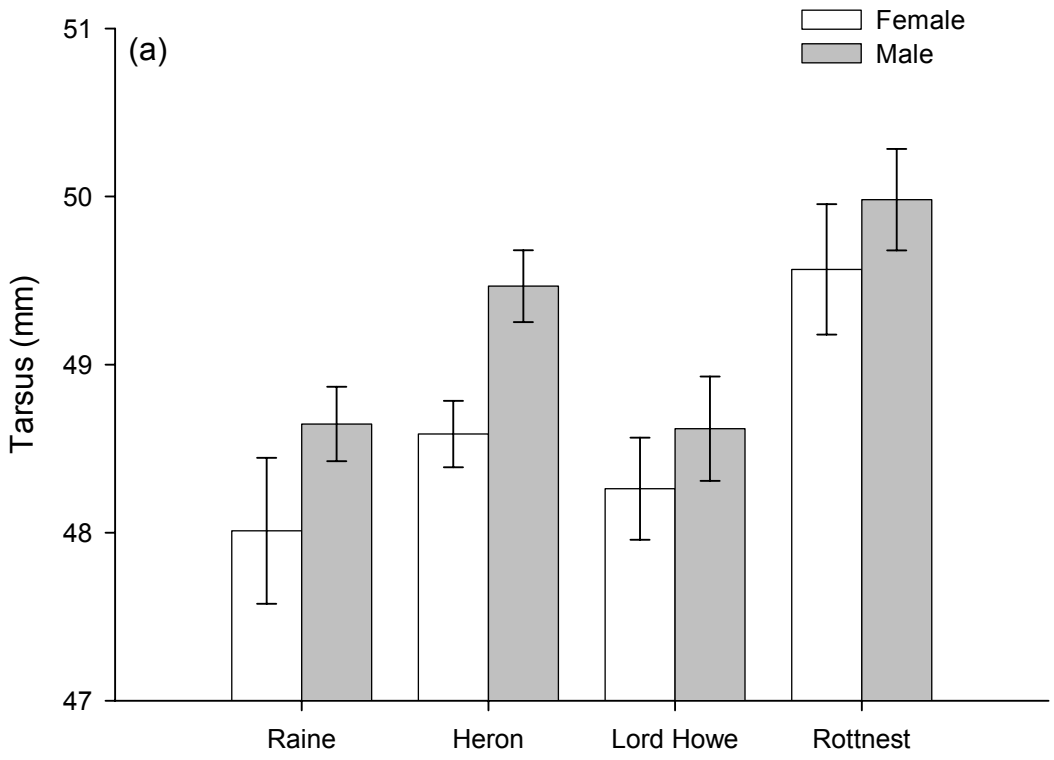
sexes for any measured trait ($p < 0.05$ in all cases). Tarsus length for Lord Howe Island fledglings in 2004 was significantly smaller than adult tarsus at Heron Island for all years combined ($t_{250} = 6.09$, $p = 0.001$).

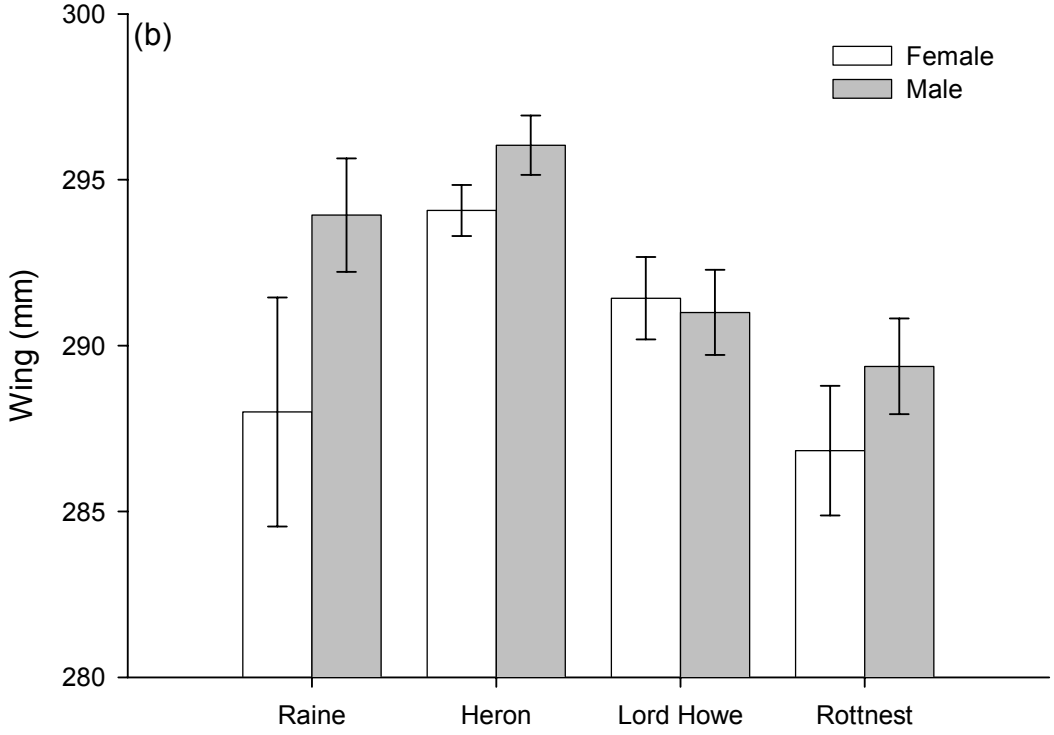
Table 7. Results from a 2-factor ANOVA examining morphological differentiation among four wedge-tailed shearwater colonies.

	Population effect		Sex effect		Interaction: Population * Sex	
Trait	*F*	*P*	*F*	*P*	*F*	*P*
Tarsus	7.01	0.0002	6.17	0.0100	0.46	0.7000
Wing	11.05	0.0001	5.71	0.0100	1.32	0.2600
Tail	22.67	0.0001	2.86	0.0900	0.52	0.6600
Culmen	24.61	0.0001	12.35	0.0006	0.34	0.7900
Bill depth	0.88	0.4100	19.23	0.0001	0.73	0.4800
Bill width	14.86	0.0001	6.70	0.0100	1.88	0.1500

Table 8. Means and CV values for morphological traits in wedge-tailed shearwaters from four breeding colonies.

Colony	Culmen		Tarsus		Wing		Tail		Bill depth		Bill width	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Raine Is.	39.26(0.45)	38.44(0.49)	48.64(0.22)	48.01(0.43)	293.93(1.71)	288(3.45)	134.46(1.15)	136.88(2.35)	*n.d*	*n.d*	*n.d*	*n.d*
Heron Is.	37.98(0.19)	36.81(0.21)	49.46(0.21)	48.58(0.19)	296.04(0.89)	294.07(0.77)	135.74(0.69)	136.57(0.52)	13.17(0.12)	12.6(0.08)	12.48(0.11)	12.12(0.14)
Lord Howe Is	36.15(0.33)	35.37(0.32)	48.61(0.31)	48.26(0.30)	291(1.28)	291.42 (1.24)	133.79(0.73)	135.52(0.83)	13.17(0.11)	12.85(0.10)	12.56(0.15)	12.04(0.12)
Rottnest Is.	37.7(0.28)	37.03(0.42)	49.98(0.30)	49.56(0.38)	289.37(1.44)	286.83(1.95)	127.93(1.02)	128.5(1.53)	13.21(0.09)	12.84(0.16)	11.6(0.07)	11.62(0.15)
CV (all pops.)	4.52	4.58	2.9	2.9	2.25	2.3	3.78	3.73	3.34	4.37	5.61	5.16


(a)
Female
Male
Tarsus (mm)
51
50
49
48
47
Raine
Heron
Lord Howe
Rottnest


(b)
Female
Male
Wing (mm)
300
295
290
285
280
Raine
Heron
Lord Howe
Rottnest

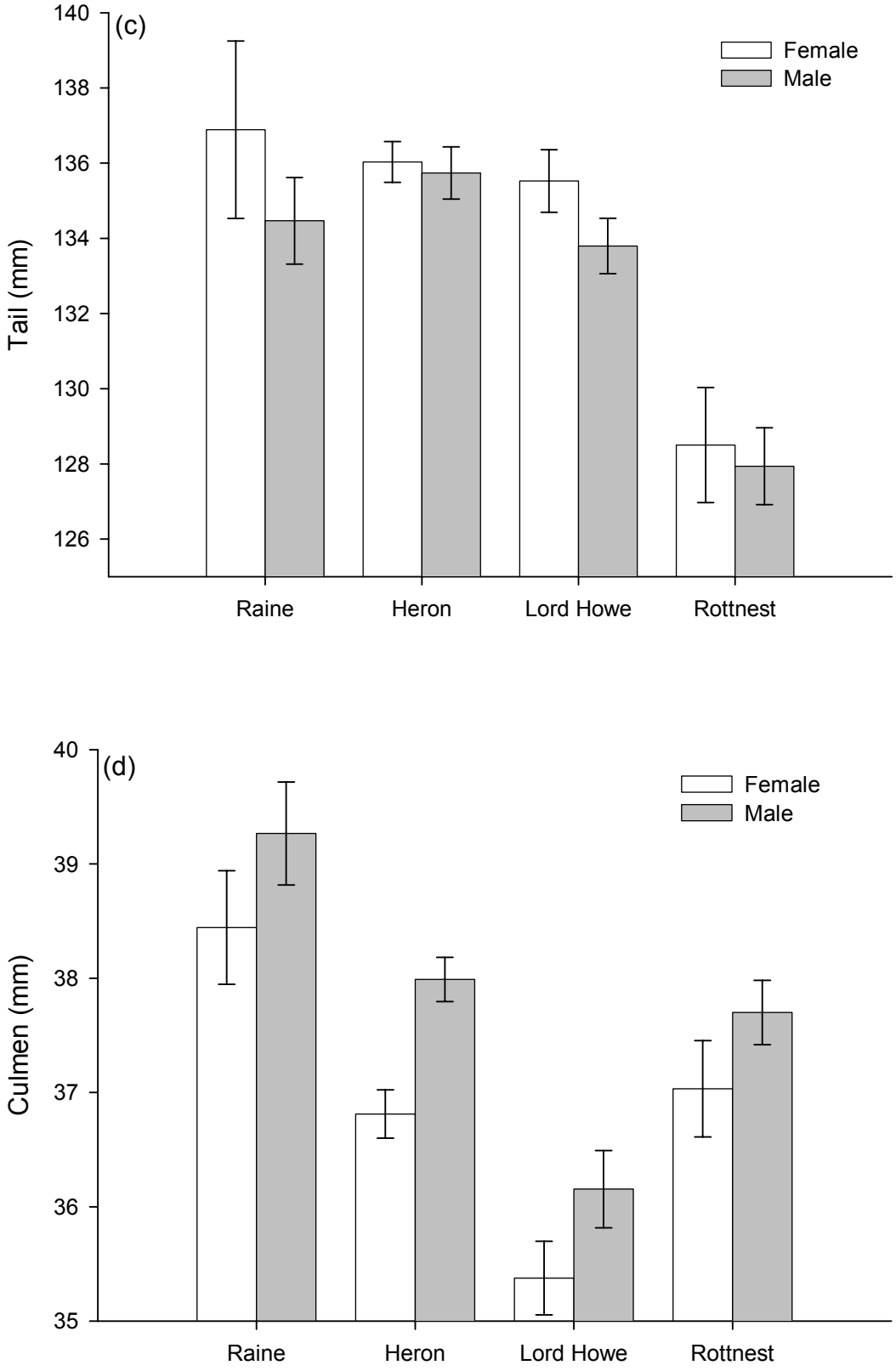


Fig. 9. Geographic variation in mean morphometric characters, and in the extent of sexual dimorphism among four breeding colonies of wedge-tailed shearwater; (a) tarsus, (b) wing, (c) tail, (d) culmen.

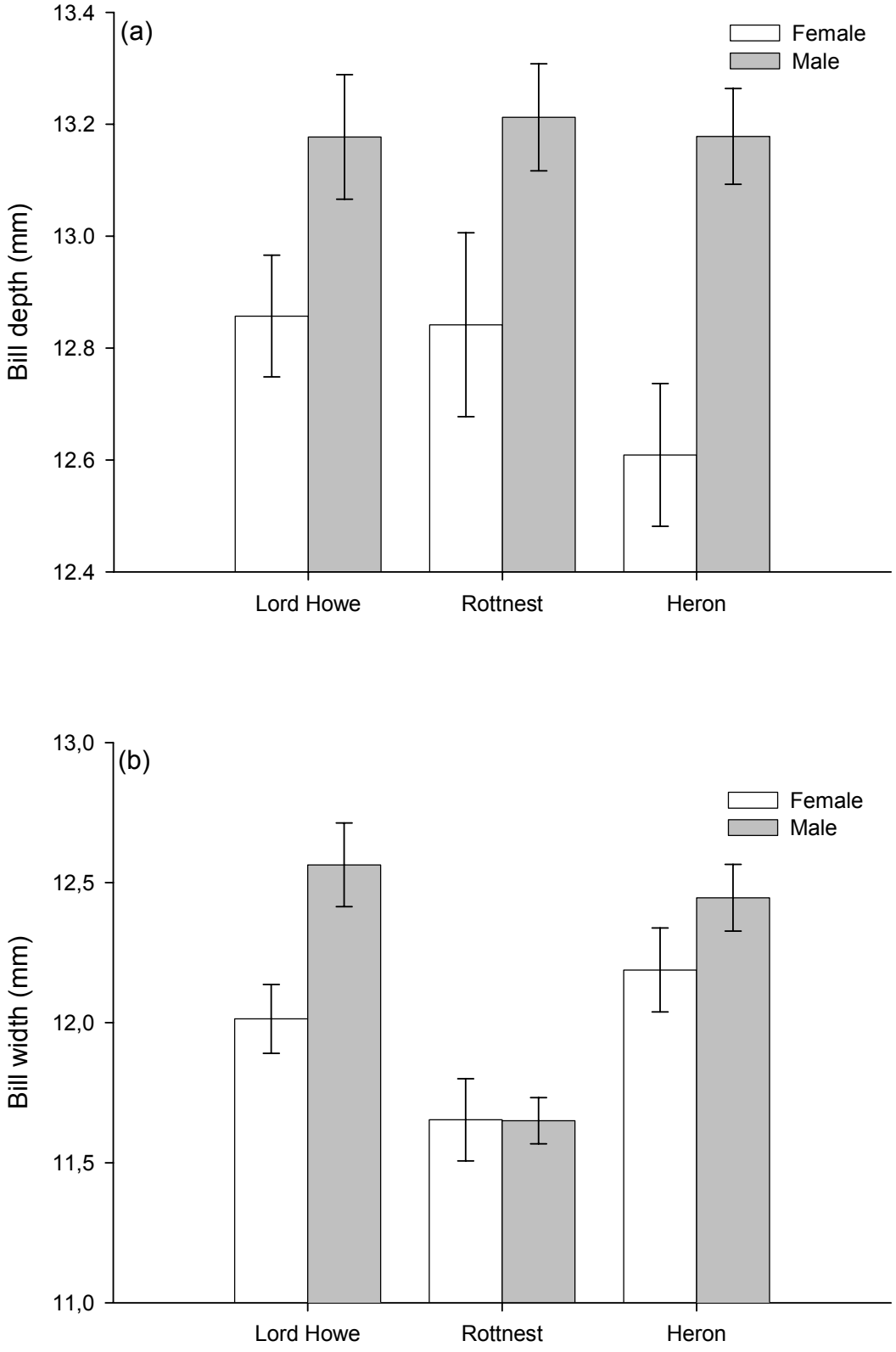


Fig. 10. Geographic variation in; (a) mean bill depth and (b), mean bill width, and in the extent of sexual dimorphism among three breeding colonies of wedge-tailed shearwater.

5.2.2 Relative differences between populations

Relative differences between sexes and among islands were analysed using two-factor ANCOVAs. However, for ease of interpretation, these data are presented using the alternative method of analysis (ANOVAs on size corrected residual scores) (Fig. 11a-e). Only 'significant' effects of the ANCOVA analyses are listed below (i.e. $p < 0.05$).

5.2.2.1 Relative wing length

The ANCOVA revealed no significant higher-order interaction effects, however wing length varied according to tarsus length ($F_{1,184} = 20.13$, $p = 0.0001$), and there was a significant effect of island ($F_{3,184} = 13.35$, $p = 0.0001$). This is because Rottnest Island birds have relatively shorter wings and Heron Island birds' relatively longer wings than elsewhere (Fig. 11a).

5.2.2.2 Relative tail length

The ANCOVA revealed no significant higher-order interaction effects, but a significant effect of island on relative tail length was detected ($F_{3,183} = 21.11$, $p = 0.0001$). Fig. 11b shows that this is due to relatively shorter tails at Rottnest Island in comparison to the other locations.

5.2.2.3 Relative culmen length

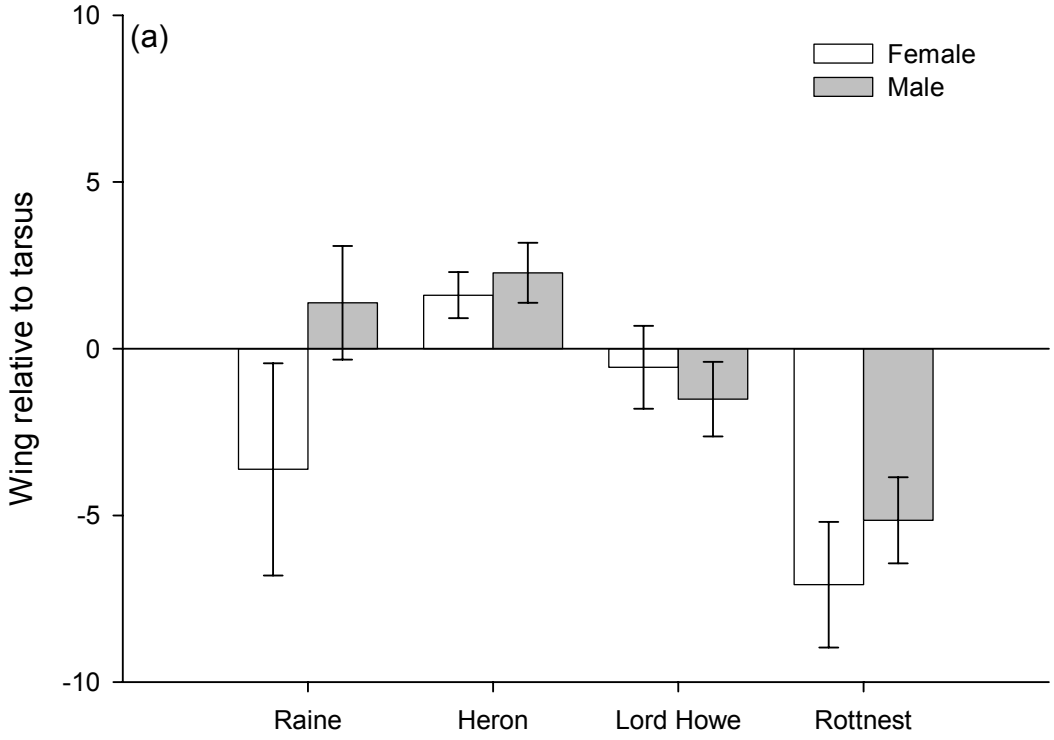
The ANCOVA revealed no significant higher-order interaction effects, however culmen length varied according to tarsus length ($F_{1,184} = 5.50$, $p = 0.02$), and there was a significant effect of island ($F_{3,184} = 25.25$, $p = 0.0001$) and sex ($F_{3,184} = 15.32$, $p = 0.0001$). Fig. 11c indicates that the island effect is because of relatively longer bills at Raine Island, and relatively shorter culmens at Lord Howe Island. The sex effect is due to longer culmens in males relative to body size (Fig. 11c).

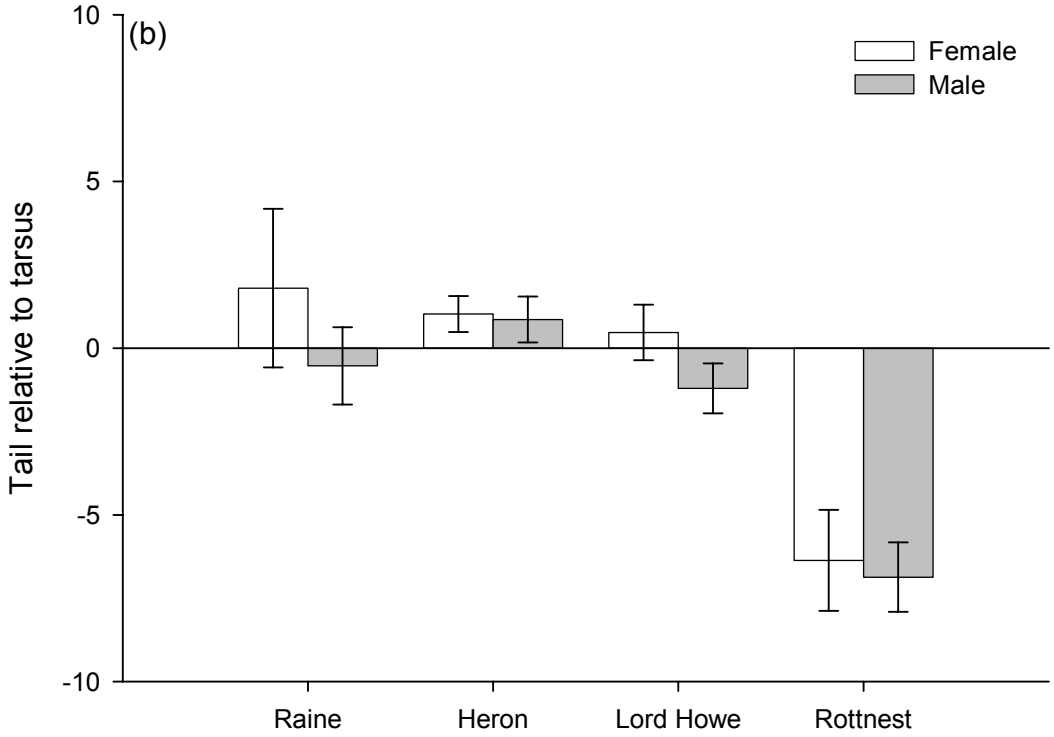
5.2.2.4 Relative bill depth

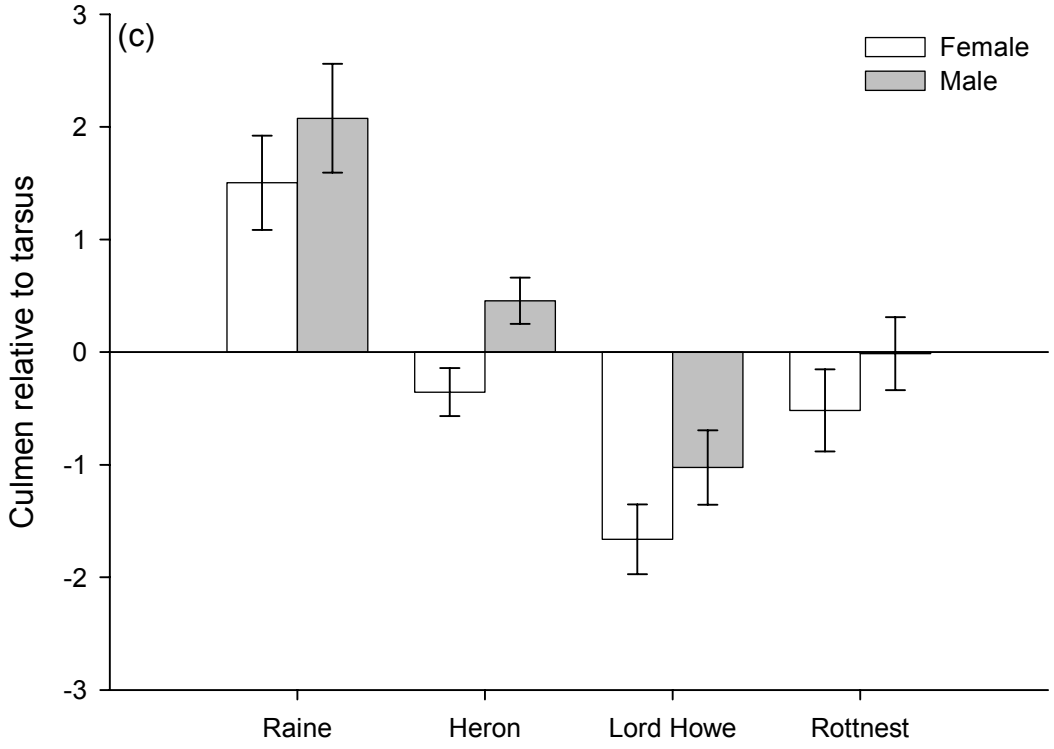
The ANCOVA revealed no significant higher-order interaction effects, however bill depth varied according to tarsus length ($F_{1,110} = 7.28$, $p = 0.02$), and there was a significant effect of sex ($F_{1,110} = 15.52$, $p = 0.0001$): male bill depth is relatively deeper than that of females (Fig. 11d).

5.2.2.5 Relative bill width

The ANCOVA revealed no significant higher-order interaction effects. Bill width did not vary according to tarsus length ($F_{1,110} = 1.55$, $p = 0.21$). The main factors of island ($F_{2,110} = 18.51$, $p = 0.0001$), and sex ($F_{1,110} = 8.64$, $p = 0.004$) were significant. Males had relatively wider bills compared to females, and birds at Rottnest Island had significantly narrower bills relative to tarsus length compared to the other two colonies (Fig. 11e).




(b)
Female
Male
Tail relative to tarsus
10
5
0
-5
-10
Raine
Heron
Lord Howe
Rottnest


(c)
Female
Male
Culmen relative to tarsus
3
2
1
0
-1
-2
-3
Raine
Heron
Lord Howe
Rottnest

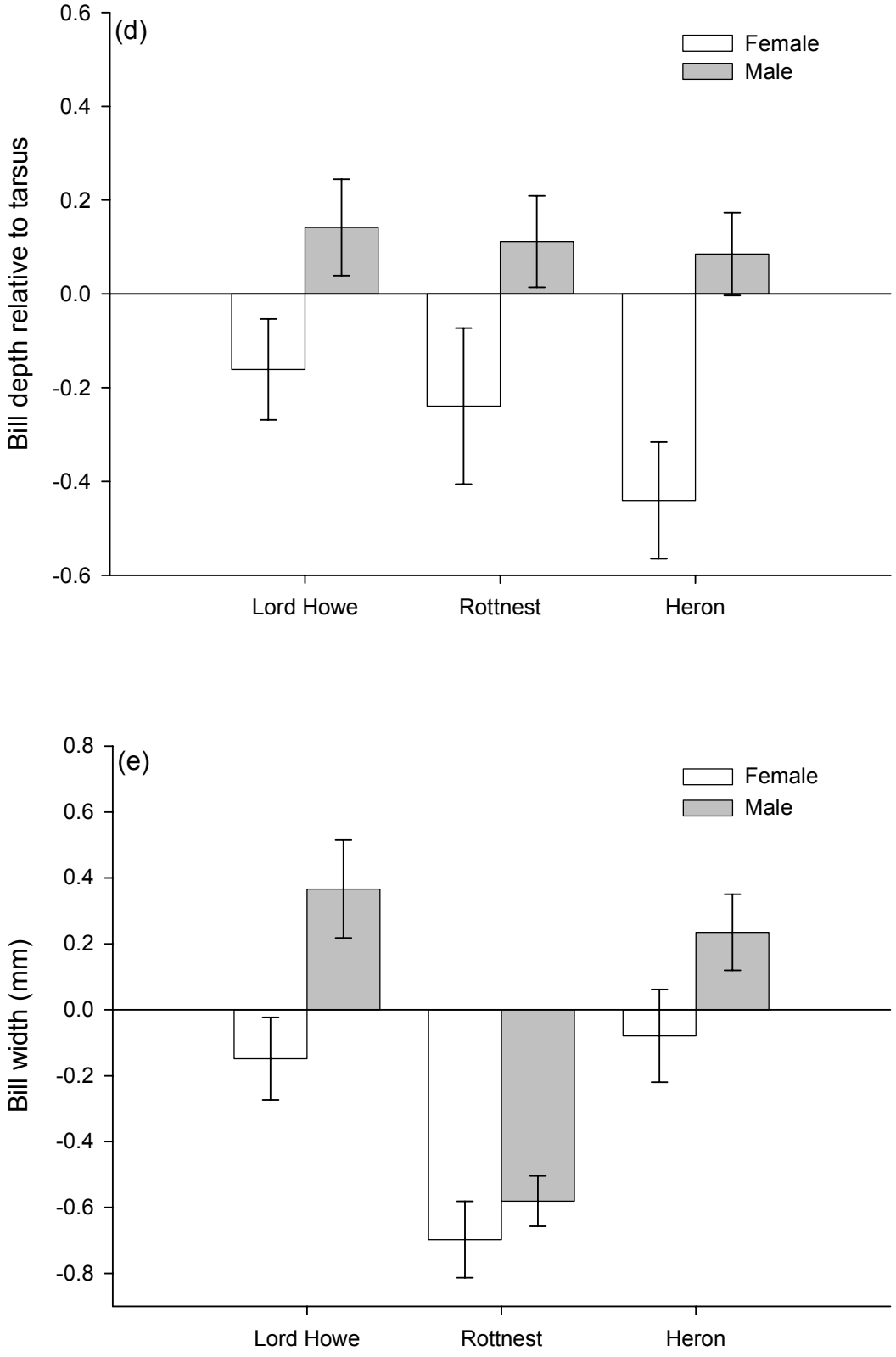


Fig. 11. Geographic variation in morphology relative to body size (tarsus), and in the extent of sexual dimorphism among wedge-tailed shearwater breeding colonies; (a) wing, (b) tail, (c) culmen, (d) bill depth, (e) bill width.

5.2.3 Multivariate analyses

The discriminant functions analysis correctly assigned individual birds to their respective populations in 53.38 % of cases. At the population level, discriminant functions indicated that Rottnest Island birds were the most distinct from other groups, being correctly classified in 76.66 % of cases. Lord Howe Island birds were also relatively distinct, (63.89 % of cases correctly classified), with Heron Island birds the least distinct (40.54 %) (Wilk's $\Lambda = 0.48$, d.f. = 12, MANOVA; $p < 0.0001$).

CV1 explained 57.46 % of the variation among groups and was correlated most strongly with tarsus (a measure of skeletal size) followed by tail (Fig. 12a). CV2 explained a further 38.30 % and was strongly correlated with culmen (Fig. 12a). Other traits were poorly correlated (Fig. 12a). The mean CV scores (± 1 SE) for each population are represented in Fig. 12b. This figure clearly indicates that the Rottnest Island population is differentiated strongly along the CV1 axis, and is significantly different in CV1 from all other populations. Lower values of CV1 indicate larger tarsi and smaller tail measurements (Fig. 12a). Therefore, birds from Rottnest Island have larger tarsi and smaller tails than all other populations.

High CV2 scores indicate longer culmen length (Fig. 12a). The distribution of points along this axis suggests that a latitudinal gradient in culmen length among Pacific Ocean populations exists with Raine Island birds having the longest culmen and Lord Howe Island birds the smallest (Fig. 12b).

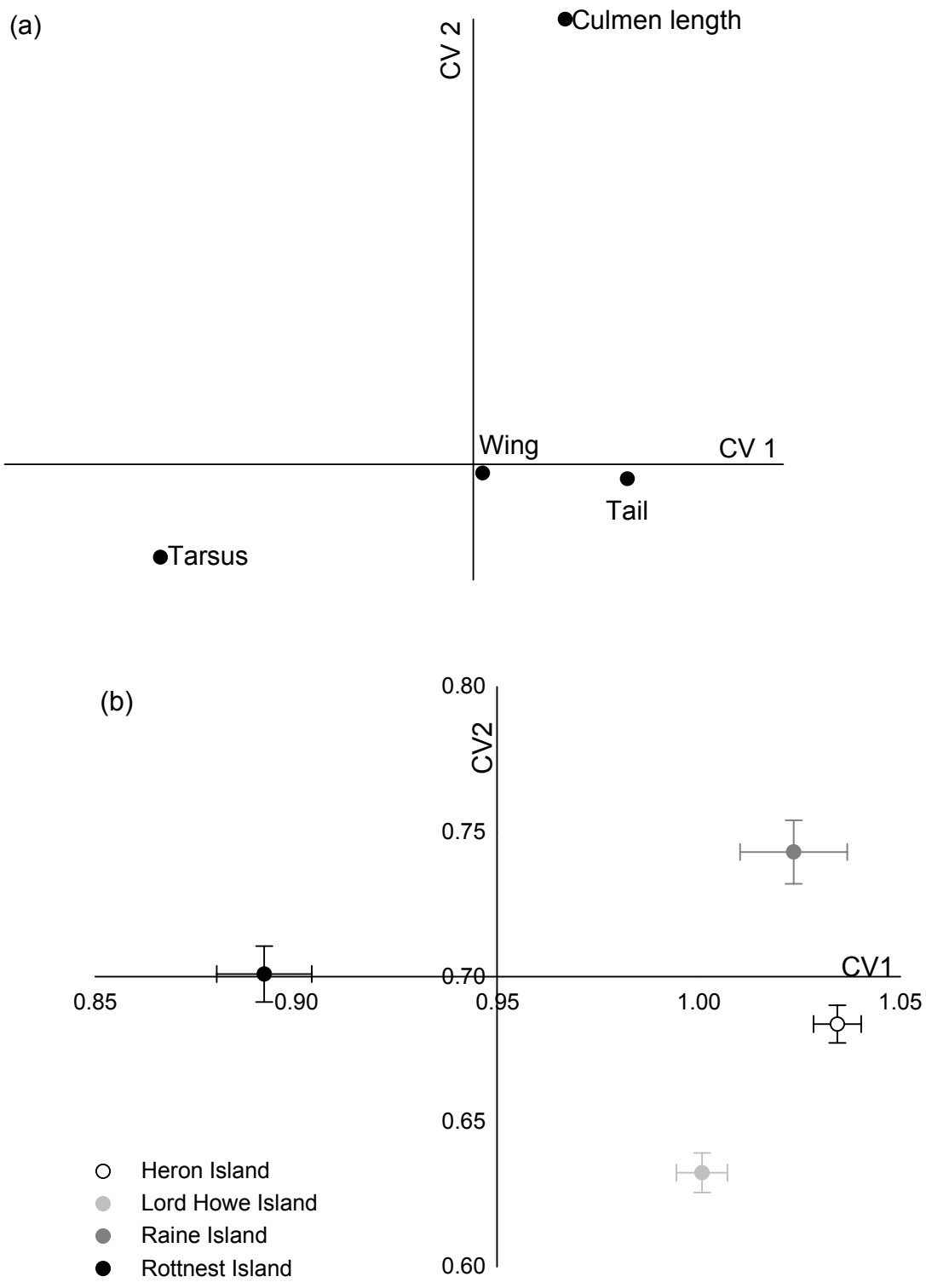


Fig. 12. Discriminant functions analysis of wedge-tailed shearwater morphology. (a) Loadings for four morphological variables. (b) Mean discriminant function scores (± SE).

As for univariate analyses, sex-specific variation was also observed using multivariate tests. The discriminant functions analysis for sex correctly assigned individual birds to their respective sex in 71.10 % of cases. Discriminant functions indicated that male birds were the most distinct, being correctly classified in 76.27 % of cases, as compared to 67.27 % in females. CV1 explained 46.80 % of the variation among sexes and was correlated most strongly with bill depth (eigenvalue = 0.12) followed by bill width (eigenvalue = 0.03) and culmen length (eigenvalue = 0.01) (F = 2.45, p = 0.04). Larger CV1 scores in males represented, deeper, wider and longer bills. CV2 could not explain any more of the variation. Bill shape is significantly different between the sexes, but cannot be used as a reliable clue to sex birds.

5.2.4 Differences in the degree of SSD among populations

Traits varied in the degree and direction of sexual dimorphism across populations and between the sexes (Table 9). For example, wing length at Lord Howe Island was female biased as opposed to male biased at the other three locations (Table 9). In addition, sexual dimorphism in tarsus/body size was an order of magnitude lower at Lord Howe Island and Rottnest Island compared to the other two colonies (Table 9). However, none of these differences were significant as evidenced by the non-significant interaction terms in the ANCOVA analyses.

Table 9. Geographic variation in sexual dimorphism for individual morphometric characters of wedge-tailed shearwaters. Positive values represent a male bias and negative values equate to a female bias in sexual dimorphism.

	n	Culmen	Tarsus	Wing	Tail	Bill depth	Bill width
Heron Is.	253	0.032	0.018	0.007	-0.002	0.045	0.030
Lord Howe Is.	72	0.022	0.007	-0.001	-0.013	0.025	0.043
Raine Is.	44	0.021	0.013	0.021	-0.018	nd	nd
Rottnest Is.	50	0.018	0.008	0.009	-0.004	0.029	-0.002

In order to assess to influence of sexual selection in maintaining any observable sexual dimorphism, the level of allometry between body size and traits that showed significant proportional differences between the sexes was examined (i.e. bill measurements only: see above). Positive allometry is expected between sexually selected traits and overall body size, if sexual selection is maintaining sexual dimorphism (Green 1992, 2000, Gonzális-Solís 2004). To test this, the first canonical variate (CV1) was used as the measure of body size. This was because CV1 is a composite of all commonly used size measurements (i.e. tarsus, wing and tail: see

results above). Bivariate reduced major axis (RMA) models on log-transformed data were used to examine allometry because both axes are equally subject to error (McArdle 1988, Sokal and Rohlf 1995). RMA regressions were conducted with RMA software for reduced major axis regression (http://www.bio.sdsu.edu/pub/andy/rma.html, Bohonak 2002). Differences in the intercept of the lines between males and females were deduced from the overlap of the 95% confidence intervals. Allometry is indicated where the slope of the relationship differs from isometry. A significant difference of a reduced major axis slope (b) from a known slope (ß) can be performed with the test statistic (McArdle, 1988):

$$T = | log\ b - log\ ß | / ((1 - r^2) / (n - 2))^{1/2}$$

The test statistic T is approximately t-distributed with $2+ ((n - 2)/(1 + 0.5\ r^2))$ degrees of freedom. Where ß equals one (the scaling exponent for an isometric relationship), a significant difference ($p < 0.05$) between b and ß indicates significant allometry in the trait tested.

Analyses of allometry indicated that only culmen length was related to body size (Table 10). This was evident for both sexes. However, culmen did not deviate significantly from isometry (1/3) for either Sex (Table 10).

Table 10. Slope and constant obtained by reduced major axis (RMA) regression of bill traits versus tarsus in wedge-tailed shearwaters. None of the slopes deviated significantly from the expected value under isometry (1/3) in either sex.

	Slope	CI slope 95%	Intercept	CI Intercept 95%	r^2
Females					
Culmen	-0.559	-0.645, -0.473	1.542	1.537, 1.546	0.465
Bill-width	-0.616	0.784, 0.447	1.054	1.045, 1.063	0.001
Bill-depth	-0.529	-0.674, -0.385	1.084	1.077, 1.092	0.027
Males					
Culmen	-0.567	-0.653, - 0.481	1.544	1.538, 1.550	0.417
Bill-width	0.662	0.834, 0.489	1.126	1.114, 1.138	0.027
Bill-depth	-0.500	-0.630, -0.370	1.092	1.083, 1.101	0.031

5.2.5 Assortative mating

Bill-width among all mated pairs was negatively correlated ($F_{1,44} = 21.82$, $r^2 = -0.32$, $p = 0.0001$, Fig.13). Wide-billed males preferentially mated narrow-billed females and vice-versa. This pattern ('disassortative" mating) has rarely been documented. The form of this relationship was consistent between Heron Island and Lord Howe Islands (ANCOVA; $F_{1,44} = 0.25$, $p = 0.61$). None of the other morphological traits examined showed significant associations between mated pairs (Table 11).

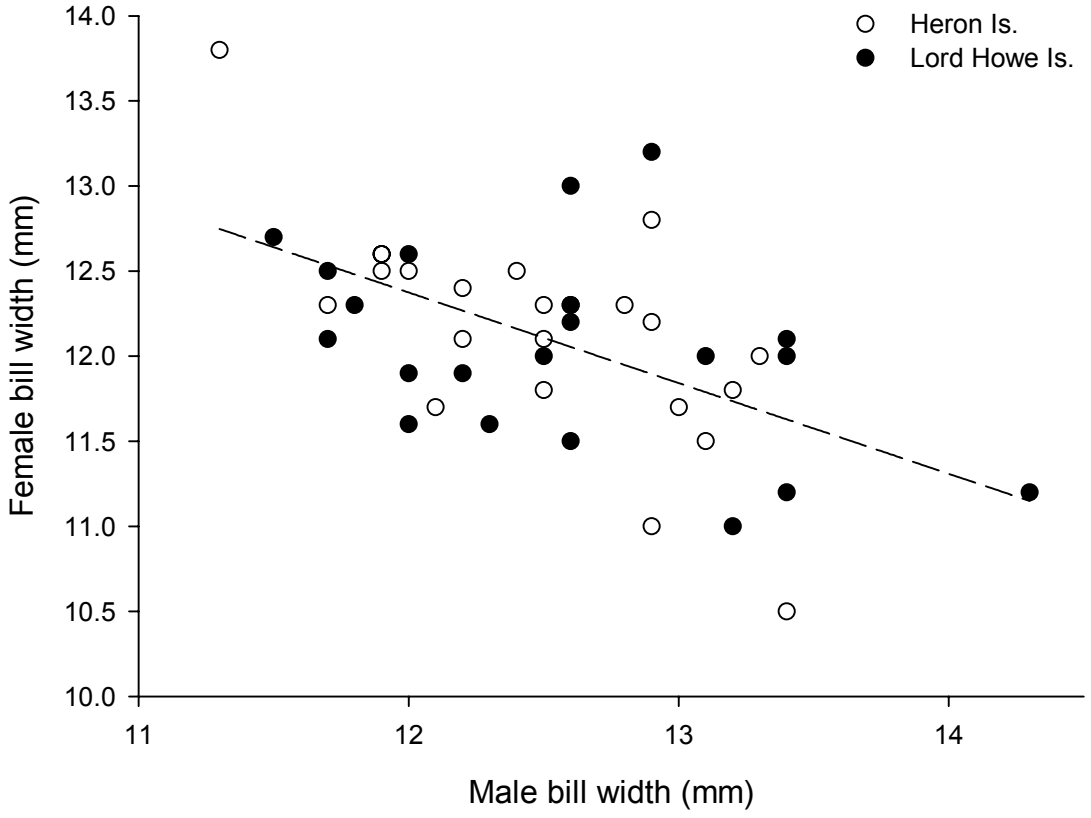


Fig. 13. Bill-width of males plotted against that of females in known pairs of wedge-tailed shearwater breeding at Heron Island (open circles) and Lord Howe Island (filled circles).

Table 11. Correlation coefficients and significance levels among measurements of forty-five known pairs of wedge-tailed shearwater breeding at Heron Island (n = 25) and Lord Howe Island (n=20).

Trait	r^2	P
Tarsus (mm)	-0.01	0.96
Wing (mm)	-0.02	0.72
Tail (mm)	0.02	0.15
Culmen (mm)	0.01	0.16
Bill-depth (mm)	-0.02	0.92
Bill-width (mm)	-0.32	0.0001

5.2.6 Heritability estimates

In spite of a small sample size, it is evident that tarsus length is heritable ($h^2 = 0.61$, $F_{1,9} = 16.68$, $p = 0.002$, Fig. 14). This value is consistent with heritability estimates for this trait in other seabirds (Barbraud 2000).

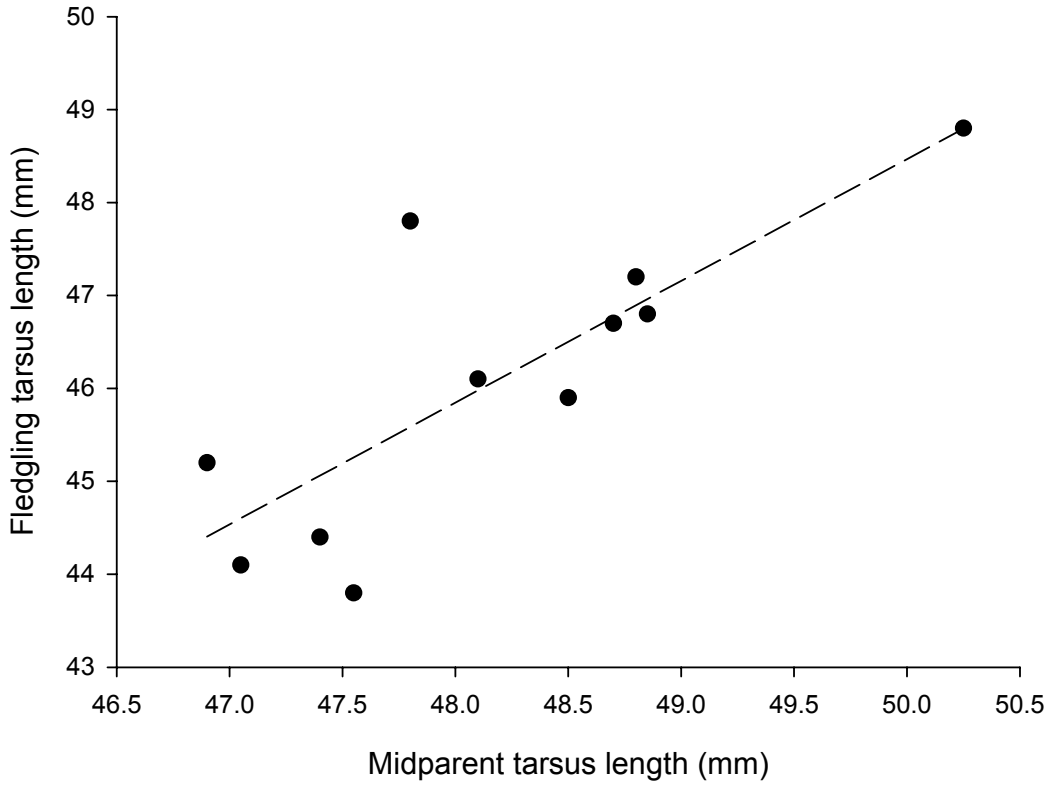


Fig. 14. The relationship between skeletal body size (tarsus) of offspring and parents among wedge-tailed shearwaters breeding at Lord Howe Island in 2004. The relationship was still significant after the removal of the upper right outlier (Outlier removed; $h^2 = 0.41$, $F_{1,8} = 7.43$, $p = 0.02$).

5.3 DISCUSSION

Theoretical and empirical work maintains that phenotypic variation among populations is influenced by numerous processes (Price et al. 2003, Aubret et al. 2004). These can be broadly classified as: (1) genetically based mechanisms such as selection (ecological and/or sexual) and drift, and (2) phenotypic plasticity in response to temporal fluctuations in local environmental pressures (Smith and Skúlason 1996, Price et al. 2003, Aubret et al. 2004). Alternatively, phenotypic variation in different traits may involve a combination of the above processes (Aubret et al. 2004).

5.3.1 Variation in body size (CV1 and tarsus)

Little information exists regarding mechanisms of body size divergence among populations of seabirds. In one of the few studies of its kind, Waugh et al. (1999) observed a relationship between differences in body size between populations of albatrosses of the genus *Diomedea*, and the distance of each population to foraging grounds. Waugh et al. (1999) proposed that inter-population differences in the distance travelled by adults to foraging grounds lowers provisioning rates and subsequent chick growth rates, differentially impacting on adult size at each colony. This implies that if food could be obtained nearer to the colony, growth rates and fledgling size would increase as a plastic response. It follows that seasonal changes in provisioning rates could cause the same pattern. Barbraud et al. (1999) proposed a mechanism similar to Waugh et al. (1999) for snow petrels (*Pagodroma nivea*). However, in this case, Barbraud et al. (1999) discovered that body size was primarily inherited despite a significant environmental component.

The 'provisioning rate' hypothesis outlined above predicts that the shearwaters from Rottnest Island (being substantially larger than all other populations) should have higher provisioning rates than those in the Pacific off eastern Australia. Unfortunately, information on the breeding/foraging ecology from this colony is not yet available, however preliminary evidence suggests that a uni-modal foraging strategy (short trips to local foraging grounds) is used during the breeding season (R.D. Wooller *pers com.*), which leads to an increase in provisioning rates as compared to colonies that use a bi-modal (mixed short and long trips) foraging strategy (i.e. Heron Island)(Chapter 3). Thus, it is possible that increased provisioning rates at Rottnest Island are contributing to the larger body size of adult birds at that location.

However, if differences in provisioning rates between populations determine body size in wedge-tailed shearwaters, then fledgling size at Lord Howe Island in 2004

(when provisioning and growth rates were significantly higher than at Heron Island in 2003) and adult size in general should be greater at Lord Howe Island compared to Heron Island (Chapter 3). However, the opposite is true: tarsi measurements for Heron Island adults are significantly larger than those of both Lord Howe Island fledglings and adults measured in 2004, with CV1 showing no significant difference between the two colonies (Table 8). Thus it would appear that provisioning rates alone do not explain all of the variation in wedge-tailed shearwater body size.

Phenotypic variation can be maintained if selection acts in opposite directions under consistently different environmental conditions (Endler 1986). For this to occur phenotypic variation has to be heritable and there must be an advantage, relative to the local environment, in possessing a particular trait (in fitness/future reproduction). Body size in wedge-tailed shearwaters is heritable (refer results this chapter), so the first requirement is met. In addition, local foraging conditions at Heron and Lord Howe Islands, as measured by chlorophyll *a* concentrations, are stable over the longer term (Chapter 3). Therefore, the prerequisites for natural selection to cause body size divergence between these two colonies exist. Two possible selective mechanisms are discussed below.

One possibility is that the vertical distribution of prey at each colony causes selection on body size as a function of dive capability (Oka et al. 1999). Procellariid seabirds obtain a substantial component of prey by diving, and body size is positively related to dive capability in animals (Costa 1991, Mori 2002). Therefore, if prey is consistently deeper, selection for larger individuals could occur. Interestingly, dive depths at Heron Island are shallower than at Lord Howe Island (Chapter 3); a fact that directly opposes the prediction made by this hypothesis. However, as this analysis only compared dive depths during one year at each location, and selection operates over much longer time-scales, it is not possible to exclude this hypothesis until further data is obtained.

Another potential selective mechanism relates to the impact that body size has on flight efficiency in birds. Since smaller birds have lower wing loadings than larger ones, and flight speed decreases with wing loading (Pennycuick 1989), small birds have lower flight efficiency than large ones (Pennycuick 1989, Barbraud et al. 1999). Therefore it would be beneficial for birds at Heron Island to be larger and have proportionally larger wings given they are required to forage at distance from the

colony in order to provision both themselves and their chicks (i.e. bi-modal foraging strategy Chapter 3). Thus, selection on both body size and wing length (both functions of flight efficiency), could explain the larger body size as well as the proportionally longer wings at Heron Island (Fig. 9a, 11a). Future work using satellite telemetry will provide the detail required to fully explore this hypothesis.

5.3.2 Variation wing and tail

Evidence to date suggests that wing and tail morphology in birds is primarily under the influence of natural selection (Brown and Brown1998, Spear and Ainley 1998). Spear and Ainley (1998) proposed that longer wings and tails in tropical Procellariids are most likely an adaptation to foraging in an environment where resources are distributed patchily. Since longer wings and tails are known to influence flight efficiency by enabling individuals to cover more distance with less effort, these traits would be an advantage when foraging over large areas with disjunct prey distributions (Spear and Ainley 1998).

In line with this hypothesis, wing and tail measurements of wedge-tailed shearwaters at the more temperate localities (Rottnest Island and Lord Howe Island) are smaller than elsewhere (Fig. 9c). This pattern was consistent even when taking into account relative size differences among populations (Fig. 11b), and contrasts with the pattern in tarsus where Rottnest Island birds were the largest. Therefore, the fact that wedge-tailed shearwaters at tropical locations probably travel greater distances in search of food (Chap 3), means that longer wings and tails are advantageous at these locations, and therefore have a selective advantage (Ashmole 1963, 1971). Unfortunately, heritability estimates of wing or tail length for wedge-tailed shearwaters were unavailable. However recent work on other seabird species has shown that wing at least has high heritability (Barbraud 2000). There is presently no information on the heritability of tail length in seabirds but work on swallows suggests that it is also high (Cuervo and MØller 2001).

As with tarsus, wing and tail morphology can theoretically be influenced by non-genetic indirect environmental pressures via increased provisioning rates (described above). If so, tail and wing lengths should be greater at colonies with increased provisioning rates. However, as with tarsus length, the observed patterns conflict with predictions, with smaller wings and tails at colonies where higher provisioning rates

are either documented (Lord Howe Island) or predicted based on foraging behaviour (Rottnest Island).

5.3.3 Variation in culmen

The majority of theoretical and empirical work concerning mechanisms of morphological divergence among avian populations has been documented using bill size and shape. This has been achieved through detailed studies such as those conducted by Grant and Grant (1989) on Darwin's finches (*Geospiza spp.*). In this case, bill shape was directly related to trophic niche (seed size). In addition, bill morphology is highly heritable, and genetic homogeneity among different forms is evident (Freeland and Boag 1999). Together, this work suggests that selection may be the driving force in maintaining bill shape in this species. A number of other studies have subsequently shown that natural selection is the most likely cause of observed divergence in avian bill dimensions (eg. Bensch et al. 1999, Marquiss and Rae 2002, Clegg et al. 2002).

However, there is also evidence that bill morphology can vary in a plastic way (James 1982). For example, James (1982) conducted a reciprocal transplant experiment on red-winged blackbirds (*Agelaius phoeniceus*). Bill shape in chicks that were raised in the 'foster environment' matched those of adults from the same location, suggesting that a large component of geographic variation in bill shape is explained via plastic responses by chicks to local foraging conditions and provisioning rates experienced by adults.

Culmen morphology in wedge-tailed shearwaters was idiosyncratic to each population, and showed a pattern of variation substantially different to body size, wing and tail variation (Fig. 9d, 11c). Lord Howe Island birds have significantly shorter culmens than all other populations (Fig. 9d). In addition, significant differences in culmen length exist between all Pacific Ocean populations (Fig. 9d, 11c). As with wing and tail measurements, culmen length appears to group according to the latitude of each colony (Fig. 9d, 11c).

My results are consistent with the species level discussion by Spear and Ainley (1998) of Procellariid morphology that suggests larger culmens in tropical/lower latitude taxa are an adaptation to aid in the capture of more vagile prey species found in tropical locales. Thus, the two 'temperate' wedge-tailed shearwater colonies (Lord Howe and

Rottnest) may forage on similar resource bases and experience similar selection pressures on trophic morphology. Moreover, as with tarsus, wing and tail characteristics, divergence in adult culmen length is unlikely to be a plastic response driven by differences in provisioning rates among colonies because colonies with known or putatively high provisioning rates do not have longer culmens. In addition, heritability of bill length (as well as bill width and bill depth) has been documented previously in the snow petrel (*Pagodroma nivea*), another Procellariid, raising the possibility that heritability may also be high in wedge-tailed shearwaters (eg. Barbraud 2000).

Bill width was also significantly divergent among populations, with narrower bills at Rottnest Island (Fig. 10b). The pattern of bill width variation does not fit with a selective mechanism involving a latitudinal difference in prey characteristics. Bill width at Heron Island and Lord Howe Island was the same, but Rottnest Island birds were substantially divergent (Fig. 10b). This does not preclude an adaptive explanation. Bill width has an impact on the size of prey targeted as well as handling efficiency (Spear and Ainley 1998). Thus, differences in prey type between the Pacific and Indian Oceans may select for divergent bill width. Unfortunately, until extensive data is available on the prey used by wedge-tailed shearwaters at all of the colonies in this study, adaptive explanations for bill size/shape will remain un-tested.

5.3.4 Sexual selection and variation in SSD

The trajectories of morphological variation among populations can be influenced by differences in mating systems, sex-specific niche partitioning or other mechanisms that maintain sexual size dimorphism (eg.sexual selection) (Selander 1966, Shine 1989). If sex-specific processes are influencing morphological variation differently in alternative populations, then patterns of sexual dimorphism are expected to differ among populations (Shine 1989). The consistent pattern of male biased sexual dimorphism in wedge-tailed shearwater morphology among populations implies that the underlying mechanism is the same at all locations, and therefore, observable population level divergence cannot be explained using this hypothesis (Table 7, 9).

Processes responsible for inter-sexual morphological divergence may be similar to those responsible for inter-population morphological divergence (Turelli et al. 2001). It is therefore worthwhile assessing the potential mechanisms that underpin sexual dimorphism, in order to highlight possible mechanisms maintaining population level

morphological divergence. As measures of culmen morphology (length, width, depth) were the only traits that differed significantly between the sexes after taking into account the effect of body size (i.e. allometry), only mechanisms that maintain morphological variation in culmen will be discussed.

Sex-specific differences in bill dimensions have been attributed to four possible theoretical mechanisms; (1) sexual selection (by one or both sexes) because some aspect of bill size indicates fitness (Darwin 1871, Andersson 1994), (2) selection for increased trait size because of the benefits during contest-competition for mates (Owens and Hartley 1998), (3) ecological niche separation and selection on trophic morphology that enhances separation (Shine 1989, Andersson 1994). Based on the following arguments, ecological niche separation (3) appears to the most likely explanation.

Firstly, variation in sexually selected traits is predicted to be between three and five times greater than that of naturally selected traits (Rowe and Houle, 1996, Cuervo and MØller 2001, Radford and Du Plessis 2004). This was not the case for the bill characters measured. The variation in bill characters for both sexes was similar to that seen in the other morphological measurements (i.e. between 1 % and 6 %)(Table 2).

Secondly, a positive allometric relationship is expected between sexually selected traits and overall body size, if sexual selection is maintaining the observed dimorphism (Green 1992, 2000). This is because if sexual selection promotes further exaggeration of a trait (by increasing attractiveness or enhancing the bearer's competitive ability during contests over mates), then larger individuals have more to gain by investing more (proportionately) than smaller individuals (Green 1992, 2000). There was no such relationship between measures of overall size and bill measurements in either sex for our sample of wedge-tailed shearwaters (Table 4). Moreover, confidence intervals for the slopes of each sex overlapped for all bill traits except bill-width (Table 4). Slopes should be divergent between the sexes (males having a steeper slope) if sexual selection is operating (Green 1992). The lack of overlap between the sexes for bill-width is a function of the fact that no relationship exists between CV1 (body size) and bill-width in this species ($p > 0.05$).

Thirdly, because males of this species (or genus) have never been recorded using physical contests to determine the outcome of mating rights, and preliminary findings

indicate no evidence of polygyny (and by extension male-to-male combat for females), sexual selection via contest-competition seems very unlikely (Peck *unpub. data,* Warham 1990, Marchant and Higgins 1990).

An alternative hypothesis is that ecological niche-divergence between the sexes has occurred with concomitant divergence in trophic (bill) morphology (Darwin 1871, Hedrick and Temeles 1989, Shaffer et al. 2001). Ecological mechanisms have been shown to be important in promoting size dimorphic trophic structures in a number of terrestrial taxa (Selander 1966, Shine 1989, Butler et al. 2000). They are also considered to be potentially important in seabirds (Fairbairn and Shine 1993, Shaffer et al. 2001, González-Solís 2004). The unpredictable nature of the marine environment means that competition for food between sexes in seabirds is likely to be strong (Ashmole 1971, Lewis et al. 2001). By sexes selecting different prey types or foraging niches, inter-sexual competition for food resources can be diminished (Selander 1966). As a result, divergent trophic morphology may evolve (Darwin 1871, Shine 1989).

Modifications of trophic morphology unrelated to body size have in the past been considered reliable (though indirect) evidence for an ecological cause of sexual dimorphism (Darwin 1871, Shine 1989, González-Solís 2004). This is because if niche partitioning has occurred with associated selection on trophic morphology, then sexual dimorphism in trophic morphology is expected to be greater than that accounted for by body size. Therefore the relatively larger bill dimensions in wedge-tailed shearwaters suggest that natural selection rather than sexual selection is maintaining the observed dimorphism. In support of this possibility, evidence of sex-specific foraging behaviour exists for this species that appears to be related to competition between the sexes (Peck and Congdon 2006).

However, the novel pattern of mate choice documented is a form of sexual selection that involves one or both sexes choosing partners based on bill width. Standard selection models can therefore not be invoked in this system. Instead it appears that a form of ‘correlational’ selection exists between pair formation and bill width in wedge-tailed shearwaters and is driving sexual dimorphism in this species (Schluter and Nychka 1994). If so, differences in bill-width between pairs would serve to reduce intra-specific competition for limited resources and thus enable adults to provision their chick more efficiently. However, there is still the need to establish the

link between foraging niche-divergence and bill dimensions, and to examine the relative success of pairs with different levels of dimorphism before this hypothesis can be fully explored.

5.3.5 Conclusions: Morphological variation

There is no evidence that a difference in the degree of sexual dimorphism is the proximate cause for the observed divergence in wedge-tailed shearwater morphology among colonies. Genetic processes (i.e. selection and/or drift) as opposed to phenotypic plasticity are more likely the primary cause based on the following; (1) A substantial body of work has shown that morphometric traits in birds is heritable. In addition, at least one trait (tarsus/body size) has high heritability in wedge-tailed shearwaters (refer results). (2) Patterns of character divergence in all traits fit with selective scenarios; tropical colonies have character traits that are predicted to enhance foraging efficiency in patchy low-productivity habitats, and thus result in a higher probability of offspring survival. (3) The pattern of trait divergence does not fit predictions of a facultative shift towards larger body dimensions in colonies where provisioning rates are higher. Instead the opposite is true implying that if facultative change exists, genetic processes override it. (4) Sex-specific bill morphology in wedge-tailed shearwaters (males larger) and a novel form of mate choice (disassortative mating) based on bill-width provides in-direct evidence that natural selection may be involved in the evolution and maintenance of bill dimorphism in this species. By extension, the results imply that inter-population level morphological diversity in this species may also be driven by a selective mechanism.

Information on gene flow among colonies is required to determine the relative importance of the alternative genetic mechanisms (i.e. selection versus drift) that may underpin the observed morphological diversity in this species. This will be addressed in Chapter 6.

CHAPTER 6

6.0 INTRODUCTION

A critical stage in the speciation process is the evolution of genetic divergence between populations. By examining patterns of genetic diversity, in conjunction with morphological, environmental and behavioural characteristics, considerable insight into historical and contemporary micro-evolutionary processes can be gained.

For example, concordance of neutral genetic and morphological divergence among populations is expected if they are determined by the same neutral mechanism (i.e. a non-selective one). Because genetic markers primarily reflect drift, correlated genetic and morphological divergence provides indirect evidence that drift is the cause of any observed differences among populations (Wright 1931, Mayr 1942, 1963, Barrowclough 1983, Reed and Frankham 2001, Clegg et al. 2002). Alternatively, if selection or phenotypic plasticity is responsible for the observed patterns, then discordance between genetic and morphological divergence is expected (Wright 1931, Barrowclough 1983, Smith et al. 1997, Chan and Arcese 2003).

Assessing levels of genetic divergence and variation among populations can also provide valuable insight into past demographic events that may have impacted on present day patterns of morphological and genetic variation (Hewitt 1996, Merilä et al. 1997, Joseph et al. 2002, Peck and Congdon 2004). In some cases, long-term climate oscillations (Milankovitch cycles) and/or glaciation events such as those that occurred during the Pleistocene and Pliocene (Eyles 1993, Hewitt 1996, Hewitt 2000) impact significantly on genetic variation among populations, and may lead to an underestimation regarding the relevance of historical processes in causing observed patterns (Friesen et al. 1996, 1997, Congdon et al. 1990, Peck and Congdon 2004). Information on past demographic events is therefore crucial when evaluating random versus non-random causes of population divergence.

Seabirds present a paradox. Despite a bewildering array of diversity, present-day allopatric barriers to gene flow are limited (Avise et al. 1992, Steeves et al. 2005). This suggests that non-allopatric mechanisms of population divergence, possibly in conjunction with historical events, may be involved during speciation (eg. Congdon et al. 2000).

The aims of this chapter were;
(1) To document levels of gene flow and genetic population structure among wedge-tailed shearwater colonies breeding around the Australian coastline.

(2) To examine levels of concordance among morphological and genetic measures in order to assess the relative importance of drift in causing the observed patterns of morphology (Chapter 3) and life history (Chapter 3) variation.

(3) To examine the effects of historical processes in shaping genetic structure.

6.1 METHODS

6.1.1 Study sites

Five populations were sampled representing tropical (Raine Island), sub-tropical (Heron Island, Hawaii) and temperate (Lord Howe Island, Rottnest Island) locales and are separated by varying degrees of geographical distance (Fig. 1). Further details on study locations and sample sizes can be found in Chapter2.

6.1.2 Genetic population structure

All laboratory materials and methods used for measuring genetic population structure are contained in section 2.3 (Chapter 2). Tests of assumptions, analyses that allow past demographic events to be detected and the effects of isolation by distance are contained in section 2.3.5.

6.1.3 Morphological and genetic divergence

Morphological divergence between populations was estimated using Mahalanobis D^2, calculated between populations in canonical variable space. Methods relating to the multivariate analysis and measurement of morphological variables and the traits themselves are described fully in sections 5.1.2 and 5.1.3. (Chapter 5). Mahalanobis distance was used instead of other distance measures (Euclidean or Pythagorean)

because potential correlations between variables are taken into account, and Mahalanobis distance measures incorporate the effects of variable correlations (Campbell and Atchley 1981, Chan and Arcese 2003). Genetic divergence measures are described in section 2.3.5 (Chapter 2).

6.1.4 Matrix correlations

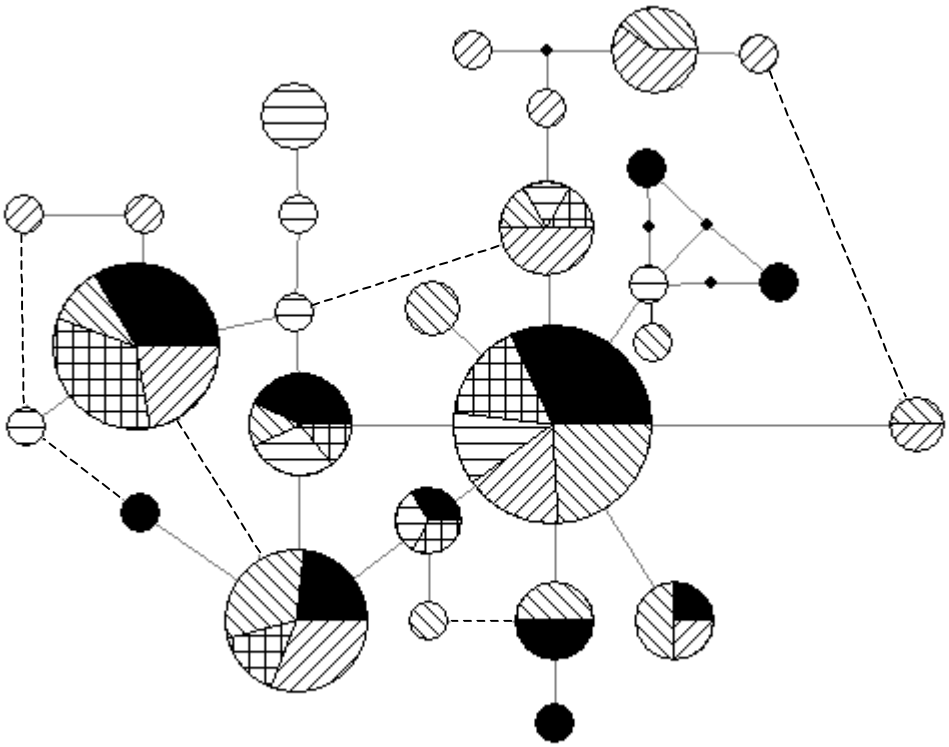
Relationships between morphological, genetic and geographical distance matrices were examined with Mantel tests (Mantel 1967). In order to explore the role of environmental differentiation in causing patterns of genetic and/or morphological divergence, sea surface temperature (SST) (http://poet.jpl.nasa.gov/) values were obtained. SST is known to influence prey availability and accessibility to foraging seabirds and other marine vertebrates, and also influences chick developmental patterns (Fullard et al. 2000, Chapter 3). Thus, variation in SST among colonies may indirectly alter foraging behaviour (Chapter 3), encourage selection on physiological and/or morphological traits, and ultimately promote genetic population divergence in wedge-tailed shearwaters (Chapter 3). Initially, genetic and morphological distance matrices were compared, and then geographical and SST matrices individually. All values were log transformed prior to analysis.

6.2 RESULTS

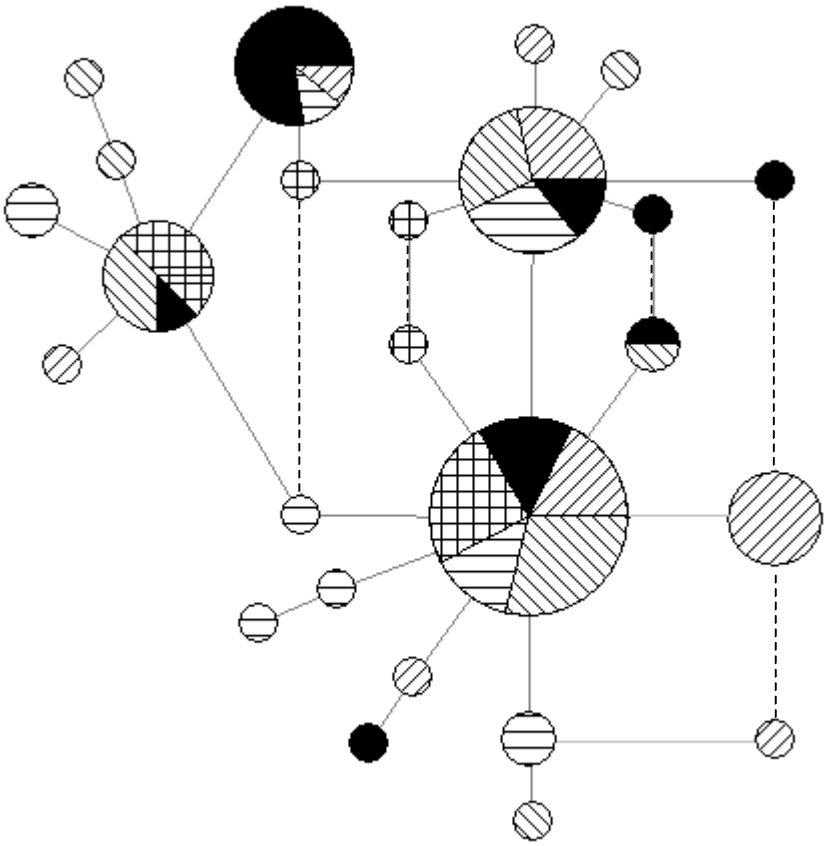
6.2.1 Patterns of Variation: Introns.

No insertions or deletions were observed in intraspecific comparisons of any intron sequenced in this study. The three introns used were variable, with the number of alleles per locus ranging from 4 to 27 (Fig. 15a-c, 16). For all intron loci, substitution relationships generally described a star-shaped pattern with most loci differing from all other loci by one substitution, although some were separated by as many as four substitutions (Fig. 15a-c).

(a)



(b)



(c)

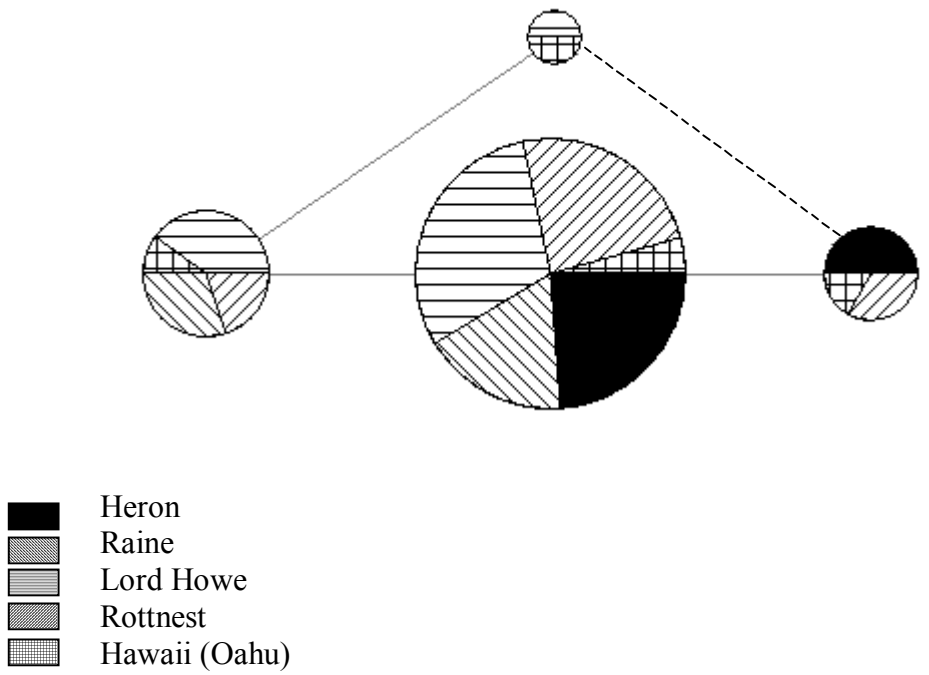


Fig. 15. Median joining networks for (a) Gapd, (b) Lamin and (c) Adolase in wedge-tailed shearwaters sampled across five populations. The size of each haplotype is proportional to its frequency (Table.). Dotted lines represent potential homoplasies and dots represent median vectors, which are extant un-sampled haplotypes or extinct ancestral haplotypes (Bandelt et al. 1999).

Allele/	*Glyceraldehyde-3-phosphate dehydrogenase*									
/Site	69	74	80	86	92	165	202	220	233	246
1	G	G	G	G	T	G	T	C	T	A
2	G	G	G	G	T	G	C	A	C	A
3	G	G	G	G	T	G	T	A	T	A
4	G	G	G	G	C	A	T	C	C	A
5	G	G	G	G	C	A	T	C	T	C
6	G	G	G	G	T	G	C	C	T	A
7	G	G	G	G	T	G	C	A	T	A
8	G	G	G	G	T	G	T	C	T	C
9	G	G	G	G	T	G	C	A	T	C
10	G	G	G	G	T	G	T	T	T	A
11	G	G	G	G	T	G	T	T	T	C
12	G	G	G	G	T	G	T	C	C	A
13	G	G	G	G	T	G	C	A	C	C
14	G	G	G	A	T	G	T	A	C	C
15	G	G	G	G	T	G	T	A	C	A
16	G	G	G	G	T	A	T	C	T	A
17	G	G	G	A	T	G	T	A	C	A
18	A	A	G	G	T	G	T	C	T	A
19	G	A	G	G	T	A	T	C	T	A
20	G	G	G	G	T	G	C	T	T	A
21	G	G	A	G	T	G	T	C	T	A
22	G	A	G	G	T	G	T	C	T	A
23	A	G	G	G	T	G	T	C	C	A
24	A	A	G	G	T	G	C	C	C	A
25	G	G	G	A	T	G	C	A	C	C
26	G	G	G	A	T	G	C	A	C	A
27	A	A	G	G	T	G	T	C	T	C

Allele/	*Lamin*										
/Site	30	53	63	77	114	136	139	165	167	176	179
1	C	C	C	C	G	C	T	G	C	C	G
2	C	G	C	C	A	C	C	G	T	T	G
3	C	G	C	C	G	A	C	G	C	C	G
4	T	G	C	C	G	A	C	G	C	C	G
5	C	G	C	C	G	C	C	A	C	C	G
6	C	G	C	C	G	C	C	A	C	C	C
7	C	G	C	C	G	C	C	A	C	C	A
8	C	G	C	C	G	C	C	A	T	T	C
9	C	G	C	C	G	C	C	A	T	T	G
10	C	G	C	C	G	C	C	C	C	C	G
11	C	G	C	C	G	C	C	G	C	C	A
12	C	G	C	C	G	C	C	G	C	C	C
13	C	G	C	C	G	C	C	G	C	C	G
14	C	G	C	C	G	C	C	G	C	T	G
15	C	G	C	C	G	C	C	G	T	T	G
16	C	G	C	C	G	C	C	G	T	C	G
17	C	G	C	C	G	C	T	C	C	C	G
18	C	G	C	C	G	C	T	G	C	C	A
19	C	G	C	C	G	C	T	G	C	C	G
20	C	G	C	C	G	C	T	G	C	T	G
21	C	G	C	C	G	C	T	G	T	C	G
22	C	G	C	C	G	C	T	G	T	T	C
23	C	G	C	C	G	C	T	G	T	T	G
24	C	G	G	C	G	C	C	G	C	C	C
25	C	G	C	T	G	C	C	G	T	T	G

Allele/	*Aldolase*	
/Site	77	178
1	T	C
2	C	C
3	T	T
4	C	T

Fig. 16. Base changes (and positions) for the three intron loci sequenced from wedge-tailed shearwaters. Positions are numbered relative to those in Congdon et al. (2000).

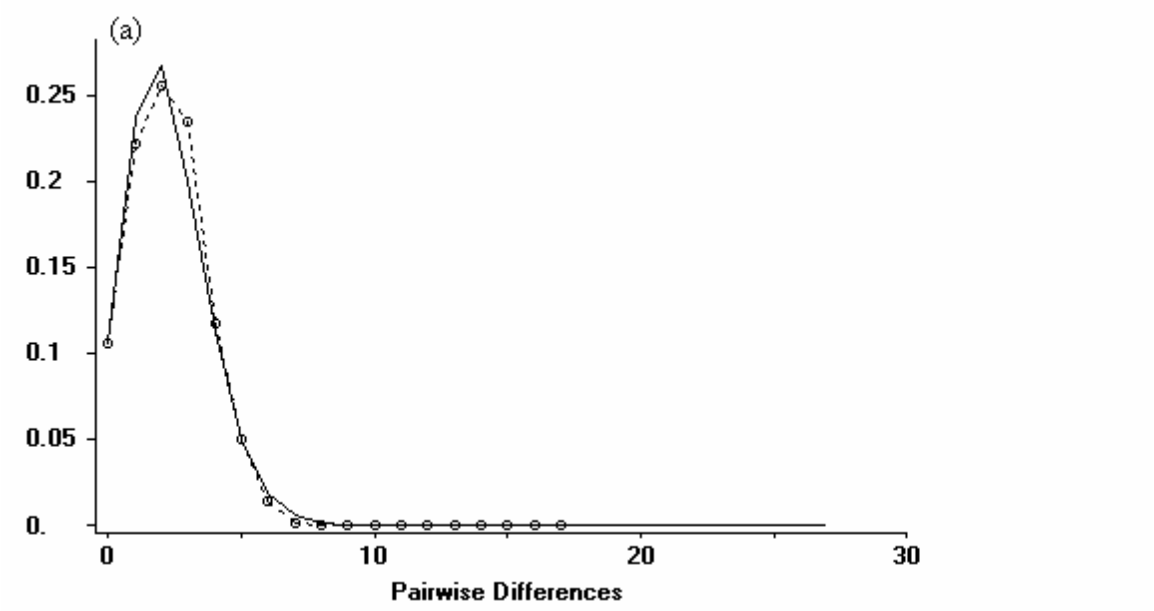
6.2.2 Tests of Assumptions

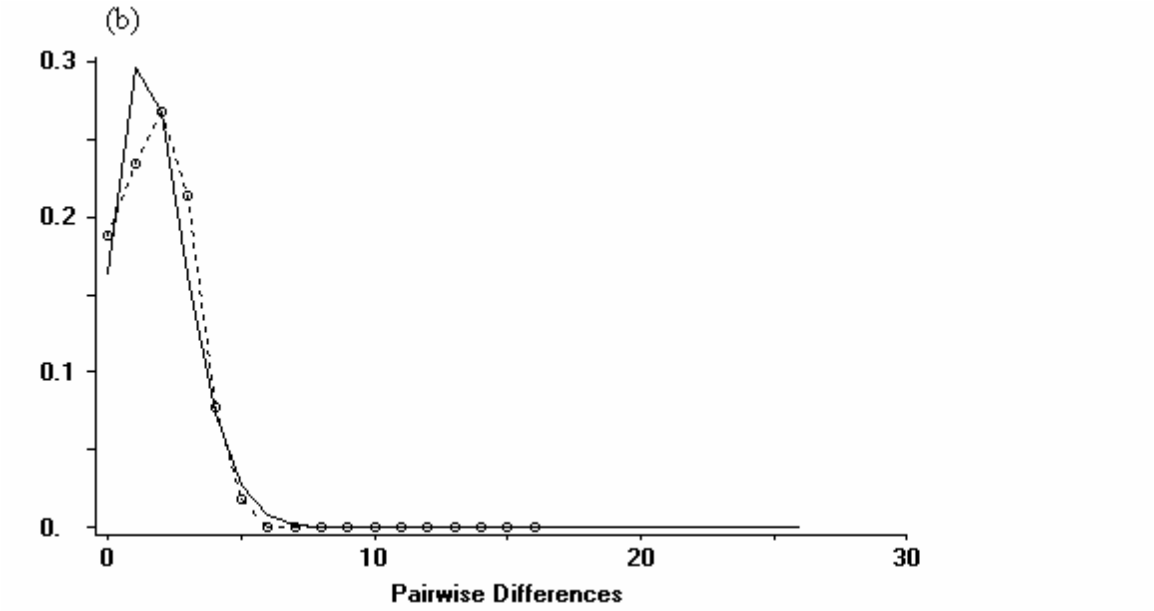
No significant cases of linkage disequilibrium were found within sampling sites ($P > 0.05$ after Bonferroni corrections) and recombination rates were an order of magnitude lower than mutation rates for all loci (range = 0.008 – 0.010 μ). Genotype frequencies did not differ from Hardy-Weinberg expectations at any of the intron loci ($P > 0.05$).

Tajima's *D* for the whole sample was negative but not significant. Estimates of Tajima's *D* for individual intron loci are listed in Table 12. Mismatch distributions produced a unimodal distribution in all three introns (Fig. 17a-c). The shape of the distributions contrasted with that predicted for populations at mutation-drift equilibrium (Fig. 18a-c)(Slatkin and Hudson 1991; Aris-Brosou and Excoffier 1996), and suggests that the samples may have undergone a recent bottleneck and subsequent expansion (Rogers and Harpending 1992). An alternative explanation is mutation rate heterogeneity among the variable sites (Slatkin and Hudson 1991, Rogers and Harpending 1992). The latter seems unlikely given that Tajima's D is negative for all population level calculations, and that previous studies have shown no evidence of mutation rate heterogeneity for these introns (Congdon et al. 2000). Fu's F_S statistic was significantly negative for both Gapd and Lamin (Table 12). No other measures of neutrality were significant at the $P = 0.05$ level (Table 12).

Table 12. Neutrality and diversity indices for the three Introns used in this study. Significant values (P < 0.05) are in bold.

				Expectation under	
	Gpd	*Lamin*	*Aldolase*	Selection	Range expansion
Nucleotide diversity (%)	0.91	0.92	0.30	Low	Low
Expansion coefficient (*S/d*)	4.44	6.67	2.74	Low	High
Tajima's D	0.19	-.90	0.96	Significant	Significant
Fu & Li's (1993) F*	1.17	-.61	0.97	Significant	Not Significant
Fu & Li's (1993) D*	1.41	-.29	0.79	Significant	Not Significant
Fu's (1997) F_S	**-18.56**	**-20.79**	-.024	Not Significant	Significant


(a)
0.25
0.2
0.15
0.1
0.05
0.
0
10
20
30
Pairwise Differences


(b)
0.3
0.2
0.1
0.
0
10
20
30
Pairwise Differences

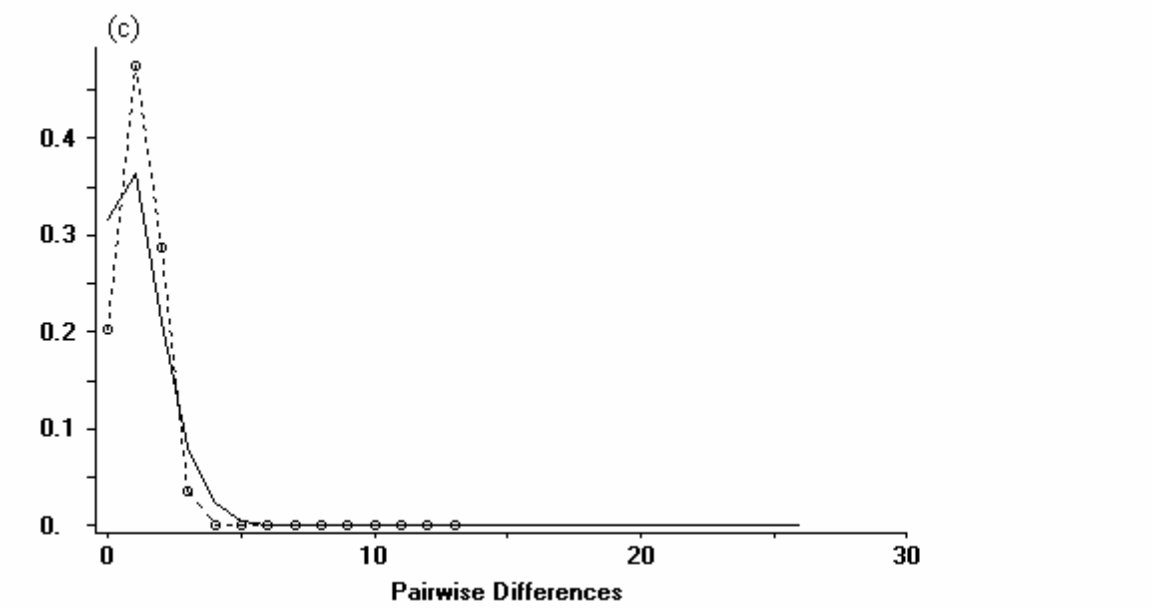
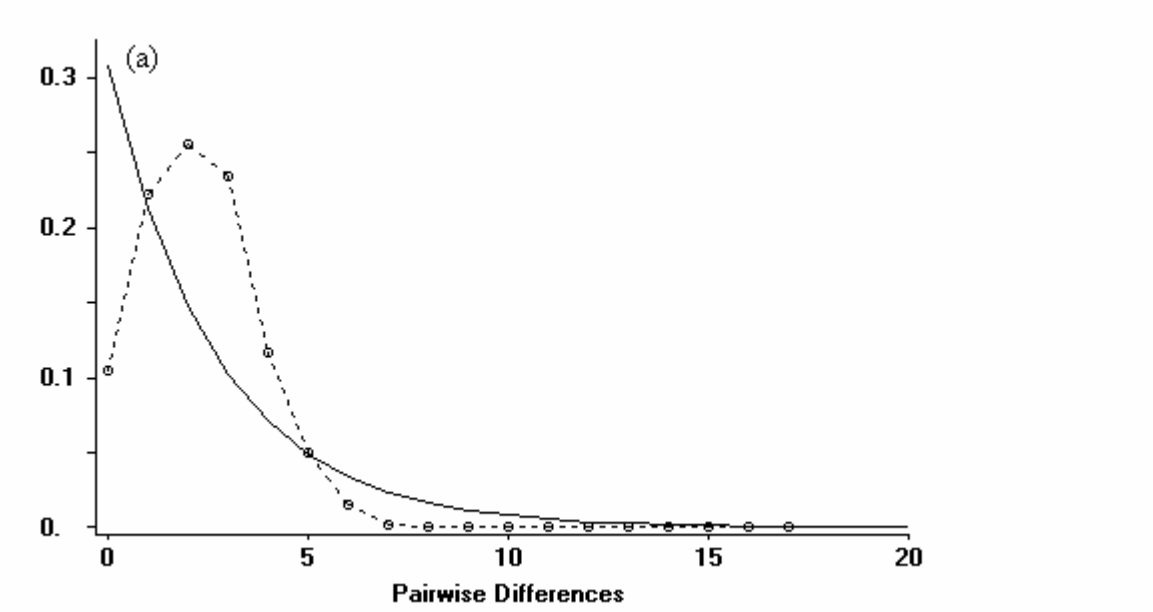


Fig. 17. Pairwise mutation differences in wedge-tailed shearwaters (dashed line) and expectation (solid line) under a population expansion in three introns; Gapd (a), Lamin (b) and Aldolase (c).



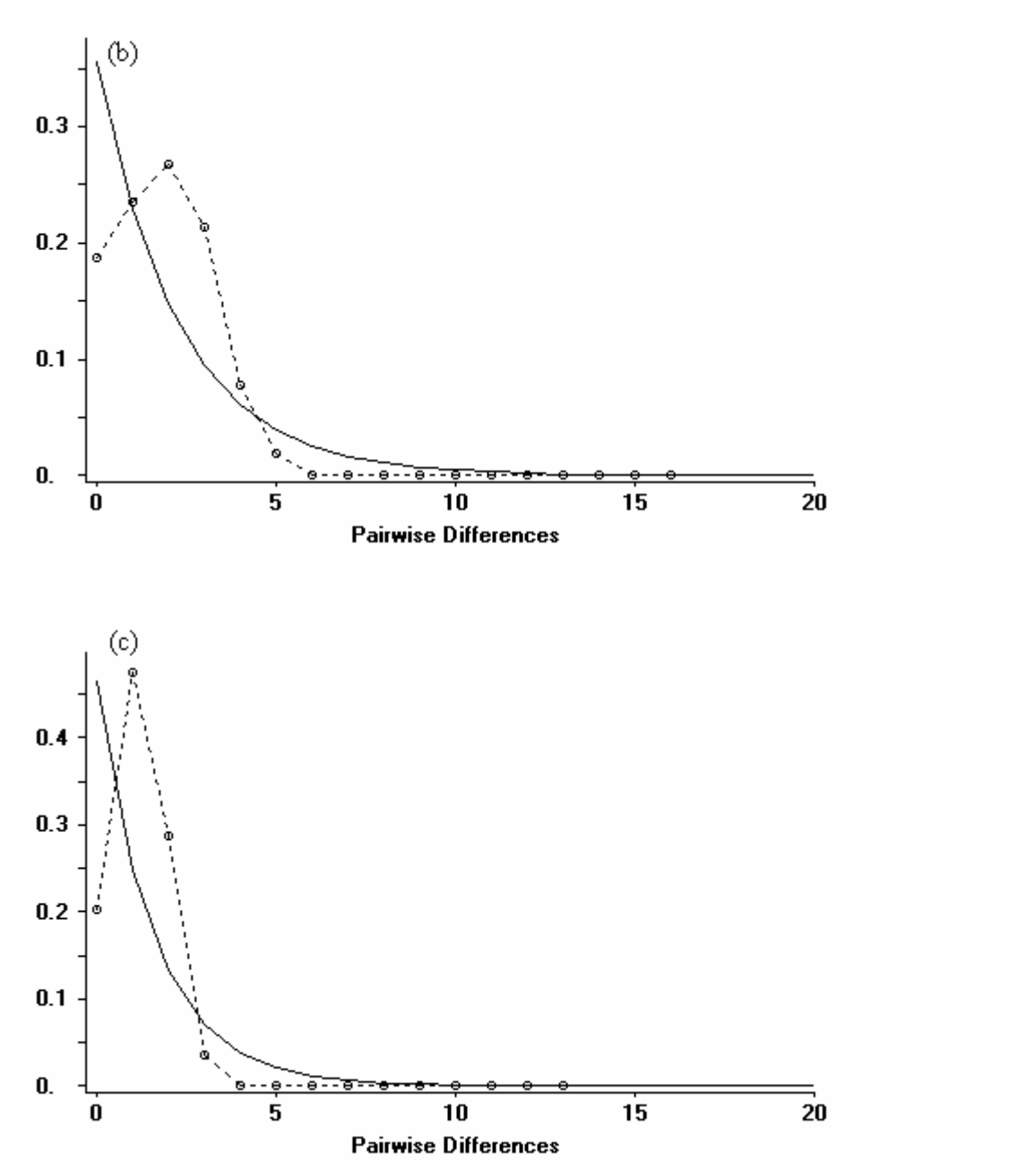


Fig.18. Pairwise mutation differences in wedge-tailed shearwaters (dashed line) and expectation (solid line) for a stable population (equilibrium) in three introns; Gapd (a), Lamin (b) and Aldolase (c).

6.2.3 Population Genetic Structure

6.2.3.1 Phylogenetic analyses

Preliminary analysis of the relationships between populations of intron alleles indicated that there was fairly strong support for divergence between all populations except between Heron Island and Raine Island (Fig. 19). Lord Howe Island was substantially differentiated from all other Australian populations, as was Hawaii from the Australian colonies (Fig. 19).

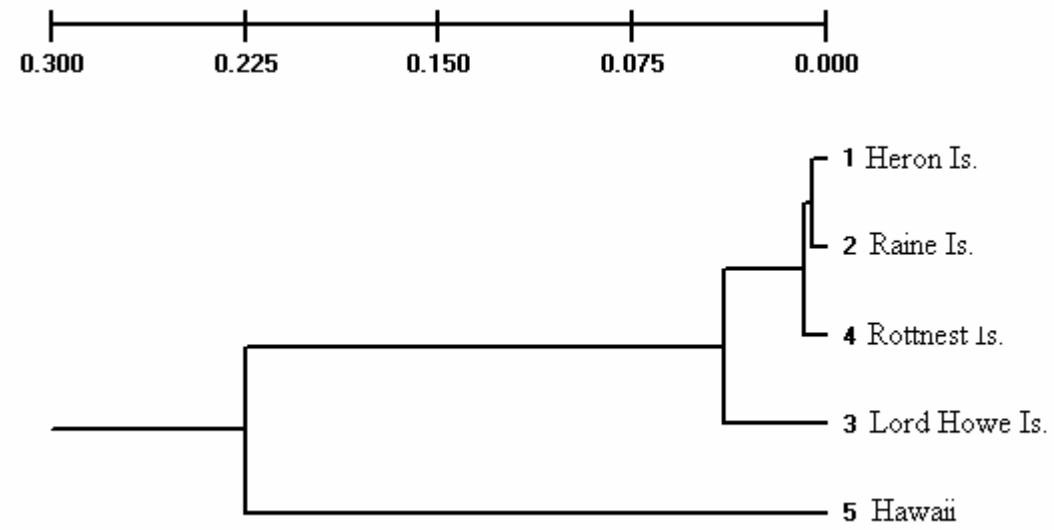


Fig. 19. Distance tree (UPGMA) based on pairwise modified coancestry coefficients (Reynolds et al. 1983) among wedge-tailed shearwater populations showing relative genetic distances. All bootstrap values were >80% except for the Raine Is. Vs. Heron Is. node.

6.2.3.2 Analysis of molecular variance

As described in the methods section, sequencing intron loci results in two overlapping sequences; a function of being nuclear genetic markers. As a result, in some instances, sequenced individuals showed base position changes at more than one base position (> 1 bp heterozygotes), thus making allele assignment difficult. This occurred in only a small proportion of the sequenced individuals as follows; Gapd = 18%, Lamin = 8.7%, Aldolase = 12.5%. The unknown heterozygotes were evenly spread across the sampled populations. In these cases, the program PHASE was used to assign unknown heterozygotes using a Bayesian approach (Stephens et al. 2001, 2003). An AMOVA was conducted on a dataset excluding and including the assigned individuals. The outcome ($F_{ST,}$ pairwise F_{ST}, and significance levels) did not differ between the two analyses. The results presented below are from the dataset including assigned individuals.

AMOVA on intron variation with all sampling sites treated as one group indicated significant differentiation among populations (Table 13). Φ_{ST} estimates for all pairwise comparisons with Lord Howe Island indicated that this population was significantly divergent from all others (Table 16). No other pairwise comparisons were significant (Table 14). However, the small sample size from the Hawaii population means that results for this population need to be interpreted with caution.

Table 13. AMOVA results for three intron loci across five wedge-tailed shearwater populations. *P*- value is based on 5000 permutations.

Source of variation	d.f.	Sum of squares	Percentage of variation	*F*- Statistics	*P*
Among populations	4	9.51	2.95	$F_{ST} = 0.029$	0.02
Within populations	91	136.82	97.05		

Table 14. Matrix of pairwise comparisons of Φ_{ST} (upper figure) and p values (lower figure)(Wright 1978) for five wedge-tailed shearwater populations. Asterisks indicate significant comparisons at p = < 0.05.

	Population			
	Heron Is.	Raine Is.	Lord Howe Is.	Rottnest Is.
Raine Is.	0.000			
	0.889			
Lord Howe Is.	0.068	0.108		
	0.013*	0.003*		
Rottnest Is.	0.021	0.015	0.133	
	0.156	0.250	<0.001*	
Hawaii	0.000	0.000	0.097	0.000
	0.998	0.995	0.030*	0.985

6.2.4 Patterns of variation: Microsatellites

The three microsatellite loci used were variable, with the number of alleles per locus ranging from 4 to 6 (Table 15). The three loci had an overall mean heterozygosity across all samples and loci of 0.68. The average number of private alleles across all loci was highest (1) at Lord Howe Is. followed by Heron Is. (0.68) (Table 18). Private alleles were not observed at the other locations (Table 15).

Table 15. Estimates of the number of alleles per locus (*Na*), private alleles (*PA*) and the expected (H_E) and observed (H_O) heterozygosities for three microsatellite loci from wedge-tailed shearwaters.

Population	Paequ3	Paequ8	Paequ13	Average
Heron Is. (n = 21)				
Na	6	4	5	5.00
H_E	0.72	0.71	0.77	0.73
H_O	0.62	0.62	0.46	0.56
PA				0.67
Raine Is. (n = 22)				
Na	5	3	5	4.33
H_E	0.69	0.66	0.76	0.70
H_O	0.75	0.25	0.50	0.50
PA				0.00
Lord Howe Is. (n = 18)				
Na	5	6	6	5.67
H_E	0.65	0.68	0.74	0.69
H_O	0.45	0.73	0.27	0.48
PA				1.00
Rottnest Is. (n = 20)				
Na	4	3	4	3.67
H_E	0.51	0.61	0.69	0.61
H_O	0.33	0.83	0.33	0.50
PA				0.00

Analysis of Hardy-Weinberg equilibrium indicated that all populations were in equilibrium ($p>0.05$), and all tests for linkage disequilibrium were not significant ($p>0.05$). AMOVA indicated that all of the observed variation was attributable to that calculated as within populations. As a result, significant population structuring was not observed (Table 16).

Table 16. AMOVA results from three microsatellite loci across four wedge-tailed shearwater populations. p- value is based on 5000 permutations.

Source of variation	d.f.	Sum of squares	Percentage of variation	*F*- Statistics	*P*
Among populations	3	0.50	-1.91	F_{ST} = -0.02	0.98
Within populations	158	109.47	101.91		

The first two axes of the PCA analysis accounted for 100% of the total inertia (83.38% for PC1) (Fig. 20). The PCA indicates that Rottnest Island is distinguished from all other populations along the first axis, with Lord Howe Island being distinguished along the second (Fig. 20).

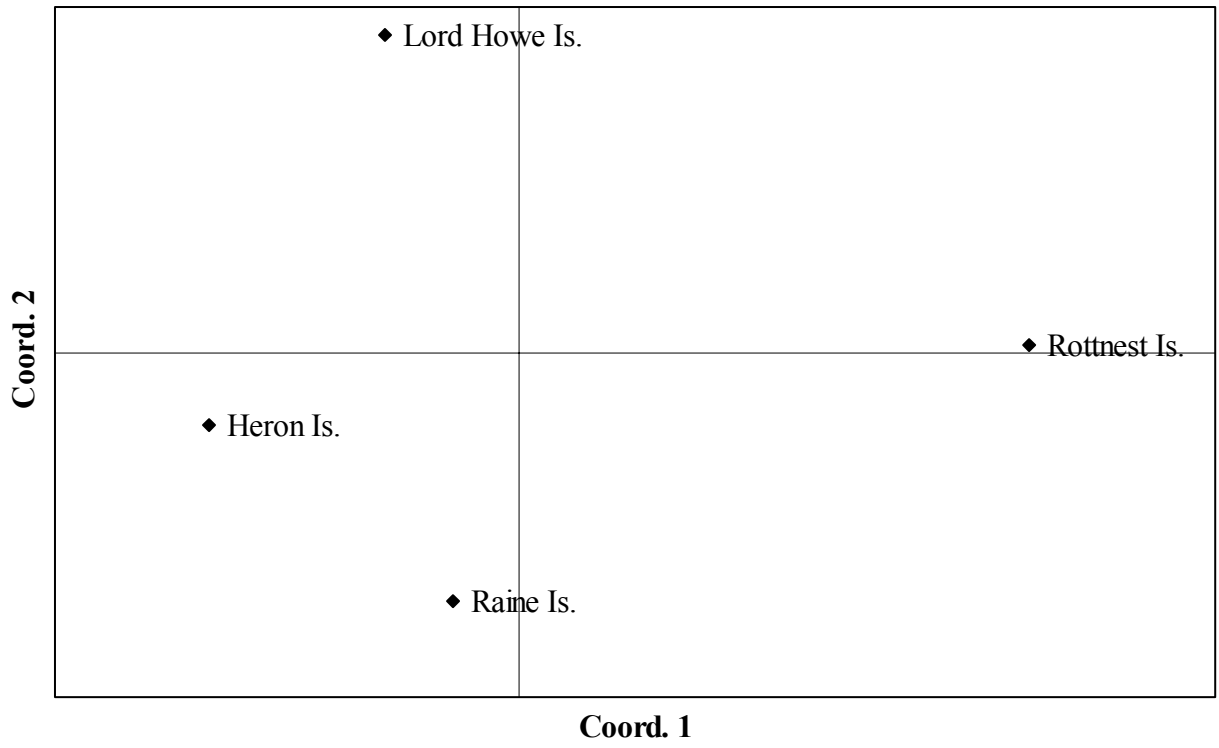


Fig. 20. Principal coordinate analysis of three microsatellite loci from wedge-tailed shearwaters. Coordinate 1 accounted for 83.38% of the total inertia.

6.2.5 Combined Analyses: Introns + Microsatellites

An AMOVA combining data from both molecular marker types showed no evidence of significant population structuring (Table 17), suggesting the sampled population are panmictic.

Table 17. AMOVA results from all six loci across four wedge-tailed shearwater populations. p- value is based on 5000 permutations.

Source of variation	d.f.	Sum of squares	Percentage of variation	*F*- Statistics	*P*
Among populations	3	2.52	0.90	$F_{ST} = 0.009$	0.23
Within populations	158	97.15	99.10		

However, gene flow estimates calculated with MIGRATE differed according to the genetic marker used. In accordance with the AMOVA, intron results suggest that gene flow between all population pairs is generally high (range 0 – 81 individuals/generation) (Table 18). However, results from microsatellite loci indicate that gene flow between population pairs is less than 1 individual per generation in most cases (Table 18). Despite the difference in the scale of migration estimates between the two molecular markers, patterns of gene flow were congruent ($r^2 = 0.73$, $p = 0.01$).

Table 18. Maximum likelihood estimates and approximate 95% confidence intervals (parentheses) for migration rates calculated with MIGRATE from introns (normal font) and microsatellites (bold font) across four populations of wedge-tailed shearwater (Hawaiian samples omitted because microsatellite data was unavailable.

		Source Populations			
Recieving		Heron	Lord Howe	Raine	Rottnest
Populations	θ ($4N_e\mu$)				
Heron	0.81 (0.44-1.77)		17.13 (9.27-668.70)	81.04 (62.15-872.06)	1.92 (1.68-575.99)
	0.26 (0.22-0.31)		**0.35 (0.20-0.57)**	**6.27 (5.56-7.05)**	**0.29 (0.16-0.48)**
Lord Howe	0.09 (0.05-0.15)	0 (0-7.33)		81.44 (51.55-121.11)	53.6 (29.75-87.10)
	1.29 (1.10-1.52)	**0.12 (0.06-0.23)**		**0.67 (0.49-0.89)**	**1.51 (1.24-1.83)**
Raine	3.46 (0.73-14.58)	66.45 (14.36-687.23)	2.66 (2.32-470.34)		37.19 (26.66-546.04)
	0.6 (0.55-0.74)	**3.96 (3.50-4.47)**	**0.55 (0.38-0.75)**		**0.42 (0.28-0.60)**
Rottnest	0.06 (0.04-0.07)	2.85 (2.50-715.77)	2.83 (2.48-715.77)	0 (0-761.54)	
	1.21 (1.00-1.47)	**0.19 (0.10-0.30)**	**1.56 (1.29-1.86)**	**0.22 (0.13-0.35)**	

6.2.6 Relationships between morphology, environment, genetics and distance

Isolation by distance analyses clarified the pattern, indicating that a clear outlier (Heron vs. Lord Howe) from the null model (decreasing gene flow with distance) exists (Fig. 21). When this outlier is removed, mantel tests support the isolation by distance model in both markers (Introns; r^2 = -0.92, p = 0.0001, Microsatellites; r^2 = -0.99, p = 0.0001). Migration rates among populations were not significantly unidirectional (as indicated by 95% confidence limit overlap), except between Lord Howe Island and Heron Island (Table 18). In this instance, migration appears to be into Heron Island from Lord Howe, with introns showing no overlap in 95 % confidence intervals and microsatellites indicating borderline significance (Table 18).

There was no significant isolation-by-distance effect on morphological divergence between populations as measured by Mahalanobis D2 (p = > 0.05). There was also no relationship between pair-wise estimates of gene flow (M) calculated using MIGRATE and morphological (Mahalanobis D2) distance among populations (Fig. 22, p = >0.05).

Although there was substantial variation in pair-wise SST differences among the four populations (range 1oC – 4.5 co), no relationship between SST differences and M was found using either marker (Fig. 23a, b)(p = >0.05 in both cases). Chlorophyll a concentrations were also not significantly correlated with levels of gene flow between/among colonies (p = >0.05 for both markers).

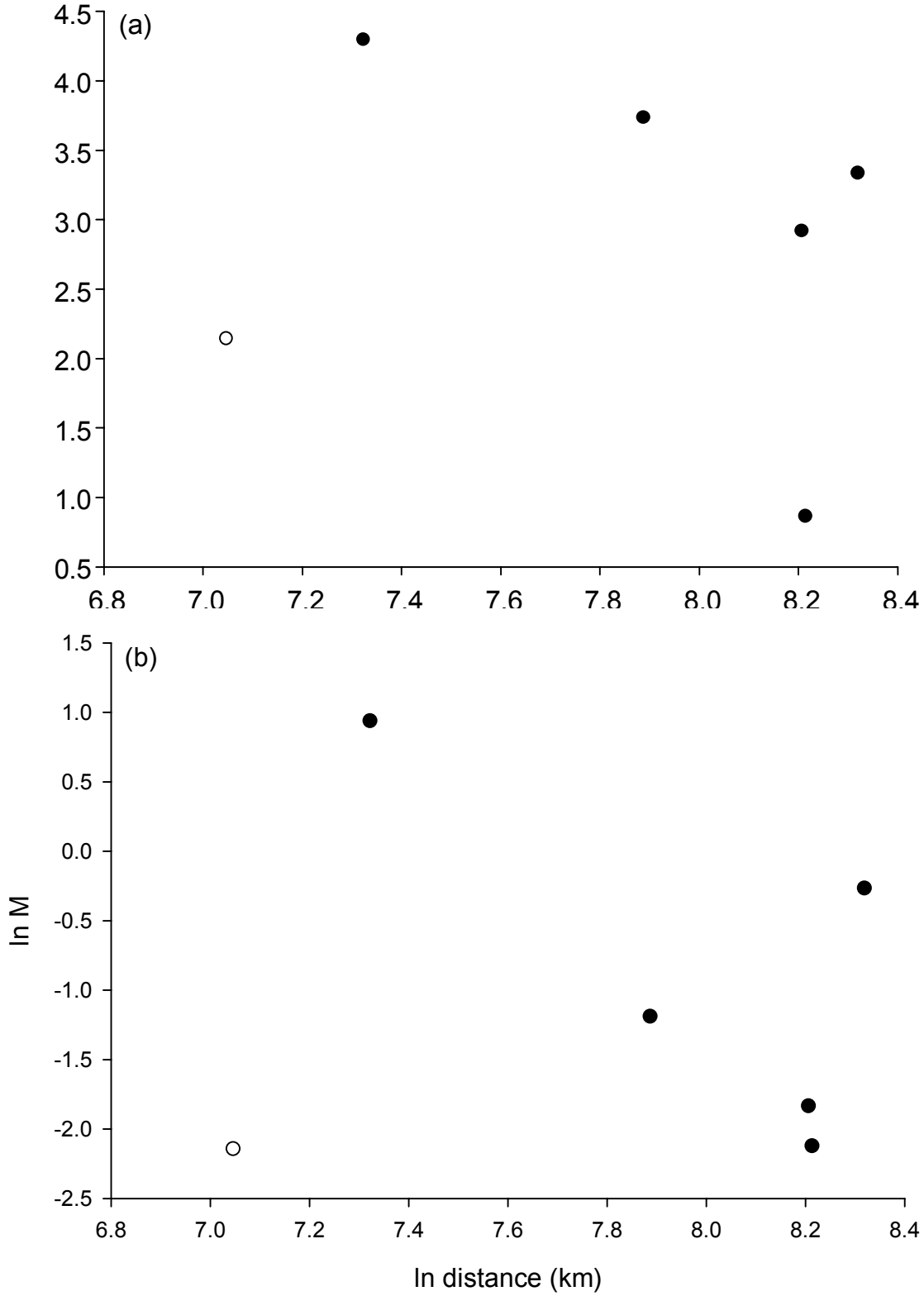


Fig. 21. Pairwise comparisons of logarithm gene flow (M) versus logarithm geographic distance between pairs of wedge-tailed shearwater colonies using (a) introns and (b) microsatellites. The outlier (open circle) to the null model is the Lord Howe Island vs. Heron Island comparison. When this outlier is removed, the isolation by distance analysis is significant in both cases.

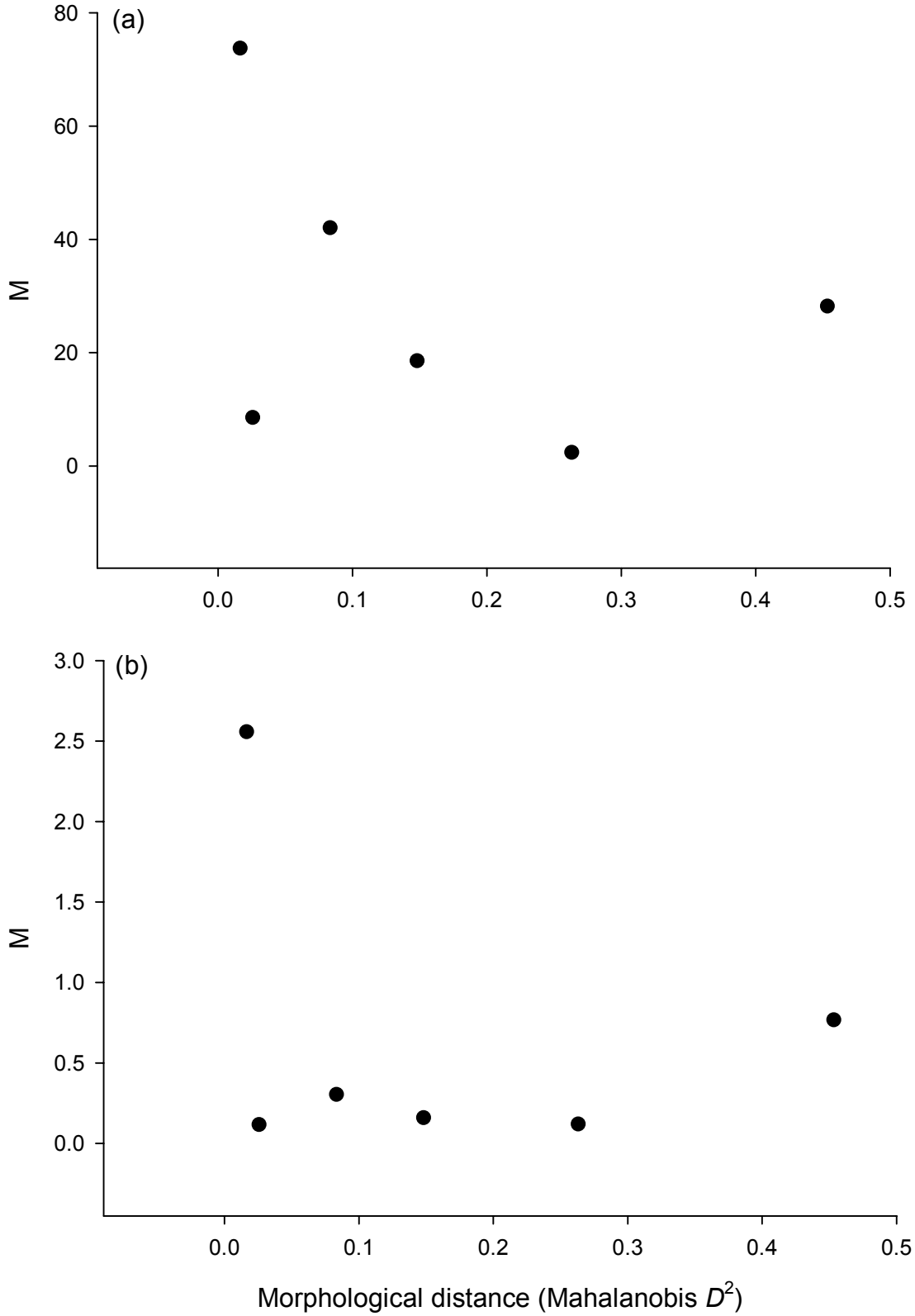


Fig. 22. Pairwise gene flow (M) estimates versus morphological distance (Mahalanobis D2) using (a) introns and (b) microsatellites among four wedge-tailed shearwater populations.

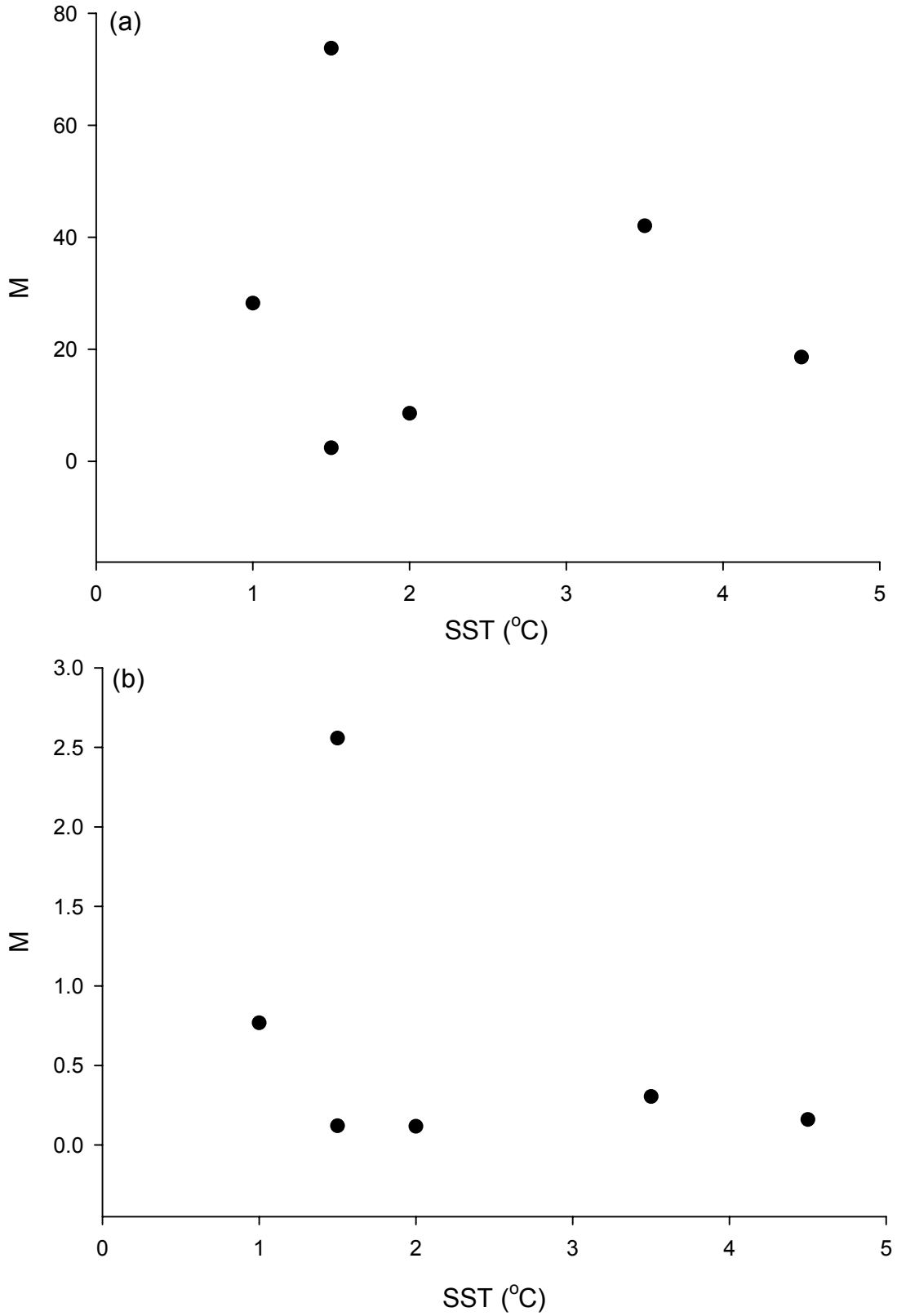


Fig. 23. Pairwise gene flow (M) estimates versus pairwise differences in SST (o C) among wedge-tailed shearwater populations. SST relationships are presented for both (a) intron and (b) microsatellite estimates of M.

6.3 DISCUSSION

The F_{ST} based AMOVA analysis of all six molecular markers combined implies that significant gene flow occurs among the wedge-tailed shearwater colonies sampled. However, F_{ST} based analyses are not appropriate for this system because the assumption that population sizes have remained stable over long periods of time has been violated. Three lines of evidence support this. Firstly, mismatch analysis produced a unimodal distribution, with the shape of the distribution contrasting with that predicted for populations at mutation-drift equilibrium (Fig. 17, 18)(Slatkin and Hudson 1991, Aris-Brosou and Excoffier 1996). Secondly, the signature of a recent expansion event can be seen in the star-shaped haplotype networks that consist primarily of 1 base pair changes among haplotypes (Fig. 15a-c). Finally, patterns of significance (or lack thereof) in neutrality indices all point to a relatively recent population expansion (Table 15). Given these findings, a coalescent-based approach should provide more accurate estimates of gene flow among shearwater populations. Therefore, the following discussion will focus on gene flow estimates obtained using MIGRATE rather than the traditional F_{ST} based analysis.

MLE estimates of gene flow from MIGRATE suggest that populations of wedge-tailed shearwater exchange migrants. However, the estimated number of migrants depends on the marker used. Intron results imply panmixis whereas microsatellites suggest that populations generally exchange less then one individual per generation (Table 18). Most likely these differences reflect differences in the mutation rate of the markers used, and as such, provide information on the relationships between colonies at different temporal scales (Bosch et al 1999, Banke and McDondald 2005). As microsatellites generally evolve quicker than intron sequences (Palumbi and Barker 1994; Bittner and King 2003), they will reveal more contemporary patterns of association, whereas the slower evolving introns will reflect residual historical connections.

If so, then the pattern of variation revealed by the introns suggests an expansion of wedge-tailed shearwaters across the Indo-Pacific; possibly during the last glacial maxima (~18000-20000 ybp) when new breeding habitat became available after sea levels rose (Peck and Congdon 2004). The neutrality indices and haplotype networks

support this interpretation (refer above), as do studies on tropical seabirds with similar contemporary distributions (Peck and Congdon 2004, Steeves et al. 2005).

Conversely, the microsatellite MIGRATE analysis suggests that more recently, gene flow between shearwater colonies has been restricted and that most populations may now be evolving independently. This analysis also implies that non-physical barriers to gene flow are important in isolating wedge-tailed shearwater populations. Two lines of evidence support this.

Firstly, isolation by distance effects are clearly evident among shearwater colonies despite a relatively small intercolony distances and a lack of physical barriers between most population pairs (Fig 21). Secondly, a clear outlier in the isolation by distance analysis (Fig. 21) suggests that gene flow is restricted between the two closest (Lord Howe vs. Heron Island) populations significantly more than expected based on the observed isolation by distance relationship. Combined these results imply that large-scale physical barriers and/or distance alone cannot explain the observed patterns of reduced gene flow among colonies.

Consequently, the results add to a growing body of seabird research that suggests a role for non-physical barriers in the creation and maintenance of population structuring (Congdon et al. 2000, Dearborn et al. 2003, Steeves et al. 2005). The micro-evolutionary processes responsible for such divergence have so far remained unclear (Congdon et al. 1998, Steeves et al. 2005). However, relationships between genetic, morphological and environmental variation can be used to examine the potential contribution of different micro-evolutionary processes further.

6.3.1 Reconciling morphological divergence and patterns of gene flow

As outlined in Chapter 5, morphological divergence among shearwater populations may be associated with philopatric isolation and genetic drift, selective divergence or developmental plasticity.

Firstly, morphological divergence may be the result of genetic drift mediated by either physical or non-physical (e.g. philopatry) barriers to gene flow. There is some (though limited) evidence that drift is involved in causing the observed patterns in this study.

Isolation-by-distance exists if the outlier from the null model is removed (Fig. 21). This means that geographic distance between colonies could be lowering dispersal/gene flow, thereby allowing morphological variation to drift randomly towards different 'peaks' in different populations. However discordance between gene flow and morphological divergence (Fig. 22) undermines a drift explanation because congruence is expected if drift were involved (Wright 1931, Mayr 1942, 1963, Barrowclough 1983, Clegg et al. 2002).

Alternatively, morphological variation may simply be a 'plastic' response to background environmental conditions. However, based on the arguments presented in Chapter 5, plasticity alone is not sufficient in explaining all of the morphological variation observed. The final possibility is that selection on morphological variation is reducing gene flow among populations via. reinforcement. Few studies on vertebrates have been able to illustrate that selection alone can reduce gene flow among populations (but see Smith et al. 1997), and data to directly test this hypothesis is required. Nonetheless, based on the arguments presented above and in Chapter 5, it is difficult to invoke drift or plasticity alone as the only explanations for morphological variation in this species. I therefore propose that selection must also be operating either alone, or synergistically with drift. Direct measures of adult and chick fitness relative to background environmental conditions, and data from further colonies and genetic markers (to expand the isolation by distance analyses), will be required to test this possibility.

6.3.2 Conclusions and Implications

I aimed to evaluate the applicability of DWGF models of speciation to pelagic seabirds. The results of this work can be summarised as follows:

(1) In general, wedge-tailed shearwaters (adults and by extension chicks) appear to be sensitive to fluctuations in sea surface temperature via. impacts on prey distribution. This means that given their widespread breeding range, they are likely to evolve adaptive responses (e.g. foraging behaviours, chick developmental patterns) to the divergent oceanographic regimes they face. Furthermore, for a given increase in SST, chick growth decreases more at Lord Howe Island than at Heron Island. This could potentially enhance reinforcement between the two colonies.

(2) Wedge-tailed shearwaters at Heron Island and Lord Howe Island are constrained to breed under quantifiably divergent productivity/oceanographic regimes. This translates into different foraging strategies and chick developmental patterns at the two locations. The fact that specific foraging strategies have been recorded numerous times in at least one location (Heron Island) indicates that foraging behaviours may be an obligate rather than facultative response in this species at some locations. Chick developmental patterns are novel and have not been recorded previously in any other seabird species. They do not fit with predictions based on previous facultative responses and would therefore appear to be an obligate response.

(3) In general, congruent sex-specific foraging and provisioning patterns were observed in wedge-tailed shearwaters breeding at Heron and Lord Howe Islands, except that at Lord Howe Island, females made significantly longer foraging trips than males and dived to significantly shallower depths whereas no difference in these two parameters was observed between the sexes at Heron Island. The most likely explanation for sex specific foraging patterns at Lord Howe Island is that niche partitioning has evolved as a way of reducing competition between the sexes. The difference in trip length and dive depth between the sexes at Lord Howe but not at Heron Island suggests that competition may be stronger at the former location.

(4) Morphological divergence among wedge-tailed shearwater colonies is substantial and the pattern of variation does not appear to fit with what would be expected if traits were a plastic response to background environmental conditions.

(5) Male biased sexual-size dimorphism is also evident and patterns are consistent among colonies. However, only bill dimensions are significantly different once overall body size is accounted for. Pair-formation based on bill-width exists and is also consistently observed. These patterns suggest that selection for pair-formation that minimises niche overlap maintains the bill size difference. The extent of sexual dimorphism among colonies means that morphological differences observed at the colony level are not driven by differences in sexual dimorphism.

(6) There are low levels of gene flow among all colonies of wedge-tailed shearwater sampled in this study however, gene flow between the two closest colonies is the lowest. This fact, combined with a lack of morphological and genetic congruence among colonies, strongly suggests a role for selection rather than drift in causing some of the observed morphological variation.

Considered as a whole, results from the work presented in this thesis would suggest that DWGF models of speciation might indeed be applicable to seabirds. Divergent patterns of chick development relative to background environmental conditions (1,2) and subtle differences in sex-specific foraging patterns (3) suggest that reinforcement between colonies is likely. Moreover, morphological variation fits with predicted selective scenarios (rather than phenotypic plasticity) and cannot be reconciled with patterns of gene flow in a way expected of a drift-mediated response (5,6). These findings are novel and provide substantial evidence that selection may play an important role during the evolution of seabird diversity. This study provides an important step towards a full understanding of the role of ecology in population divergence and speciation in seabirds.

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