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Phylogeny and Phylogeography of four Southern Ocean Petrels

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Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
In the Department of Molecular and Cell Biology
UNIVERSITY OF CAPE TOWN
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DECLARATION

This thesis reports the results of the original research I conducted under the auspices of the Molecular and Cell Biology Department, University of Cape Town, between 2002 and 2006. All the assistance that I received has been acknowledged. This work has not been submitted for a degree at any other university.

Signed by candidate

NMS Mareile Techow

ABSTRACT

This thesis investigates the phylogeography of four southern ocean petrel species in an attempt to resolve taxonomic uncertainties and phylogeography in these species. A large proportion of petrel and albatross species are listed as threatened under Red List criteria, in many cases as a result of threats at sea. Most albatrosses and petrels breed in discrete island colonies and exhibit strong natal philopatry. They may thus be expected to show population divergence, but published studies show that this is not always the case. Most studies to date have concentrated on northern hemisphere species, with mostly albatrosses studied within the southern oceans. White-chinned (*Procellaria aequinoctialis*), Spectacled (*P. conspicillata*) and giant petrels (*Macronectes giganteus* and *M. halli*) are southern ocean species of Procellariiformes. All four species are threatened by accidental mortality in longline and other fisheries, as well as by introduced predators at their breeding colonies. In order to adequately conserve these species, species limits need to be resolved. Taxonomic uncertainties are an important issue in conservation because often only recognised species receive protection. In addition, islands of origin for birds killed at sea need to be identified.

This thesis examines the species status of the Spectacled Petrel (*Procellaria conspicillata*), which has been separated from the White-chinned Petrel (*P. aequinoctialis*) based on morphology and vocalisations, as well as examining the taxonomic status of the two forms of giant petrel, and their phylogeography.

Cytochrome b was used to confirm the species rank of the Spectacled Petrel. The decision to support separate species status was based on the lack of shared haplotypes, six fixed mutational differences between the closest haplotypes of the White-chinned and Spectacled Petrel and a sequence divergence of 1.74%. Within *Procellaria*, White-chinned and Spectacled Petrels are sister species, closely related to the wide-ranging Grey Petrel. Within the White-chinned Petrel, two regional populations were found corresponding to colonies in the New Zealand region and the Indian/Atlantic Ocean. Evidence of population expansions were detected in both species and both regional populations of the White-chinned Petrel. Between these two regional populations, the greatest genetic diversity was within the New Zealand regional population. This result is consistent with the White-chinned Petrel originating in the New Zealand area.

A microsatellite DNA library was constructed to allow a more detailed investigation of White-chinned Petrel population structure. The two regional populations were confirmed and further population division was found within the Atlantic/Indian ocean regional population based on differences in allele frequencies as measured by F_{ST}. Bycatch was analysed and assigned to putative natal colonies. The success rate varied, but analysis suggested that long-distance dispersal is rare and, because no shared haplotypes were found between regional populations within cytochrome b, male biased movement is suggested. Foraging distributions seemed to overlap slightly as some Atlantic Ocean birds were caught in the New Zealand region, but most birds killed off southern Africa apparently derived from adjacent colonies in the Indian Ocean.

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Separate species status for the two giant petrels was confirmed based on analysis of both cytochrome b sequence and microsatellite DNA, although sequence divergence in cytochrome b between the two forms was low. The British Ornithologists' Union has provided guidelines to resolve species ranks. Accordingly, the decision to promote species rank for both giant petrel forms is based on morphological differences, one fixed mutational difference within cytochrome b between the Northern and Southern Giant Petrels as well as evidence of no gene flow with nuclear microsatellite DNA. Perhaps most convincing is the very limited gene flow between sympatric breeding populations at several islands.

Within the Southern Giant Petrel two lineages were found: one on Marion Island, Iles Crozet and Macquarie, and a second comprising the remaining colonies. However as Iles Crozet and Marion Island also contain haplotypes of this second lineage, secondary contact of lineages after separation is suggested. Preliminary microsatellite DNA analysis detected further genetic structuring between colonies. The Northern Giant Petrel colonies sampled form a monophyletic clade which could be divided into further populations with microsatellite DNA analysis. Clade relationships suggest that speciation occurred after fragmentation into three phylogroups perhaps the result of climatic changes during ice ages, with two lineages forming the Southern Giant Petrel. The lineage comprising the Northern Giant Petrel developed isolating mechanisms preventing gene flow after conditions, which had resulted in the initial fragmentation, changed.

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OVERVIEW

A large proportion of seabird species is threatened worldwide (BirdLife-International, 2006). Major threats contributing to population decreases at breeding sites include introduced predators such as cats and rats and human disturbance. The impacts of these threats are fairly easily identified and can be quantified at breeding sites. However, many species also face threats offshore, such as pollution and especially, accidental mortality in fisheries. In order to estimate the impacts of these threats, it is necessary to know the full extent of the problem as well as to identify foraging ranges at sea for each population. Phylogeography can provide insights into some of these issues. Long term tracking and marking studies are time consuming and difficult and most species breed in remote locations. In addition, because most species mature late, immature birds spend four to eight years at sea and movements are therefore hard to track. Genetic studies can be invaluable for inferring phylogeographic structure and dispersal rates of oceanic seabirds, as well as identifying source populations of birds killed during longline fishing. This research can then be used to complement banding, tracking and behavioural studies in order to establish conservation priorities and subsequently management strategies.

Seabirds, especially pelagic species, have traditionally been difficult to study with respect to phylogeography and taxonomy because of their philopatry to discrete oceanic colonies. Concepts such as the Biological Species Concept are thus hard to apply because of this allopatric breeding behaviour. In addition, phylogeographic studies have identified several cryptic species within seabird species. Within Procellariiformes, taxonomy is therefore under constant revision and uncertainties have become a conservation issue, because only recognised species are afforded protection in most countries.

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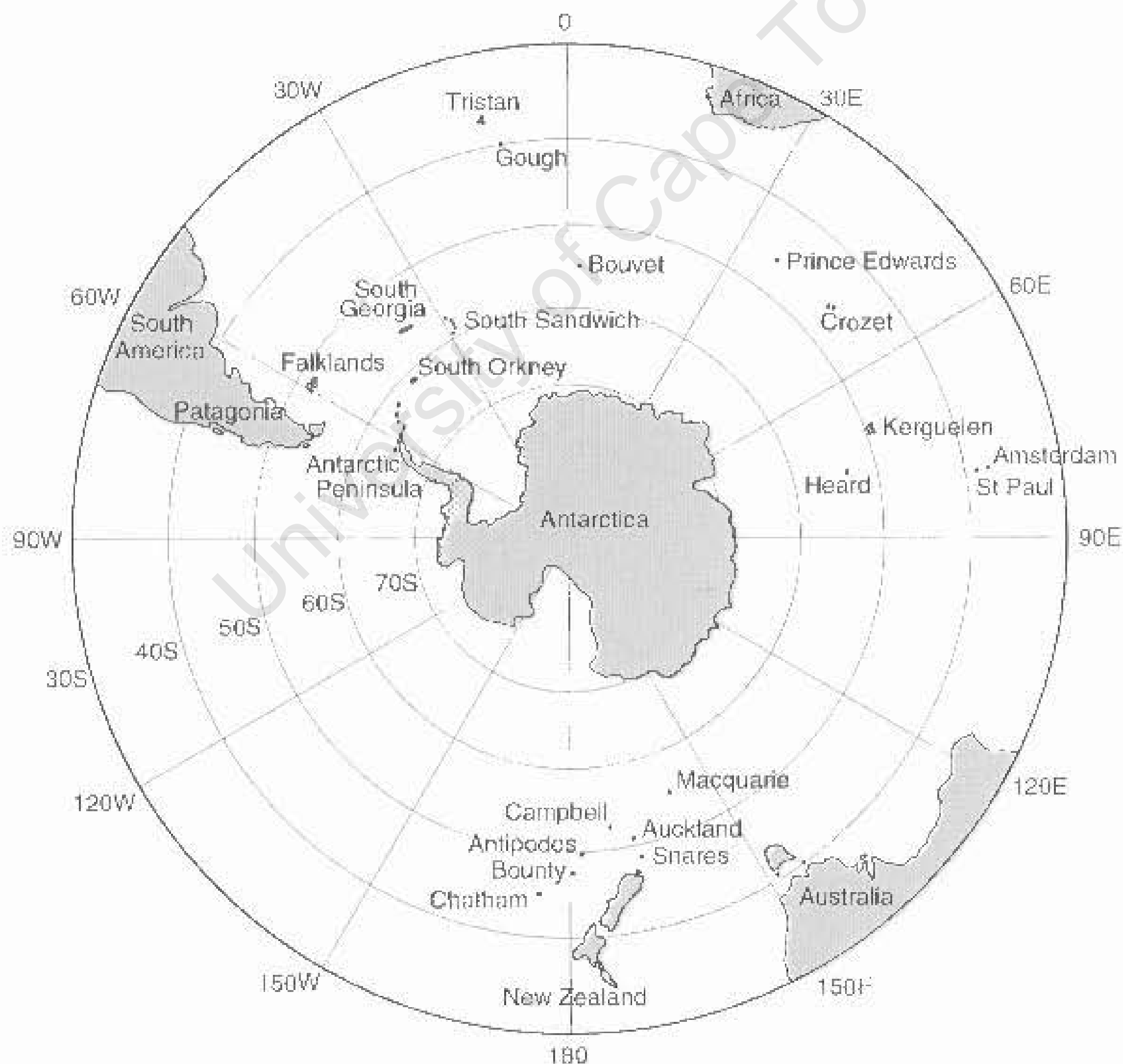
Physical barriers to gene flow such as continents and climatic changes during past ice ages in the Pliocene and Pleistocene, but also non-physical barriers such as behaviour, shape the evolutionary history of species. In the southern hemisphere oceans, physical barriers such as continents are of less importance than in northern hemisphere oceans. However, it has been found that in the absence of these physical barriers, behaviour such as natal philopatry is the cause of speciation. By investigating the forces that shape the evolutionary history of lineages and species, taxonomic uncertainties may be resolved.

Figure 1: Map showing the Southern Ocean



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Research aims

This thesis addresses the phylogeography of four species of southern hemisphere (Figure 1) petrels, the White-chinned Petrel (*Procellaria aequinoctialis*), the Spectacled Petrel (*P. conspicillata*), the Southern Giant Petrel (*Macronectes giganteus*) and the Northern Giant Petrel (*M. halli*), using molecular markers. Partial mitochondrial DNA (mtDNA) cytochrome b sequences were used to resolve taxonomic uncertainties and to establish maternal population structure. Biparentally inherited microsatellite markers are used to corroborate the mtDNA data and to achieve a higher resolution of spatial organization of genetic variation within each species. A practical application addresses the biggest threat to these species: accidental mortality in the longline fishing industry. Identification of the regions of origin of most casualties will facilitate identifying those populations most at risk and thus facilitate management plans.

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Organisation of this thesis

The thesis is divided into five Chapters. The first Chapter contains a more detailed introduction, providing details on the species studied and the threats facing them, and methodology common to both species complexes, explaining the choice and background of analysis. A third part of Chapter one describes the construction of a microsatellite DNA library in the White-chinned Petrel and cross-species amplification of designed primers in several other Procellariiformes.

Chapter 2 deals with the species status of the Spectacled Petrel in relation to the White-chinned Petrel and the other *Procellaria* species using cytochrome b DNA sequences. Analysis shows that the Spectacled Petrel is a distinct species with possible origins in the New Zealand region. The Chapter also addresses the phylogeography of the White-chinned Petrel. Two distinct regional populations are identified, one comprising colonies in New Zealand and the other comprising colonies in the Atlantic and Indian Oceans. No maternal gene flow is identified between these distinct regional populations.

Chapter 3 analyses the fine-scale structure of the White-chinned Petrel using microsatellites. Genetic structure between colonies is defined with the greatest differences between colonies in different oceans. Analysis also corroborates the cytochrome b hypothesis of a possible species origin in New Zealand.

Chapter 4 investigates the feasibility of using assignment methods to assign birds caught at sea as longline fishing mortalities to possible source populations. Two methods are compared using microsatellite DNA as well as using a single nucleotide polymorphism within cytochrome b as identified in Chapter 2. The success of the microsatellite based methods is comparable to other studies using this marker. Both microsatellite and cytochrome b analyses indicate that foraging ranges of different colonies only overlap marginally. In addition, the small number of immigrants identified confirms philopatry to natal colonies.

Chapter 5 discusses the phylogeography of the giant petrels, *Macronectes giganteus* and *M. halli*. Both cytochrome b DNA sequences and a preliminary microsatellites DNA analysis are used to describe present population structure, as well as species status and possible modes of speciation. Both DNA markers confirm separate species status for these two forms. Two lineages are identified in the Southern Giant Petrel and it is hypothesised that speciation occurred through fragmentation and isolation. Secondary contact of the two lineages within the Southern Giant Petrels is shown and gene flow between these is confirmed by microsatellite analysis. Further genetic structure is identified in preliminary microsatellite analysis.

The thesis is concluded with a synthesis highlighting the important findings of all Chapters. Each Chapter contains an appendix, including allele frequency data, sequence alignments and details on samples and locations relevant to this particular Chapter.

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CHAPTER 1

INTRODUCTION AND METHODOLOGY

SUMMARY

This Chapter has three parts. Part I provides a general introduction to the study organisms and the threats facing them. Part II describes the techniques used to describe the phylogeny and phylogeography of the *Procellaria* and *Macronectes* species investigated. Techniques that are specific to species or markers are outlined in the respective Chapters rather than at this point. Tissue, blood or feathers from sampled individuals were used to generate haplotypes for cytochrome b DNA and allelic profiles at microsatellite loci. Phylogenetic and phylogeographic analyses were applied to populations to investigate population structure and relationships between populations and species. This was achieved by constructing trees with genetic distance, cladistic and Bayesian methods using cytochrome b haplotypes. Microsatellite loci were analysed for linkage disequilibrium and deviation from Hardy-Weinberg equilibrium. Partitioning of variation was analysed through Principal Component Analysis. Part III describes the development of microsatellite primers and tests for cross-species amplification. Cross-species amplification of microsatellite loci isolated for *Procellaria aequinoctialis* was investigated for several other Procellariiformes.

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PART I: INTRODUCTION

John Avise, arguably the father of phylogeography, has defined phylogeography as "a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species" (Avise, 2000, p. 3). Thus phylogeography considers spatial distributions of many phenotypic traits such as morphology and behaviour, but in most cases the phylogenetic relationships of alleles or haplotypes are investigated (Avise, 2000). Mitochondrial DNA haplotypes are of special interest because their descent can be traced along generations, with no recombination because of its uniparental mode of inheritance. Several factors govern the evolution of closely related lineages such as mutation, genetic drift and gene flow. As the relationships of lineages within populations and closely related species can be investigated through phylogenetics, their spatial distributions can be explained by the processes that shaped them. The resolution of phylogeographic boundaries between populations is an integral part of establishing conservation priorities and thereafter management strategies. Biodiversity, defined as the variety and genetic diversity within species and populations (Frankham *et al.*, 2002), may be lost if distinct evolutionary populations are not recognised (Frankham *et al.*, 2002). Therefore, phylogeographic studies have been used to identify cryptic species, recent introductions and their origins, and range expansions (Avise, 2004).

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The Procellariiformes are an order of tube nosed pelagic seabirds with a worldwide distribution. Most species are capable of covering vast distances. Foraging is achieved through a combination of surface seizing and pursuit diving (Warham, 1990; Brooke, 2004). The group is generally divided into four families (Marchant and Higgins, 1990): (i) Diomedeidae, the albatrosses and mollymawks; (ii) Procellariidae, the petrels; (iii) Hydrobatidae, the storm petrels; and (iv) Pelecanoididae, the diving petrels, which have now been included in the

Procellariidae on genetic evidence (Nunn and Stanley, 1998). However, this division is being updated and revised constantly (e.g. Paterson *et al.*, 1995; Nunn and Stanley, 1998; Kennedy and Page, 2002; Penhallurick and Wink, 2004; Rheindt and Austin, 2005). The genera *Procellaria* and *Macronectes* belong to the family Procellariidae. The family has a worldwide distribution with 13 genera comprising approximately 76 species, mostly in the southern hemisphere (Brooke, 2004). Based largely on morphology, the family has been split into four main groups with *Procellaria* and *Bulweria* uncertain; genetic data has promoted five divisions: i) the fulmarine petrels (*Macronectes*, *Fulmarus*, *Thalassoica*, *Daption*, *Pagodroma*, *Lugensa*), ii) the gadfly petrels (*Pterodroma*), iii) prions (*Pachyptila*, *Halobaena*), iv) shearwaters (*Calonectris*, *Puffinus*), v) and *Bulweria* and *Procellaria* (Sibley and Ahlquist, 1990; Warham, 1990; Brooke, 2004). In an attempt to resolve their taxonomy, several phylogenetic studies have been conducted, which largely confirm these groupings, with some anomalies and uncertainties (see Paterson *et al.*, 1995; Nunn *et al.*, 1996; Nunn and Stanley, 1998; Kennedy and Page, 2002; see Austin *et al.*, 2004; Penhallurick and Wink, 2004; Rheindt and Austin, 2005).

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Within the Procellariiformes, phylogeography has mainly been studied in albatrosses and shearwaters. Most of this research involves resolving taxonomic uncertainties (Randi *et al.*, 1989; Austin *et al.*, 1994; da Silva and Granadeiro, 1999; Rabouam *et al.*, 2000; Burg and Croxall, 2001; Abbott and Double, 2003, 2003; Burg and Croxall, 2004; Walsh and Edwards, 2005; Gomez-Diaz *et al.*, 2006). Albatrosses (like petrels) are philopatric to their natal colonies and this has led to the belief that most Procellariiformes will show high population structure (Weimerskirch *et al.*, 1985; Ovenden *et al.*, 1991; Abbott and Double, 2003; Newton, 2003; Van Bekkum *et al.*, 2006). Most species' breeding populations are fragmented as birds breed on islands. This is especially marked in the sub-Antarctic, where there are only a handful of islands scattered around the Southern Ocean. Therefore, little or no gene flow could lead to the formation of subspecies or species. For example, a recent genetic study has shown that

Grey-headed Albatrosses (*Thalassarche chysostoma*), which forage along continental systems and travel widely outside the breeding season, have a globally panmictic distribution, whereas Black-browed Albatrosses (*T. melanophris*), which forage on frontal shelves and prefer to be inshore, display strong population structure (Burg and Croxall, 2001; Phillips *et al.*, 2005). This difference in foraging range and habitat seems to be the reason for the differences in population structure. One could therefore hypothesise that despite displaying philopatry, the overlapping foraging ranges promotes the occasional dispersal between breeding islands; although the mixing at foraging locations outside the breeding season may not necessarily result in gene flow.

In a study involving the wandering albatross complex, which is known for inter-colony movements (Inchausti and Weimerskirch, 2002), the wide-ranging *Diomedea exulans*, was shown to have a panmictic population structure whereas three other species of the complex with more restricted distributions showed strong structuring (Burg and Croxall, 2004). Further studies on other albatross species also showed either very little or no structure (Van Bekkum *et al.*, 2006), or distinct geographic structure between populations and regions (Walsh and Edwards, 2005).

The few published studies of petrel phylogeography have mostly investigated northern hemisphere species (Wink *et al.*, 1993; Austin *et al.*, 1994; Paterson and Snyder, 1999; Rabouam *et al.*, 2000; Burg *et al.*, 2003; Cagnon *et al.*, 2004; Genovart *et al.*, 2005; Gomez-Diaz *et al.*, 2006). A few studies have investigated southern hemisphere petrels: Short-tailed Shearwaters (*Puffinus tenuirostris*) (Austin *et al.*, 1994), Fairy Prion (*Pachyptila turtur*) (Ovenden *et al.*, 1991), and the South Georgian Diving Petrel (*Pelecanoides georgicus*) (Paterson and Wallis, 2000). Two studies involved taxonomy in the tropical dark-rumped petrels *Pterodroma phaeopygia* and *P. sandwichensis* (Browne *et al.*, 1997; Friesen *et al.*, 2006). The two species were elevated from subspecies to species and within the Galapagos Petrel (*P. phaeopygia*) structure amongst islands within the

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Galapagos archipelago was observed. No common pattern explaining genetic structuring was seen in the above studies as both structure and genetic homogeneity was observed, in addition to signals of historic population fragmentation and recent range expansions.

The major difference between southern hemisphere oceans above 40°S and northern hemisphere oceans at productive high latitudes is that the northern oceans are confined to ocean basins with landmasses as barriers, whereas in the south, the oceans are circumpolar with no large continents as barriers to seabird movements. Thus far, none of the larger southern hemisphere petrels with a wide distribution has been investigated to test if this has implications on the phylogeography of these species in comparison to the northern hemisphere petrels, and to see if a similar range from structured to panmictic populations is seen as in albatrosses.

Threats facing petrels and albatrosses: Longline fishing

Almost all albatross species (21 out of a currently recognised 24 species) are listed as threatened with extinction under the IUCN Red List criteria (BirdLife-International, 2006). The main threat to their continued survival is longline fishing, and this threat also impacts *Macronectes* and *Procellaria*, with six out of seven species listed as threatened or near threatened (Table 1.1). An international convention, the Agreement on the Conservation of Albatrosses and Petrels (ACAP – available online at www.acap.aq) has been established to conserve Southern Ocean albatrosses and petrels. One of its key tasks is to resolve taxonomic ambiguities. *Procellaria* and *Macronectes* have both been listed under ACAP as deserving special attention due to the threats facing them and their current debated taxonomic status.

The threats facing these birds are not confined to interactions with fishing vessels at sea. Introduced predators such as rats and feral cats, eat the chicks or eggs, although introduced predators have been successfully eradicated from some

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islands (e.g. Bester *et al.*, 2002). Other dangers include the degradation of the breeding sites due to expansion of the Antarctic fur seal, *Arctocephalus gazella* (BirdLife International, 2006). Human disturbance can also be a problem at some breeding sites. Global warming is a further issue that might threaten seabird populations in general due to warming of the oceans and changing of currents (Croxall *et al.*, 2002). A change in water temperature may influence foraging grounds by changing prey composition and availability (Newton, 2003).

However, the biggest threat to many of these seabird species remains longline fishing and other fishery interactions such as trawling (Sullivan *et al.*, 2006). Many petrel and albatross species are opportunistic foragers, and scavenge at fishing boats. They are attracted to fishing vessels for their bait and offal, so much so, that some seabird distribution patterns have been altered (Biodiversity Group-Environment Australia, 1998; Wienecke and Robertson, 2002). In the southern hemisphere, longline fishing is concentrated between 30°S and 60°S, which coincides with the major foraging areas of many seabird species including the White-chinned Petrel, *Procellaria aequinoctialis* (Woehler, 1996; Weimerskirch *et al.*, 1999; Tuck *et al.*, 2003). Seabirds attracted to the bait, either swallow the hook or get entangled in lines and are then dragged underwater where they drown; others are caught during the hauling process (Brothers, 1991; Agnew, 2000; Tuck *et al.*, 2001; Wienecke and Robertson, 2002; Lewison *et al.*, 2004).

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Table 1.1: Albatross and petrel species listed under ACAP according to their Red List status with estimated world population breeding pairs (single numbers are estimates) and trends according to BirdLife International (www.birdlife.org). Species highlighted are studied in this thesis.

Species	Common Name	World Population	Population Trend
	Critically Endangered		
Diomedea amsterdamensis	Amsterdam Albatross	18-25	decreasing
Thalassarche eremita	Chatham Albatross	3,200-4,200	stable
Procellaria conspicillata	Spectacled Petrel	2,500-10,000	increasing?
Phoebastria irrorata	Waved Albatross	15,600-18,200	decreasing
	Endangered		
Diomedea dabbenena	Tristan Albatross	1,500-2,400	decreasing
Diomedea sanfordi	Northern Royal Albatross	6,500-7,000	decreasing
Phoebetria fusca	Sooty Albatross	12,500-19,000	decreasing
Thalassarche chlororhynchos	Atlantic Yellow-nosed Albatross	32,200-46,200	decreasing
Thalassarche carteri	Indian Yellow-nosed Albatross	36,500	decreasing
Phoebastria nigripes	Black-footed Albatross	109,500	decreasing
Thalassarche melanophrys	Black-browed Albatross	680,000	decreasing
	Vulnerable		
Phoebastria albatrus	Short-tailed Albatross	2,052 total individuals	increasing
Diomedea antipodensis	Antipodean Albatross	4,600-5,760	unknown
Diomedea epomophora	Southern Royal Albatross	8,200-8,600	stable
Diomedea exulans	Wandering Albatross	8,500	decreasing
Thalassarche impavida	Campbell Albatross	19,000-26,000	stable
Thalassarche salvini	Salvin's Albatross	30,750	stable
Thalassarche bulleri	Buller's Albatross	32,000	stable
Thalassarche chrysostoma	Grey-headed Albatross	92,300	decreasing
Phoebastria immutabilis	Laysan Albatross	437,000	decreasing
Procellaria parkinsoni	Black Petrel	2,500	stable
Procellaria westlandica	Westland Petrel	2,000	stable
Macronectes giganteus	Southern Giant-petrel	31,000	decreasing
Procellaria aequinoctialis	White-chinned Petrel	2,400,000	decreasing
	Near Threatened		
Phoebetria palpebrata	Light-mantled Albatross	19,000-24,000	unknown
Thalassarche steadi	White-capped Albatross	75,100	
Thalassarche cauta	Shy Albatross	85,000-95,000	unknown
Macronectes halli	Northern Giant Petrel	11,500	decreasing
Procellaria cinerea	Grey Petrel	unknown >1,000,000	unknown

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Since the introduction of commercial fisheries, fishing efforts have increased, intensifying the impact on marine species such as seabirds. Longline fishing has been killing seabirds since at least the 1970s, but it was only in the late 1980s that it was recognized that the impact on some species is considerable (Croxall, 1987, 1990; Brothers, 1991). The longline fishing industry targets pelagic and demersal finfish species and sharks (Gales, 1993) and most seabird mortality is associated with fisheries for tuna (*Thunnus* spp) (Brothers, 1991; Polacheck and Tuck, 1995; Gales *et al.*, 1998; Brothers *et al.*, 1999; Ryan *et al.*, 2002; Kellian, 2003), hake (*Merluccius spp*) (Barnes *et al.*, 1997) and Patagonian toothfish (*Dissostichus eleginoides*) (Ryan and Boix-Hinzen, 1999; Agnew, 2000; Wienecke and Robertson, 2002; Nel *et al.*, 2003). More recently, it has been discovered that trawl fisheries also kill significant numbers of seabirds when they are drowned on the trawl cables (Sullivan *et al.*, 2006).

The incidental by-catch is of particular concern given the conservative life history strategies of albatrosses and petrels. Pairs lay one egg per season, with no replacement clutch. They breed annually or even biennially (Warham, 1990) and have delayed sexual maturity (Moloney *et al.*, 1994; Weimerskirch *et al.*, 1999; Tuck *et al.*, 2003). This life history strategy requires a long lifespan and high adult survival rate (Gales *et al.*, 1998). Because of their longevity, their wide foraging ranges and exclusively pelagic feeding patterns, petrels and albatrosses are sensitive bioindicators of the state of ocean systems (e.g. Weimerskirch *et al.*, 2003). Thus seabird populations may decline for a number of years before the effects of fishery mortality are seen (Croxall and Rothery, 1991), because the effects of the loss of juveniles may not be reflected in the populations for several years, when the birds would have reached maturity. The long generation times of these birds make them especially vulnerable to population declines. Juvenile birds are mostly susceptible to being killed in the longline fishing process, although this depends on the target fish species (Gales *et al.*, 1998; Ryan and Boix-Hinzen, 1998). There may also be a sex bias in mortality that varies spatially and temporally, which further complicates the impact on population

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dynamics (Croxall and Prince, 1990; Ryan and Boix-Hinzen, 1999; Mills and Ryan, 2005).

The direct impact of the longline fisheries remains difficult to assess. Fishery induced mortality is poorly documented in most areas such as South America and international waters, but fairly good data is available in countries where observer programmes have been implemented (Australia, New Zealand, and to some extend South Africa) albeit not enough to quantify seabird bycatch with reasonable precision. Crude estimates of bycatch are approximately 100,000 to 200,000 birds annually (Tuck *et al.*, 2003). Data on population level impacts vary, with some data available in the Southern Ocean for surface nesting species such as albatrosses and giant petrels, but data is scant on burrow nesters such as the White-chinned Petrel where monitoring of breeding numbers is more difficult.

The habitat of the White-chinned Petrel covers the Subantarctic Zone, and less commonly the Antarctic Zone south to the edge of pack ice. On some islands birds are present all year while on other islands they desert them in winter when they move to subtropical waters (Marchant and Higgins, 1990). The species breeds in small numbers at Falkland Islands, South Georgia (2,000,000 pairs), Marion Island (10s of thousand pairs), Iles Crozet (many thousand pairs), Iles Kerguelen (100,000 – 300,000 pairs), Auckland Islands (100,000 pairs), Campbell (10,000 pairs) and Antipodes Islands (100,000 pairs). Berrow and colleagues (2000) studied the status of White-chinned Petrels at South Georgia by comparing burrow occupancy in 1998 from 1981. They estimated a decrease in occupancy and thus breeding population on this island of 28%, stating the most likely factor of populations decrease as longline fishing (Berrow *et al.*, 2000). An estimated 10-20% of the breeding population of the White-chinned Petrel at the Prince Edward Islands were killed during sanctioned and IUU (illegal, unregulated and unreported) longline fishing for Patagonian toothfish around the Prince Edward Islands between 1996 and 2000 (Nel *et al.*, 2003). Catard *et al.* (2000) estimated a total of 45 000 White-chinned Petrels killed each

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year in legal and IUU fisheries. Other studies confirm that the most common bird following the fishing vessels and drowning on the attempts to dive (up to 6m) for the baited hooks is the White-chinned petrel (Cherel *et al.*, 1996; Barnes *et al.*, 1997; Weimerskirch *et al.*, 1999; Ryan *et al.*, 2002). The species closely follows the fishing boats at night (Ashford and Croxall, 1995; Cherel *et al.*, 1996). In addition, White-chinned Petrels have been observed being killed in Patagonian toothfish trawl fishing, but fatal incidences are rarer occurring usually through collision with the gear (Wienecke and Robertson, 2002). As the species is widespread, information on bycatch origin would facilitate identification of populations most at risk and thus aid in implementing management plans.

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The Spectacled Petrel, *P. conspicillata*, has been awarded separate species status from the White-chinned Petrel based on differences in vocalisation and plumage (Ryan, 1998). The species is known to breed only on a single island, Inaccessible Island off Tristan da Cunha. Large numbers are killed annually mainly in the fisheries off Brazil (Olmos, 1997). In order to conserve the species efficiently and to assess the impact of mortality on this species, separate species status needs to be confirmed with the use of a molecular marker.

The giant petrels, *Macronectes*, consist of two closely related species. The Southern Giant Petrel, *M. giganteus*, breeds on remote islands throughout the Southern Ocean (details on breeding locations and numbers are given in Chapter 5) with most breeding colonies located in the Atlantic. The Northern Giant Petrel, *M. halli*, is concentrated in New Zealand and the Indian Ocean (distribution and numbers are detailed in Chapter 5). Both species are under threat due to birds being killed in the longline fishing industry, with an estimated 2000-4000 giant petrels being killed in 1997/98 in the Patagonian toothfish (*Dissostichius eleginoides*) industry alone, and an estimated 4% of the Southern Giant Petrel breeding population at the Prince Edward Islands were killed during sanctioned and IUU (illegal, unregulated and unreported) longline fishing for Patagonian toothfish around the Prince Edward Islands between 1996 and 2000 (Nel *et al.*,

2003). Further population declines are attributed to human disturbances at breeding sites, and introduced predators such as rats and cats. In order to ensure the species' future survival, two issues need to be addressed. Firstly, separate species status of the two forms need to be confirmed as well as the species association of several colonies and the existence of possible cryptic taxa, are under debate and need to be confirmed. A further issue is the easy species identification of bycatch. Genetic markers provide the means to address these issues.

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PART II: GENERAL METHODS AND ANALYSIS

Collection of samples

Samples were collected by field researchers stationed on the breeding islands. Blood was collected from the vein on either leg or wing and stored in 96% ethanol. Growing feathers were collected from chicks and a number of adults and stored in 96 % ethanol. Tissue samples were taken from dead birds and stored in 96% ethanol. The following researchers were responsible for collecting samples in the White-chinned Petrel: Deon Nel (Marion Island), Henri Weimerskirch (Iles Crozet), Richard Phillips (South Georgia), and Kath Walker, Graeme Elliott, and Erica Sommer (New Zealand). Spectacled Petrel samples (Inaccessible Island) were collected by Peter Ryan and Cliff Dorse. The following researchers collected samples of giant petrels: Richard Phillips (South Georgia, Falklands, South Orkney), Flavio Quintana (Staten Island, Isla Arce, Gran Robredo), Scott Dreischman and David Oehler (Isla Noir), Markus Ritz (King George/Two Summit Island), Richard Cuthbert and Erica Sommer (Gough), Deon Nel and Peter Ryan (Marion Island), Henri Weimerskirch and Emmanuel Milot (Iles Crozet/Pointe Basse), Rosemary Gales (Macquarie), Brian Bell (Chatham Islands/Fourty Fours), David Thompson (Campbell Islands), and Kath Walker (Auckland - Enderby/Adams, Antipodes),.

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Samples from longline mortalities were collected by fishery observers on board fishing vessels. Tissue samples were collected during routine examinations of carcasses and frozen. Upon return to port these samples were thawn and stored in 96% ethanol. New Zealand and Chatham Rise bycatch samples were collected by Chris Robertson.

Extraction of DNA from Tissue, Blood and Feathers

Tissue extraction

For DNA extractions, approximately 500 mg of tissue was cut into small pieces and subjected to a standard overnight Proteinase K digestion in extraction buffer (50 mM Tris-HCl, 0.1% SDS, 10 mM EDTA pH 8, 100 mM NaCl), which was then followed by a standard phenol: chloroform extraction (Ausubel *et al.*, 1989; Sambrook *et al.*, 1989). DNA was precipitated by 7.5 M ammonium acetate (1/2 volume) and absolute alcohol (2 volumes) and collected by centrifugation. The DNA pellets were washed in 70% ethanol, dried and re-suspended in Tris-EDTA (TE - pH 8.0).

Blood extraction

Two protocols were followed. Extractions for *P. aequinoctialis* followed the same protocol as extraction from tissue starting with 100-200 µl of blood. Blood from *P. conspicillata* and both *Macronectes* species was extracted using the DNeasy Tissue Extraction Kit (Qiagen), by following the manufacturer's instructions.

Feather extraction

Growing feathers were cut into pieces and washed with 0.9% NaCl. Thereafter the pieces were incubated at 37°C for 16 hours in 1 mL of 200 mM Tris-HCl (pH 8) and 390 Units collagenase. After the incubation period the solution was centrifuged at 1500 g for 5 min and the supernatant discarded. The pellet was re-dissolved in 0.5 mL TE (pH 8), 0.1% SDS and 30 units Proteinase K for 16 hours at 37°C. Subsequently, the DNA was extracted using a standard phenol: chloroform extraction. DNA was precipitated by 5 M NaCl (0.1 volumes) and absolute ethanol (2.5 volumes) (Eguchi and Eguchi, 2000). The pellet was washed in 70% ethanol, dried and re-suspended in TE (pH 8.0).

Mitochondrial cytochrome b analysis

Most studies investigating phylogeography in animals use mitochondrial DNA sequence data, because it shows maternal inheritance and is thus less complex

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to analyse than nuclear markers. However, as they have become more available, neutral microsatellites have been used increasingly (Avise, 2004). Cytochrome b is often chosen as a molecular marker to construct phylogenetic relationships between avian species (Moore and DeFilippis, 1997). The gene is attractive as a rapid rate of silent substitutions increases the probability that the gene will contain synapomorphies even between recently shared ancestries. Birds characteristically show low levels of divergence in both nuclear and mitochondrial genes, therefore this is an important feature. However, it has been shown that the cytochrome b gene performs best when used to infer intraspecific relationships and phylogenetic relationships between closely related species (Moore and DeFilippis, 1997). In birds, cytochrome b sequences accumulate transitions at a rapid but constant rate to the level of distinct genera and transversions to the level of superfamilies. These substitutions are primarily synonymous and appear to be neutral or only weakly under selection. These positions, which are mostly third codon positions, become saturated quickly whereas positions under selection are constrained and therefore unlikely to be substituted, making cytochrome b unsuited to resolving higher taxonomic levels (Meyer, 1994).

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Polymerase Chain Reaction (PCR) and sequencing for cytochrome b analysis
Details of primer design are discussed in the relevant Chapters. Thermal cycling was performed using the GeneAmp® PCR System 2700 (Applied Biosystems) under the following conditions: 2 min at 94°C, 30 cycles at 94°C for 45 sec, T_a°C for 45 sec (T_a°C = 45°C for *Procellaria* and T_a°C = 55°C for *Macronectes*) and 72°C for 1 min, with a final extension at 72°C for 5 min. Reactions contained 0.4 pmol/µl of each primer, 2 mM $MgCl_2$, 200 µM of each dNTP, 0.02 U/µl Promega GoTaq® Flexi DNA Polymerase and 1x reaction buffer in a total volume of 20µl. PCR products were electrophoresed on 2% agarose gels. Size of products was determined according to a DNA ladder (Promega 100 bp DNA ladder). Bands were excised with a sterile razor blade and purified with the Promega Wizard® SV Gel and PCR Clean-up System. Of the resulting purified PCR products, 3 µl

were electrophoresed on 2% agarose to determine the volume to be used as template in 10 μl BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reaction products were purified using Centrisep columns (Princeton) and resolved by electrophoresis on an ABI 3130 Genetic Analyser.

Sequence editing and numts

Sequences were edited using CHROMAS Lite version 2.0 (Technelysium Pty Ltd, available online: http://www.technelysium.com.au/chromas.html) and BIOEDIT version 5.0.9 (Hall, 1999), and aligned using CLUSTAL X (Thompson *et al.*, 1997).

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Numts (nuclear copies of mtDNA genes or segments) present a challenge to sequencing studies involving mtDNA. The word was coined by Lopez and colleagues (Lopez *et al.*, 1994) while working on the domestic cat. Since then numts have been discovered in a variety of eukaryotes (Quinn, 1997). Avian studies are particularly challenging as blood, which is frequently used in studies, has nucleated blood cells and is therefore rich in nuclear DNA relative to mtDNA (Quinn, 1997). Several studies have shown that primers designed for mtDNA will often also amplify a nuclear copy. Sequences were confirmed as mitochondrial cytochrome sequences by comparing them to previously published sequences (Nunn *et al.*, 1996), and by translating sequences into amino acid profiles and looking for stop codons.

Phylogeographic and phylogenetic analyses of cytochrome b

Basic statistical analysis of the sequences was performed using DNASP version 4.0 (Rozas et al., 2003). Sequence divergence was estimated using uncorrected nucleotide divergence (k for species or Dxy for populations) implemented in the programme. Uncorrected distances were calculated as sequence divergence was expected to be low and therefore homoplasy not a problem. Phylogenetic

analysis was performed using haplotypes from all sequences and using an appropriate outgroup for rooting (details in Chapters) the tree.

There are several different methods of phylogeny construction with hotly debated strengths and weaknesses associated with each method (see e.g. Weir, 1996; see e.g. Nei and Kumar, 2000; Avise, 2004; Steel, 2005). Methods can be divided into i) distance methods, ii) parsimony methods, iii) maximum likelihood methods and iv) Bayesian methods. Phylogeography deals with lineages and their distribution in time and space. It therefore has a phylogenetic component which is dealt with in terms of gene trees (Avise, 2000). However, often networks are better in depicting relationships between genes or haplotypes in closely related species or populations than trees. Nonetheless, trees are useful in describing relationships amongst populations and closely related species within the framework of phylogeography.

For the purpose of this mitochondrial cytochrome b work, trees were constructed using Neighbour Joining, Maximum Parsimony and Bayesian Analysis. The trees were then compared for robustness and congruence. More than one method was used as each has a different algorithm and different assumptions. By comparing among methods, recurring relationships can be assumed to be true. The theory behind the computations is discussed below.

Intraspecific mtDNA analysis has the advantage that small genetic distances suggest that sequences are not saturated with superimposed nucleotide substitutions and small branch lengths negate possible problems with rate heterogeneity (mutation rates may vary from one nucleotide site to the other, creating 'mutational hotspots' (Aris-Brosou and Excoffier, 1996)).

Neighbour Joining (NJ) is a distance based tree method (Saitou and Nei, 1987). It uses an arithmetic mean to derive a tree from a distance matrix that is derived from the sequence alignment. NJ is based on the Minimum Evolution model but

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uses less computing time than some other methods, which makes it more popular. It allows for different rates of molecular change amongst lineages and is probably the most popular distance method used today (Avise, 2004). The algorithm starts with a star-like tree under the assumption that there is no clustering of taxa. Then the sum of all branches is calculated with the assumption that this sum should be bigger than the sum of all branches from the true tree. All pairs of taxa are considered and the sum of all branch lengths is considered. The pair with the smallest sum is considered a true pair. It then creates a new matrix by using the node instead of the two taxa, where the distance of each taxon to the node that joins them is calculated (Nei and Kumar, 2000; Avise, 2004; Hall, 2004).

There exist several distance measures that can be used to construct a NJ tree. Often these measures are quite complex and although a Goodness of Fit can be calculated they do not necessarily produce better trees. Nei and Kumar (2000) suggest using a simpler method as the variances of the more complicated methods is often greater. The Kimura-2-Parameter corrected distance (K2P - Kimura, 1980) estimates nucleotide substitution by taking into account that transitions tend to occur more often than transversions but is otherwise similar to one of the simplest measures, the Jukes-Cantor method (Nei and Kumar, 2000). This method is also called the one-parameter model as it only considers the nucleotide substitution rate and assumes that the probabilities of any nucleotide to change to another are equal (Hall, 2004). In this study, a NJ tree was constructed using the K2P corrected distances implemented in the program Mega version 3.1 (Kumar *et al.*, 2004). NJ produces an unrooted tree. Appropriate outgroups were used and both unrooted and rooted trees are presented. Reliability of the trees was tested by bootstrapping 1000 times.

Maximum Parsimony (MP - Eck and Dayhoff, 1966; Fitch, 1971) searches for trees that require the minimum number of evolutionary changes to explain the data. Often several equally parsimonious trees are generated. In parsimony

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analysis not all character states in an alignment are useful; invariant sites and sites that occur only in one taxon are not informative and are thus ignored. Several parsimony approaches are available with slightly different assumptions (Avise, 2004). For example, weighted MP methods give different importance to certain substitution such as transitions over transversions. Homoplasy may be a problem in constructing MP trees and any trees constructed in a situation where homoplasy is suspected are unreliable. As neither sister taxa pair in this study is assumed to have a high divergence, a large amount of homoplasy was not expected and thus MP should give reliable results. If taxa number is small an exhaustive tree search can be used but computations are lengthy if more than ten taxa are used. In this study, close neighbour interchange (CNI) with search factor 1 and the mini-mini heuristic search with level 100 was employed to find the most parsimonious trees. Reliability was tested by 1000 bootstraps as implemented in Mega version 3.1 (Kumar et al., 2004). CNI means that branches on a tree are swapped around and compared to the current most parsimonious tree. The mini-mini branch and bound search refers to the order branches are added to an initial tree of three taxa. The minimum number of substitutions is calculated for each tree and taxon and the taxon with the smallest value is selected and added (Nei and Kumar, 2000).

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Bayesian inference (Rannala and Yang, 1996) means that the posterior distribution of parameters is calculated, i.e. the probability of a tree is calculated given the data and an evolutionary model. The method is a variant of likelihood methods such as Maximum Likelihood (ML). Bayesian inference has become popular in recent years (for a review see Beaumont and Rannala, 2004). In genetics, probability models often include many interdependent variables that are tied to a particular range of values. ML inference needs to carry out a constrained multidimensional maximization in order to find the combined set of parameter values that maximise the likelihood function. Mostly, this requires a large computational effort. Furthermore, calculation of confidence intervals involves approximations such as testing that the distribution of the maximum likelihood

estimate follows a normal distribution. In contrast, in Bayesian inference the prior automatically imposes constraints on the parameters. In addition, inferences about parameters are made on the basis of the posterior distribution, which requires integration rather than maximization, and no further approximation is needed. Further, the integration of Monte Carlo Markov Chains and the availability of more powerful computers have facilitated the evaluation of posterior probabilities.

When used for tree building, Bayesian inference as the following advantages. In contrast to ML where a single tree with the highest likelihood is chosen, Bayesian inference produces a best set of trees and produces probability distributions of likelihoods given the data and the evolutionary model specified, thereby giving a true probability rather than a likelihood value. Similarly to ML it searches the landscape of innumerable possible trees moving from one likely tree to a more likely tree. ML can get trapped on a local 'hill', i.e. a set of likely trees in the landscape (Avise, 2004) if there are more than one set of more likely trees. MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001) was used to construct a Bayesian inference tree. The program uses the Metropolis-coupled Monte Carlo Markov Chain method, with four chains running independently and exchanging information every so often which allows the Bayesian approach to leap a 'valley'. This is achieved by the four independent chains starting on different trees. The so called 'cold chain' is the source of the tree saved every 100 generations. In each generation there is a chance for two chains to swap states. Therefore if the cold chain is trapped it has a chance to escape through being exchanged with another chain. In addition, the Bayesian approach will consider the same tree more than one time (Hall, 2004). The algorithm starts by choosing a random tree and a new chain is defined by the branch length, substitution parameter and rate variation. A new state is then proposed and the probability of this, given the old state, is calculated (Hall, 2004). A random number between 0 and 1 is drawn and compared to the calculated value; if the number is smaller then the new state the tree is accepted. This is called one

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generation. In this study 13,000,000 generations were computed with a burn-in of 13,000 trees. The evolutionary model chosen for this analysis was the General Time Reversible model, which uses six different rates of nucleotide substitutions. The model also assumes that the rate of change from a base *i* to *j* is the same as from *j* to *i* (Nei and Kumar, 2000; Hall, 2004).

As mentioned earlier, the construction of a network can often recover relationships between haplotypes more accurately than phylogenies. Networks may present the relationships between haplotypes by including cycles in the data or alternative connections. These alternative connections can represent evolutionary events such as hybridization, genetic recombination or horizontal gene transfer. This feature makes networks more appropriate to intraspecific data than phylogenies which are bifurcating. In addition, assumptions about relationships of alleles or haplotypes are different depending on the analysis of intra- or inter-specific data. In inter-specific analysis alleles or haplotypes are hierarchical because they are the result of reproductive isolation and population differentiation over a long time period. In contrast intraspecific analysis assumes relationships to be the result of sexual reproduction, recombination and a small number of relatively recent mutations. Traditional tree building methods have reduced statistical power because fewer phylogenetic characters are present in intraspecific data as conspecific individuals are closely related. Further ancestral alleles or haplotypes are likely to still be present within populations and therefore genealogies are expected to be multifurcating rather than bifurcating. Lastly large sample sizes are needed to overcome low divergence (Lowe *et al.*, 2004). A Minimum Spanning Network (MSN) is often used for phylogeographic analysis (Lowe *et al.*, 2004) as it minimizes the length of the branches connecting all haplotypes. ARLEQUIN version 3.1 (Schneider *et al.*, 2000; Excoffier *et al.*, 2005) was used to construct a MSN using pairwise differences.

In addition to constructing phylogenies, partitioning of variation was examined. When a species is subdivided into different populations, it shows less

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heterozygosity or gene diversity (in the case of sequence data) than if all the individuals were allowed to mate randomly and there was no subdivision. Wright's fixation index (or *F* statistic) calculates the difference between the mean heterozygosity/gene diversity among the subdivided populations and the potential frequency if there was no division. This fixation index can be calculated at different hierarchical levels. Molecular variance analysis (AMOVA, (Excoffier *et al.*, 1992) is used to test significance between these levels and shows at which hierarchical level most of the variation is contained. If divergence between defined groupings is significant than most of the gene diversity should be between these and not within groupings. ARLEQUIN version 3.1 (Schneider *et al.*, 2000; Excoffier *et al.*, 2005) was used to test for between species partitioning of variance as well as within group partitioning within species. Variance is partitioned into covariance components according to differences within and between the groups, colonies and individuals.

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An analysis of the historical demography of sister species pairs (White-chinned and Spectacled Petrel, and Southern and Northern Giant Petrels) and different geographical regions within single species was investigated with the use of mismatch distributions (Rogers and Harpending, 1992). A unimodal distribution of haplotypes in a plot based on pairwise sequence differences is expected in a species that has undergone an historic range expansion whereas a more "ragged" distribution is expected if populations have been stable over a long period of time. However, in a population that has undergone a recent demographic expansion one can expect a larger number of haplotypes with single point mutations and low frequencies with the distribution appearing smooth with a peak indicating the time of expansion (Harpending *et al.*, 1993; Harpending, 1994). ARLEQUIN version 3.1 (Schneider *et al.*, 2000; Excoffier *et al.*, 2005) was used to test the model of sudden range expansion (Rogers, 1995) using Harpending's raggedness index *r* (Harpending, 1994) of the observed distribution and the sum of the squared deviation (SSD - Schneider *et al.*, 2000) between the observed and expected distributions. SSD is a measure to

statistically test the difference between these two distributions. The sum of square deviations is used to test between expected and observed statistics where the null hypothesis is that there has been a demographic expansion and the observed values fit the model. The raggedness statistic is a quantification of the observation that the distribution is smooth for a population that has experienced an expansion, thus the null hypothesis is for smoothness. By viewing a histogram, the old distribution can be observed on the right of the graph because the number of differences accumulates with time. Usually a peak is present in a histogram denoting the expansion event. The statistic Tau can be used to calculate time of expansion. Tau is measured in unit mutation time and equals two times mutation rate multiplied by time in generations (Rogers and Harpending, 1992; Harpending *et al.*, 1993). In addition Fu's *Fs* (Fu, 1997) test of neutrality was computed. Fu's *Fs* (Fu, 1997) is a test of neutrality, testing a deviation from the neutral Wright-Fisher model. The model tests if a population is in mutation-drift equilibrium and if mutations are neutral. *Fs* specifically tests for population growth and has been shown to perform well (Ramos-Onsins and Rozas, 2002). The statistic calculates the probability of observing a random neutral sample with the same or larger number of haplotypes as compared to the observed value. If an excess of low frequency alleles/haplotypes is present compared to an expected value under neutrality, the statistic produces a strong negative value. ARLEQUIN version 3.1 (Schneider *et al.*, 2000; Excoffier *et al.*, 2005) was used to calculate Fu's *Fs* and significance levels were generated by comparison of the test statistic to the distribution of 1000 random samples that were generated under mutation-drift equilibrium and neutrality.

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Microsatellite analysis

Expected and observed heterozygosities, departures from Hardy-Weinberg and linkage equilibrium were calculated using GenePop version 3.1c (Raymond and Rousset, 1995). Significance values for deviations from Hardy-Weinberg expectations were obtained using Fisher's method of combining exact test probabilities. In order to get exact probabilities a Markov chain method was used

with the following parameters: dememorisation 1000, batches 100, and iterations per batch 1000. Critical significance levels for multiple tests was corrected by applying a sequential Bonferroni procedure (Rice, 1989).

Several different genetic distance measures can be used to resolve evolutionary relationships when utilising microsatellite data. Delta μ^2 (Goldstein *et al.*, 1995), chord distance D_{CE} (Cavalli-Sforza and Edwards, 1967) and D_A (Nei *et al.*, 1983) can be used for microsatellite data. Delta μ^2 was specifically designed for the use of microsatellite markers, and is based on the Stepwise Mutation Model (SMM), which is the proposed mechanism for microsatellite mutation taking into account the high mutation rate in microsatellite evolution (Primmer *et al.*, 1998). In addition, it makes use of the size differences between alleles, considers the high variance characteristic to microsatellite data and is robust regarding sample sizes (Goldstein *et al.*, 1995). The two other genetic distance measures D_{CE} and D_A, do not assume a model of mutation but instead make use of the sum of the products of allele frequencies shared between samples. Both have been demonstrated to perform well in constructing trees from microsatellite data when operational taxonomic units are closely related (Takezaki and Nei, 1996; Goldstein and Pollock, 1997). Takezaki and Nei (1996) observed that for very recently separated populations, distance measures that make use of the sum of the products of allele frequencies shared between populations are the most accurate. This is especially true when allelic variance and thus mutation rate is high as for microsatellites. Furthermore the authors note that of the three measures, D_{CE} has the highest chance of recovering the correct tree topology due to it not making assumptions concerning equal mutation rates over loci or constant population size.

Genetic distances were calculated in POPULATIONS v1.2.28 (Langella, 2001) from microsatellite allele frequency data. This program is able to calculate both distance matrices and representative trees. Trees were constructed using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA, Sneath and

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Sokal, 1973), which is a distance-based tree method, which uses arithmetic means to derive a tree from a distance matrix that was derived from allele frequencies. UPGMA assumes equal rates of evolution between lineages and often introduces errors when the number of nucleotides is small when using sequences (Nei and Kumar, 2000; Hall, 2004), but not when using allele frequencies. However, this cluster analysis is considered well suited to reflect similarity between operational taxonomic units. The method relies on the rate of evolution among different lineages being roughly equal, which is the case for microsatellite evolution within a species (Goldstein and Pollock, 1997; Schlotterer, 1998). UPGMA, however, is not considered a good algorithm for constructing phylogenetic trees and therefore no phylogenetic inferences should be drawn from clustering patters seen. However, it is considered a good algorithm for inferring relationships between closely related taxonomic units (Nei and Kumar, 2000; Lowe *et al.*, 2004). TREEVIEW version 1.6.6 (Page, 1996) was used to view and edit trees.

Population structure can only develop in cases when there is limited or no gene flow between populations because extensive gene flow may counteract divergence via genetic drift and local adaptation and thus divergence. Using data from wing morphology, mark-recapture studies and satellite telemetry, it has been shown that many seabird species have the capacity to travel enormous distances, but most species exhibit strong natal site fidelity (Dearborn *et al.*, 2003), including most albatrosses and at least some petrels (Warham, 1990; Brooke, 2004).

Population differentiation was measured with the traditional measurements of F_{ST} and R_{ST}, which measure changes in levels of heterozygosity and variance in allele size relative to that expected for an equivocal single population, respectively. Wright's F_{ST} (Wright, 1951) is based on the infinite allele model (IAM, (Ohta and Kimura, 1973)), whilst its analogue, Slatkin's R_{ST} (Slatkin, 1995), is based on the stepwise mutation model (SMM) and was developed to

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accommodate data from microsatellite markers (McDonald and Potts, 1997). F_{ST} and R_{ST} behave differently with regard to drift and mutation, thus making them ideal when compared to explain the factors responsible for population differentiation.

Fixation indices were developed by Wright (Wright, 1951, 1965) for use with allozymes. They demonstrate how genetic variation, in terms of expected and observed heterozygosity, can be separated into hierarchical population level components. These then reflect genetic structure and allow deduction of various population parameters (Jarne and Lagoda, 1996).

F-statistics estimate the relationship of alleles within and between individuals and describe the proportion of variation within individuals relative to that expected in the sub-population (F_{is}) and the total population (F_{it}). F_{ST} describes the proportion of variation in sub-populations in relation to the total variance in the population sampled. Fixation indices at a higher hierarchical level, however, do not take into account any further subdivision that is due to behaviourally isolated groups, i.e. through philopatry, dispersal and territorial exclusion, although F_{ST} can be used to describe sub-structure level once it is predetermined. F_{is} and F_{it} are not dependant upon mutation models and describe deviations from expectations under Hardy-Weinberg equilibrium (Jarne and Lagoda, 1996). F_{is} is an inbreeding coefficient and is a measure describing the deviation of genotype frequencies from panmictic frequencies as a result of the divergence of observed heterozygosity to expected heterozygosity (Lowe *et al.*, 2004). F_{is} essentially measures the probability that two alleles in an individual are identical by descent. The values for F_{is} range from –1 to +1 where a negative value describes heterozygote excess or outbreeding and a positive value describes heterozygote deficiency or inbreeding relative to Hardy-Weinberg expectations (Ridley, 1996). In practice F_{ST} values up to 0.05 usually indicate negligible genetic differentiation and values greater than 0.25 mean great genetic differentiation (Ridley, 1996; Hartl and Clark, 1997). FSTAT version 2.9.3 (Goudet, 1995, 2001) was used to

calculate F_{is} and F_{ST} values and to obtain significance values. ARLEQUIN version 2.0 (Schneider *et al.*, 2000) was used to obtain R_{ST} values and their significance.

The program BOTTLENECK v1.2.01 (Cornuet and Luikart, 1996) was used to ascertain if there have been recent bottlenecks within colonies or populations. The program is based on the hypothesis that a population that has experienced a recent bottleneck, thereby experiencing a reduction in effective population size, would show a related reduction in allele numbers and gene diversity at polymorphic loci. However, allele numbers are expected to be lost at a faster rate than gene diversity at equilibrium. Therefore, the observed gene diversity is higher than the expected equilibrium gene diversity, which is computed from the observed number of alleles, under the assumption of a constant-size equilibrium population (Luikart and Cornuet, 1997). This phenomenon is only observed under the Infinite Allele Model (IAM) (Maruyama and Fuerst, 1985). In cases where the locus evolves under the Stepwise Mutation Model (SMM) gene diversity excess may not be observed (Cornuet and Luikart, 1996). Nevertheless, only few loci follow the SMM strictly and as soon as they depart only slightly towards the IAM, gene diversity excess as a result of a recent bottleneck can be observed. The authors of the program have included another model for the use with microsatellite markers – the Two-phased model of mutation (TPM). This model is intermediate to the IAM and SMM and consists mostly of one-step mutations, but a small percentage (5-10%) of multi-step changes. In a population at mutation-drift equilibrium there is an approximately equal chance that a locus is at gene diversity excess or deficit. In order to determine that a population shows a sufficient number of loci with a gene diversity excess, the program proposes three tests: a sign test, a standardized differences test and a Wilcoxon Sign-Rank test (Cornuet and Luikart, 1996). The first test suffers from low statistical power (Cornuet and Luikart, 1996), the second test requires at least 20 polymorphic loci and is therefore not very useful in this study (Cornuet and Luikart, 1996). The Wilcoxon sign-rank (Luikart and Cornuet, 1997) test can be

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used with as few as four polymorphic loci (recommended 10-15), any number of individuals (recommended 15-40) and has high statistical power. Furthermore a qualitative descriptor of the allele frequency distribution has been implemented. This 'mode-shift' indicator discriminates between bottlenecked and stable populations, but needs at least 30 individuals to have any real statistical power (Cornuet and Luikart, 1996).

A principal component analysis (PCA) was performed at interpopulation level using allele frequency data as implemented in PCA-GEN version 1.2.1 (Goudet, 1999). PCA is a multivariate statistical method that looks at total variation within a data set and attempts to use as few factors or components as possible to describe this variation thereby simplifying a dataset. As data present many dimensions the statistic reduces these but tries to maintain the original structure. Structure is then expressed as a number of principal components. In the case of highly structured data, the first two principal components should account for the majority of variation (>50%). Each sample is depicted as a component score and reflects the relationship between the variable and the principal component. This may then be plotted as a factor map. If there is structure in the data, allele frequencies for each locus should be correlated within colonies or populations. The program PCA-GEN version 1.2.1 links this to Wright's F_{ST}. It accounts for substructure in the data set by a linear combination of those alleles that express most of the variance in allele frequency. The model has been shown through simulations of several genetic models of gene flow to perform well and to show a consistent relationship between the two statistics.

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PART III: MICROSATELLITE LIBRARY CONSTRUCTION FROM *PROCELLARIA AEQUINOCTIALIS* AND CROSS-SPECIES AMPLIFICATION

Introduction

The estimated density of avian microsatellites within the avian genome is every 20-39 kb and a $(CA)_{10}$ repeat every 136-150 kb, whereas in comparison the respective densities for the human genome is 6 kb and 30 kb, respectively (Review on nuclear volume and DNA content in birds: Cavalier-Smith, 1978; Burton *et al.*, 1989; Van den Bussche *et al.*, 1995; Review on nuclear volume and DNA content in birds: Primmer *et al.*, 1997; Hedges and Kumar, 2002). To avoid extensive screening, an enriched library approach is therefore more practical. Most recent studies have used an enriched library in order to locate avian microsatellites in genomes not yet studied (Li *et al.*, 1997; Piertney and Dallas, 1997; Primmer *et al.*, 1997; Piertney *et al.*, 1998; Burg, 1999; Cabe and Marshall, 2001; Lieckfeldt *et al.*, 2001; Piertney and Hoglund, 2001; Russello *et al.*, 2001; Sefc *et al.*, 2001; Zane *et al.*, 2002).

The enriched method used for the library construction closely follows the approach outlined in Cabe and Marshall (2001). It makes use of a degenerate primer eliminating the use of restriction enzymes and subsequent linkers. This eliminates the time that has to be spent on the correct linker design and ligation as well as the cloning effort. The enrichment process involves streptavidin magnetic beads and a biotinylated probe, which circumvents the use of radioactive screening and labelling. Final screening of the colonies eliminates radioactive use by using PCR in the same step as predetermining the insert size, thus reducing the risk of sequencing non-microsatellite inserts. However, in order to increase sensitivity, a Southern Blot was included in the final screening. Compared to the traditional enrichment using linkers, this method is also less time-consuming. Studies using standard techniques (Sambrook *et al.*, 1989)

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would have to screen several thousand clones to achieve a similar result. Furthermore, after optimising the technique, a genomic microsatellite library can be constructed in much less time than a traditional library.

Microsatellite DNA library construction

A degenerate oligonucleotide primer (DOP) was used to obtain random fragments from the genomic DNA in a PCR reaction (K6-MW: CCGAGGTACCNNNNNNATGTGG - Cabe and Marshall, 2001). The cycle conditions on a Hybaid Thermal Reactor were as follows, one cycle at 95°C for 2 min, 5 cycles at 95°C for 30 sec, 30°C for 50 sec, 72°C for 2 min, 30 cycles at 95°C for 30 sec, 56°C for 1.5 min, 72°C for 3 min and a final extension at 72°C for 20 min. In a 50 µl PCR, conditions were as follows: 1 pmol/µl of the primer, 3 mM $MgCl_2$, 200 µM of each dNTP, 1.25 units Taq polymerase (Bioline) and 168 ng DNA (four individuals pooled). PCR product were confirmed on a 2% agarose gel and products containing fragments of the length 200-1500 bp were chosen and subjected to an enrichment technique. A biotinylated microsatellite probe, $(CA)_{12}$ containing a 3' amino C7 modification, is hybridised to the DNA fragments in a 65 µl reaction containing 10 pmol/µl probe, 20 µl of DOP PCR in 6x SSC. The mixture was then incubated for 5 min at 98°C followed by 25 min incubation at 67°C. The probe was then captured with streptavidin coated magnetic beads by adding the hybridisation mixture to 35 µl of 6x SSC to the beads. This mixture was agitated at room temperature for at least 20 minutes. The captured DNA was washed twice with 1 mL 2x SSC/01% SDS and 1x SSC for 5 minutes. Thereafter, the beads were washed twice with 1 mL of 67°C 1x SSC for 2 minutes and 5 minutes. Washes were discarded. The DNA was eluted by incubation for 20 minutes in 20 µl of 0.1 M NaOH and 0.1 M NaCl. After the incubation the mixture was neutralised by adding 10 µl 0.2 M HCl and 2.2 µl 0.1 M Tris-HCl (pH 7.5). The eluate was removed and further purified using a Qiagen PCR Purification Kit and 4 µl of eluted DNA was used as template to repeat the PCR, and additional enrichment and PCR steps. The PCR product was cloned using the pGEM-T-easy Vector System (Promega). After selection of the clones containing an insert,

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the clones were stored in microtitre plates containing 100µl of LB-Amp medium. Screening was performed in two ways for *P. aequinoctialis*: Clones were dot-blotted onto Hybond N^+ membranes (Amersham Pharmacia) and screened for inserts using a γ-^{32}P labelled CA probe (O'Ryan *et al.*, 1999). Positives were then amplified in 10 µl PCR reactions using M13 forward and reverse primers and an additional $(CA)_{12}$ primer following the principle outlined in Cabe and Marshall (2001). The reactions contained 1.5 pmol/µl of the M13 primers, 1 pmol/µl of the $(CA)_{12}$ primer, 200 µM dNTP, 1.5 mM $MgCl_2$, 0.5 U of Taq polymerase (Bioline) and 1 µl of bacterial cell suspension. Thermal cycling was performed using a Hybaid Thermal Reactor under the following conditions: 3 min at 95°C, 5 cycles at 95°C for 30 sec, 60°C for 30 sec, and 45 sec at 72°C, 30 cycles at 92°C for 30 sec, 60°C for 30 sec and 72°C for 55 sec, and a final extension at 72°C for 30 min. PCR products were subsequently electrophoresed on 2% agarose gels. Plasmids for sequencing were selected on the basis of having a large enough insert and showing two bands on an agarose gel representing the entire insert amplified by the M13 primers and the fragment amplified by the $(CA)_{12}$ primer and the reverse M13 primer (signifying the presence of a microsatellite repeat). After this identification process, suitable plasmids were isolated using a standard plasmid mini-prep isolation protocol (Sambrook *et al.*, 1989). Sequencing was performed on a MegaBACE 500 Molecular Dynamics (AmershamBiosciences). The DYEnamic ET Dye Terminator Cycle Sequencing Kit for MegaBACE was used for the reactions and all reactions were performed according to the manufacturer's instructions and cycle sequenced on a GeneAmp PCR System 9700, Perkin Elmer, Applied Biosystems.

Table 1.2 shows the success of the method in constructing a genomic microsatellite library in *Procellaria aequinoctialis* - less than five hundred putative positive clones were screened to yield six polymorphic loci.

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Table 1.2: Details of primer construction in terms of numbers and percentages indicating the success of the enriched library method

	Number	%
Number of putative positive clones after blue/white selection	466	100
Number of putative positive clones after Southern Blotting	53	11.4
Number of putative positive clones after PCR screening	25	5.4
Number of clones containing a microsatellite after sequencing	17	3.6
Number of primer sets designed	10	2.2
Number of polymorphic primers	6	1.3

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Of the 25 putative positive clones after PCR screening four were small in size and therefore deemed too small to produce enough base pairs to design primers. Of 21 recombinant plasmids sequenced for *Procellaria aequinoctialis*, 17 contained microsatellites, and two loci were sequenced twice. For the 10 microsatellites that had sufficient flanking DNA, primers were designed. The reason why only 10 primer sets were designed from 17 sequences containing microsatellites is that the remaining clones were asymmetric, meaning that the DNA fragment was either within or too close to the microsatellite sequences thereby producing insufficient flanking region for the design of primers. It was found during the study that the most success could be achieved in sequencing only fragments in the range of 400-800bp. Also closer inspection of the second smaller band of the two PCR products in the PCR screen showed that only clones with a second band bigger than 300 bp should be selected and the distance between the bands should not too large.

Sequence editing and primer design

Sequences were edited using Chromas (Technelysium Pty. Ltd.) and BioEdit v5.0.9. (Hall, 1999), and primers were designed for all sequences having sufficient flanking DNA using DNAMan version 4.13 (Lynnon BioSoft).

Microsatellite genotyping with γ-^{32}P-dATP

Radioactive microsatellite genotyping was used for the *Procellaria aequinoctialis* samples. Using microsatellite primers developed in the microsatellite library genomic DNA was amplified in 10 μl reactions using a PTC-100 Programmable Thermal Controller. The forward primer of each pair was labelled with γ-^{32}P-dATP prior to amplification using T4 polynucleotide kinase (New England Biolabs). Optimum annealing temperatures and $MgCl_2$ concentrations are listed in Table 1.3. Reactions contained 1.25μM of each primer (forward primer labelled), 200 μM dNTP, 0.25 U/μl Taq polymerase (Bioline), the optimum concentration of $MgCl_2$ and 70-300 ng of DNA. Cycling conditions were as follows: 94°C for 45 sec, T_a for 45 sec, 72°C for 1 min for 30 cycles and a final extension at 72°C for 5 min. PCR products were electrophoresed on a 6% polyacrylamide denaturing gel using a formamide loading dye. Two sequenced size ladders of M13 ssDNA (USB Sequenase version 2.0 DNA sequencing Kit) was run on each gel as a molecular size standard. Each polyacrylamide gel was transferred to blotting paper after electrophoresis and exposed to x-ray film (autoradiograph) for 3 h to 5 days depending on the intensity of the incorporated γ-^{32}P-dATP. Primers developed for polymorphic microsatellite loci were tested by genotyping at least 68 individuals at each microsatellite locus and six out of ten loci were found to be polymorphic in the source species. The remaining four loci were monomorphic, one of which was polymorphic in the two Giant Petrel species.

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Table 1.3: Characteristics of microsatellite loci in *Procellaria aequinoctialis*. The annealing temperature (T_a) is in °C and the $MgCl_2$ concentration in mM. Number of individuals tested, *n*; number of alleles, N_a; size range, bp; observed heterozygosity, H_O; expected heterozygosity, H_E; heterozygosity values were calculated using individuals from three Southern Ocean islands only: Marion Island (21), South Georgia/Bird Island (16) and Antipodes Island (17). Cloned sequences have been deposited with GenBank under Accession numbers AY371070-AY371076

Locus		Primer sequence (5'-3')	Repeat type	*n*	bp	N_a	T_a	$MgCl_2$	Ho	H_e
Paequ2	**F:**	GCCTACTCCATCTTAATTGTG	$(CA)_2TT(CA)_{10}$	94	196-212	5	54	1	0.60	0.58
	R:	GGTTCATACAGTTTCCTAGGTC								
Paequ3	**F:**	TGTGGGTGCAGTAGAGCA	$(GA)_{19}$	93	228-262	9	55	1.5	0.67	0.79
	R:	CAATAAGAAGATCAGCAGAACAGAC								
Paequ4	**F:**	TGTACTTCCGTTTCCAGCCTC	$(CA)_3GT(CA)_7$	102	249	1	61	1	-	-
	R:	CCAAGTCCAAGGTACCACATTG								
Paequ7	**F:**	TGCAGACCTGACTTTCACAGCTC	$(GT)_{12}$	94	184-192	5	64	1.5	0.29**	0.54
	R:	CCTCCAAACATCCAGCCATC								
Paequ8	**F:**	TATTCTGAGACTTGCGTTATCC	$(CA)_{11}$	77	227-231	3	58	2	0.22	0.24
	R:	GTGATCCATTAGTTGATGTCTACTG								
Paequ10	**F:**	GAAGCTGCACTGGAACTG	$(CA)_8$	69	182-196	8	55	1	0.66*	0.64
	R:	CATGTGGTAAGAATCCAGATG								
Paequ13	**F:**	GACCTGCAGCAATAGCACGAC	$(GT)_9$	68	143-149	4	62	1	0.54	0.49
	R:	TGCCTTCATCAGAATCCTCCTG								

*Significant deviation from HWE in one of the three populations tested
**Significant deviation from HWE in two of the three populations tested

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Cross-species amplification

Six other Procellariiform species were tested to establish whether the White-chinned Petrel (*P. aequinoctialis*) primers amplified polymorphic loci in related species. The protocol for genotyping remained the same as for the source population. The species tested were Spectacled Petrel (*P. conspicillata*), Southern Giant Petrel (*M. giganteus*), Northern Giant Petrel (*M. halli*), Black-browed Albatross (*Thalassarche melanophris*), Grey-headed Albatross (*T. chrysostoma*) and Wandering Albatross (*Diomedea exulans*). Table 1.4 lists the results of the five other procellariiform species used to test cross-species amplification on the designed primers.

Table 1.4: Cross-species amplification using primers developed in *P. aequinoctialis*. P, polymorphic, M, monomorphic, '-', unsuccessful amplification, (n), number of individuals amplified

Locus	*Procellaria conspicillata* n = 5	*Macronectes giganteus* n = 5	*M. halli* *n* = 6	*Thalassarche melanophris* *n* = 6	*T. chrysostoma* *n* = 6	*Diomedea exulans* *n* = 6
Paequ2	P	M	M	P	P	P
Paequ3	P	P	P	P	P	-
Paequ4	M	P	P	-	-	-
Paequ7	P	P	M	M	M	-
Paequ8	P	M	M	M	P	-
Paequ10	P	P	P	P	P	-
Paequ13	P	-	-	P	P	-

Results show six polymorphic loci in the Spectacled Petrel, four loci polymorphic in the Southern Giant Petrel and three loci in the Northern Giant Petrel, four polymorphic loci in the Black-browed Albatross, five polymorphic loci in the Grey-headed Albatross and only one polymorphic locus in the distantly related Wandering Albatross (Kennedy and Page, 2002) where six out of seven loci tested failed to amplify. At this point only polymorphism versus monomorphism was tested in these species and not differences in allele sizes or frequencies.

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CHAPTER 2

PHYLOGEOGRAPHY OF WHITE-CHINNED AND SPECTACLED PETRELS: TAXONOMIC IMPLICATIONS AND POPULATION GENETICS

SUMMARY

The genus *Procellaria* traditionally consists of four species, two that are restricted to the New Zealand region and two that are widespread in the Southern Ocean. All four species are endangered because of the incidental mortality associated with longline fishing. Within the widespread White-chinned Petrel (*Procellaria aequinoctialis*), a spectacled form exists that has recently been elevated to species status, Spectacled Petrel (*P. conspicillata*), based on differences in morphometrics and vocalisations. The Spectacled Petrel is only known to breed on Inaccessible Island, in the Tristan da Cunha archipelago, and its status is considered 'critical' by the IUCN. In this Chapter, the species status of the Spectacled Petrel is investigated using molecular techniques. In addition, the intraspecific phylogeography of the White-chinned Petrel is investigated using partial cytochrome b sequences. Results confirm separate species status of the Spectacled Petrel with a sequence divergence of 1.74% and Nested Clade Analysis suggests that allopatric fragmentation was the process of speciation. Within *Procellaria*, the Spectacled Petrel is a sister taxon to the White-chinned Petrel with the Grey Petrel (*P. cinerea*) as the closest relative. Phylogeographic study of the White-chinned Petrel found that lineages are divided into two distinct geographic regions, a New Zealand regional population and another regional population in the South Atlantic and southern Indian Ocean. Within geographic region variation was small, but sequence divergence between the two regions (0.95%) was as high as between many subspecies within the Procellariiformes. Thus these two regions should be considered as two separate Evolutionary Significant Units. The highest genetic diversity was found in the New Zealand regional population, which is significant for conservation management because the population in this region is relatively small. It appears that the species has expanded its range in the past few hundred thousand years from the New Zealand sector of the Southern Ocean to the current breeding range.

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INTRODUCTION

Taxonomic uncertainties have become a conservation issue because often only recognised species are awarded protection as a result of scarce resources (Frankham *et al.*, 2002). In addition, to implement management plans and to assess impact of disturbances such as incidental mortality in longline fishing, knowledge of the distribution of genetic diversity within a species is important. Natal philopatry and site-fidelity can produce partitioning of genetic variation and produce population sub-structuring (Riffaut *et al.*, 2005). This could make populations vulnerable to local extinctions and potential recolonization or translocations to reintroduce the species difficult (Frankham *et al.*, 2002).

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The genus *Procellaria* belongs to the Procellariidae and traditionally four species have been recognised; *P. aequinoctialis* (White-chinned Petrel - pelagic species with a circumpolar distribution, Figure 2.1), *P. cinerea* (Grey Petrel – pelagic species with a circumpolar distribution), *P. westlandica* (Westland Petrel - breeds in an area of forested hills near the coast at Punakaiki, South Island, New Zealand) and *P. parkinsoni* (Black or Parkinson's Petrel - breeds on Little and Great Barrier Islands off North Island, New Zealand). Ryan (1998) proposed elevating the spectacled form of the White-chinned Petrel to species status (*P. conspicillata*). Of the five *Procellaria*, the Spectacled Petrel is considered 'Critical' (a proposal has been made to lower the status to 'Vulnerable' based on the 2004 census (Ryan *et al.*, 2006), PG Ryan personal communication), the Grey Petrel 'Near Threatened', and the remaining three species 'Vulnerable' under IUCN listings (BirdLife International, 2006).

The IUCN Red List status of *Procellaria aequinoctialis* is 'Vulnerable' as populations are declining and it has the highest incidental mortality risk of all seabirds (Phillips *et al.*, 2006). However, no reliable estimates of historical populations exist and there are few monitoring studies in place to detect current changes in numbers. Birds at sea in Prydz Bay decreased by 86% during 1981-1993 (Woehler, 1996), but the significance at a population level is unknown. *P.*

aequinoctialis is the most common species found near the trawlers off south-eastern Brazil, and constitutes almost all the recorded by-catch from the South African hake longline fishery (Barnes *et al.*, 1997). Recent observations indicate large numbers killed on hake longliners off Namibia (M. Goven personal communication). Several thousands are killed in the Patagonian toothfish (*Dissostichus eleginoides*) fishery (Weimerskirch *et al.*, 1999; Berrow *et al.*, 2000), and an estimated 31,000-111,000 and 50,000-89,000 seabirds were killed in illegal fishery in 1997 and 1998, respectively of which approximately 80% were *P. aequinoctialis* (BirdLife International, 2006). In addition, more than 800 are estimated to be killed annually in the Australian fishing zone (Gales *et al.*, 1998) by the Japanese tuna longline fishery alone. (For a summary of seabird bycatch per fishery and region see Tuck *et al.*, 2003). The current global population estimate for White-chinned Petrels is about 5,000,000 birds (BirdLife-International, 2006), but the population is thought to be declining and the global level of longline mortality is very high (Sullivan, 2003).

Procellaria conspicillata, the Spectacled Petrel, has only recently been separated as a species from *P. aequinoctialis* as a result of differences in plumage, morphometrics and vocalizations (Ryan, 1998; 1999). The species is listed as critical because it is only known to breed on one small island (Figure 2.1) and there is evidence that a proportionally large number of birds are caught as bycatch in the longline fisheries. The only known breeding colony is on the western plateau of Inaccessible Island, Tristan da Cunha Group (Ryan and Moloney, 2000; Ryan *et al.*, 2006). Birds disperse throughout the temperate South Atlantic, with most heading west to the waters off southern Brazil (Enticott and O'Connell, 1985; Olmos, 1997; Olmos *et al.*, 2000). Nests are built in burrows along the banks of river valleys and in adjacent marshy areas above 380 m (Ryan and Moloney, 2000). Recent estimates of breeding pairs on Inaccessible Island are approximately 10,000 pairs indicating population numbers are increasing (Ryan *et al.*, 2006). The extend of incidental mortality is not known. In Brazilian waters in excess of 200 birds were killed per year in the

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1980s, which decreased in the early 1990s, but increased to over 700 birds annually in the late 1990s. Fewer birds are killed in more recent years. In addition, there are anecdotal reports of bycatch mortality in experimental fisheries off Uruguay and no estimates are known for high-seas pelagic fisheries operating in international waters (Ryan, 1999; Ryan and Moloney, 2000; Ryan *et al.*, 2006).

This Chapter has two primary objectives; to infer the evolutionary history of the White-chinned Petrel and the Spectacled Petrel in relation to the other species in the genus *Procellaria*, and to investigate White-chinned Petrel phylogeography to consider the possibility of assigning bycatch to natal colonies.

The following hypotheses are tested:

(i) The Spectacled and White-chinned Petrels are reciprocally monophyletic sister taxa.

(ii) The White-chinned Petrel exhibits natal and site philopatry, and thus this has resulted in genetic partitioning into separate lineages corresponding to colonies or regional populations.

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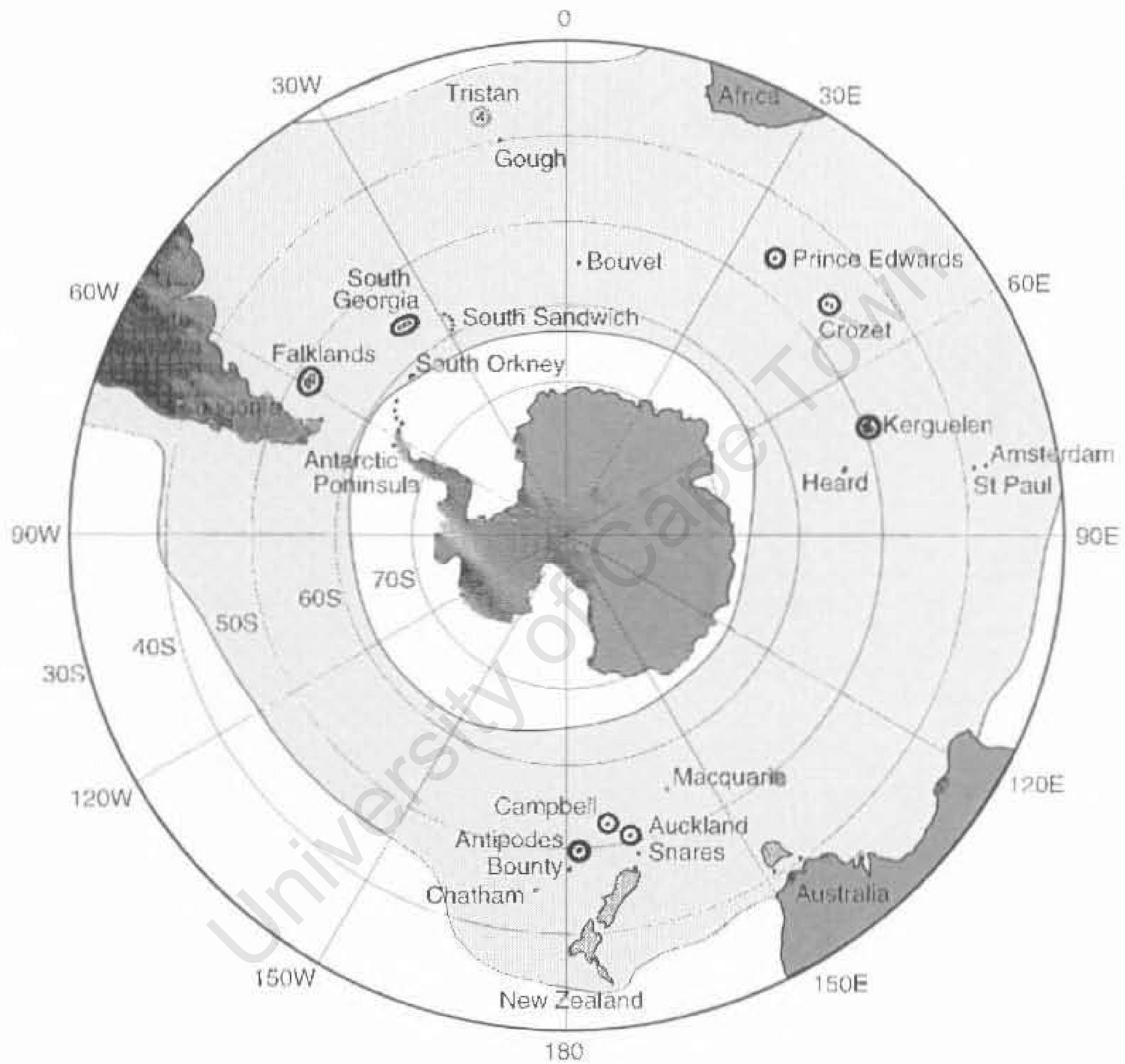
Figure 2.1: The distribution (shaded) and breeding locations of the White-chinned (black circles) and Spectacled Petrel (grey circle).



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MATERIALS AND METHODS

Samples

Tissue and blood samples were collected from 89 White-chinned Petrels from South Georgia (15), Marion Island (19), Iles Crozet (11), Antipodes Island (22), Auckland Island/Disappointment Island (22), as well as 23 Spectacled Petrels from Inaccessible Island (Chapter 2 appendix). Details on all White-chinned Petrel samples with respect to sample locations and whether DNA was extracted from blood, muscle or feathers are available in the appendix (Chapter 4).

Polymerase Chain Reaction (PCR) and sequencing

Internal primers for the *Procellaria* spp. cytochrome b gene were designed by searching for conserved regions in a comparison of *Procellaria aequinoctialis* (Genbank accession number: U74350) and *P. westlandica* (GenBank accession number AF076078) to increase specificity. Primers for polymerase chain reaction (PCR) were designed using DNAMan version 4.13 (Lynnon BioSoft): WCPcytbF (5' AAT CGA CCT ACC TAC TCC 3') and WCPcytbR (5' GAG GAA TAT GAG TGT GAA TC 3') starting at 57 bp of the published *P. aequinoctialis* cytochrome b sequence (Genbank accession number: U74350) and ending at 714 bp resulting in a 658 bp fragment.

Phylogeographic analysis

A nested clade analysis (NCA - Crandall and Templeton, 1993; Templeton and Sing, 1993; Templeton, 1998) was performed on both the White-chinned Petrel and Spectacled Petrels to examine population structure and histories of these two species. NCA recently has become the method of choice for the analysis of intraspecific data (e.g. Templeton, 1998; Gomez-Zurita *et al.*, 2000; Templeton, 2001; Paulo *et al.*, 2002; e.g. Abbott and Double, 2003; Demboski and Sullivan, 2003; Bowie *et al.*, 2004; Lowe *et al.*, 2004; Steeves *et al.*, 2005; Burns and Barhoum, 2006) as well as resolving taxonomic difficulties between sister taxa (Templeton, 2001). Restricted gene flow, range expansion and fragmentation can

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be difficult to differentiate and NCA can provide some insight to distinguish between them, as well as providing an indication of inadequate sampling. The advantage of the method is that a null hypothesis can be tested statistically (Templeton, 2001; Lowe *et al.*, 2004). Haplotypes of both White-chinned and Spectacled Petrels were used to construct a 95% confidence network using the program TCS version 1.21 (Clement *et al.*, 2000) and nesting rules were applied (Templeton *et al.*, 1987; Templeton and Sing, 1993). The program GeoDis version 2.4 (Posada *et al.*, 2000) was used to test for significant associations between haplotypes and geography. The first test is a contingency test which determines if there is a significant association between haplotypes and geography without incorporating geographic distances. The null hypothesis tested is that of no geographical association. The second test incorporates geographical distances and calculates two distance measures, the clade distance (Dc) and the nested clade distance (Dn). The first statistic Dc calculates the weighted average distance of haplotypes from the geographic centre of the clade thus giving the geographic spread of that clade. The second statistic Dn investigates the individuals within a clade, calculating their average distance to the geographic centre of the next immediately inclusive higher clade. The program also investigates the relationship of tip clades versus interior clades. It assumes that tip clades are evolutionary younger than interior clades. Dc and Dn are also calculated for those comparisons (I-T). A modified key to the one presented in appendix of Templeton *et al.,* (1987) was then used to evaluate these results. The modified key is available at zoology.byu.edu/Crandall_lab/geodis.htm.

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RESULTS

A 599 bp sequence of the amplified 658 bp fragment of the mitochondrial cytochrome b gene was analysed. The 599 bp fragment was aligned to the published full sequence of the White-chinned Petrel gene (U74350) from 107 bp to 705 bp of this published sequence. Within the 89 White-chinned and 23 Spectacled Petrel sequences, 25 nucleotide changes were identified, with 15 parsimony informative sites comprising 20 haplotypes (Table 2.1). No insertions or deletions were observed. All changes were point mutation transitions only. Twenty of these changes were synonymous and five non-synonymous. Eighteen substitutions were made at the third codon position, five at the first and two at the second codon position. Average base composition was biased with a deficiency of guanine (G 14.5%, A 26.9%, T 27.8%, C 30.8%) as expected for mitochondrial DNA (mtDNA).

Genetic diversity was measured using haplotype diversity (h), which refers to the uniqueness of a particular haplotype in a given population, and nucleotide diversity (π), which measures the degree of polymorphism in a population (Table 2.2). Individual haplotype diversity ranged from 0 to 0.831 across colonies and nucleotide diversity ranged from 0 to 0.00294, with the highest diversity in the New Zealand islands of Antipodes and Auckland Island (Table 2.2). The Spectacled Petrel had haplotype and nucleotide diversities similar to the Atlantic and Indian Ocean colonies of the White-chinned Petrel. The New Zealand islands had the greatest number of haplotypes (13), which were mostly shared between Antipodes and Auckland and including seven haplotypes represented by a single individual. All eleven individuals from the Crozets had the same haplotype which was also found in high frequency at South Georgia and Marion Island. South Georgia had three haplotypes, one of which was represented by a single individual. Marion Island also had three haplotypes with two represented by a single individual. The Spectacled Petrel had three haplotypes, which were unique and not shared by any White-chinned Petrel.

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Table 2.1: Variable sites in the cytochrome b fragment for the White-chinned Petrel (WCP) and Spectacled Petrel (SP). Nucleotide positions are relative to the published *P. aequinoctialis* sequence (Genbank Accession number U74350). *N* total number of individuals sharing that particular haplotype. In cases where haplotypes were specific to a sampling location it was indicated as follows: South Georgia (SG), Marion Island (M), New Zealand regional population (NZL), Antipodes (An), Auckland (Au). Grey shaded mutations are fixed between the two regional populations of the White-chinned Petrel, yellow shaded mutations are fixed between the White-chinned and Spectacled Petrels.

Haplotype	104	117	135	159	182	164	1[illegible]	207	226	285	306	333	336	375	384	393	453	4[illegible]	462	46[illegible]	481	540	543	576	595	Total *N*
Atlantic-Indian Ocean																										
wcp1	T	C	G	C	A	A	T	T	A	T	A	G	G	G	C	A	T	T	T	A	C	T	C	A	A	37
wcpSG2															T											2
wcpSG3				T																						3
wcpSG4						G																				1
wcpM5										C																1
wcpM6														A												1
New Zealand Islands																										
wcpNZL7								C								G		C			T					11
wcpNZL8								C								G				G	T					4
wcpNZL9								C								G		C			T				G	16
wcpAn10								C				A				G				G	T					1
wcpAn11								C								G		C			T	C			G	1
wcpNZL12								C					A			G		C			T					2
wcpAn13							C	C								G		C			T					2
wcpNZL14								C								G					T					3
wcpAu15								C								G	C	C			T				G	1
wcpAu16								C	G				A			G		C			T					2
wcpAu17				T				C								G				G	T					1
Inaccessible Island (*Procellaria conspicillata*)																										
sp1	C	T						C			G	A							C		T		T	G		15
sp2	C	T			G			C			G	A							C		T		T	G		4
sp3	C	T	A					C			G	A							C		T		T	G		4

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Table 2.2: Diversity indices calculated for colonies of White-chinned and Spectacled Petrels, including the two regional populations of White-chinned Petrels. Marion Island and Iles Crozet are combined as Indian Ocean for comparison.

	Number of polymorphic sites	Number of haplotypes	Average number of Nucleotide Differences between sequences (k)	Nucleotide Diversity π ± SD	Haplotype Diversity Hd ± SD
P. aequinoctialis					
S.Georgia	3	4	0.72	0.00121 ± 0.003	0.62 ± 0.12
Marion Is	2	3	0.21	0.00035 ± 0.00021	0.21 ± 0.119
Iles Crozet	0	1	0	0	0
Antipodes	7	8	1.52	0.0025 ± 0.00044	0.79 ± 0.069
Aukland Is	6	8	1.76	0.0029 ± 0.00047	0.83 ± 0.053
Regions					
New Zealand	11	11	1.63	0.0027 ± 0.00033	0.8 ± 0.043
Indian Ocean	2	3	0.13	0.00022 ± 0.00014	0.13 ± 0.082
Atlantic/Indian Ocean	5	6	0.04	0.00058 ± 0.00017	0.32 ± 0.089
P. conspicillata	2	3	0.53	0.00088 ± 0.00026	0.49 ± 0.126

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There were six fixed mutational sequence differences and no shared mutations between haplotypes of White-chinned and Spectacled Petrels. The Spectacled Petrel showed an average number of nucleotide differences ((k) = 0.53 and π = 0.0009 ± 0.0003), whereas the White-chinned Petrel is significantly more diverse with a k of 2.92 and π of 0.0049 ± 0.0006. The haplotype composition showed clear geographical structure (Figures 2.2-4). Within *P. aequinoctialis* there was a split between the New Zealand islands and the three islands located in the south Atlantic and the south Indian Oceans. This is illustrated by the lack of shared haplotypes between the above mentioned regions and three mutational steps between the nearest haplotypes. The region comprising the Atlantic and Indian Ocean populations could not be further separated. There is a significant but small difference between South Georgia and Marion Island (Gst 0.09, $p<0.05$), but the two colonies share one haplotype, which occurs at a high frequency. G_{ST} values (Table 2.3) ranged from zero (Marion Is vs. Iles Crozet, and Antipodes Is vs. Auckland Is) to 0.97 (*P. conspicillata* vs. Iles Crozet, $p<0.0001$) with the highest significant values being comparisons of *P. conspicillata* to all of the *P. aequinoctialis* colonies. Second highest values were comparisons of the two Indian Ocean islands to the two New Zealand islands. South Georgia was significantly different from Marion Island but not from Iles Crozet. There was no significant difference between colonies within an oceanic region.

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Table 2.3: Matrix of pairwise comparisons of G_{ST} (above diagonal) and corresponding *p*-values indicating significant difference from zero when $p<0.05$ for colonies of White-chinned and Spectacled Petrels (*P. conspicillata*) below diagonal.

	SG	Marion	Crozet	Antipodes	Auckland	*P. conspicillata*
SG		0.088	0.06418	0.76431	0.72928	0.93599
Marion	0.0079		0	0.80831	0.77552	0.9616
Crozet	0.1527	0.9997		0.78808	0.74885	0.96514
Antipodes	0.001	0.001	0.001		0.001	0.88951
Auckland	0.001	0.001	0.001	0.5055		0.87547
P. conspicillata	0.001	0.001	0.001	0.001	0.001	

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Sequence divergence (Table 2.4) for colonies of the White-chinned Petrel ranged from 0.02% to 0.82%, with the highest divergence between colonies from different oceans and in particular between New Zealand colonies and those in the Atlantic and Indian Oceans. When the New Zealand islands were grouped together and compared to the combined south Atlantic and south Indian Ocean colonies, sequence divergence was 0.96%. The lowest values were obtained between colonies within an oceanic region, but in contrast to G_{ST} values Antipodes and Auckland Island differed more than any of the colonies of the other two ocean basins.

Sequence divergence between the White-chinned Petrel and Spectacled Petrel (Table 2.5) was higher than any within species sequence divergence for the White-chinned Petrel (1.74%) but marginally lower than other comparisons between *Procellaria* species (Table 2.5), which ranged from 2.5% (*conspicillata/cinerea*) to 4.5% (*cinerea/pakinsoni*).

Table 2.4: Nucleotide divergence (Dxy) among colonies of the White-chinned Petrel given as percentage.

	S Georgia	Marion Is	Iles Cozet	Antipodes	Aukland Is
S Georgia					
Marion Is	0.084				
Iles Cozet	0.067	0.018			
Antipodes	0.841	0.792	0.774		
Aukland Is	0.819	0.774	0.756	0.267	

Table 2.5: Nucleotide divergence (k) between *Procellaria* species given as percentage. Sequences other than the White-chinned and Spectacled Petrels were taken from GenBank.

	P. aequinoctialis	*P. conspicillata*	*P. cinerea*	*P. westlandica*	*P. parkinsoni*
P. aequinoctialis					
P. conspicillata	1.74				
P. cinerea	3.47	2.5			
P. westlandica	3.38	2.67	3.17		
P. parkinsoni	4.39	3.78	4.51	3.01	

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Total molecular variance within and between White-chinned and Spectacled Petrels was partitioned into hierarchical components using separate analysis of molecular variance. AMOVA results suggest that 75.1% ($p < 0.0001$) of variation can be explained by variance between the two petrel species. Within the White-chinned Petrel 79.8% ($p<0.0001$) of variance was explained between the two regional populations, with 20.19% residing within regional populations, 70.6% ($p<0.0001$) between colonies and 29.4% occurring within colonies. The high percentage between colonies can be explained by the comparisons between colonies in the different regional populations, making it likely that divergence is a result of geographical distance and genetic drift.

Gene trees (Figure 2.3-4) constructed using Bayesian Inference and Neighbour Joining, show that haplotypes are clearly partitioned into three clusters corresponding to *P. conspicillata* and the two regional populations of *P. aequinoctialis*. Cladistic (MP) and Bayesian methods resulted in similar tree topologies. Bootstrap support for the MP tree and probability support for the Bayesian tree were similar, both indicating strong support for the above topology. A minimum spanning network shows the same structuring into three groups, with *P. conspicillata* and *P. aequinoctialis* separated by eight mutational steps whereas the two regional populations of *P. aequinoctialis* are separated by a maximum of three steps (Figure 2.2).

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A NJ tree (Figure 2.4) was constructed with all five *Procellaria* species in which there is a high bootstrap support for three lineages of the White-chinned Petrel, one showing the Atlantic and Indian Ocean haplotypes as a monophyletic group and two groups for the New Zealand colonies. The Spectacled Petrel forms a monophyletic group branching off from the White-chinned Petrel and Grey Petrel clade. The Westland Petrel and Parkinson's Petrel are the two species restricted to the Pacific and are most closely related to each other. The same topology was recovered by rooting the tree with *Bulweria bulwerii* (Bulwer's Petrel, closest relative to *Procellaria* (Nunn and Stanley, 1998)) and the more distant Northern Fulmar *Fulmarus glacialis* (data not shown). A Bayesian tree (data not shown) including all *Procellaria* species showed similarly high probability support and topology to the NJ tree with the closest relative to the White-chinned Petrel and Spectacled Petrel being the Grey Petrel.

In the nested clade analysis, data were clustered into one 4-step clade and two 3-step clades that separated the Spectacled Petrel from the White-chinned Petrel (Figure 2.5). The null hypothesis of no geographical association could only be rejected for the clades 2-1 (X^2= 14.0, p=0.015), 3-1 (X^2=17.6, p=0.031) and the total cladogram (X^2=17.0, p=0.04), but clade 1-1 had significant distances within

the clade although the null hypothesis could not be rejected. Using the inference key, clade 1-1 showed range expansion but lacked sufficient sampling to distinguish between contiguous expansion, long distance colonization or past fragmentation. The inference key for both the 2-1 and 3-1 clades suggested restricted gene flow with isolation by distance. The total cladogram consisted of two 3-step clades. Clade 3-1 included all the White-chinned Petrels and clade 3-2 included all Spectacled Petrels. Clade 3-1 had both significantly small Dc and Dn values whereas clade 3-2 had a large significant Dn. No tip-interior relationship could be inferred as both clades were assumed to be tip clades. However, the results suggest allopatric fragmentation.

Mismatch Distributions (Figure 2.7) were constructed for the White-chinned Petrel and the Spectacled Petrel as well as for the two regional groups identified in the White-chinned Petrel. The distribution for the Spectacled Petrel shows a fit of the model with both SSD ($p = 0.148$) and Harpending's r ($p = 0.362$), but Fu's Fs shows no significant deviation from neutrality ($p = 0.325$). This discrepancy could be explained by the fact that the distribution for the Spectacled Petrel is based on only three haplotypes. Similarly, the distribution for the White-chinned Petrel fits the model of expansion for SSD and r, but also has a non-significant although negative Fs value ($p< 0.103$). When regional populations of the White-chinned Petrel were examined, each fit the model of a recent expansion in all three statistics but the Atlantic/Indian group seems to lack the characteristic peak.

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Figure 2.2: Minimum Spanning Network from 599 bp of partial cytochrome b sequence from 20 White-chinned Petrel and Spectacled Petrel haplotypes (details regarding locations and haplotypes coding are described in Table 2.1). Small circles represent haplotypes found in one individual only. The size of ovals and larger circles is approximately proportional to the number of individuals sharing that haplotype. Single lines connecting haplotypes indicate one mutational difference; crossing lines indicate a number different to one.

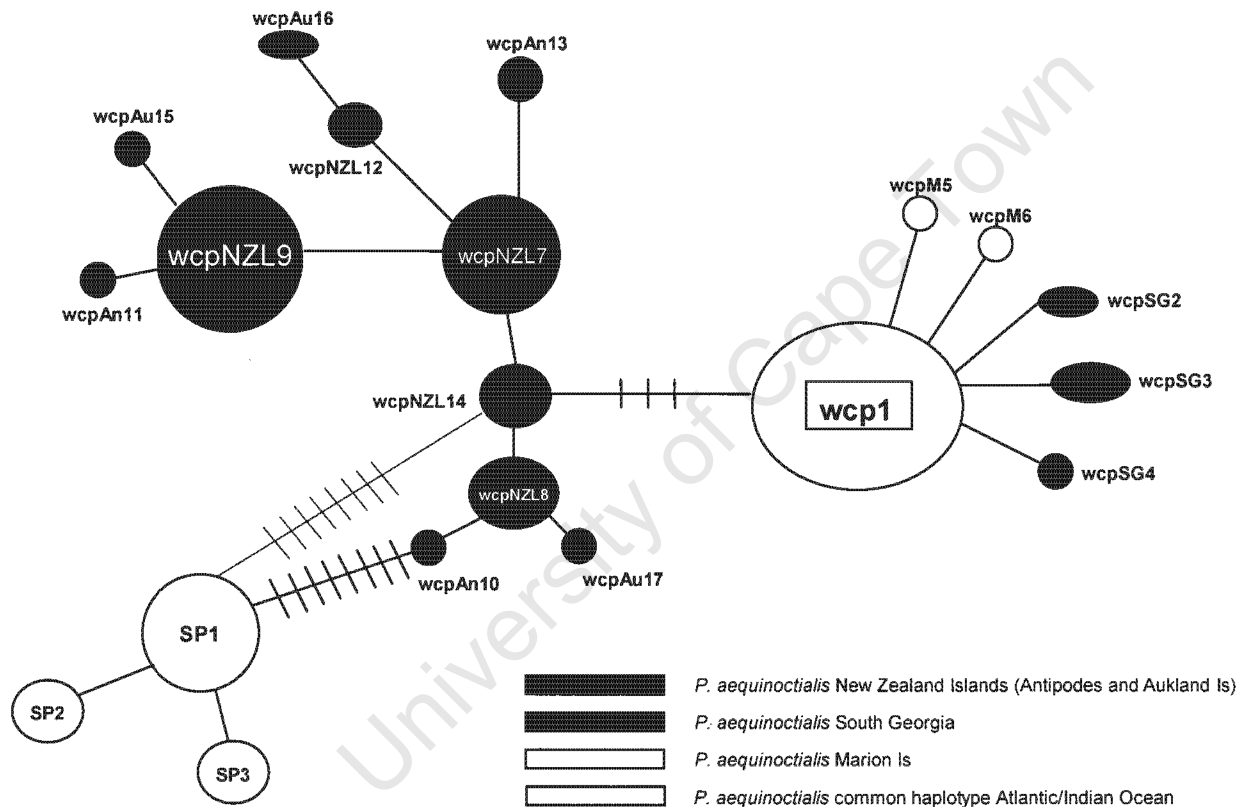

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Figure 2.3: Unrooted tree constructed using Bayesian Inference with haplotypes from the White-chinned Petrel and the Spectacled Petrel. The Grey Petrel *P. cinerea* was included as reference point to show relationships between clades. Probability values are given indicating support for the topology. The same topology was shown by trees constructed with Maximum Parsimony and Neighbor Joining. Red, Spectacled Petrel; blue, White-chinned Petrel haplotypes found in the Atlantic/Indian Ocean regional population; green, White-chinned Petrel haplotypes found in the New Zealand regional population; black *P. cinerea*.

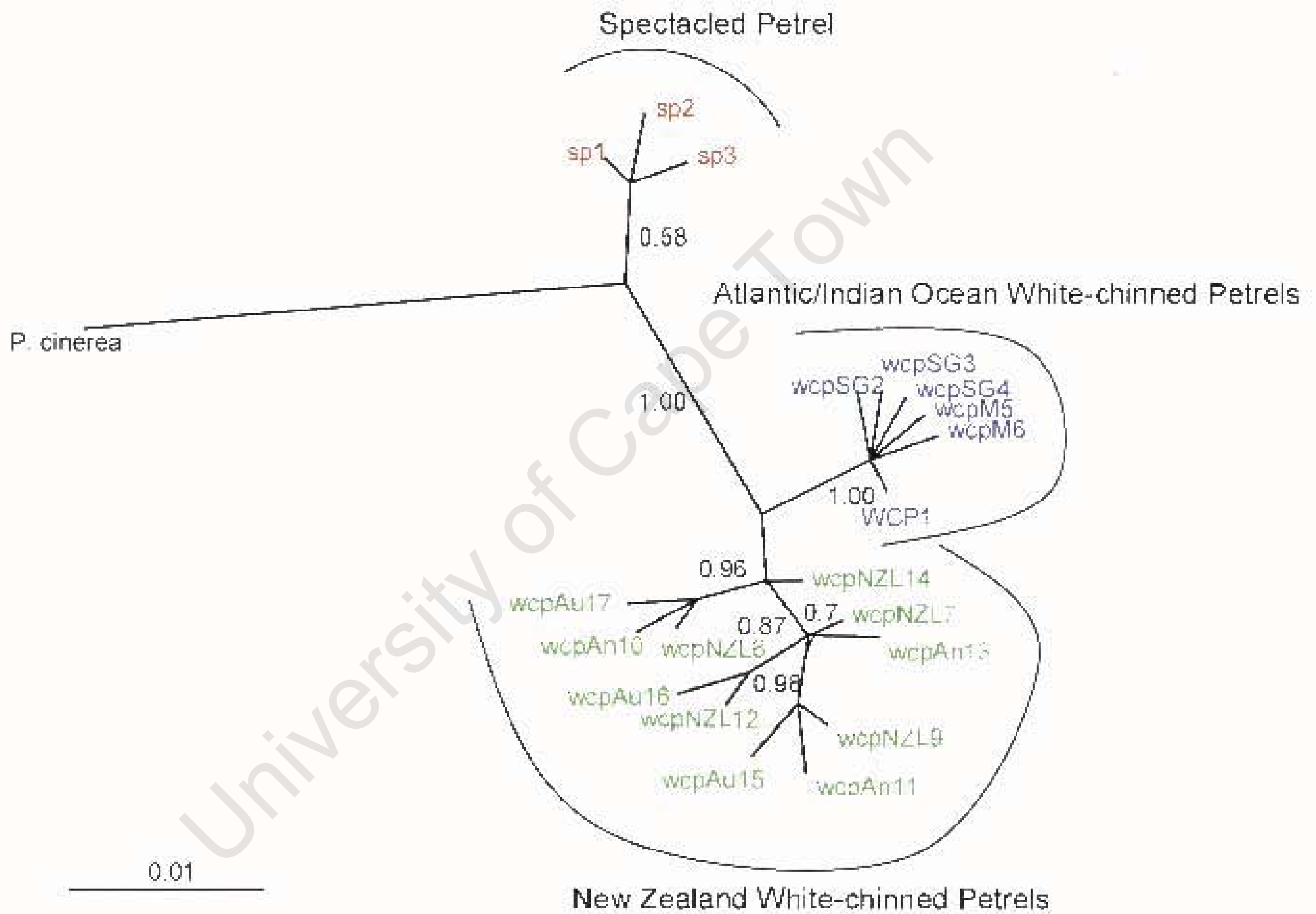

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Figure 2.4: Neighbour Joining reconstruction of the phylogenetic relationships and bootstrap values (after 1000 replicates) within the *Procellaria* genus based on 599 bp of the mitochondrial cytochrome b gene and using Kimura-2-Parameter corrected distances as the model. White-chinned Petrel haplotypes are shown as circles, with non-solid circles being haplotypes found in the New Zealand regional population and solid circles being haplotypes found in the other regional population. Triangles represent haplotypes found within the Spectacled Petrel.

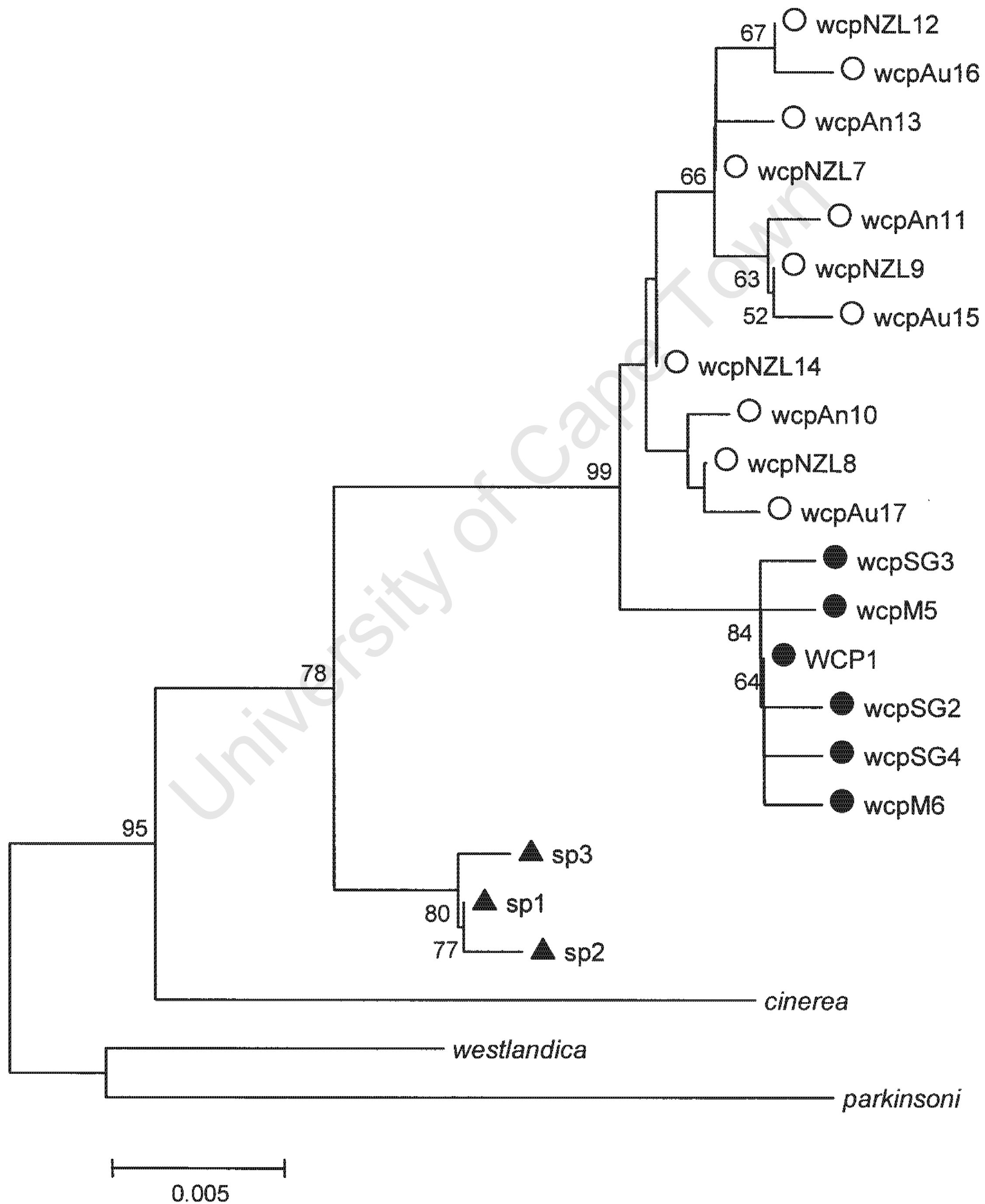

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Figure 2.5: Unrooted network of cytochrome b haplotypes (*P. aequinoctialis*) inferred using statistical parsimony and associated nested clade design. Lines connecting haplotypes represent single mutational steps; black dots represent unsampled haplotypes, circles represent haplotypes sampled in not more than two individuals; squares represent haplotypes sampled in more than two individuals, green represents haplotypes found in the Spectacled Petrel; red represents haplotypes found in the White-chinned Petrel Atlantic/Indian Ocean regional population; blue represents haplotypes found in the New Zealand regional population

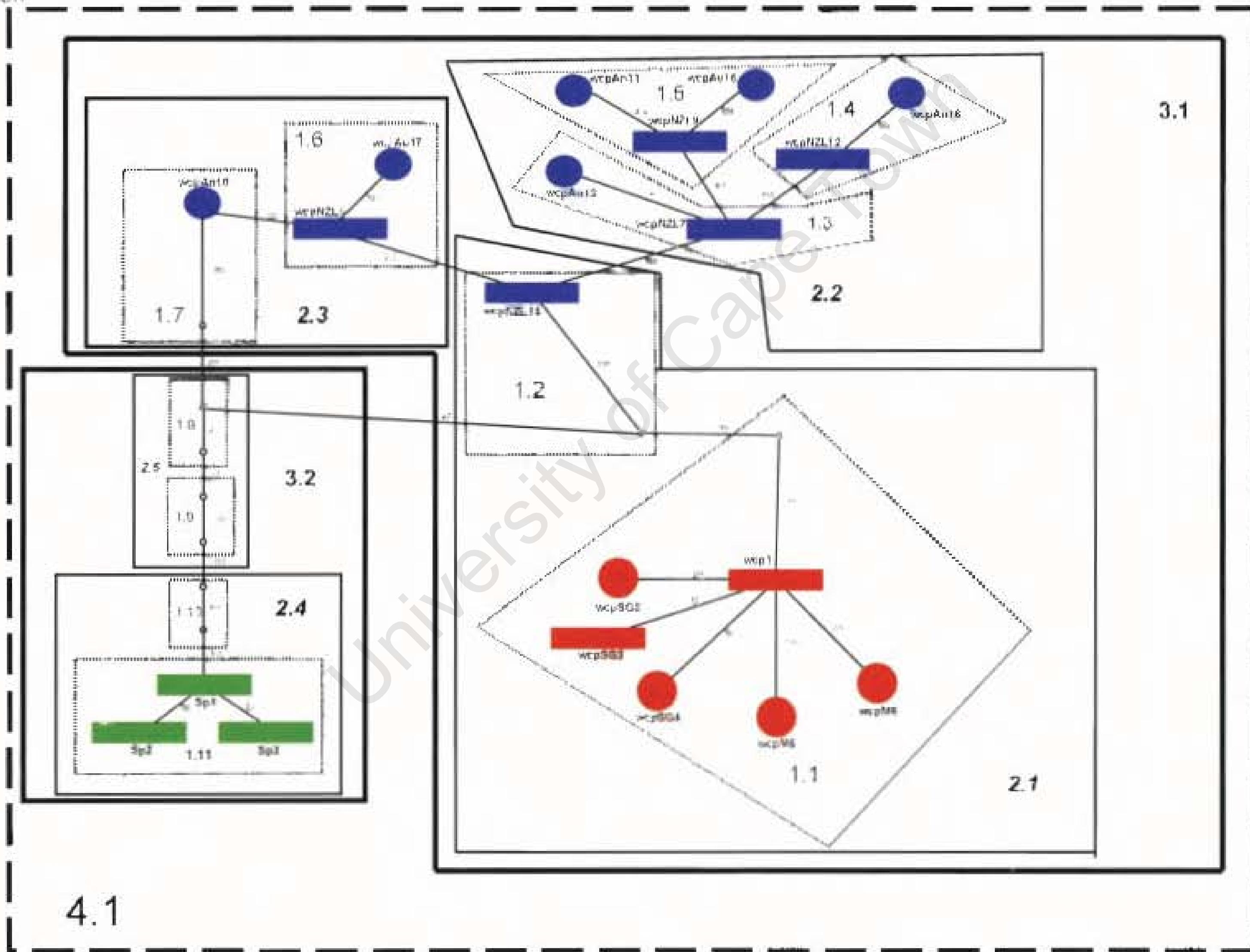

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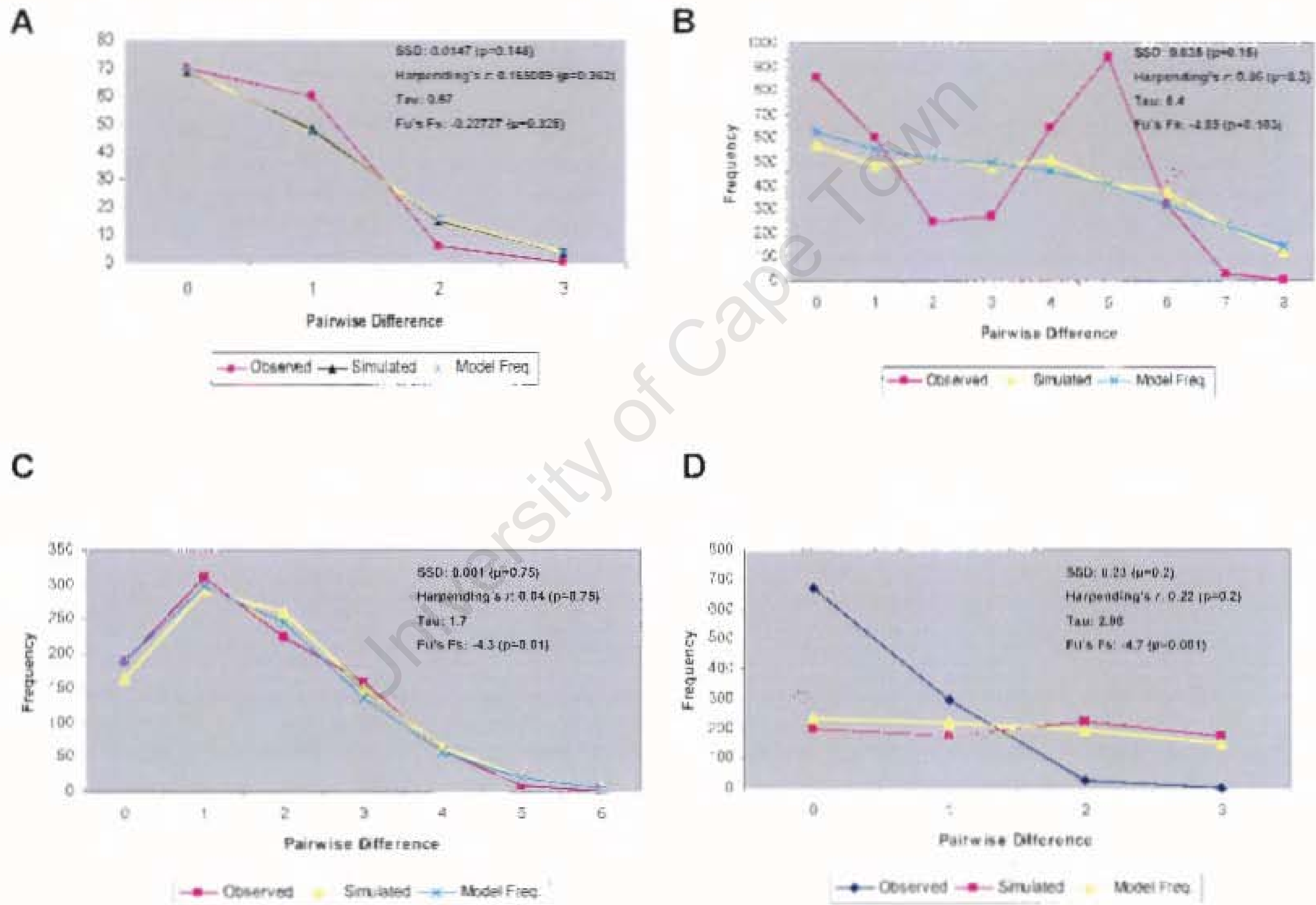
Figure 2.6: Mismatch Distributions for the Spectacled Petrel (A), the White-chinned Petrel (B), and regional populations of White-chinned Petrel from New Zealand (C) and Atlantic/Indian Ocean (D)



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DISCUSSION

Spectacled Petrel species status

Within the Procellariiformes, cytochrome b sequences have been used to investigate the intraspecific relationships of the Black-footed Albatross, *Phoebastria nigripes* (Walsh and Edwards, 2005), the European Storm Petrel, *Hydrobates pelagicus* (Cagnon *et al.*, 2004), and the interspecific relationship between the Yelkouan, *Puffinus yelkouan* and the Balearic Shearwaters, *P. mauretanicus* (Genovart et al., 2005), all of which are northern hemisphere species. In the investigation by Genovart *et al.,* (2005), sequence divergence was found to be 1.6% between sympatric species, which is slightly lower than that found between the Spectacled Petrel and the White-chinned Petrel (1.7%). The Spectacled Petrel and White-chinned Petrel form monophyletic clades in all three tree algorithms and have six fixed differences between them. Also the Minimum Spanning Network and highly significant G_{ST} values show distinct structuring between the two species. The lack of shared haplotypes indicates that females do not migrate between the colonies in which the two forms occur. The Spectacled Petrel is endemic to the Atlantic Ocean, although there may have been colonies in the Indian Ocean in the past (Ryan, 1998). The Minimum Spanning Network and G_{ST} values show that the Spectacled Petrel is more closely related to the New Zealand population of White-chinned Petrels. Two other *Procellaria* species, the Westland Petrel and the Parkinson's Petrel, are confined to the New Zealand region. The highest genetic diversity of the White-chinned Petrel resides within the New Zealand colonies. Taken together these results suggest that the genus originated, or at least diversified, around New Zealand. Although interpreted with some caution, especially in the case of the Spectacled Petrel where only three haplotypes were used for the analysis, mismatch distributions for both White-chinned and Spectacled Petrel show recent expansions within populations, with two waves of dispersal from the New Zealand region; one to subantarctic islands in the Atlantic and Indian Oceans and one to more temperate regions resulting in the Spectacled Petrel. This is supported by the peaks of the mismatch distribution curves, which indicate the

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time of expansion, differing slightly for both species, with the White-chinned Petrel expansion from New Zealand preceding that of the Spectacled Petrel.

Species status for the Spectacled Petrel was originally proposed based on consistent differences in vocalisations, morphometrics and plumage (Ryan, 1998). There are several concepts that attempt to define what constitutes a species (e.g. Frankham *et al.*, 2002; Avise, 2004). Several concepts have been proposed (Frankham *et al.*, 2002) with the most used concept being the Biological Species Concept (Frankham *et al.*, 2002). In essence the concept defines a species on the grounds of reproductive isolation (Dobzhansky, 1937; Mayr, 1963). In the case of the White-chinned Petrel and Spectacled Petrel, it is debatable whether these two species are isolated in a physiological sense, but different mating seasons are considered a valid isolating mechanism (Lowe *et al.*, 2004). One of the versions of the Phylogenetic Species Concept (Cracraft, 1983) describes a species as the minimum diagnosable unit and thus the smallest group of individuals that are distinguishable by a unique combination of character states (Avise, 2004; Lowe *et al.*, 2004). Six fixed mutational differences between sequences of the two forms in the cytochrome b gene qualify as unique character states, and thus under this concept, the two species would be recognised. Another version of the concept considers monophyletic groups as the basis of distinguishing species (Avise, 2004). As all three tree algorithms demonstrate both species to be monophyletic groups, this provides further evidence for species status. The Evolutionary Species Concept (Simpson, 1951) defines a species as an entity that can maintain its evolutionary history through time and space (Avise, 2004; Lowe *et al.*, 2004). The Spectacled Petrel appears to have had an independent evolutionary history and several fixed differences are apparent, as well as female philopatry. Therefore within the framework of the Biological, Phylogenetic and Evolutionary Species Concepts, species status is confirmed for the Spectacled Petrel.

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Interspecies relationships

In relation to the other *Procellaria*, the Spectacled Petrel is a sister taxon to the White-chinned Petrel. Sequence divergence between the two species is low (1.7%) compared to other established species and also compared to within genus pairwise comparisons. The Grey Petrel emerges as the closest relative to the two taxa in all gene trees. A rooted tree shows a common ancestor for the two New Zealand endemics, the Westland Petrel and the Parkinson's Petrel. These species share a common ancestor with the Grey Petrel, which is basal to the White-chinned Petrel and Spectacled Petrel, again suggesting a New Zealand origin for the genus. It also shows that within the genus, the White-chinned Petrel and the Spectacled Petrel are young species, which is supported by the lack of transversions within sequence comparisons. Allopatry was suggested as the driving force for speciation in the White-chinned and Spectacled Petrels. Range contraction during the last ice age could have led to local adaptations to specific oceanographic conditions (shown in shearwaters, Gomez-Diaz *et al.*, 2006), different foraging locations (Phillips *et al.*, 2006) and this, coupled with strong site fidelity, resulted in speciation.

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Phylogeography of the White-chinned Petrel

Within the White-chinned Petrel, phylogeographic structure was observed that corresponded to distinct ocean basins and two distinct regional populations could be identified that shared no common haplotypes. The New Zealand regional population contained the greater genetic diversity but has fewer breeding pairs than the other regional population. Sequence divergence between the two regional populations is 0.955% which is comparable to other studies of subspecies (Avise, 2004; Cagnon *et al.*, 2004; Walsh and Edwards, 2005). In these studies partial cytochrome b sequences were used to define two regional populations in the Black-footed albatross (Walsh and Edwards, 2005) and to define subspecies in the Storm Petrel *Hydrobates pelagicus* (Cagnon *et al.*, 2004) with a sequence divergence of 0.8% in the latter. Within each region of the White-chinned Petrel, small non-significant G_{ST} values were observed with the

exception of the South Georgia to Marion Island pairwise comparison (G_{ST} 0.088, p=0.0079). G_{ST} between regions however, was highly significant. In addition three fixed differences were found between the two regions.

The Nested Clade Analysis (NCA) detected patterns of geographic associations that were similar to the pattern shown by G_{ST} values. The inference key suggested restricted gene flow by isolation-by-distance for the within species groups. For the between species clades allopatric fragmentation was suggested. It has been suggested that NCA is not always reliable in establishing differences between recurrent gene flow and historical processes (Knowles and Maddison, 2002), and results should be viewed with caution. It is always important to compare these results with other evidence to draw inferences of population structure. For the White-chinned Petrel, the data indicate two separate regions. Petrels are strongly philopatric to natal sites (e.g. Weimerskirch *et al.*, 1985; Warham, 1990) and immature birds spend six to ten years at sea before returning to breed. G_{ST} values, sequence divergence and tree algorithm suggest that females do not migrate from one region to the other to breed. Therefore the NCA suggestion of isolation-by-distance is likely. Allopatric fragmentation is a common form of speciation (Frankham *et al.*, 2002). Seabirds are restricted to islands to breed and are dependant on certain ocean currents for foraging grounds. The New Zealand region is a centre of Procellariiform diversity (Abbott and Double, 2003) and thus it is a likely source of expansion to other areas of the Southern Ocean. This is also supported by the following evidence; haplotype numbers for both the Atlantic/Indian Ocean group of the White-chinned Petrel and the Spectacled Petrel are much lower than for the New Zealand group. In addition, more mutational steps are required to connect New Zealand haplotypes than are required to connect haplotypes in the other regional population, making it older in terms of evolution. Prevailing winds would have facilitated dispersal from New Zealand towards South Georgia and from there to Kerguelen and finally Marion Island and Iles Crozet in a stepping stone fashion. Taken together,

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this supports the hypothesis of range expansion from the New Zealand region and later allopatric speciation.

Currently the 'stronghold' of the White-chinned Petrels in terms of population numbers, is South Georgia with approximately 2,000,000 breeding pairs. However, most genetic diversity resides in the New Zealand regional population, where numbers are decreasing (Taylor, 2000). The reduced level of genetic diversity in the other regional population is consistent with expansion events and founder effects. In terms of management, it is important to maintain the genetic diversity of the New Zealand population.

Evolutionary Significant Units (Ryder, 1986) are defined as genetically differentiated populations within a species. Initially the definition included populations with a shared evolutionary history and reproductive isolation as well as adaptive distinctions from other populations. It was then suggested that molecular markers should be used to distinguish between populations (Moritz, 1995). When using mtDNA markers, a significant divergence and monophyly with regard to other populations was searched for; and with nuclear markers a significant divergence of allele frequencies (Avise, 2004). Recently the definition of an ESU has been debated (e.g. Pennock and Dimmick, 1997; Paetkau, 1999; Crandall *et al.*, 2000) mainly because of contradictory species concepts that underlie its definitions. Therefore one of the recommendations is one of flexibility in designating ESU status and including lineages that show restricted gene flow to other such lineages and some form of lineage sorting (Fraser and Bernatchez, 2001). Thus in conclusion, since the two regions of White-chinned Petrel have a comparable sequence divergence to recognised subspecies and show monophyly, they should be considered two separate ESUs. This hypothesis can then be corroborated by nuclear markers.

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Gene flow and feasibility of assigning bycatch within the White-chinned Petrel

Strong natal philopatry and site fidelity can make populations vulnerable to local extinctions, this is further complicated by the high adult survival rate of adults and low annual fecundity (Riffaut *et al.*, 2005). Mortality at sea shows a male bias (Ryan and Boix-Hinzen, 1999) introducing skews in sex ratios that may take a long time to rectify. Seabirds are difficult to monitor once they leave colonies to forage and making it difficult to predict the population structure in foraging ranges. Impacts of the above mentioned dangers are difficult to assess. Assignment tests have been used for a variety of reasons (discussed in more detail in Chapter 3), including the study of dispersal (Paetkau *et al.*, 1995). The phylogeography of the White-chinned Petrel showed two regional populations but no fine-scale structure. Birds caught as bycatch in New Zealand and south of the African continent were shown to either have haplotypes found in known birds or derived haplotypes. As three fixed mutational differences can be found between the two regional populations, an easy test could be designed to screen bycatch samples to determine from which region they originated. If one or more of these differences includes a restriction enzyme site that cuts in one region but not the other, quick and easy identification could be achieved (discussed in more detail in Chapter 4). Twenty-three birds caught as bycatch were sequenced. Results show that all birds caught within the New Zealand region possessed a haplotypes found or derived from haplotypes within the New Zealand regional population. The same was shown to be the case for birds killed in the Atlantic or Indian Ocean, and all were assigned to this regional population and none to New Zealand. It indicates that foraging locations are not shared by females of this species. This has been shown in at least White-chinned Petrels from South Georgia, which seem to remain in the southwest Atlantic (Phillips *et al.*, 2006). In order to test this hypothesis, a greater number of samples need to be screened and a nuclear marker must be used to see if this pattern also holds true for males.

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Concluding remarks

This part of the thesis investigated the species status of the Spectacled Petrel and the phylogeography of its sister species, the White-chinned Petrel. Cytochrome b data was used to investigate the separate species status from the White-chinned Petrel. Six fixed mutational differences and a sequence divergence of 1.7% was found between the two sister species. Data indicated that the Spectacled Petrel has had an independent evolutionary history and is likely to maintain its genetic independence in the future. Therefore, independent species status is merited. Cytochrome b data also indicated that the White-chinned and Spectacled Petrel most likely originated within the New Zealand region and speciated after the last glacial cycles expanding into the other ocean basins.

Within the White-chinned Petrel, cytochrome b identified two distinct regional populations; the New Zealand population and the Atlantic/Indian Ocean population. No female mediated gene flow between these two regional populations was apparent using cytochrome b DNA, and pending nuclear DNA analysis, it was suggested that these two regions should be managed as separate Evolutionary Significant Units.

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APPENDICES

Table A1: List of Spectacled Petrel samples used in genetic analysis showing location and collection details.

Sample ID	Origin	Sample Material
795501	Inaccessible Island	Blood
795504	Inaccessible Island	Blood
795506	Inaccessible Island	Blood
795507	Inaccessible Island	Blood
795543	Inaccessible Island	Blood
795544	Inaccessible Island	Blood
795545	Inaccessible Island	Blood
795546	Inaccessible Island	Blood
795547	Inaccessible Island	Blood
795548	Inaccessible Island	Blood
795550	Inaccessible Island	Blood
795552	Inaccessible Island	Blood
795553	Inaccessible Island	Blood
795554	Inaccessible Island	Blood
795555	Inaccessible Island	Blood
795556	Inaccessible Island	Blood
795557	Inaccessible Island	Blood
795569	Inaccessible Island	Blood
795570	Inaccessible Island	Blood
795586	Inaccessible Island	Blood
795587	Inaccessible Island	Blood
795609	Inaccessible Island	Blood
795613	Inaccessible Island	Blood

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Table A2: Mitochondrial cytochrome b alignment using the published sequence of the White-chinned Petrel and found haplotypes as well as outgroups used in the analysis. Published sequences are in lower case, lower case letters within haplotypes indicate nucleotides edited by visual inspection and/or reverse sequencing.

published WCP	1	ctctcctagg	catctgccta	ataacccaga	tcctaactgg	40
WCP1	1	CTCTCCTAGG	CATCTGCCTA	aTAACCCAGA	TCCTAACTGG	40
wcpSG2	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpSG3	1	CTCTCCTAGG	catCTGCCTA	ATAACCCAGA	TcCTAACTGG	40
wcpSG4	1	CTCTCcTAGG	CATCTGCCTA	ATAACCCAGA	TcCTAACTGG	40
wcpM5	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpM6	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpNZL7	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpNZL8	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpNZL9	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAn10	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAn11	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpNZL12	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAn13	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpNZL14	1	CTCTCcTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAu15	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAu16	1	cTcTCCtAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpAu17	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TcCTAACTGG	40
wcpB18	1	CTCTCCTAGG	CAtCtgCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpB19	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpB20	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpB21	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
wcpB22	1	CTCTCCTAGG	CATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
sp1	1	CCCTCCTAGG	TATCTGCCTA	ATAACCCAGA	TCCTAACTGG	40
sp2	1	CcCTCCTAGG	TATCTGCCTA	atAaCCCAGA	TCCTAaCTGG	40
sp3	1	CCcTCCTAGG	TaTCTGCCTA	ATAACCCAAA	TCCTAACTGG	40
westlandica	1	ccctcctagg	tatctgccta	ataacccaaa	tcctaactgg	40
cinerea	1	ccctcctagg	tatctgccta	ataacccaaa	tcctaactgg	40
parkinsoni	1	ccctcctagg	tatctgctta	ataacccaga	tcctaactgg	40
Bulweria bulwerii	1	ccctcctagg	tatctgccta	ataactcaaa	tcctaaccgg	40
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wcpSG2	41	TCTACTACTA	GCTATACACt	AtACCGCTGA	TACAACCCTA	80
wcpSG3	41	tCTACTACTA	GtTATAcAct	aTACcGCTGA	TACAACCCTA	80
wcpSG4	41	TCTACTACTA	GcTATACGct	AtacCGCTGA	TACAACCCTA	80
wcpM5	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
wcpM6	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
wcpNZL7	41	TCTACTACTA	GcTaTACAcT	ATACCGCTGA	TACAACCCTA	80
wcpNZL8	41	TCTACTACTA	GcTATACACt	ATACCGCTGA	TACAACCCTA	80
wcpNZL9	41	TCTACTACTA	GCTATACACT	AtACCGCTGA	TACAACCCTA	80
wcpAn10	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
wcpAn11	41	TCTACTACTA	GcTATaCACT	ATACCGCTGA	TACAACCCTA	80
wcpNZL12	41	TCTACTACTA	gcTATacACt	ATACCGCTGA	TACAACCCTA	80
wcpAn13	41	TCTACTACTA	GCTATacacT	ATACCGCTGA	CACAACCCTA	80
wcpNZL14	41	TCTACTACTA	GcTATACAcT	ATACCGCTGA	TACAACCCTA	80
wcpAu15	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
wcpAu16	41	TCTACTACTA	gcTATACAct	ATAccGCTGA	TACAACCCTA	80
wcpAu17	41	TCTACTACTA	GtTATAcACT	AtaCCGCTGA	TACAACCCTA	80
wcpB18	41	TCTACTACTA	GcTATAcaCt	aTACCGCTGA	TACAACCCTA	80
wcpB19	41	TCTACTACTA	GCTaTACACT	ATACCGCTGA	TACAACCCTA	80
wcpB20	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
wcpB21	41	TCTACTACTA	GCTaTACACT	ATACCGCTGA	TACAACCCTA	80
wcpB22	41	TCTACTACTA	GcTATACACT	ATACCGCTGA	TACAACCCTA	80
sp1	41	TCTACTACTA	GCTATACACT	ATACCGCTGA	TACAACCCTA	80
sp2	41	tCTACTACTA	GcTATGCACT	ATACCGCTGa	TACAACCCTA	80
sp3	41	TCTACTACTA	gctAtAcACt	AtACCGCTGA	TACAACCCTA	80
westlandica	41	cctactacta	gctatacact	ataccgctga	tacaacttta	80
cinerea	41	tctactacta	gctatacact	acactgctga	tacaacctta	80
parkinsoni	41	cctactacta	gctatacact	ataccgctga	tacaacctta	80
Bulweria bulwerii	41	cctactatta	gccatacact	ataccgctga	cacaacccta	80
published WCP	81	gctttttcat	ccgttgctca	tacatgtcga	aacgtacaat	120

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Table A2 continued

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WCP1               81   GCTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpSG2             81   GCTTTTTCAT  CCgTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpSG3             81   GCTTTTTCAT  CCgTTgcTcA  TAcAtGTCGA  AACGTACAAT   120
wcpSG4             81   GCTTTTTCAT  CCgTTgCTCA  TACATGTCGA  AACGTACAAT   120
wcpM5              81   GCTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpM6              81   GCTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpNZL7            81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpNZL8            81   GCTTTTTCAT  CCGTTgCTCA  CACATGTCGA  AACGTACAAT   120
wcpNZL9            81   GCTTTTTCAT  CCGTtgCTCA  CACATGTCGA  AACGTACAAT   120
wcpAn10            81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpAn11            81   GCTTTTTCAT  CCGTTGcTCA  CACATGtCGA  AACGTACAAT   120
wcpNZL12           81   GCTTTTTCAT  CCGTTGCTcA  CACATGTCGA  AACGTACAAT   120
wcpAn13            81   GCTTTTTCAT  CCGTTGctCA  cACATGTCGA  AACGTACAAT   120
wcpNZL14           81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpAu15            81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpAu16            81   GCTTTTTCAT  CCGTTGcTCA  CACATGTCGA  AACGTACAGT   120
wcpAu17            81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpB18             81   GCTTTTTCAT  CCGTTGcTCA  CAcAtGTCGA  AACGTACAAT   120
wcpB19             81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
wcpB20             81   ACTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpB21             81   GCTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
wcpB22             81   GCTTTTTCAT  CCGTTGCTCA  TACATGTCGA  AACGTACAAT   120
sp1                81   GCTTTTTCAT  CCGTTGCTCA  CACATGTCGA  AACGTACAAT   120
sp2                81   GCTTTTTCAT  CCGTtGCTCA  CACATGTCGA  aaCGtACaAT   120
sp3                81   GCTTTTTCAT  CCgTTGcTcA  cacATGTCGA  AACGTACAAT   120
westlandica        81   gcttttttcat ccgttgctca  cacatgccga  aacgtacaat   120
cinerea            81   gctttttcat  ccgttgctca  cacatgtcga  aacgtacaat   120
parkinsoni         81   gctttttcat  ccgttgctca  cacatgtcga  aacgtacaat   120
Bulweria bulwerii  81   gcctttttcat ccgtcgccca  tacatgccga  aacgtacagt   120

published WCP      121  acggctgact  aatccgaaat  ctacatgcaa  atggagcctc   160
WCP1               121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpSG2             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpSG3             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpSG4             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpM5              121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpM6              121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpNZL7            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
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wcpAn11            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpNZL12           121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpAn13            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpNZL14           121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpAu15            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpAu16            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpAu17            121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpB18             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpB19             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpB20             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpB21             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
wcpB22             121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
sp1                121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
sp2                121  ACGGcTGACT  AATCCGAAAT  CTaCaTGCAA  ATGGAGCCTC   160
sp3                121  ACGGCTGACT  AATCCGAAAT  CTACATGCAA  ATGGAGCCTC   160
westlandica        121  acggctgact  aatccgaaat  ctacatgcaa  atggagcctc   160
cinerea            121  acggctgatt  aatccgaaat  ctacatgcaa  atggagcctc   160
parkinsoni         121  acggctgact  aatccgaaat  ctacatgcaa  atggagcctc   160
Bulweria bulwerii  121  acggctgact  aattcgaaac  ctacatgcaa  acggagcctc   160

published WCP      161  attcttcttc  atctgtattt  accttcacat  tggacgagga   200
WCP1               161  ATTCTTCTTC  ATCTGTATTT  ACCTTCACAT  TGGACGAGGA   200
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Table A2 continued

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wcpSG3	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpSG4	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpM5	161	ATTCTTCTTC	ATCTGTATCT	ACCTTCACAT	TGGACGAGGA	200
wcpM6	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpNZL7	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
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wcpNZL9	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpAn10	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpAn11	161	ATTCTTCTTC	aTCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpNZL12	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpAn13	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpNZL14	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
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wcpAu16	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpAu17	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
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wcpB20	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpB21	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
wcpB22	161	ATTCTTCTTC	ATCTGTATTT	ACCTTCACAT	TGGACGAGGA	200
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sp3	161	ATTCTTCTTC	aTCTGTATTT	ACCTTCACAT	TGGGCGAGGA	200
westlandica	161	attcttcttc	atctgtatct	accttcacat	tggacgagga	200
cinerea	161	attcttcttc	atctgtattt	accttcacat	tggacgagga	200
parkinsoni	161	attcttcttc	atctgtattt	acctccacat	cggacgaggg	200
Bulweria bulwerii	161	attcttcttc	atctgcatct	acttccacat	tggacgagga	200
published WCP	201	ttctactacg	gctcatacct	atacaaggag	acctgaaaca	240
WCP1	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpSG2	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpSG3	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpSG4	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACcTGAAACA	240
wcpM5	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpM6	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpNZL7	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpNZL8	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpNZL9	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpAn10	201	TTCTACTACG	GCTCATACCT	ATACAAAGAG	ACCTGAAACA	240
wcpAn11	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
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wcpB20	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpB21	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
wcpB22	201	TTCTACTACG	GCTCATACCT	ATACAAGGAG	ACCTGAAACA	240
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sp2	201	TTCTACTACG	GCTCATACCT	ATACAAAGAG	ACCTGAAACA	240
sp3	201	TTCTACTACG	GCTCATACCT	ATACAAAGAG	ACCTGAAACA	240
westlandica	201	ttctactacg	gctcatacct	gtacaaagag	acctgaaaca	240
cinerea	201	ttctactacg	gctcatacct	gtacaaagag	acctgaaaca	240
parkinsoni	201	ttctactacg	gctcatacct	gtacaaagag	acctgaaaca	240
Bulweria bulwerii	201	ttctattacg	gctcctacct	gtacaaagaa	acttgaaaca	240
published WCP	241	caggagttat	tctcttactc	accctcatag	caactgcctt	280
WCP1	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpSG2	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCTTT	280

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Table A2 continued

wcpSG3	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpSG4	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpM5	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpM6	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAA	CAACTGCCTT	280
wcpNZL7	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpNZL8	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
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wcpAn10	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpAn11	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpNZL12	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpAn13	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
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wcpAu15	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpAu16	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpAu17	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpB18	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpB19	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpB20	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpB21	241	CAGGGGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
wcpB22	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
sp1	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
sp2	241	CAGGAGTTAT	TCTCTTACTC	aCCCTCATAG	CAACTGCCTT	280
sp3	241	CAGGAGTTAT	TCTCTTACTC	ACCCTCATAG	CAACTGCCTT	280
westlandica	241	caggagttat	tcttttactc	accctcatag	caactgcctt	280
cinerea	241	caggagttat	tcttttactc	accctcatag	caactgcctt	280
parkinsoni	241	caggagttat	tcttttactc	actcttatag	caactgcctt	280
Bulweria bulwerii	241	caggagttat	tctcctactc	accctcatag	caaccgcctt	280
published WCP	281	cgtaggatac	gtcttaccct	gaggccaaat	atcattctga	320
WCP1	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpSG2	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpSG3	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpSG4	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpM5	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpM6	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpNZL7	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpNZL8	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpNZL9	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAn10	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAn11	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpNZL12	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAn13	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpNZL14	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAu15	281	CGTAGGGTAC	GTCCTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAu16	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpAu17	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpB18	281	CGTAGGGTAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
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wcpB20	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpB21	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
wcpB22	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
sp1	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
sp2	281	CGtAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
sp3	281	CGTAGGATAC	GTCTTACCCT	GAGGCCAAAT	ATCATTCTGA	320
westlandica	281	cgtagggtac	gtcttaccct	gaggccaaat	atcattctga	320
cinerea	281	cgtaggatac	gtcttaccct	gaggccaaat	atcattctga	320
parkinsoni	281	cgtagggtat	gtcttaccct	gaggccaaat	atcattttga	320
Bulweria bulwerii	281	cgtagggtat	gtccttccct	gaggccaaat	atcattctga	320
published WCP	321	ggggctacag	tcatcaccaa	tctattttca	gctattccct	360
WCP1	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpSG2	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpSG3	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360

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Table A2 continued

wcpSG4	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpM5	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpM6	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpNZL7	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpNZL8	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpNZL9	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpAn10	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpAn11	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpNZL12	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpAn13	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpNZL14	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpAu15	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpAu16	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpAu17	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpB18	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCCT	360
wcpB19	321	GGGGCTACAG	TCATCACCAA	TCTATTCTCA	GCTATTCCTT	360
wcpB20	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpB21	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
wcpB22	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATTCCCT	360
sp1	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATCCCCT	360
sp2	321	GGGGCTaCAG	TCATCaCCAA	TCTATTTTCA	GCTATCCCCT	360
sp3	321	GGGGCTACAG	TCATCACCAA	TCTATTTTCA	GCTATCCCCT	360
westlandica	321	ggggctacag	tcatcaccaa	tctattttca	gctatcccct	360
cinerea	321	ggggctacag	tcatcaccaa	tctattttcg	gctattccct	360
parkinsoni	321	ggggctacag	tcatcaccaa	tctattttca	gccatcccct	360
Bulweria bulwerii	321	ggggctacag	tcatcaccaa	cctattctca	gccattccct	360

published WCP	361	acattggcca	aaccctagta	gaatgagcct	gaggggggctt	400
WCP1	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpSG2	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpSG3	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpSG4	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpM5	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpM6	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpNZL7	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpNZL8	361	ACGTTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpNZL9	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAn10	361	ACGTTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAn11	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpNZL12	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAn13	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpNZL14	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAu15	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAu16	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpAu17	361	ACGTTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpB18	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpB19	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpB20	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpB21	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
wcpB22	361	ACATTGGCCA	AACCCTAGTA	GAATGAGCCT	GAGGGGGCTT	400
sp1	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
sp2	361	ACATTGGCCA	AaCCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
sp3	361	ACATTGGCCA	AACCTTAGTA	GAATGAGCCT	GAGGGGGCTT	400
westlandica	361	acattggcca	aaccttagta	gaatgagcct	gaggaggctt	400
cinerea	361	acattggcca	agccttagta	gaatgagcct	gaggggctt	400
parkinsoni	361	acattggcca	aaccttagta	gaatgagcct	gaggaggctt	400
Bulweria bulwerii	361	atatcggcca	aaccctcgta	gaatgagcct	gaggggatt	400

published WCP	401	ctcagtggat	aaccccacac	taactcgatt	ctttgcccta	440
WCP1	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpSG2	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpSG3	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpSG4	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440

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Table A2 continued

wcpM5	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpM6	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpNZL7	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpNZL8	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpNZL9	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAn10	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAn11	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTCGCCCTA	440
wcpNZL12	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAn13	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpNZL14	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAu15	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAu16	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpAu17	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpB18	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpB19	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpB20	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpB21	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
wcpB22	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCCCTA	440
sp1	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCTCTA	440
sp2	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCTCTA	440
sp3	401	CTCAGTGGAT	AACCCCACAC	TAACTCGATT	CTTTGCTCTA	440
westlandica	401	ctcagtggat	aaccccacac	taacccgatt	cttcgcccta	440
cinerea	401	ctcagtggat	aaccctacac	taacccgatt	ctttgctcta	440
parkinsoni	401	ctcagtggat	aaccccacac	taacccgatt	cttcgcccta	440
Bulweria bulwerii	401	ctcagtagac	aaccccacac	taacccgatt	ctttgctcta	440
published WCP	441	cacttcctcc	ttccctttat	aatcgcagga	cttaccctag	480
WCP1	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpSG2	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpSG3	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpSG4	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpM5	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpM6	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpNZL7	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpNZL8	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpNZL9	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAn10	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAn11	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpNZL12	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAn13	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpNZL14	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAu15	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAu16	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpAu17	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpB18	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpB19	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpB20	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpB21	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
wcpB22	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGA	CTTACCCTAG	480
sp1	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGG	CTTACCCTAG	480
sp2	441	CACTTCcTCC	TTCCCTtTAT	AATCGCAGGG	CTTACCCTAG	480
sp3	441	CACTTCCTCC	TTCCCTTTAT	AATCGCAGGG	CTTACCCTAG	480
westlandica	441	cacttcctcc	tcccttttat	aatcgcaggg	cttaccctag	480
cinerea	441	cacttcctcc	ttccctttat	aatcgcaggg	cttaccctag	480
parkinsoni	441	cacttcctcc	ttccctttat	aatcgcaggg	cttaccctag	480
Bulweria bulwerii	441	cacttcctcc	tccccttcat	aatcgcagga	cttagcctaa	480
published WCP	481	tccatctcac	cttcctccac	gaatcaggtt	caaataaccc	520
WCP1	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpSG2	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpSG3	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpSG4	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpM5	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520

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Table A2 continued

wcpM6	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpNZL7	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpNZL8	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpNZL9	481	TCCATCTCGC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAn10	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAn11	481	TCCATCTCGC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpNZL12	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAn13	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpNZL14	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAu15	481	TCCATCTCGC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAu16	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpAu17	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpB18	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpB19	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpB20	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpB21	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
wcpB22	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
sp1	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
sp2	481	TcCATCTCAC	CTtCcTCCAC	GAaTCAGGtT	CaAATAaCCc	520
sp3	481	TCCATCTCAC	CTTCCTCCAC	GAATCAGGTT	CAAATAACCC	520
westlandica	481	tccatctcac	cttcctccac	gaatcaggtt	caaataaccc	520
cinerea	481	tccatctcac	cttcctccac	gaatcaggtt	caaataaccc	520
parkinsoni	481	tccatctcac	cttcctccac	gaatcaggtt	caaataaccc	520
Bulweria bulwerii	481	tccatctcac	cttcctccac	gaatcaggct	caaacaaccc	520

published WCP	521	cctcggcatc	gtatcaaact	gtgataaaat	cccattccac	560
WCP1	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpSG2	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpSG3	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpSG4	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpM5	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpM6	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpNZL7	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpNZL8	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpNZL9	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAn10	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAn11	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpNZL12	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAn13	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpNZL14	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAu15	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAu16	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpAu17	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpB18	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpB19	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpB20	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpB21	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
wcpB22	521	CCTCGGCATC	ATATCAAACT	GTGATAAAAT	CCCATTCCAC	560
sp1	521	CCTCGGCATC	GTATCAAACT	GTGATAAAAT	CCCATTCCAC	560
sp2	521	CCTCGGcATC	GTAtCAAACT	GTGATAAAAT	CCCATTCcAC	560
sp3	521	CCTCGGCATC	GtATCAAACT	GTGATAAAAT	CCCATTCCAC	560
westlandica	521	cctcggcatc	gtatcaaact	gtgataaaat	cccattccac	560
cinerea	521	cctcggcatc	gtatcaaact	gtgataaaat	cccattccac	560
parkinsoni	521	ccttggcatc	gtatcaaact	gtgataaaat	cccattccac	560
Bulweria bulwerii	521	cctaggtatc	gtatcaaact	gtgacaaaat	cccattccac	560

published WCP	561	ccttacttca	ccctaaaaga	catcctagga	ttcacactc	599
WCP1	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpSG2	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpSG3	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpSG4	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpM5	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpM6	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599

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Table A2 continued

wcpNZL7	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpNZL8	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpNZL9	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpAn10	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTc	599
wcpAn11	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpNZL12	561	CCTTACTTCA	CCCtAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpAn13	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpNZL14	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpAu15	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpAu16	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpAu17	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpB18	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpB19	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpB20	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTc	599
wcpB21	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
wcpB22	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTc	599
sp1	561	CCTTACTTCA	CCCTAAAaGA	cATCCTAGGA	TtCAcactC	599
sp2	561	CCTTACTTCA	CCcTAAaaGA	cATCcTAgGA	TtCAcaCTC	599
sp3	561	CCTTACTTCA	CCCTAAAAGA	CATCCTAGGA	TTCACACTC	599
westlandica	561	ccttacttca	ccctaaaaga	catcctagga	ttcacactc	599
cinerea	561	ccctacttta	ccctaaaaga	catcctagga	ttcacactc	599
parkinsoni	561	ccttacttca	ccctaaaaga	tatcctagga	ttcacactt	599
Bulweria bulwerii	561	ccctacttca	ccctaaaaga	catcctaggc	ttcacactt	599

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Table A3: Table based on Table 2.1 including details on geographic origin of individuals sharing haplotypes. Cr, Iles Crozet, Mar, Marion Island, SG, South Georgia, Auk, Auckland Island, Dis, Disappointment Island, Ant, Antipodes.

Haplotype	Nucleotide Position																									Total N	Individuals sharing that haplotype
			1	1	1	1	1	2	2	2	3	3	3	3	3	3	4	4	4	4	4	5	5	5	5		
	5	7	3	5	5	6	7	0	2	8	0	3	3	7	8	9	0	9	5	6	8	4	4	7	9		
	8	7	5	8	2	4	7	7	5	5	0	3	5	6	4	2	0	3	2	9	1	0	3	5	5		
Atlantic Indian Ocean																											
wcp1	T	C	G	C	A	A	T	T	A	T	A	G	G	G	C	A	T	T	T	A	C	T	C	A	A	37	Cr (n=11), Mar (n=17), SG21, 26, 29, 32, 33, 34, 35, 30, 36
wcpSG2															T											2	SG17, 27
wcpSG3				T																						3	SG18, 24, 28
wcpSG4						G																				1	SG20
wcpM5										C																1	Mar55
wcpM6														A												1	Mar63
New Zealand Islands																											
wcpNZL7								C								G		C			T					11	Auk19, 23, 24, 57, 62, Ant2, 6, 11, 14, 20, Dis4
wcpNZL8								C								G				G	T					4	Ant15, 23, Dis8, Auk4
wcpNZL9								C								G		C			T				G	16	Ant3, 6, 7, 12, 17, 19, 21, 24, 3, 32, Dis3, 5, Auk3, 17, 25, 74
wcpAnt10								C				A				G				G	T					1	Ant1
wcpAnt11								C								G		C			T	C			G	1	Ant5
wcpNZL12								C					A			G		C			T					2	Auk18, Ant9
wcpAnt13							C	C								G		C			T					2	Ant10, 13
wcpNZL14								C								G					T					3	Auk26; Ant16, Dis1
wcpAuk15								C								G	C	C			T				G	1	Dis6
wcpAuk16								C	G				A			G		C			T					2	Auk20, 72
wcpAuk17				T				C								G				G	T					1	Auk22
Inaccessible Island (*Procellaria conspicillata*)																											
sp1	C	T						C			G	A							C		T		T	G		15	SP (n=15)
sp2	C	T			G			C			G	A							C		T		T	G		4	SP2, 3, 20, 49
sp3	C	T	A					C			G	A							C		T		T	G		4	SP13, 15, 32, 75

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CHAPTER 3

POPULATION STRUCTURE OF THE WHITE-CHINNED PETREL

SUMMARY

Microsatellite DNA diversity is used to infer population structure in the White-chinned Petrel (*Procellaria aequinoctialis*). Eight polymorphic loci were genotyped, six of which had been isolated in the White-chinned Petrel. Five colonies were sampled including South Georgia (Bird Island), Marion Island, Iles Crozet, Antipodes and Auckland Island (Disappointment Island). Heterozygosity values range from 0.41 to 0.58, and allelic richness per locus ranges from 2.1 to 9.2. The same regional populations identified with cytochrome b are also identified with microsatellites. Population differentiation measured by F_{ST} was found between all colonies except Antipodes and Auckland Island, whereas R_{ST} showed differentiation only in pairwise comparisons with South Georgia. There is no correlation between the differentiation measures, F_{ST} and R_{ST}, indicating that genetic drift, dispersal and mutation affect each colony in a different way. Colonies within ocean basins were either weakly or not differentiated, whereas structuring was observed between ocean basins. Patterns of allele frequencies and private alleles corroborate the hypothesis, based on cytochrome b, that founder events are responsible for establishing colonies in the Indian Ocean and the Atlantic Ocean from the New Zealand region. Regional population specific alleles and differentiation between the two populations also supports the suggestion of recognising two Evolutionary Significant Units in the White-chinned Petrel in order to conserve diversity.

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INTRODUCTION

Although microsatellites have become the molecular tool of choice in population level studies (Frankham *et al.*, 2002), they have only recently been used in avian species (e.g. Primmer and Ellegren, 1998; Avise, 2004) with the most comprehensive studies carried out in passerine birds (Primmer *et al.*, 1995, 1996; Primmer *et al.*, 1997; Pruett and Winker, 2005). Microsatellites have been used to resolve questions of parentage and mating systems (e.g. Sunnucks, 2000; Tarop *et al.*, 2001; Blouin, 2003; Carew *et al.*, 2003; Dawson *et al.*, 2005), individual identification and kinship (Piertney and Dallas, 1997; Sunnucks, 2000), colony formation, integrity, isolation and gene flow (Piertney *et al.*, 1998; Estoup and Clegg, 2003), and genetic structuring in relation to morphology (Bensch *et al.*, 1997). There are few studies using microsatellites in seabirds (Goostrey *et al.*, 1998; Genovart *et al.*, 2003; Pons *et al.*, 2004; Riffaut *et al.*, 2005). Microsatellites have been used in Procellariiformes on their own (Van Bekkum *et al.*, 2006), or together with a mitochondrial marker such as ATPase 6/8 (Friesen *et al.*, 2006) or control region (Burg and Croxall, 2001; Abbott and Double, 2003; Burg and Croxall, 2004).

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This Chapter aims to corroborate the cytochrome b gene findings of the previous Chapter using nuclear microsatellite DNA because of the importance of establishing population structure in petrels to identify the origin of bycatch. As mitochondrial cytochrome b is maternally inherited, only female lineages are observed. However, microsatellite DNA is biparentally inherited thus providing an opportunity to investigate previous findings in both males and females. In addition, microsatellite DNA has a faster mutation rate than coding DNA, enabling a more detailed look at population structure.

MATERIALS AND METHODS NOT DISCUSSED IN CHAPTER 1

Microsatellite loci isolated in the White-chinned Petrel (Chapter 1), together with two loci originally characterised in albatrosses (Burg, 1999) were used (Dc5 and De33). The appendix at the end of Chapter 4 contains a list of individuals typed and their locations. Samples were obtained from adults at five breeding localities: Antipodes (38 individuals), Auckland Island (92), South Georgia (39), Marion Island (85), and Iles Crozet (14). The majority of samples from Auckland Island (85) and Antipodes (22) were caught as longline mortalities. All were confirmed breeders and were caught within 100 km of each island during the breeding season and were assigned to either Auckland Island or the Antipodes.

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Table 3.1: Details of microsatellite loci used on the White-chinned Petrel including repeat type. bp, base pairs of PCR products; Na, number of alleles found at that locus; T_a, annealing temperature; $MgCl_2$, concentration (mM) of Magnesium Chloride used in the reaction.

Locus	Repeat type	bp	N_a	T_a	$MgCl_2$
Paequ2	$(CA)_2TT(CA)_{10}$	194-214	7	54	1
Paequ3	$(GA)_{19}$	231-259	15	55	1.5
Paequ7	$(GT)_{12}$	182-194	6	64	1.5
Paequ8	$(CA)_{11}$	226-234	5	58	2
Paequ10	$(CA)_8$	179-197	10	55	1
Paequ13	$(GT)_9$	144-150	4	62	1
Dc5	$(AC)_5G(CAC)_2G(CA)_{12}$	173-180	7	57	1
De33	$(TCT)_{29}TTT(TCT)_3(TCA)_5CCA(TCA)_3$	146-188	12	55	1.5

Eight polymorphic loci were used to genotype 268 White-chinned Petrels from known locations. The De33 locus (Table 3.1) has been found to be sex linked in the wandering albatross species complex (Burg and Croxall, 2004). To determine whether this was the case in White-chinned Petrels, known females from New

Zealand (the only colonies where sex was known) were inspected using the program CERVUS version 2.0 (Marshall *et al.*, 1998). Polymorphic loci are expected to be located on the larger sex chromosome, which is the Z chromosome in the Z-W system found in birds. As females have the ZW genome a sex-linked locus will display only homozygotes in females and thus an observed heterozygosity (H_O) of zero. In males the locus will show both homozygotes and heterozygotes, whereas the expected heterozygosity (H_E) would be similar in both sexes. The New Zealand female population showed a H_E of 0.712 for De33 and H_O of 0.679. Further indicating that the locus was not sex-linked is the low frequency of null alleles. De33 showed an expected null allele frequency of 0.0074, whereas a sex-linked locus is expected to have a high frequency (>0.02) of null alleles. These results are sufficient evidence that the locus was not sex-linked in the White-chinned Petrel and therefore were included in further analyses.

Linkage disequilibrium was tested for all pairs of loci both within and across all colonies. No significant values were observed and thus all loci were included in further analysis.

Estimation of first generation immigrants

First generation immigrants to source populations were estimated by a Bayesian inference method (Paetkau *et al.*, 2004) implemented in GeneClass2 (Piry *et al.*, 2004). In this method the distribution of genotype likelihood in real residents is approximated and then compared to the calculated likelihood of sampled individuals. The method is similar to the partial Bayesian assignment method described in more detail in Chapter 4 (Rannala and Mountain, 1997). However, the partial Bayesian method has been shown to have a larger type 1 error (resident individuals falsely assigned as immigrants). The new algorithm obtains a simulated individual during Monte Carlo resampling by drawing with replacement multilocus gametes from randomly chosen individuals in each population of the data set (Paetkau *et al.*, 2004), as opposed to drawing alleles

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according to allele frequency (Rannala and Mountain, 1997). In addition, type 1 errors are reduced by calculating the likelihood statistics on a simulated data set of equal genotypes as the original data set, and then repeating the process until 10000 genotypes are generated (Paetkau *et al.*, 2004), as opposed to generating a larger data set of individuals than the original data set for calculating likelihood statistics, and then repeating the process until 10000 genotypes are reached.

As a large number of samples assigned to either New Zealand colony (Antipodes and Auckland Island) were caught as bycatch, this method was used to justify using them as known samples. No first generation immigrants were identified in the South Georgia colony. On Marion Island one immigrant was identified with the most likely origin being Iles Crozet. Similarly one immigrant found on Iles Crozet most likely originated in Marion Island. Two immigrants were found on Antipodes and one on Auckland Island. For all three the most likely origin was identified as South Georgia. In order to avoid biasing analysis these individuals were removed from further analysis. As the immigrants identified within the New Zealand colonies were caught as bycatch, they are most likely birds visiting these foraging locations from the Atlantic Ocean.

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RESULTS

Intrapopulation genetic variation

Deviations from Hardy-Weinberg equilibrium were tested for all pairs of loci and colonies. Even after sequential Bonferroni corrections, several loci deviated from Hardy-Weinberg expectations (Table 3.2), although no single locus deviated from Hardy-Weinberg expectations for all colonies. Overall, this resulted in all but one (Iles Crozet) colony being outside expectations. This was mostly due to positive F_{is} values. Deviations from Hardy-Weinberg Equilibrium due to an excess of homozygotes may exist for several reasons, which include non-random mating, selection, null alleles, inbreeding and population substructure (Wahlund effect) (Ridley, 1996). When colonies were combined into regions identified in Chapter 2, this could not be improved. It was not possible to investigate further colony substructure as their exact nest locations within islands was not known. As six loci had been isolated in this species, null alleles would seem unlikely, although not impossible.

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A total of 66 alleles were found over the eight loci from all individuals (Table 3.2). Similar allele numbers characterised all colonies with Iles Crozet having the fewest (31 alleles) and Marion Island having the most alleles (50). When taken together, the New Zealand island colonies had most of the private alleles (three in Antipodes and five in Auckland Island) while only two were found in South Georgia, one in Marion Island and none in Iles Crozet (data shown in Appendix 1). These data are similar to the cytochrome b data in Chapter 2 where the New Zealand islands were identified as having the greatest diversity when compared to the other regional populations. Allelic richness showed that locus Paequ3 had on average the most alleles (corrected for sample size = 13 (Iles Crozet)), with locus De33 having 6.6 and Paequ2 having 5.2, whereas the remaining loci had on average only two alleles.

Table 3.2: Estimate of the number of alleles (*Na*), expected heterozygosity (H_E) and observed heterozygosity (H_O), the probability of deviation from Hardy-Weinberg Equilibrium (HWE *p*) and F_{is} for eight microsatellite loci in five colonies (*N* number of individuals sampled) of White-chinned Petrel. Significant deviations from Hardy-Weinberg expectations after Bonferroni corrections are marked with an asterix.

	Locus								Overall
	Paequ2	*Paequ3*	*Paequ7*	*Paequ8*	*Paequ10*	*Paequ13*	*Dc5*	*De33*	
South Georgia (*N*=39)									
Na	5	10	4	3	7	2	3	9	43
Allelic Richness	4.5	7.5	2.3	2.1	5.9	2	2.6	7.2	
H_O	0.784	0.758	0.316	0.143	0.714	0.605	0.444	0.310	0.58
H_E	0.691	0.829	0.466	0.163	0.785	0.509	0.502	0.082	0.58
(HWE) *p*	0.545	0.299	0.00115*	1	0.20055	0.2055	0.0221	0*	highly sign.
Fis (W&C)	-0.137	0.087	0.296	-0.049	0.091	-0.219	0.117	0.619	0.134
Marion Island (*N*=84)									
Na	6	11	5	3	9	4	3	9	50
Allelic Richness	5.5	8.8	2.3	2.1	7.03	2.4	2.2	6.4	
H_O	0.464	0.808	0.247	0.238	0.723	0.407	0.709	0.549	0.59
H_E	0.743	0.881	0.454	0.249	0.776	0.435	0.507	0.756	0.69
(HWE) *p*	0*	0.363	0*	0.697	0*	0.016	0.0002*	0*	highly sign.
Fis (W&C)	0.376	0.081	0.457	0.048	0.063	0.064	-0.402	0.275	0.136
Iles Crozet (*N*=13)									
Na	4	7	3	2	5	2	2	6	31
Allelic Richness	3.9	6.7	2.9	2	4.5	2	2	6	
H_O	0.571	0.643	0.143	0.333	0.571	0.5	0.357	0.583	0.53
H_E	0.648	0.802	0.373	0.471	0.587	0.516	0.389	0.819	0.66
(HWE) *p*	0.14	0.053	0.023	1	1	1	1	0.092	0.15
Fis (W&C)	0.05	0.19	0.556	0.154	0.028	0.032	0.085	0.297	0.165
Antipodes Island (*N*=36)									
Na	3	11	4	3	6	4	4	7	42
Allelic Richness	2.3	8.9	3.2	2.3	4.9	3.3	2.6	6.1	
H_O	0.271	0.861	0.132	0.108	0.595	0.444	0.395	0.444	0.41
H_E	0.316	0.896	0.419	0.222	0.677	0.537	0.406	0.795	0.53
(HWE) *p*	0.437	0.845	0*	0.0403	0.153	0.266	0.5241	0*	highly sign.
Fis (W&C)	0.146	0.036	0.671	0.458	0.122	0.175	0.028	0.438	0.23
Aukland Island (*N*=91)									
Na	5	12	4	3	8	3	5	7	47
Allelic Richness	3.7	8.6	3.2	2.3	4.9	3.3	2.6		
H_O	0.233	0.865	0.143	0.173	0.573	0.244	0.489	0.589	0.41
H_E	0.397	0.884	0.301	0.211	0.727	0.503	0.423	0.708	0.52
(HWE) *p*	0*	0.746	0*	0.295	0*	0*	0.5401	0*	highly sign.
Fis (W&C)	0.411	0.02	0.512	0.137	0.213	0.505	-0.158	0.198	0.198
Total									
Na	7	15	6	5	10	4	7	12	66
Allelic Richness	5.2	9.2	2.9	2.1	6.5	2.5	2.4	6.6	
H_O	0.465	0.787	0.196	0.199	0.635	0.440	0.479	0.495	0.50
H_E	0.559	0.858	0.403	0.263	0.710	0.500	0.445	0.632	0.59
(HWE) *p*	0	0.357	0	0.4543	0	0	0.003	0	highly sign.

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Population structure

F_{ST} values (Table 3.3) were all significantly different from zero except for the comparison between Antipodes and Auckland Island, which represent the New Zealand region already identified in Chapter 2. However, this should be viewed with some caution due to using bycatch birds as known provenance individuals. The highest values were found to be comparisons between Crozet with either of the two New Zealand colonies. However, this could be a function of the small sample size of Crozet (14). Significant R_{ST} values (Table 3.3) were only found in comparisons of South Georgia with the other colonies (except Antipodes). All other comparisons were not significantly different from zero. There was no correlation between F_{ST} and R_{ST} (Mantel test: p=0.31).

Table 3.3: Matrix of pairwise comparisons of R_{ST} (Slatkin, 1995) values (above diagonal) and F_{ST} values (below diagonal) for all five White-chinned Petrel colonies. Values significant at $p < 0.05$ are in bold.

	South Georgia	Marion Is	Iles Crozet	Antipodes	Auckland Is
South Georgia		**0.066**	**0.115**	0.011	**0.040**
Marion Is	**0.029**		0.009	0.015	0.015
Iles Crozet	**0.060**	**0.051**		0.059	0.050
Antipodes	**0.048**	**0.068**	**0.136**		-0.005
Auckland Is	**0.059**	**0.076**	**0.147**	0.005	

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Bottleneck

None of the colonies showed a significant deviation from the null hypothesis of stable populations and all colonies showed normal L-shaped distributions. This result should be interpreted with caution as at least four loci are needed to have a chance of finding a significant result and the optimum number of loci is ten to fifteen (Cornuet and Luikart, 1996), which is more than the eight analysed. On the other hand, the lack of a recent bottleneck is not unexpected as there is no

evidence to the contrary. Given the long life span of White-chinned Petrels (in excess of 30 years) the recent declines would extend over less than one to two generations and therefore it is unlikely to be already detectable in neutral genetic markers. Furthermore, generations are overlapping and since predominantly adults were sampled it is possible that adults, born before any population declines, would not carry any genetic signatures relating to a recent bottleneck.

Genetic distance measures

Three trees were constructed using the distance measures Nei's D_A (Nei *et al.*, 1983), Cavalli-Sforza and Edward's D_{CE} (Cavalli-Sforza and Edwards, 1967) and the microsatellite specific Goldstein's Delta μ^2 (Goldstein *et al.*, 1995) and the tree building method of UPGMA. The use of trees constructed from microsatellites has been shown to be useful in clarifying evolutionary relationships (Takezaki and Nei, 1996). The tree constructed using Nei's D_A was best supported with the highest bootstrap values (Figure 3.1). Cavalli-Sforza and Edward's D_{CE} had the same topology with slightly less well-supported branches. The microsatellite specific distance measure had the least supported topology with bootstrap supports ranging around 50%. It has been shown in simulations that Nei's D_A and Cavalli-Sforza and Edward's D_{CE} often perform better than other distance methods under either mutation model but Goldstein's Delta μ^2 is stronger in estimating evolutionary times (Takezaki and Nei, 1996). As the goal was to establish relationships between colonies, the performance of the various distance measures is not surprising. The trees show a strongly supported divide between the two regional populations identified in Chapter 2. Iles Crozet has the closest relationship of the Atlantic/Indian Ocean population to the New Zealand population. However, this relationship was not well supported by Principal Component Analysis (Figure 3.2).

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Figure 3.1: UPGMA phenogram of the five colonies of the White-chinned Petrel using Nei's Da (Nei *et al.*, 1983). The same topology was resolved using Cavalli-Sforza and Edward's D_{CE} (Cavalli-Sforza and Edwards, 1967). Bootstrap (1000, given as percentage) support is shown as numbers on branches.

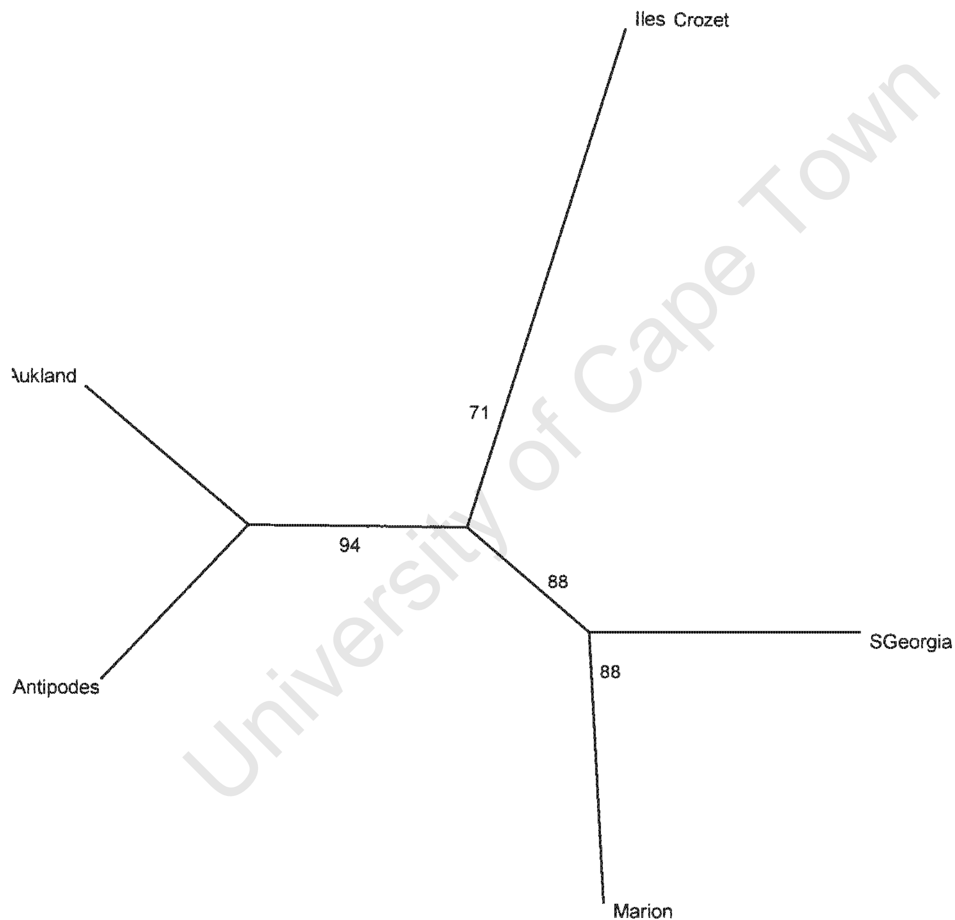

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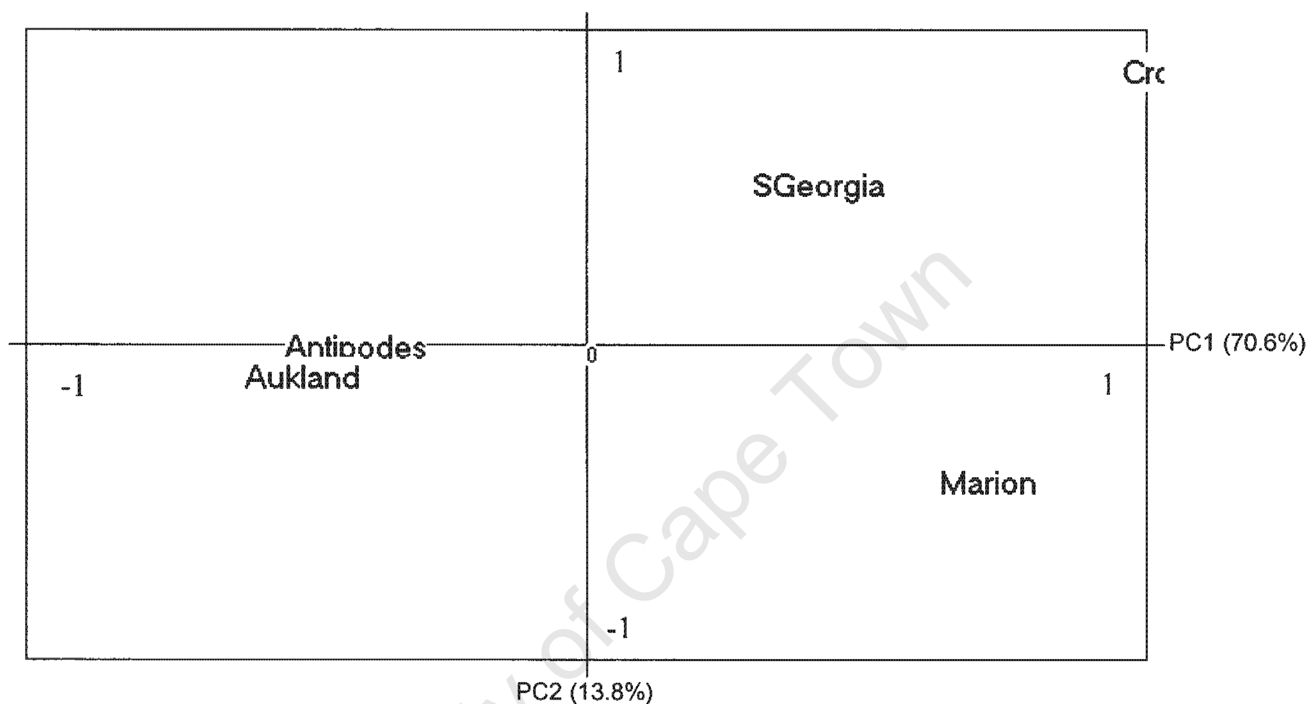
Figure 3.2: Factor map of the two main axes of principal component analysis for White-chinned Petrel colonies; green, Atlantic Ocean, red, New Zealand, black, Indian Ocean. The first two main axes account for 84.4% of inertia.



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DISCUSSION

Many seabirds, and Procellariiformes in particular, are philopatric to natal and breeding sites (Weimerskirch *et al.*, 1985; Edwards *et al.*, 2001; Newton, 2003). This behaviour is conducive to the formation of population differentiation over time. Cytochrome b analysis in the White-chinned Petrel (Chapter 2) found two distinct regional populations, one around New Zealand and another in the Atlantic and Indian Oceans. This result is supported by the microsatellite DNA data. Microsatellite DNA analysis showed highest divergence between colonies of different regional populations. Together with the occurrence of regional specific alleles, this supports the recommendation made in Chapter 2 which discussed the possibility of recommending two Evolutionary Significant Units (ESUs - Ryder, 1986) for both regional populations based on cytochrome b analysis.

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Estimates of genetic differentiation

Genetic differentiation, measured by F_{ST} and R_{ST}, should increase as a function of the accumulation of spatially localised allelic similarity due to philopatry. Overall F_{ST} and R_{ST} values were high enough to indicate some barrier to gene flow between breeding colonies. Pairwise values ranged from 0.005 to 0.147 for F_{ST} and from -0.005 to 0.115 for R_{ST}, indicating differences in the amount of gene flow within and between regional populations. This is comparable to other microsatellite DNA studies in Procellariiformes. For example, the Galapagos Petrel, *Pterodroma phaeopygia*, formed three populations over five islands with F_{ST} values ranging from 0.09 to 0.26 (Friesen *et al.*, 2006), although all islands were at most 100 km apart. In contrast, White-chinned Petrel colonies sampled here are at least 800 km apart (Marion Island/Iles Crozet). Pairwise comparisons of F_{ST} were highest between colonies of different regional populations, confirming the earlier results obtained for cytochrome b. The only non-significant value for F_{ST} was found in comparisons between Auckland and Antipodes, indicating ongoing gene flow between these two colonies although separated by more than 800 km. Pairwise R_{ST} values were only significant for three comparisons which all

involve South Georgia. As expected, a Mantel test found no correlation between F_{ST} and R_{ST}. Because the two statistics measure different characteristics of microsatellite DNA, they are useful in investigating the different effects mutation and genetic drift have on shaping population structure. F_{ST} is based on allele frequency data and thus measures the effects of genetic drift and dispersal. Allele frequencies can change quickly in response to different factors and F_{ST} thus measures current demographic dynamics. R_{ST} is based on the differences in allele length. The length can only be changed by mutation or lost through genetic drift, which takes longer than changes in allele frequencies. Significant F_{ST} values show that there is limited gene flow between islands of different ocean basins and thus enough of a barrier to allow differences in allele frequencies to appear. In contrast, there is enough gene flow between the New Zealand colonies (900km) to prevent genetic drift from differentiating colonies from each other despite smaller population sizes. Within the Indian Ocean, Marion Island and Iles Crozet are differentiated despite their relatively close proximity. The presence of differentiation between different colonies as shown by F_{ST} is supported by the estimation of first generation immigrants within colonies. Only five immigrants were identified within all colonies, and three of these can be assumed to be visiting birds rather than residents.

R_{ST} values indicate differentiation between South Georgia and the Indian Ocean colonies, as well as Auckland Island, although the latter differentiation is weak. No other significant comparisons exist, indicating that different forces are acting on mutation and drift within the South Georgia population. South Georgia is the largest colony with an order of magnitude more birds (BirdLife-International, 2006) and therefore genetic drift is less evident than in other colonies. As R_{ST} measures allele length variation, it is difficult to investigate differences between colonies if some gene flow exists especially when measuring loci with few alleles. In the smaller populations, genetic drift might be responsible for losing alleles. Samples from South Georgia originate from Bird Island, which is situated at the west end of South Georgia. Although this colony is small, it may well be

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maintained through gene flow of the large colonies on South Georgia, and therefore genetic drift may be countered. Differences between R_{ST} and F_{ST} are to be expected as allele frequencies may change rapidly. If impacts, such as mortality from longline fishing are strong, genetic drift together with restricted dispersal may rapidly change allele frequencies. In contrast, allele lengths, as measured by R_{ST}, may still be present throughout the species for a longer time span.

Inferences about species origin

In Chapter 2, it was hypothesised that White-chinned Petrels as well as the other *Procellaria* probably originated or at least diversified around New Zealand. Founder events are often associated with range expansions. As a result, a general loss of diversity and genetic variation is expected in these founder populations (McRae *et al.*, 2005) together with a reduction in effective population size, allele numbers and heterozygosity (Nei *et al.*, 1975; Chakraborty and Nei, 1977). Further, it is expected that mostly high frequency alleles reach new colonies. Weak differentiation and therefore genetic similarities may be due to recent separation and founder events. Even in the complete absence of contemporary gene flow residual similarities will remain, especially in large colonies. It is therefore unlikely that new distinguishing mutations arising in colonies will have reached high frequencies at the time of sampling (Edwards *et al.*, 2001). Heterozyosities were similar in all colonies and also total number of alleles within colonies was comparable. The exception is Iles Crozet which had the smallest number of alleles but this could be due to the small sample size. New Zealand had 11 region specific alleles (colony and regional population specific alleles per locus can be found in Appendix I) and the combined Atlantic and Indian Ocean regional population had 12 region specific alleles, which is similar. However, when colony specific alleles were compared, New Zealand, which was pooled due to the lack of differentiation, had far more private alleles than South Georgia (2), Marion Island (1) and Iles Crozet (0). This suggests that enough time without gene flow between regional populations has passed for

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specific alleles to occur. On the other hand, it could suggest that founder effects limited diversity in the Atlantic and Indian Ocean colonies. Results further suggest that enough gene flow within regional populations exists to allow new alleles to spread. It was found that all region specific alleles had low frequencies, whereas alleles found in both regional populations had higher frequencies. This supports the hypothesis that the White-chinned Petrel originated in New Zealand.

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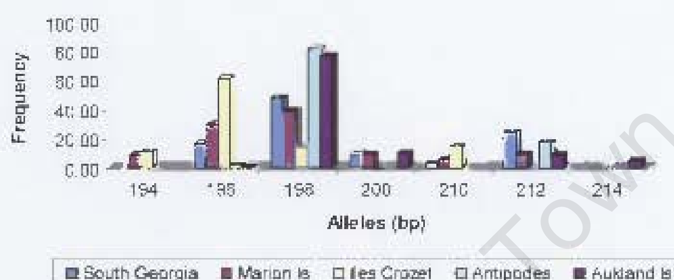
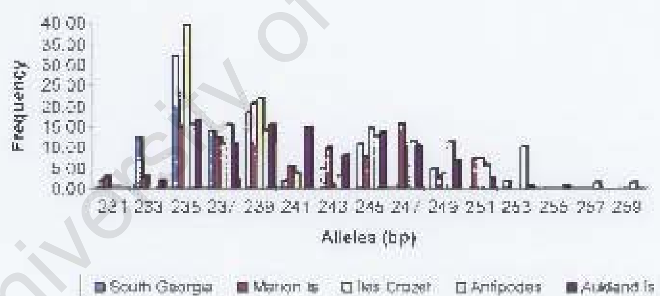
APPENDICES

Figure A1: Allele frequencies of microsatellite loci in the White-chinned Petrel

Locus Paequ2



Locus Paequ3



Locus Paequ7

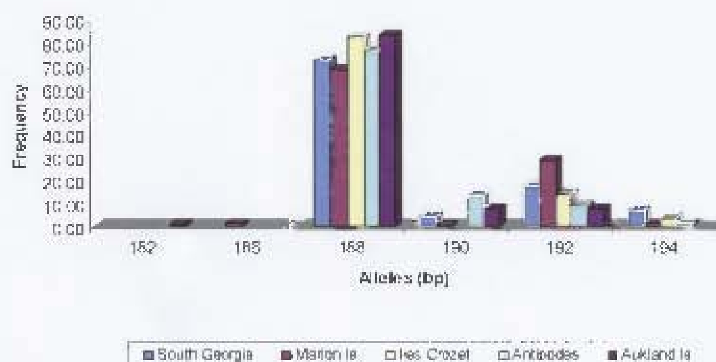


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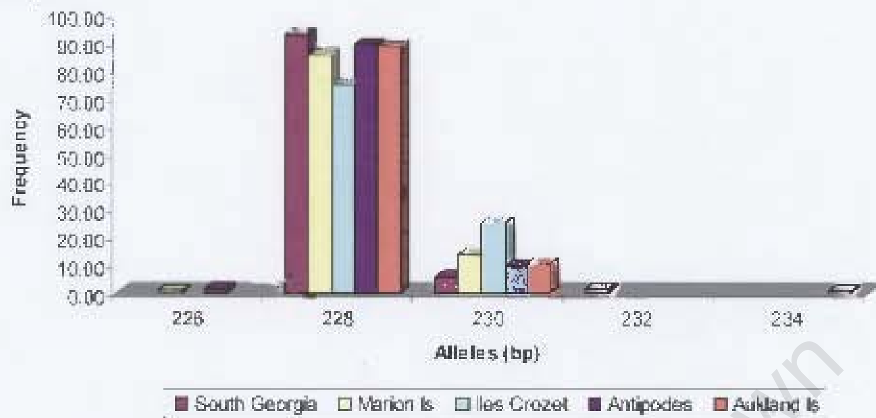
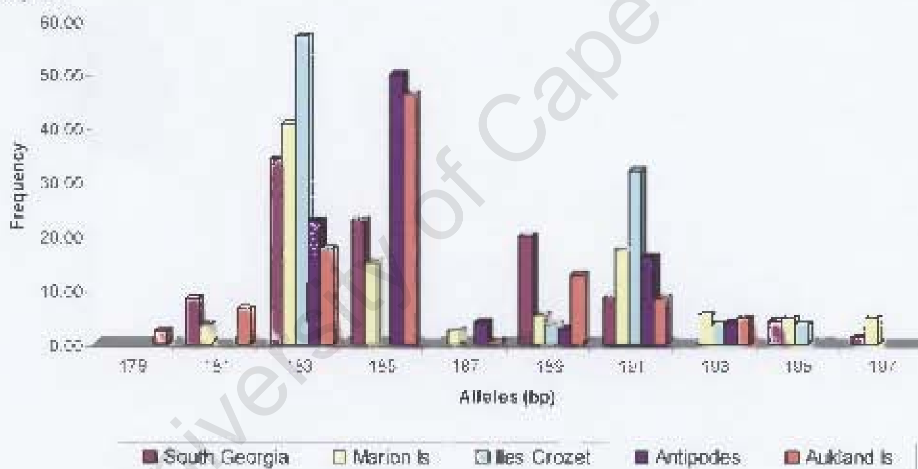
Figure A1 continued





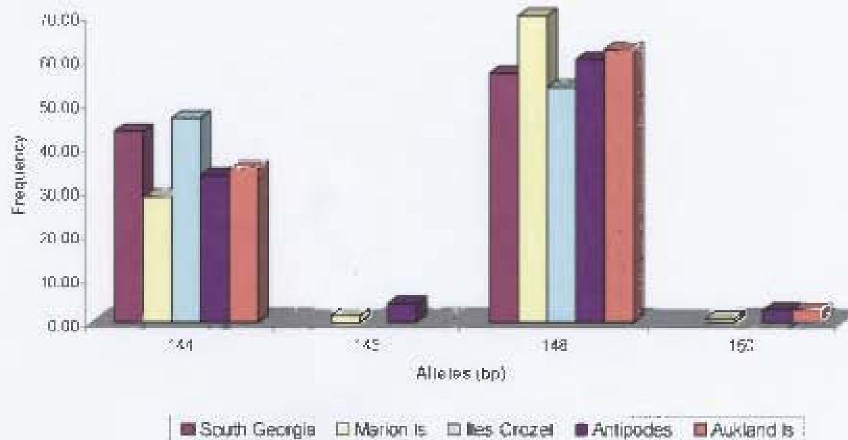


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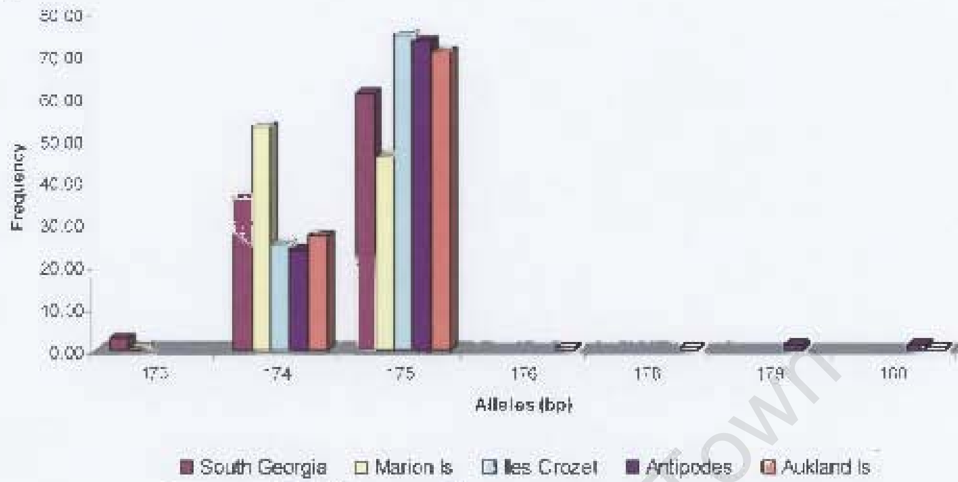
Figure A1 continued

Locus Dc5



Locus De33

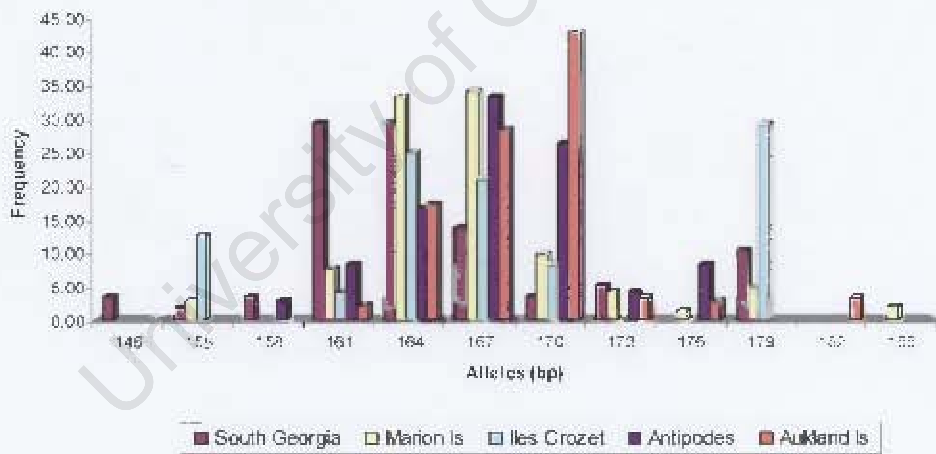


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Table A1: Alleles presented as letters to illustrate private alleles to colonies of the White-chinned Petrel. Colony names are abbreviated as follows: SG South Georgia, M Marion Island, C Iles Crozet, An Antipodes, Au Auckland Island. *Na* number of alleles. Regional populations are i) New Zealand and ii) Marion Island, Iles Crozet and South Georgia

Locus	Colony	Alleles															*Na*	No. of colony specific alleles	No. of regional population specific alleles
Paequ2	SG		B	C	D					I	J						5	0	2
	M	A	B	C	D					I	J						6	0	
	C	A	B	C						I							4	0	
	An		B	C							J						3	0	1
	Au		B	C	D						J	K					5	1	
Paequ3	SG	A	B	C	D	E	F	G	H		J		L	M			11	0	1
	M	A	B	C	D	E	F	G	H	I	J	K					11	0	
	C			C	D	E	F		H		J	K					7	0	
	An			C	D	E		G	H	I	J	K	L	M	N	O	12	2	2
	Au		B	C	D	E	F	G	H	I	J	K	L	M			12	0	
Paequ7	SG					E	F	G	H								4	0	1
	M				D	E	F	G	H								5	1	
	C					E		G	H								3	0	
	An					E	F	G	H								4	0	1
	Au	A				E	F	G									4	1	
Paequ8	SG		B	C	D												3	0	1
	M	A	B	C													3	0	
	C		B	C													2	0	
	An	A	B	C													3	0	1
	Au		B	C		E											3	1	
Paequ10	SG		B	C	D		F	G		I	J						7	0	2
	M		B	C	D	E	F	G	H	I	J						9	0	
	C			C			F	G	H	I							5	0	
	An			C	D	E	F	G	H								6	0	1
	Au	A	B	C	D	E	F	G	H								8	1	
Paequ13	SG	A		C													2	0	0
	M	A	B	C	D												4	0	
	C	A		C													2	0	
	An	A	B	C	D												4	0	0
	Au	A		C	D												3	0	
Dc5	SG	A	B	C													3	0	1
	M	A	B	C													3	0	
	C		B	C													2	0	
	An		B	C				G	H								4	1	4
	Au		B	C	D		F		H								5	2	
De33	SG	A			D	E	F	G	H	I	J		L			O	9	2	4
	M				D		F	G	H	I	J	K	L				9	0	
	C				D		F	G	H	I			L				6	0	
	An					E	F	G	H	I	J	K					7	0	1
	Au						F	G	H	I	J	K		M			7	1	
Totals	SG																44	2	12
	M																50	1	
	C																31	0	
	An																43	3	11
	Au																47	7	

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CHAPTER 4

MOLECULAR PROVENANCE ANALYSIS OF WHITE-CHINNED PETRELS KILLED IN FISHERIES INTERACTIONS

SUMMARY

This Chapter investigates the feasibility of using assignment tests to identify the natal colonies of White-chinned Petrels (*Procellaria aequinoctialis*) caught at sea. Four methods were used, with one making use of a single nucleotide polymorphism (SNP) in cytochrome b identified in Chapter 2, a second sequencing individuals at the cytochrome b gene, and two making use of microsatellite DNA. The SNP analysis showed only local birds in respective fisheries, suggesting that foraging locations are largely separate for each ocean basin, with fisheries catching local birds rather than birds from different ocean basins. Cytochrome b sequencing identified an additional five haplotypes to the 17 identified in Chapter 2. Three of these haplotypes belonged to birds caught in the South African fisheries and were related to haplotypes found within the Atlantic and Indian Ocean regional population. The remaining two were of birds caught within the New Zealand region, and were related to haplotypes found in this regional population. For microsatellite data, a partial Bayesian exclusion method, where not all possible source populations have to have been sampled, and a direct Bayesian approach, which assumes that all populations have been sampled as well as Hardy-Weinberg proportions within populations, were used. Self assignments designed to test the success of each method performed similarly for each of the microsatellite based methods. For provenance analysis, the two New Zealand colonies were combined and left separate, based on morphometrics (separate) and earlier microsatellite analysis (combined). In the microsatellite based direct analysis, results showed that most individuals caught within a certain area also originate within that ocean basin, with only a few individuals possibly originating from a different ocean basin. The microsatellite based exclusion method for both analyses excluded almost all possible source populations and could thus not be used to corroborate findings.

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INTRODUCTION

Longline fishing affects large numbers of seabird species (e.g. Tuck *et al.*, 2003; Lewison *et al.*, 2004; e.g. BirdLife-International, 2006). In petrels and albatrosses mortality associated with longline fishing occurs on a global scale. Several mitigation measures have been put into place (Brothers *et al.*, 1999; Ryan and Watkins, 2002; Lokkeborg, 2003) and observers on ships help to reduce bycatch. However population specific information is lacking for most species, hindering the assessment of magnitude and significance of seabird-fishery interactions. Population level differences in dispersal and foraging areas may cause variation in the susceptibility to fishery bycatch (Berrow *et al.*, 2000; Catard *et al.*, 2000; BirdLife-International, 2006). As indicated in previous Chapters, the White-chinned Petrel is the species most frequently killed by fisheries (Phillips *et al.*, 2006). The species is monotypic with few if any morphological differences to distinguish populations, population genetic markers may provide a tool to identify regions or colonies most at risk. Some colonies may be impacted more strongly than others by virtue of greater overlap between foraging and fishing areas. As a result they may have a larger number of birds killed in the longline process than is expected given their population size, increasing the risk of local extinction. In addition, genetic diversity may be lost within species if some populations become locally extinct.

Microsatellites have previously been used to assign individuals to populations or breeding localities in several species. Endangered whales (Dalebout *et al.*, 1998; Dalebout *et al.*, 2002), and sharks (Shivji *et al.*, 2005) have been identified in markets, and populations from which elephant tusks were poached (Wasser *et al.*, 2004). Microsatellites have also been used to identify rookeries of captive tortoises (Burns *et al.*, 2003), to prove the illegal translocation of red deer (*Cervus elaphus* - Frantz *et al.*, 2006); and to identify the source population of dispersed rock-wallabies (*Petrogale lateralis* - Eldridge *et al.*, 2001). In seabirds, mtDNA has been used to assign Black-footed albatross (*Phoebastria nigripes*) bycatch to regions (Walsh and Edwards, 2005) and microsatellites to distinguish

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Shy-type albatrosses (*Thalassarche cauta*) within bycatch carcasses (Abbott, 2004; Abbott *et al.*, 2006). One study using microsatellites attempted to locate the geographic origins of the Common Guillemot (*Uria aalge*) after oil spills, but was unsuccessful due to a lack of intrinsic structure in Common Guillemot populations (Riffaut *et al.*, 2005).

The aim of this Chapter is to identify source populations of White-chinned Petrels (*Procellaria aequinoctialis*) caught at sea using a variety of methods using cytochrome b and microsatellite DNA. Furthermore, assignment tests are used to consider if foraging locations are shared as well as investigate current gene flow.

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MATERIALS AND METHODS

White-chinned Petrels were collected from longline fishing vessels off South Africa (PG Ryan) and New Zealand (CJ Robertson). Individuals included males and females from a range of ages. Age classifications were based on gonad size and bill scarring. However, due to uncertainties in classifying all birds correctly, age class distributions were not pursued further. Most birds were sexed, but as these data were not available for all individuals, sex was not used for analysis. Tissue samples were collected from all birds and stored in 96% ethanol. Extractions were performed as described in Chapter 1.

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Restriction enzyme analysis of a single nucleotide polymorphism

158 individuals (Table 4.1) were subjected to a restriction enzyme digest. Three fixed mutational differences were found between two regional populations in the White-chinned Petrel (Chapter 2). Of these, one single nucleotide polymorphism (SNP) is recognised by the restriction enzyme *Csp6I* in sequences originating in the New Zealand regional population. Cytochrome b gene fragments of the New Zealand regional population had two *Csp6I* sites resulting in fragment sizes of 311 bp, 173 bp and 114 bp whereas gene fragments of the Atlantic/Indian Ocean regional population had one *Csp6I* site resulting in two fragments of the sizes 484 bp and 114 bp. PCR amplification of cytochrome b was as described in Chapter 2. Products were confirmed by electrophoresis on 2% agarose. Digests conditions were as follows: 5 units of *Csp6I*, 1x digestion buffer B, 5 µl of PCR product in a total volume of 14.5 µl. Digests were incubated at 37°C for 2 hours and inactivated by incubating at 65°C for 20 min. Products were electrophoresed on 3% agarose. Each agarose gel contained a control sample of known origin.

Cytochrome b sequencing

Twenty-three individuals were sequenced for the cytochrome b gene as described in Chapter 2. Eleven of these individuals were caught as bycatch at Chatham Rise, four around New Zealand, three in the South African tuna fishery and five in the Prince Edward Islands toothfish fishery. Haplotypes were

compared to those identified in Chapter 2. A Minimum Spanning Network (MSN) as well as an unrooted Bayesian tree was constructed using haplotypes identified in this Chapter and Chapter 2, to identify associations of bycatch haplotypes and haplotypes of known provenance.

Assignment tests

A genetic assignment test was carried out on all individuals genotyped for microsatellites, including both known individuals from colonies and unknown bycatch samples (Table 4.1), which were genotyped as described previously (Chapter 3). However, the majority of individuals used for Antipodes and Auckland Islands were caught within the breeding season within 100 km of the islands (Chapter 3). Several methods are available to attempt to assign individuals to their natal colonies using microsatellites. The first tests were based on allele frequencies and calculate the probability of obtaining a single multilocus genotype from a potential source population through observation of allele frequencies at each locus and each potential population (Paetkau *et al.*, 1995). Another method is the partial Bayesian assignment test (Rannala and Mountain, 1997), which uses Bayesian inference to estimate allele frequencies followed by a frequentist approach to assess the statistical significance of genotypes. Another method is full Bayesian inference (Pritchard *et al.*, 2000) which yields a posterior probability for each potential source population and which can be interpreted as the probability of an individual belonging to a given population. However, the underlying assumption of this inference is that all potential source populations have been sampled (Manel *et al.*, 2002). All these methods assume populations to be in Hardy-Weinberg proportions as well as linkage equilibrium and independence between loci. However, simulations have shown that slight deviations from Hardy-Weinberg proportions (excess of homozygotes) have little effect on assignment test performance (Cornuet *et al.*, 1999; Hauser *et al.*, 2006). It also has been shown that the full Bayesian method (Pritchard *et al.*, 2000) performed better and assigned more individuals correctly than the partial Bayesian method (Rannala and Mountain, 1997) using several data sets (Manel

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et al., 2002). However, the method is sensitive to sample size and certainty of success is difficult to assess (Hauser *et al.*, 2006).

Not all potential source populations of the White-chinned Petrel were sampled in this study (Table 4.1), violating the underlying assumption of the full Bayesian method. As this method assigns individuals to the population with the highest likelihood, false assignments can be expected. Therefore two methods were used and compared; the exclusion method is based on partial Bayesian inference (Rannala and Mountain, 1997) with simulation of probability according to Cornuet *et al.* (1999), and the direct assignment method as implemented in GENECLASS2 (Cornuet *et al.*, 1999; Piry *et al.*, 2004). The exclusion method does not require the assumption that the actual source population has been sampled and has therefore proven useful in several poaching cases (e.g. Primmer *et al.*, 2000). Instead of comparing populations as is done in the full Bayesian method, the exclusion method treats all populations separately. Assignment/exclusion of individuals to reference populations was calculated using the 'leave one out' option, so that the reference allele frequencies are calculated without introducing a bias by including the individual in question being assigned. Probability of belonging to a population is calculated by simulating a frequency distribution of 10 000 genotypes for this population by means of a Monte Carlo resampling algorithm. It then counts how many times the genotype in question is likely to occur within the 10 000 randomly sampled genotypes. If the genotype is observed less than once in 1000 randomly sampled genotypes, it is considered outside the tail of the distribution or significance threshold and the population can thus be excluded as a source population with $p<0.001$. This method has been shown to perform best with isolated populations described by high F_{ST} values (Manel *et al.*, 2002).

Direct assignment of individuals does not make use of Monte Carlo resampling and therefore does not compare the genotype in question to a generated distribution. Allele frequencies are calculated for each population. The

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programme then calculates the likelihood of this genotype belonging to each population by generating a larger data set than the original population data set, this is then repeated until 10000 genotypes have been generated. It then assigns the individual to the source population with the highest likelihood value (Cornuet *et al.*, 1999).

In wildlife forensics a stringent threshold for assignment confidence is used ($p<0.001$), as these values are used for convictions (Cornuet *et al.*, 1999; Manel *et al.*, 2002; Frantz *et al.*, 2006). However, in ecological studies a higher threshold is often used such as $p<0.01$ (e.g. Eldridge *et al.*, 2001; e.g. Pruett and Winker, 2005; Riffaut *et al.*, 2005) or even $p<0.05$ (e.g. Abbott *et al.*, 2006). The latter two thresholds were used in this study as this study has ecological rather than forensic implications.

In the analysis the two New Zealand colonies, Antipodes and Auckland, were treated in two ways; for one analysis they were combined into one population, as both F_{ST} and R_{ST} found no significant differentiation between the two colonies (Chapter 3). The second analysis treated them separately as morphometrical differences have been found to exist between the two colonies (C.J. Robertson, personal communication).

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Table 4.1: Origins of samples used in this Chapter. Samples are divided into birds from known breeding colonies (a) including colonies that have not been sampled, and birds caught at sea (b). Note: as explained in Chapter 3, the majority of birds assumed as belonging to either Antipodes or Auckland Island were caught within 100 km of the islands during the breeding season.

(a) Known-provenance White-chinned Petrel samples from breeding colonies

Breeding Colony	Approximate colony size* (pairs)	Number of samples for microsatellites	Number of samples for SNP
South Georgia	2,000,000	39	15
Marion Island	10s of 1000s	85	19
Iles Crozet	many tens of thousands	14	11
Antipodes	100,000	38	22
Auckland Islands	100,000	92	16
Kerguelen	100,000 - 300,000	0	0
Campbell Island	10 000	0	0

(b) Samples of White-chinned Petrels caught at sea

Area	Source	Number of samples for microsatellites	Number of samples for SNP	Number of samples for cytochrome b
South Africa	Tuna and Hake Longline Fisheries	23	102	3
Prince Edwards	Toothfish Longline Fishery	20	10	5
New Zealand	Longline Fishery around NZL	31	46	4
	Chatham Rise	14	0	11

*** Population estimates according to BirdLife International (2006) and Brooke (2004).**

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RESULTS

Assignment using restriction enzyme analysis and cytochrome b sequencing

Twenty-three individuals caught at sea and of unknown provenance were sequenced for the cytochrome b gene. In addition to these individuals and the known provenance samples sequenced for the mitochondrial cytochrome b gene (Chapter 3), 158 individuals of unknown origin were tested for the presence or absence of a restriction cutting site within the cytochrome b gene at one single nucleotide polymorphism (SNP) site identified in Chapter 2. The site had been shown fixed in 83 known individuals (Chapter 2). One individual sequenced, which had been caught within the New Zealand region showed an absence of the cutting site (as would be expected for the Atlantic/Indian Ocean regional population) but contained the two fixed mutations indicative of the New Zealand regional population. It was therefore assigned to New Zealand. All birds caught within the New Zealand regional population were assigned to this population and all individuals caught within the Atlantic or Indian Ocean were assigned to that regional population (Table 4.2). This suggests that little if any mixing occurs at foraging locations of birds from other regional populations. However, some individuals assigned to the Atlantic/Indian population could be birds visiting from New Zealand due to the individual found to contain a SNP site indicative of the Atlantic/Indian Ocean population but containing the mutations found in New Zealand at the other two fixed sites. As neither of the other two fixed sites identified in Chapter 2 contained sites recognised by restriction enzymes, mismatch primers (FitzSimmons *et al.*, 1997) for either site could be designed to add an additional SNP test for certainty.

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Cytochrome b sequencing of the 23 additional unknown birds revealed an additional five haplotypes to the seventeen haplotypes identified previously (Chapter 2). Haplotype B19 contains the mutation at the SNP cutting site, but the other two fixed mutations indicative of the New Zealand regional population (Table 4.3). Figures 4.1 and 4.2 show the Minimum Spanning Network and

unrooted Bayesian tree depicted in Chapter 2 respectively with the addition of the five new haplotypes.

Table 4.2: Summary of bycatch cytochrome b genes digested with *Csp6I* and assigned to either regional population. *N* number of individuals tested.

Fishery	*n*	Atlantic/Indian Ocean population	New Zealand population
South African tuna and hake fisheries	102	102	0
Prince Edwardstoothfish fishery	10	10	0
New Zealand longline fisheries	46	0	46

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Assignment using microsatellites

Firstly, assignment tests were evaluated treating the two New Zealand Islands as one population. Overall, the two assignment methods performed similarly in the self test in assigning 73% (direct) and 72% (exclusion) of individuals to the correct breeding colony (Table 4.4). However, the direct method performed better in correctly assigning 91% of individuals to the Indian Ocean/Atlantic regional population and 83% to the New Zealand regional population (as identified in Chapter 2), as opposed to 80% and 73% respectively for the exclusion method. A further difference was the accuracy with which each method assigned individuals to specific colonies (Table 4.4). As overall both methods were similar in performance, both were used to assign White-chinned Petrels killed at sea. The exclusion method excluded all possible source populations for each individual (Table 4.4). The direct assignment method identified several birds that did not belong into the region they were caught (Table 4.4) indicating some mixing of colonies at foraging locations. However, of the eleven individuals caught off South Africa and assigned to New Zealand, two contained one allele specific to the Atlantic/Indian Ocean regional population. The same allele (De33, allele 179) was found in one of the four individuals caught in the toothfish fishery that had

Table 4.3: Variable sites in the cytochrome b fragment for the White-chinned Petrel. Nucleotide positions are relative to the published *P. aequinoctialis* sequence (Genbank Accession number U74350). *N* is the total number of individuals sharing that particular haplotype. In cases were haplotypes were specific to a sampling location it was indicated as follows: South Georgia (SG), Marion Island (M), New Zealand regional population (NZL), Antipodes (An), Auckland Island (Au), samples found exclusively in bycatch samples were indicated by a *B* after the species identification. Grey positions identify fixed mutations (exception wcpB19).

Haplotype	108	117	135	158	162	164	177	187	207	226	285	291	300	333	335	351	376	384	393	400	453	462	465	469	481	540	543	576	595	637	Total *N*
Atlantic/ Indian Ocean																															
wcp1	T	C	G	C	A	A	T	G	T	A	T	T	A	G	G	A	G	C	A	T	T	T	C	A	C	T	C	A	A	G	36
wcpSG2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	2
wcpSG3	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
wcpSG4	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
wcpM5	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
wcpM6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	1
wcpB20	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
wcpB21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
wcpB22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	1
New Zealand islands																															
wcpNZL7	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	T	.	.	.	.	.	13
wcpNZL8	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	T	.	.	.	.	.	5
wcpNZL9	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	T	.	.	.	G	.	22
wcpAn10	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.	.	.	.	G	.	.	.	.	G	T	.	.	.	.	.	1
wcpAn11	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	T	C	.	.	G	.	1
wcpNZL12	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	G	.	C	.	.	.	T	.	.	.	.	.	3
wcpAn13	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	T	.	.	.	.	.	2
wcpNZL14	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	4
wcpAu15	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	C	C	.	.	.	T	.	.	.	G	.	1
wcpAu16	.	.	.	.	.	.	.	.	C	G	.	.	.	.	A	.	.	.	G	.	C	.	.	.	T	.	.	.	.	.	2
wcpAu17	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	G	T	.	.	.	.	.	1
wcpB18	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	G	.	C	.	.	.	T	.	.	.	.	.	1
wcpB19	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	T	.	.	.	.	.	1

Figure 4.1: Minimum Spanning Network of 25 haplotypes identified in the White-chinned Petrel. Details of original haplotypes can be found in Chapter 2 (Figure 2.2). Haplotypes found in the New Zealand regional population are in black, haplotypes found in the Atlantic and Indian Ocean regional population are in grey. Haplotypes found in bycatch are in white. wcpB18 and wcpB19 were caught in the New Zealand longline fishery, whereas wcpB20-22 were caught off South Africa.

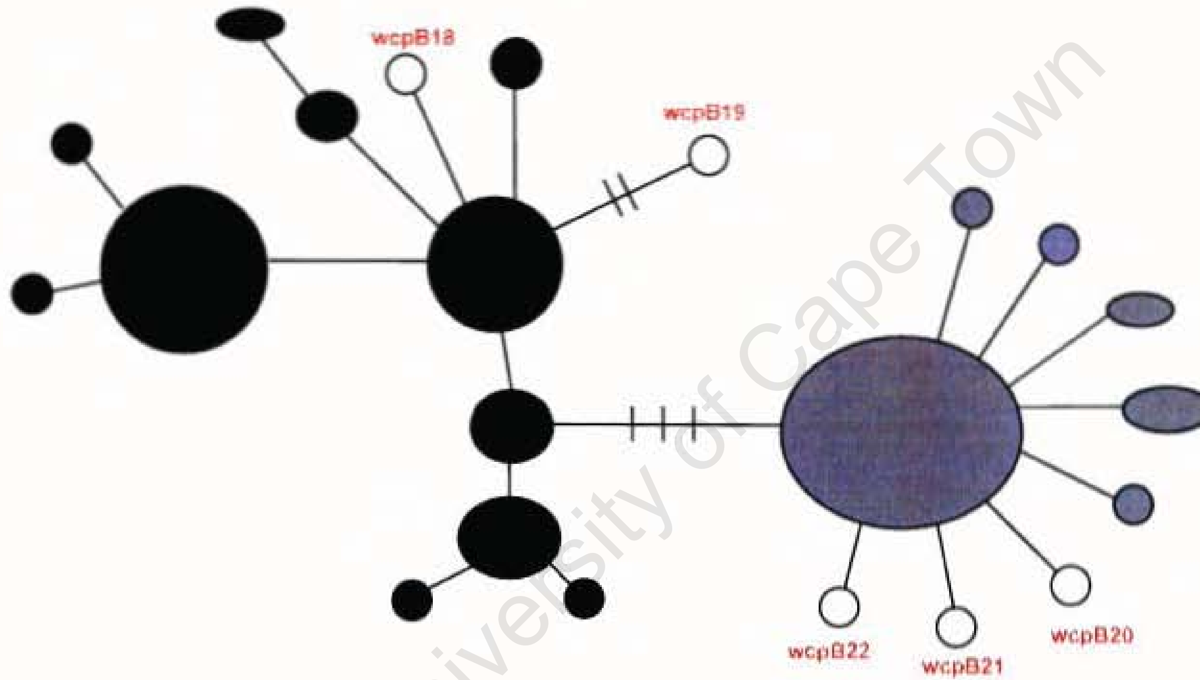

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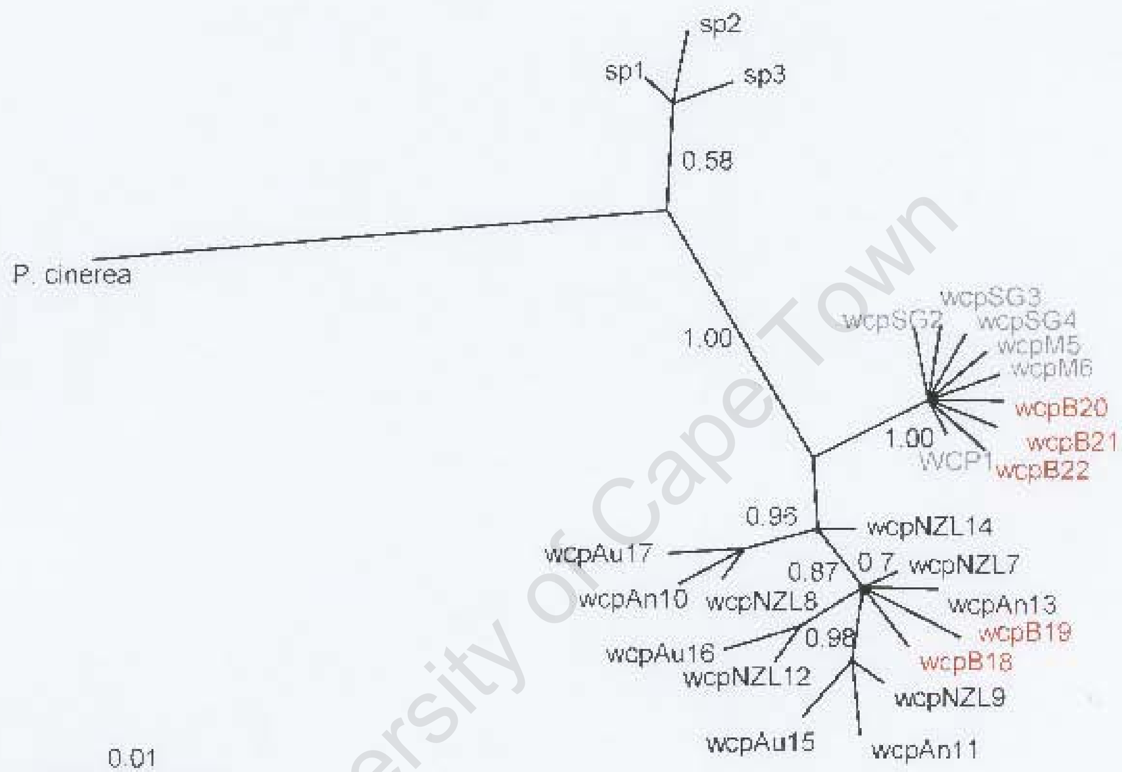
Figure 4.2: Unrooted tree constructed from Bayesian inference using haplotypes from the White-chinned Petrel and Spectacled Petrel (see Figure 2.3 for details). Haplotypes found in bycatch are represented in red. WcpB18 and wcpB19 were caught in the New Zealand longline fishery, whereas wcpB20-22 were caught off South Africa.



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had been assigned to New Zealand indicating that these birds have been misclassified. In contrast, 3 of the 24 individuals caught in New Zealand that had been assigned to colonies in the other regional population contained alleles specific to the regional population they had been assigned to (Paequ2, allele 210, and Paequ8, allele 232). In addition two individuals caught in New Zealand but assigned elsewhere each contained one allele specific to New Zealand (Paequ2, allele 214 and De33, allele 182) as well as one or more alleles specific to the Atlantic/Indian Ocean regional population (Paequ2, alleles 210 and 194, De33, allele 179).

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When New Zealand colonies were treated separately, the assignment of known provenance individuals compared to the results above (Table 4.4), except that for both direct and exclusion methods, each colony alone had a lower percentage success of correct self assignment than the two colonies combined (Table 4.5). When the exclusion method was used to assign bycatch, again most colonies were excluded as possible source populations and only a small percentage of birds was assigned. The direct method suggested that most birds caught off South Africa and at the Prince Edward Islands, originated in the Indian Ocean. Similarly, only one bird caught at Chatham Rise was assigned to South Georgia, with the remaining individuals being assigned to either of the two New Zealand colonies. These results therefore support the findings of cytochrome b. On the other hand, a large percentage of birds caught around the New Zealand were assigned mostly to Marion Island (53%) and Auckland Island (38%). Of the six individuals caught off South Africa and assigned to either New Zealand colony, none contained region specific South African alleles. Of the three individuals caught in the Prince Edward Island toothfish fishery that were assigned to New Zealand, one contained an allele specific to the Atlantic/Indian Ocean regional population (Paequ10, allele 197) indicating probable mis-assignment. The one individual caught in Chatham Rise and assigned to South Georgia did not have a region specific allele. In contrast, four out of the eight individuals caught in New Zealand that had been assigned to colonies in the other regional population

contained alleles specific to the regional population they had been assigned to (Paequ2, allele 194 and 210, and De33, allele 179). In addition, of these four, two contained each one allele specific to New Zealand (Paequ2, allele 214 and De33, allele 182) as well as one or more alleles specific to the Atlantic/Indian Ocean regional population (Paequ2, alleles 210 and 194, De33, allele 179).

The above observed disagreement between methods as well as the observation that several individuals were assigned to one colony or regional population when indeed they contained specific alleles to another colony or regional population, indicates that the microsatellite assignment is not robust. Robustness could be improved by increasing sample sizes of known provenance birds as well as by increasing the number of polymorphic loci. A further problem is that many 'known' provenance samples from New Zealand are in fact bycatch samples. Resolution, as well as confidence of results, between these two colonies could be improved by sampling chicks in breeding colonies at these islands.

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Table 4.4: Results of microsatellite based provenance assignment testing using partial Bayesian Inference with simulation (a) and direct assignment (b). Known-origin White-chinned Petrels were used to assess success of assignments and to compare distributions to bycatch caught in different fisheries. The two New Zealand colonies, Antipodes and Auckland Island were combined for analysis. Numbers in bold within the self-assignment indicate correctly assigned birds. *n*, number of samples. 1, South Georgia, 2, Marion Island, 3, Iles Crozet, 4 both New Zealand colonies, 5 colonies of the Atlantic and Indian Ocean combined.

(a) Partial Bayesian inference

Colony	*n*	*n* unambiguously assigned to					all source colonies excluded	% correctly assigned
		1	2	3	4	5		
1	39	**17**	12	1	2	30	7	43.6
2	85	3	**63**	1	7	74	11	74.1
3	14	1	10	**2**	0	13	1	14.3
4	130	5	18	0	**95**	23	12	73.1
5	138					**110**	19	79.7
Total correct	*268*							*72.0*
Bycatch								
New Zealand	13	0	0	0	0	0		-
Chatham Rise	18	0	0	0	0	0		-
Prince Edwards	20	0	0	0	0	0		-
South Africa	23	0	0	0	0			-

(b) Direct assignment

Colony	*n*	1	2	3	4	5	all source colonies excluded	% correctly assigned
1	39	**25**	8	2	4	35	-	64.1
2	85	12	**50**	14	9	76	-	58.8
3	14	0	2	**12**	0	14	-	85.7
4	130	8	14	0	**108**	22	-	83.1
5	138					**125**	-	90.6
Total correct	*268*							*72.8*
Bycatch								
New Zealand	13	6	2	2	3	10		-
Chatham Rise	18	8	6	0	4	14		-
Prince Edwards	20	13	1	2	4	16		-
South African	23	5	5	2	11	12		-

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Table 4.5: Results of microsatellite based provenance assignment testing using partial Bayesian Inference with simulation (a) and direct assignment (b). Known-origin White-chinned Petrels were used to assess success of assignments and to compare distributions to bycatch caught in different fisheries. The two New Zealand colonies, Antipodes and Auckland Island were treated separate for analysis. Numbers in bold within the self-assignment indicate correctly assigned birds. *n*, number of samples. 1, South Georgia, 2, Marion Island, 3, Iles Crozet, 4 Antipodes, 5 Auckland Island, 6 both New Zealand colonies combined, 7 colonies of the Atlantic and Indian Ocean combined.

a) Partial Bayesian inference

Colony	*n*	*n* unambiguously assigned to							all source colonies excluded	% correctly assigned
		1	2	3	4	5	6	7		
1	39	**17**	13	1	3	2	5	31	3	43.6
2	85	4	**65**	2	3	4	7	71	7	76.5
3	14	3	8	**2**	0	0	0	13	1	14.3
4	38	3	5	0	**19**	7	26	8	4	50.0
5	92	2	13	0	24	**49**	73	15	4	53.3
6	130						**111**	23		85.4
7	138						12	**115**		83.3
Total correct	268									*60.8*
Bycatch										
New Zealand	13	6	1						6	
Chatham	18	9	1						8	
Prince Edwards	20	6	0	0	0	0			13	
South African	23	2							20	

b) Direct assignment

Colony	*n*	1	2	3	4	5	6	7	all source colonies excluded	% correctly assigned
1	39	**24**	7	2	4	2	6	33		61.5
2	85	10	**48**	14	7	6	13	72		56.5
3	14	0	2	**12**	0	0	0	14		85.7
4	38	4	3	0	**21**	10	31	7		55.3
5	92	3	7	0	21	**61**	82	10		66.3
6	130						**113**	17		86.9
7	138						19	**119**		86.2
Total correct	268									61.9
Bycatch										
New Zealand	13	1	7	0	0	5	5	8		
Chatham	18	1	0	0	7	10	17	1		
Prince Edwards	20	2	14	1	2	1	3	17		
South African	23	5	12	0	4	2	6	17		

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DISCUSSION

Performance of assignment tests

Differences in allele frequencies, as measured by F_{ST}, show that in the recent past gene flow has been reduced between ocean basins (Chapter 3). Studies of albatrosses have shown that oceanic species show no or little structuring with microsatellite DNA, whereas species feeding primarily in shelf waters show stronger population structuring (Burg and Croxall, 2001; Abbott and Double, 2003; Burg and Croxall, 2004). Cytochrome b analysis clearly identified two regional populations (Chapter 2), which is supported by the microsatellite data (Chapter 3). Consequently the probability of correctly assigning individuals to colonies increased if only the correct regional population was required. Other studies using genotypes for correct assignment achieved success rates of 60% in polar bears (*Ursus maritimus*, Paetkau *et al.*, 1995), 6% in the common guillemot (*Uria aalge*, Riffaut *et al.*, 2005), 64-69% in wild Turkey (*Meleagris gallopavo silvestris*, Latch and Rhodes, 2005), and 50-80% in African Elephants (*Loxodonta africana*, Wasser *et al.*, 2004). This study's results are well in the range of published studies, and even better than many bird studies. This study shows success of self assignment ranging from 14% to 86% across both microsatellite based methods. When these methods were compared, both the exclusion and direct assignment methods had similar success in the self assignment of known individuals. Not all possible source colonies for the White-chinned Petrel had been sampled, violating one of the assumptions of the direct method. It is therefore surprising that both methods performed similarly in the self test. When it came to assigning unknown birds, however, the exclusion method either excluded most or all possible source colonies.

A discrepancy was found between assignment using microsatellite DNA and a SNP test based on a fixed difference in the cytochrome b gene between the two regional populations. The SNP test showed that birds were caught in their region of origin (albeit interpreted with some caution), whereas assignment based on microsatellite DNA showed some dispersal. Further examination found some

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individuals that were inferred to have moved between regions, containing alleles specific to the region in which they were caught. As self tests showed, assignment was not 100% accurate and therefore these individuals may have been misclassified. In contrast, some individuals assigned to the opposite regional population indeed contained alleles specific to this opposite population and can therefore be assumed to have originated there.

Success of assignments could be increased in two ways. To increase confidence of microsatellite assignment, more loci could be used. Some of the microsatellite loci used in this study contained only two to three alleles. By increasing the number of loci, more resolution between colonies could be achieved. In addition, the majority of birds used as known provenance samples from the New Zealand islands were caught as bycatch within a 100 km radius of islands during the breeding season (discussed in Chapter 3). The validity of this assumption is difficult to assess (attempted in Chapter 3), but assignment of these can only be attempted if a larger source population is presented. Therefore, in order to increase confidence as well as success rate in other colonies, more samples need to be included in future research. In order to resolve the ambiguity of the one fixed mutation within the cytochrome b SNP site, one or two of the other fixed mutations could be included. However, neutral markers may not always reflect adaptive variation, as is seen with birds from Antipodes and Auckland, where differences could be found using morphometrics (C.J. Robertson, personal communication) but not with either DNA marker. This is especially so, when some gene flow exists between colonies. To improve assignments a genetic marker could be used that reflects adaptive variation such as genes involved in immune response. Seabirds often have a high frequency of parasites and thus there is potential for local adaptations (McCoy *et al.*, 2003). The major histocompatibility complex (MHC) might be an ideal marker as these loci are variable and under strong selection.

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Distribution and gene flow at sea

Information about the provenance of White-chinned Petrels caught at sea because fishery bycatch would facilitate identification of those colonies most affected of bycatch mortality. The molecular data suggest that most (if not all) White-chinned Petrels stay within the ocean basins where their breeding colonies are located. Mitochondrial gene analysis shows that females are philopatric and no gene flow occurs between regional populations. Microsatellite assignment shows several birds away from natal colonies, although the presence of visiting birds only increases the chance of but does not necessarily result in gene flow. Higher genetic differentiation can be expected in a uniparentally inherited mitochondrial (mtDNA) marker, than with a biparentally inherited nuclear marker (Durand *et al.*, 2005). MtDNA markers have a four-fold smaller effective population size than nuclear DNA. This makes mtDNA more susceptible to genetic drift. Genetic models, however, show, that larger differences between these two types of markers are unlikely to simply reflect the differences in effective population sizes and can be indicative of sex-biased dispersal (Mossman and Waser, 1999). Chapter 2 and 3 reported that cytochrome b G_{ST} values between regional populations are up to ten times higher than corresponding microsatellite values, thus gene flow is likely to be male mediated, at least between regional populations.

It has been hypothesised that species that remain near their breeding colonies or feed inshore, are genetically more structured than species feeding offshore or undergoing distant winter migrations (Friesen, 1997; Burg and Croxall, 2001). The White-chinned Petrel forages primarily in coastal shelf waters and is capable of flying considerable distances (Weimerskirch *et al.*, 1999). As most seabirds nest on remote locations tracking studies are difficult and costly and therefore only a small number of birds are followed (but see Nicholls *et al.*, 1995; Gonzalez-Solis *et al.*, 2002; Phillips *et al.*, 2005; Phillips *et al.*, 2006). Thus information about inter-colony movements is mostly incidental or comes from banding studies. So far only three recoveries have been made of chicks banded

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on Iles Crozet that have been recovered on shores of South Africa and Namibia after 12 and 14 month and eight years respectively (Marchant and Higgins, 1990; Hockey *et al.*, 2006). Satellite tagging has been used to track breeding birds from Iles Crozet and South Georgia as well as non-breeding adults from South Georgia using GLS (Hockey *et al.*, 2006). Neither study has found large movements of birds away from colonies. It is not known if foraging grounds of different populations or colonies of the White-chinned Petrel overlap during breeding and non-breeding season, as relatively few White-chinned Petrels have been banded and recoveries are insufficient to confidently infer dispersal patterns (e.g. Marchant and Higgins, 1990). The handbook of Australian, New Zealand and Antarctic birds (HANZAB) mentions possible movements from Iles Crozet to Australian waters as well as movements from New Zealand to Chile. However, no banding studies have been conducted to support these observations (Marchant and Higgins, 1990). One study tracking adult White-chinned Petrels from South Georgia indicates that overlap may be restricted throughout the year (Phillips *et al.*, 2006). Adults equipped with GLS loggers remained in the waters off the Southwest Atlantic Ocean year round. The only other colony where tracking has been attempted is the Crozets, where breeding adults dispersed to waters off South Africa (Catard *et al.*, 2000). However, their non-breeding range is unknown. Genetic studies offer the possibility of indirectly studying population structure and gene flow (Edwards *et al.*, 2001). Assignment tests show that foraging ranges of colonies in the different ocean basins mostly do not overlap supporting the tracking data. This observation can serve as the explanation to the genetic structuring seen in an offshore seabird.

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Conservation implications

White-chinned Petrels mainly forage in highly productive coastal and upwelling regions. This inevitably results in overlap with fishing vessels (Phillips *et al.*, 2006). The impact of the continued incidental mortality of seabirds is hard to assess as petrels have long life spans. Current disturbances to populations may not be seen for several years even though effective mitigation measures are now

in place to decrease mortality. Diversity at neutral microsatellite markers is generally low in seabirds (e.g. Burg and Croxall, 2001; Abbott and Double, 2003; Van Bekkum *et al.*, 2006), which was also found in this study (Chapter 3). As incidental bycatch is a recent disturbance and White-chinned Petrel generation time is more than six years, effects on colony level may not yet be evident in neutral markers. Knowledge of dispersal routes can help establish management plans for colonies at risk. This study has shown that although dispersal events happen, the majority of individuals are philopatric and gene flow across ocean basins is limited. In addition, foraging ranges do not seem to overlap across ocean basins. Therefore, the impact of a given fishery is likely to be mainly confined to nearby breeding colonies. Birds killed off South Africa probably originate in the Indian Ocean, whereas those killed in New Zealand waters probably originate there. This is of concern for local populations, given the largest breeding population occurs at South Georgia. Populations in the Indian and New Zealand sectors are a magnitude smaller than the South Atlantic population, increasing the risks of local extinctions. Furthermore, the New Zealand population contains most of the genetic diversity, yet supports only 10% of the total population. However, foreign birds were found in different fisheries, as well as immigrants to distant colonies, making petrel conservation a global as well as a local issue.

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APPENDICES

Table A1: List of White-chinned Petrel samples used in genetic analysis from South Georgia

Sample ID	Origin	Sample material	Marker	
bird1	Bird Is/South Georgia	blood	microsat	
bird2	Bird Is/South Georgia	blood	microsat	
bird3	Bird Is/South Georgia	blood	microsat	
bird4	Bird Is/South Georgia	blood	microsat	
bird5	Bird Is/South Georgia	blood	microsat	
bird6	Bird Is/South Georgia	blood	microsat	
bird7	Bird Is/South Georgia	blood	microsat	
bird8	Bird Is/South Georgia	blood	microsat	
bird9	Bird Is/South Georgia	blood	microsat	
bird10	Bird Is/South Georgia	blood	microsat	
bird11	Bird Is/South Georgia	blood	microsat	
bird12	Bird Is/South Georgia	blood	microsat	
bird13	Bird Is/South Georgia	blood	microsat	
bird14	Bird Is/South Georgia	blood	microsat	
bird15	Bird Is/South Georgia	blood	microsat	
bird16	Bird Is/South Georgia	blood	microsat	
bird17	Bird Is/South Georgia	Blood	microsat	cyt b
bird18	Bird Is/South Georgia	Blood	microsat	cyt b
Bird19	Bird Is/South Georgia	Blood	microsat	
Bird20	Bird Is/South Georgia	Blood	microsat	cyt b
Bird21	Bird Is/South Georgia	Blood	microsat	cyt b
Bird22	Bird Is/South Georgia	Blood	microsat	
Bird23	Bird Is/South Georgia	Blood	microsat	
Bird24	Bird Is/South Georgia	Blood	microsat	cyt b
Bird25	Bird Is/South Georgia	Blood	microsat	
Bird26	Bird Is/South Georgia	Blood	microsat	cyt b
Bird27	Bird Is/South Georgia	Blood	microsat	cyt b
Bird28	Bird Is/South Georgia	Blood	microsat	cyt b
Bird29	Bird Is/South Georgia	Blood	microsat	cyt b
Bird30	Bird Is/South Georgia	Blood	microsat	
Bird31	Bird Is/South Georgia	Blood	microsat	
Bird32	Bird Is/South Georgia	Blood	microsat	cyt b
Bird33	Bird Is/South Georgia	blood	microsat	cyt b
Bird34	Bird Is/South Georgia	Blood	microsat	cyt b
Bird35	Bird Is/South Georgia	Blood	microsat	cyt b
Bird36	Bird Is/South Georgia	Blood	microsat	cyt b
Bird37	Bird Is/South Georgia	Blood	microsat	
Bird38	Bird Is/South Georgia	Blood	microsat	
Bird39	Bird Is/South Georgia	Blood	microsat	cyt b
Bird40	Bird Is/South Georgia	Blood	microsat	
Bird41	Bird Is/South Georgia	blood	microsat	

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Table A2: List of White-chinned Petrel samples used in genetic analysis from Marion Island

Sample ID	Date of sampling	Origin	Location	Sample material	Marker	
marion17	06/10/1996		Marion Island	tissue	microsat	
marion18	06/10/1996		Marion Island	tissue	microsat	cyt b
marion19	06/10/1996		Marion Island	tissue	microsat	
marion20	06/10/1996		Marion Island	tissue	microsat	
marion21	11/10/1996		Marion Island	tissue	microsat	
marion22	04/11/1996		Marion Island	tissue	microsat	cyt b
marion23	01/12/1996		Marion Island	tissue	microsat	
marion24	13/12/1996		Marion Island	tissue	microsat	
marion25	14/12/1996		Marion Island	tissue	microsat	cyt b
marion26	14/12/1996		Marion Island	tissue	microsat	
marion27	19/12/1996		Marion Island	tissue	microsat	
marion28	19/12/1996		Marion Island	tissue	microsat	
marion29	19/12/1996		Marion Island	tissue	microsat	
marion30	03/01/1997		Marion Island	tissue	microsat	
marion31	03/01/1997		Marion Island	tissue	microsat	
marion32	07/01/1997		Marion Island	tissue	microsat	
marion33	07/01/1997		Marion Island	tissue	microsat	
marion34	12/01/1997		Marion Island	tissue	microsat	
marion35	28/01/1997		Marion Island	tissue	microsat	
marion36	28/01/1997		Marion Island	tissue	microsat	
marion37	01/02/1997		Marion Island	tissue	microsat	
marion38	5/02/2004		Marion Island	tissue	microsat	
marion39	10/04/2004	carcass Archway	Marion Island	tissue	microsat	
marion40	10/04/2004	carcass Trypot	Marion Island	tissue	microsat	
marion41	10/03/2004		Marion Island	tissue	microsat	
marion42			Marion Island	feather	microsat	
marion43 WCP9	4/12/1990	Koryo Maru		tissue	microsat	
marion44 WCP18	20/11/1998	Koryo Maru		tissue	microsat	
marion45 WCP26	28/11/1998	Koryo Maru		tissue	microsat	
marion46 WCP13	19/11/1998	Koryo Maru		tissue	microsat	
marion47 WCP15	19/11/1998	Koryo Maru		tissue	microsat	
marion48 WCP7	19/11/1998	Koryo Maru		tissue	microsat	
marion49 WCP6	3/12/1998	Koryo Maru		tissue	microsat	cyt b
marion50 WCP11	19/11/1998	Koryo Maru		tissue	microsat	
marion51 WCP23	28/11/?	Koryo Maru		tissue	microsat	cyt b
marion52 WCP4	3/12/1998	Koryo Maru		tissue	microsat	cyt b
marion53 WCP14	19/11/1998	Koryo Maru		tissue	microsat	cyt b
marion54 WCP30	29/11/1998	Koryo Maru		tissue	microsat	cyt b
marion55 WCP28	29/11/1998	Koryo Maru		tissue	microsat	
marion56 WCP12	19/11/1998	Koryo Maru		tissue	microsat	
marion57 WCP21	28/11/?	Koryo Maru		tissue	microsat	cyt b
marion58 WCP2	19/11/1998	Koryo Maru		tissue	microsat	cyt b
marion59 WCP19	27/11/1998	Koryo Maru		tissue	microsat	cyt b
marion60 WCP29	29/11/1998	Koryo Maru		tissue	microsat	cyt b
marion61 WCP17	20/11/1998	Koryo Maru		tissue	microsat	
marion62 WCP20	17/11/1998	Koryo Maru		tissue	microsat	
marion63 WCP8	3/12/1998	Koryo Maru		tissue	microsat	
marion64 WCP10	19/11/1998	Koryo Maru		tissue	microsat	
marion65 WCP16	19/11/1998	Koryo Maru		tissue	microsat	cyt b
marion66 WCP27	29/11/1998	Koryo Maru		tissue	microsat	
marion67				tissue	microsat	cyt b
marion68				tissue	microsat	cyt b
marion69				tissue	microsat	cyt b
marion70				tissue	microsat	cyt b
marion71				tissue	microsat	
marion72				tissue	microsat	
marion73				tissue	microsat	
marion74				tissue	microsat	
marion75				tissue	microsat	
marion76				tissue	microsat	
marion77				tissue	microsat	
marion78				tissue	microsat	
marion79				tissue	microsat	
marion80				tissue	microsat	
marion81				tissue	microsat	
marion82				tissue	microsat	
marion83				tissue	microsat	
marion84				tissue	microsat	
marion85				tissue	microsat	
marion86				tissue	microsat	
marion87				tissue	microsat	
marion88				tissue	microsat	
marion89				tissue	microsat	

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Table A2 continued

Sample ID	Date of sampling	Origin	Location	Sample material	Marker	
marion90				tissue	microsat	cyt b
marion91				tissue	microsat	
marion92				tissue	microsat	
marion93				tissue	microsat	cyt b
marion94				tissue	microsat	
marion95				tissue	microsat	
marion96				tissue	microsat	
marion97				tissue	microsat	
marion98				tissue	microsat	
marion99				tissue	microsat	
marion100				tissue	microsat	
marion101				tissue	microsat	
marion102				tissue	microsat	
marion103				tissue	microsat	
marion104				tissue	microsat	

Table A3: List of White-chinned Petrel samples used in genetic analysis from Iles Crozet

Sample ID	Date of sampling	Origin	Location	Sample material	Marker	
Crozet1			Crozet Island	muscle	microsat	
Crozet2			Crozet Island	muscle	microsat	
Crozet3	17/03/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet4	26/03/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet5	16/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet6	5/02/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet7	29/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet8		Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet9	2/03/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet10	24/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet11	18/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	
Crozet12	23/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet13		Ile Bourbon	Crozet Island	tissue	microsat	cyt b
Crozet14	25/01/2004	Ile Bourbon	Crozet Island	tissue	microsat	cyt b

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Table A4: List of White-chinned Petrel samples used in genetic analysis from Antipodes

Sample ID	Date of sampling	Location	Sex	Sample material	Marker	
A1	20/01/2003	Antipodes		liver	microsat	cyt b
A2	21/01/2003	Antipodes		blood	microsat	cyt b
A3	16/02/2003	Antipodes		blood	microsat	cyt b
A4	17/02/2003	Antipodes		blood	microsat	
A5	18/02/2003	Antipodes		blood	microsat	cyt b
A6	25/02/2003	Antipodes		blood	microsat	cyt b
A7	26/02/2003	Antipodes		blood	microsat	cyt b
A8	26/02/2003	Antipodes		blood	microsat	cyt b
A9	1/03/2003	Antipodes		blood	microsat	cyt b
A10	1/03/2003	Antipodes		blood	microsat	cyt b
A11	2/03/2003	Antipodes		blood	microsat	cyt b
A12	5/03/2003	Antipodes		blood	microsat	cyt b
A13	5/03/2003	Antipodes		blood	microsat	
A14	5/03/2003	Antipodes		blood	microsat	cyt b
A15	7/03/2003	Antipodes		blood	microsat	cyt b
A16	8/03/2003	Antipodes		blood	microsat	cyt b
A17	9/03/2003	Antipodes		liver	microsat	cyt b
A18/011025	30/11/2000	Antipodes	Female	liver	microsat	cyt b
A19/011042	26/11/2000	Antipodes	Male	liver	microsat	cyt b
A20/011048	9/12/2000	Antipodes	Male	liver	microsat	cyt b
A21/011049	2/12/2000	Antipodes	Male	liver	microsat	
A22/011050	9/12/2000	Antipodes	Male	liver	microsat	
A23/011053	9/12/2000	Antipodes	Male	liver	microsat	cyt b
A24/011054	5/12/2000	Antipodes	Male	liver	microsat	cyt b
A25/011058	27/11/2000	Antipodes	Male	liver	microsat	cyt b
A26/011059	27/11/2000	Antipodes	Male	liver	microsat	
A27/011353	17/12/2000	Antipodes	Male	liver	microsat	
A28/011354	17/12/2000	Antipodes	Male	liver	microsat	
A29/022892	16/11/2002	Antipodes	Female	liver	microsat	
A30/022900	14/11/2002	Antipodes	Female	liver	microsat	
A31/022907	14/11/2002	Antipodes	Male	liver	microsat	
A32/022909	14/11/2002	Antipodes	Female	liver	microsat	cyt b
A33/022911	14/11/2002	Antipodes	Male	liver	microsat	
A34/022915	15/11/2002	Antipodes	Male	liver	microsat	
A35/022919	14/11/2002	Antipodes	Male	liver	microsat	
A36/022921	15/11/2002	Antipodes	Female	liver	microsat	
A37/022929	14/11/2002	Antipodes	Male	liver	microsat	
A38/033044	24/11/2002	Antipodes	Male	liver	microsat	

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Table A5: List of White-chinned Petrel samples used in genetic analysis from Auckland Island (Disappointment Island)

Sample ID	Date of sampling	Location	Sex	Sample material	Marker	
Dis1		Auckland		feather	microsat	cyt b
Dis2		Auckland		feather	microsat	
Dis3		Auckland		feather	microsat	cyt b
Dis4		Auckland		feather	microsat	cyt b
Dis5		Auckland		feather	microsat	cyt b
Dis6		Auckland		feather	microsat	cyt b
Dis7		Auckland		feather	microsat	
Dis8		Auckland		muscle	microsat	cyt b
AU1/997	8/12/2000	Auckland	Male	liver	microsat	
AU2/11012	5/12/2000	Auckland	Female	liver	microsat	
AU3/011013	8/12/2000	Auckland	Male	liver	microsat	cyt b
AU4/011014	8/12/2000	Auckland	Male	liver	microsat	cyt b
AU5/011033	16/12/2000	Auckland	Male	liver	microsat	
AU6/011078	15/11/2000	Auckland	Male	liver	microsat	
AU7/011080	17/11/2000	Auckland	Female	liver	microsat	
AU8/011081	17/11/2000	Auckland	Male	liver	microsat	
AU9/011082	19/11/2000	Auckland	Female	liver	microsat	
AU10/011083	20/11/2000	Auckland	Male	liver	microsat	
AU11/011084	17/11/2000	Auckland	Male	liver	microsat	
AU12/011085	20/11/2000	Auckland	Female	liver	microsat	
AU13/011086	20/11/2000	Auckland	Male	liver	microsat	
AU14/011087	19/11/2000	Auckland	Male	liver	microsat	
AU15/011088	19/11/2000	Auckland	Female	liver	microsat	
AU16/011089	19/11/2000	Auckland	Male	liver	microsat	
AU17/011090	20/11/2000	Auckland	Male	liver	microsat	cyt b
AU18/011091	17/11/2000	Auckland	Female	liver	microsat	cyt b
AU19/011092	20/11/2000	Auckland	Male	liver	microsat	cyt b
AU20/011093	19/11/2000	Auckland	Male	liver	microsat	cyt b
AU21/011094	27/11/2000	Auckland	Female	liver	microsat	
AU22/011097	15/11/2000	Auckland	Male	liver	microsat	cyt b
AU23/011098	27/11/2000	Auckland	Male	liver	microsat	cyt b
AU24/011099	27/11/2000	Auckland	Female	liver	microsat	cyt b
AU25/011100	15/11/2000	Auckland	Male	liver	microsat	cyt b
AU26/011101	15/11/2000	Auckland	Female	liver	microsat	cyt b
AU27/011102	27/11/2000	Auckland	Male	liver	microsat	
AU28/011103	19/11/2000	Auckland	Male	liver	microsat	
AU29/011104	19/11/2000	Auckland	Male	liver	microsat	
AU30/011105	19/11/2000	Auckland	Male	liver	microsat	
AU31/011106	19/11/2000	Auckland	Female	liver	microsat	
AU32/011107	19/11/2000	Auckland	Male	liver	microsat	
AU33/011108	19/11/2000	Auckland	Male	liver	microsat	
AU34/011109	19/11/2000	Auckland	Male	liver	microsat	
AU35/011110	19/11/2000	Auckland	Male	liver	microsat	
AU36/011111	19/11/2000	Auckland	Male	liver	microsat	
AU37/011112	19/11/2000	Auckland	Male	liver	microsat	
AU38/011113	19/11/2000	Auckland	Male	liver	microsat	
AU39/011114	17/11/2000	Auckland	Female	liver	microsat	
AU40/011115	27/11/2000	Auckland	Male	liver	microsat	
AU41/011116		Auckland	Male	liver	microsat	
AU42/011117		Auckland	Female	liver	microsat	
AU43/011119		Auckland	Male	liver	microsat	
AU44/011120		Auckland	Male	liver	microsat	
AU45/011121		Auckland	Female	liver	microsat	
AU46/011126		Auckland	Male	liver	microsat	
AU47/011127		Auckland	Female	liver	microsat	
AU48/011128		Auckland	Female	liver	microsat	
AU49/011131		Auckland	Male	liver	microsat	
AU50/011241		Auckland	Male	liver	microsat	
AU51/011242		Auckland	Male	liver	microsat	
AU52/011243		Auckland	Male	liver	microsat	cyt b
AU53/011311		Auckland	Male	liver	microsat	
AU54/011312		Auckland	Male	liver	microsat	
AU55/011313		Auckland	Male	liver	microsat	
AU56/011336		Auckland	Female	liver	microsat	
AU57/011339		Auckland	Female	liver	microsat	cyt b
AU58/011345		Auckland	Male	liver	microsat	
AU59/022041		Auckland	Male	liver	microsat	
AU60/022042		Auckland	Male	liver	microsat	
AU61/022043		Auckland	Female	liver	microsat	
AU62/022044		Auckland	Male	liver	microsat	cyt b
AU63/022045		Auckland	Male	liver	microsat	
AU64/022046		Auckland	Male	liver	microsat	
AU65/022047		Auckland	Male	liver	microsat	

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Table A5 continued

Sample ID	Date of sampling	Location	Sex	Sample material	Marker	
AU66/022048		Auckland	Male	liver	microsat	
AU67/022049		Auckland	Male	liver	microsat	
AU68/022050		Auckland	Male	liver	microsat	
AU69/022051		Auckland	Male	liver	microsat	
AU70/022052		Auckland	Female	liver	microsat	
AU71/032930		Auckland	Male	liver	microsat	
AU72/032932		Auckland	Female	liver	microsat	cyt b
AU73/032954		Auckland	Female	liver	microsat	
AU74/032958		Auckland	Female	liver	microsat	cyt b
AU75/032970		Auckland	Male	liver	microsat	
AU76/032972		Auckland	Male	liver	microsat	
AU77/032974		Auckland	Male	liver	microsat	
AU78/032976		Auckland	Male	liver	microsat	
AU79/032978		Auckland	Male	liver	microsat	
AU80/032980		Auckland	Female	liver	microsat	
AU81/033004		Auckland	Male	liver	microsat	
AU82/033006		Auckland	Male	liver	microsat	
AU83/033008		Auckland	Female	liver	microsat	
AU84/033014		Auckland	Male	liver	microsat	
AU85/033016		Auckland	Male	liver	microsat	

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Table A6: List of White-chinned Petrel bycatch samples used in genetic analysis

Sample	Date of Sampling	Fishery	Location	Vessel	Sex	**Sample material**	**Marker**
1		Hake Longline Fishery	Cape Point			tissue	SNP
2		Hake Longline Fishery	Cape Point			tissue	SNP
3		South African Tuna Fishery		Dong Wong 619	Male	tissue	SNP
4		South African Tuna Fishery		Dong Wong 620	Female	tissue	SNP
7		South African Tuna Fishery		Dong Wong 632	Male	tissue	SNP
8		South African Tuna Fishery		Dong Wong 633	Male?	tissue	SNP
9		South African Tuna Fishery		Dong Wong 619	Female?	tissue	SNP
13		South African Tuna Fishery		Dong Wong 217	Female	tissue	SNP
16		South African Tuna Fishery			Male	tissue	SNP
23		South African Tuna Fishery		Dong Wong 217	Male	tissue	SNP
30		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
31		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
32		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
33		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
35		South African Tuna Fishery		Dong Wong 635	Male?	tissue	SNP
38		South African Tuna Fishery		Dong Wong 217	Female	tissue	SNP
40		South African Tuna Fishery		Dong Wong 217	Male	tissue	SNP
41		South African Tuna Fishery		Dong Wong 217	Female	tissue	SNP
42		South African Tuna Fishery		Dong Wong 217	Male	tissue	SNP
43		South African Tuna Fishery		Dong Wong 217	Male	tissue	SNP
44		South African Tuna Fishery		Dong Wong 217	Male	tissue	SNP
81		South African Tuna Fishery				tissue	SNP
82		South African Tuna Fishery		Dong Wong 632	Male	tissue	SNP
86		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
87		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
88		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
89		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
92		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
93		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
94		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
95		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
96		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
266		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
267		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
270		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
271		South African Tuna Fishery		Dong Wong 619	Male	tissue	SNP
273		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
274		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
276		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
277		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
278		South African Tuna Fishery		Dong Wong 619	Male	tissue	SNP
279		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
280		South African Tuna Fishery		Dong Wong 619	Female	tissue	SNP
293		South African Tuna Fishery		Dong Wong 630	Female	tissue	SNP
294		South African Tuna Fishery		Dong Wong 630	Male	tissue	SNP
299		South African Tuna Fishery		Saxon	Male?	tissue	SNP
300		South African Tuna Fishery		Dong Wong 635	Male?	tissue	SNP
302		South African Tuna Fishery		Dong Wong 635	Female	tissue	SNP
303		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP
304		South African Tuna Fishery		Dong Wong 635	Male	tissue	SNP

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Table A6 continued

Sample	Date of Sampling	Fishery	Location	Vessel	Sex	**Sample material**	**Marker**		
305		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
306		South African Tuna Fishery		Dong Wong 635	Female	tissue			SNP
307		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
308		South African Tuna Fishery		Dong Wong 635	Female	tissue			SNP
309		South African Tuna Fishery		Dong Wong 635	Female	tissue			SNP
311		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
312		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
313		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
314		South African Tuna Fishery		Dong Wong 635	Female	tissue			SNP
315		South African Tuna Fishery		Dong Wong 635	Female	tissue			SNP
317		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
319		South African Tuna Fishery		Or Yung 731	Male	tissue			SNP
320		South African Tuna Fishery		Or Yung 731	Female	tissue			SNP
324		South African Tuna Fishery			Female	tissue			SNP
398		South African Tuna Fishery		Dong Wong 630	Female	tissue			SNP
399		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
400		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
401		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
411		South African Tuna Fishery		Dong Wong 630	Female	tissue			SNP
412		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
413		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
415		South African Tuna Fishery		Dong Wong 630	Male	tissue			SNP
1045		South African Tuna Fishery		Atalanta	Male	tissue			SNP
1046		South African Tuna Fishery		Atalanta	Male	tissue			SNP
1047		South African Tuna Fishery		Dong Wong 635	Male	tissue			SNP
1054		South African Tuna Fishery		Dong Wong 622	Female	tissue			SNP
1060		South African Tuna Fishery		Dong Wong 217	Male	tissue			SNP
1085		South African Tuna Fishery		Tonina 3	Male	tissue			SNP
1086		South African Tuna Fishery		Tonina 3	Male	tissue			SNP
1087		South African Tuna Fishery		Tonina 3	Male	tissue			SNP
1088		South African Tuna Fishery		Tonina 3	Female	tissue			SNP
1090		South African Tuna Fishery		Dong Wong 632	Male	tissue			SNP
1091		South African Tuna Fishery		Dong Wong 632	Male	tissue			SNP
1092		South African Tuna Fishery		Dong Wong 632	Male	tissue			SNP
1	26/10/1999	South African Tuna Fishery		Chung Yong 62	Female	muscle	microsat		SNP
2	13/02/2001	Marion Toothfish Fishery		Koryo Maru		muscle	microsat		
3	9/03/2001	Marion Toothfish Fishery		Koryo Maru		muscle	microsat		
4	10/10/1999	South African Tuna Fishery		Chung Yong 62	Male	muscle	microsat		SNP
5	8/02/2001	Marion Toothfish Fishery		Koryo Maru	Female	muscle	microsat		SNP
6	Jun-00	South African Tuna Fishery		Prinz Willem1		muscle	microsat	cyt b	
7	12/02/2001	Marion Toothfish Fishery		Koryo Maru	Male	muscle	microsat		SNP
8	15/03/2001	Marion Toothfish Fishery		Koryo Maru	Female	muscle	microsat		SNP
9	10/03/2001	Marion Toothfish Fishery		Koryo Maru		muscle	microsat		
10	8/02/2001	Marion Toothfish Fishery		Koryo Maru		muscle	microsat	cyt b	
11	13/02/2001	Marion Toothfish Fishery		Koryo Maru	Female	muscle	microsat		SNP
12	17/10/1999	South African Tuna Fishery		Chung Yong 62	Male	muscle	microsat		SNP
13	13/2/2001	Marion Toothfish Fishery		Koryo Maru		muscle	microsat		
14	7/03/2001	Marion Toothfish Fishery		Koryo Maru	Male	muscle	microsat		SNP
15	10/08/2001	Marion Toothfish Fishery		Koryo Maru	Male	muscle	microsat		SNP
16	4/05/2001	South African Tuna Fishery		Anneliese		muscle	microsat		SNP

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Table A6 continued

Sample	Date of Sampling	Fishery	Location	Vessel	Sex	Sample material	Marker		
17	28/08/2001	South African Tuna Fishery	35°24'07"S 18°40'066"E			muscle	microsat		SNP
18	8/02/2001	South African Tuna Fishery		Prinz Willem1		muscle	microsat	cyt b	
19	15/10/2001	South African Tuna Fishery		Amoria		muscle	microsat		SNP
22	2/08/2001	South African Tuna Fishery	34°11'S 17°98'E	P. Mullins PDA		muscle	microsat		SNP
23	26/09/2002	South African Tuna Fishery		Canna		muscle	microsat		SNP
24	24/09/2002	South African Tuna Fishery	Agulhas Bank	T.V. Thinte		muscle	microsat		SNP
25	16/09/2002	South African Tuna Fishery	29°07'S 34°49'E			muscle	microsat		SNP
26	28/09/2002	South African Tuna Fishery		T.V. Thinte		muscle	microsat	cyt b	
27	14/09/2002	South African Tuna Fishery		Christina de Boer		muscle	microsat		SNP
28	16/09/2002	South African Tuna Fishery	29°07'S 34°50'E			muscle	microsat		SNP
29	16/09/2002	South African Tuna Fishery	29°07'S 34°50'E			muscle	microsat		
30	15/09/2002	South African Tuna Fishery	29°07'S 34°50'E	Allison		muscle	microsat		SNP
31	16/09/2002	South African Tuna Fishery	29°07'S 34°50'E			muscle	microsat		SNP
32	2002	South African Tuna Fishery				muscle	microsat		
33	2002	South African Tuna Fishery				muscle	microsat		SNP
34	2002	South African Tuna Fishery				muscle	microsat		SNP
35	2002	South African Tuna Fishery				muscle	microsat		SNP
36	2002	South African Tuna Fishery				muscle	microsat		SNP
37	2002	South African Tuna Fishery				muscle	microsat		SNP
38	21/11/1996	Marion Toothfish Fishery	46°1'S 37°37'E			muscle	microsat		SNP
39	22/11/1996	Marion Toothfish Fishery	46°18'S 37°22'E			muscle	microsat		SNP
40	23/11/1996	Marion Toothfish Fishery	46°19'S 37°25'E			muscle	microsat		SNP
41	25/11/1996	Marion Toothfish Fishery	46°17'S 37°32'E			muscle	microsat	cyt b	
42	25/11/1996	Marion Toothfish Fishery	46°17'S 37°32'E			muscle	microsat	cyt b	
43	12/03/1996	Marion Toothfish Fishery	46°16'S 37°32'E			muscle	microsat	cyt b	
44	12/09/1996	Marion Toothfish Fishery	46°21'S 38°29'E			muscle	microsat	cyt b	
45	14/12/1996	Marion Toothfish Fishery	47°02'S 37°56'E			muscle	microsat		SNP
47	17/12/1996	Marion Toothfish Fishery	47°06'S 37°53'E			muscle	microsat		
653						liver	microsat		
654						liver	microsat		
655						liver	microsat		
656		NZL			Male	liver	microsat		SNP
657		NZL			Male	liver	microsat		SNP
679		NZL			Male	liver	microsat	cyt b	SNP
759		NZL			Female	liver	microsat	cyt b	SNP
760		NZL			Male	liver	microsat	cyt b	SNP
761		NZL			Male	liver	microsat		SNP
762		NZL			Female	liver	microsat		SNP
878		NZL			Male	liver	microsat		SNP
879						liver	microsat		
880		NZL			Female	liver	microsat		SNP
11478		NZL			Female	tissue			SNP
33087		NZL			Male	tissue			SNP
33089		NZL			Male	tissue			SNP
33091		NZL			Male	tissue			SNP
33093		NZL			Male	tissue			SNP
33095		NZL			Male	tissue			SNP
33097		NZL			Male	tissue			SNP
33099		NZL			Male	tissue			SNP
33101		NZL			Male	tissue			SNP
33103		NZL			Male	tissue			SNP

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Table A6 continued

Sample	Date of Sampling	Fishery	Location	Vessel	Sex	Sample material	Marker		
33105		NZL			Male	tissue			SNP
33107		NZL			Female	tissue			SNP
33109		NZL			Male	tissue			SNP
567		NZL			Female	tissue			SNP
568		NZL			Male	tissue			SNP
569		NZL			Male	tissue			SNP
570		NZL			Male	tissue			SNP
867		NZL			Female	tissue			SNP
868		NZL			Male	tissue			SNP
869		NZL			Female	tissue			SNP
11465		NZL			Female	tissue			SNP
11470		NZL			Male	tissue			SNP
11472		NZL			Male	tissue			SNP
11625		NZL			Male	tissue			SNP
11627		NZL			Male	tissue			SNP
11628		NZL			Male	tissue			SNP
11629		NZL			Male	tissue			SNP
11630		NZL			Female	tissue			SNP
11644		NZL			Female	tissue			SNP
11646		NZL			Male	tissue			SNP
11647		NZL			Male	tissue			SNP
11648		NZL			Male	tissue			SNP
22505		NZL			Male	tissue			SNP
22552		NZL			Male	tissue			SNP
22553		NZL			Male	tissue			SNP
22688		NZL			Male	tissue			SNP
22753		NZL			Male	tissue			SNP
C1/000685	1/12/1999		Chatham Rise		Female	liver	microsat	cyt b	
C2/000687	1/12/1999		Chatham Rise		Male	liver	microsat		
C3/022175	19/11/2001		Chatham Rise		Male	liver	microsat		
C4/022190	20/11/2001		Chatham Rise		Male	liver	microsat		
C5/022205	14/11/2001		Chatham Rise		Male	liver	microsat		
C6/022210	14/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C7/022250	26/11/2001		Chatham Rise		Female	liver	microsat	cyt b	
C8022255	26/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C9/022260	26/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C10/022265	20/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C11/022271	18/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C12/022275	18/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C13/022285	17/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C14/022380	10/12/2001		Chatham Rise		Male	liver	microsat	cyt b	
C15/022390	26/11/2001		Chatham Rise		Male	liver	microsat	cyt b	
C16/022400	26/11/2001		Chatham Rise		Male	liver	microsat		
C17/990406	19/11/1998		Chatham Rise		Male	liver	microsat		
C18/990448	23/11/1998		Chatham Rise		Male	liver	microsat		

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CHAPTER 5

GIANT PETREL PHYLOGEOGRAPHY

SUMMARY

There are two species of giant petrels, (*Macronectes*) both of which are endangered mainly because of longline fishing. Species status of the two forms has been debated, and it has been concluded that their divergence is recent. In addition, the specific status of several northerly colonies, remain unclear, although they are generally associated with the Southern Giant Petrel (*M. giganteus*). This Chapter used mitochondrial cytochrome b DNA sequences and microsatellite diversity to assess their evolutionary history and phylogeography. Cytochrome b sequences confirmed that species divergence is indeed recent, with a low sequence divergence (0.78%). Northern Giant Petrels (*M. halli*) form a monophyletic group, but the Southern Giant Petrel is paraphyletic, with a clade basal to both Northern Giant Petrels and most Southern Giant Petrel haplotypes confined to the South Indian Ocean. However, preliminary microsatellite analysis shows complete nuclear segregation between the two species, indicating gene flow is largely confined to within currently-defined species. The Falklands population belongs to the Southern Giant Petrel clade and is monophyletic with colonies found in Patagonia and the southwest Atlantic. The Gough colony is separated from this group by high allele frequency differences at microsatellite loci, as well as being divergent at cytochrome b. Birds breeding on Gough belong to the Southern Giant Petrel, but are different enough to warrant further investigation. Microsatellite analysis shows that within both species further population differentiation occurs. Mismatch distribution analysis shows population expansions in both species. Phylogeography indicates that speciation and clade formation could be due to past refugia and later expansion events.

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INTRODUCTION

Separate species status for the two giant petrels (*Macronectes*) was originally suggested in 1966 by Bourne and Warham on the basis of morphological and behavioural differences as well as evidence of sympatric breeding on Macquarie Island without hybridization. Subsequent studies have found occasional hybridisation (Voisin and Bester, 1981; Hunter, 1983, 1987) on at least South Georgia and Marion Island (Hunter, 1983; Cooper *et al.*, 2001), and hybrids are known in South Georgia (Brooke, 2004) with some mixed pairs returning to breed in successive seasons, successfully raising chicks (R. Phillips, personal communication). The viability of these hybrid offspring is unknown.

Studies investigating the phylogeny of Procellariiformes subsequently treated them as two separate species (Nunn and Stanley, 1998; Kennedy and Page, 2002). Penhallurick and Wink (2004) analysed cytochrome b sequences and concluded that the sequence divergence of 0.61% between the two taxa was insufficient to retain species status, given interbreeding (but see Rheindt and Austin, 2005). Debate has been ongoing as to the validity of separate species status. BirdLife International (2006) consider the two forms of giant petrel as two separate species, but the Taxonomic Working Group to the Advisory Committee of the Agreement on the Conservation of Albatrosses and Petrels (ACAP – available online at www.acap.aq) has included them on their list for review in their next meeting.

The two species breed sympatrically on several islands including South Georgia, Marion Island, Crozet and Macquarie. Most Northern Giant Petrel (*M. halli*) colonies are concentrated around New Zealand (Figure 5.1a), whereas Southern Giant Petrels (*M. giganteus*) are concentrated in the Atlantic sector of the Southern Ocean (Figure 5.1b). Gough Island is the colony furthest north and the identity of the birds breeding on this island and Falklands has been debated (Voisin and Bester, 1981; Penhallurick and Wink, 2004) and remains unclear (Brooke, 2004). Originally they were referred to as Northern Giant Petrels

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(Bourne and Warham, 1966). Subsequent observations showed that the birds breeding on Gough have several features that differentiate them from other giant petrels (Voisin and Bester, 1981), including smaller size and darker colouration than typical Southern Giant Petrels, and their chicks often resemble those of Northern Giant Petrels. Furthermore, birds on Gough build nests in sheltered rather than open places. Voisin and Bester (1981) considered them Southern Giant Petrels, but suggested subspecies status for them as they differed from other Southern Giant Petrels except birds breeding on the Falklands.

The IUCN Red List status of *M. giganteus* is vulnerable and the status decreasing; the world population count in 2003 was 36,000 breeding pairs (Sullivan, 2003). A total of 2000-4000 giant petrels are estimated to have been killed in illegal or unregulated Southern Ocean longline fisheries for Patagonian toothfish, *Dissostichius eleginoides*, in 1997-1998 (BirdLife-International, 2006). In 2001 the total estimate for total seabird bycatch had increased from 32800-85800 to 36300-90100 (Tuck *et al.*, 2003) leading to the assumption that the bycatch numbers of giant petrels killed also increased. Other decreases have been attributed to human disturbance and persecution (such as extirpation of giant petrels on Tristan following human settlement), as well as the reductions in southern elephant seal *Mirounga leonina*, which present an important source of carrion. The international status for *M. halli* is near threatened (lower risk) and the species is protected in most waters. The estimated global population consists of 7000-12000 breeding pairs and the status of the species is decreasing (see ACAP (2006) and Sullivan, 2003).

The primary aim of this Chapter is to investigate the phylogeography of the giant petrels. Species status had previously been investigated using one cytochrome b sequence per species from Marion Island (Nunn and Stanley, 1998). Here, more extensive sampling throughout the range of the giant petrel was used to attempt to solve the current ambiguity surrounding their taxonomic status and to investigate geographic genetic variation. The species association of the two

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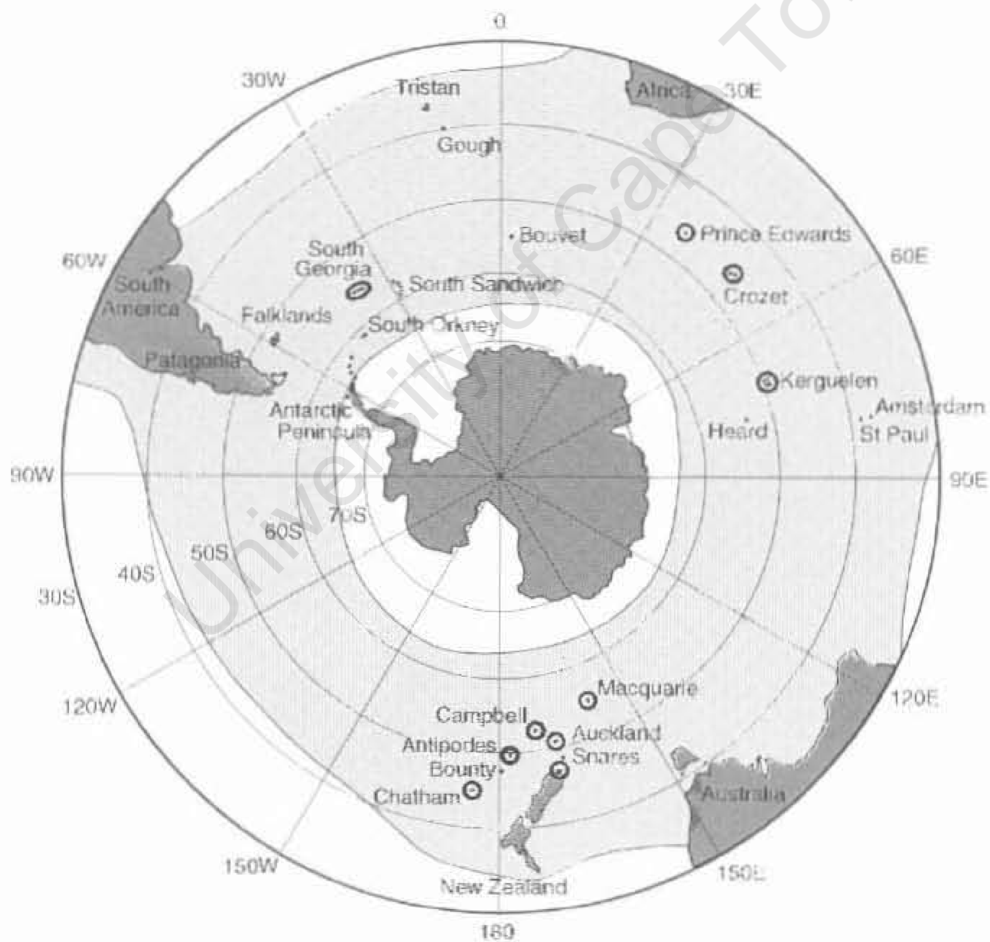
breeding colonies on Gough and Falklands is investigated using cytochrome b sequences, and microsatellite DNA is used in a preliminary study to investigate finer scale population structure and gene flow within the two species.

Figure 5.1: Map showing the distribution and breeding locations (circled) of the Northern Giant Petrel (A) and Southern Giant Petrel (B)

A



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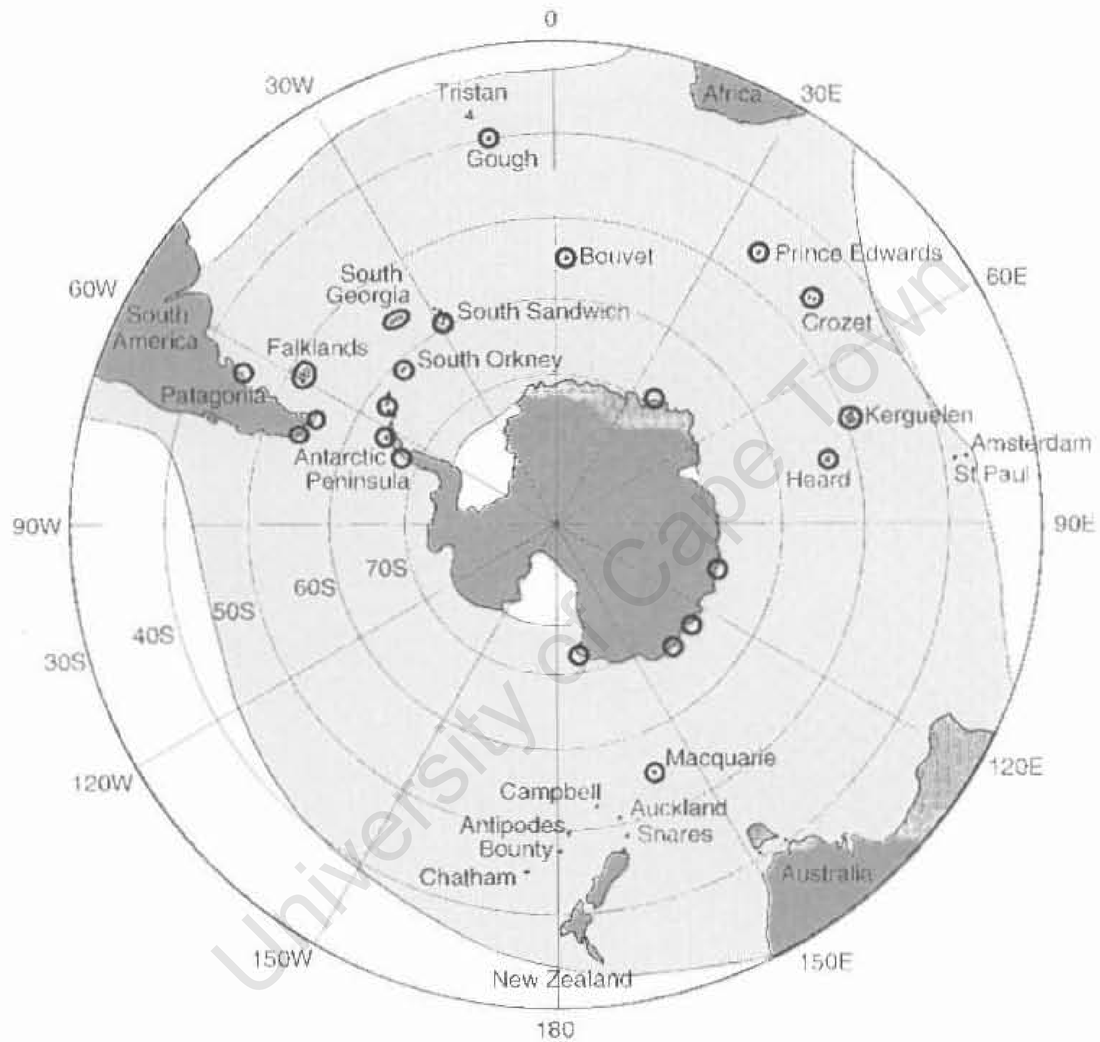
B



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MATERIALS AND METHODS

Giant petrel samples

Samples were collected mostly from chicks (except Marion Island where non-breeding adults were sampled) at colonies by various collaborators (a list of individual and colony information is provided in the appendix): Scott Dreischman and David Oehler (Isla Noir), Flavio Quintana (Staten Island, Isla Arce and Gran Robredo), Markus Ritz (King George), Richard Phillips (Falklands and South Georgia), Richard Cuthbert and Peter Ryan (Gough), Peter Ryan (Marion Island), Graham Robertson (Heard), Henri Weimerskirch (Iles Crozet and Kerguelen), Rosemary Gales (Macquarie), Brian Bell (Chatham Islands), David Thompson (Campbell), and Kath Walker (Auckland and Antipodes). Samples for cytochrome b analysis were as follows for the Southern Giant Petrel: Isla Arce (5), Isla Noir (5), Staten Island (4), Gran Robredo (4), South Georgia (5), Iles Crozet (10), Marion Island (10), Falklands (10), Gough (7), South Shetland (King George, 4), Heard Island (1), and Macquarie (9). For the Northern Giant Petrel the following samples were sequenced: Antipodes (5), Auckland Island (Adams, 4 and Enderby, 4), Campbell Island (5), Chatham Island (5), Macquarie (5), Iles Crozet (5), Marion Island (5), Kerguelen (7) and South Georgia (6). Microsatellite analysis was preliminary and thus did not include all colonies. The following colonies were genotyped for the Southern Giant Petrel: South Georgia (23), Gough (20), Iles Crozet (26), Falklands (30), Macquarie (23), and Gran Robredo (20). For the Northern Giant Petrel individuals from South Georgia (32), Iles Crozet (25) and Chatham Islands (30) were genotyped at six microsatellite loci.

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PCR and sequencing cytochrome b DNA

Internal primers to the *Macronectes* spp. cytochrome b gene were designed by searching for conserved regions in a comparison of two sequences of *Macronectes giganteus* (GenBank accession numbers AF076060 and U48941) and one sequence of *M. halli* (GenBank accession number AF076061). Primers for polymerase chain reaction (PCR) were designed using DNAMan version 4.13 (Lynnon BioSoft): GPcytbF (5' GCC TAA TAA CCC AAA TCC TAA CCG 3') and

GPcytbR (5' GCC GAT GAT GAT GAA TGG ATG 3') starting at 122 bp of the published *Macronectes* cytochrome b sequence and ending at 1056 bp providing a 935 bp fragment. Seventy-seven samples were sequenced with both forward and reverse primers to test for accuracy of sequence data collection.

Cytochrome b analysis

Genetic diversity was measured for the Southern and Northern Giant Petrels using haplotype diversity (Hd), which measures the number and frequency of different variants at a locus and nucleotide diversity (π), which is the weighted sequence divergence between individuals in a population, regardless of the number of haplotypes (Table 5.2).

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Time of expansion can be estimated with the statistic Tau where t (time of expansion in generations) equals Tau (τ) divided by two times mutation rate (u). The mutation rate was estimated with the formula $u = \mu k$, where μ is the mutation rate per nucleotide and k the number of nucleotides essayed (Rogers and Harpending, 1992; Qu *et al.*, 2005) Generation time is difficult to estimate in giant petrels as these birds are long-lived. Banding has shown that birds can live up to forty years and one can expect them to active breeders up to this time. Average age of first breeding is approximately ten years (BirdLife-International, 2006), therefore a more conservative generation time of twelve years was used in calculations.

Microsatellite genotyping with fluorescently labelled primers

Fluorescent genotyping was used for the two giant petrel species, *Macronectes giganteus* and *M. halli*. The following loci were used for both species, Paequ3 and Paequ4 (Techow and O'Ryan, 2004, Chapter 2), De11, Dc16 and Dc26 (Burg, 1999); De37 was used to genotype the Southern Giant Petrel and Dc5 to genotype the Northern Giant Petrel as the loci were found to be polymorphic in one but not the other species (Burg, 2000). Genomic DNA was amplified in 10µl reactions using an Applied Biosystems GeneAmp® PCR System 2700. The

forward primer of each pair was labelled with fluorescent dye. Optimum annealing temperatures and $MgCl_2$ concentrations are listed in Table 5.1. Reactions contained 1.25 µM of each primer, 200 µM dNTP, 0.25 U/µl Taq polymerase (GoTaq® Flexi DNA Polymerase, Promega), the optimum concentration of $MgCl_2$, 1x Promega GoTaq reaction buffer and 70-300 ng of DNA. *Cycling conditions were as follows: 94°C for 45 sec, T_a for 45 sec, 72°C for* 1 min for 30 cycles and a final extension at 72°C for 5 min. PCR products were run on an ABI 373 Sequencer. Each lane contained Rox350 (ABI) as standard, and if possible two loci were combined and run together. Lanes were analysed using ABI GeneScan Software version 1.2.

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Table 5.1: Details of microsatellite loci including repeat type; *bp* base pairs of PCR products; *$MgCl_2$* concentration of Magnesium Chloride used in the reaction.

Locus	bp (SGP)	bp (NGP)	T_a	$MgCl_2$
Paequ3	234-256	240-256	55	1.5
Paequ4	249-265	247-261	58	1
De11	188-204	188-202	53	1
Dc16	109-119	109-117	58	1
Dc26	184-200	186-188	53	1
De37	211-219	217	55	1.5
Dc5		173	*	1

* PCR conditions for Dc5 were as in Burg (2000) with two annealing temperatures: T_{a1} 60°C and T_{a2} 50°C

RESULTS

Cytochrome b analysis

A 935 bp fragment of the mitochondrial cytochrome b gene was amplified of which 752 bp were analysed. The 752 bp fragment was aligned to the published full sequence of the gene (AF076060) and aligned from 185 bp to 936 bp. A total of 125 sequences was analysed. Over all sequences 23 polymorphic sites with 24 mutations were identified with 16 parsimony informative sites comprising 22 haplotypes (Table 5.2). No insertions or deletions were observed. The transition to transversion ratio was 22:2 with both transversions among Northern Giant Petrels. Nineteen mutations were synonymous and five non-synonymous and most substitutions were made at the 3rd codon position. Average base composition was biased with a deficiency of guanine (G 13.9%, A 26.8%, T 26.6%, C 32.7%).

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Table 5.2: Cytochrome b diversity indices calculated for both species of Giant Petrel and combined.

	Southern Giant Petrel	Northern Giant Petrel	Total
Sample size (number of colonies)	74 (12)	51 (9)	125 (21)
Number of haplotypes	13	9	22
Number of polymorphic sites	16	8	24
Number of mutations	16	8	24
Number of parsimony informative sites	11	5	16
Transition:Transversion	16:0	6:2	22:2
Synonymous changes	13	6	19
Non-synonymous changes	3	2	5
Substitutions in:			
1st codon	2	1	3
2nd codon	1	1	2
3rd codon	13	6	19

Haplotype diversity (Hd) was similar for the Southern Giant (0.78 ± 0.0014) and the Northern Giant Petrels (0.73 ± 0.05). This is reflected in the number of

haplotypes found in both species, with 13 haplotypes in the Southern Giant Petrel and nine in the Northern Giant Petrel. Nucleotide diversity (π) differed slightly with 0.005 ± 0.0006 for the Southern Giant Petrel and 0.002 ± 0.0003 for the Northern Giant Petrel. The relationship of haplotype diversity to nucleotide diversity can be used to interpret the demographic history of a population (Grant and Bowen, 1998). High Hd (>0.5) and high π (>0.5%) such as in the Southern Giant Petrel describes a population with a stable history and secondary contact between lineages. However, high Hd and low π (<0.5%) such as for the Northern Giant Petrel, indicates a population that has undergone a bottleneck or founder event, followed by rapid population growth and mutation accumulation. The average number of nucleotide differences (k) for the Southern Giant Petrel was 3.8 and for the Northern Giant Petrel 1.5. This shows that although a similar number of haplotypes was found in both species, more nucleotide differences were found between haplotypes in the Southern Giant Petrel. Only one fixed mutational difference was found between the two petrel species and the two closest haplotypes from each species were separated by only this fixed difference (Figure 5.2). There was one shared haplotype (Table 5.3), one Southern Giant Petrel from Marion Island was found to have the most common haplotype found in the Northern Giant Petrel (gp1). Marion Island has both species breeding sympatrically. Morphologically this bird appeared to be a Southern Giant Petrel but it was a non-breeder and may have been misidentified. Also, successful hybrids have been reported in giant petrels breeding on South Georgia (Hunter, 1983, 1987) and Marion Island (Cooper *et al.*, 2001). As it would only be possible to test this by using a nuclear marker, for example microsatellite DNA, this individual was excluded in diversity, AMOVA and G_{ST} analysis as not to introduce an error in calculations. The individual was included on tree algorithms.

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Table 5.3: Variable sites in the cytochrome b fragment for the Northern and Southern Giant Petrels. Nucleotide positions are in relation to the published sequence (Genbank Accession number AF076060). *N* total number of individuals sharing that particular haplotypes; Ts transition, Tv transversion; S synonymous change, NS non-synonymous change. In cases where haplotypes were specific to a sampling location it was indicated as follows: South Georgia (SG), Chatham Islands (Cha), Marion Island (Mar), Campbell (Ca), Kerguelen (K), Gough (Gou), Patagonia (Pat), Iles Crozet (Cr), Falklands (Fa) and Macquarie (Mac). The highlighted nucleotide position shows the fixed difference between the two species.

Haplotype	Nucleotide Position																							Total *n*
	188	228	231	240	270	279	294	352	354	465	501	579	582	700	709	732	786	822	861	870	887	897	927	
	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Tv/Ts	Ts	Ts	Ts	
	NS	S	S	S	S	S	S	NS	S	S	S	S	S	NS	NS	S	S	S	S	S	NS	S	S	
Macronectes halli (Northern Giant Petrel)																								
gp1	C	T	T	C	C	C	T	A	T	C	G	T	C	A	G	T	G	T	T	T	C	A	C	23
ngp1	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	3
ngp2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	T	4
ngp3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	15
ngpCh4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	1
ngpSG5	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	G	T	3
ngpMar6	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	1
ngpCa7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	1
ngpK8	G	.	.	.	.	.	.	.	.	T	A	.	.	.	A	.	.	.	.	.	.	G	T	1
Macronectes giganteus (Southern Giant Petrel)																								
sgp1	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	30
sgpPat2	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	.	4
sgpGou3	.	.	.	.	T	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	T	7
sgp4	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	C	.	C	.	.	.	.	T	14
sgpMac5	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	1
sgpPat6	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	T	1
sgpSG7	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	A	C	.	.	.	.	T	1
sgpMar8	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	C	.	C	.	C	.	.	T	3
sgp9	.	.	C	.	T	.	C	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	7
sgpFa10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	2
sgpCr11	.	.	.	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	1
sgpCr12	.	.	.	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	T	.	T	1
sgpCr13	.	.	C	T	T	.	C	.	C	.	.	.	.	G	.	.	.	C	C	.	.	.	T	1

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The Northern Giant Petrel network (Figure 5.2) is simple with most haplotypes connected through one mutational difference and two common haplotypes (gp1 and ngp3). The Southern Giant Petrel haplotypes on the other hand have a more complex network. The clade separated by three mutational differences contains three haplotypes exclusively found on Marion Island, Iles Crozet and Macquarie. The other clade contains the remaining haplotypes found in all other colonies including three haplotypes found on Iles Crozet and one haplotype found on both Iles Crozet and Marion Island. Sequence divergence between species was 0.78% (uncorrected). A NJ tree was rooted with both Fulmar species, *Fulmarus glacialis* and *F. glacialoides*, as these have been previously identified as the closest taxa to the giant petrels (Nunn and Stanley, 1998). Northern Giant Petrels formed a monophyletic clade, whereas the Southern Giant Petrel was paraphyletic, with one clade (sgpMac5, sgp4 and sgpMar8) basal to both Northern Giant Petrels and most Southern Giant Petrel haplotypes confined to the South Indian Ocean. Trees were constructed using NJ, Bayesian and MP methods (Figures 5.3-5). All three algorithms showed the same well supported three groups identified with the MSN. Only the NJ and Bayesian tree are presented here as they have the strongest branch support. Both species are sympatric at some islands, but it is noteworthy that they do not form sister clades, instead clustering with their respective species. Southern Giant Petrels form two clades. One clade is formed by Southern Giant Petrels from Iles Crozet, Marion Island and Macquarie. A second clade consists of individuals from all remaining colonies as well as some individuals from Iles Crozet and Marion Island.

Within the Southern Giant Petrel (Table 5.4) G_{ST} values confirm the relationships shown within trees. Birds from Gough Islands differed significantly from all other colonies. Similarly, Macquarie was genetically different to all colonies except Iles Crozet. The MSN showed several haplotypes found on Iles Crozet amongst the other clade, which is supported by non-significant differentiation if the Iles Crozet colony to Staten Island and Marion Island. All other pairwise comparisons did not significantly differ from zero. Within the Northern Giant Petrel, G_{ST} values (Table

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5.5) did not show any differentiation between the two colonies on Auckland Island and therefore they were combined in analysis. G_{ST} did indicate some differentiation between the other colonies, mainly between colonies in different ocean basins, with the exception of Chatham Islands and Auckland, which were differentiated.

Analysis of molecular variance (AMOVA) showed that 52.7% (F_{ST} = 0.52, p<0.0001) of variance could be explained between species, with 47.4% of variance within species. No obvious groupings were suggested by either the trees or MSN for the Northern Giant Petrel, and analysis of variance suggested that 26.4% (F_{ST} = 0.26, p<0.001) could be explained between colonies with the majority of variance being within colonies (73.6%). Within Southern Giant Petrels, the two clades result in a greater proportion of variance between colonies 51.4% (F_{ST} =0.51, p<0.00001). When the Southern Giant Petrel was divided into those two clades, 84.1% (F_{ST} = 0.84, p<0.00001) of variation was explained between the two clades.

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Table 5.4: Southern Giant Petrel G_{ST} values between colonies. Values in bold are significant at *p*<0.05. Heard Island was excluded as only one individual was sequenced. Samples sizes are shown in brakets.

	Isla Arce (5)	Isla Noir (5)	Gran Robredo (4)	Staten Island (4)	Falklands (10)	King George (4)	South Georgia (5)	Gough (7)	Marion Island (10)	Iles Crozet (10)	Macquarie (9)
Isla Arce	0										
Isla Noir	0.25	0									
Gran Robredo	-0.26	0.39	0								
Staten Island	0.10	0.06	0.10	0							
Falklands	0.11	0.01	0.14	-0.15	0						
King George	0.19	0	0.33	0	-0.03	0					
South Georgia	0.17	0	0.25	0.04	0.03	-0.05	0				
Gough	**0.90**	**1**	**0.91**	**0.58**	**0.60**	**1**	**0.93**	0			
Marion Island	0.20	0.20	0.17	0.03	0.16	0.16	0.19	**0.31**	0		
Iles Crozet	**0.44**	**0.44**	**0.41**	0.25	**0.39**	**0.40**	**0.43**	**0.41**	0.07	0	
Macquarie	**0.96**	**0.98**	**0.96**	**0.88**	**0.88**	**0.98**	**0.97**	**0.98**	**0.54**	0.24	0

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Table 5.5: Northern Giant Petrel G_{ST} values. Values in bold are significant at *p*<0.05.

	South Georgia (6)	Marion Island (5)	Iles Crozet (5)	Kerguelen (7)	Macquarie (5)	Auckland (8)	Campbell (5)	Antipodes (5)	Chatham Islands (5)
South Georgia	0								
Marion Island	0.34	0							
Iles Crozet	0.41	0.08	0						
Kerguelen	0.06	0.04	-0.01	0					
Macquarie	0.50	-0.09	0.41	0.23	0				
Auckland	**0.43**	-0.11	0.25	0.15	-0.14	0			
Campbell	**0.56**	0.13	0.60	**0.35**	0.00	0.03	0		
Antipodes	0.44	-0.18	0.11	0.11	-0.14	-0.13	0.17	0	
Chatham Islands	0.03	0.33	0.33	-0.06	**0.56**	**0.45**	**0.64**	0.44	0

Figure 5.2: Minimum Spanning Network showing haplotypes found within the Southern and Northern Giant Petrels. For a key to haplotypes please refer to Table 4.2 and the appendix. Haplotypes found in the Northern Giant Petrel are in red, Southern Giant Petrel haplotypes are in black. The size of the circles is representative of the number of individuals that shared the haplotype, which is also indicated in brackets after the haplotype name, the smallest circles show haplotypes represented by a single individual.

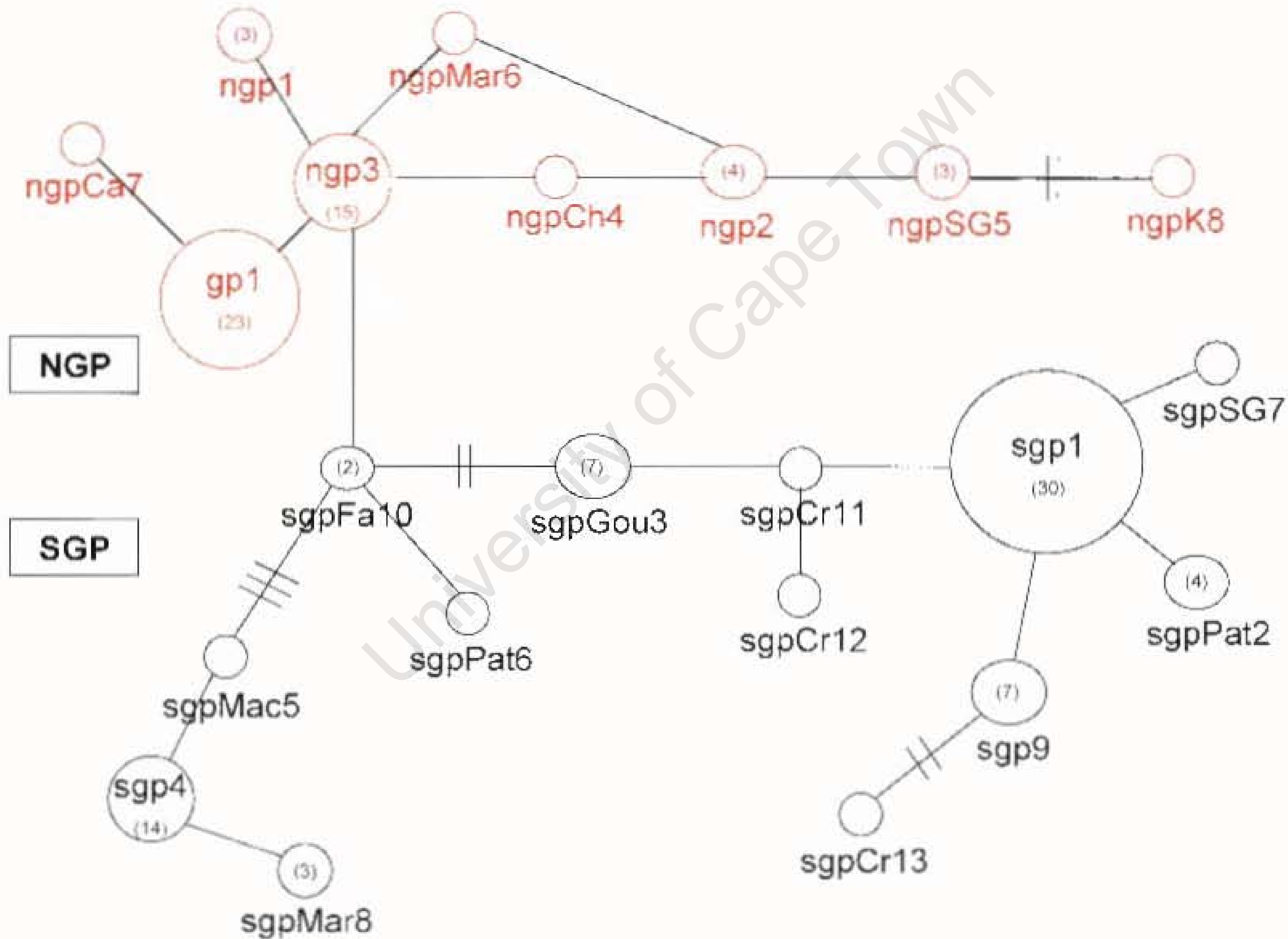

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Figure 5.3: Unrooted tree constructed from Bayesian Inference using haplotypes (Table 5.3) from both Giant Petrel species. Probability values are given indicating support for the topology. The same topology was shown by trees constructed by Maximum Parsimony. Green, Northern Giant Petrels; blue, Southern Giant Petrels; red, Southern Giant Petrels from Iles Crozet, Marion Island and Macquarie.

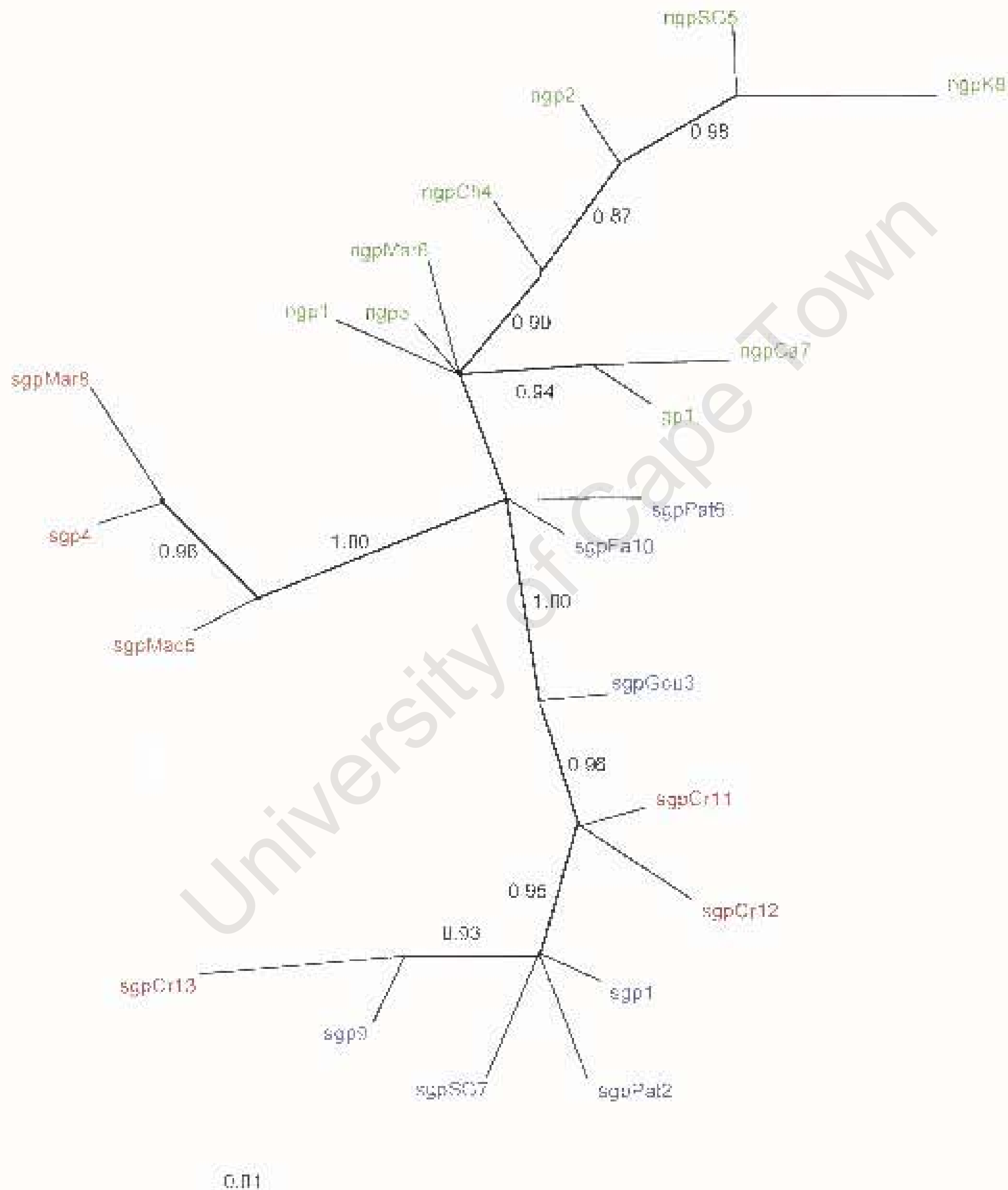

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Figure 5.4: Neighbor Joining reconstruction of phylogenetic relationships and bootstrap values (after 1000 replicates, only values above 50% reported) between haplotypes of the two giant petrel species based on 752 bp of the mitochondrial cytochrome b gene and using Kimura-2-Paramter as model. The tree is rooted on the two fulmar species *Fulmarus glacialis* and *F. glacialoides*. Closed circles represent haplotypes found in the Southern Giant Petrel, open circles represent haplotypes from the Northern Giant Petrel.

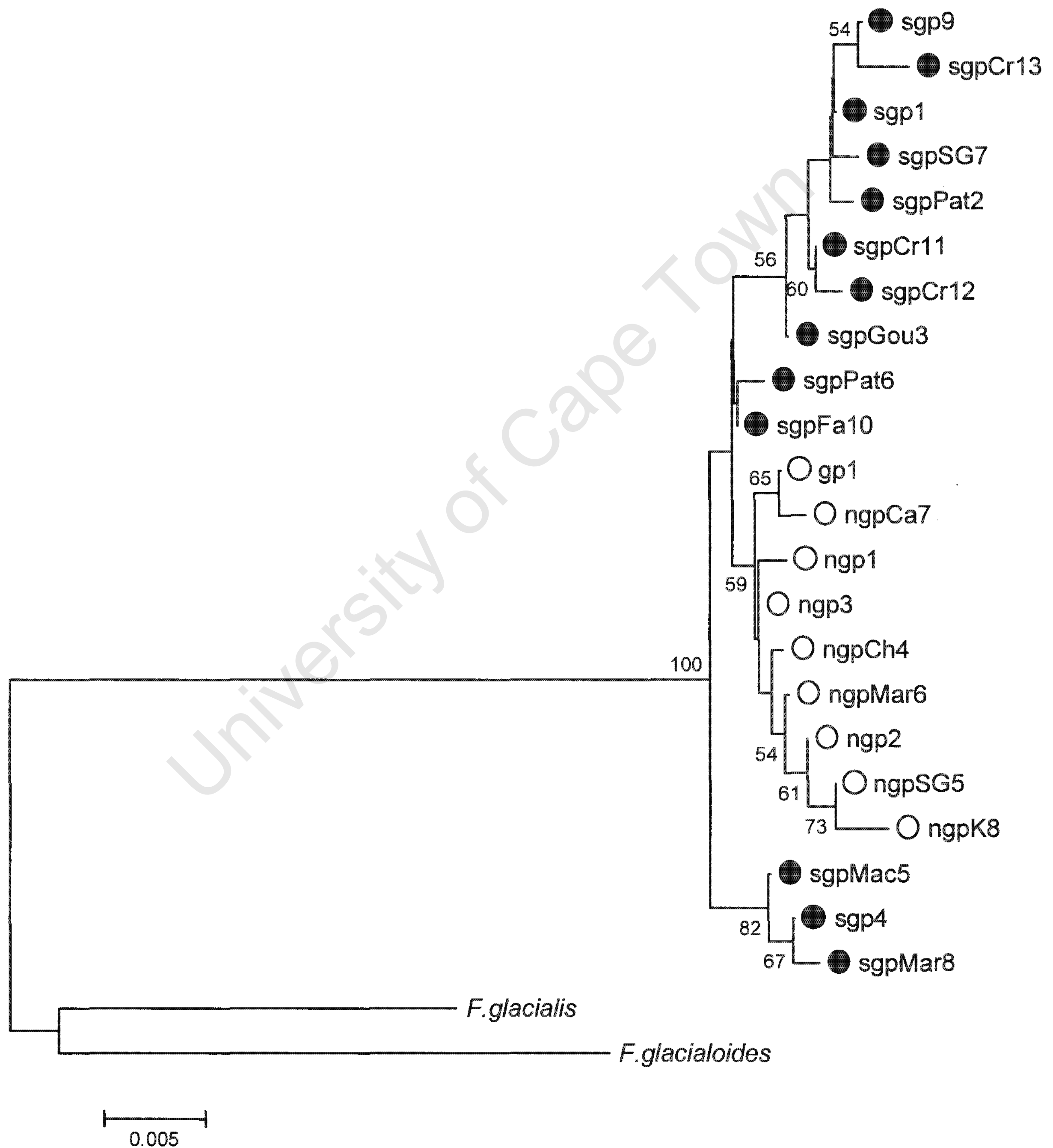

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Figure 5.5 shows mismatch distributions for both species. The Northern Giant Petrel fits the model of a population expansion with both SSD and Harpending's Raggedness Index but does not deviate from Fu's Fs statistic of neutrality. The statistic however, is negative, albeit not significantly different from zero. What this indicates is that the expansion was relatively small. The Tau value shows a time of expansion of 1.45 units of mutational time, which is recent. Similarly, the Southern Giant Petrel also fits the model of expansion using SSD and *r* and with a non-significant negative Fs statistic. The calculated Tau value indicates an expansion at 9.27 units of mutational time, which is considerably older than the one of the Northern Giant Petrel. As two clades were identified in the Southern Giant Petrel, the species was split into these clades. The clade comprising haplotypes found exclusively on Iles Crozet, Marion Island and Macquarie does not fit the model of expansion using SSD and Fs. The raggedness index *r* shows an expansion, but the calculated Tau value shows an expansion time of zero. Therefore the null hypothesis of expansion was rejected. The remaining clade however, fit the model of expansion with both statistics. Fu's Fs was negative but still not different from zero. An estimated 1.52 units of mutational time were given as time of expansion.

The general mutation rate for cytochrome b in birds has been estimated at 2% per million years (e.g. Avise *et al.*, 1987; e.g. Qu *et al.*, 2005) but recent estimates suggest it may be as little as 0.64% (Pereira and Baker, 2006). Different mutation rates for Procellariiformes have been suggested (Nunn and Stanley, 1998), but this has been contested (Lovette, 2004; Pereira and Baker, 2006). It was not possible to calibrate a molecular clock for giant petrels, therefore estimated times of expansion are only crude. The original expansion of Southern Giant Petrels is estimated to have occurred 3.7 to 12.3 myr ago; but with the major radiation 06 – 2.0 myr, similar to the expansion of Northern Giant Petrels 0.6 to 1.9 myr ago.

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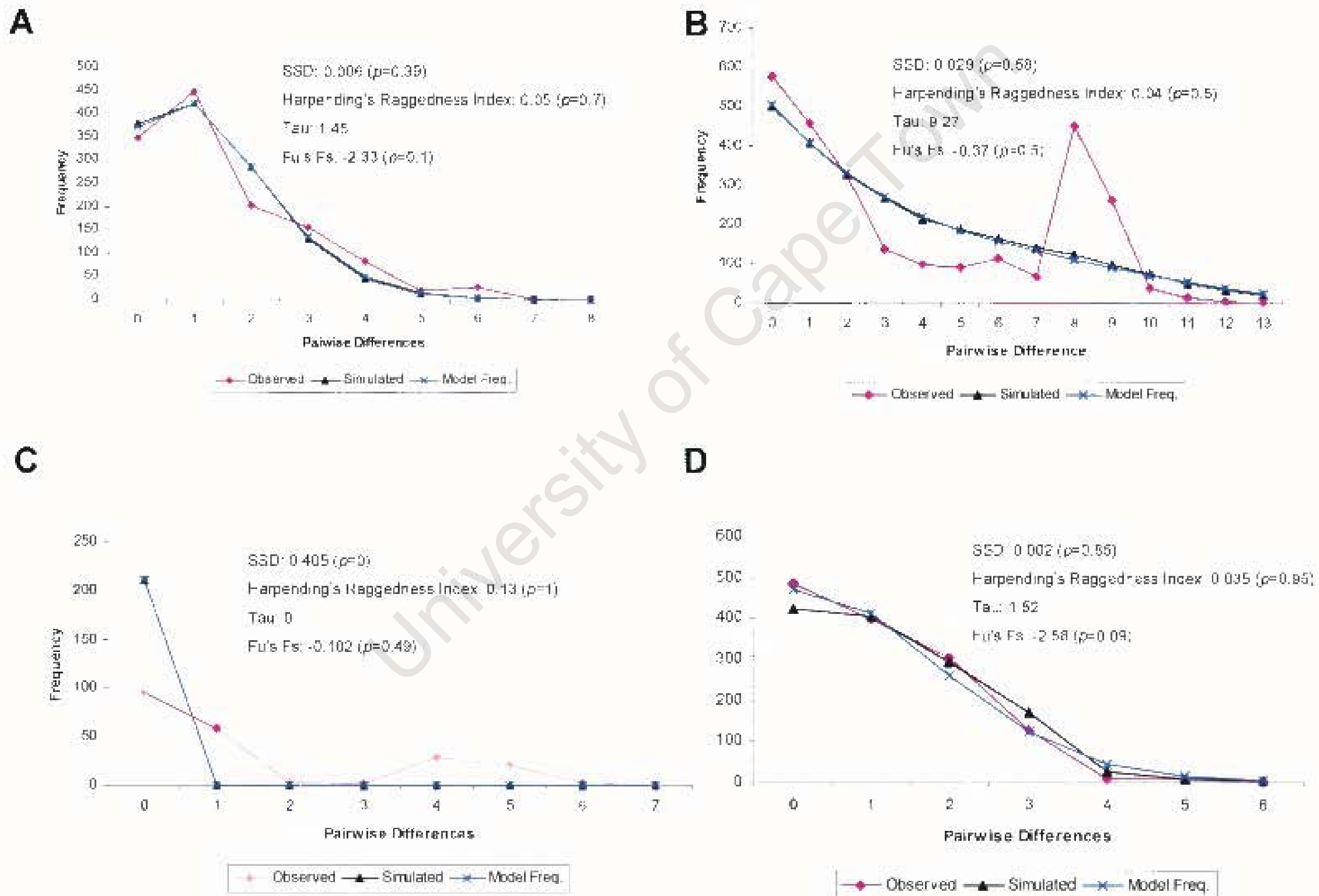
Figure 5.5: Mismatch distributions for the Northern Giant Petrel (A), Southern Giant Petrel (B), Southern Giant Petrel clade Iles Crozet/Marion Island/Macquarie (C), and Southern Giant Petrel excluding previous clade (D).



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Microsatellite analysis

Six microsatellite loci were used to type 142 Southern Giant Petrels and 87 Northern Giant Petrels from six and three colonies, respectively. Dc5 had been found to be polymorphic in the Northern Giant Petrel previously (Burg, 2000) but was monomorphic in the three colonies genotyped in this study. Linkage disequilibrium was tested for all pairs of loci within all colonies and overall. No significant values were observed and thus all loci were included. Thirteen colony/loci pairs deviated from Hardy-Weinberg equilibrium in the Southern Giant Petrel (Table 5.6) even after sequential Bonferroni corrections, but only two in the Northern Giant Petrel. For the Southern Giant Petrel this could not be improved by grouping colonies according to clades identified with cytochrome b. Most of these deviations were due to a positive F_{is} value suggesting an excess of homozygotes. In all these instances heterozygosity was less than expected under Hardy-Weinberg expectations. Reasons for this may include non-random mating, overlapping generations and also the effects of genetic drift on small populations. In this respect it is surprising that the smaller colonies of the Northern Giant Petrel do not deviate from Hardy-Weinberg expectations. In the Southern Giant Petrel, deviations from Hardy-Weinberg expectations together with higher expected heterozygosity may indicate a Wahlund effect and thus finer structure. Overall heterozygosity was low with the Southern Giant Petrel showing the lesser heterozygosity of 0.33 as opposed to 0.44 of the Northern Giant Petrel.

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Table 5.6: Estimate of the number of alleles (*Na*), expected heterozygosity (H_E) and observed heterozygosity (H_O), the probability of deviation from Hardy-Weinberg Equilibrium (HWE *p*) and F_{is} for seven microsatellite loci in three colonies (*N* number of individuals sampled) of Northern Giant Petrel and six colonies of Southern Giant Petrel. Significant deviations from Hardy-Weinberg expectations after Bonferroni corrections are marked with an asterix. Locus Dc5 was genotyped but monomorphic in both species and therefore excluded in the table.

	Locus						Overall
	Paequ3	*Paequ4*	*De11*	*Dc16*	*Dc26*	*De37*	
				Northern Giant Petrel			
South Georgia (N=32)							
# of alleles	5	4	7	4	2	1	23
H_O	0.625	0.406	0.839	0.367	0.323	Monomorphic	0.512
H_E	0.498	0.348	0.795	0.374	0.451		0.493
(HWE) *p*	0.1288	1	0.336	0.768	0.222		0.457
Fis (W&C)	-0.26	-0.17	-0.055	-0.056	0.233		-0.064
Iles Crozet (N=25)							
# of alleles	5	8	6	4	2	1	26
H_O	0.5	0.48	0.76	0.375	0.04*	Monomorphic	0.423
H_E	0.649	0.746	0.824	0.363	0.223		0.561
(HWE) *p*	0.149	0.012	0.029	1	0.007		0.001
Fis (W&C)	0.217	0.338	0.07	-0.144	0.786		0.201
Chatham Islands (N=30)							
# of alleles	7	5	5	3	2	1	23
H_O	0.621	0.467*	0.621	0.333	0.367	Monomorphic	0.388
H_E	0.835	0.720	0.704	0.297	0.446		0.600
(HWE) *p*	0.032	0	0.085	1	0.656		Highly Sign.
Fis (W&C)	0.247	0.356	0.12	-0.126	0.114		0.188
Total							
# of alleles	8	8	8	5	2	1	32
H_O	0.582	0.295*	0.740	0.358	0.230		0.441
H_E	0.661	0.605	0.774	0.344	0.373		0.552
(HWE) *p*	0.015	Highly Sign.	0.026	0.997	0.031		Highly Sign.

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Table 5.6 continued.

	Locus						Overall
	Paequ3	*Paequ4*	*De11*	*Dc16*	*Dc26*	*De37*	
Southern Giant Petrel							
South Georgia (N=23)							
# of alleles	5	7	6	6	3	3	30
H_O	0.261	0.857	0.696	0.870	0.087*	0.435	0.520
H_E	0.485	0.777	0.682	0.715	0.457	0.531	0.608
(HWE) *p*	0.025	0.447	0.462	0.011	0.000	0.666	Highly Sign.
Fis (W&C)	0.431	-0.166	-0.02	-0.222	0.8	0.116	0.098
Gough (N=20)							
# of alleles	5	4	4	4	1	3	21
H_O	0.375*	0.263*	0.471	0.684	Monomorphic	0.053*	0.192
H_E	0.708	0.673	0.437	0.679		0.664	0.527
(HWE) *p*	0.001	0.0001	0.14	0.521		0	Highly Sign.
Fis (W&C)	0.478	0.615	-0.08	-0.009		0.923	0.423
Falklands (N=30)							
# of alleles	4	7	7	2	2	4	26
H_O	0.375*	0.263	0.471	0.654	0.138*	0.053	0.240
H_E	0.742	0.673	0.437	0.509	1.322	0.714	0.733
(HWE) *p*	0.003	0.646	0.625	0.233	0.0002	0.081	0
Fis (W&C)	-0.008	0.027	0.105	-0.292	0.704	0.068	0.086
Iles Crozet (N=26)							
# of alleles	6	8	9	5	3	5	36
H_O	0.269*	0.682	0.846	0.615	0.2*	0.346	0.415
H_E	0.730	0.882	0.870	0.759	0.534	0.436	0.702
(HWE) *p*	0	0.013	0.136	0.021	0.002	0.119	Highly Sign.
Fis (W&C)	0.624	0.21	0.027	0.193	0.601	0.153	0.282
Maquarie (N=23)							
# of alleles	5	6	7	6	3	4	31
H_O	0.4	0.476*	0.682*	0.55	0.1*	0.696	0.274
H_E	0.496	0.768	0.751	0.812	0.537	0.545	0.651
(HWE) *p*	0.041	0.004	0.003	0.102	0	0.026	0
Fis (W&C)	0.198	0.381	0.094	0.328	0.818	-0.351	0.256
Gran Robredo (N=20)							
# of alleles	2	6	3	5	3	3	22
H_O	0.55	0.684	0.35	0.385*	0.2*	0.55	0.356
H_E	0.512	0.683	0.353	0.831	0.531	0.612	0.587
(HWE) *p*	1	0.365	1	0.0002	0.005	0.248	0.001
Fis (W&C)	-0.077	-0.047	-0.127	0.54	0.592	0.103	0.204
Total							
# of alleles	8	9	9	6	5	5	42
H_O	0.202*	0.414*	0.472*	0.567*	0.000	0.347*	0.333
H_E	0.612	0.742	0.588	0.717	0.563	0.584	0.635
(HWE) *p*	Highly Sign.	0.000009	0.00933	0.0000	Highly Sign.	Highly Sign.	Highly Sign.

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Within the Southern Giant Petrel colonies, Crozet showed the greatest number of alleles (36) whereas Gough had the least (21) (Table 5.6). This was also reflected in Iles Crozet having the largest number of colony specific alleles (3). Within the Northern Giant Petrel the number of alleles per colony was similar with South Georgia having 22, Iles Crozet 25 and Chatham Island 22, although again Iles Crozet had the largest number. For the Southern Giant Petrel, Crozet had the largest number of colony specific alleles (4) with Chatham (2) following. In comparison more private alleles were found in Northern Giant Petrel colonies than in Southern Giant Petrel colonies. The Southern Giant Petrel had a four-fold greater number of species-specific alleles than the Northern Giant Petrel. This number mostly derives from one locus, De37, which is monomorphic in the Northern Giant Petrel. It has been reported that some loci are fixed in the Northern Giant Petrel, while being polymorphic in the Southern Giant Petrel (Chapter 2, Burg, 2000).

Population structure is reflected in F_{ST} and R_{ST} values (Table 5.7) both within and between species. Within the Northern Giant Petrel both F_{ST} and R_{ST} show significant values between all three colonies, however there is no correlation between the two statistics (Mantel test p=1). Gough had been identified in the cytochrome b study as different to the remaining colonies. Both F_{ST} and R_{ST} show highly significant differentiation with R_{ST} values being up to 10 times greater than any other pairwise comparisons, thus confirming this observation. In addition the birds on Gough were identified as belonging to the Southern Giant Petrel species, this was also evident in microsatellites as between species comparisons were highest between Gough and any Northern Giant Petrel colony. Within the Southern Giant Petrel no evidence of South Indian Ocean divergence was found, which is in contrast to cytochrome b results. Surprisingly, seeing the differences of significance between statistics, Mantel tests show a correlation between them ($p<0.05$). Between species comparisons in both statistics are all highly significant.

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Table 5.7: Matrix of pairwise R_{ST} values (above diagonal) and G_{ST} values (below diagonal) for the Southern Giant Petrel (A) and Northern Giant Petrel (B). Values significant at $p < 0.05$ are in bold. Within species colony values were calculated separately to values comparing between species.

A

Macronectes giganteus **- Southern Giant Petrel**

	Gough	Gran Robredo	Falklands	South Georgia	Crozet	Macquarie
Gough		**0.605**	**0.462**	**0.447**	**0.349**	**0.418**
Gran Robredo	**0.252**		**0.061**	**0.037**	0.006	**0.087**
Falklands	**0.212**	**0.067**		-0.007	-0.015	-0.009
South Georgia	**0.177**	**0.063**	0.033		0.005	-0.021
Crozet	**0.196**	**0.137**	**0.0703**	**0.073**		-0.002
Macquarie	**0.197**	**0.155**	0.019	0.022	**0.053**	

B

Macronectes halli **- Northern Giant Petrel**

	South Georgia	Crozet	Chatham Is
South Georgia		**0.0984**	**0.0642**
Crozet	**0.0477**		**0.0341**
Chatham Is	**0.1159**	**0.0602**	

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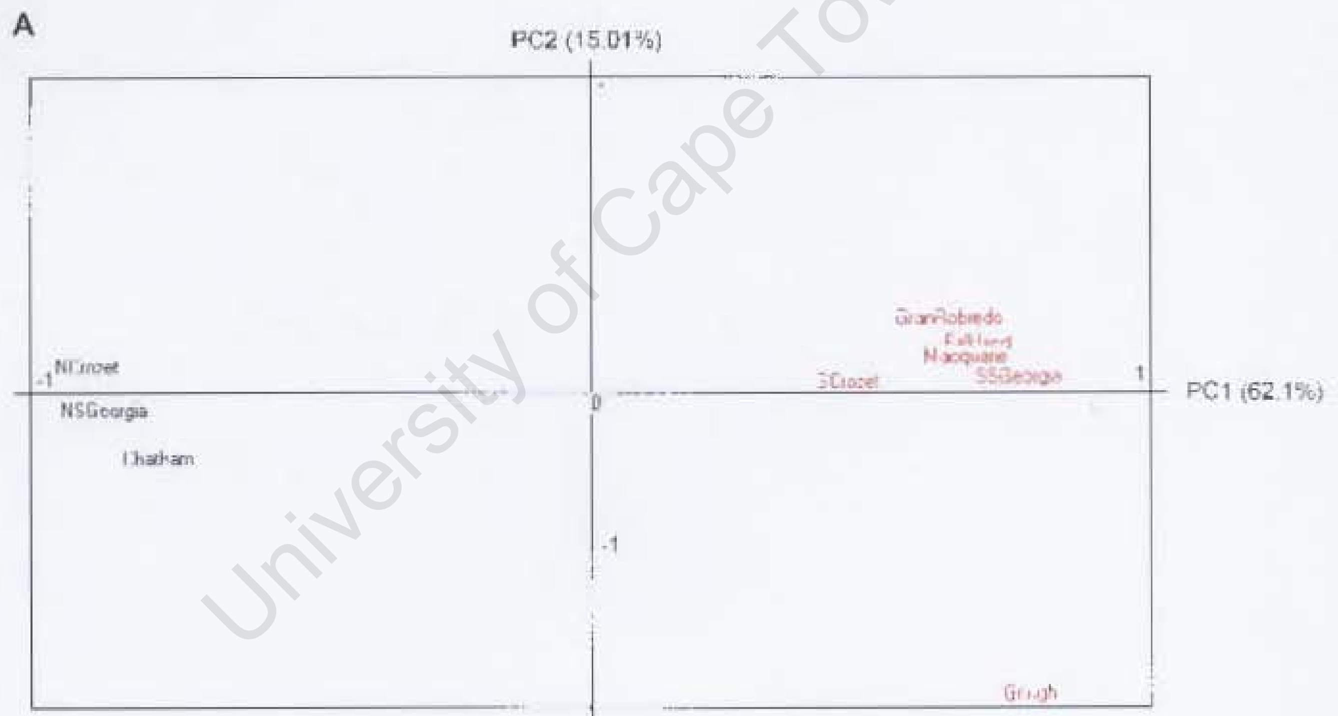
The principal component analysis at species level showed clear clustering of colonies belonging to either species (Figure 5.7A). Noteworthy is that colonies were not clustered according to geographic location but according to species arrangement. When compared to the Northern Giant Petrel, Gough was the only Southern Giant Petrel colony showing a negative value along the second axis and was separate from all other colonies. Similarly when structure was investigated at the individual level (Figure 5.7B), individuals of each species clustered with each other rather than with individuals of the other species, except two Southern Giant Petrels (Gough 57 and Gran Robredo 255), which clustered

with the Northern Giant Petrel. Further, within each species, additional clusters were shown that did not correspond to colonies. Additional colonies need to be genotyped to explain this pattern. Principal Component Analysis at the colony level for each species showed Gough to be separate from all other Southern Giant Petrel colonies (Figure 5.8).

Figure 5.7: Factor map of the two main axes of the Principal Component Analysis comparing the two Giant Petrel species: (A) colonies and (B) individuals of the Southern Giant Petrel (red) and Northern Giant Petrel (black).

A



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B

PC1 (24.7%)
PC2 (10.6%)

Figure 5.8: Factor map of the two main axis of Principal Component Analysis for the Southern Giant Petrel.

A

Cruzet
Macquarie
Gough
SGeorgia
Falklands
Gran Robredo
PC1 (46.8%)
PC2 (26.03%)

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The Northern Giant Petrel showed no deviations from mutation-drift equilibrium, but Southern Giant Petrels showed significant deviations as a species and for each colony (Table 5.8), suggesting they have experienced recent bottlenecks.

Table 5.8: Deviation from mutation-drift equilibrium for Southern Giant Petrel colonies. The occurrence of a recent bottleneck was tested for under the Infinite-allele model (IAM) and Step-wise Mutation Model (SMM) as well as allele frequency distribution (mode-shift or L-shaped distribution). Wilcoxon sign-ranked test was used as the standardized differences test. Only significant values are shown.

Colony	Model	*p* (2-tail test for H excess or deficiency)	*p* (1-tail test for H excess)	Allele frequency distribution
South Georgia	SMM	0.031		
Gough				mode-shift
Falklands	IAM	0.031	0.016	
Iles Crozet	IAM		0.039	
Macquarie	IAM		0.039	
Gran Robredo	IAM	0.047	0.023	mode-shift

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DISCUSSION

Species status of the giant petrels

Gene trees are evolutionary reconstructions of the history and genetic variation of single genes that have experienced little or no recombination. They have the potential to bridge intra- and inter-specific evolution, at which point speciation occurs, because haplotype trees may explore both genetic variation within species and between closely related species (Templeton, 2001). However, there is no theoretical basis for associating gene trees with population lineages (Avise, 2000). Recently evolved species present a problem for interpreting neutral variation because lineage sorting is driven by genetic drift. Therefore the equivalence between organismal and gene phylogenies, is dependant on time (Spaulding *et al.*, 2006). Thus there may be a lack of diagnostic lineage sorting even in the presence of barriers to gene flow. Demographic events such as expansions can preserve lineages and slow lineage sorting (Rogers and Harpending, 1992). In this event, separate species may be detected by differences in allele frequencies (Moritz, 1994). Using both mitochondrial and nuclear data, therefore yields valuable insights otherwise lost to gene trees alone (e.g. Oyler-McCance *et al.*, 1999; Bench *et al.*, 2006).

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Southern and Northern Giant Petrels are phylogenetically distinct, but the Southern Giant Petrel is paraphyletic, with one clade basal to the other Southern Giant Petrel clade and to the Northern Giant Petrel using cytochrome b data. However, nuclear microsatellite data shows clear separation of these two forms. Evidence thus suggests that the Northern Giant Petrel evolved recently from one Southern Giant Petrel lineage, which then expanded. Southern Giant Petrels remained a cohesive nuclear gene pool, but retained ancestral mitochondrial lineages. Molecular variance is significantly partitioned between species, but a large percentage lies within species. No shared mitochondrial haplotypes were found between the two species, with the exception of one bird caught on Marion Island. This individual had the Southern Petrel typical morphology but had the highest frequency haplotype of the Northern Giant Petrels. As hybrids have been

described, this seems the most logical explanation, but this bird could also have been misidentified. One fixed mutational difference was found between the two species. Sequence divergence was 0.78%, greater than the previous estimate (Penhallurick and Wink, 2004). The lack of shared haplotypes and the presence of one fixed mutation, however does suggest, that there is no female gene flow between species. Giant petrels sampled on islands where the two species occur sympatrically clustered with their respective species, not with each other, suggesting there is little if any gene flow. This should be interpreted with some caution, though, because sample sizes within colonies were low. Support for separate species status is given by the consistent separation through Minimum Spanning Network and G_{ST} values of both genetic markers. The occurrence in a coding gene of transitions in 1st and 2nd codon positions and thus non-synonymous changes as well as the presence of two transversion in the cytochrome b gene in the Northern Giant Petrel indicates that enough time has passed for these to occur between species. In addition, morphological differences and reproductive isolation exists (Hunter, 1987).

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An approach used in taxonomy is to compare the genetic distances to 'good' species within the same group or family. Cytochrome b was used to separate Manx *Puffinus puffinus* and Mediterranean Shearwaters *P. yelkouan* given a sequence divergence of 6.6% (Wink *et al.*, 1993). A subsequent study further divided Mediterranean Shearwaters into Yelkouan and Balearic Shearwaters *P. mauretanicus* based on a sequence divergence of 1.6% and more than 10 fixed differences (Genovart *et al.*, 2005). A 1% difference between two colour morphs of the Herald Petrel (*Pterodroma heraldica*) was found in addition to differences in vocalisations, and the authors recommended species status for both forms (Brooke and Rowe, 1996). Similarly, I found six fixed differences and a sequence divergence of 1.7% between the White-chinned and Spectacled Petrels and thus support their separate species status (Chapter 3). The sequence divergence of 0.78% found between the two giant petrel species is considerably lower.

However, the presence of mechanisms for reproductive isolation as well as distinct morphological features argues for maintaining species status.

The British Ornithologists' Union guidelines for assigning species rank to bird species ask two questions: i) are the taxa diagnosable and ii) are they likely to retain their genetic and phenotypic integrity in the future (Helbig *et al.*, 2002, p. 519)? These guidelines have been adopted by ACAP and encompass the General Lineage Concept (de Queiroz, 1999). Species delineations in petrels and albatrosses are often difficult as different populations or colonies mostly do not come into contact because their strong the philopatry. In addition, both petrels and albatrosses show unusually low levels of genetic divergence (Nunn *et al.*, 1996; Nunn and Stanley, 1998; Penhallurick and Wink, 2004), which reduces the power of genetic analysis to resolve taxonomic uncertainties in closely related species (Burg and Croxall, 2001; Abbott and Double, 2003; Burg and Croxall, 2004).

Giant petrels qualify on both of the above-mentioned criteria. Individuals of the Southern and Northern Giant Petrel can be diagnosed by one or more qualitative differences in the fixed differences of cytochrome b sequences. In addition individuals can be identified by a combination of two or more functionally independent characters through differences in plumage and bill colour. Reproductive isolation can be tested in the giant petrels as both forms occur sympatrically on some islands and it is shown here that individuals breeding sympatrically cluster with individuals of their species and not with individuals of the other species. In addition, nuclear microsatellite allele frequencies are markedly different in the two forms. Hybridization has been observed on South Georgia and Marion Island but frequencies are low (Hunter, 1983) and hybridisation to some degree has been allowed even in the biological species concept (O'Brien and Mayr, 1991; Mayr, 1992) as low frequencies of hybridisation are unlikely to cause gene pools to merge (Helbig *et al.*, 2002). Although speciation has occurred recently (estimated around 0.39 to 1.2 myr

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ago), future integrity can be assumed as reproductive isolation mechanisms have been formed. Thus all the data from this study are consistent with two separate giant petrel species being recognised and conserved.

Evolutionary history

Mismatch distributions of the giant petrels identified two expansions in the Southern Giant Petrel, one old (3.7 – 12.3 myr ago) and the other more recent (0.6 – 2.0 myr years ago), occurring at the same time as the expansion in the Northern Giant Petrel, the caveat being that this lineage does not have a calibrated molecular clock. This seems to indicate that the Southern Giant Petrel experienced fragmentation perhaps through climatic changes. It has been argued that the pattern shown within a mismatch distribution may also be indicative of a bottleneck or founder event (Slatkin and Hudson, 1991; Rogers and Harpending, 1992; Rogers *et al.*, 1996) and the two are hard to distinguish. This expansion was followed by secondary contact of the two clades of Southern Giant Petrel as shown by haplotypes geographically overlapping even though one clade has a high sequence divergence (1.1%) towards the other. Nuclear data indicates ongoing gene flow between these two clades indicating that although population differentiation exists, no isolation mechanisms have formed despite historical separation. At the same time, one Southern Giant Petrel clade was separated long enough to form isolating mechanisms as well as morphological adaptations, so that when the two forms came into contact as shown in islands on which the two forms occur sympatrically, barriers to gene flow existed forming the Northern Giant Petrel.

Several lines of evidence suggest that Southern Giant Petrel was the founding species: it has a greater diversity within both cytochrome b and microsatellites and a greater number of alleles as well as more private alleles. Overall heterozygosity was higher in the Northern Giant Petrel, but this should be viewed with caution, because fewer colonies were genotyped for microsatellite DNA. One locus polymorphic in the Southern Giant Petrel was monomorphic in the

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Northern Giant Petrel. Microsatellite data also showed the greatest number of alleles in Iles Crozet. Allelic diversity is thought to be an indicator of bottlenecks or founder effects as low frequency alleles are often lost in founder populations due to genetic drift in small populations (Nei *et al.*, 1975). This effect is found in the low genetic diversity of the Northern Giant Petrel. With the exception of a few private alleles most alleles are a subset of the Southern Giant Petrel. However, investigation of recent bottlenecks also showed that all colonies of the Southern Giant Petrel have experienced reductions in population sizes.

Within species structure

Most Northern Giant Petrels colonies are concentrated around New Zealand and the Indian Ocean, whereas most Southern Giant Petrel colonies are concentrated in the Atlantic and Indian Ocean sector of the Southern Ocean. The identity of birds breeding on Gough, the colony furthest north (giant petrels previously bred further north at Tristan da Cunha, but was extirpated in the 19th century) as well as birds breeding on Falklands has been debated (Voisin and Bester, 1981; Penhallurick and Wink, 2004). Genetic analysis indicates that the birds breeding on Gough indeed belong to the Southern Giant Petrel. However, cytochrome b (Table A4, appendix) and especially microsatellite DNA analysis show the colony to be differentiated from the other colonies within the Southern Giant Petrel. As the colony is small (ca. 260 pairs, PG Ryan personal communication) and geographically apart from the others, the data suggest that gene flow is limited and allele frequencies have differentiated mainly due to genetic drift. Because of this, sequence divergence is not high and no fixed mutational differences were observed between birds breeding on Gough and elsewhere. Thus the population on Gough does not merit subspecies status. Despite this, microsatellite data shows highly significant G_{ST} and R_{ST} values in all pairwise comparisons, and further investigation is required with a larger sample size for both DNA markers to investigate possible status as an Evolutionary Significant Unit. By comparison, the Falklands and Patagonian birds are indistinguishable from the remaining

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Southern Giant Petrels breeding at South Georgia and on the Antarctic Peninsula using cytochrome b and microsatellites.

Within the Northern Giant Petrel, cytochrome b analysis showed no substructuring, but there was evidence for population structure within microsatellites. As only three widely-spread colonies were genotyped for preliminary analysis, no pattern could be identified and more colonies need to be investigated.

Concluding remarks

This Chapter investigated species status and phylogeography of the giant petrels. Results for microsatellite DNA are preliminary as greater sample numbers are needed to confirm observed patterns. The two forms of giant petrel differ only by a sequence divergence of 0.78% in cytochrome b indicating recent divergence. However, reproductive isolation and morphological differences in addition to genetic evidence are enough to suggest that these lineages have had separate evolutionary histories and will continue to maintain their integrity through time and space. Preliminary microsatellite DNA analysis shows further fine scale structuring within both giant petrel species and is worth further investigation.

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APPENDICES

Table A1: List of Southern Giant Petrels used in the genetic analysis detailing sampling and marker information. *Cyt b*, mitochondrial cytochrome b; *microsat*, microsatellite DNA.

Sample ID	***Date of Sampling***	***Origin***	***Location***	***Adult/ Chick***	***Sample Material***	***Marker***	
3		South Georgia	Bird Island	C	Blood	microsat	cyt b
5		South Georgia	Bird Island	C	Blood	microsat	cyt b
6		South Georgia	Bird Island	C	Blood	microsat	
8		South Georgia	Bird Island	C	Blood	microsat	
9		South Georgia	Bird Island	C	Blood	microsat	
11		South Georgia	Bird Island	C	Blood	microsat	cyt b
12		South Georgia	Bird Island	C	Blood	microsat	cyt b
13		South Georgia	Bird Island	C	Blood	microsat	
14		South Georgia	Bird Island	C	Blood	microsat	
15		South Georgia	Bird Island	C	Blood	microsat	
16		South Georgia	Bird Island	C	Blood	microsat	
18		South Georgia	Bird Island	C	Blood	microsat	
19		South Georgia	Bird Island	C	Blood	microsat	
20		South Georgia	Bird Island	C	Blood	microsat	
21		South Georgia	Bird Island	C	Blood	microsat	
22		South Georgia	Bird Island	C	Blood	microsat	
25		South Georgia	Bird Island	C	Blood	microsat	
26		South Georgia	Bird Island	C	Blood	microsat	
27		South Georgia	Bird Island	C	Blood	microsat	
28		South Georgia	Bird Island	C	Blood	microsat	
29		South Georgia	Bird Island	C	Blood	microsat	
30		South Georgia	Bird Island	C	Blood	microsat	
32		South Georgia	Bird Island	C	Blood	microsat	cyt b
Gou50	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou51	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou52	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou53	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou54	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou55	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou56	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou57	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou58	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b

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Table A1 continued

Sample ID	***Date of Sampling***	***Origin***	***Location***	***Adult/Chick***	***Sample Material***	***Marker***	
Gou59	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou60	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou61	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou62	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou63	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou64	7.10.2003	Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou65		Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	cyt b
Gou1		Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou2		Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou3		Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Gou4		Gough *Low Hump*	40° 20'5'E; 09° 56'5'W	A	Blood	microsat	
Falk1	Feb-03	Falkland Islands	George Island	C	Blood	microsat	cyt b
Falk2	Feb-03	Falkland Islands	George Island	C	Blood	microsat	
Falk3	Feb-03	Falkland Islands		C	Blood	microsat	
Falk4	Feb-03	Falkland Islands	George Island	C	Blood	microsat	
Falk5	Feb-03	Falkland Islands		C	Blood	microsat	
Falk6	Feb-03	Falkland Islands	George Island	C	Blood	microsat	cyt b
Falk7	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk8	Feb-03	Falkland Islands	George Island	C	Blood	microsat	cyt b
Falk9	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk10	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk11	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk12	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk13	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk14	Feb-03	Falkland Islands		C	Blood	microsat	cyt b
Falk15	Feb-03	Falkland Islands		C	Blood	microsat	
Falk16	Feb-03	Falkland Islands		C	Blood	microsat	
Falk17	Feb-03	Falkland Islands		C	Blood	microsat	
Falk18	Feb-03	Falkland Islands		C	Blood	microsat	
Falk19	Feb-03	Falkland Islands		C	Blood	microsat	
Falk20	Feb-03	Falkland Islands		C	Blood	microsat	
Falk21	Feb-03	Falkland Islands		C	Blood	microsat	
Falk22	Feb-03	Falkland Islands		C	Blood	microsat	
Falk23	Feb-03	Falkland Islands		C	Blood	microsat	
Falk24	Feb-03	Falkland Islands		C	Blood	microsat	
Falk25	Feb-03	Falkland Islands		C	Blood	microsat	
Falk26	Feb-03	Falkland Islands		C	Blood	microsat	
Falk27	Feb-03	Falkland Islands		C	Blood	microsat	
Falk28	Feb-03	Falkland Islands		C	Blood	microsat	
Falk29	Feb-03	Falkland Islands		C	Blood	microsat	
Falk30	Feb-03	Falkland Islands		C	Blood	microsat	

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Table A1 continued

Sample ID	*Date of Sampling*	*Origin*	*Location*	*Adult/Chick*	*Sample Material*	*Marker*	
Croz1		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz2		Crozet	Pointe Basse	C	Blood	microsat	
Croz3		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz4		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz5		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz6		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz7		Crozet	Pointe Basse	C	Blood	microsat	
Croz8		Crozet	Pointe Basse	C	Blood	microsat	
Croz9		Crozet	Jardin Japonais	C	Blood	microsat	
Croz10		Crozet	Jardin Japonais	C	Blood	microsat	
Croz11		Crozet	Jardin Japonais	C	Blood	microsat	
Croz31/CF40231		Crozet	Pointe Basse	C	Blood	microsat	
Croz32/CF40232		Crozet	Pointe Basse	C	Blood	microsat	
Croz33/CF40233		Crozet	Pointe Basse	C	Blood	microsat	
Croz34/CF40234		Crozet	Pointe Basse	C	Blood	microsat	
Croz35/CF40235		Crozet	Pointe Basse	C	Blood	microsat	
Croz36/CF40236		Crozet	Pointe Basse	C	Blood	microsat	
Croz37/CF40237		Crozet	Pointe Basse	C	Blood	microsat	
Croz38/CF40238		Crozet	Pointe Basse	C	Blood	microsat	
Croz17/CF42004		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz19/CF42005		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz21/CF42006		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz23/CF42007		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz25/CF42008		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz27/CF42009		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz29/CF42010		Crozet	Pointe Basse	C	Blood	microsat	
King17/055178		S. Shetland/King George		C	Blood		cyt b
King25/055186		S. Shetland/King George		C	Blood		cyt b
King5162		S. Shetland/King George		C	Blood		cyt b
King5182		S. Shetland/King George		C	Blood		cyt b
King5183		S. Shetland/King George		C	Blood		cyt b
Mac1		Macquarie			Blood	microsat	cyt b
Mac2		Macquarie			Blood	microsat	cyt b
Mac3		Macquarie			Blood	microsat	cyt b
Mac4		Macquarie			Blood	microsat	cyt b
Mac5		Macquarie			Blood	microsat	
Mac6		Macquarie			Blood	microsat	
Mac7		Macquarie			Blood	microsat	cyt b
Mac8		Macquarie			Blood	microsat	
Mac9		Macquarie			Blood	microsat	cyt b
Mac10		Macquarie			Blood	microsat	cyt b
Mac11		Macquarie			Blood	microsat	
Mac12		Macquarie			Blood	microsat	
Mac13		Macquarie			Blood	microsat	cyt b
Mac14		Macquarie			Blood	microsat	

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Table A1 continued

Sample ID	***Date of Sampling***	***Origin***	***Location***	***Adult/Chick***	***Sample Material***	***Marker***	
Mac15		Macquarie			Blood	microsat	
Mac16		Macquarie			Blood	microsat	
Mac18		Macquarie			Blood	microsat	
Mac19		Macquarie			Blood	microsat	
Mac20		Macquarie			Blood	microsat	cyt b
Mac21		Macquarie			Blood	microsat	
Mac22		Macquarie			Blood	microsat	
Mac23		Macquarie			Blood	microsat	
Staten Is10		Argentina	Staten Island (Isla de los Est.)	A	Blood		cyt b
Staten Is15		Argentina	Staten Island (Isla de los Est.)		Blood		cyt b
Staten Is16		Argentina	Staten Island (Isla de los Est.)		Blood		cyt b
Staten Is26		Argentina	Staten Island (Isla de los Est.)		Blood		cyt b
Arce1		Argentina	Isla Arce		Blood		cyt b
Arce3		Argentina	Isla Arce		Blood		cyt b
Arce9		Argentina	Isla Arce		Blood		cyt b
Arce11		Argentina	Isla Arce		Blood		cyt b
Arce17		Argentina	Isla Arce		Blood		cyt b
Gran2		Argentina	Gran Robredo		Blood	microsat	cyt b
Gran4		Argentina	Gran Robredo		Blood	microsat	cyt b
Gran5		Argentina	Gran Robredo		Blood	microsat	
Gran9		Argentina	Gran Robredo		Blood	microsat	cyt b
Gran12		Argentina	Gran Robredo		Blood	microsat	cyt b
Gran18		Argentina	Gran Robredo		Blood	microsat	
Gran20		Argentina	Gran Robredo		Blood	microsat	
Gran251		Argentina	Gran Robredo		Blood	microsat	
Gran255		Argentina	Gran Robredo		Blood	microsat	
Gran256		Argentina	Gran Robredo		Blood	microsat	cyt b
Gran257		Argentina	Gran Robredo		Blood	microsat	
Gran258		Argentina	Gran Robredo		Blood	microsat	
Gran259		Argentina	Gran Robredo		Blood	microsat	
Gran260		Argentina	Gran Robredo		Blood	microsat	
Gran262		Argentina	Gran Robredo		Blood	microsat	
Gran263		Argentina	Gran Robredo		Blood	microsat	
Gran270		Argentina	Gran Robredo		Blood	microsat	
Gran401		Argentina	Gran Robredo		Blood	microsat	
Gran402		Argentina	Gran Robredo		Blood	microsat	
Gran403		Argentina	Gran Robredo		Blood	microsat	
Chile1		Isla Noir	Isla Noir	A	Blood		cyt b
Chile2		Isla Noir	Isla Noir	A	Blood		cyt b
Chile3		Isla Noir	Isla Noir	A	Blood		cyt b
Chile4		Isla Noir	Isla Noir	A	Blood		cyt b
Chile5		Isla Noir	Isla Noir	A	Blood		cyt b
Heard5		Heard Island			Tissue		cyt b

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Table A1 continued

Sample ID	*Date of Sampling*	*Origin*	*Location*	*Adult/Chick*	*Sample Material*	*Marker*
Mar1	2006	Marion Island			Blood	cyt b
Mar2	2006	Marion Island			Blood	cyt b
Mar3	2006	Marion Island			Blood	cyt b
Mar4	2006	Marion Island			Blood	cyt b
Mar5	2006	Marion Island			Blood	cyt b
Mar6	2006	Marion Island			Blood	cyt b
Mar7	2006	Marion Island			Blood	cyt b
Mar8	2006	Marion Island			Blood	cyt b
Mar9	2006	Marion Island			Blood	cyt b
Mar10	2006	Marion Island			Blood	cyt b

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Table A2: List of Northern Giant Petrels used in the genetic analysis detailing sampling and marker information. *Cyt b*, mitochondrial cytochrome b; *microsat*, microsatellite DNA.

Sample ID	Date of Sampling	Origin	Location	Adult/Chick	Sample Material	Marker	
Y1		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y2		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y3		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y4		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y5		South Georgia	Bird Island	C	Blood	microsat	
Y6		South Georgia	Bird Island	C	Blood	microsat	
Y7		South Georgia	Bird Island	C	Blood	microsat	
Y8		South Georgia	Bird Island	C	Blood	microsat	
Y9		South Georgia	Bird Island	C	Blood	microsat	
Y10		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y11		South Georgia	Bird Island	C	Blood	microsat	
Y12		South Georgia	Bird Island	C	Blood	microsat	
Y13		South Georgia	Bird Island	C	Blood	microsat	
Y14		South Georgia	Bird Island	C	Blood	microsat	
Y15		South Georgia	Bird Island	C	Blood	microsat	
Y16		South Georgia	Bird Island	C	Blood	microsat	
Y17		South Georgia	Bird Island	C	Blood	microsat	
Y18		South Georgia	Bird Island	C	Blood	microsat	
Y19		South Georgia	Bird Island	C	Blood	microsat	
Y20		South Georgia	Bird Island	C	Blood	microsat	cyt b
Y21		South Georgia	Bird Island	C	Blood	microsat	
Y22		South Georgia	Bird Island	C	Blood	microsat	
Y23		South Georgia	Bird Island	C	Blood	microsat	
Y24		South Georgia	Bird Island	C	Blood	microsat	
Y25		South Georgia	Bird Island	C	Blood	microsat	
Y26		South Georgia	Bird Island	C	Blood	microsat	
Y27		South Georgia	Bird Island	C	Blood	microsat	
Y28		South Georgia	Bird Island	C	Blood	microsat	
Y29		South Georgia	Bird Island	C	Blood	microsat	
Y30		South Georgia	Bird Island	C	Blood	microsat	
Y31		South Georgia	Bird Island	C	Blood	microsat	
Y32		South Georgia	Bird Island	C	Blood	microsat	
Cam1		Campbell Island			Blood		cyt b
Cam2		Campbell Island			Blood		cyt b
Cam3		Campbell Island			Blood		cyt b
Cam4		Campbell Island			Blood		cyt b
Cam5		Campbell Island			Blood		cyt b
Chat1		Chatham Islands	Fourty Fours	C	Blood	microsat	cyt b
Chat2		Chatham Islands	Fourty Fours	C	Blood	microsat	cyt b
Chat3		Chatham Islands	Fourty Fours	C	Blood	microsat	cyt b
Chat4		Chatham Islands	Fourty Fours	C	Blood	microsat	cyt b

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Table A2 continued

Sample ID	Date of Sampling	Origin	Location	Adult/Chick	Sample Material	Marker	
Chat6		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat7		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat8		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat9		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat10		Chatham Islands	Fourty Fours	C	Blood	microsat	cyt b
Chat11		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat12		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat13		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat14		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat15		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat16		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat17		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat18		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat19		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat20		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat21		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat22		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat23		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat24		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat25		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat26		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat27		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat28		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat29		Chatham Islands	Fourty Fours	C	Blood	microsat	
Chat30		Chatham Islands	Fourty Fours	C	Blood	microsat	
Mar1		Marion Island	Prince Edwards		Blood		cyt b
Mar2		Marion Island	Prince Edwards		Bloodr		cyt b
Mar9A04756		Marion Island	Prince Edwards		Blood		cyt b
Mar973529		Marion Island	Prince Edwards		Blood		cyt b
Mar968254		Marion Island	Prince Edwards		Blood		cyt b
Croz1		Crozet	Pointe Basse	C	Blood	microsat	
Croz2		Crozet	Pointe Basse	C	Blood	microsat	
Croz3		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz4		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz5		Crozet	Pointe Basse	C	Blood	microsat	
Croz6		Crozet	Pointe Basse	C	Blood	microsat	
Croz7		Crozet	Pointe Basse	C	Blood	microsat	
Croz8		Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz9		Crozet	Pointe Basse	C	Blood	microsat	
Croz10		Crozet	Pointe Basse	C	Blood	microsat	
Croz11	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat	cyt b
Croz12	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat	
Croz13	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat	
Croz14	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat	
Croz15	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat	cyt b

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Table A2 continued

Sample ID	*Date of Sampling*	*Origin*	*Location*	*Adult/Chick*	*Sample Material*	*Marker*
Croz16	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz17	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz18	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz19	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz20	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz21	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz22	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz23	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz24	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Croz25	13.01.2004	Crozet	Pointe Basse	C	Blood	microsat
Mac2		Macquarie			Blood	cyt b
Mac3		Macquarie			Blood	cyt b
Mac4		Macquarie			Blood	cyt b
Mac8		Macquarie			Blood	cyt b
Mac9		Macquarie			Blood	cyt b
Adams1		Auckland	Adams	C	Blood	cyt b
Adams2		Auckland	Adams	C	Blood	cyt b
Adams3		Auckland	Adams	C	Blood	cyt b
Adams4		Auckland	Adams	C	Blood	cyt b
Adams5		Auckland	Adams	C	Blood	cyt b
End1		Auckland	Auckland	C	Blood	cyt b
End2		Auckland	Auckland	C	Blood	cyt b
End3		Auckland	Auckland	C	Blood	cyt b
End4		Auckland	Auckland	C	Blood	cyt b
End5		Auckland	Auckland	C	Blood	cyt b
Antip2		Antipodes			Feather	cyt b
Antip3		Antipodes			Feather	cyt b
Antip4		Antipodes			Blood	cyt b
Antip5		Antipodes			Blood	cyt b
Antip6		Antipodes			Blood	cyt b

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Table A3: Mitochondrial cytochrome b alignments of haplotypes found in the two forms of Giant Petrels (*Macronectes giganteus* and *M. halli*) and the fulmars (*Fulmarus glacialis* and *F. glacialoides*) used as outgroups in phylogenetic analysis. Published sequences are in lower case, lower case letters within haplotypes indicate nucleotides edited by visual inspection and/or reverse sequencing.

M. giganteus	1	tagctttctc	atccgttgcc	catacatgcc	gaaatgtaca	40
gp1	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngp1	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngp2	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngp3	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngpCh4	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngpSG5	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngpMar6	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngpCa7	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
ngpK8	1	TAGgTTTcTC	ATCCGTTGcC	cATAcaTGcc	GAAATGTACA	40
sgp1	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTAcA	40
sgpPat2	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpGou3	1	TAGCTTTcTC	ATcCGTTGcc	CATACATGCC	GAAAtGTaCA	40
sgp4	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpMac5	1	TAGCTTTCTC	ATccGTTgcc	CATACATGCC	GAAAtGTaCA	40
sgpPat6	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpSG7	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpMar8	1	TAGcTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgp9	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpFa10	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpCr11	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpCr12	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
sgpCr13	1	TAGCTTTCTC	ATCCGTTGCC	CATACATGCC	GAAATGTACA	40
F.glacialis	1	tagccttctc	atccgttgcc	cacacatgcc	gaaacgtaca	40
F.glacialoides	1	tagccttctc	atccgttgct	catacatgcc	gaaacgtaca	40
M. giganteus	41	atatggctga	ctcatccgaa	atctacatgc	aaatggagcc	80
gp1	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngp1	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngp2	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngp3	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngpCh4	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngpSG5	41	AtAtGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAgCC	80
ngpMar6	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngpCa7	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
ngpK8	41	ATATGGtTGA	CTCATCCGAA	ATcTACATGC	AAATGGAGCC	80
sgp1	41	AtAtgGctGA	CTCATCCGAA	ATCTACATGC	AAATGGAgCC	80
sgpPat2	41	ATATGGCTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpGou3	41	ATATgGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCc	80
sgp4	41	ATACGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpMac5	41	ATaCGgtTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpPat6	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpSG7	41	ATATGGCTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpMar8	41	ATACGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgp9	41	ATATGGCTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpFa10	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpCr11	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpCr12	41	ATATGGTTGA	CTCATCCGAA	ATCTACATGC	AAATGGAGCC	80
sgpCr13	41	ATATGGCTGA	CTCATTCGAA	ATCTACATGC	AAATGGAGCC	80
F.glacialis	41	gtacggctga	ctcattcgaa	atctacatgc	aaacggagcc	80
F.glacialoides	41	atatggctga	ctcattcgaa	atctacatgc	aaacggagcc	80
M. giganteus	81	tcattttttt	tcatctgcat	ttacctacac	attggacgag	120
gp1	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngp1	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngp2	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngp3	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngpCh4	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngpSG5	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngpMar6	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngpCa7	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
ngpK8	81	TCATTCTTTT	TCaTCTGCAT	TTaCcTACAT	ATTGGACGAG	120
sgp1	81	tCaTTTTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120

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Table A3 continued.

sgpPat2	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpGou3	81	TCATTtTTTt	TCATCTGCAT	TtACCTACAT	ATtGGACGAG	120
sgp4	81	TCATTCTTTT	TCATTTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpMac5	81	TCAtTCTttt	TCATTTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpPat6	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpSG7	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpMar8	81	TCATTCTTTT	TCATTTGCAT	TTACCTACAT	ATTGGACGAG	120
sgp9	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAC	ATTGGACGAG	120
sgpFa10	81	TCATTCTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpCr11	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpCr12	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAT	ATTGGACGAG	120
sgpCr13	81	TCATTTTTTT	TCATCTGCAT	TTACCTACAC	ATTGGACGAG	120
F.glacialis	81	tcattctttt	tcatctgcat	ttacctacat	attgggcgag	120
F.glacialoides	81	tcattctttt	tcatctgcat	ttacctacat	attggacgag	120
M. giganteus	121	gattctacta	tggctcctac	ctttacaaag	aaacctgaaa	160
gp1	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngp1	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngp2	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngp3	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngpCh4	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngpSG5	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngpMar6	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngpCa7	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
ngpK8	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgp1	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGaAA	160
sgpPat2	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpGou3	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgp4	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpMac5	121	GATTCTACTA	TGGCTCCTAC	CtTTACAAAG	AAACCTGAAA	160
sgpPat6	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpSG7	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpMar8	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgp9	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpFa10	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpCr11	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpCr12	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
sgpCr13	121	GATTCTACTA	TGGCTCCTAC	CTTTACAAAG	AAACCTGAAA	160
F.glacialis	121	gattctacta	tggctcctac	ctttacaaag	aaacctgaaa	160
F.glacialoides	121	gattctacta	tggctcctac	ctttacaaag	aaacctgaaa	160
M. giganteus	161	cacaggaatc	atccttctac	tcaccctcat	agcaaccgcc	200
gp1	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngp1	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngp2	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngp3	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngpCh4	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngpSG5	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngpMar6	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngpCa7	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
ngpK8	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgp1	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpPat2	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpGou3	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgp4	161	CACAGGAGTT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpMac5	161	CACAGGAGTT	ATCCTTCTaC	TcACCCTCAT	AGCAaCCGCc	200
sgpPat6	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpSG7	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpMar8	161	CACAGGAGTT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgp9	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpFa10	161	CACAGGAATT	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpCr11	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
sgpCr12	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200

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Table A3 continued.

sgpCr13	161	CACAGGAATC	ATCCTTCTAC	TCACCCTCAT	AGCAACCGCC	200
F.glacialis	161	cacaggaatt	atccttctac	tcaccctcat	agcaaccgcc	200
F.glacialoides	161	cacaggaatt	atcctcctac	tcaccctcat	agcaaccgcc	200
M. giganteus	201	ttcgtaggat	atgtcttacc	ctgaggccaa	atatcattct	240
gp1	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngp1	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngp2	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngp3	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngpCh4	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngpSG5	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngpMar6	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngpCa7	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
ngpK8	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgp1	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpPat2	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpGou3	201	tTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgp4	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpMac5	201	tTcGTAGGAT	ATGTCTTaCC	ctGAGgCCAA	ATATCATTCT	240
sgpPat6	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpSG7	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpMar8	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgp9	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpFa10	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpCr11	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpCr12	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
sgpCr13	201	TTCGTAGGAT	ATGTCTTACC	CTGAGGCCAA	ATATCATTCT	240
F.glacialis	201	ttcgtagggt	atgtcctacc	ctgaggccaa	atatcattct	240
F.glacialoides	201	ttcgtggggt	atgtcctacc	ctgaggccaa	atatcattct	240
M. giganteus	241	gaggggccac	agtcatcacc	aatctattct	cggccattcc	280
gp1	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngp1	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngp2	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngp3	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngpCh4	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngpSG5	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngpMar6	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngpCa7	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
ngpK8	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgp1	241	GAGGGGCCAC	AGtCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpPat2	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpGou3	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgp4	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpMac5	241	GAGGGGcCaC	AGTCATCAcC	AATCTATTcT	CGgCCATTCC	280
sgpPat6	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpSG7	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpMar8	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgp9	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpFa10	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpCr11	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpCr12	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
sgpCr13	241	GAGGGGCCAC	AGTCATCACC	AATCTATTCT	CGGCCATTCC	280
F.glacialis	241	gaggggccac	agtaatcact	aatctattct	cggccattcc	280
F.glacialoides	241	gaggggccac	agtgatcact	aatctattct	cggccattcc	280
M. giganteus	281	ctatattggc	cagaccctcg	tagaatgggc	ctgaggggga	320
gp1	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
ngp1	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
ngp2	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGAGGA	320
ngp3	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
ngpCh4	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
ngpSG5	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGAGGA	320

Table A3 continued.

ngpMar6	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGAGGA	320
ngpCa7	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
ngpK8	281	TTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGAGGA	320
sgp1	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpPat2	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpGou3	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgp4	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpMac5	281	CTATATTGGC	CAGACCCTcG	TAGAATGGGC	CTGAGGGGGA	320
sgpPat6	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpSG7	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpMar8	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgp9	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpFa10	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpCr11	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpCr12	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
sgpCr13	281	CTATATTGGC	CAGACCCTCG	TAGAATGGGC	CTGAGGGGGA	320
F.glacialis	281	ctatattggc	caaaccctcg	tagaatgagc	ttgaggggga	320
F.glacialoides	281	atatattggt	caaaccctcg	tagagtgagc	ctgaggggga	320
M. giganteus	321	ttttcagtag	ataaccccac	actaacccga	ttctttgccc	360
gp1	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngp1	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngp2	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngp3	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngpCh4	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngpSG5	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngpMar6	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngpCa7	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
ngpK8	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgp1	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpPat2	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpGou3	321	TTTTCAGTAG	ATAACCCCAC	ACTAAcCCGA	TTCTTTGCCC	360
sgp4	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpMac5	321	TTTTCAGTAG	ATAACCCCAC	ACTAAcCcGA	TTCTTTGCCC	360
sgpPat6	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpSG7	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpMar8	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgp9	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpFa10	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpCr11	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpCr12	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
sgpCr13	321	TTTTCAGTAG	ATAACCCCAC	ACTAACCCGA	TTCTTTGCCC	360
F.glacialis	321	ttttcggtag	ataaccccac	actaacccga	ttctttgccc	360
F.glacialoides	321	ttttcagtag	ataatcccac	actaacccga	ttctttgcct	360
M. giganteus	361	tacacttcct	cctccccttt	gcaattgcag	gacttaccct	400
gp1	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngp1	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACTCT	400
ngp2	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngp3	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngpCh4	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngpSG5	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngpMar6	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngpCa7	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
ngpK8	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgp1	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpPat2	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpGou3	361	TACACTTCCT	CCTCCCCTtt	GCAATtGCAG	GACTTACCcT	400
sgp4	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpMac5	361	TACACTTCCT	CCTCcCcttt	GCAATtgCAG	GACTTACCcT	400
sgpPat6	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTCACCCT	400
sgpSG7	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpMar8	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400

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Table A3 continued.

sgp9	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpFa10	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpCr11	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpCr12	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
sgpCr13	361	TACACTTCCT	CCTCCCCTTT	GCAATTGCAG	GACTTACCCT	400
F.glacialis	361	tacacttcct	ccttcccttt	gcaattgcag	ggcttaccct	400
F.glacialoides	361	tacacttcct	ccttcccttt	gcaattgcag	gacttacctt	400
M. giganteus	401	aattcacctc	accttccttc	acgaatcagg	ctcaaacaac	440
gp1	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngp1	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngp2	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngp3	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngpCh4	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngpSG5	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngpMar6	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngpCa7	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
ngpK8	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgp1	401	AATTCACCTC	ACCTTCCTTC	AcGAATCAGG	CTCAAACAAC	440
sgpPat2	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpGou3	401	AATTCACCTC	ACCTTCCTTC	aCGAATCAGG	CTCAAACAAC	440
sgp4	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpMac5	401	AATTCACCTC	ACCTTCCTTC	aCGAATCAGG	CTCAAaCAAC	440
sgpPat6	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpSG7	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpMar8	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgp9	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpFa10	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpCr11	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpCr12	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
sgpCr13	401	AATTCACCTC	ACCTTCCTTC	ACGAATCAGG	CTCAAACAAC	440
F.glacialis	401	aattcacctt	accttccttc	acgaatcagg	ctcaaacaac	440
F.glacialoides	401	aattcacctt	accttccttc	acgagtcagg	ctcaaacaac	440
M. giganteus	441	cccctaggca	tcgtatcaaa	ctgtgacaaa	atcccattcc	480
gp1	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngp1	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngp2	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngp3	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngpCh4	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngpSG5	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngpMar6	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngpCa7	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
ngpK8	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgp1	441	CCCCTAGGCA	TCGTATCAAA	CTGtGACAAA	ATCCCATTCC	480
sgpPat2	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpGou3	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgp4	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpMac5	441	cccctAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpPat6	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpSG7	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpMar8	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgp9	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpFa10	441	CCCCTAGGcA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpCr11	441	CCCCTAGGCA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpCr12	441	CCCCTAGGcA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
sgpCr13	441	CCCCTAGGcA	TCGTATCAAA	CTGTGACAAA	ATCCCATTCC	480
F.glacialis	441	cccctaggca	tcgtatcaaa	ctgtgacaaa	atcccatttc	480
F.glacialoides	441	cccctaggca	tcgtatcaaa	ctgtgacaaa	atcccattcc	480
M. giganteus	481	atccctactt	caccctaaaa	gacatcctag	gcttcgcact	520
gp1	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngp1	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520

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Table A3 continued.

ngp2	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngp3	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngpCh4	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngpSG5	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngpMar6	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngpCa7	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
ngpK8	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
sgp1	481	ATCCCTACTT	CACCCTAAAA	GACaTCCTAG	GCTTCGCACT	520
sgpPat2	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgpGou3	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgp4	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
sgpMac5	481	ATCCCTACTT	CACCCTAAAA	GACATCcTAG	GCTTCaCACT	520
sgpPat6	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
sgpSG7	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgpMar8	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCACACT	520
sgp9	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgpFa10	481	ATCCCTACTT	CACCCTAAAA	GACATCcTAG	GCTTCACACT	520
sgpCr11	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgpCr12	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
sgpCr13	481	ATCCCTACTT	CACCCTAAAA	GACATCCTAG	GCTTCGCACT	520
F.glacialis	481	acccctactt	caccctaaaa	gacatcctag	gttttacact	520
F.glacialoides	481	acccctactt	caccctaaaa	gacatcctag	gcttcacact	520
M. giganteus	521	catagtcctc	ccactaacat	ccctagctct	attctcccct	560
gp1	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngp1	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngp2	521	CATAATCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngp3	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngpCh4	521	CATAATCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngpSG5	521	CATAATCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngpMar6	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngpCa7	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
ngpK8	521	CATAATCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgp1	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpPat2	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpGou3	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgp4	521	CATAGTCCTC	CCACTAACAT	CCCTAGCCCT	ATTCTCCCCT	560
sgpMac5	521	CATAGTCCTC	CCACtAACAT	CCCTAGCTCT	aTTCTCCCCT	560
sgpPat6	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpSG7	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpMar8	521	CATAGTCCTC	CCACTAACAT	CCCTAGCCCT	ATTCTCCCCT	560
sgp9	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpFa10	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpCr11	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpCr12	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
sgpCr13	521	CATAGTCCTC	CCACTAACAT	CCCTAGCTCT	ATTCTCCCCT	560
F.glacialis	521	catattcctc	ccactaacat	ccctagccct	attctccccc	560
F.glacialoides	521	catattcctc	ccactaacat	ccctagccct	attctccccc	560
M. giganteus	561	aacctactag	gagacccaga	aaactttacc	cccgcaaacc	600
gp1	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngp1	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngp2	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngp3	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngpCh4	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngpSG5	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngpMar6	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngpCa7	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
ngpK8	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgp1	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpPat2	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpGou3	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgp4	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600

Table A3 continued.

sgpMac5	561	AaCCTAcTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACc	600
sgpPat6	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpSG7	561	AACCTACTAG	GAGAcCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpMar8	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgp9	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpFa10	561	AACCTACTAG	GAGAcCCAgA	AAACTTTAcC	CCCGCAAACC	600
sgpCr11	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAAcC	600
sgpCr12	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAACC	600
sgpCr13	561	AACCTACTAG	GAGACCCAGA	AAACTTTACC	CCCGCAAAcC	600
F.glacialis	561	aatctactgg	gagacccaga	aaactttacc	cccgcaaacc	600
F.glacialoides	561	aatctactgg	gagacccaga	aaactttacc	cctgcaaacc	600

M. giganteus	601	cgctagttac	accccctcat	atcaaaccag	agtggtactt	640
gp1	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngp1	601	CgCTAGTTAC	ACCCCCtCaT	ATCAAACCAG	AGTGGTATTT	640
ngp2	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngp3	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngpCh4	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngpSG5	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngpMar6	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGtATTT	640
ngpCa7	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
ngpK8	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTATTT	640
sgp1	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgpPat2	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgpGou3	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAaCCAG	AGTGGTACTT	640
sgp4	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgpMac5	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAaCCAG	AGTGGTACTT	640
sgpPat6	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAAcCAG	AGTGGTACTT	640
sgpSG7	601	CACTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AgtGGtACTT	640
sgpMar8	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgp9	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgpFa10	601	CGCTAGTTAC	ACCCCCTCaT	ATCAAACCAG	AGTGGTACTT	640
sgpCr11	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGTGGTACTT	640
sgpCr12	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGtGGTACTT	640
sgpCr13	601	CGCTAGTTAC	ACCCCCTCAT	ATCAAACCAG	AGtGGtACTT	640
F.glacialis	601	cactagtcac	acctccccat	atcaaaccag	aatggtactt	640
F.glacialoides	601	cactagtcac	acctccccat	attaaaccag	aatggtactt	640

M. giganteus	641	cctattcgca	tacgccatcc	tacgctcaat	ccccaataaa	680
gp1	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngp1	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngp2	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngp3	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngpCh4	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngpSG5	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngpMar6	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngpCa7	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
ngpK8	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgp1	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpPat2	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpGou3	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgp4	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpMac5	641	CCTATTCGCA	TACGCCATCC	TaCGCTCAAT	CCCCAATAAA	680
sgpPat6	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAAtaAA	680
sgpSG7	641	cCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCaATAAA	680
sgpMar8	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgp9	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpFa10	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpCr11	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAATAAA	680
sgpCr12	641	CCTATTCgCA	TACGCCATCC	TACGCTCAAT	CCCCAAtAAA	680
sgpCr13	641	CCTATTCGCA	TACGCCATCC	TACGCTCAAT	CCCCAAcAAA	680
F.glacialis	641	cttattcgca	tatgctatcc	tacgctcaat	ccccaacaaa	680
F.glacialoides	641	cctattcgca	tatgccattc	tacgctcaat	ccccaacaaa	680

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Table A3 continued.

M. giganteus	681	ttaggtggag	tattagctct	agcggcctcc	gtactagtcc	720
gp1	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngp1	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngp2	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngp3	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngpCh4	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngpSG5	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTGCTAGTCC	720
ngpMar6	681	tTAGGTGGAG	tATTAGCTCT	AGCGGCCTCC	GtACTAGTCC	720
ngpCa7	681	TTAGGGGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
ngpK8	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTGCTAGTCC	720
sgp1	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpPat2	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpGou3	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgp4	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpMac5	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpPat6	681	TTAGGtGGAG	TATTAGCTCT	AGcGGCCTCC	GTACTAGTCC	720
sgpSG7	681	tTAGGtGGAG	tATTAGCTCT	AGcGGcCTCC	GtActAGTCC	720
sgpMar8	681	TTAGGCGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgp9	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpFa10	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpCr11	681	TTAGGTGGAG	TATTAGCTCT	AGCGGCCTCC	GTACTAGTCC	720
sgpCr12	681	TTAGGTGGAG	TATTAGCTCT	AGtGGCCTCC	GtACTAGTCC	720
sgpCr13	681	tTAGGtGGAg	TATTAGCTCT	AGcGGcCTCC	GTACTAGTCC	720
F.glacialis	681	ttaggcggag	tattagcttt	agctgcctcc	gtactagtcc	720
F.glacialoides	681	ctaggtggag	tattagcttt	agctgcctct	gtactagtcc	720

M. giganteus	721	tattcctatc	cccattcctc	cataaagcca	aa	752
gp1	721	TATTCCTATC	CCCATTCCTC	CACAAAGCCA	AA	752
ngp1	721	TATTCCTATC	CCCaTTCCTC	CATAAAGCCA	AA	752
ngp2	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
ngp3	721	TATTCCTATC	CCCATTCCTC	CATAAAGcCA	AA	752
ngpCh4	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
ngpSG5	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
ngpMar6	721	TATTCCTATC	CCCATTCCTC	CaTAAAGCCA	AA	752
ngpCa7	721	TAtTCCTATC	CCcATTCCTC	CaCAAAGCCA	AA	752
ngpK8	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgp1	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpPat2	721	TATTCCTATC	CCCATTCCTC	CACAAAGCCA	AA	752
sgpGou3	721	TATTcCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgp4	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpMac5	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpPat6	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpSG7	721	TATTCCTATC	CCCATTCCtC	CAtAaAGCCA	AA	752
sgpMar8	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgp9	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpFa10	721	TATTCCTATC	CCCATTCCTC	CATAAAGCCA	AA	752
sgpCr11	721	TATTCCTATC	CCCATTCCtC	CAtAAAGCCA	AA	752
sgpCr12	721	TATTCCTATC	CCCATTCCtC	CATAAAGCCA	AA	752
sgpCr13	721	TATTCCTATC	CCCATTCCTC	CaTAAAGCcA	AA	752
F.glacialis	721	tattcctaac	cccattcctc	cataaagcca	aa	752
F.glacialoides	721	tattcctatc	tccattcctt	cacaaggcca	aa	752

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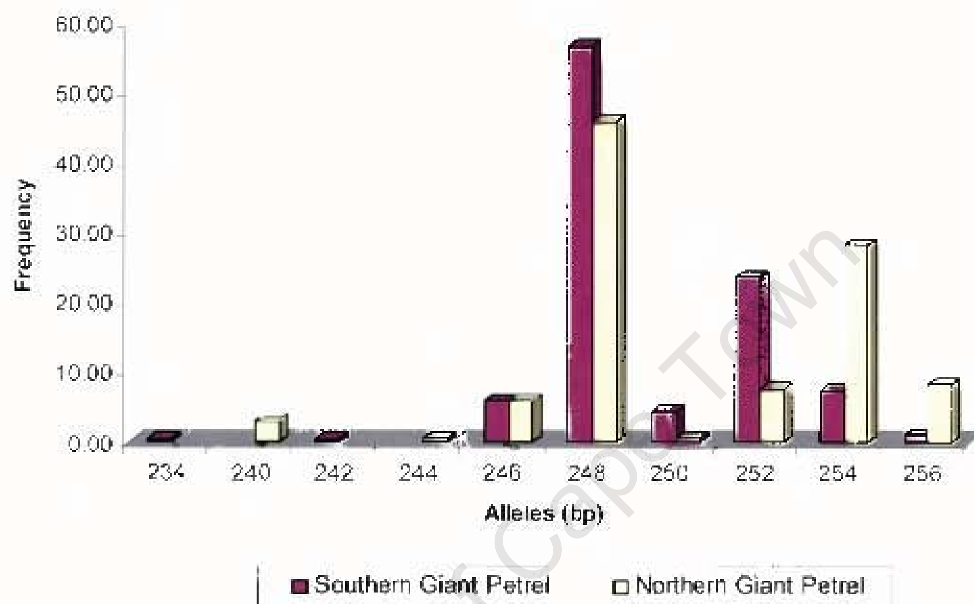
Table A4: Haplotypes found within the giant petrels. Table is based on Figure 5.3 but includes details on individuals which share a particular haplotype.

Haplotype	Nucleotide Position																							Total *n*	Individuals carrying that haplotypes
Macronectes halli (Northern Giant Petrel)																									
gp1	C	T	T	C	C	C	T	A	T	C	G	T	C	A	G	T	G	T	T	T	C	A	C	23	SMarion5, Kerguelen4, Adams1, 3, 4, NMacquarie2, 4, 8, 9, Campbell1, 2, 4, 5, Enderby3, 4, Antipodes3, 4, 5; NSGeorgia4; NMarion1, 973, 968; NCrozet15
ngp1													T										T	3	Adams2, Enderby1, 5
ngp2											A				A								T	4	Kerguelen1, Chatham1, 3, NSGeorgia20
ngp3																							T	15	Kerguelen5, 7, 11, 12, Chatham2, 10, NMacquarie3, Antipodes2, 6; NSGeorgia1, NMarion2, NCrozet3, 4, 8, 11
ngpCh4															A								T	1	Chatham4
ngpSG5											A				A							G	T	3	NSGeorgia2, 3, 10
ngpMar6											A												T	1	NMarion9A0
ngpCa7																				G				1	Campbell3
ngpK8	G									T	A				A							G	T	1	Kerguelen6
Macronectes giganteus (Southern Giant Petrel)																									
sgp1			C		T				C					G				C					T	30	IslaArce1, 3, 17; Chile1, 2, 3, 4, 5, GranRobredo2, 12, StatenIsland10, 15, 16, SSGeorgia3, 5, 11, 12; Falklands1, 7, 8, 9, 11, 12, 13, 14, KingGeorge5182, 5179, 5182, 5186; Heard2
sgpPat2			C		T				C					G				C						4	IslaArce9, 11; GranRobredo4, 9
sgpGou3					T									G				C					T	7	Gough51, 53, 54, 57, 58, 60, 63
sgp4		C				T		G								C		C					T	14	SMacquarie2, 3, 7, 9, 10, 13, 20, SCrozet 1, 3, 4, 5, 23, 25
sgpMac5		C				T		G										C					T	1	SMacquarie 4
sgpPat6												C						C					T	1	StatenIsland26
sgpSG7			C		T				C					G			A	C					T	1	SSGeorgia32
sgpMar8		C				T		G								C		C		C			T	3	SMarion1, 9, 10
sgp9			C		T		C		C					G				C					T	7	SMarion2, 3, 4, 6, 7, 8; SCrozet27
sgpFa10																		C					T	2	Falklands6, 10
sgpCr11					T				C					G				C					T	1	SCrozet19
sgpCr12					T				C					G				C			T		T	1	SCrozet17
sgpCr13			C	T	T		C		C					G				C	C				T	1	SCrozet21

Figure A1: Allele frequency comparisons between Southern and Northern Giant Petrels for each microsatellite locus.

Locus Paequ3



Locus Paequ4

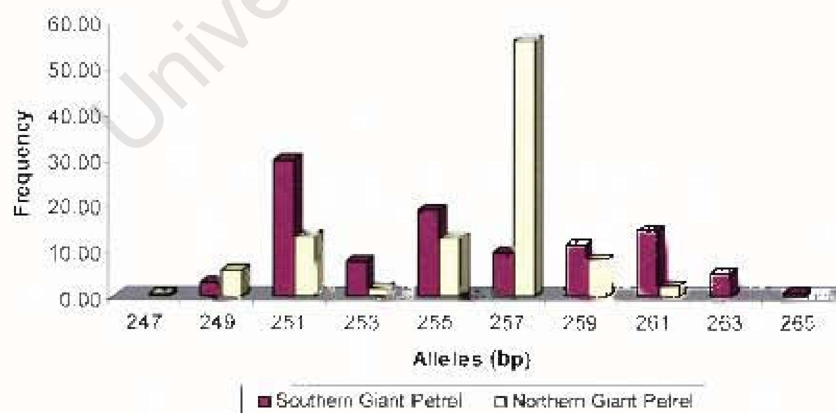


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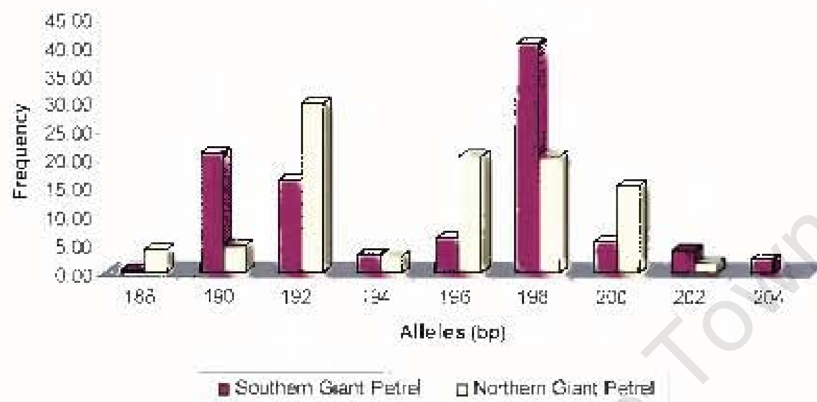
Figure A1 continued.

Locus De11



Locus D16

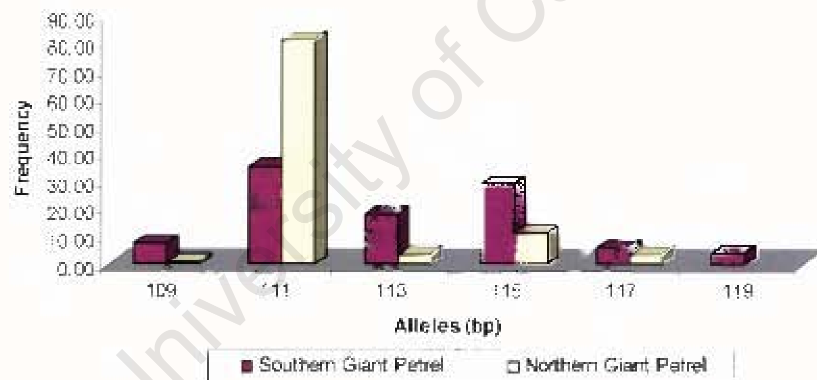


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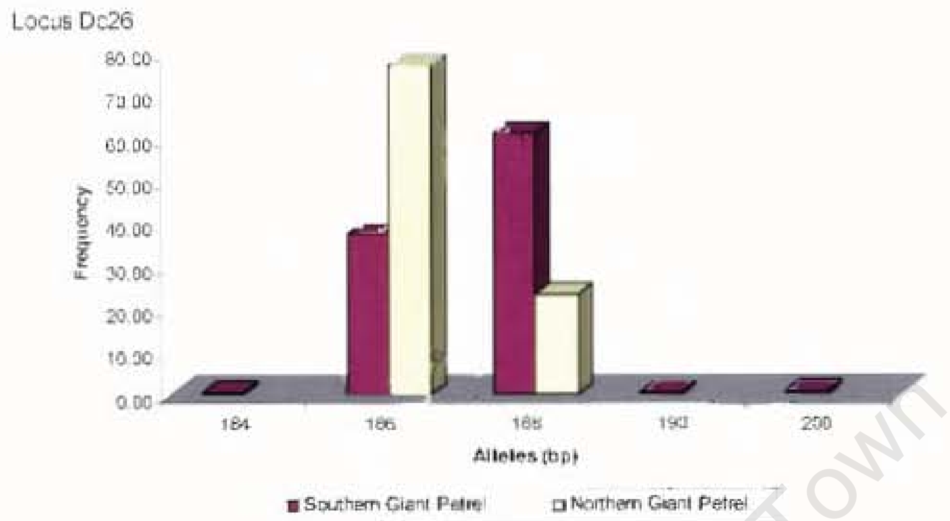
Figure A1 continued.

Locus Dc26



Locus De37

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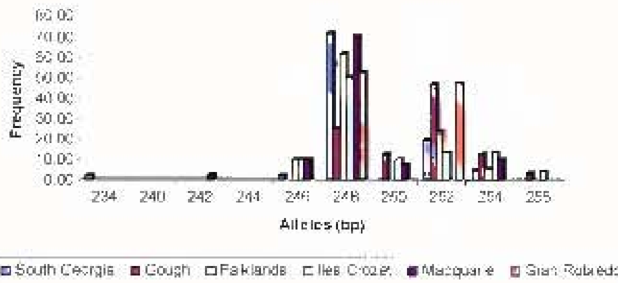
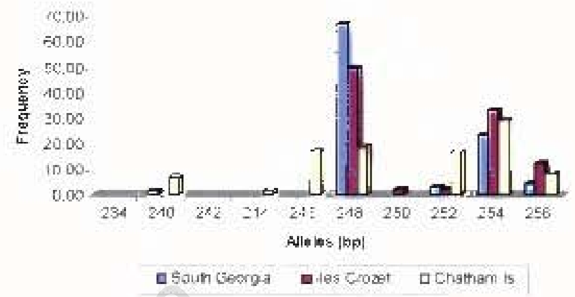
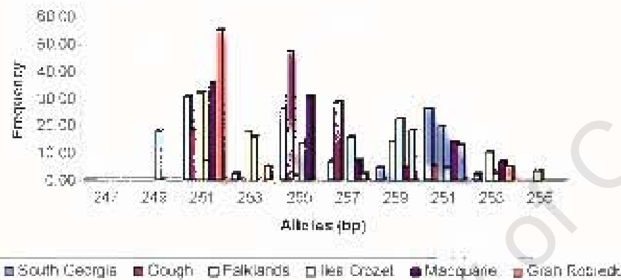
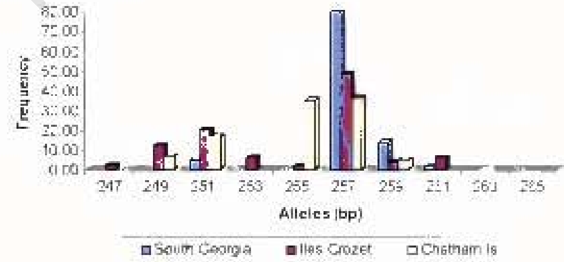
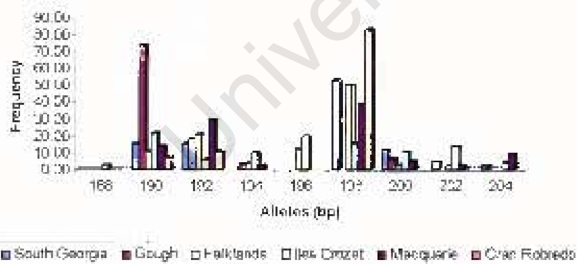
Figure A2: Microsatellite allele frequencies for both species of giant petrels per colony. SGP Southern Giant Petrel, NGP Northern Giant Petrel

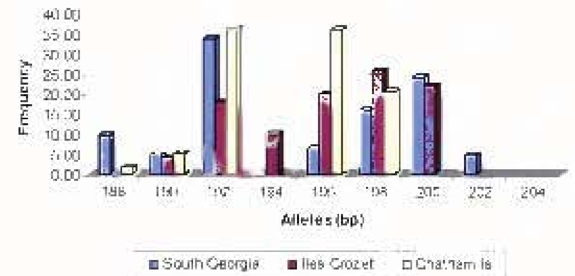


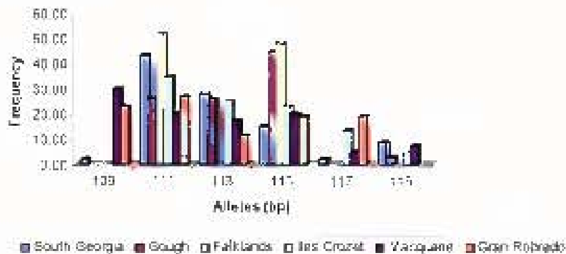












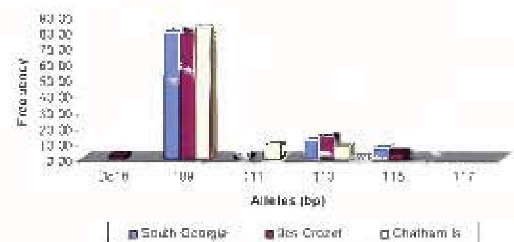


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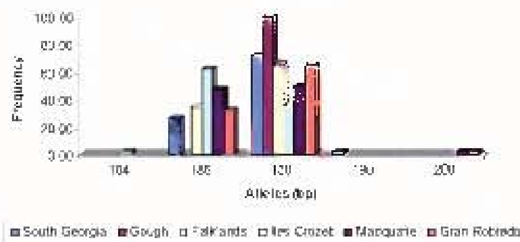
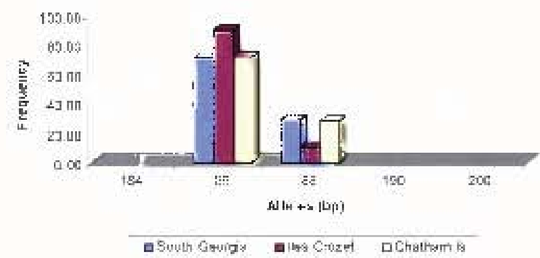
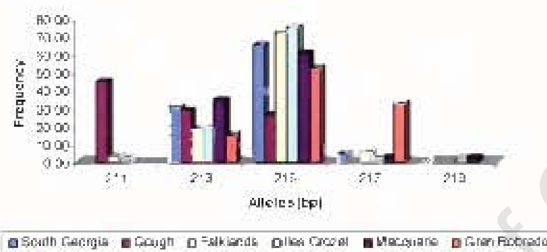
Figure A2 continued.







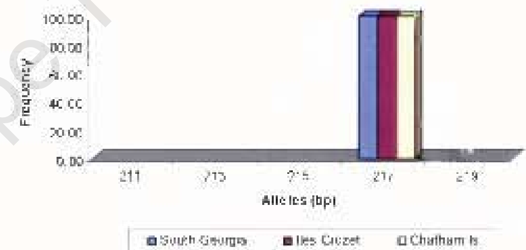


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Table A5: Microsatellite alleles shown as letters in the Southern and Northern Giant Petrels as comparisons between colonies and species.

Southern Giant Petrel

Locus	Colony	Alleles												*Na*	No. of colony specific alleles
Paequ3	SG	A						G	H		J	K		5	1
	G								H	I	J	K	L	5	0
	Falk							G	H		J	K		4	0
	Cro							G	H	I	J	K	L	6	0
	Mac					E		G	H	I		K		5	1
	GR								H		J			2	0
Paequ4	SG			C	D	E	F	G	H	I				7	0
	G			C		E	F		H					4	0
	Falk			C	D	E		G	H	I	J			7	1
	Cro		B	C	D	E	F	G	H	I				8	1
	Mac			C		E	F	G	H	I				6	0
	GR			C	D		F	G	H	I				6	0
De11	SG		B	C			F	G	H	I				6	0
	G		B	C	D			G						4	0
	Falk		B	C	D	E	F	G	H					7	0
	Cro	A	B	C	D	E	F	G	H	I				9	1
	Mac		B	C	D		F	G	H	I				7	0
	GR		B	C			F							3	0
Dc16	SG	A	B	C	D	E	F							6	0
	G		B	C	D		F							4	0
	Falk		B		D									2	0
	Cro		B	C	D	E	F							5	0
	Mac	A	B	C	D	E	F							6	0
	GR	A	B	C	D	E								5	0
Dc26	SG		B	C	D									3	1
	G			C										1	0
	Falk		B	C										2	0
	Cro	A	B	C										3	1
	Mac		B	C						I				3	0
	GR		B	C						I				3	0
De37	SG		B	C	D									3	0
	G	A	B	C										3	0
	Falk	A	B	C	D									4	0
	Cro	A	B	C	D	E								5	0
	Mac		B	C	D	E								4	0
	GR		B	C	D									3	0
Total	SG													30	2
	G													21	0
	Falk													26	1
	Cro													36	3
	Mac													31	1
	GR													22	0

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Table A5 continued.

Northern Giant Petrel

Locus	Colony	Alleles												*Na*	No. of colony specific alleles
Paequ3	SG				D				H		J	K	L	5	0
	Cro								H	I	J	K	L	5	1
	Chat				D		F	G	H		J	K	L	7	2
Paequ4	SG			C			F	G	H					4	0
	Cro	A	B	C	D	E	F	G	H					8	1
	Chat		B	C		E	F	G						5	0
De11	SG	A	B	C		E	F	G	H					7	1
	Cro		B	C	D	E	F	G						6	1
	Chat	A	B	C		E	F							5	0
Dc16	SG		B	C	D	E								4	0
	Cro	A	B		D	E								4	1
	Chat		B	C	D									3	0
Dc26	SG		B	C										2	0
	Cro		B	C										2	0
	Chat		B	C										2	0
Total	SG													22	1
	Cro													25	4
	Chat													22	2

Species specific alleles

Locus	Species	Alleles												*Na*	No. of species specific alleles
Paequ3	SGP	A				E		G	H	I	J	K	L	8	2
	NGP				D		F	G	H	I	J	K	L	8	2
Paequ4	SGP		B	C	D	E	F	G	H	I	J			9	2
	NGP	A	B	C	D	E	F	G	H					8	1
De11	SGP	A	B	C	D	E	F	G	H	I				9	1
	NGP	A	B	C	D	E	F	G	H					8	0
Dc16	SGP	A	B	C	D	E	F							6	1
	NGP	A	B	C	D	E								5	0
Dc26	SGP	A	B	C	D					I				5	3
	NGP		B	C										2	0
De37	SGP	A	B	C	D	E								5	4
	NGP				D									1	0
Total	SGP													42	13
	NGP													32	3

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SYNTHESIS

Seabirds have a high proportion of threatened species (BirdLife-International, 2006). Taxonomic uncertainties have become a major conservation priority especially in Procellariiformes, because only recognised species are generally afforded protection (Frankham *et al.*, 2002). In addition to ecology, knowledge of the phylogeographic distribution and population structure is essential for appropriate management plans to conserve threatened species and subspecies (Gaston, 2001; Avise, 2004; Beebee and Rowe, 2004). To this end, the main aims of this thesis were to resolve the taxonomic ranks of two species of *Procellaria*, the White-chinned and Spectacled Petrels, and the two forms of *Macronectes*, the Southern and Northern Giant Petrels. In addition, the phylogeography of the White-chinned and giant petrels was investigated, in part to test whether birds killed at sea could be assigned to specific colonies or regions.

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Taxonomy

Current taxonomic status, although contested, for all four species is separate species rank (Ryan, 1998; Hockey *et al.*, 2006; Ryan *et al.*, 2006). Analyses of mitochondrial cytochrome b sequence data support these rankings. The White-chinned and Spectacled Petrels diverged relatively recently as shown by limited sequence divergence and the fixation of only six mutations within cytochrome b. Within *Procellaria* they are sister species, with the closest other species being the Grey Petrel, which has a similar wide-ranging distribution as the White-chinned Petrel. Phylogenetic trees show that White-chinned and Spectacled Petrels have an ancestor that evolved from a common ancestor with the Grey Petrel. The two New Zealand endemic *Procellaria* species, *P. westlandica* and *P. parkinsoni* are sister taxa within the genus. The New Zealand region probably is where the genus evolved and from which recent expansions took place in the White-

chinned and Spectacled Petrels. In terms of management implications this means that the Spectacled Petrel should continue to be recognised as a separate species, receiving separate attention in terms of species management. Recent reports show that the numbers are increasing (Ryan *et al.*, 2006), however, the species is known to breed only on Inaccessible Island and is vulnerable to threats at sea.

Analyses of the two forms of giant petrels also supported separate species rank, although sequence divergence in cytochrome b is limited to only one mutational difference. This suggests a more recent speciation event than that between the White-chinned and Spectacled Petrels. The recent timing of speciation is further indicated in the paraphyly of the Southern Giant Petrel within cytochrome b. If it were not for the fact that the two forms breed sympatrically on several islands they could well be considered subspecies. Tree topologies show that individuals cluster with their species rather than with heterospecific individuals breeding on the same island, and microsatellite data shows a clear separation of gene pools between the two forms. The separation of the two forms is further supported by sexual isolation and morphological differences. Genetic findings together with the ecological data thus indicate that the two forms should be managed separately as two different species.

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Evolutionary history of giant petrels

Speciation and phylogeography of northern hemisphere species are often characterized by glacial cycles of the Pleistocene and Pliocene (Avise, 2000) and expansions in high latitude bird species following these periods are common (Pearce *et al.*, 2002). Highly mobile species such as seabirds often present an enigma regarding the importance of physical barriers to gene flow. Physical barriers in the case of seabirds can be continental landmasses (e.g. Steeves *et al.*, 2005) or glaciers during ice ages resulting in allopatric refugia during the Pleistocene (e.g. Jouventin and Viot, 1985; e.g. Kidd and Friesen, 1998; Moum and Árnason, 2001). Although seabirds have great dispersal potential, most

choose to breed in natal colonies (Ovenden *et al.*, 1991). If philopatry is very strong, it may create a non-physical barrier to gene flow. However, even strongly philopatric species such as wandering albatrosses sometimes move between colonies (Inchausti and Weimerskirch, 2002) and only one effective movement per generation is sufficient to maintain genetic continuity between populations (Ridley, 1996).

Southern and northern hemisphere oceans differ in that northern hemisphere oceans are divided by landmasses that create barriers to gene flow whereas the southern hemisphere oceans are more continuous, although only at 50-60° S, with a bottleneck at the Drake Passage between Cape Horn and the Antarctic Peninsula which may have presented a barrier prior to ca. five million years ago. In the northern hemisphere these barriers to gene flow are mostly responsible for causing differentiation within species. The phylogeography of northern seabirds therefore often exhibits structure (e.g. Goostrey *et al.*, 1998; Liebers and Helbig, 2002; Pons *et al.*, 2004). In contrast it has been found that at least some pelagic, widespread seabird species in the southern hemisphere have diverged in the absence of such physical barriers (e.g. Burg and Croxall, 2004; Steeves *et al.*, 2005).

As mentioned earlier, events leading to speciation often initiated in the Pliocene, but a small percentage originated in the Pleistocene. These are usually characterised by small sequence divergences (Avise, 2000). The speciation event separating Southern and Northern Giant Petrels has been recent, an estimated 0.39 to 1.2 million years ago, which places it during recent glaciation cycles (Avise and Walker, 1998). These climatic events also may have produced phylogroups within the Southern Giant Petrel. Secondary contact probably occurred after climatic changes, and although no isolating mechanisms have evolved, ancient lineages still persist in cytochrome b within the Southern Giant Petrel. In contrast, reproductive isolation occurred between the Southern and Northern forms thereby leading to the formation of two species. In the giant

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petrels ancient vicariance events seem to have shaped current geographic distributions and speciation rather than current physical barriers.

Within species phylogeography

Of the few studies of Procellariiform phylogeography in the southern hemisphere, most have investigated albatrosses (Burg, 2000; Burg and Croxall, 2001; Abbott and Double, 2003, 2003; Abbott, 2004; Burg and Croxall, 2004; Abbott *et al.*, 2006; Van Bekkum *et al.*, 2006), with only a few studies of petrel species (Jouventin and Viot, 1985; Ovenden *et al.*, 1991; Austin *et al.*, 1994; Austin, 1996; Browne *et al.*, 1997; Paterson and Wallis, 2000; Friesen *et al.*, 2006). Within northern and southern hemisphere Procellariiformes both population differentiation (e.g. Abbott and Double, 2003; Cagnon *et al.*, 2004; Walsh and Edwards, 2005) and homogeneity (e.g. Burg and Croxall, 2001, 2004) have been observed. One explanation is that species with overlapping foraging locations and a predominantly oceanic lifestyle will show little to no structure, whereas species which have populations with more discrete inshore foraging locations will show population differentiation possibly leading to the formation of subspecies (Burg and Croxall, 2001, 2004). Data on White-chinned Petrel foraging ranges are scant, but it appears that foraging locations of colonies in different ocean basins do not overlap (Phillips *et al.*, 2006). The species is partially oceanic in lifestyle and has the capability to fly great distances, but mainly forages in highly productive coastal and upwelling regions. Therefore, genetic structure between populations can be expected within the White-chinned Petrel if foraging locations indeed are discrete. Giant Petrels range further north as immature birds but have more constrained ranges as adults although this differs slightly between the two species (Marchant and Higgins, 1990). Banding recoveries indicate that Southern Giant Petrel immatures occasionally visit other breeding colonies and sometimes stay to breed (Marchant and Higgins, 1990). It can be expected that the two species exhibit population genetic structuring at least between distant colonies. On the other hand, this scenario has prompted the supported hypothesis, that juvenile dispersal rates may provide sufficient gene flow to promote

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metapopulation structure throughout the range (Inchausti and Weimerskirch, 2002; Burg and Croxall, 2004).

Within the White-chinned Petrel, two distinct regional populations were discovered. However, within ocean basins only weak differentiation was found. The existence of two strong regional populations supports the observation of effective regional but not colony-level philopatry in these birds. Assignment tests indicated that short distance dispersal occurs at least to such an extent that colonies within ocean basins remain connected but such that weak differentiation is maintained as shown by microsatellite DNA and F_{ST}. Long distance dispersal occurs less commonly as is shown by higher F_{ST} and G_{ST} values of comparisons of colonies further apart than colonies geographically closer. As no shared haplotypes were found between regional populations and differentiation was higher in cytochrome b than with microsatellite DNA, long distance dispersal is likely to be male biased. Such male-biased dispersal has been detected through differences in nuclear and mitochondrial DNA markers (Rassmann *et al.*, 1997) and has been shown in Black-browed Albatrosses (Burg and Croxall, 2001). The hypothesis of structured populations is supported in this species due to discrete foraging locations and philopatry. By investigating dispersal and gene flow as well as population structuring, it is possible to contribute to assessment studies of human induced impacts such as longline associated mortality. My study has shown that impacts of longlining on local populations can be considered serious as bycatch from local fisheries originates locally rather than being a mixture of global populations. The presence of population differentiation also means that local extinctions are more serious than in a metapopulation, because of the loss of associated genetic diversity.

Within the Southern Giant Petrel, two separate lineages were identified in cytochrome b corresponding to birds found at Iles Crozet, Marion Island and Macquarie, and a second lineage comprising birds of all remaining colonies as well as some individuals from Iles Crozet and Marion Island. In contrast, nuclear

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microsatellite DNA showed that although divergence is high between these two lineages, gene flow still exists. Microsatellites indicated further genetic structuring within the Southern Giant Petrel, but in order to identify relationships between colonies, more colonies need to be genotyped and included in analysis. Within the Northern Giant Petrel, no further sub-structuring could be found with cytochrome b, but preliminary microsatellite DNA analysis showed some differentiation between colonies. Recent expansions, as indicated by cytochrome b, often mean that populations/colonies are still tightly connected even if no contemporary gene flow occurs. This would explain the lack of differentiation found in cytochrome b. As only three colonies were genotyped in the Northern Giant Petrel no further conclusions could be reached. Future studies are planned to include more colonies. However, analysis does show that the hypothesis of genetic structuring between colonies is supported rather than one of a single metapopulation in giant petrels. Conservation implications are therefore similar to the White-chinned Petrels; even more so, as breeding populations of giant petrels are considerably smaller.

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Conclusions and future work

In the White-chinned Petrel, two distinct regional populations were defined by both mitochondrial and nuclear markers. It was further shown that local colonies are at risk from longline fishing and local loss of genetic diversity may become a conservation issue. Genetic analysis identified the New Zealand region as having the greatest genetic diversity. However, this region does not contain the greater number of breeding pairs. Management plans need to consider this information in order to conserve the species main source of diversity.

Within the giant petrels, genetic analysis has confirmed species status of the two forms as well as showing population differentiation between colonies within species. The species identity of the Falkland and Gough populations has been clarified, which will aid in the local conservation of these colonies. Further nuclear

analysis is required in order to complete phylogeographic analysis of the two species.

From this, it is clear then that the study of phylogeography and population genetics can greatly contribute to conservation management by providing insights into threatened species. By examining the processes that have shaped the geographical distribution of species, one can develop management plans to conserve their future.

I would recommend for future work the application of the genetic findings in these species. In this thesis, microsatellite as well as cytochrome b sequence data was used to assess the population composition of birds caught in Indian Ocean and New Zealand bycatch. In order to fully assess the impact of fisheries on all colonies, I would recommend including the remaining two colonies (Kerguelen and Campbell Island), as well as a larger sample size for the other source colonies, in further analysis in order to investigate the colony composition of other fishing industries.

Similarly, the two giant petrel species are often hard to distinguish in bycatch samples. As the world population of giant petrels is only approximately 31,000 and 11,500 breeding pairs of Southern and Northern Giant Petrels, respectively, the species identity of bycatch is important. An easy identification based on the fixed mutational difference in cytochrome b as well as microsatellite DNA, similar to that developed for the White-chinned Petrel, could aid in the assessment of bycatch. In order to do so, the preliminary microsatellite DNA analysis included in this thesis needs to be expanded to include more colonies of both species. This data would further aid in the characterisation of the two species phylogeography facilitating the implication of any management plans to ensure the continued future of giant petrels.

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