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Speciation and phylogeography of giant petrels *Macronectes* ☆

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ABSTRACT

We examine global phylogeography of the two forms of giant petrel *Macronectes* spp. Although previously considered to be a single taxon, and despite debate over the status of some populations and the existence of minimal genetic data (one mitochondrial cytochrome *b* sequence per form), the current consensus based on morphology is that there are two species, Northern Giant Petrel *M. halli* and Southern Giant Petrel *M. giganteus*. This study examined genetic variation at cytochrome *b* as well as six microsatellite loci in giant petrels from 22 islands, representing most island groups at which the two species breed. Both markers support separate species status, although sequence divergence in cytochrome *b* was only 0.42% (corrected). Divergence was estimated to have occurred approximately 0.2 mya, but with some colonies apparently separated for longer (up to 0.5 my). Three clades were found within giant petrels, which separated approximately 0.7 mya, with the Southern Giant Petrel paraphyletic to a monophyletic Northern Giant Petrel. There was evidence of past fragmentation during the Pleistocene, with subsequent secondary contact within Southern Giant Petrels. The analysis also suggested a period of past population expansion that corresponded roughly to the timing of speciation and the separation of an ancestral giant petrel population from the fulmar *Fulmarus* clade.

1. Introduction

Gene trees are evolutionary reconstructions of the historic relationships amongst groups of single genes that have experienced little or no recombination. They have the potential to reflect both intra- and inter-specific evolution, and the points at which speciation occurred; haplotype trees explore both genetic variation within species and between closely related species (Templeton, 2001). There has been much debate recently on the suitability of mitochondrial DNA (mtDNA) phylogenies for discerning avian taxonomy, as 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. The equivalence between organismal and gene phylogenies is thus dependent on time (Spaulding et al., 2006), so there may be a lack of diagnostic lineage sorting even in the presence of barriers to gene flow. Demographic events such as population 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).

One of the goals of phylogeography is to determine whether species consist of one or several phylogroups, which are independently evolving units, and to determine their relationships. If the

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objective is to determine taxonomic limits of recently derived groups, then mtDNA is still preferred over nuclear markers due to its high variability and shorter coalescence time. In a recent review, Zink and Barrowclough (2008) compared the congruence of mtDNA and nuclear DNA phylogenies, the latter mostly based on microsatellites. The authors observed that the mutation rate of the marker determines the density, and therefore resolution, of observed changes, but does not affect the timescale reflected by a particular gene tree. It also affects the ease with which the tree can be rooted. Therefore, for microsatellites, which have a fast mutation rate, rooting is often impossible due to homoplasy. MtDNA is therefore useful for detecting phylogeographic patterns, but nuclear DNA is required to reduce the error rate in determining evolutionary processes. Also, mtDNA reflects only the maternal side of evolution and inferences from mtDNA may therefore conflict with nuclear DNA due to unidirectional introgression. We present a case of incomplete lineage sorting in mtDNA that is not found in microsatellites in an evolutionarily young group of seabirds, the giant petrels.

Separate species status for two morphologically distinct forms of giant petrel (*Macronectes* spp.) was originally suggested by Bourne and Warham (1966) on the basis of differences in morphology (bill tip colour, presence of white morph in only one taxon) and behaviour (six week difference in mean laying date), as well as evidence for sympatric breeding on Macquarie Island without hybridization. Subsequent studies found occasional hybridisation at South Georgia, Marion Island, and (very rarely) at Macquarie Island, with the same partners breeding in mixed pairs over successive seasons (Burger, 1978; Johnstone, 1978; Voisin and Bester, 1981; Hunter, 1983, 1987; Cooper et al., 2001). Studies investigating the phylogeny of Procellariiformes subsequently treated giant petrels as two separate species (Nunn and Stanley, 1998; Kennedy and Page, 2002). Penhallurick and Wink (2004) recently 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). However, both BirdLife International (www.birdlife.org), which is the official Red List Authority for birds for the International Union for Conservation of Nature (IUCN) and the Taxonomic Working Group to the Advisory Committee of the Agreement on the Conservation of Albatrosses and Petrels (ACAP; www.acap.aq) currently consider them to be separate species. This is largely based on the morphological differences listed in Bourne and Warham (1966) and the original molecular study by Nunn and Stanley (1998).

Both species have circumpolar breeding distributions in the Southern Ocean, and occur sympatrically at South Georgia, the Prince Edward Islands, Crozet Islands and Macquarie Island. More island groups are occupied by Northern than Southern Giant Petrels in the New Zealand region, whereas the reverse is true in the South Atlantic (Fig. 1). Southern Giant Petrels breed both further north (at Gough Island), and further south (South Shetland Islands, Antarctic Peninsula region and continental Antarctica) than Northern Giant Petrels. The species identity of the birds breeding at Gough and at the Falklands/Malvinas Islands has been debated, as they apparently possess several intermediate characteristics (Bourne and Warham, 1966; Voisin and Bester, 1981; Brooke, 2004; Penhallurick and Wink, 2004). Indeed, Voisin and Bester (1981) suggested that they should be given subspecies status, as *M. giganteus solanderi*; however, this has not been generally accepted. There are further difficulties with birds nesting in Argentina and Chile, where adults seem to be small, as at Gough and the Falklands/Malvinas Islands, and none are white phase (Quintana et al., 2005; Copello et al., 2006; Copello and Quintana, 2009a). In addition, the Northern Giant Petrels breeding at the

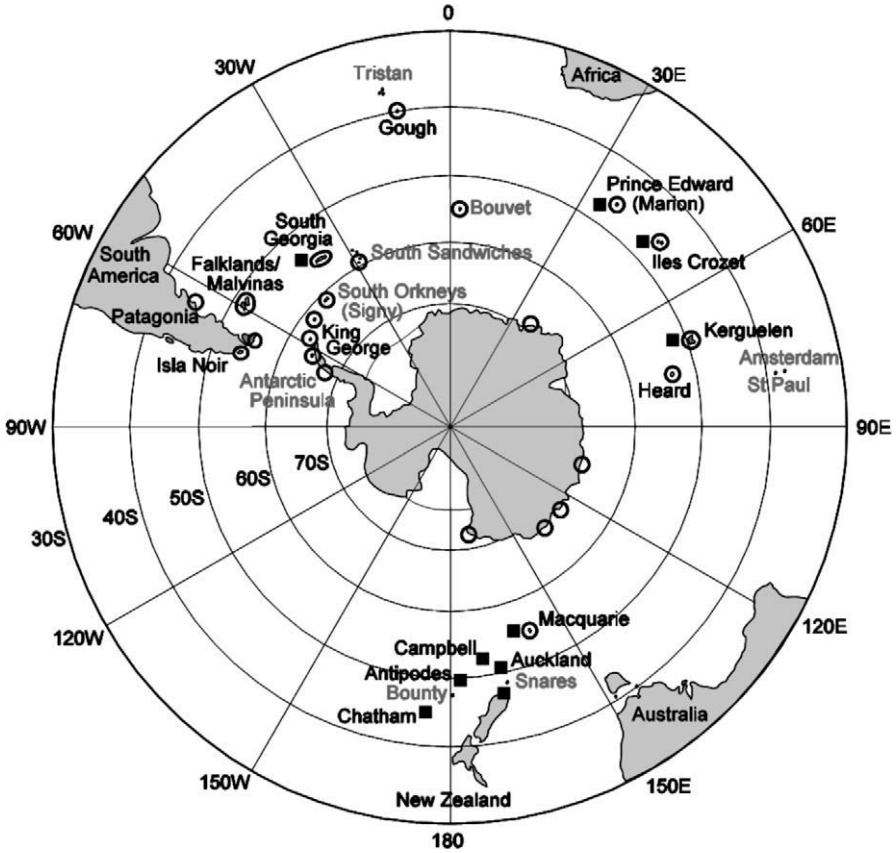

Fig. 1. Map showing the breeding locations of Northern (squares) and Southern Giant Petrels (circles). Sampled colonies are indicated in bold.

Chatham Islands are somewhat distinctive in being relatively small, and often breeding in dark, or even black, plumage, similar to that of juveniles at other locations (Warham, 1990). There has been no attempt until now to resolve these issues genetically.

Both Northern and Southern Giant Petrel are currently considered to be Near Threatened by BirdLife International (www.birdlife.org), the Red List authority for birds for the IUCN. Decreases at some colonies have been attributed to human disturbance and persecution such as extirpation of giant petrels on Tristan following human settlement, as well as reductions in southern elephant seals *Mirounga leonina*, which represent an important source of carrion. A total of 2000–4000 giant petrels are estimated to have been killed by illegal or unregulated Southern Ocean longline fisheries for Patagonian toothfish, *Dissostichus eleginoides*, in 1997–1998 (BirdLife International, 2008). Giant petrels are also killed in many other fisheries worldwide (e.g. Gales et al., 1998; Favero et al., 2003; Gandini and Frere, 2006; Copello and Quintana, 2009b). Identifying the provenance of bycaught birds through genetic means therefore offers considerable potential for better resolving the threats from fisheries to particular populations.

This study investigated the phylogeography of the giant petrels, based on analysis of cytochrome *b* sequences, and microsatellite DNA from blood samples collected during a uniquely comprehensive sampling programme for such widely distributed seabirds. The study aimed to resolve the current ambiguity surrounding their taxonomic status and to investigate geographic genetic variation including fine scale population structure and gene flow. This included an examination of the genetic identity of the contentious populations at Gough, the Falkland/Malvinas Islands and the Chatham Islands.

2. Methods

2.1. Sampling

Blood samples were collected mostly from chicks with the exception of Marion and Gough islands, where non-breeding and breeding adults, respectively, were sampled. Sample locations, number of breeding pairs at locations, and number of samples analysed for cytochrome *b* and microsatellites are given in Table 1. At the Auckland Islands, two colonies were sampled (Enderby to the north and Adams to the south of the main island), which are separated by approximately 40 km. All samples were stored in 96% ethanol at room temperature. Total genomic DNA was extracted using the DNeasy Tissue Extraction Kit (Qiagen) following manufacturer's instructions.

2.2. Cytochrome b amplification and sequencing

Internal primers for the giant petrel cytochrome *b* gene were designed by searching for conserved regions between two published Southern Giant Petrel sequences (Genbank Accession Nos. AF076060 and U48941) and one Northern Giant Petrel (Genbank Accession No. AF076061). Primers for polymerase chain reaction (PCR) were designed using DNAMan version 4.13 (Lynnon BioSoft) and chosen by visual inspection: 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 giant petrel sequence and ending at 1056 bp providing a 935 bp fragment. Thermal cycling was performed using the GeneAmp® PCR System 2700 (Applied Biosystem) under the following conditions: 2 min at 94 °C, 30 cycles at 94 °C for 45 s, 55 °C for 45 s 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 1× reaction buffer (Promega) in a total volume of 20 μl. PCR products were electrophoresed on 2% agarose gels. Size of amplified products was determined using a DNA ladder (Promega 100 bp DNA ladder). Correctly sized bands were excised using a sterile razor blade and purified using the Wizard® SV Gel and PCR Clean-up System (Promega). The purified cytochrome *b* fragment was used as template in 10 μl BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified using Centrisep columns (Princeton)

Table 1
Sampling locations for Northern and Southern Giant Petrels indicating sampling numbers (*N*) for cytochrome *b* (cyt *b*) and microsatellite (microsat) data analyses. Breeding pairs relate to the island groups rather than the single island from which birds were sampled.

Colony	Island Group	Northern Giant Petrel			Southern Giant Petrel			Reference for breeding pairs
		Breeding pairs	cyt *b*	microsats	Breeding pairs	cyt *b*	microsats	
Isla Arce	North Patagonia, Argentina				448	5	20	Quintana et al. (2005, 2006)
Isla Noir	Chile				1000	5	9	BirdLife International (unpubl.)
Isla Gran Robredo	North Patagonia, Argentina				1883	4	20	Quintana et al. (2005, 2006)
Isla Observatorio (Isla de los Estados)	South Patagonia, Argentina				269	4	13	Quintana et al. (2005)
Bird Island	South Georgia	17,000	6	32	8500	5	23	South Georgia Surveys and British Antarctic Survey (unpubl.)
Low Hump	Gough				260	7	20	Cuthbert and Sommer (2004)
George Island	Falklands/Malvinas Islands				19,529	10	30	Reid and Huin (2008)
King George	South Shetland Islands				5409	4	18	Pattersen et al. (2008)
	Heard Island				3600	1		Baker et al. (2002)
Pointe Basse	Iles Crozet	1155–1200	5	25	1100–1250	10	26	Delord et al. (2008)
Marion Island	Price Edward Islands	350	5	25	1759	10	27	Crawford et al. (2003)
	Kerguelen	1450–1800	7	16	4			Weimerskirch et al. (1988)
	Macquarie Island	1400	5	14	2100–2300	9	23	Trebilco et al. (2008)
Forty Fours	Chatham Islands	2000	5	30				Brooke (2004)
	Campbell Islands	230	5	18				Brooke (2004)
Adams and Enderby	Auckland Islands	50	8	13				Brooke (2004)
	Antipodes Islands	320	5	20				Brooke (2004)

and resolved by electrophoresis on an ABI 3130 Genetic Analyser. Sequences were edited using CHROMAS LIGHT 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). Sequences were confirmed as mitochondrial by comparing sequences to the published sequences, translating into amino acids and looking for stop codons and ambiguous sites indicative of heterozygosity (no numts were found).

2.3. Microsatellite genotyping

Six microsatellite loci were used to genotype 229 Southern Giant Petrels and 192 Northern Giant Petrels. Paequ3 and Paequ4 were previously isolated in the White-chinned Petrel (Techow and O'Ryan, 2004), and De11, Dc16, Dc26 and De37 have been characterised in albatross species (Burg, 1999). Thermal cycling was performed using the GeneAmp® PCR System 2700 (Applied Biosystem) under the following conditions: 2 min at 94 °C, 30 cycles at 94 °C for 45 s, T_a (De11, Dc26 at 53 °C; Paequ3, De37 at 55 °C; Paequ4, Dc16 at 58 °C) for 45 s 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, the optimum $MgCl_2$ concentration (1 mM except Paequ3 and De37, which was 1.5 mM), 200 μM of each dNTP, 0.02 U/μl Promega GoTaq® Flexi DNA Polymerase and 1× reaction buffer (Promega) in a total volume of 20 μl. The forward primer of each pair was labelled with fluorescent dye (Hex or Fam). PCR products were electrophoresed on an ABI 373 Sequencer. Each lane contained Rox350 (Applied Biosystems) as standard, and if possible two loci labelled with a different dye were run in one lane. Lanes were analysed using ABI GeneScan Software version 1.2.

2.4. Data analyses for cytochrome b

Basic statistical analyses were performed using DNASP version 4 (Rozas et al., 2003). Genetic diversity was measured using haplotype diversity (h) and nucleotide diversity (π).

2.4.1. Phylogeography

Phylogenetic analysis was performed using the fulmars *Fulmarus glacialis* and *F. glacialoides*, the closest relatives to the giant petrels (Nunn and Stanley, 1998), as outgroups. Trees constructed using Maximum Likelihood (ML) and Bayesian Analysis (BA) were compared for robustness and congruence. A ML tree was constructed using TreePuzzle v. 5.2 (Schmidt et al., 2002) and involved the tree algorithm quartet puzzling (Strimmer and von Haeseler, 1996) implemented in the program. Bootstrapping was performed using 100 replicates. Appropriate outgroups were used and both unrooted and rooted trees inferred. The BA tree was constructed with 13,000,000 generations being computed with a burn-in of 13,000 trees as implemented in MRBAYES v. 3.1.2 (Huelsenbeck and Ronquist, 2001). The evolutionary model chosen for both ML and BA analysis was HKY+G. The model was chosen as having the best-fit to the data using MRMODELTEST 3.06 (Posada and Crandall, 1998). A parsimony network was constructed using TCS v. 1.21 (Clement et al., 2000).

Partitioning of variation was examined using Wright's fixation index (Wright, 1951). Molecular variance analysis (AMOVA—Excoffier et al., 1992) was used to test significance at the hierarchical level within and between taxa, colonies and individuals using ARLEQUIN v. 3.1. Not every possible grouping of colonies could be tested; therefore potential groupings of colonies were investigated using a SAMOVA (Dupanloup et al., 2002; version 1.2.02). Differentiation between groups was tested using F_{ST} and 1000 permutations as well as an exact test (Raymond and Rousset, 1995) using 100,000 Markov chain steps and a burn-in of 10,000 steps.

2.4.2. Estimates of divergence and demographic history

Percent sequence divergence between populations not sharing haplotypes (both uncorrected [π_{xy}] and corrected for sequence variation within populations [δ]) was estimated and used to calculate time since divergence using the equation $t = \delta/r$ (where t is divergence time in years and r is the sequence divergence rate) (Wilson et al., 1985). The general mutation rate for cytochrome b in birds has been estimated at 2% per million years (e.g. Avise et al., 1987; 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). The most recent estimate for cytochrome b in Procellariiformes is 1.89 ± 0.35% per million years (Weir and Schluter, 2008), which was used in this study. Divergence times between clades was estimated through the Bayesian approach as implemented in BEAST version 1.2.1 (Drummond et al., 2002; Drummond and Rambaut, 2003). Searches were 10^7 generations long and sampling was done every 1000 genealogies, discarding the first 10^6. The program TRACER was used to ensure that the search had reached stationary distribution and that the parameter space had been searched efficiently (ESS for each clade always >200).

The historical demography of giant petrels was investigated using mismatch distributions in ARLEQUIN v. 3.1. The model of sudden range expansion (Rogers, 1995) was tested using Harpending's raggedness index r (Harpending, 1994). Fu's test on neutrality (*Fs*—Fu, 1997) was computed to test for deviations from the neutral Wright–Fisher model (Ramos-Onsins and Rozas, 2002). Significance levels were obtained by comparing the test statistics to the distribution of 1000 generated samples under mutation-drift equilibrium and neutrality. In case of a unimodal mismatch distribution and assuming a molecular clock (Wilson et al., 1985), we used the equation $t = \tau/2\mu$ (where τ is the mode of the mismatch distribution, and μ is the mutation rate per year of the mitochondrial fragment, calculated by dividing the divergence rate [1.89% million years^{-1}] by 2 and multiplying by the length of the sequence, 752 bp) to estimate approximate time of expansion (Rogers and Harpending, 1992). The 95% confidence interval around τ was calculated with parametric bootstrapping as implemented in ARLEQUIN v. 3.1.

2.5. Data analyses for microsatellite genotyping

2.5.1. Basic statistics

Linkage disequilibrium, departure from Hardy–Weinberg Equilibrium (HWE) at each locus, expected (H_E) and observed (H_O) heterozygosities as well as allele frequencies and mean number of alleles per locus were tested in GENEPOP v. 4 (Raymond and Rousset, 1995; Rousset, 2007). HWE was tested at each locus via exact tests using a Markov chain algorithm (Guo and Thompson, 1992) in GENEPOP v. 4. Sequential Bonferroni corrections were used to adjust obtained p values for possible type 1 errors (Rice, 1989).

2.5.2. Population genetic structure

Estimates of Wright's F_{ST} (Wright, 1951) and Slatkin's R_{ST} (Slatkin, 1995) were used to determine the degree of colony subdivision within giant petrel species in ARLEQUIN v. 3.1 (Schneider et al., 2000; Excoffier et al., 2005) and R_{ST} CALC (Goodman, 1997), respectively. It has recently been shown that Wright's (1951) G_{ST} and its derivatives such as F_{ST} should not be used as a measure of differentiation (for a review see Jost, 2008) if gene diversity is high. For highly polymorphic loci such as microsatellites, G_{ST} is not monotonic with respect to increasing differentiation and therefore cannot provide information about the actual degree of differentiation of subpopulations. Jost (2008) showed that if within-subpopulation heterozygosity is high and differentiation is at

100%, G_{ST} will incorrectly give differentiation at nearly 0%. Jost (2008) therefore proposed a new differentiation statistic *D*, which was calculated using SMOGD v. 1.2.2 (Crawford, accepted for publication). The assignment test implemented in STRUCTURE version 2.2 (Pritchard et al., 2000) was used as an indirect means to assess population structure and the presence of two species. The method is based on Bayesian inference and depends on the detection of departures from Hardy–Weinberg and linkage equilibria, i.e. detection of a Wahlund-type effect. Tested values of *k* clusters ranged from 1 (one panmictic species) to 20 (the total number of colonies sampled) with 500,000 iterations of Monte Carlo Markov chain after a burn-in period of 50,000 iterations. All simulations were run under correlated allele frequencies and allowing for admixture between clusters. A priori knowledge on the investigated taxa and populations indicate that a hierarchical island model may best describe the situation in giant petrels. Therefore, the most likely number of genetic clusters was additionally calculated after Evanno et al. (2005). Principle component analyses (PCA) were performed at the inter-colony and individual levels with PCA-GEN (Goudet, 1999).

2.5.3. Demographic history

The null hypothesis of no geographical correlation to genetic divergence was tested using a nonparametric Mantel test in GENEPOP v. 4 (Rousset, 2007). Correlations were examined between the logarithm of Euclidean geographical distance in kilometres and $F_{ST}/(1 - F_{ST})$ in (i) all sampled colonies of giant petrels and (ii) within Northern and Southern giant petrels respectively (Rousset, 2007). BOTTLENECK v. 1.2.01 (Cornuet and Luikart, 1996) was used to determine recent bottlenecks at the colony level. All three models were implemented: the infinite alleles model (IAM), the stepwise mutation model (SMM) and the two-phased model of mutation (TPM). Multi-step changes were estimated to occur at a frequency of 30% as suggested by Cornuet and Luikart (1996). A Wilcoxon sign rank test (Luikart and Cornuet, 1998) was used to ascertain whether a colony showed a significant number of loci with a heterozygosity excess. Further, the qualitative descriptor of allele frequency distributions, the mode-shift indicator, was used as implemented in BOTTLENECK. This indicator discriminates between stable (normal L-shaped) and bottlenecked (shifted) populations.

3. Results

3.1. Cytochrome b molecular analyses

3.1.1. Basic statistics

We compared a 752 bp fragment of the cytochrome *b* gene from 125 giant petrels (74 Southern Giant Petrels and 51 Northern Giant Petrels). Over all sequences 23 polymorphic sites with 24 mutations were identified with 16 parsimony informative sites comprising 22 haplotypes (Table 2; Genbank Accession Nos. GQ120455–GQ120476). No insertions or deletions were observed. The transition to transversion ratio was 22:2 with both transversions amongst Northern Giant Petrels. Nineteen mutations were synonymous and five were non-synonymous with most substitutions located 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%). Haplotype diversity (h) was similar for the Southern (0.78 ± 0.0014) and Northern Giant Petrels (0.73 ± 0.05). This is reflected in the number of haplotypes found in both species, with 13 in the Southern Giant Petrel and nine in the Northern Giant Petrel. Nucleotide diversity (π) was 0.005 ± 0.0006 for the Southern Giant Petrel and 0.002 ± 0.0003 for the Northern Giant Petrel. Sequence divergence within Southern Giant Petrels (0.5%) was greater than within Northern Giant Petrels (0.2%), despite a similar number of haplotypes. Sequence divergence between the two species was 0.42% (corrected) with only one fixed mutational difference (Fig. 2). There was one shared haplotype (Table 2). A supposed Southern Giant Petrel from Marion Island was found to have the most common haplotype found in the Northern Giant Petrel (ngp1). At Marion Island, both species breed sympatrically and hybridisation has been reported (Cooper et al., 2001). Microsatellite data indicated that this individual had mixed ancestry, with a 30% probability of being a Southern Giant Petrel. Furthermore, the possibility remains that this individual, caught as a non-breeding adult, was assigned to the wrong species at the time of sampling. It was therefore excluded from analyses.

3.1.2. Phylogeography

The Northern Giant Petrel network (Fig. 2) is simple with most haplotypes connected through one mutational difference, and two abundant haplotypes (ngp1 and ngp4). The Southern Giant Petrel haplotypes on the other hand formed a more complex network. One clade separated by three mutational differences contained three haplotypes found exclusively on Marion Island, Iles Crozet and Macquarie Island in the southern Indian Ocean. The other clade contains the remaining haplotypes found in all other colonies including three haplotypes found on Iles Crozet only (sgpCr1, sgpCr2 and sgpCr3) and one haplotype found on both Iles Crozet and Marion Island (sgp3). Northern Giant Petrels formed a monophyletic clade, whereas the Southern Giant Petrel was paraphyletic.

Within the Southern Giant Petrel (Table 3) θ_{ST} values confirmed the relationships shown within trees. Birds from Gough Island differed significantly from all other colonies. Similarly, Macquarie Island was genetically distinct from all colonies except Iles Crozet. The network showed several haplotypes found on Iles Crozet amongst the other clade, which is supported by non-significant differentiation of the Iles Crozet colony to Isla Observatorio (Isla de los Estados) and Marion Island. All other pairwise comparisons did not differ significantly from zero. Within the Northern Giant Petrel, θ_{ST} values (Table 3) did not show any differentiation between the two colonies at the Auckland Islands and therefore they were combined in analysis. θ_{ST} did indicate some differentiation between the other colonies, mainly between colonies in different ocean basins, with the exception of Chatham Islands and Auckland Islands, which were differentiated.

Analysis of molecular variance (AMOVA) showed that 52.7% ($F_{ST} = 0.52$, $p < 0.0001$) of variance could be explained by the species grouping. No obvious populations were suggested by either the trees or network 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%). However, SAMOVA analysis indicated that the variance component could be increased ($F_{CT} = 0.39$) when splitting the Northern Giant Petrels into three groupings. This division treated South Georgia as one population, Chatham Island and Kerguelen as a second, and the remainder of colonies as a third population. Within Southern Giant Petrels, the two clades result in a greater proportion (51.4%) of variance between colonies ($F_{ST} = 0.51$, $p < 0.00001$). 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.

3.1.3. Estimates of divergence and demographic history

The three major phylogroups found within the giant petrels (Fig. 3) diverged approximately 0.8 mya. Corrected percent sequence divergence values suggested that speciation of Northern and Southern Giant Petrels did not finalise before 0.2 mya,

Table 2
Variable sites in the cytochrome *b* fragment for the Northern and Southern Giant Petrels and their originating colonies. Nucleotide positions are in relation to the published sequence (Genbank Accession No. 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 Islands (Ca), Kerguelen (K), Gough (Gou), Patagonia (Pat), Iles Crozet (Cr), Falklands/Malvinas Islands (Fa) and Macquarie Island (Mac). The highlighted nucleotide position shows the fixed difference between the two species. Haplotypes have been submitted to Genbank Accession Nos. GQ120455–GQ120476.

Haplotype	Nucleotide position																							Total *n*	Colonies sharing haplotype
	1	2	2	2	2	2	2	3	3	4	5	5	5	7	7	7	7	8	8	8	8	8	9		
	8	2	3	4	7	7	9	5	5	6	0	7	8	0	0	3	8	2	6	7	8	9	2		
	8	8	1	0	0	9	4	2	4	5	1	9	2	0	9	2	6	2	1	0	7	7	7		
	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)																									
ngp1	C	T	T	C	C	C	T	A	T	C	G	T	C	A	G	T	G	T	T	T	C	A	C	23	sMarion Island, Marion Island, Iles Crozet, Kerguelen, Adams, Enderby, Macquarie, Campbell, Antipodes, South Georgia
ngp2	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	3	Adams, Enderby
ngp3	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	T	4	Kerguelen, Chatham, South Georgia
ngp4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	..	.	.	.	T	15	Antipodes, Chatham, Macquarie, South Georgia, Marion Island, Iles Crozet, Kerguelen
ngpCh	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	T	1	Chatham
ngpSG	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	G	T	3	South Georgia
ngpMar	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	1	Marion Island
ngpCa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	1	Campbell
ngpK	G	.	.	.	.	.	.	.	.	T	A	.	.	.	A	.	.	.	.	.	.	G	T	1	Kerguelen
Macronectes giganteus (Southern Giant Petrel)																									
sgp1	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	30	Isla de los Estados, Isla Arce, Isla Noir, Gran Robredo, South Georgia, Falklands/ Malvinas, King George, Heard
sgpPat1	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	.	4	Isla Arce, Gran Robredo
sgpGou	.	.	.	.	T	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	T	7	Gough
sgp2	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	C	.	C	.	.	.	.	T	14	Macquarie, Iles Crozet
sgpMac	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	1	Macquarie
sgpPat2	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	T	1	Isla de los Estados
sgpSG	.	.	C	.	T	.	.	.	C	.	.	.	.	G	.	.	A	C	.	.	.	.	T	1	South Georgia
sgpMar	.	C	.	.	.	T	.	G	.	.	.	.	.	.	.	C	.	C	.	C	.	.	T	3	Marion Island
sgp3	.	.	C	.	T	.	C	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	7	Marion Island, Iles Crozet
sgpFa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	2	Falklands/Malvinas
sgpCr1	.	.	.	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	.	.	T	1	Iles Crozet
sgpCr2	.	.	.	.	T	.	.	.	C	.	.	.	.	G	.	.	.	C	.	.	T	.	T	1	Iles Crozet
sgpCr3	.	.	C	T	T	.	C	.	C	.	.	.	.	G	.	.	.	C	C	.	.	.	T	1	Iles Crozet

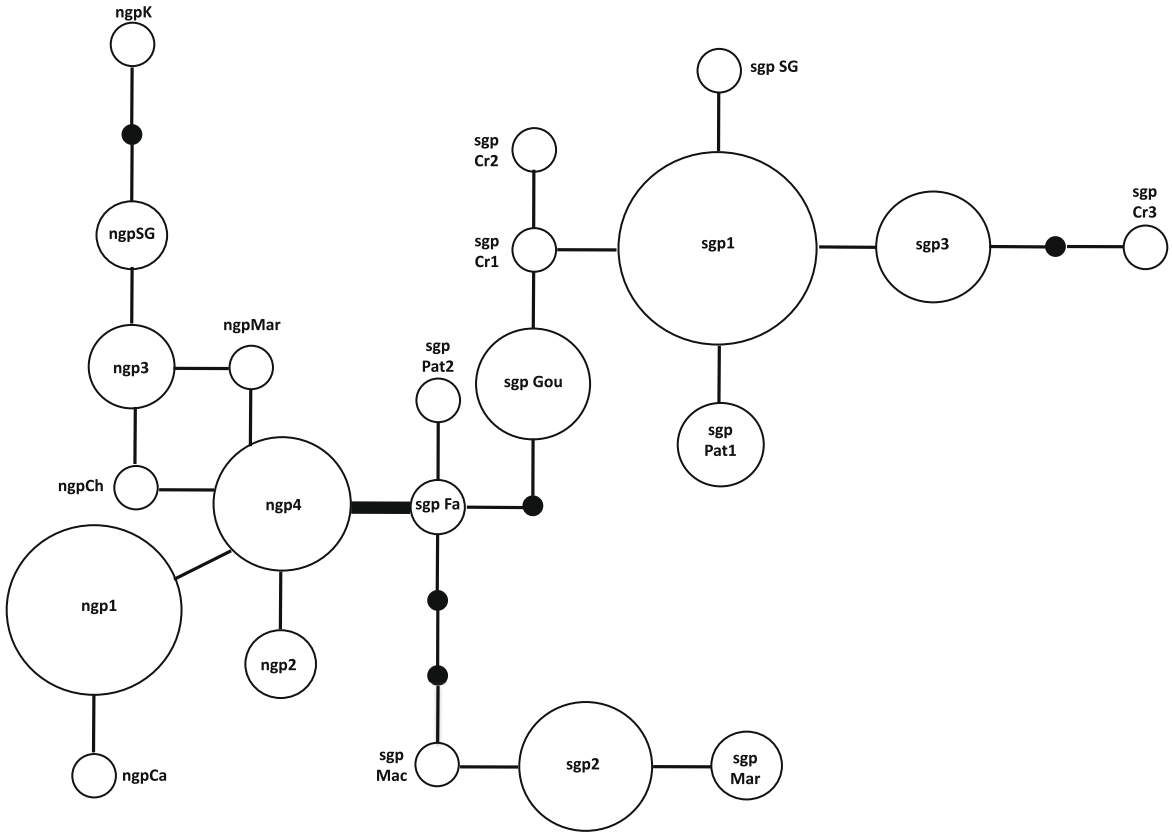

Fig. 2. TCS Network showing cytochrome *b* haplotypes found within the Southern and Northern Giant Petrels. For a key to haplotypes see Table 2. Sizes of circles represent the number of individuals sharing that haplotype, black small circles represent missing haplotypes. The line connecting haplotypes of the two species of giant petrels is represented in bold.

although colonies in different ocean basins seem to have been separated for up to 0.5 my (Table 3).

Fig. 4 shows mismatch distributions for both species and different ocean basins (Gough Island was excluded as the population only contains one haplotype). The Northern Giant Petrel fits the model of a population expansion/fragmentation with both SSD and Harpending's Raggedness Index, but does not deviate from Fu's Fs statistic of neutrality. The Tau value shows a time of expansion of 1.45 units of mutational time, which equates to c. 0.11 mya (0–0.2 mya). The Southern Giant Petrel also fits the model of expansion using SSD and *r* and with a non-significant negative Fs statistic. However, the calculated Tau value indicates an earlier expansion/fragmentation at 9.27 units of mutational time, some 0.7 mya (0–4.4 mya). Similarly ocean basins for both species all fit the model of a population expansion/fragmentation with both SSD and Harpending's Raggedness Index, but did not deviate from Fu's Fs statistic of neutrality. Time estimates had extremely wide confidence intervals. Time estimates for the south Atlantic and south Pacific were around 0.15–0.27 mya; the south Indian Ocean showed an older demographic change for the Southern Giant Petrel populations (0.7 mya [0.1–1.2 mya]) and the most recent for the Northern Giant Petrel (0.07 [0.02–0.17]).

3.2. Microsatellite genetic analyses

3.2.1. Basic statistics

The six microsatellite loci were polymorphic in samples from all nine Northern Giant Petrel colonies and nine of the Southern Giant Petrel colonies (Dc26 monomorphic in Gough, and Paequ3 monomorphic in Isla Observatorio (Isla de los Estados); Supplementary material). Even after sequential Bonferroni corrections, several loci deviated from Hardy–Weinberg expectations (Northern Giant Petrel: Dc26 Iles Crozet, Paequ4 Iles Crozet and Chatham Islands, Paequ3 Campbell Islands; Southern Giant Petrel: Dc16 Isla Gran Robredo, Dc26 South Georgia, Falklands/Malvinas, Isla Gran Robredo, Iles Crozet, Macquarie Island, Paequ4 Gough, Macquarie Island, De11 Macquarie Island, De37 Gough, Paequ3 Falklands/Mavinas, Gough, Iles Crozet, Marion Island; Supplementary material), although no single locus deviated for all colonies. More Northern than Southern Giant Petrel colonies were in Hardy–Weinberg equilibrium. No evidence of linkage disequilibrium was shown between loci comparisons across all colonies and genetic independence was thus assumed. Sixty alleles were found in both species, with 49 in Northern Giant Petrels and 51 in Southern Giant Petrels, 11 species-specific alleles in Southern Giant Petrels and 8 species-specific alleles in Northern Giant Petrels.

3.2.2. Population structure

F_{ST} values (Table 4) were significantly different from zero between most colonies except some that were geographically close (e.g. Northern Giant Petrel colonies at the Auckland and Antipodes Islands, and Marion Island and Kerguelen, respectively). R_{ST} values (Table 4) showed that Northern Giant Petrels breeding on Macquarie Island were significantly different from all other Northern Giant Petrels, and Southern Giant Petrels breeding on Gough were very different from all other populations. *D* values showed a much higher level of differentiation much more closely resembling θ_{ST} as shown by cytochrome *b*. *D* values also more closely resembled the pattern of differentiation as shown by θ_{ST}.

The cluster based assignment test (Pritchard et al., 2000) gave the best-fit for $k = 6$ clusters (Fig. 5; Supplementary material) with higher *K*s not much less likely but with higher variance. When *k*

Table 3
Estimates of θ_{ST} (upper number above diagonal), t (lower number above diagonal) for colonies/populations not sharing haplotypes and δ (below diagonal) for global giant petrel colonies using cytochrome *b*. Values in bold are significant at $p < 0.05$. Heard Island was excluded as only one individual was sequenced.

	nSouth Georgia	nIles Crozet	nMarion	Kerguelen	nMacquarie	Chatham	Campbell	Adams	Enderby	Antipodes	Isla Arce	Isla Noir	Gran Robredo	Isla de los Estados	sSouth Georgia	Gough	Falklands	King George	sMarion	sIles Crozet	sMacquarie
nSouth Georgia		**0.41**	0.34	0.06	**0.5**	0.03	**0.56**	**0.43**	**0.43**	0.44	**0.81** 419,810	**0.84** 422,155	**0.8** 422,155	**0.65** 281,436	**0.82** 422,155	**0.82** 281,436	**0.73** 315,834	**0.83** 422,155	**0.47** 265,020	**0.48** 265,801	**0.87** 406,519
nIles Crozet	0.12		0.08	0	0.41	0.33	**0.6**	0.25	0.25	0.11	**0.91** 347,574	**0.96** 351,796	**0.91** 349,450	**0.7** 211,077	**0.93** 351,796	**0.95** 211,077	**0.76** 245,475	**0.96** 351,796	**0.39** 194,191	**0.41** 195,442	**0.94** 336,160
nMarion	0.11	0.01		0.04	0	0.33	0.13	0	0	0	**0.86** 346,167	**0.91** 372,903	**0.85** 342,415	**0.67** 232,185	**0.88** 372,903	**0.89** 232,185	**0.74** 266,583	**0.9** 372,903	**0.4** 209,670	**0.43** 216,550	**0.91** 357,268
Kerguelen	0.02	0.01	0.01		0.23	0	**0.35**	0.15	0.15	0.11	**0.77** 357,491	**0.8** 358,497	**0.76** 360,172	**0.58** 217,778	**0.78** 358,497	**0.75** 217,778	**0.68** 252,176	**0.78** 358,497	**0.41** 201,696	**0.42** 202,143	**0.83** 342,861
nMacquarie	**0.18**	0.04	−0.01	0.06		**0.56**	0	0	0	0	**0.91** 356,017	**0.97** 394,011	**0.91** 349,450	**0.74** 253,293	**0.93** 394,011	**0.96** 253,293	**0.78** 287,691	**0.96** 394,011	**0.43** 227,964	**0.46** 237,658	**0.95** 378,376
Chatham	0.01	0.05	0.07	−0.01	**0.13**		**0.64**	**0.45**	**0.45**	**0.44**	**0.86** 386,975	**0.9** 379,939	**0.85** 391,666	**0.66** 239,221	**0.87** 379,939	**0.88** 239,221	**0.74** 273,619	**0.89** 379,939	**0.42** 225,149	**0.43** 223,586	**0.91** 364,304
Campbell	0.22	0.08	0.01	0.11	0	**0.19**		0.03	0.03	0.17	**0.91** 372,903	**0.97** 422,155	**0.91** 363,522	**0.76** 281,436	**0.94** 422,155	**0.96** 281,436	**0.8** 315,834	**0.96** 422,155	**0.46** 249,071	**0.49** 265,801	**0.95** 406,519
Adams	0.17	0.027	−0.013	0.05	−0.013	**0.12**	0		0	0	**0.87** 351,796	**0.93** 386,975	**0.86** 345,933	**0.67** 246,257	**0.89** 386,975	**0.91** 246,257	0.75 **280655**	**0.92** 386,975	**0.39** 221,631	**0.42** 230,622	**0.92** 371,340
Enderby	0.16	0.02	0	0.04	0.02	0.1	0.04	−0.02		0	**0.87** 354,141	**0.93** 375,248	**0.86** 351,796	**0.67** 234,530	**0.89** 375,248	**0.91** 234,530	**0.75** 268,928	**0.92** 375,248	**0.39** 213,422	**0.42** 218,895	**0.92** 359,613
Antipodes	**0.15**	0.008	−0.02	0.03	−0.008	0.09	0.013	−0.01	0.004		**0.89** 346,167	**0.95** 372,903	**0.89** 342,415	**0.7** 232,185	**0.91** 372,903	**0.93** 232,185	**0.76** 266,583	**0.94** 372,903	**0.41** 209,670	**0.44** 216,550	**0.94** 357,268
Isla Arce	**0.79**	**0.66**	**0.65**	**0.68**	**0.67**	**0.73**	**0.7**	**0.66**	**0.67**	**0.65**		0.25	0	0.1	0.17	**0.9** 147,754	0.11	0.19	0.2 85,838	**0.44** 216,550	**0.96** 554,274
Isla Noir	**0.8**	**0.66**	**0.7**	**0.68**	**0.74**	**0.72**	**0.8**	**0.73**	**0.71**	**0.7**	0.01		0.39	0.06	0	**1** 140,718	0.01	0	0.2 84,431	**0.44** 209,514	**0.98** 547,238
Gran Robredo	**0.8**	**0.66**	**0.65**	**0.68**	**0.66**	**0.74**	**0.69**	**0.65**	**0.66**	**0.65**	−0.02	0.02		0.1	0.25	**0.91** 152,445	0.14	0.33	0.17 89,122	**0.41** 221,240	**0.96** 558,964
Isla de los Estados	**0.53**	**0.4**	**0.44**	**0.41**	**0.48**	**0.45**	**0.53**	**0.47**	**0.44**	**0.44**	0.01	0	0.02		0.04	**0.58** 70,359	0	0	0.03 28,144	0.25 118,047	**0.88** 406,519
sSouth Georgia	**0.8**	**0.66**	**0.7**	**0.68**	**0.74**	**0.72**	**0.8**	**0.73**	**0.71**	**0.7**	0.01	0	0.02	0		**0.93** 140,718	0.03	0	0.19 84,431	**0.43** 209,514	**0.97** 547,238
Gough	**0.53**	**0.4**	**0.44**	**0.41**	**0.48**	**0.45**	**0.53**	**0.47**	**0.44**	**0.44**	**0.28**	**0.27**	**0.29**	**0.13**	**0.27**		**0.6** 90,685	**1** 140,718	**0.31** 112,575	**0.41** 153,227	**0.98** 406,519
Falklands	**0.6**	**0.46**	**0.5**	**0.48**	**0.54**	**0.52**	**0.6**	**0.53**	**0.51**	**0.5**	0.03	0.01	0.03	−0.04	0.01	**0.17**		0	0.16 45,655	**0.39** 142,595	**0.88** 440,917
King George	**0.8**	**0.66**	**0.7**	**0.68**	**0.74**	**0.72**	**0.8**	**0.73**	**0.71**	**0.7**	0.01	0	0.02	0	0	**0.27**	0.012		0.16 84,431	**0.4** 209,514	**0.98** 547,238
sMarion	**0.5**	**0.37**	**0.4**	**0.38**	**0.43**	**0.43**	**0.47**	**0.42**	0.4	0.4	0.16	0.16	0.17	0.05	0.16	0.21	0.09	0.16		0.07	**0.54**
sIles Crozet	**0.5**	**0.37**	**0.41**	**0.38**	**0.45**	**0.42**	**0.5**	0.44	**0.41**	**0.41**	0.41	0.4	0.42	0.22	0.4	**0.29**	**0.27**	0.4	0.06		0.24
sMacquarie	**0.77**	**0.64**	**0.68**	**0.65**	**0.72**	**0.69**	**0.77**	**0.7**	**0.68**	**0.68**	**1.05**	**1.03**	**1.06**	**0.77**	**1.03**	**0.77**	**0.83**	1.03	**0.46**	0.12	

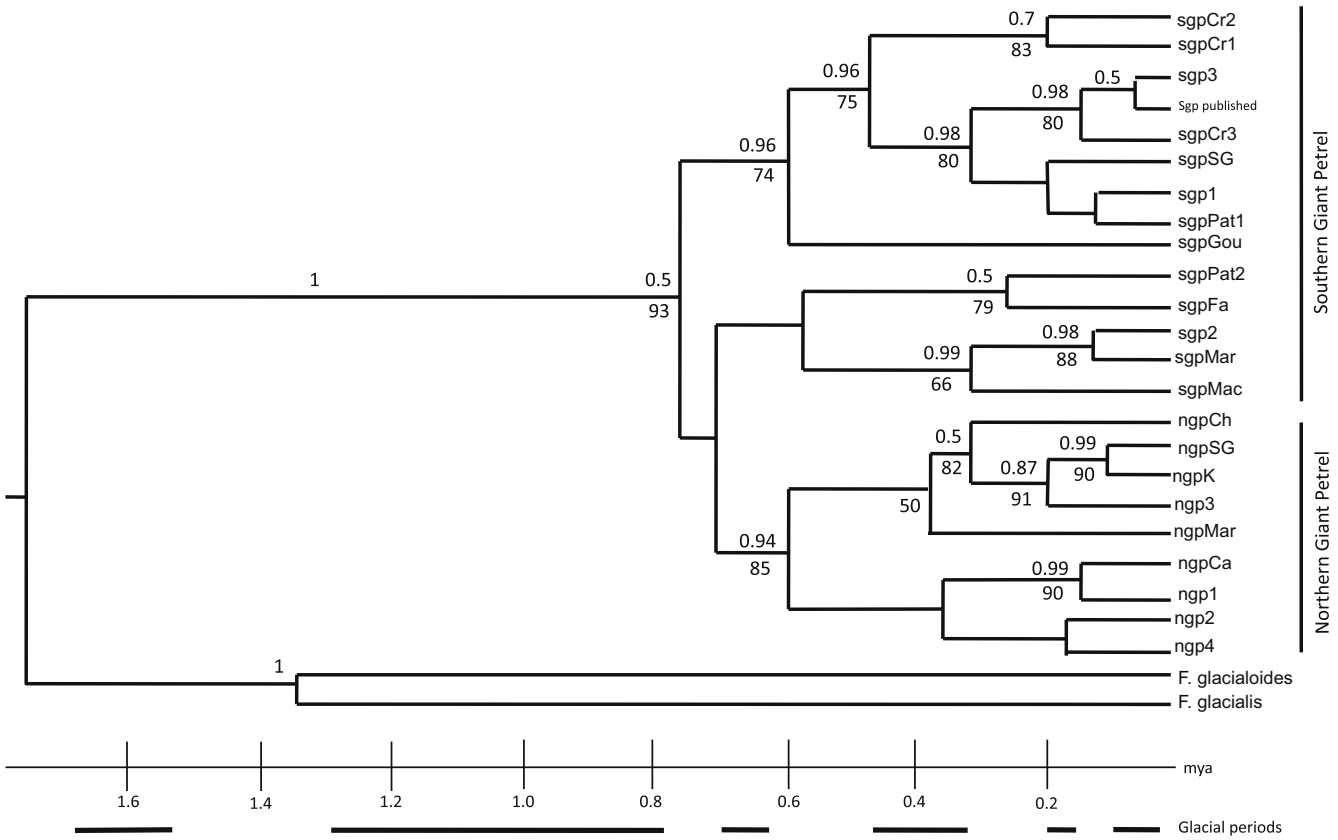

Fig. 3. Bayesian Inference tree drawn from giant petrel cytochrome *b* haplotypes and two fulmar species as outgroups. Clade divergence times are indicated to the right of clades in million years. Probability support for braches (>0.5) is indicated to the left of nodes.

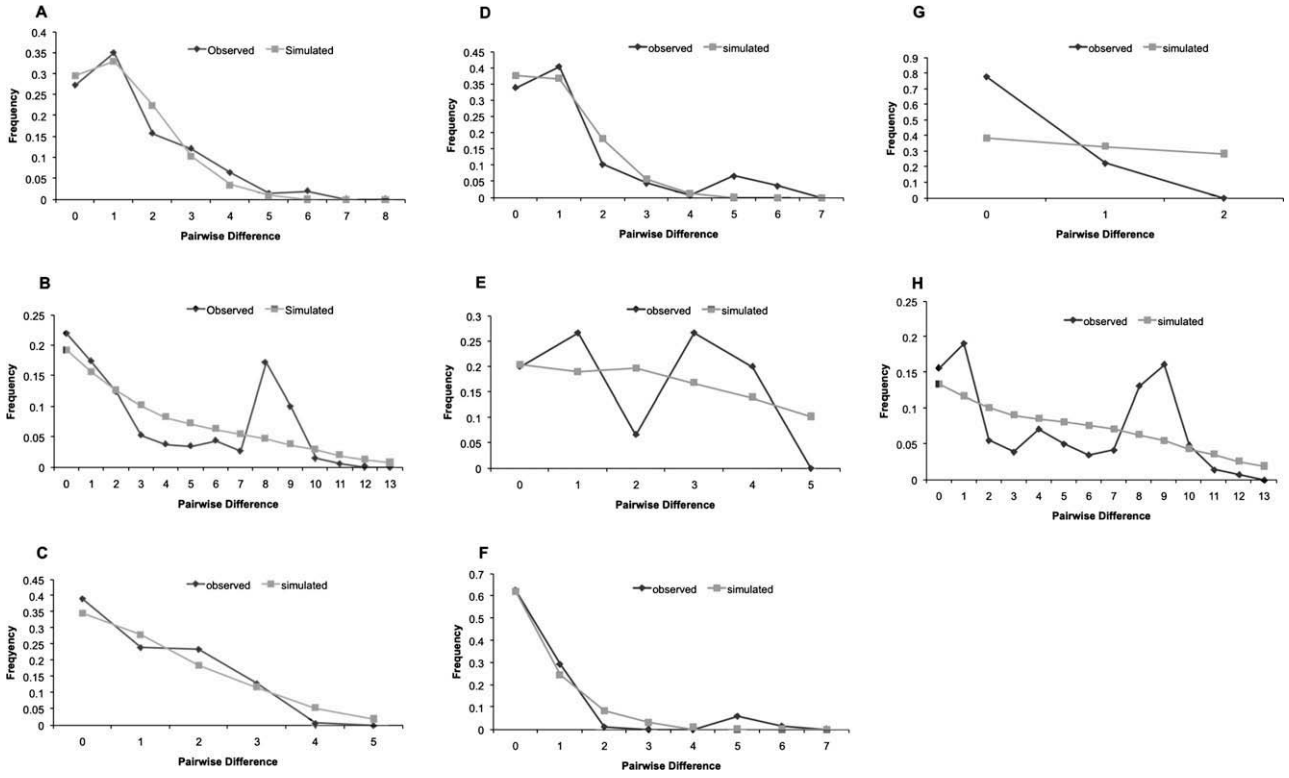

Fig. 4. Mismatch distributions computed from cytochrome *b* for Northern (A), Southern Giant Petrels (B), Northern Giant Petrel colonies in the south Pacific (C), Northern Giant Petrel colonies in the south Indian (D), Northern Giant Petrel colonies in the south Atlantic (E), Southern Giant Petrel colonies in the South Atlantic (F), Southern Giant Petrel colonies in the south Pacific (G) and Southern Giant Petrel colonies in the south Indian (H). All distributions indicate past population size changes and/or geographic fragmentation.

Table 4
R_{ST} (above diagonal), F_{ST} (upper number below diagonal) and $D_{estimated}$ (lower number below diagonal, italic) values for global Giant Petrel colonies using microsatellites. Values that are significant at $p < 0.05$ for R_{ST} and F_{ST} are indicated in bold.

	nSouth Georgia	nIles Crozet	nMarion	Kerguelen	nMacquarie	Chatham	Campbell	Adams	Enderby	Antipodes	Isla Arce	Isla Noir	Gran Robredo	Isla de los Estados	sSouth Georgia	Gough	Falklands	King George	sMarion	sIles Crozet	sMacquarie
nSouth Georgia		**0.1**	0.02	0.02	**0.24**	**0.06**	0	**0.27**	**0.11**	**0.08**	−0.01	**0.17**	**0.12**	**0.28**	**0.07**	**0.28**	**0.04**	**0.09**	**0.06**	**0.08**	**0.06**
nIles Crozet	**0.04** *0.05*		**0.09**	**0.08**	**0.36**	**0.04**	0.03	**0.35**	0.04	**0.16**	**0.08**	**0.14**	**0.05**	**0.25**	**0.1**	**0.34**	**0.11**	**0.09**	**0.1**	**0.05**	**0.09**
nMarion	**0.18** *0.3*	**0.12** *0.26*		−0.02	**0.09**	**0.09**	−0.03	0.14	0.02	**0.06**	−0.05	**0.07**	**0.07**	**0.18**	0.02	**0.24**	0.01	**0.06**	0.02	**0.04**	0.02
Kerguelen	**0.2** *0.29*	**0.15** *0.3*	0 *0.05*		**0.2**	**0.03**	−0.04	0	0.02	0.02	−0.01	0.04	**0.07**	**0.15**	0.01	**0.22**	0.01	0.01	−0.01	0.01	0
nMacquarie	**0.19** *0.31*	**0.12** *0.26*	**0.06** *0.15*	**0.09** *0.19*		**0.37**	**0.21**	**0.19**	**0.18**	**0.32**	**0.19**	**0.34**	**0.32**	**0.43**	**0.24**	**0.47**	**0.24**	**0.33**	**0.23**	**0.26**	**0.26**
Chatham	**0.12** *0.18*	**0.07** *0.12*	**0.1** *0.21*	**0.13** *0.26*	**0.08** *0.19*		**0.03**	**0.31**	**0.11**	**0.03**	0.01	**0.06**	**0.1**	**0.21**	**0.1**	**0.22**	**0.09**	0.01	**0.07**	**0.07**	**0.06**
Campbell	**0.22** *0.36*	**0.11** *0.28*	**0.03** *0.03*	**0.07** *0.1*	**0.05** *0.12*	**0.09** *0.2*		0.08	0	**0.06**	−0.01	0.02	**0.07**	**0.13**	0.01	**0.29**	**0.04**	**0.04**	0.02	0	0.01
Adams	**0.18** *0.34*	**0.1** *0.27*	0 *0*	0.01 *0.05*	0.02 *0.06*	**0.1** *0.21*	0.05 *0*		0.16	0.06	0.03	−0.04	**0.3**	0.18	**0.19**	**0.33**	**0.22**	0.07	0.08	**0.23**	0.11
Enderby	**0.25** *0.39*	**0.15** *0.3*	**0.06** *0.07*	**0.09** *0.17*	**0.06** *0.15*	**0.14** *0.26*	0.05 *0.02*	0 *0*		**0.14**	0.03	0.05	0.01	**0.17**	0.01	**0.35**	0.02	0.05	0.01	−0.03	0.02
Antipodes	**0.23** *0.32*	**0.17** *0.32*	**0.04** *0.09*	**0.04** *0.06*	**0.12** *0.23*	**0.11** *0.2*	**0.07** *0.1*	0.06 *0.11*	**0.11** *0.18*		0.03	**0.06**	**0.18**	**0.2**	**0.11**	**0.19**	**0.1**	0.03	**0.06**	**0.1**	**0.07**
Isla Arce	**0.29** *0.48*	**0.27** *0.47*	**0.12** *0.25*	**0.19** *0.29*	**0.15** *0.28*	**0.23** *0.45*	**0.24** *0.27*	**0.15** *0.17*	**0.27** *0.32*	**0.24** *0.32*		0.06	**0.06**	**0.14**	0	**0.24**	0	0.03	0.01	0.02	0
Isla Noir	**0.26** *0.44*	**0.17** *0.34*	**0.06** *0.18*	**0.12** *0.23*	**0.1** *0.25*	**0.13** *0.32*	**0.13** *0.23*	0.05 *0.16*	**0.12** *0.25*	**0.15** *0.23*	**0.23** *0.35*		**0.12**	−0.02	**0.05**	**0.36**	**0.08**	−0.04	0	0.05	0.03
Gran Robredo	**0.27** *0.39*	**0.25** *0.38*	**0.17** *0.29*	**0.25** *0.43*	**0.2** *0.36*	**0.26** *0.43*	**0.27** *0.36*	**0.14** *0.24*	**0.2** *0.28*	**0.28** *0.43*	**0.21** *0.33*	**0.24** *0.36*		**0.18**	0.01	**0.36**	**0.04**	**0.05**	**0.03**	0	0.02
Isla de los Estados	**0.3** *0.41*	**0.23** *0.32*	**0.1** *0.18*	**0.14** *0.22*	**0.14** *0.26*	**0.18** *0.3*	**0.16** *0.23*	**0.11** *0.15*	**0.19** *0.25*	**0.17** *0.23*	**0.26** *0.36*	0 *0*	**0.3** *0.39*		**0.13**	**0.48**	**0.17**	0.03	**0.07**	**0.15**	**0.09**
sSouth Georgia	**0.19** *0.29*	**0.19** *0.29*	**0.15** *0.31*	**0.19** *0.4*	**0.16** *0.29*	**0.18** *0.31*	**0.25** *0.38*	**0.11** *0.25*	**0.21** *0.34*	**0.24** *0.43*	**0.17** *0.31*	**0.18** *0.31*	**0.06** *0.12*	**0.24** *0.3*		**0.3**	−0.01	0.02	−0.02	0.01	−0.01
Gough	**0.29** *0.53*	**0.29** *0.57*	**0.22** *0.55*	**0.28** *0.62*	**0.29** *0.56*	**0.26** *0.45*	**0.38** *0.68*	**0.23** *0.54*	**0.29** *0.53*	**0.33** *0.63*	**0.28** *0.54*	**0.28** *0.56*	**0.24** *0.42*	**0.33** *0.56*	**0.15** *0.31*		**0.26**	**0.2**	**0.21**	**0.25**	**0.26**
Falklands	**0.19** *0.3*	**0.17** *0.27*	**0.11** *0.22*	**0.15** *0.34*	**0.12** *0.23*	**0.17** *0.31*	**0.19** *0.27*	**0.07** *0.14*	**0.14** *0.21*	**0.18** *0.32*	**0.15** *0.24*	**0.15** *0.28*	**0.06** *0.11*	**0.21** *0.29*	**0.04** *0.07*	**0.18** *0.3*		0.05	−0.01	0	0.01
King George	**0.25** *0.43*	**0.21** *0.42*	**0.07** *0.22*	**0.12** *0.25*	**0.07** *0.16*	**0.14** *0.27*	**0.18** *0.27*	**0.07** *0.19*	**0.15** *0.27*	**0.18** *0.29*	**0.1** *0.21*	**0.08** *0.15*	**0.17** *0.33*	**0.1** *0.14*	**0.12** *0.2*	**0.16** *0.32*	**0.11** *0.24*		0.01	0.02	−0.01
sMarion	**0.24** *0.43*	**0.22** *0.4*	**0.09** *0.19*	**0.09** *0.21*	**0.08** *0.15*	**0.16** *0.32*	**0.18** *0.23*	0.04 *0.05*	**0.14** *0.17*	**0.17** *0.28*	**0.09** *0.15*	**0.09** *0.2*	**0.15** *0.27*	**0.14** *0.22*	**0.11** *0.21*	**0.16** *0.35*	**0.09** *0.19*	**0.03** *0.07*		−0.01	−0.02
sIles Crozet	**0.12** *0.23*	**0.08** *0.17*	**0.09** *0.27*	**0.07** *0.28*	**0.1** *0.28*	**0.09** *0.21*	**0.13** *0.32*	0.02 *0.21*	**0.1** *0.23*	**0.13** *0.34*	**0.18** *0.37*	**0.1** *0.31*	**0.14** *0.29*	**0.17** *0.3*	**0.08** *0.19*	**0.14** *0.37*	**0.07** *0.19*	**0.11** *0.29*	**0.09** *0.24*		0.01
sMacquarie	**0.18** *0.28*	**0.16** *0.28*	**0.14** *0.32*	**0.16** *0.39*	**0.16** *0.34*	**0.16** *0.29*	**0.23** *0.39*	**0.08** *0.25*	**0.18** *0.34*	**0.2** *0.37*	**0.22** *0.4*	**0.15** *0.29*	**0.1** *0.14*	**0.21** *0.28*	**0.03** *0.05*	**0.17** *0.37*	**0.06** *0.13*	**0.13** *0.25*	**0.12** *0.26*	**0.05** *0.19*	

was set to two, probabilities of belonging to either cluster were much higher. All Northern Giant Petrel colonies were assigned to one cluster and nine Southern Giant Petrel colonies assigned to the second cluster. Two Southern Giant Petrel colonies in southern South America (Isla Observatorio (Isla de los Estados) and Isla Noir) had equal probabilities of being assigned to either cluster. Delta *K* found that $k = 3$ is the most likely setting: (i) Northern Giant Petrel colonies South Georgia, Iles Crozet, Chatham Islands. (ii) Southern Giant Petrel colonies South Georgia, Falklands/Malvinas Islands, Isla Gran Robredo, Gough, Iles Crozet and Macquarie Island and (iii) Northern Giant Petrel colonies Marion Island, Kerguelen, Campbell Island, Auckland Islands, Antipodes, Macquarie Island and Southern Giant Petrel colonies Isla Observatorio (Isla de los Estados), Isla Arce, Isla Noir, King George Island and Marion Island. At $k = 4$ the third cluster split into Northern and Southern Giant Petrel colonies with Isla Observatorio (Isla de los Estados) and Isla Noir having equal probability belonging to either cluster. When each species was investigated separately, the Northern Giant Petrel best-fit $k = 2$ with South Georgia (97%), Iles Crozet (83%) and Chatham Islands (62%) in one cluster, and the remaining colonies assigned to a second cluster (all >91% except Macquarie Island 85%). Southern Giant Petrel colonies best-fit $k = 4$, but $k = 3$ gave higher assignment probabilities: South Georgia (68%), Falklands/Malvinas Islands (68%), Isla Gran Robredo (89%), and Macquarie Island (54%) in one cluster, Gough (77%) and Iles Crozet (63%) in a second cluster, and the remaining colonies (Isla Observatorio (Isla de los Estados) and Isla Arce 89%, Isla Noir 79%, King George Island 73% and Marion Island 69%) in the third (which was divided at $k = 4$). The two Northern Giant Petrel clusters in addition to four Southern Giant Petrel clusters were recovered by PCA (Fig. 6). The four Southern Giant Petrel clusters differed to the Structure clusters by grouping Gough with Macquarie Island, South Georgia, Falklands/Malvinas Islands and Isla Gran Robredo, whereas Iles Crozet was separated. Closer investigation of Southern Giant Petrels from Iles Crozet indicated some birds had a higher probability of belonging to a Northern Giant Petrel cluster, with the remainder having a probability of >95% of belonging to Southern Giant Petrels.

3.2.3. Demographic history

Although the null hypothesis of no geographical correlation to genetic divergence could not be rejected in the Southern Giant Petrel ($p = 0.78$; points were scattered with no trend, data not shown), a positive relationship was observed in the Northern Giant Petrel ($p = 0.049$, Fig. 7). Most colonies in both species as well as clusters showed signs of a recent reduction in population size based on at least one mutation model as well as a mode-shift (Supplementary material). Recent data indicate that Southern Giant Petrels are increasing in numbers at Gough, Isla Arce and Isla Gran Robredo, Argentina, Isla Noir and Falklands/Malvinas Islands and Northern Giant Petrels at least at South Georgia (Quintana et al., 2005, 2006). Microsatellite data may reflect prior bottlenecks of these colonies.

4. Discussion

4.1. Species status of the giant petrels

Microsatellites are useful makers to determine geographical variation and population structure between different populations based on their high mutation rate. These approaches are based on differences in allele frequencies and departures from Hardy–Weinberg equilibrium, i.e. the detection of Wahlund-type effects (Zink and Barrowclough, 2008). In phylogeographic studies microsatellites may provide additional information on evolutionary processes and patterns. However, a direct comparison with mtDNA may not always be appropriate due to several reasons, e.g. the difficulty of superposition of a gene tree onto geography (reviewed in Zink and Barrowclough, 2008).

If two populations differ in size at the time of vicariance, it is expected that the smaller population should coalesce first, leaving the other population paraphyletic due to incomplete lineage sorting. For neutral markers, coalescent time is determined by the effective size of populations (N_e) and is independent of the mutation rate of the marker. For nuclear markers, N_e is four times that of mitochondrial markers. Consequently, mtDNA markers will be informative in the intermediate past and are relatively 'leading' indicators, while nuclear markers are informative in the distant past and are 'lagging' indicators, irrespective of their mutation rate (Zink and Barrowclough, 2008). It is therefore possible that in some cases, lineage sorting is complete for mtDNA but incomplete for nuclear markers for some vicariance events. Our nuclear microsat-

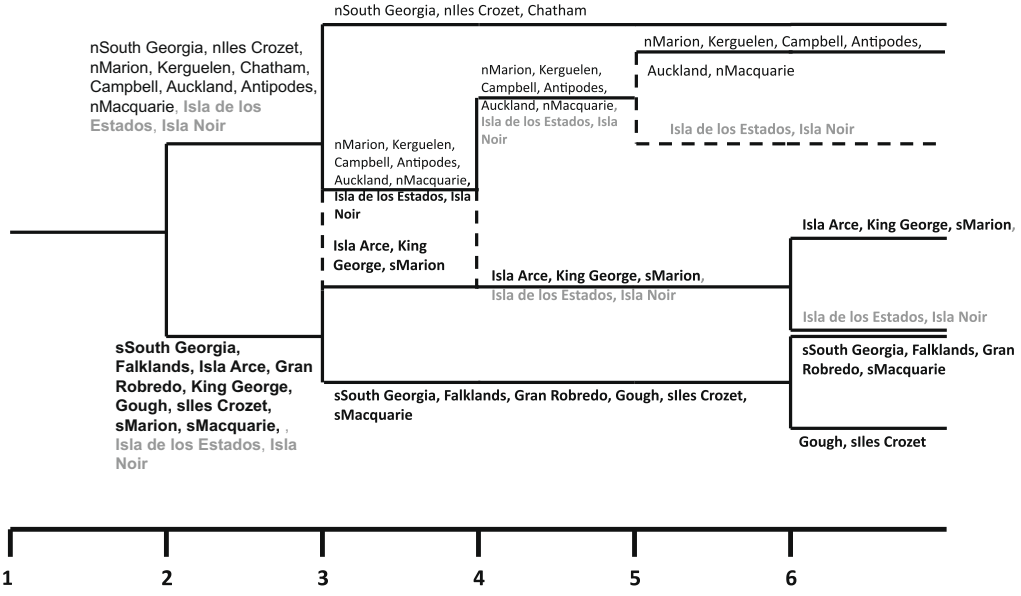

Fig. 5. Diagram showing how colonies split into clusters inferred from six microsatellite loci by STRUCTURE as the value of k increases (based on Delta *K*). Reticulations (dotted line) indicate that the provenance of some original groups is not consistent across all *k* values. The dotted line indicates the most likely solution, based on clustering at lower *k* values. Colonies having similar probabilities of occurring in either of two clusters are indicated in grey (see text). Southern Giant Petrel colonies are shown in bold.

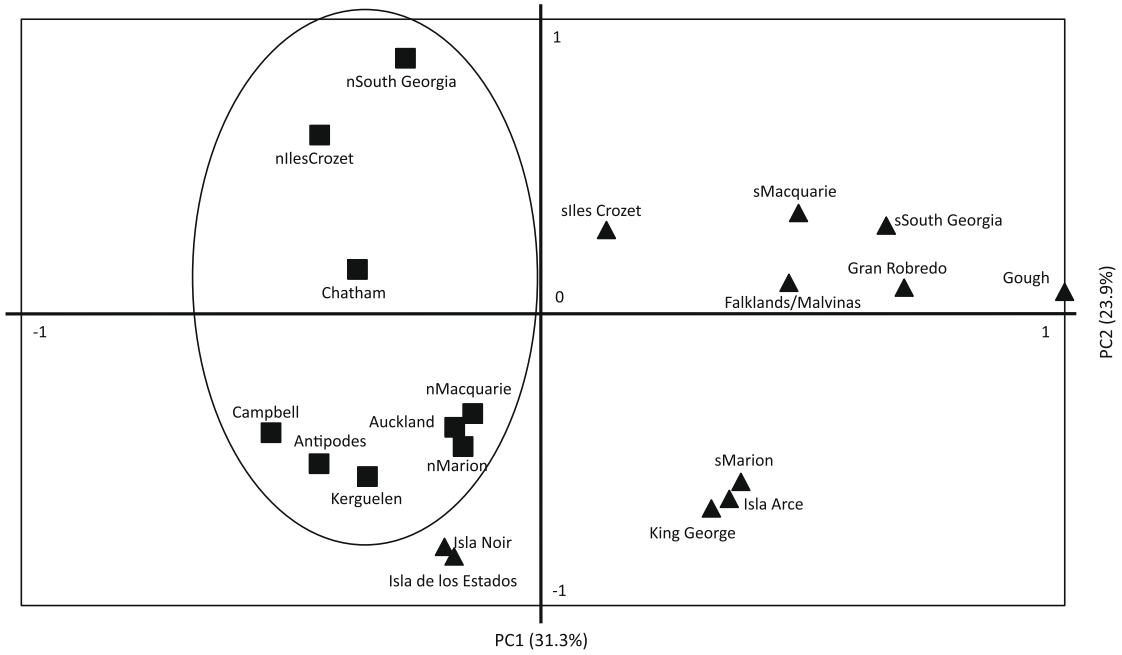

Fig. 6. Factor map of the two main axes of principle component analysis using six microsatellite loci for giant petrel colonies. The first two axes account for 55.2% inertia; three axes were significant to account for 66.5% inertia.

ellite data show incomplete separation of these two forms in F_{ST}, R_{ST}, D as well as in the STRUCTURE analysis. This can be explained by the relatively recent speciation, which occurred around 0.2 mya.

No shared mitochondrial haplotypes were found between the two species, with the exception of one bird caught on Marion Island. This individual had the greenish bill tip typical for Southern Petrel but possessed the highest frequency haplotype of the Northern Giant Petrels and showed evidence of hybrid origin in its microsatellite DNA. Hybrids between the two species have been described and this bird being a hybrid seems the most likely explanation. However, the possibility that this bird was misidentified cannot be excluded entirely. Hybridisation is most likely too rare to be seen in mtDNA and there are no confirmed observations showing that hybrids are fertile. The lack of shared haplotypes and the presence of one fixed mutation suggest that there is little, if any, gene flow between species. This ties in with observations that mixed pairs invariably consist of male Southern Giant Petrels and female Northern Giant Petrels. Giant petrels sampled on islands where the two species occur sympatrically clustered with their respective species, and we estimate that these populations separated 0.2–0.4 mya. This should be interpreted with caution, however, because sample sizes within colonies were quite small.

One approach in taxonomic studies is to compare the genetic distances between well-defined species within the same group or family. Cytochrome *b* was used to separate Manx *Puffinus puffinus* and Mediterranean Shearwater *P. yelkouan* based on a sequence divergence of 6.6% (Wink et al., 1993). A subsequent study further divided Mediterranean Shearwater into Yelkouan and Balearic Shearwater *P. mauretanicus* based on a sequence divergence of 1.6% and more than 10 fixed differences (Genovart et al., 2005). In the Herald Petrel *Pterodroma heraldica* a 1% difference between two colour morphs in agreement with differences in vocalisations was found and the authors recommended species status for both forms (Brooke and Rowe, 1996). Similarly, six fixed differences

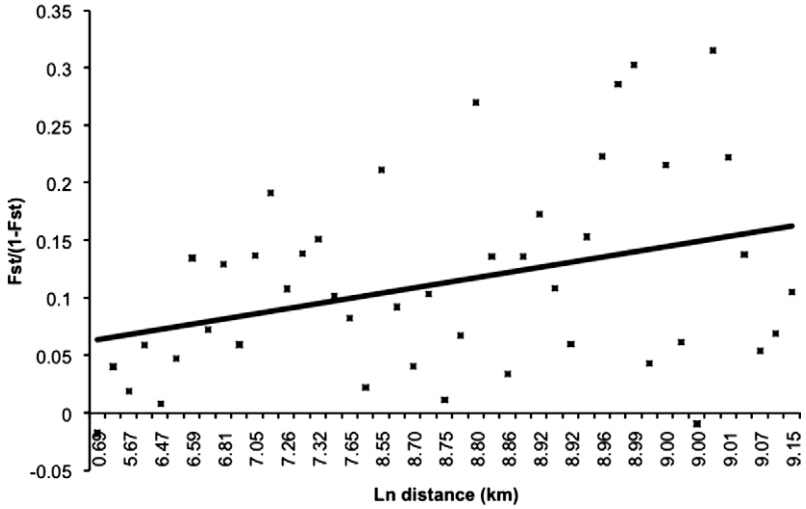

Fig. 7. Correlation between geographical and genetic distance amongst colonies of Northern Giant Petrels ($r2 = 0.151$, $p = 0.049$) using microsatellites.

and a sequence divergence of 1.6% between White-chinned *Procellaria aequinoctialis* and Spectacled *P. conspicillata* Petrels support their separate species status (Techow et al., 2009). Given that the two species of giant petrels rarely interbreed in sympatry and are distinguishable based on morphology, it is surprising that cytochrome *b* sequence divergence between the two species is only 0.42%. There are nevertheless strong arguments for retaining the species status.

The British Ornithologists' Union (BOU) as well as the Agreement on the Conservation of Albatrosses and Petrels (ACAP—see Document 11 of AC2 Meeting documents available at http://www.acap.aq/en/index.php?option=com_docman&task=cat_view&gid=37&Itemid=33) 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). Species delineations in petrels and albatrosses are often difficult given the very high level of natal philopatry, which greatly reduces mixing of geographically widely-separated colonies. 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 (Burg and Croxall, 2001, 2004; Abbott and Double, 2003). Giant petrels qualify on both of the BOU criteria. Individuals can be diagnosed by fixed differences of cytochrome *b* sequences as well as functionally independent plumage and bill colour characters, and there is no overlap in timing of egg laying (at Bird Island, South Georgia, the last female Northern Giant Petrel always lays before the first Southern Giant Petrel; Hunter, 1984a and Phillips unpublished data). Still, reproductive isolation is incomplete, with mixed pairings always occurring between male Southern Giant Petrels and female Northern Giant Petrels (Burger, 1978; Johnstone, 1978; Hunter, 1983, 1987; Cooper et al., 2001). Infrequent hybridisation is allowed even in the biological species concept (O'Brien and Mayr, 1991; Mayr, 1992) if it is unlikely to cause gene pools to merge (Helbig et al., 2002). Although speciation has occurred recently, future integrity can be assumed given the existence of a reproductive isolation mechanism. Thus all the data support the recognition of two giant petrel species.

4.2. Demographic history

In several high latitude seabirds, climate change, with concomitant effects on degree of glaciation and sea level height is postulated to have had a major influence on phylo-structure within species, and to have led to speciation (e.g. Ritchie et al., 2004; Ritz et al., 2008). The availability of breeding habitat for giant petrels will certainly have changed according to the extent and location of ice sheets on oceanic islands and the South American and Antarctic continents. The appearance of new breeding locations during periods of warming, together with changes in ocean currents and availability of productive up-wellings would have facilitated long distance dispersal and population expansion. Antarctica, large parts of South America, and some New Zealand islands would have had large ice sheets throughout the Pleistocene (approximately 1.8–0.011 mya). Mismatch distributions identified two demographic changes, one in Southern Giant Petrels ca. 0.7 mya (Pastonian interglacial, 0.8–0.6 mya) and one in Northern Giant Petrels ca. 0.11 mya (interglacial period 0.13–0.11 mya). During these interglacials Antarctica was least covered by ice, thus providing a large amount of available breeding habitat. Giant petrels and fulmars separated around 1.8 mya at the Pliocene–Pleistocene border, with giant petrels diverging into separate clades ca. 0.8 mya, following a period of glaciation (1.3–0.8 mya, Pre-Pastonian glaciation). This seems to indicate that a giant petrel ancestral population experienced fragmentation co-incident with climatic changes. This expansion was followed by secondary contact between some populations (two clades) of Southern Giant Petrel. The oldest demographic change is indicated in the south Indian Ocean within Southern Giant Petrels following the split ca. 0.7 mya. Confidence intervals are very large, which seems to be a result of this area's demographic flux. Nuclear data indicate ongoing gene flow between populations of these two clades. Within one clade some populations became separated for long enough to evolve isolating mechanisms and morphological adaptations. These barriers to gene flow resulted in the divergence of the Northern Giant Petrel.

Several lines of evidence suggest that the Northern Giant Petrels is the derived species: Southern Giant Petrels have a greater diversity within both cytochrome *b* and microsatellites and a greater number of alleles, as well as more private alleles. The relationship of haplotype diversity to nucleotide diversity can be used to interpret the demographic history of a population (Grant and Bowen, 1998). High h (>0.5) and high π (>0.5%), such as in the Southern Giant Petrel, is typical of a population with a stable history and secondary contact between lineages. However, high *h* 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. One locus that was polymorphic in the Southern Giant Petrel was monomorphic in the Northern Giant Petrel. The low allelic diversity in Northern Giant Petrels is an indicator of bottlenecks or founder effects (Nei et al., 1975). With the exception of a few private alleles, most Northern Giant Petrel alleles are a subset of those found in the Southern Giant Petrel. However, investigation of genetic data also showed that all colonies of the Southern Giant Petrel had experienced a reduction in population size (bottleneck) in the past.

Isolation by distance is expected in seabirds (Friesen et al., 2007), as colonies are often geographically well separated. However, although there was significant isolation by distance, neither species shows strong isolation by distance, suggesting other factors are responsible for the observed population structure. Historical isolation from other colonies may have been influenced by variable climate conditions during Pleistocene glacial cycles. Changes in sea level as well as changing regions of up-welling may have influenced population sizes and connectivity. Population specific foraging areas decrease the chances of intermixing of birds from different populations. These factors would have been enhanced by the philopatry of giant petrels. There are only a few ringing studies available for giant petrels, but they indicate that only few fledglings disperse to other islands or ocean basins. This is despite tracking and ringing data showing that juveniles roam widely (South Georgia birds have been recovered in Australasia and birds banded on Macquarie Island, Antarctic, Heard Island and South Orkneys have been recovered to the west coast of South America (Hunter, 1984b; Voisin, 1990; Trivelpice and Trivelpice, 1998; Gonzalez-Solis et al., 2008); Southern Giant Petrel juveniles banded at the Patagonian colonies of Argentina have been recovered in neighbouring Exclusive Economic Zones such as Brazil and Chile but also in New Zealand and Australian marine areas (Copello et al., 2009). Tracking data have also shown that there is some overlap in foraging ranges but most breeding adults generally stay within ocean basins also in the non-breeding period (e.g. Quintana and Dell' Arciprete, 2002; Gonzalez-Solis et al., 2000, 2008; Trebilco et al., 2008; Copello and Quintana, 2009b). The presence of phylogeographic and population structure within giant petrels supports these observations, despite the fact that fulmarines are amongst the most dispersive Procellariiformes, as evidenced by the rapid spread of the Northern Fulmar *F. glacialis* (Snow et al., 1998; BirdLife International, 2008).

4.3. Phylogeography of giant petrels

Although many marine species exhibit less phylogeographic structure than terrestrial species presumably due to high mobility and the lack of physical barriers, non-physical barriers seem to play an important role in many seabirds (see review by Friesen et al., 2007). Late Pleistocene glacial activity has been linked to both intra- and inter-specific diversification in seabirds and birds in general (Avise and Walker, 1998). Within giant petrels, most changes in population and phylogeographic genetic structure appear to pre-date the last Ice Age, which extended from approximately 70,000–15,000 years before present. 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 sectors of the Southern Ocean.

The identity of birds breeding on Gough, the colony presently furthest north (giant petrels previously bred further north at Tristan da Cunha, but were extirpated in the 19th century) as well as birds breeding on the Falklands/Malvinas Islands 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* and microsatellite DNA analysis show the colony to be differentiated from other populations. As the colony is small (ca. 260 pairs, Cuthbert and Sommer, 2004; PGR unpublished data) and geographically isolated, gene flow is limited and allele frequencies are likely to have changed due to genetic drift. Sequence divergence is not higher than other pairwise comparisons and no fixed mutational differences were observed. However, microsatellite data show highly significant F_{ST} and R_{ST} and a large percentage of differentiation in all pairwise comparisons. Hence, although the population on Gough does not merit subspecies status, it could be considered an Evolutionary Significant Unit (Ryder, 1986; for a review see Avise, 2004).

By comparison, the Falklands/Malvinas population resembles Southern Giant Petrels breeding at South Georgia and on the Antarctic Peninsula in terms of cytochrome *b*. Microsatellites indicate a close relationship with Southern Giant Petrels of South Georgia, Isla Gran Robredo (Argentina), the Indian Ocean and Macquarie Island. Further, cytochrome *b* indicates no differentiation between Patagonian birds and the remaining Atlantic colonies. However, microsatellites indicate that birds from Isla Noir and Isla Observatorio (Isla de los Estados) are differentiated from the remaining Southern Giant Petrel colonies and intermediate between the two species. The evolutionary implications of this are not clear: as there is no apparent difference in mitochondrial DNA, it may result from the retention of historical fragmentation in the nuclear genome. As mentioned above, nuclear microsatellites do not show a clear separation of species; structure analysis indicated that giant petrels are equally likely to be described by three clusters. Two of which consist either entirely of Northern or Southern Giant Petrels, respectively. A third cluster consists of Northern Giant Petrel colonies located in New Zealand (except Chatham Islands), Kerguelen Island and Marion Island and Southern Giant Petrel colonies located close to South America (Isla de los Estados, Isla Noir, Isla Arce and King George Island) as well as Marion Island.

Further subpopulation structuring was suggested with six clusters, which roughly corresponds to ocean basins. As this pattern was exclusive to the nuclear DNA, it could be an indication of connectivity during times of past fragmentation. Within the Northern Giant Petrel, analysis of microsatellites and cytochrome *b* showed contrasting evidence of population structuring. Evidence for structure was weak using mitochondrial DNA but three groups could be deduced through analysis of molecular variance. South Georgia was identified as one population, Chatham Island and Kerguelen as a second, and the remaining colonies as a third. Nuclear microsatellites on the other hand showed that all sampled New Zealand colonies with the exception of the Chatham Islands clustered with Kerguelen and Marion Island, whereas the Chatham Islands birds clustered with South Georgia and Iles Crozet. Once again this discrepancy may be the result of the nuclear retention of historical fragmentation and indicate changes in gene flow post colonisation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.09.005.

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