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Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites

T. M. BURG*‡ and J. P. CROXALL†
*Department of Zoology, University of Cambridge, Cambridge, CB2 3EJ, UK, †British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

Abstract

The population structure of black-browed (*Thalassarche melanophris* and *T. impavida*) and grey-headed (*T. chrysostoma*) albatrosses was examined using both mitochondrial DNA (mtDNA) and microsatellite analyses. mtDNA sequences from 73 black-browed and 50 grey-headed albatrosses were obtained from five island groups in the Southern Ocean. High levels of sequence divergence were found in both taxa (0.55–7.20% in black-browed albatrosses and 2.10–3.90% in grey-headed albatrosses). Black-browed albatrosses form three distinct groups: Falklands, Diego Ramirez/South Georgia/Kerguelen, and Campbell Island (*T. impavida*). *T. melanophris* from Campbell Island contain birds from each of the three groups, indicating high levels of mixture and hybridization. In contrast, grey-headed albatrosses form one globally panmictic population. Microsatellite analyses on a larger number of samples using seven highly variable markers found similar population structure to the mtDNA analyses in both black-browed and grey-headed albatrosses. Differences in population structure between these two very similar and closely related species could be the result of differences in foraging and dispersal patterns. Breeding black-browed albatrosses forage mainly over continental shelves and migrate to similar areas when not breeding. Grey-headed albatrosses forage mainly at frontal systems, travelling widely across oceanic habitats outside the breeding season. Genetic analyses support the current classification of *T. impavida* as being distinct from *T. melanophris*, but would also suggest splitting *T. melanophris* into two groups: Falkland Islands, and Diego Ramirez/South Georgia/Kerguelen.

Keywords: albatross, microsatellite, mtDNA, phylogenetics, speciation, *Thalassarche*

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Introduction

Grey-headed (*Thalassarche chrysostoma*) and black-browed albatrosses (*T. melanophris* and *T. impavida*) breed on small, remote islands in cool temperate and sub-Antarctic regions of the Southern Ocean (Tickell 1976; Waugh *et al*. 1999a). Recent taxonomic revision of the albatrosses (family Diomedeidae) has resulted in the recognition of four genera. Black-browed and grey-headed albatrosses have been assigned to the largest genus *Thalassarche* comprising the smaller, exclusively Southern Hemisphere species (Nunn *et al*. 1996; Robertson & Nunn 1998). Within the black-browed albatross group, the two subspecies *Diomedea melanophris melanophris* and *D. m. impavida* were raised to species rank as *T. melanophris* and *T. impavida*, respectively.

The black-browed albatross (*T. melanophris*) is the most abundant albatross species with large breeding populations on the Falkland Islands (521 000–576 000 pairs), South Georgia (96 000 pairs), Chile (32 000 pairs) and Kerguelen Island (3000 pairs) (Gales 1998). Data from banding studies and satellite-tracking indicate that the species is characteristically associated with continental and coastal

Correspondence: T. M. Burg. ‡Present address: Department of EPO Biology, N122 Ramaley, University of Colorado, Boulder, CO. 80309-0334, USA. Fax: + 044 (0) 1223 336676; E-mail: burgt@spot.colourado.edu

shelves and shelf slope systems (Cherel & Weimerskirch 1995; Prince *et al*. 1998; Waugh *et al*. 1999b). Falkland Island birds appear to be largely resident within the Patagonian Shelf system (Tickell 1976; Huin 2000a), whereas South Georgia birds migrate eastwards mainly to South Africa, where they overwinter in large numbers, and occasionally to Australia (Prince *et al*. 1998).

T. impavida is endemic to Campbell Island, a subantarctic island to the south of New Zealand. It is characterized by yellow irises, thicker black eye-brows and smaller body size than *T. melanophris* (Marchant & Higgins 1990; Waugh *et al*. 1999a). The population numbers 26 000 breeding pairs annually (Gales 1998). Interspersed in some *T. impavida* colonies, birds resembling *T. melanophris* have been observed and limited hybridization has been reported (Moore *et al*. 1997; Moore *et al*., in press).

Grey-headed albatrosses are generally similar to black-browed albatrosses in terms of size, overall distribution and behaviour. However, grey-headed albatrosses are an oceanic species and forage near frontal systems or zones associated with upwelling phenomena (Prince *et al*. 1994, 1998). The main grey-headed albatross breeding sites are: South Georgia (54 000 pairs annually), Diego Ramirez (10 000), Kerguelen (8000), Marion (7700), Campbell (6400) and Crozet (6000) (Gales 1998). Outside the breeding season they are widely distributed at high latitudes. Birds from South Georgia have been recovered, albeit in much smaller numbers than black-browed albatrosses, throughout the Indian Ocean and east to Australasia (Marchant & Higgins 1990; Prince *et al*. 1998).

Both black-browed and grey-headed albatrosses, like all albatross species, are thought to exhibit strong natal philopatry. Most juveniles return to their colony of birth and almost all of the remaining birds were recaptured at a nearby colony; a few chicks banded at Bird Island have been recaptured on the mainland of South Georgia and some may go further afield (Prince *et al*. 1994). Levels of site fidelity are also high. Movements of breeding birds between adjacent colonies (e.g. a few hundred metres) are rare, with only two recorded instances in more than 20 years of study (Prince *et al*. 1994).

Although grey-headed albatrosses are biennial breeders and black-browed albatrosses are annual breeders, all other aspects of their breeding cycle are very similar (Prince *et al*. 1994; Croxall *et al*. 1998). They start breeding as young as 6–8 years of age with an average age of 10 years and, if successful, a pair will produce one chick per breeding attempt (Prince *et al*. 1994; Croxall *et al*. 1998; Waugh *et al*. 1999c). Both grey-headed and black-browed albatrosses show variation in the length of chick-rearing between islands. The chick-rearing period of black-browed albatross lasts 116–118 days at South Georgia (*T. melanophris*) compared to 130 days at Campbell Island (*T. impavida*); in grey-headed albatross it ranges from 138 to 144 days at South Georgia to 152 days at Campbell Island (Tickell & Pinder 1975; Croxall *et al*. 1988; Moore & Moffat 1990; Prince *et al*. 1994; Weimerskirch *et al*. 1997).

Black-browed and grey-headed albatrosses are vulnerable to longline fisheries, particularly those operating near the breeding grounds. At South Georgia, grey-headed and black-browed albatrosses are currently declining at rates of 1.4–1.9% and 7.0% annually, respectively (Croxall *et al*. 1998). In grey-headed albatrosses, populations have decreased by 19–30% in the last 20 years at South Georgia and by 79–85% at Campbell Island since the 1940s (Moore & Moffat 1990; Croxall & Gales 1998; Croxall *et al*. 1998; Waugh *et al*. 1999c).

Both mitochondrial DNA (mtDNA) and microsatellites have been used in recent avian studies to address questions on mating systems, population structure and speciation (Burke *et al*. 1989; Ball & Avise 1992; Ellegren 1992; Wenink *et al*. 1994; Freeman & Zink 1995; Double *et al*. 1997; Petrie & Kempenaers 1998). mtDNA, while limited to female lineages, is a highly variable marker that is relatively easy to use. Microsatellites are biparentally inherited and provide a quick and efficient method for screening large numbers of samples. One limitation of microsatellites is the presence of homoplasy where two alleles of the same size are in fact different alleles, but scored as the same allele (Estoup *et al*. 1995; Primmer *et al*. 1995; Grimaldi & Crouau-Roy 1997; Primmer & Ellegren 1998; Taylor *et al*. 1999).

The aims of this study are twofold. First, if albatrosses are as philopatric as has been suggested, then high levels of population structure should be present between the islands. A second objective is to examine the new classification of black-browed albatrosses based on samples from a larger geographical distribution than used by Robertson & Nunn (1998) and using both nuclear and mitochondrial markers.

Materials and methods

Sampling

Blood samples were collected from albatrosses breeding on six islands (Fig. 1, Tables 1 and 2). The island groups where these samples were collected represent 99.87% and 97.30% of the world's population of black-browed and grey-headed albatrosses, respectively. Genomic DNA was isolated from ethanol preserved whole blood using a modified Chelex extraction (Walsh *et al*. 1991). Ten microlitres of blood ethanol mix was placed in a 1.5-mL microcentrifuge tube and incubated at 55 °C for 30 min to allow the ethanol to evaporate. A 300 μL solution of low TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) containing 5% w/v Chelex, 500 μg proteinase K and 250 μg RNase was added and the sample was incubated at 55 °C for a further 90 min. The solution was left overnight on a rotating wheel at 37 °C.

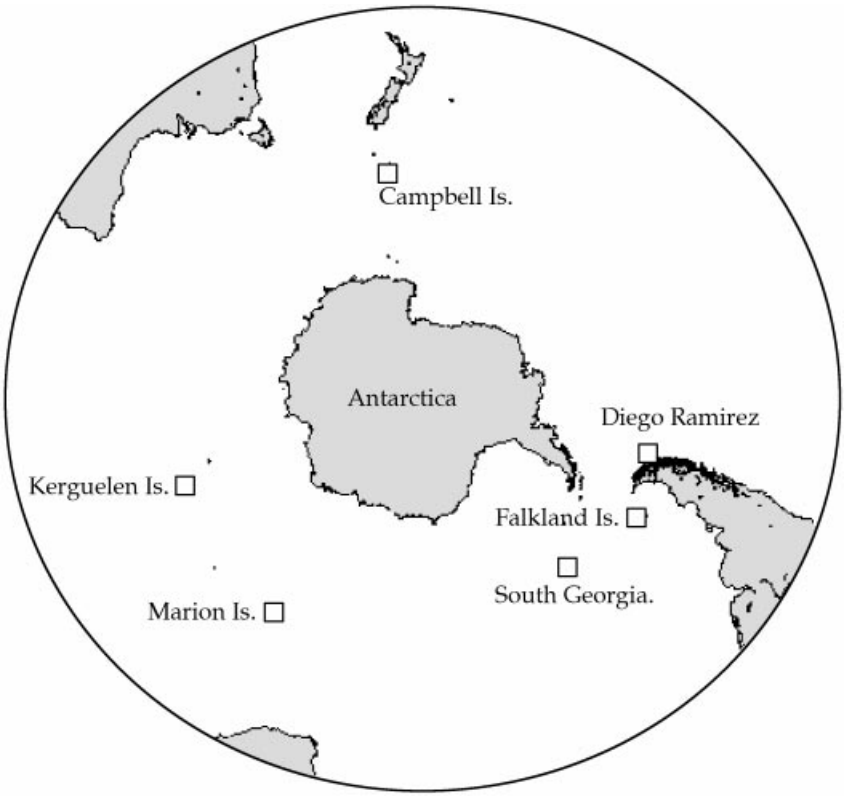

Fig. 1 Distribution of sampling sites for black-browed and grey-headed albatrosses in the subantarctic.

Table 1 List of sampling sites including identification (Id.), number of samples (NmtDNA) used for each taxa in mtDNA analysis, nucleotide diversity within each island (*n*) and haplotype diversity (h)

Sampling Site	Id.	N mtDNA	*n*	h
Black-browed				
Diego Ramirez	DR	10	0.0145	0.844
Falkland Islands	FI	17	0.0212	0.993
South Georgia	BI	14	0.0248	0.985
Kerguelen Island	K	7	0.0079	0.714
Campbell Island	mC	10	0.0419	1.000
Campbell Island	iC	15	0.0280	0.985
Grey-headed				
Diego Ramirez	DR	10	0.0278	1.000
South Georgia	BI	10	0.0287	0.978
Marion Island	M	11	0.0396	1.000
Kerguelen Island	K	9	0.0322	1.000
Campbell Island	C	10	0.0218	0.867

mtDNA

Approximately, 100 ng of genomic DNA was amplified using 200 μM dNTP, 1.5 μM $MgCl_2$, 0.2 U Taq (Hybaid), and 5 pmol of each primer (ND6* = L16406 5′-CCACCCCATAATACGGCGAAGG-3′, modified from Quinn & Wilson 1993; H505 5′-GAAAGAATGG-TCCTGAAGC-3′) in 1× reaction buffer [75 mM Tris-HCl pH 9, 20 mM $(NH_4)_2SO_4$, 0.01% Tween20] in a 25 μL reaction volume. Amplification consisted of one cycle of 120 s at 94 °C, 45 s at 50 °C, 120 s at 72 °C; six cycles of 60 s at 94 °C, 45 s at 50 °C, 90 s at 72 °C; 27 cycles of 60 s at 93 °C, 30 s at 55 °C, 60 s at 72 °C and one final cycle for 5 min at 72 °C. Polymerase chain reaction (PCR) products were visualized on a 0.7% agarose gel. Ten microlitres of the PCR product was incubated with 2 U SAP and 5 U exonuclease I at 37 °C for 30 min. Enzymes were heat inactivated and 3 μL of the treated PCR product was sequenced using BigDye ABI sequencing system and 2.5 pmol H505 primer.

Sequences were visually aligned using Seqed (Applied Biosystems) and any differences were confirmed by rechecking chromatograms. Sequences were analysed using PAUP* based on the HKY model of nucleotide substitution and neighbour-joining, maximum likelihood and Minspnet to create phylogenetic trees (Excoffier *et al*. 1992; Swofford 1999). AMOVA was used to calculate F_{ST} and hierarchical population structure (Excoffier *et al*. 1992).

For black-browed albatrosses, several fixed differences between the three mtDNA types were found and an additional 20 Falkland Island, 30 South Georgia and nine Campbell Island *Thalassarche melanophris* and 15 *T. impavida* were screened using the group-specific primers described in Moore *et al*. (in press).

Table 2 Allelic variation (A), expected (H_E) and observed (H_O) heterozygosities at microsatellite loci used to screen black-browed and grey-headed albatross samples. Abbreviations for sampling sites are: Diego Ramirez (DR); Falkland Islands (FI); South Georgia (BI); Marion (M); and Campbell (C, *Thalassarche chrysostoma*; mC, *T. melanophris*; iC, *T. impavida*). Loci with significant departures from Hardy–Weinberg equilibrium after Bonferroni correction are indicated (*)

Sampling Site	Dc5	Dc9	De11	Dc21	Dc22	Dc27	De35	Average
Black-browed								
DR (n = 10)								
A	4	6	4	2	4	4	8	4.6
H_E	0.28	0.72	0.60	0.48	0.79	0.48	0.94	0.61
H_O	0.30	0.70	0.50	0.20	0.80	0.50	1.00	0.57
FI (n = 37)								
A	5	5	6	2	3	3	9	4.7
H_E	0.30	0.60	0.78	0.51	0.59	0.35	0.81	0.56
H_O	0.27	0.48	0.78	0.44	0.69	0.27	0.94	0.55
BI (n = 660)								
A	6	13	6	4	4	6	12	7.3
H_E	0.26	0.61	0.81	0.53	0.67	0.46	0.77	0.59
H_O	0.24	0.59	0.79	0.47	0.68	0.50	0.79	0.58
mC (n = 19)								
A	4	6	7	3	4	4	9	5.3
H_E	0.38	0.58	0.86	0.68	0.64	0.29	0.74*	0.61
H_O	0.42	0.58	0.84	0.63	0.53	0.26	0.58	0.55
iC (n = 30)								
A	4	6	4	4	4	2	7	4.4
H_E	0.13	0.53	0.69*	0.62	0.61	0.46	0.80	0.57
H_O	0.10	0.53	0.47	0.57	0.57	0.47	0.87	0.51

Sampling site	De3	Dc5	Dc9	De11	Dc26	Dc27	De35	Average
Grey-headed								
DR (n = 10)								
A	2	4	8	8	9	4	8	6.1
H_E	0.39	0.59	0.84	0.91	0.94	0.46	0.90	0.72
H_O	0.30	0.60	0.80	0.80	0.90	0.30	0.90	0.66
BI (n = 699)								
A	5	8	12	15	14	6	22	11.7
H_E	0.46	0.41	0.72	0.81*	0.75	0.49	0.89	0.65
H_O	0.43	0.41	0.72	0.68	0.69	0.51	0.82	0.61
M (n = 18)								
A	2	3	6	7	7	2	10	5.3
H_E	0.52	0.43	0.81	0.92	0.71*	0.12	0.85	0.62
H_O	0.21	0.30	0.64	0.83	0.36	0.11	0.75	0.46
C (n = 19)								
A	3	3	7	9	8	6	13	7.0
H_E	0.41	0.41	0.54	0.83	0.87	0.47*	0.95	0.64
H_O	0.37	0.47	0.58	0.74	0.77	0.32	0.94	0.60

Microsatellites

Details of microsatellite primers and PCR conditions are listed in Burg (1999) with the exception of De35 (De35a: 5′-CAAACCTGAAACCTTCCAAAAC-3′ and De35b: 5′-CCCCCTGTTTCTACTCTGGTC-3′, 48/52 °C annealing temperature). Seven of the more variable loci were used for population analysis for each taxa (Table 2). A total of 765 black-browed and 756 grey-headed albatrosses were used for this analysis (Table 2). The sample sizes for South Georgia are larger than the other sites as these were used in another study. GENEPOP3 was used to test for linkage disequilibrium, deviations from Hardy–Weinberg equilibrium and population differentiation (Raymond & Rousset 1995). F_{ST} and R_{ST} were calculated using GENEPOP3 and RSTCALC, respectively (Raymond & Rousset 1995; Goodman 1997).

Results

mtDNA

Black-browed albatross. Sequencing 219 bp of mtDNA control region (domain I) from 73 black-browed albatrosses revealed 57 variable sites (Fig. 2). These variable sites defined 57 haplotypes (GenBank accession numbers AY016052–AY016108). Eight haplotypes were shared

	Variable sites	FI	DR	BI	K	mC	iC
	1111111111111111111111111111111111111112						
	2223556777777788999000022222222233333344555566677777888991						
	3688237023456903568234502345678901235935023426824782791 31						
TmFI1	CTATGTAGATGTTGTTAATTTGCAATTGTCAGCGAGTTGAGAAGCACTGTRYRTGGT	1					
TmFI2	.....C.........C.....................C..............Y...	1					
TmFI4	.....CG........C....Y.TR.........A..........T.....GCGA..Y	1					
TmFI6	...C.C.........C.....................CR...........GC.....	1					
TmFI8	.....C.........C..................T....C.............C.....	1					
TmFI9	.....C.........C.....................R...........C.....	1					
TmFI10	.....C.....................................C.....	1					
TmFI21	.....C..G......C.....................G..........TCT....	1					
TmFI23	.....C.....C...C......T.............C.............C.....	1					
TmFI26	.....C.................................G..........GTG....	1					
TmFI27	.....C.........C.....................................C.....	1					
TmFI29	.....C.......A.CG.....................................C.....	1					
TmFI30	.....C..G......Y.....................G...........C.....	1					
TmDR3	............C.........Y.........................C.....		1				
TmDR8	T.................G...AT.....CTGA.....		1				
TmDR9	T....C.........C.G...AT.....CTGA............T.....		1				
TmBIS3	.....C..............AT.....CTGA..G..............C.....			1			
TmBIS5	T..............C.....AT......TG.................A.ACA....			1			
TmBIQ1	T....C.........C.....AT....AC.GA.....C.............CG....			1			
TmBIQ2	.....C.........C.....AT.....CTGA.....C.....			1			
TmBIQ3	T.GC.G.........C..C..AT..C..CTGA....G..........G...CG....			1			
TmBI109b	.....C.........C.....AT.....CTGA..................ATG....			1			
TmBI98b	..............C.....AT.....CTGA.....................G....			1			
TmBI99b	T....C.......A.C.....AT.....CTGA..................ACG....			1			
TmBIG1	.....C.........C.....AT.....CTGA.....................G....			1			
TmBIG2	.....C....A....C.....AT.....CTGA..G.....A.........GC.....			1			
TmK3	TY...C.........C.....AT.....CTG...................AC..R..				1		
TmK4	T...CC........CC.....AT.....CTGA...................CG....				1		
TmC2	T....C.......A.C.G...AT.....CTGA..................ACG....					1	
TmC4	........G......C.....AT.GCA.CTG...GA......G.T...A.GTA....					1	
TmC5	..............C.....AT.....CTGA.................AC.....					1	
TmC6	...........C...C.....AT.GCG.CTG...G.....AG..T.....GT.....					1	
TmC19	T......A.......C...C.AT......TGA............T....C.C.....					1	
TmC22	..............C..............G......C.............G....					1	
TmC24	T..............C.....AT.....CTGA..................C.....					1	
TmC28	.....C.........C.....AT.....CTGA...........A......GC...A.					1	
TiC1	.....C..G......C.....AT.GCA.CTG...GAC.....G.T...A.GTA....						1
TiC2	.....C.....C...Y.....AT.GCA.GTG...G.........T...A.GTA....						1
TiC14	.....C..G......C.....AT.GCA.CTG...GA...R..G.T...A.GTA....						1
TiC15	.....C..G......C.....AT.GCG.CTG...G.......G.T...A.GTA....						1
TiC16	.....C...G.C...C.....AT.GCA.CTG...GA........T...A.GTA....						1
TiC20	........G......C.....AT.GCA..TG...G.......G.T...A.GTA....						1
TiC21	.....C...G.C...C.....AT.GCA.CTG...GA........T...A.GTG....						1
TiC26	.....C.....C...C.....AT.GCA.C.G...G.........TG..A.GTG....						1
TiC101	.....C.....C...C.....AT.GCA.CTG...G.........T...A.GTA....						1
TiC102	.....C..G......C.....AT.GCA.CTG...G.C.....G.T...A.GTA....						1
TiC104	.....C.....C...C.....AT.GCA.CTG...GA........T...A.GTG....						1
TiC105	.....C..G......C.....AT.GCA.CTG...G.......G.T...A.GTA....						1
TiC106	.....C.....C...C.....AT.GCA.CTG...G.........TGT.A.GTG....						1
A (n=3)	T..............C.....AT.....CTGA..................GC.....	1	1		1		
B (n=7)	T....C.........C.....AT.....CTGA.....................C.....		2		4	1	
C (n=2)	T....C...........G...AT.....CTGA.....................C.....			2			
D (n=4)	T....C.........C.G...AT.....CTGA.....................C.....		4				
E (n=2)	..............C.....................................G....	1				1	
F (n=2)	.....C.........C.................G.....	2					
G (n=2)	........G......Y.....AT.GCG.CTG...G.......G.T...A.GTA....						2
H (n=2)	.....C.........C.....AT.....CTGA.....						2
Tc	.....C.....C....G....AT.CC...T....G.C...A.GAT.....GTA....						

Fig. 2 Variable sites in black-browed albatross mtDNA sequence. Aligned grey-headed albatross (Tc) is shown for comparison. Sequence identity to TmFI1 is indicated by a '.' and shared haplotypes are given a single letter designation followed by the sample size. Mixed bases are indicated by 'Y' (= C / T) and 'R' (= A/G).

between two or more islands, while 49 were unique to single individuals. MST (minimum spanning tree) (Fig. 3), neighbour-joining and maximum likelihood (Fig. 4) analyses showed the same three distinct groups: (1) Falkland Islands; (2) Diego Ramirez, South Georgia and Kerguelen; and (3) Campbell Island (*Thalassarche impavida*). Black-browed albatrosses from the Falkland Islands were separated from the other *T. melanophris* by six fixed mutations (356 / 500 bootstrap replicates from neighbour-joining analysis). *T. impavida* was separated from the larger

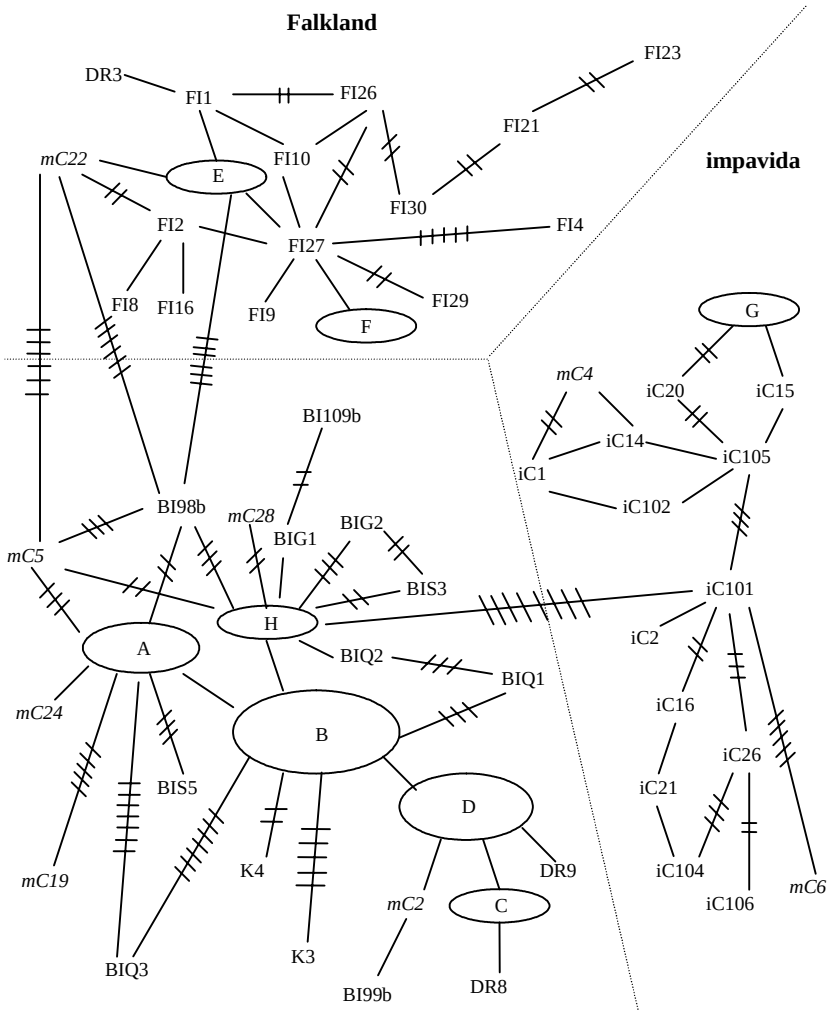

Fig. 3 Minimum spanning network from 219 bp of control region sequence from 73 black-browed albatrosses. The size of the ovals are proportional the number of birds sharing that haplotype (see Fig. 2) and the number of crosshatches indicates the number of nucleotide substitutions.

group of *T. melanophris* by eight fixed mtDNA differences (500/500 bootstrap replicates from neighbour-joining analysis). Average pairwise sequence divergence within each group (1.80–2.06%) was lower than divergence between groups (4.16–7.16%). The largest difference was 14 fixed differences between the Falkland Islands (*T. melanophris*) and *T. impavida*. F_{ST} estimates ranged from 0.0675 to 0.7408 (Table 3) and showed significant structuring between the three groups ($P < 0.001$) with 57.95% of the variance due to among group variation and only 3.56% among populations within a group.

Grey-headed albatrosses. mtDNA sequences from 220 bp of control region sequence from 50 grey-headed albatrosses revealed 39 haplotypes (GenBank accession numbers AF326413–AF326458) containing 40 variable sites and one insertion/deletion (Fig. 5). Two haplotypes were shared between two or more birds, but both were restricted to a single island (Haplotype A: South Georgia; and Haplotype B: Campbell Island). Average levels of sequence divergence were higher in grey-headed albatrosses than within group black-browed albatross (2.99% compared to 1.80–2.06%), but showed no population structure ($F_{ST} = 0.000$, $P = 0.08$; Figs 6 and 7; Table 4).

Both species contained ambiguous bases at several positions (Figs 2 and 5). PCR amplification of the downstream portion of the control region, and subsequent cloning and sequencing, revealed that this was due to high levels of heteroplasmy, a condition present in other avian taxa (Berg *et al*. 1995; Crochet & Desmarais 2000). Similar results have been found in other petrels (shy albatross *T. cauta* and white-capped albatross *T. steadi*; M. Double, personal communication, northern fulmar *Fulmarus glacialis*; T. Burg, unpublished data).

Microsatellite analyses

Of the 37 loci isolated from albatrosses, seven were used for large scale screening of each taxon (Table 2). These seven

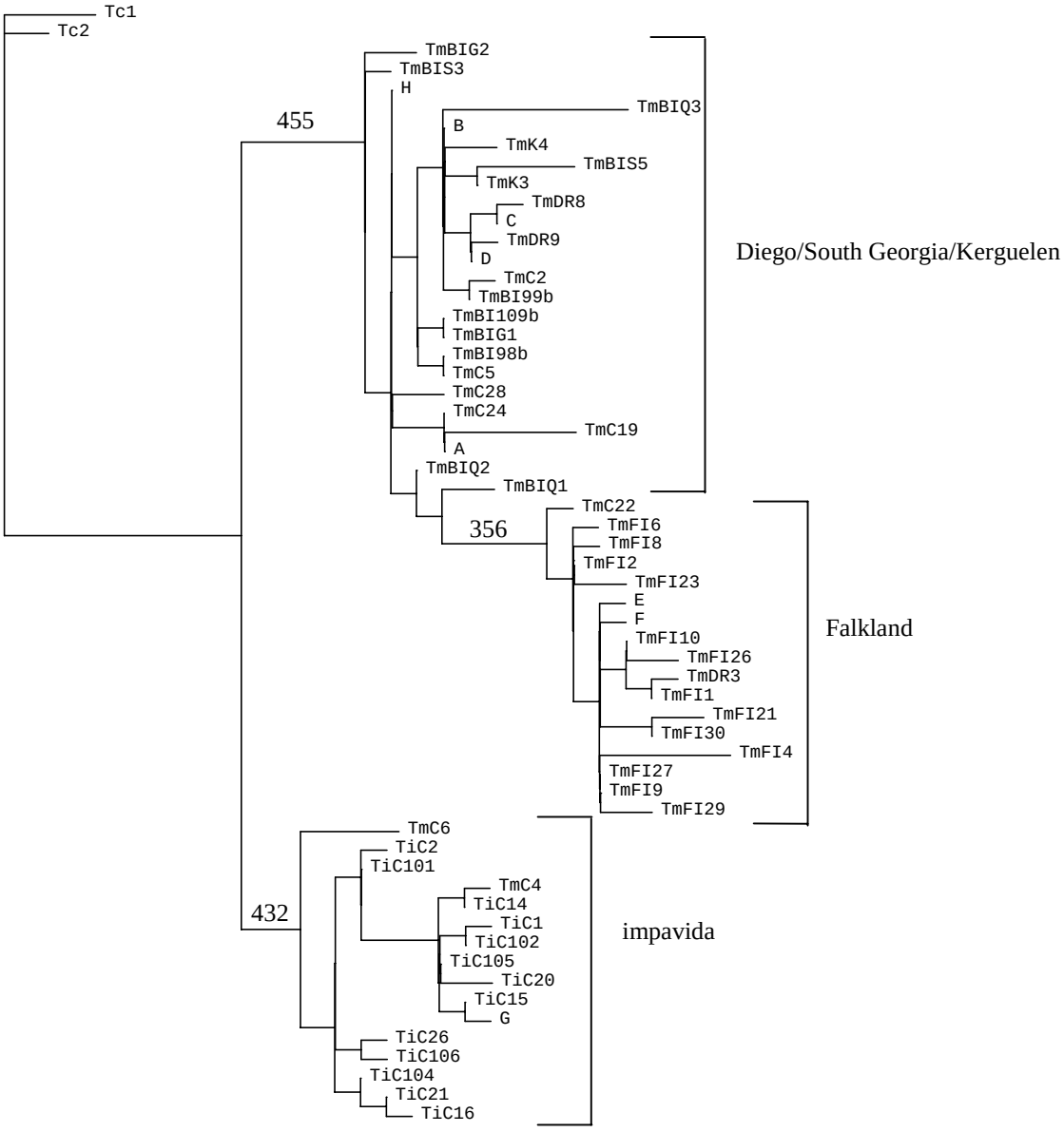

Fig. 4 Black-browed albatross maximum likelihood tree constructed using HKY model of nucleotide substitution. The same three major clades were produced using neighbour-joining and bootstrap values from 500 replicates are shown for each of the major clades. Two grey-headed albatross sequences (Tc1 and Tc2) were used as outgroups.

microsatellite loci contained 4–13 alleles for black-browed albatrosses and 5–22 alleles for grey-headed albatrosses (Table 2). Expected heterozygosity was lower in black-browed albatrosses (0.56–0.61) compared with grey-headed albatrosses (0.63–0.72). After Bonferroni correction for multiple tests (Rice 1989), three significant heterozygote deficiencies were present: *T. impavida* at locus De11 and grey-headed albatross from Marion Island at locus Dc26 and from Campbell Island at locus Dc27 (Table 2). Microsatellite analyses of seven variable loci in black-browed albatrosses showed significant population structure between the same three groups as mtDNA analyses with F_{ST} estimates ranging from 0.0123 to 0.1494 ($P < 0.001$) (Table 3). In comparison, the grey-headed albatrosses F_{ST} estimates were considerably lower (0.0059–0.0614) and not significant ($P = 0.08$) (Table 4).

Discussion

While grey-headed albatrosses were found to be globally panmictic using both mtDNA and microsatellite markers, three distinct groups of black-browed albatrosses were found (Falkland, Diego Ramirez/South Georgia/Kerguelen, and *Thalassarche impavida*). The different nature of population

	Falkland	Diego	S. Georgia	Kerguelen	Campbell	impavida
Falkland		0.0339	**0.0220**	—	0.0404	**0.1344**
Diego	**0.5838**		0.0225	—	0.0584	**0.1484**
S. Georgia	**0.5390**	0.0675			0.0123	**0.1494**
Kerguelen	**0.6489**	0.1463	**0.0987**		—	—
Campbell	**0.3654**	0.0934	0.0598	**0.1441**		**0.1191**
impavida	**0.7221**	**0.7046**	**0.6498**	**0.7408**	**0.4851**	

Table 3 Black-browed albatross F_{ST} for mtDNA (below diagonal) and microsatellites (above diagonal). Values significant at $P = 0.05$ are indicated in bold

```
                                  Variable sites
                                  1111111111111111111111111222222
                  1224555678899900122223333344555567778880011123
                  7356349753535768946780123556034721390795812 33
TcBIZ2            ACACCCGATTCCGATGTTCAAGGGGCACGGG-TTTTTATTCTCCG
TcBIZ3            ........C....G.A.........T...A.....G....T...A
TcBIT1            ........A....G.A..T.....................T...R
TcBIT3            ........C....G.A..T.......GTA.A............A
TcBIX1            ........C....G.A..T....A..GTA.AA...........A
TcBIX3            ........C.T..G.A..T.G.......A..............A
TcBI18b           ............AG.A..T....A.....A..............A
TcBI19a           ........C.T..G.A..T.G.......A.A............A
TcDR1             ........C....G.A........R.....A............A
TcDR2             ............G.A..T....A....A.A............A
TcDR3             ....T.......AG.A..T....A...................YA
TcDR4             ........C....G.A..T..A.A..GTA.A.........T...A
TcDR5             ........C....G.A...G..........A............A
TcDR6             .........C..AG.A.CT....A...................A
TcDR7             .......GG....G.A..T.............C.......T...A
TcDR8             ............G.A..T....A..GTA...............A
TcDR9             ............AGCA..T....A...................A
TcDR10            .........C..AG.A..T....A................T...A
TcK1              ............G.A..T...AAA................T...A
TcK2              ....T...C.T..G.A..T.G.....................A
TcK3              ........C...AG.A..T.G..A......A............A
TcK4              ....T...G....G.A.........................T...A
TcK6              ...T....C....G.A..............A............A
TcK7              .T..........AG.A..T.G..A.............G..T...A
TcK8              ............G.A........A....A......C........
TcK9              ........C....G.A............A...............
TcK10             ............G.A............A...............
TcM2              ....T........G.A..T..A.A....AAC.............A
TcM3              ........CC...G.A.............A..C...........
TcM4              ....TT..C....G.A...R.......................
TcM5              ....T...C....G.A..T....A...................A
TcM6              ..G.....C....G.A..T....A....RAC.Y........CT.A
TcM8              ........C....G.A.........................T...A
TcM9              ........C....G.A.......................C.....
TcM10             ......A.C....G.A..T....A..GTA....CC...C.....A
TcM11             G..........TAG.AC.T.G..A.....CA.....G...T....
TcM15             ........C....G.A..T....A..GTA.A............A
TcM17             ........C....G.A..T....A..G.A.A.........T...A
TcC7              ........C....G.A..T....A.T....C............R
TcC9              ........C....G.A..T..A.A..GTA.A............A
TcC119            ....T.......AG.A..T.G..AA..............T...A
TcC120            ....T...C....G.A..T....A......A............A
TcC121            ............G.A...G.........A...............
TcC122            ........C....G.A..T....AA...A.C............A
A (n=2)           ............AG.A..T....A...................A
B (n=4)           ........C....G.A..T....A....A.C............A
Tm                ........C...AG.....TGCA..T...A..C...G...T...A
```

Fig. 5 Variable sites in grey-headed albatross mitochondrial control region. Aligned black-browed albatross sequence (Tm) is shown for comparison. Sequence identity to TmFI1 is indicated by a '.' and shared haplotypes are given a single letter designation followed by the sample size. Haplotype A is found on South Georgia and haplotype B on Campbell Island. Mixed bases are R = G/A and Y = A/T.

structure in black-browed and grey-headed albatrosses is perhaps surprising given the high degree of site fidelity and natal philopatry associated with albatrosses. Also unexpected is the amount of divergence between the *T. melanophris* from the Falklands and those from other islands.

Black-browed albatross

The foraging grounds for the three black-browed albatross groups are distinct. Black-browed albatrosses from the Falklands forage mostly within the Patagonian shelf region, whereas *T. melanophris* from the other islands forage near Africa and Australia during the nonbreeding season. *T. impavida* is largely restricted to the region around Campbell Island, at least during the breeding season (Tickell 1967; Croxall *et al*. 1996; Waugh *et al*. 1999d; Grémillet *et al*. 2000; Huin 2000a).

Despite high levels of natal philopatry, there is still sufficient migration occurring between birds from Diego Ramirez, South Georgia and Kerguelen to prevent population differentiation. However, differences in foraging and wintering grounds of the Falkland Island black-browed albatrosses appear to have isolated these birds from the other *T. melanophris*, such that sufficient time has elapsed to allow fixed genetic differences to accumulate between these two groups. These differences are more noticeable with the mtDNA than microsatellites. This could be due to more recent male-mediated gene flow or to differences in mutation rates between the two markers. Male-mediated gene flow is possible as *T. melanophris* at Campbell Island show a male bias (Moore *et al*. 1997); however, other studies have found that both sexes appear to disperse at similar rates (British Antarctic Survey, unpublished data, Prince *et al*. 1994). Alternatively, this could be attributed to differences in effective population size of the mtDNA and nuclear markers. As mtDNA is uniparentally inherited, it has a smaller effective population size and as a result is more strongly influenced by genetic drift. However, the male bias on Campbell Island combined with the genetic data suggest that male-mediated migration is most likely the explanation.

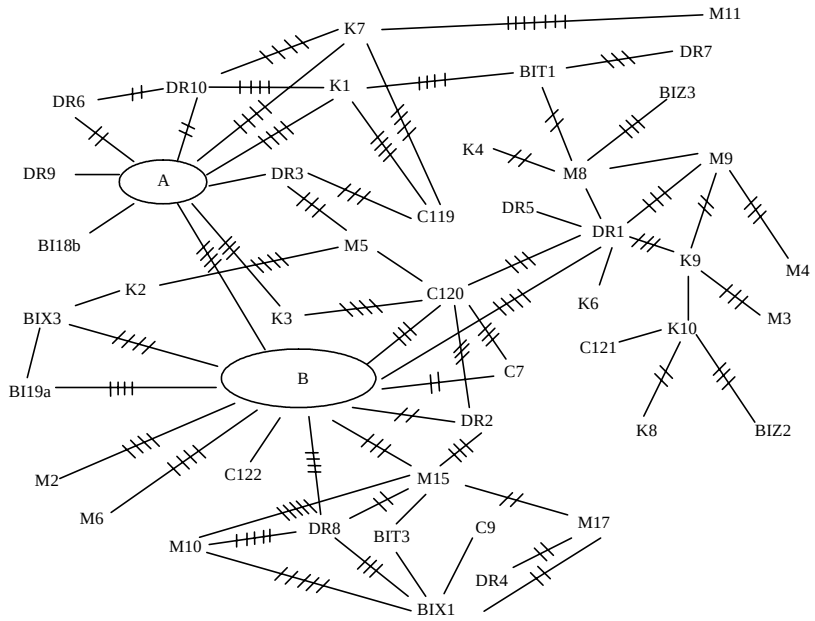

Fig. 6 Minimum spanning network based on 220 bp of control region sequence from 50 grey-headed albatrosses. The size of the ovals are proportional the number of birds sharing that haplotype and the number of crosshatches indicates the number of nucleotide substitutions.

Campbell Island T. melanophris

T. melanophris breeding on Campbell Island are interesting for two reasons. First, they are present in each of the three mtDNA groups and second *T. melanophris* and *T. impavida* appear to be hybridizing on Campbell Island.

One possible explanation for the Campbell Island *T. melanophris* population comprising mtDNA from all three groups is the formation of a new *T. melanophris* colony by birds from different breeding sites. Despite earlier surveys, no *T. melanophris* were reported breeding on Antipodes Island prior to 1950 (Warham & Bell 1979) or on Campbell Island prior to the 1970s (Moore *et al*. 1997). However, after this time, *T. melanophris* has been reported breeding on both islands in low numbers. The closest colonies to Campbell Island are those at Macquarie Island (725 km to the southwest, 60–80 breeding pairs) and Antipodes Island (744 km to the northeast, 115 breeding pairs) (Moore *et al*. 1997; Tennyson *et al*. 1998). Birds banded on Campbell Island have been reported on Macquarie Island and further away off Chile (Moore *et al*. 1997; Moore *et al*., in press). While data from Antipodes Island are limited due to the inaccessibility of the colonies, data from *T. melanophris* on Campbell Island show a male bias (10 males: 2 females, excluding known hybrids), suggesting possible male-biased dispersal.

Several mixed pairs of *T. melanophris* and *T. impavida* have been observed breeding on Campbell Island and have successfully reared chicks (Moore *et al*. 1997). Evidence of hybridization between *T. melanophris* and *T. impavida* on Campbell Island was confirmed using mtDNA analysis. Of the 19 *T. melanophris* sampled from Campbell Island that were classified based on iris colour (brown in *T. melanophris* and yellow in *T. impavida*), six had *T. impavida* mtDNA. As there appears to be a male bias in the non-hybrid *T. melanophris* at Campbell Island, hybrids are more likely to result from matings between *T. melanophris* males and *T. impavida* females. Therefore, most of the hybrids should contain *T. impavida* mtDNA and would have been detected with the mtDNA screening primers. It is known that the success rate of mixed pairs on Campbell Island is lower than that of *T. impavida*, though mixed pairs are successful at fledging chicks (Moore *et al*. 1997). However, 50% of the *T. melanophris* breeding on Campbell have dark-eyed partners. As there is an abundance of *T. impavida* (26 000 pairs compared to 30 pairs of *T. melanophris*), this suggests that breeding between the two types is not random and *T. melanophris* are selecting dark-eyed mates when possible (Moore *et al*., in press).

This lack of differentiation in Campbell *T. melanophris* is in agreement with morphological data (Waugh *et al*. 1999a). Assignment tests based on morphological data failed to assign correctly any *T. melanophris* from Campbell Island to Campbell Island.

Falkland Island black-browed albatross

Of the 37 black-browed albatrosses analysed from the Falkland Islands, 32 contained haplotypes that were distinct from *T. melanophris* breeding on other islands. The levels of differentiation were as high as those found between *T. impavida* and the other group of *T. melanophris*. Black-browed albatrosses from the Falklands are known

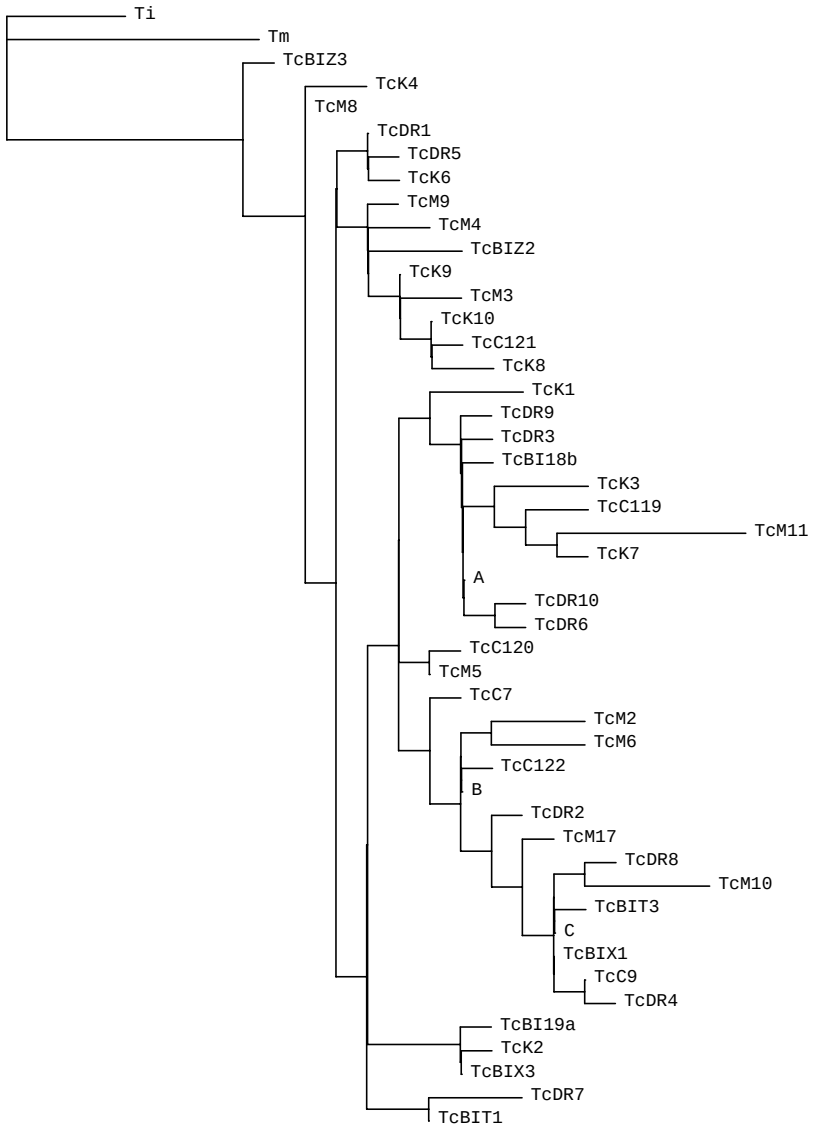

Fig. 7 Grey-headed albatross maximum likelihood tree constructed using HKY model of nucleotide substitution. Two black-browed albatross sequences (Tm and Ti) were used as outgroups.

Table 4 Grey-headed albatross F_{ST} for mtDNA (below diagonal) and microsatellites (above diagonal). Values significant at $P = 0.05$ are indicated in bold

	Diego	S. Georgia	Marion	Kerguelen	Campbell
Diego		0.0059	0.0095	—	0.0084
S. Georgia	0.0207		0.0215	—	0.0116
Marion	0.0175	−0.0304		—	0.0614
Kerguelen	0.0511	−0.0094	0.0043		—
Campbell	−0.0056	−0.0125	−0.1350	**0.1129**	

to have very different foraging and wintering grounds from the South Georgia birds, which may account for the reduced gene flow between the islands (Tickell 1967; Prince *et al*. 1998; Huin 2000a). One interesting point is that despite results of satellite and geolocator tracking of Falkland black-browed albatrosses (Grémillet *et al*. 2000; Huin 2000a; Huin, unpublished data), indicating a distribution restricted to the Patagonian Shelf, some black-brows from the Falklands have obviously been recruited into the Campbell population (Figs 3 and 4; Moore *et al*., in press). This presumably happens during the juvenile period of the life history. Indeed, ringing recoveries from Falkland-banded chicks indicated that although 88% turned up as juveniles along the South American coasts, 12% were recovered in South Africa (Tickell 1967). It would not be surprising, therefore, if a small proportion of juveniles travelled as far east as Campbell Island. In addition, one *T. melanophris* from Campbell Island was shot off the Chilean coast, further suggesting movement between the two islands (P. Moore, personal communication). It may be that in the black-browed albatross, spatial differences in foraging grounds have reduced

levels of migration between the Falklands and other islands such that genetic drift has occurred. The Patagonian Shelf area is sufficiently large and productive that Falkland black-browed albatrosses could sustain themselves there year round. In contrast, other *T. melanophris* from sub-Antarctic islands (e.g. South Georgia, Kerguelen, Macquarie) may not have a large enough surrounding shelf and need to travel more widely, at least seasonally, to find food.

Grey-headed albatross

Despite a similar geographical distribution to the black-browed albatross, population structure in grey-headed albatrosses can best be described as globally panmictic. No evidence of population structure in grey-headed albatross was detected using either mtDNA or microsatellite data. Grey-headed albatross are oceanic/pelagic in contrast to black-browed albatross, which are more inshore/neritic. Outside the breeding season little is known about the distribution of grey-headed albatross, except that they occur in relatively high latitudes in the Southern Ocean. The few banding recoveries for grey-headed albatrosses, suggest that mixing of different populations may be occurring at sea. This is supported by both mtDNA and microsatellite data.

Lack of any population differentiation in grey-headed albatrosses is surprising, due to high levels of philopatry and apparent, but possibly superficial, similarity to black-browed albatrosses. Data from 25 years of behavioural observations on Bird Island, South Georgia show no large scale movement of birds between colonies (Prince *et al*. 1994). One reason for the lack of differentiation in grey-headed albatrosses, could be that they are an oceanic species and mixing is occurring between the juveniles on the feeding grounds. As grey-headed albatrosses forage along frontal systems, they cover a larger area than black-browed albatrosses which are confined to coastal shelf areas for foraging. No significant morphological differences were found between grey-headed albatrosses from four different islands, three of which were used in our genetic analyses (Waugh *et al*. 1999a). Alternatively, female-mediated gene flow in grey-headed albatross would have produced similar results to those found using genetic markers.

Sex-biased dispersal

The results from the genetic analyses are not in agreement with observational studies on albatrosses that suggest high levels of philopatry (Tickell 1967; Weimerskirch *et al*. 1985; Prince *et al*. 1994; Waugh *et al*. 1999a). Mitochondrial and microsatellite analyses showed similar levels of population differences within both albatrosses, but differences were present between the two taxa. For black-browed albatrosses, mtDNA analysis showed higher levels of differentiation between the same three groups analysed using microsatellite data. As mentioned earlier, from the sex ratios of *T. melanophris* on Campbell Island, male-biased dispersal is evident. In black-browed albatrosses, this is reflected in the different F_{ST} from mtDNA and microsatellites, where F_{ST} from mtDNA is 10-fold higher than F_{ST} from microsatellites (Table 3), with the exception of the *T. impavida* group where values are about three-fold higher, suggesting male-mediated gene flow. In contrast, F_{ST} values in grey-headed albatrosses were similar for both mtDNA and microsatellite data. This could be the result of either female-mediated gene flow or the result of movements of both males and females between islands. As little information is available from banding studies on grey-headed albatross, no firm conclusions can be made.

Taxonomy

There has been much debate recently over what constitutes a species or distinct group of animals (Moritz 1994a,b; Avise & Wollenberg 1997; Paetkau 1999). Moritz (1994a) describes the differences between management units (MU) and evolutionary significant units (ESU). ESUs are two groups that show reciprocal monophyly of mtDNA haplotypes and significant differences in allele frequencies at nuclear loci. MUs on the other hand show significant differences in allele frequencies without regard to the phylogeny of the markers. Avise & Wollenberg (1997) discuss the phylogenetic species concept (PSC) which emphasizes the criteria of phylogenetic relationships and not reproductive relationships. That is, it eliminates the biological species concept requirement for hybrid sterility (Avise & Wollenberg 1997). Based on both Moritz (1994a) and Avise & Wollenberg (1997) criteria for ESU or PSC, the three groups of black-browed albatrosses would be classified as three ESUs as they are distinct using both mtDNA and microsatellite analyses. Furthermore, if the hybrids are excluded, the branching patterns from the mtDNA analysis show that each group is monophyletic.

Similar levels of genetic differences in other avian species have been found in sage grouse (*Centrocercus urophasianus*) and bluethroat (*Luscinia svecica*), both of which exhibit morphological differentiation between subspecies (Questiau *et al*. 1998; Kahn *et al*. 1999; Oyler-McCance *et al*. 1999). Colorado sage grouse show genetic differences between the small and large bodied forms with some overlap between mtDNA haplotypes (Kahn *et al*. 1999; Oyler-McCance *et al*. 1999). In comparison, bluethroat show fixed differences in mtDNA haplotypes between the two subspecies (Questiau *et al*. 1998). Other comparable studies of control region sequence in other avian taxa exhibited lower levels of sequence divergence between subspecies (1.1–3.3% in six subspecies of dunlin *Calidris alpina*, Wenink *et al*. 1996; 0.1% in two subspecies of bluethroat *Luscinia svecica*

namnetum and *L. s. svecica*, Questiau *et al*. 1998; 1.7–4.8% in seven subspecies of common chaffinch *Fringilla coelebs*, Marshall & Baker 1998), species (5.7% between *Spizella* species, Klicka *et al*. 1999) and genera (3–3.9% between *Geospiza* and *Certhidea*, Freeland & Boag 1999) than were found between the three black-browed albatross groups (4.16%–7.16%).

In this study we sampled black-browed and grey-headed albatrosses from each of five of the seven island groups where they breed. The islands from which samples could not be obtained only contribute to 0.13% (mainly at Heard Island) and 2.7% (mainly at Crozet Island) of the world population of black-browed and grey-headed albatrosses, respectively. We are confident, therefore, that our genetic analysis is sufficiently comprehensive to test current views of the taxonomic classification of these two taxa. It confirms current views on the taxonomic classification of grey-headed albatrosses. For black-browed albatrosses, the genetic distinctiveness of *T. impavida* confirms recent views that despite hybridization, this taxon merits species recognition. The degree of genetic differentiation between the Falkland Island population and the other *T. melanophris* was unexpected. If morphological, morphometric and/or ecological distinctions from the larger *T. melanophris* population can be discerned, then this taxon might also be awarded species status. As it stands, it is a very strong candidate for distinct subspecific status and qualified using either the ESU or PSU criteria.

Conservation implications

The results of our genetic analyses lead to new insights that have implications for conservation status. The current status of the black-browed albatross is lower risk–near threatened (Birdlife International 2000), based on observed declines in many populations, particularly at South Georgia, together with the apparent stability of Falkland Island populations. However, if the Falkland Islands represent a discrete breeding population, as suggested by both mtDNA and microsatellite analyses, then the status of both taxa would need to be re-evaluated. The Falkland Island population will probably remain as near threatened, as recent declines have been reported (Huin 2000b) and there is a continuing threat from longline fisheries, which are widespread in the Patagonian Shelf region. The remaining black-browed albatross populations will probably qualify as vulnerable, given the observed rates of population decline.

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This work was part of a PhD thesis by Theresa Burg examining population structure and mating systems in three albatross taxa conducted in Bill Amos's laboratory. John Croxall is head of higher predator research at British Antarctic Survey and has been studying albatrosses at South Georgia for the past 25 years.
