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Global population structure and taxonomy of the wandering albatross species complex

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Abstract

A recent taxonomic revision of wandering albatross elevated each of the four subspecies to species. We used mitochondrial DNA and nine microsatellite markers to study the phylogenetic relationships of three species (*Diomedea antipodensis*, *D. exulans* and *D. gibsoni*) in the wandering albatross complex. A small number of samples from a fourth species, *D. dabbenena*, were analysed using mitochondrial DNA only. Mitochondrial DNA sequence analyses indicated the presence of three distinct groups within the wandering albatross complex: *D. exulans*, *D. dabbenena* and *D. antipodensis*/*D. gibsoni*. Although no fixed differences were found between *D. antipodensis* and *D. gibsoni*, a significant difference in the frequency of a single restriction site was detected using random fragment length polymorphism. Microsatellite analyses using nine variable loci, showed that *D. exulans*, *D. antipodensis* and *D. gibsoni* were genetically differentiated. Despite the widespread distribution of *D. exulans*, we did not detect any genetic differentiation among populations breeding on different island groups. The lower level of genetic differentiation between *D. antipodensis* and *D. gibsoni* should be reclassified as *D. antipodensis*. Within the context of the current taxonomy, these combined data support three species: *D. dabbenena*, *D. exulans* and *D. antipodensis*.

Keywords: *Diomedea* spp., microsatellite, mitochondrial DNA, Procellariformes, seabird, Southern Ocean

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Introduction

The taxonomic history of the wandering albatross is long and complicated. Much of the confusion regarding the taxonomy is centred on the uncertainty of the origin of the type specimen used by Linnaeus (Medway 1993). Over the last 100 years, there have been no fewer than six taxonomic revisions (see Bourne 1989; Robertson & Warham 1992; Robertson & Nunn 1998). The first four revisions (see Bourne 1989) involve naming and revising the naming of the different subspecies. Populations breeding on sub-Antarctic islands in the Atlantic and Indian Oceans have been assigned to as many as five different subspecies and the name *exulans* has been applied to populations on Tristan, Antipodes and the sub-Antarctic islands in the Atlantic and Indian Oceans. It was not until 1992 that Robertson & Warham (1992) described *Diomedea antipodensis* and *D. gibsoni* breeding off New Zealand. The next revision six years later by Robertson & Nunn (1998) listed four species: *D. antipodensis*, *D. chionoptera*, *D. dabbenena* and *D. gibsoni*. Gales (1998) adopted the Robertson & Nunn (1998) taxonomy with two exceptions; wandering albatross in the South Atlantic and Indian Ocean are referred to as *D. exulans* not *D. chionoptera* and those on Tristan are referred to as *D. dabbenena* not *D. exulans*. In accordance with the recent taxonomic revisions, we have adopted the taxonomy as outlined by Gales (1998) and we use 'wandering albatross' to refer to the entire wandering albatross complex comprising all four species: *D. antipodensis*, *D. dabbenena*, *D. exulans* and *D. gibsoni*.

The first molecular revision of albatross taxonomy by Robertson & Nunn (1998) used a combination mitochondrial DNA (mtDNA) data from a limited number of taxa and morphological and behavioural data. They recommended recognizing four genera of albatrosses and suggested that all albatross subspecies be elevated to species rank. The changes in generic nomenclature have been universally adopted and many authors have followed the

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species-level recommendations. However, adequate evaluation of the status and rank of several taxa clearly requires more data and investigation (Bourne & Warham 1999). Particular examples are the southern and northern Buller's albatrosses (*Thalassarche bulleri* and *Thalassarche* sp.) and shy and white-capped albatrosses (*T. cauta* and *T. steadi*) for which de Brooke (2004) and Tennyson (2002) concluded that the available evidence did not warrant recognition at the species level.

The nature of relationships within the wandering albatross complex is also controversial, given the proposed recognition of one widespread higher latitude taxon *D. exulans* (Marion, Prince Edward, Crozet, Macquarie and South Georgia Islands) and three taxa breeding at more northerly temperate sites (Robertson & Nunn 1998). These latter taxa are essentially endemic to single islands/island groups: *D. gibsoni* at the Auckland Islands group (95% at Adams Island), *D. dabbenena* at the Tristan da Cunha group (over 99% on Gough Island) and *D. antipodensis* at Antipodes Island (with six pairs reported to breed at Campbell Island) (Gales 1998).

Available morphological, phenological and ecological data are either insufficient or equivocal for supporting the taxonomic relationships and rankings within the wandering complex. In terms of body size, *D. exulans* is significantly larger than the others, but *D. dabbenena*, *D. antipodensis* and *D. gibsoni* overlap substantially in most measurements (Cuthbert *et al*. 2003). In respect of adult plumage, *D. exulans* is the whitest [even though significant differences exist between the populations at South Georgia (southwest Atlantic) and Crozet (Indian Ocean) (Prince *et al*. 1997)] but all four taxa show considerable overlap in plumage characteristics, to the extent that reliable discrimination by simple plumage scores is not feasible (e.g. Gibson 1967; Cuthbert *et al*. 2003). However, breeding phenology shows some site and population specific differences. Laying dates for *D. gibsoni* on Adams Island are about 1–3 weeks later than for *D. exulans* and 3 weeks later than *D. antipodensis* on Antipodes Island (Marchant & Higgins 1990; Tickell 1968, 2000; Walker & Elliott 1999).

Against this background, investigation of the genetic structure within and among wandering albatross populations was essential in order to clarify the relationships among the taxa involved. Such an investigation is also extremely timely given the globally threatened status of these taxa (BirdLife International 2000, 2003) and the fact that many, if not all, of them face major threats from longline and trawl fisheries (Brothers 1991; Klaer & Polacheck 1997; Gales *et al*. 1998; Ryan & Boix-Hinzen 1999). An understanding of relationships among different populations is thus crucial for the interpretation of population trends and to evaluate the potential impact of threats, especially from fisheries. In an attempt to elucidate these relationships, the objectives of the present study were: (i) to examine phylogenetic and population genetic structure in the wandering albatross species complex; and (ii) to assess how this structure should best be reflected in the delimitations and ranking of the taxa involved.

Materials and methods

Blood samples were obtained from adult wandering albatrosses on seven island groups in the Southern Ocean (Fig. 1). All of the samples are from a single island within each island group with one exception (Marion and Prince Edward Islands). Islands within each group are often located within 10 km of each other. In total, 772 samples were screened for either mtDNA or microsatellites; with 584 of the samples originating from South Georgia. A subset of the samples was used for mtDNA sequence analyses (n = 79, Fig. 2) and restriction site analysis (n = 115 from New Zealand). These seven island groups represent all but two of the breeding sites and contain 93% of the 21 000 breeding pairs (Gales 1998). The only breeding sites that were not sampled for *Diomedea exulans* are: Macquarie Island (10 breeding pairs) and Kerguelen (1455 breeding pairs). Genomic DNA was isolated from ethanol-preserved whole blood using a Chelex extraction modified from Walsh *et al*. (1991) as described in Burg & Croxall (2001).

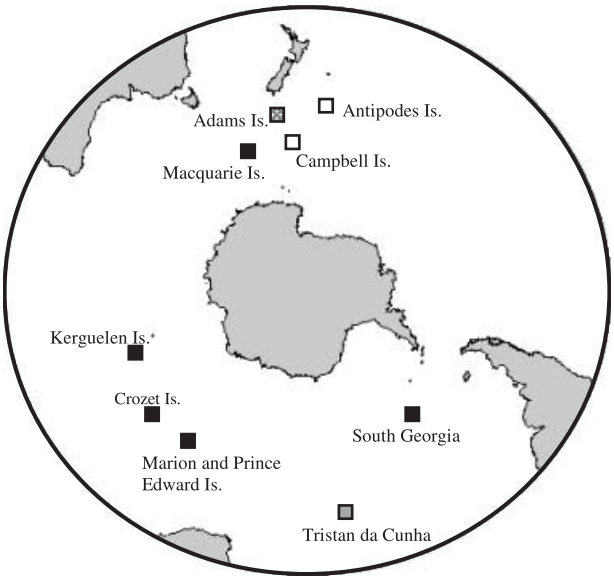

Fig. 1 Distribution of wandering albatross sampling and breeding sites. Sampling sites include *Diomedea antipodensis* (open box) on Antipodes (An) and Campbell (C) Islands, *D. gibsoni* (stippled box) on Adams (Ad), *D. exulans* (black box) on Crozet (Cr), Marion (M) and Prince Edward (PE), South Georgia (BI) Islands and *D. dabbenena* (grey box) on Tristan da Cunha (Tr). The only breeding sites not sampled are Macquarie (10 breeding pairs) and Kerguelen (1455 breeding pairs) Islands.

	Variable sites	Location						
	11111111111111122222222222222222222333333333333	Dg	Da			De		Dd
	44455577777999900022234455555788890113555666 6	Ad	An	C	Cr	M	BI	Tr
	78924834679358912608917901258745764277278035 6							
BI10m	TGTACTAGTTCACCTCATAATTCCTTACCACTGGAATATAACGTC						1	
BI19m	.....T.C.....						1	
BI103m	.....G.....T.C.....G....						1	
BI131f	.....C.....G....						1	
BI105f	.....T.C.....G.A..						1	
M1A	.....CC..G.T.....					1		
M1B	.....G.....TCC.T..T.....G....					1		
M2A	.....C.T.....G....A..					1		
M3A	.....C.....T.....G....A..					1		
M3B	.....G.....TTCC.T..T.....G....					1		
M4C	.....T.....C.....					1		
M4D	.....G.....G....A..					1		
PE5	.....C.....G.A.T					1		
PE18	.....C.....A..					1		
Cr1	.....Y.....GA..				1			
Cr2	.....T.....CG.....				1			
Cr10	.....C.....A..				1			
Cr15	.....T.C.....G....				1			
Ad3	.AC.....G.T....CG.....CCG.....T.....CG..A.T	1						
Ad12	.A.....G.T....C.....CCG.....T.....CG..A.T	1						
Ad20	.A.....G.T....C.....CCG.....T...C.CG..A.T	1						
An2	..Y.....G.T.C..C.....CCG.....T.....CG..A.T		1					
An3	.....G.T....C.R....YCG.....T.....CG..A.T		1					
An12	.....G.T.....CCG.....T.....G.AA.T		1					
An15	.....G.T.....CCG.....T.....CG..ACT		1					
An19	G.C.....G.T.....CCG.....T.....CG..T.T		1					
An21	..Y.....G.T....C.G....CCG.....T.....CG..A.T		1					
An22	..Y...G...G.T....C...C..CCG.....T.....CG..A.T		1					
C3	..Y.....G.TT...C.....CCG.....T..G..CG..A.T			1				
C4	.....G.T....Y...C..C.G.....T.....CG..A.T			1				
C5	.....G.T.....CC.....T.....CG..A.T			1				
Tr3	.....G.T..TCA.....CC....T.....G....A..							1
A	..Y.....						2	
B	.....G....				4			
C	.....T.C.....G....				1	1	1	
D	.....A.....T.C.....G....						2	
E	.....C.....A..				2	2		
F	.....G.T.....CCG.....T.....G..A.T	2						
G	.....G.T.....CCG.....T.....CG..T.T		2					
H	.....G.T.....CCG.....T.....CG..A.T	1	1					
I	.....G.T....CG.....CCR.....T.....CG..A.T		3					
J	.....G.T..T.C.....CCG.....T.....CG..A.T	7						
K	....T.....G.T....C.....C.G.....T.....CG..A.T		2					
L	.....G.T....C.....CCG.....T.....CG..A.T	6	2					
M	.....G.T....C.....C.G.....T.....CG..A.T	1		1				
N	.....G.T.C.....CCG.....T.....CG..A.T		1	1				
O	..Y.....G.T..TCA.....CC....T.....G....A..							2
SRoyal	.AG..G..CCG.T....A...C..C.G...-.TAC.....A..							
Total		20	18	5	11	12	10	3

Fig. 2 Variable sites in wandering albatross mtDNA Domain I. Sequence identity to haplotype BI10m is indicated by a '.'. Sites are numbered from the 5′-end of the control region. Haplotypes shared by multiple individuals are indicated by a single upper case letter. Geographic distribution of haplotypes is also shown (refer to Fig. 1 for abbreviations). Aligned sequence from a Southern Royal albatross is also provided for reference.

Mitochondrial DNA sequence analysis

A short 234 bp region of the hypervariable portion of the control region (Domain I) was targeted for mtDNA analyses. Genomic DNA was amplified using 200 μM dNTP, 1.5 μM $MgCl_2$, 0.2 U *Taq* DNA polymerase (Hybaid) and 5 pmol of each primer in 1x reaction buffer (75 mM Tris–HCl pH9, 20 mM $(NH_4)_2SO_4$, 0.01% Tween20) in a 25 μL reaction volume. Amplification consisted of one cycle for 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. The polymerase chain reaction (PCR) consisted of two parts. The first PCR used L16206-ND6 (5′-TAAACHG-CCCGAATYGCCCC-3′, Sorenson *et al.* 1999) and H505 (5′-GAAAGAATGGTCCTGAAGC-3′) primers. The PCR product was diluted 1 : 250 and a semi-nested PCR was performed using the same thermal profile as the initial PCR with primers H505 and L16758Glu (5′-GGYTTGAAAA-GCYGTYGTTG-3′) and sequenced using the protocols in Burg & Croxall (2001). Sequences were visually aligned using SEQED (Applied Biosystems) and any differences were confirmed by rechecking chromatograms.

Sequences were analysed using PHYLIP to estimate genetic divergence (Felsenstein 1989), MINSPNET to build minimum spanning networks (Excoffier *et al.* 1992) and AMOVA (5000 permutations) to calculate F_{ST} and estimate hierarchical population structure (Excoffier *et al.* 1992).

A nested clade analysis (NCA) was also performed on the mtDNA control region sequence data. NCA uses a permutation approach to statistically test the geographic distribution of haplotypes or groups of haplotypes (Templeton 2001). Using TCS (Clement *et al.* 2000) a statistical parsimony network was created from wandering albatross mtDNA sequences. The haplotypes were nested using the guidelines outlined in Templeton *et al*. (1987) and any network ambiguities were resolved using Crandall & Templeton (1993). GEODIS 2.0 (Posada *et al.* 2000) was used to test for significant associations among haplotypes and geographical distances and calculate clade distance (D_c, geographical spread of a clade) and nested clade distance (D_n, geographical distribution of a clade relative to other clades within the same nesting category). A revised inference key (Templeton 2004) was used to interpret the geographical association of haplotypes at the higher level clades where significant associations were found.

Mitochondrial DNA random fragment length polymorphism

One near-fixed difference corresponding to an *Alu*I site was found in the mtDNA control region for a subset of *D. antipodensis* and *D. gibsoni* samples (Burg 2000). PCR amplification conditions were as above, using the primers L436 (5′-CCTCACGAGAAAYCAGCAAC-3′) (Sorenson *et al*. 1999) and H1097 (5′-ATCGCTAGGGGAGTTTCTT-3′) located in Domains II and III, respectively. Restriction digests were performed on 115 wandering albatrosses from Adams (n = 57, *D. gibsoni*) and Antipodes (n = 58, *D. antipodensis*) Islands in 10 μL reaction volumes using 8.9 μL of PCR product, 1 μL react1 (1× buffer: 10 mM Bis Tris propane HCl, 10 mM $MgCl_2$, 1 mM DTT) and 0.8 U *Alu*I. The digest was incubated for a minimum of 90 min at 37 °C. Digests were electrophoresed on 1% agarose gel stained in ethidium bromide to visualize the DNA. If the *Alu*I site was present, two bands ~263 and 319 bp in size were present and if the restriction site was absent, a single uncut fragment (582 bp) was visible.

Microsatellites

A total of 772 wandering albatross samples, including those used for mtDNA sequence and restriction site analyses, were genotyped at nine microsatellite loci (Table 1). Details of microsatellite primers and PCR conditions are given in Burg (1999) with the exception of *De37* (De37a: 5′-ACT-CTAGAGGATCAGTTCGG-3′, De37b: 5′-AGAGCCTAA-GGAATGTGCAG-3′, 55 °C annealing temperature and no TMAC or formamide). GENEPOP 3 was used to calculate F_{ST} and to test for Hardy–Weinberg equilibrium (HWE), linkage disequilibrium and population differentiation (Raymond & Rousset 1995). De33 was found to be sex-linked and was not included in tests for population differentiation.

Results

MtDNA sequences from 79 wandering albatrosses revealed 39 variable sites in a 234 bp segment of control region domain I. Forty-seven haplotypes including 15 shared haplotypes were found (GenBank Accession nos AY016127–AY016174, Fig. 2). Six of the shared haplotypes were found on more than one island. Three distinct phylogenetic groups were present (Figs 3 and 4). The first contains *Diomedea exulans* (South Atlantic/Indian: South Georgia, Crozet, Marion and Prince Edward Islands), the second *D. gibsoni* and *D. antipodensis* (New Zealand: Adams, Antipodes and Campbell Islands) and the third *D. dabbenena* (Tristan). These three groups are separated from each other by a minimum of seven (New Zealand and Atlantic/Indian) and nine (New Zealand and Tristan) mtDNA substitutions (Fig. 3). Within-group sequence divergence was lower (0.14–1.78%) than sequence divergence among the three groups (4.53–5.22%). F_{ST} estimates between pairs of islands were high and ranged from 0.03 to 0.86. The greatest among-group variation (68.43%) was found when the albatrosses were divided into the three groups described in Fig. 3 (F_{ST} = 0.72, $P < 0.001$). This same structure was reflected in pairwise estimates of population differentiation (Table 2).

Using GEODIS, we were able to reject the null hypothesis of no geographical association of haplotypes for several of the nested clades. One one-step clade containing haplotype *L*, contained several significant values of D_c and D_n and the geographical association of haplotypes predicted restricted gene flow; however, this pattern was not significant at the higher step clades in network II. There were two significant findings at the higher level clades where we rejected the null hypothesis, specifically clade 4–1 (χ^2 = 10.37, $P < 0.03$) and the total cladogram (χ^2 = 157.00, $P < 0.001$). All clades nested within these, had at least one

Table 1 Allelic variation (A), expected (H_E) and observed (H_O) heterozygosities for the nine microsatellites used to screen wandering albatrosses. Heterozygosities that show a significant heterozygote deficit after Bonferroni correction are indicated (*)

Sampling site	*De3*	*Dc5*	*De11*	*Dc16*	*De18*	*Dc20*	*Dc27*	*De33*	*De37*	Average
Diomedea gibsoni										
Adams (n = 63)										
A	3	4	4	5	3	2	2	21	6	5.6
H_E	0.27	0.28	0.30	0.67	0.34	0.08	0.47	0.92	0.25	0.40
H_O	0.21	0.18	0.33	0.46*	0.37	0.05	0.46	0.37*	0.21	0.29
Diomedea antipodensis										
Antipodes (n = 60)										
A	4	3	5	5	2	3	2	18	7	5.4
H_E	0.35	0.52	0.48	0.20	0.28	0.10	0.48	0.84	0.58	0.43
H_O	0.28	0.36	0.38	0.22	0.27	0.10	0.49	0.41*	0.53	0.34
Diomedea exulans										
Crozet (n = 29)										
A	2	2	6	3	2	2	2	15	6	4.4
H_E	0.11	0.10	0.75	0.42	0.10	0.04	0.46	0.91	0.57	0.38
H_O	0.11	0.07	0.41*	0.48	0.10	0.03	0.52	0.65*	0.36	0.30
Marion (n = 36)										
A	2	2	4	6	2	1	2	12	6	4.1
H_E	0.06	0.07	0.64	0.36	0.06	0.00	0.49	0.91	0.80	0.38
H_O	0.03	0.07	0.44	0.28	0.06	0.00	0.34	0.46*	0.73	0.27
South Georgia (n = 584)										
A	3	4	6	7	5	3	4	15	7	6.0
H_E	0.04	0.17	0.70	0.28	0.05	0.04	0.50	0.84	0.68	0.37
H_O	0.04	0.09	0.61	0.28	0.05	0.04	0.53	0.40*	0.63	0.30

Table 2 Pairwise F_{ST} values for mtDNA (below diagonal) and microsatellites (above diagonal). Values that are significant at $P < 0.05$ after Bonferroni correction are indicated in bold. Wandering albatross samples from Campbell and Tristan Islands were not used in the microsatellite analyses

	Adams	Antipodes	Campbell	Crozet	Marion	S. Georgia	Tristan
Adams		**0.07**	—	**0.08**	**0.11**	**0.13**	—
Antipodes	**0.12**		—	**0.07**	**0.09**	**0.08**	—
Campbell	**0.23**	0.05		—	—	—	—
Crozet	**0.82**	**0.76**	**0.73**		0.02	0.02	—
Marion	**0.74**	**0.68**	**0.60**	0.03		0.02	—
S. Georgia	**0.85**	**0.79**	**0.79**	0.10	0.13		—
Tristan	**0.86**	**0.75**	**0.77**	**0.78**	**0.57**	**0.83**	

lower level clade with significantly large or small D_c and/or D_n. Using the revised inference key (Templeton 2004), the geographical pattern detected for clade 4–1 was long-distance dispersal. At the highest level, all three networks had significantly small D_c values (Networks I and II $P < 0.0001$ and Network III $P < 0.03$) and Network I had a significantly large D_n ($P < 0.001$). The inference key suggested that the pattern for the three networks (Fig. 4) is historical fragmentation.

The total number of microsatellite alleles ranged from 4 to 30 and the average expected heterozygosity ranged from 0.37 to 0.43 (Table 1). Expected heterozygosity showed considerable variation among islands for individual loci (0.04–0.92). Allelic diversity within each population was low and most loci had one or two common alleles. With the exception of *De33* (sex-linked) and two loci at individual sites (*Dc16* at Adams and *De11* at Crozet), all remaining loci were in HWE and no linkage was observed. Microsatellite analyses of the remaining eight variable loci (*De33* was excluded from analyses) revealed significant levels of population subdivision among *D. exulans*, *D. gibsoni* and *D. antipodensis* ($P < 0.001$).

Within each of the three groups (S. Atlantic/Indian Ocean, Tristan and New Zealand), significant within-group differences were found only in the New Zealand group. MtDNA random fragment length polymorphism (RFLP) analyses revealed one near-fixed difference between *D. antipodensis* and *D. gibsoni* (position 703). There was a significant difference in the presence of the *Alu*I restriction site between these

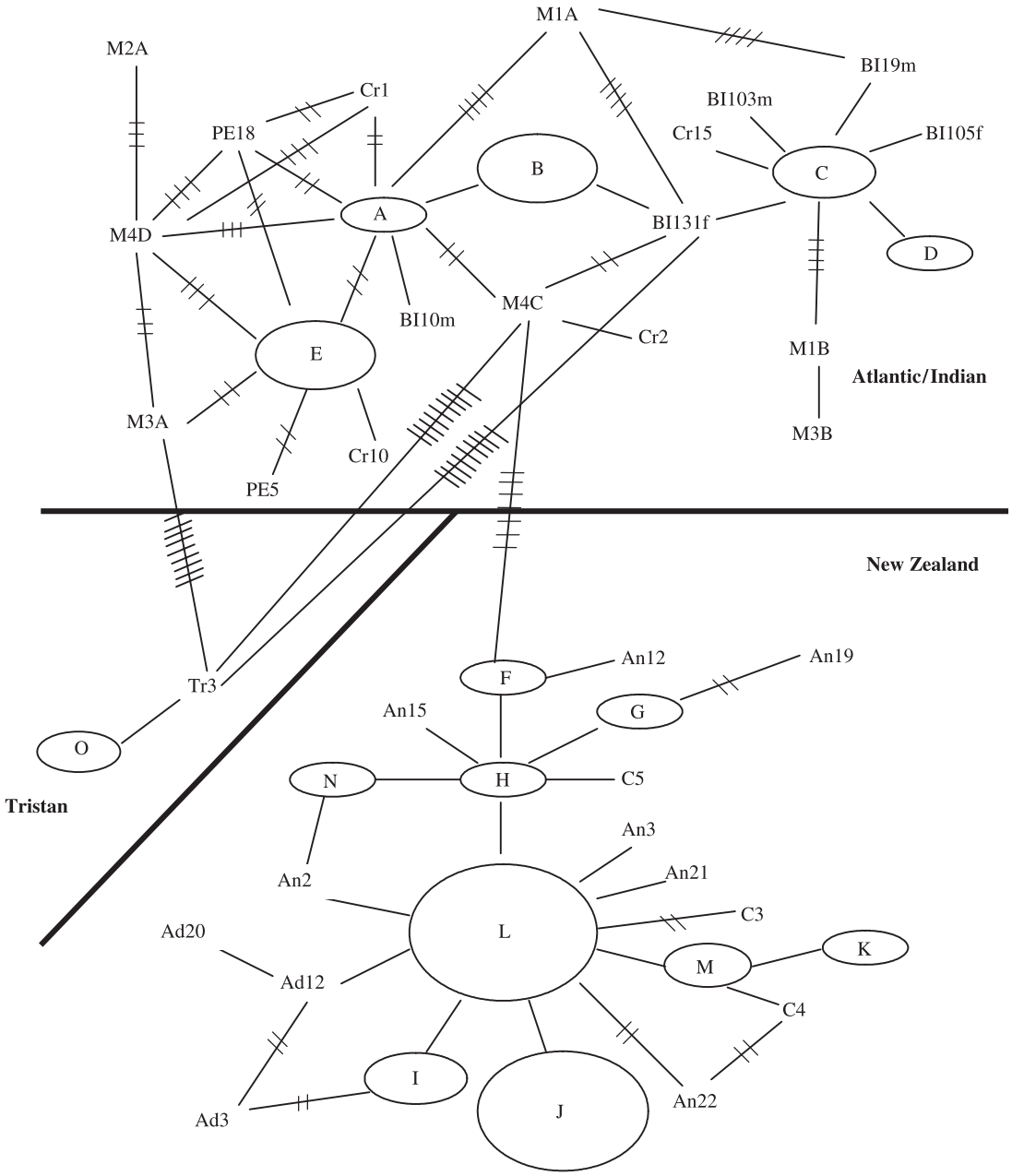

Fig. 3 Minimum spanning network of wandering albatross haplotypes based on 234 bp of Domain I control region sequence. Shared haplotypes are indicated by ovals with the area being proportional to the number of individuals sharing that haplotype (see Fig. 2 for sampling size and location). The number of nucleotide differences between haplotypes is indicated by cross-hatches.

two taxa (*D. gibsoni* 17.5%, $n = 57$, and *D. antipodensis* 72.4%, $n = 58$; exact test, $P < 0.001$). In addition, significant genetic structuring was detected in *D. gibsoni* and *D. antipodensis* using microsatellite analyses ($F_{ST} = 0.07$, $P < 0.05$, Table 2).

Discussion

Mitochondrial DNA found three distinct lineages in the wandering albatross complex comprising *Diomedea exulans*, *D. dabbenena* and *D. antipodensis*/*D. gibsoni*. Within the *D. exulans* and *D. antipodensis*/*D. gibsoni* groups, varying levels of genetic structure were present. Neither mtDNA nor microsatellite analyses were able to differentiate among *D. exulans* breeding on different sub-Antarctic islands. In contrast, both mtDNA RFLP and microsatellite analyses were able to differentiate between *D. antipodensis* and *D. gibsoni* breeding in New Zealand. However, the level of mtDNA differentiation between *D. antipodensis* and

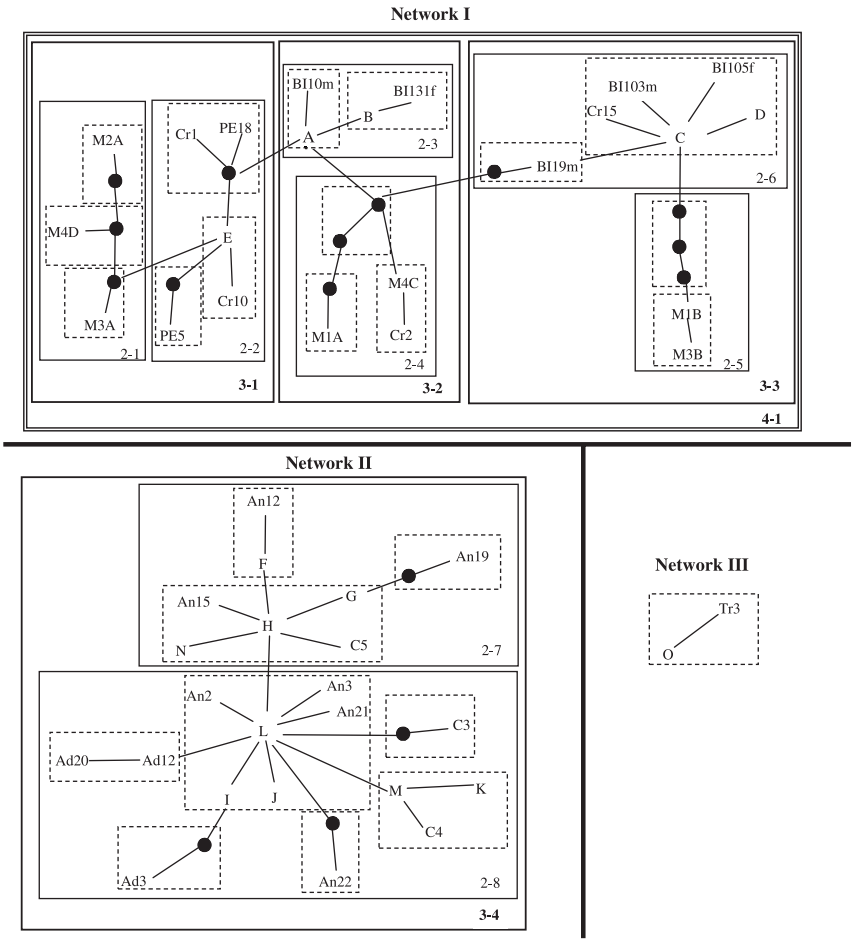

Fig. 4 Unrooted statistical parsimony networks of wandering albatross mtDNA haplotypes. Lines connecting haplotypes represent one mutational step and inferred haplotypes are indicated by filled circles. Frequency and geographical distribution of haplotypes are provided in Fig. 2. Nesting of clades is indicated by boxes. One-step clades are indicated by dashed boxes and higher level clades are represented by solid boxes and clade numbers are in the lower right corner. The three networks could not be connected within the 95% confidence limits of TCS. Network I contains individuals from the South Atlantic/Indian Ocean, Network II individuals from New Zealand and Network III all Tristan haplotypes.

D. gibsoni is lower than levels among any other species pair within the wandering albatross complex or indeed among any other albatross species (Burg & Croxall 2001; Abbott & Double 2003b). The amount of sequence divergence and the fact that haplotypes are shared between the two New Zealand species raises questions about the recently revised taxonomy.

Population differentiation in the wandering albatross complex

The three groups of wandering albatross (Tristan, S. Atlantic/Indian and New Zealand) are well supported using molecular data (this study). Behavioural and morphological data (see Introduction) show differences between birds in the South Atlantic/Indian Oceans and those found elsewhere, however, there are few morphological differences between individuals from Tristan and New Zealand. Our results from NCA suggest that this pattern was caused by historical fragmentation. Although it is difficult to envisage any physical barriers restricting gene flow in these highly mobile seabirds that inhabit vast oceans virtually free of large land masses; it is probable that nonphysical barriers are restricting gene flow among these three groups.

For *D. dabbenena* and *D. gibsoni*, current (albeit relatively limited) knowledge of their at-sea distributions suggests that their ranges are considerably less extensive than that of *D. exulans* and both show relatively little overlap with that species. Thus *D. dabbenena* is restricted to latitudes between 30° S and 50° S in the Atlantic Ocean (Cuthbert *et al*. 2003; RJ Cuthbert & P Ryan unpublished data), largely north of the range of *D. exulans*. *D. gibsoni* appears to be mainly confined to the Tasman Sea and waters around southern New Zealand (Nicholls *et al*. 2000, 2002). In contrast, *D. antipodensis* occurs mainly off eastern New Zealand during its breeding season and then migrates eastwards to the coast of Chile; its return route is apparently unknown (Nicholls *et al*. 2000, 2002).

This suggests that the main nonbreeding ranges of all four species are substantially distinct, with limited likelihood of birds approaching breeding sites of congeners, especially during the breeding season. However, this is clearly less so for *D. antipodensis* and *D. gibsoni*, especially during the breeding season. Antipodes and Adams Islands are < 1000 km apart; this is similar to the distance between Marion and Crozet Islands, sites between which persistent interchange of individuals is known to occur.

One apparent paradox arising from our findings is that both the mtDNA and microsatellite data indicate the wandering albatross populations breeding on islands separated by 7000–10 000 km show different patterns of gene flow. For example, populations at South Georgia, Marion/Prince Edward and Crozet apparently experience sufficient gene flow to counteract genetic drift, yet this interchange extends neither from South Georgia to Tristan da Cunha (based on mtDNA alone), nor from Crozet to the New Zealand sub-Antarctic islands. Furthermore, the genetic data provide evidence of greater separation between the populations on Antipodes and Adams Islands (700 km apart) than among any of the island populations within *D. exulans sensu stricto*. Some of this may be explained by considering our knowledge of the foraging range, migration and dispersal of the taxa involved. Thus, although during the breeding season the foraging ranges of *D. exulans* populations are confined to adjacent oceanic areas, e.g. the South Atlantic (for South Georgia) and the western central Indian Ocean (for Marion/Prince Edward and Crozet), eastward postbreeding migrations to Australia are well established (Jouventin & Weimerskirch 1990; Prince *et al*. 1998; Nicholls *et al*. 2000, 2002) for all populations. The Indian Ocean populations appear to return via a westward migration (Nicholls *et al*. 2000), whereas South Georgia birds complete an eastwards circumpolar journey (British Antarctic Survey unpublished data). Dispersal of juveniles is less well known but probably follows similar patterns, though doubtless involving more extensive distributions.

These patterns of movement suggest substantial potential for interchange, particularly among the populations of the Indian Ocean, but also including South Georgia. A recent model of *D. exulans* dispersal (Inchausti & Weimerskirch 2002), including all breeding sites, but using recapture data only for chicks banded at Crozet, concluded that, whereas adults are highly philopatric, juvenile dispersal rates may be sufficient to produce metapopulation structure throughout the range of this species. This theoretical evaluation is clearly supported by our genetic analyses including NCA, which predicted long-distance dispersal of *D. exulans* among the different South Atlantic and Indian Ocean islands. It is also substantiated by documented exchange of birds between Marion Island (Prince Edward Islands) and Possession Island (Crozet Islands), 1000 km apart (Cooper & Weimerskirch 2003). Using banding data from 1601 adults and 4364 chicks, there were 61 reported exchanges over more than 25 years. Of these, 57 were from Crozet to Prince Edward, including 18 fledglings banded at Possession and breeding at Marion; only four exchanges, including one fledgling banded at Possession and breeding at Marion, were in the opposite direction.

Cooper & Weimerskirch (2003) suggest, reasonably, that these two populations form a metapopulation that should be treated, for conservation purposes, as a single unit. In contrast, from the more than 8000 adults and 20 000 chicks banded at South Georgia (Prince *et al*. 1998; BAS unpublished data), there have been no recaptures reported at either Marion or Possession Islands. Similarly, in over 20 years of intensive recapture operations at South Georgia, only two wandering albatrosses, both from Possession Island, have been recorded (and only as visitors). This suggests that very low rates of interchange may be sufficient to maintain genetic homogeneity in these populations. This raises the question of what has prevented similarly low rates of interchange occurring within and among the other populations in the complex.

In contrast to *D. exulans*, wandering albatross in New Zealand show evidence of restricted gene flow among breeding sites. Genetic differentiation between *D. antipodensis* (Antipodes and Campbell Island) and *D. gibsoni* (Adams Island) was detected using both mtDNA RFLP and microsatellite DNA. In addition, NCA predicted that geographical distribution of haplotypes in one of the one-step clade is consistent with restricted gene flow. Although this finding does not extend to the entire New Zealand group, it does encompass haplotypes from almost half of the New Zealand samples.

The higher degree of population subdivision in wandering albatross off New Zealand (*D. antipodensis* and *D. gibsoni*) found using microsatellites compared with mtDNA is similar to that found in shy albatross (*Thalassarche cauta*, Abbott & Double 2003a,b). Using mtDNA control region sequences, no genetic structure was detected in shy albatross breeding on three islands off Tasmania (Abbott & Double 2003b), but significant differences were found among the same sites using microsatellite markers (Abbott & Double 2003a).

Taxonomy of wandering albatross

As indicated in the Introduction, the information available on morphometrics, timing of breeding and plumage characteristics do not, of themselves, provide unequivocal evidence either of taxonomic relationships or rankings of taxa in the wandering albatross complex. There is a clear need for the collection of further data to enable critical comparison between the morphological and ecological features of the different island populations in this complex. In the meantime, however, basing taxonomy and nomenclature extensively on the genetic evidence from our study would seem most appropriate. The new insights from our work are:

1 Strong support for earlier conclusions that the large, white-plumaged wandering albatross *D. exulans*, breeding on the more southerly sub-Antarctic islands, constitutes a single taxonomic unit.
2 Clarification that, despite size and plumage similarities, the wandering albatross population on Tristan da Cunha,

D. dabbenena, is genetically distinct from *D. exulans*. Despite a similar breeding latitude and habitat, it is almost equally distinct from the wandering albatross breeding at the New Zealand sub-Antarctic islands.

3 The populations on the New Zealand sub-Antarctic islands are very closely related.

Accordingly, it is clear that DNA-based data do not support the classification of *D. antipodensis* and *D. gibsoni* as two distinct species. The preliminary reclassification was based on incomplete sampling (Nunn *et al*. 1996; Robertson & Nunn 1998); however, Nunn and Robertson later obtained sequence data from one member of each taxon (Nunn & Stanley 1998) and found 1 bp difference in 1143 bp of cytochrome *b* sequence (GenBank Accession nos AF076047 and AF076050). In this study, we examined 18 and 20 control region sequences from *D. antipodensis* and *D. gibsoni*, respectively, and found that not only were haplotypes shared between the two species, but that the level of genetic divergence between *D. antipodensis* and *D. gibsoni* (0.97%) was lower than between any other closely related species of albatross (Burg & Croxall 2001; Abbott & Double 2003b). Compare this with levels of sequence divergence between other species of albatross: *D. exulans* and *D. dabbenena* (4.8%, this study), *D. exulans* and New Zealand wandering albatross (5.2%, this study), *D. dabbenena* and New Zealand wandering albatross (4.5%, this study), *T. cauta* and *T. steadi* (1.8%, Abbott & Double 2003b) and *T. impavida* and *T. melanophris* (7.2%, Burg & Croxall 2001). Thus, we suggest that elevation of *D. antipodensis* and *D. gibsoni* to species status was premature and recommend that they should be reclassified as *D. antipodensis*. Although our mtDNA analyses confirm that the six breeding pairs on Campbell Islands belong to the New Zealand group, and AMOVA shows that they were undifferentiated from *D. antipodensis*, our sample size ($n = 5$) was too small for microsatellite analysis and we were therefore unable to determine conclusively whether they were more similar to birds from Adams or Antipodes Islands.

Conservation and management

Longline fisheries pose a considerable threat to wandering albatrosses. Given that birds forage over large geographical areas and have low reproductive output, wandering albatross are probably one of the most susceptible seabirds to such activities (Weimerskirch 1998; Nicholls *et al*. 2000, 2002; Tuck *et al*. 2001; Prince *et al*. 1998 Croxall *et al*. 1998). Over the last four decades, wandering albatross populations have declined in excess of 20% throughout their range (Croxall & Gales 1998; Croxall *et al*. 1998) and all populations are listed as vulnerable under IUCN (Croxall & Gales 1998). As such, an accurate assessment of wandering albatross phylogenetic structure is crucial for effective management of this species complex.

Both our mtDNA, microsatellite results indicate that wandering albatrosses from the Atlantic/Indian Group (*D. exulans*) form one Evolutionarily Significant Unit (ESU; Moritz 1994) and the birds from the New Zealand group (*D. antipodensis*/*D. gibsoni*) form a second ESU. Although we only have mtDNA data from a small number of samples from the Tristan group (*D. dabbenena*; $n = 3$), the level of mtDNA divergence between it and other members of the complex suggests that these birds should be treated as a third ESU. Because wandering albatrosses from Antipodes (*D. antipodensis*) and Adams Islands (*D. gibsoni*) show significant differences from each other in terms of microsatellite allele frequencies and mtDNA data, but no fixed mtDNA differences, birds from these two islands should form two Management Units (MU, Moritz 1994). This is also logical in the light of their very different ranges at sea, especially wintering areas, which are likely to necessitate different management strategies.

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