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Postsealing genetic variation and population structure of two species of fur seal (*Arctocephalus gazella* and *A. tropicalis*)

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Abstract

Commercial sealing in the 18th and 19th centuries had a major impact on the Antarctic and subantarctic fur seal populations (*Arctocephalus gazella* and *A. tropicalis*) in the Southern Ocean. The intensive and unrestricted nature of the industry ensured substantial reductions in population sizes and resulted in both species becoming locally extinct at some sites. However, both species are continuing to recover, through the recolonization of islands across their former range and increasing population size. This study investigated the extent and pattern of genetic variation in each species to examine the hypothesis that higher levels of historic sealing in *A. gazella* have resulted in a greater loss of genetic variability and population structure compared with *A. tropicalis*. A 316-bp section of the mitochondrial control region was sequenced and revealed nucleotide diversities of 3.2% and 4.8% for *A. gazella* and *A. tropicalis*, respectively. There was no geographical distribution of lineages observed within either species, although the respective Φ_{ST} values of 0.074 and 0.19 were significantly greater than zero. These data indicate low levels of population structure in *A. gazella* and relatively high levels in *A. tropicalis*. Additional samples screened with restriction endonucleases were incorporated, and the distribution of restriction fragment length polymorphism (RFLP) and sequence haplotypes were examined to identify the main source populations of newly recolonized islands. For *A. tropicalis*, the data suggest that Macquarie Island and Iles Crozet were probably recolonized by females from Marion Island, and to a lesser extent Ile Amsterdam. Although there was less population structure within *A. gazella*, there were two geographical regions identified: a western region containing the populations of South Georgia and Bouvetøya, which were the probable sources for populations at Marion, the South Shetland and Heard Islands; and an eastern region containing the panmictic populations of Iles Kerguelen and Macquarie Island. The latter region may be a result of a pronounced founder effect, or represent a remnant population that survived sealing at Iles Kerguelen.

Keywords: *Arctocephalus*, fur seal, mtDNA, population genetics, subantarctic

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Introduction

Commercial sealing during the 18th and 19th centuries resulted in substantial declines in the number and size of fur seal populations throughout the Southern Ocean (Bonner & Laws 1964). The Antarctic and subantarctic fur seal (*Arctocephalus gazella* and *A. tropicalis*) suffered differing degrees of exploitation during this time, with the former reportedly brought to the brink of extinction (Bonner & Laws 1964). However, since the cessation of

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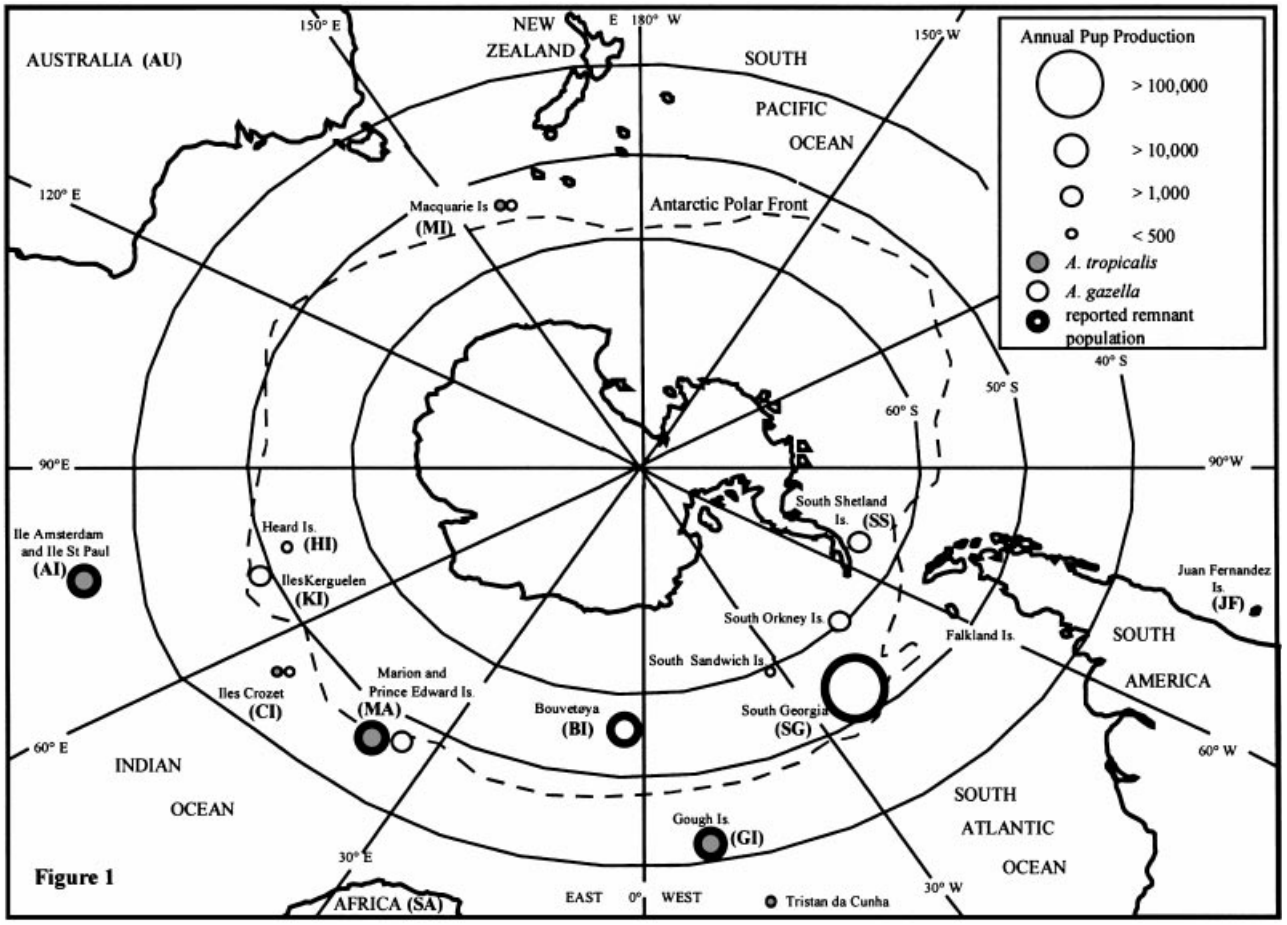

Fig. 1 Map of the islands in the subantarctic region upon which *Arctocephalus gazella* and *A. tropicalis* breed. Reported remnant populations and indications of current population size are shown through estimates of annual pup production figures (Hofmeyr *et al*. 1997; Isaksen *et al*. 1997; Shaughnessy *et al*. 1998; S. D. Goldsworthy, unpublished). Map modified from original by John Cox (Australian Antarctic Division).

sealing, these species have recolonized islands throughout their former range, with most populations experiencing rapid increases in size (Hofmeyr *et al*. 1997; Wickens & York 1997). The Antarctic fur seal currently breeds on islands predominantly to the south of the Antarctic Polar Front (APF) (Fig. 1). Approximately 97% of the species occur in populations at South Georgia (Hofmeyr *et al*. 1997), but its range has recently extended as far east as Macquarie Island. Subantarctic fur seals breed on islands to the north of the APF with the major concentrations occurring at Gough, Amsterdam and the Prince Edward Islands (Hofmeyr *et al*. 1997). Antarctic and subantarctic fur seals occur sympatrically at Iles Crozet, the Prince Edward and Macquarie Islands (Fig. 1). Hybridization has been reported at two of these sites (Condy 1978, 1983b; Kerley 1983a; Shaughnessy & Fletcher 1987; Goldsworthy *et al*. 1999).

The discovery of South Georgia in 1775 by James Cook led to the commencement of sealing in this region (Bonner 1958). The vast numbers of seals reported here and on islands further south were harvested in such an intense and indiscriminate fashion, that stocks were rapidly exhausted (Headland 1984). Such a pattern of discovery and subsequent depletion was paralleled throughout the subantarctic. As fur seal stocks declined, the focus shifted to the exploitation of southern elephant seals (*Mirounga leonina*) and southern right whales (*Eubalaena australis*), which were harvested for their blubber (Roberts 1950; Bonner & Laws 1964). Fur sealing was most intense at islands that contained, or were close to, large populations of these other species, because fur seals continued to be killed whenever they hauled out (Rand 1956; Bonner & Laws 1964). Many local extinctions resulted, and because the largest populations of elephant seals were on islands south or just north of the APF, *A. gazella* was the most severely exploited. This species suffered a major range contraction and was considered virtually extinct early in the 20th century (Bonner & Laws 1964). However, records

from this time indicate that it is probable that *A. gazella* survived in remnant populations at Bouvetøya (numbering approximately 1000–1200 in 1928; Olstad (1929) as cited in Fevoden & Sømme (1976)), and on islands off the northwest coast of South Georgia (Bird Island and the Willis group — estimated at less than 100 in the 1930s; Bonner 1968; Laws 1973). This species has since recovered with a world-wide annual pup production of approximately 400 000 (Hofmeyr *et al*. 1997; Isaksen *et al*. 1997).

Records indicate that prior to sealing, *A. tropicalis* were abundant on the Tristan da Cunha group, Ile Amsterdam and Ile St Paul (Clark 1875; Wace & Holdgate 1976). As major populations of *A. tropicalis* occur on islands north of the APF which did not support large populations of elephant seals, they were probably visited by sealers only when fur seal numbers were large enough to ensure an economic return. As such, local extinctions in this species are known to have occurred only at Tristan da Cunha and Ile St Paul (Shaughnessy 1982; Roux 1987). The postsealing status of *A. tropicalis* is questionable at a number of sites, such as on Prince Edward Island (De Villiers & Ross 1976) and Iles Crozet. There is no evidence that this species occurred at the latter site prior to sealing (Jouventin *et al*. 1982; Roux 1987), while the identity of the species occurring at Macquarie Island prior to sealing is unknown (Shaughnessy & Fletcher 1987; Richards 1994). There were, however, three remnant populations of *A. tropicalis* documented: at Gough, Amsterdam and Marion Islands (Roux 1987; Bester 1987; Kerley 1987). It is at these three islands that the bulk of this species currently resides, containing approximately 99% of the annual pup production for *A. tropicalis* occurring here (73 000 recorded between 1988 and 1994; Hofmeyr *et al*. 1997).

The aim of this study was to examine postsealing mitochondrial DNA variation in *A. gazella* and *A. tropicalis* to determine whether differences in their respective exploitation histories are reflected in the levels and distribution of observed genetic variation. We use reports of historic sealing records and contemporary data on population recovery to examine two hypotheses. First, if *A. gazella* has passed through a more intense population bottleneck, then it is expected that this species will exhibit lower levels of genetic variation compared with *A. tropicalis*. Second, given that *A. gazella* suffered a greater reduction in range due to commercial exploitation, this species will exhibit reduced population structure relative to *A. tropicalis*.

Materials and methods

Sample collection

Skin biopsies were collected from both *Arctocephalus tropicalis* and *A. gazella* from all the major populations across each species' range. These include South Georgia (SG), South Shetland Islands (Seal Island and Cape Shireff) (SS), Bouvetøya (BI), Marion Island (MA), Iles Crozet (CI), Iles Kerguelen (KI), Heard Island (HI) and Macquarie Island (MI) for *A. gazella*; and Iles Crozet (CI), Ile Amsterdam (AI), Gough Island (GI), Marion Island (MA) and Macquarie Island (MI) for *A. tropicalis*. A number of samples from this species were also available from vagrant seals found in the Juan Fernandez Islands (JF), South Africa (SA) and Australia (Melbourne Zoo) (AU) (Table 1).

For all populations, pups were targeted to ensure that members of the breeding population, and not vagrants, were being sampled. Such a protocol also ensured that the sampled individuals were not full-sibs. At populations where *A. gazella* and *A. tropicalis* occur sympatrically, efforts were made to ensure that individuals sampled were not phenotypic hybrids. At MI, where a large proportion of the pups born annually are hybrid, and the *A. tropicalis* population is small (pup production for 1997/1998 = 27; S. D. Goldsworthy *et al*., accepted) it was possible that some samples were from hybrid pups. As such, the *A. tropicalis* samples incorporated into the sequence analysis from MI were consistent with the adopted sampling regime, but this could not be guaranteed for the restriction fragment length polymorphism (RFLP) analyses (described below).

Biopsies were obtained using a 6-mm biopsy punch and stored in salt-saturated 20% dimethylsulphoxide (DMSO). Attempts were made to obtain samples from at least 20 individuals from each population for DNA sequencing, and an additional number (~ 20 per population) for RFLP analysis (Table 1). This was not possible for all sites. For HI, only seven of the 40 biopsies collected from *A. gazella* were from pups and only five of the *A. gazella* samples obtained from SS (Seal Island) were sequenced. As a result of the problem of hybridization outlined above, there were only 17 sequences obtained for *A. tropicalis* at MI, and a further 12 individuals screened for RFLPs.

Additional control region sequences were obtained from the New Zealand fur seal, *A. forsteri* to assist in examining phylogenetic relationships. Individuals from across the species' geographical distribution were included. Fifteen of these sequences were from Lento (1995), one from Slade *et al*. (1994) (GenBank Accession no. UO3576) and another from an individual at Taronga Zoo, Australia (sequenced as part of this study). A harbour seal sequence (*Phoca vitulina*) (Arnason & Johnsson 1992; GenBank Accession no. X63726 S37044) was used as an outgroup.

Laboratory analysis

Total genomic DNA was extracted from each skin biopsy using a CTAB (hexadecyltrimethylammonium bromide)/

Table 1 Populations of *Arctocephalus gazella* and *A. tropicalis* sampled. Individuals screened for restriction fragment length polymorphisms (RFLPs) were additional to those sequenced. All samples collected from pups unless otherwise stated

Population	Acronym	Sequenced	RFLP only	Comments
A. gazella				
South Shetland Island	SS	5[1]	26[2]	[1]Seal Island, [2]Cape Shireff
South Georgia	SG	20	20	
Bouvetøya	BI	20	20	
Marion Island	MA	20	34	
Iles Crozet	CI	20	20	
Iles Kerguelen	KI	20	20	
Heard Island	HI	20[3]	20	[3]Only seven are pups
Macquarie Island	MI	20	30	
		145	**190**	
A. tropicalis				
Gough Island	GI	20	19	
Marion Island	MA	20	19	
Iles Crozet	CI	20	19	
Ile Amsterdam	AI	20	20	
Macquarie Island	MI	17	12	
South Africa	SA	1		Vagrant
Australia	AU	2		Vagrants
Juan Fernandez	JF	3		Vagrants
		103	**89**	
A. forsteri				
Australia/New Zealand		17	0	

proteinase K incubation procedure and phenol–chloroform extraction adapted from Grewe *et al*. (1993). DNA was precipitated using a standard ethanol precipitation protocol (Grewe *et al*. 1993) and resuspended in sterile distilled water. A 457-bp fragment of the maternally inherited mitochondrial $tRNA^{thr}$ control region was amplified using polymerase chain reaction (PCR). In a 25-μL reaction volume: 17.775 μL of milliQ water, 0.125 μL of 10 mM dNTPs, 1.5 μL of 25 mM $MgCl_2$, 2.5 μL of 10× buffer (500 mM KCl, 100 mM Tris pH = 9.0, 1% Triton X), 1.0 μL each of 10 μM primers: TDKD (Slade *et al*. 1994) and L15926 (Kocher *et al*. 1989), 0.1 μL of *Taq* polymerase (5–10 units), 1.0 μL of extracted DNA and overlaid with oil. The amplification parameters are as outlined in Slade *et al*. (1994). The product was purified by gel purification using 1.5% agarose in Tris acetate EDTA.

A 316-bp fragment of the PCR product was sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer) using internal primers: Thr/Pro (5′-TCCCTAAGACTCAAGGAAGAG-3′) and Cent (5′-GAGCGAGAAGAGGTACACTTT-3′). Both internal primers were designed for this study using an *A. forsteri* sequence (Slade *et al*. 1994). The fragment was sequenced initially from the 5′ end and only sequenced from the 3′ end if the first sequence was too short and/or there were too many ambiguous sites. The sequenced product corresponds to sites 68–373 of the GenBank sequence for *A. forsteri* (UO3576).

Additional samples from each population were screened with a series of restriction endonucleases in order to enable a frequency-based analysis for the examination of population structure. The sequences obtained above were used as a template to identify a series of restriction sites that could recognize species haplotypes as well as group individuals into the clades identified from the sequence analysis. DNA was extracted and amplified as above using internal primers before digestion with each enzyme. *Nde*I and *Tsp*509I were the enzymes employed to identify species haplotypes by targeting specific differences in the sequences. Additional enzymes, namely *Bcl*I, *Ssp*I and *Hin*fI were employed to classify further each individual into one of the major clades identified by the neighbour-joining (NJ) tree (for *A. tropicalis* only). All enzymes were obtained from New England Biolabs. Digestion of the PCR product proceeded as directed by the manufacturer, but using 4 units of enzyme per reaction instead of 5 for all enzymes except *Nde*I, which required 6 units. Digests were run out on 2% NuSieve 3:1 agarose (FMC BioProducts) stained with ethidium bromide and scored over an ultra-violet illuminator.

Data analysis

Sequences were examined using SEQED (version 1.0.3; Applied Biosystems) to ascertain quality and to verify the scoring. Sequences were aligned using CLUSTAL W

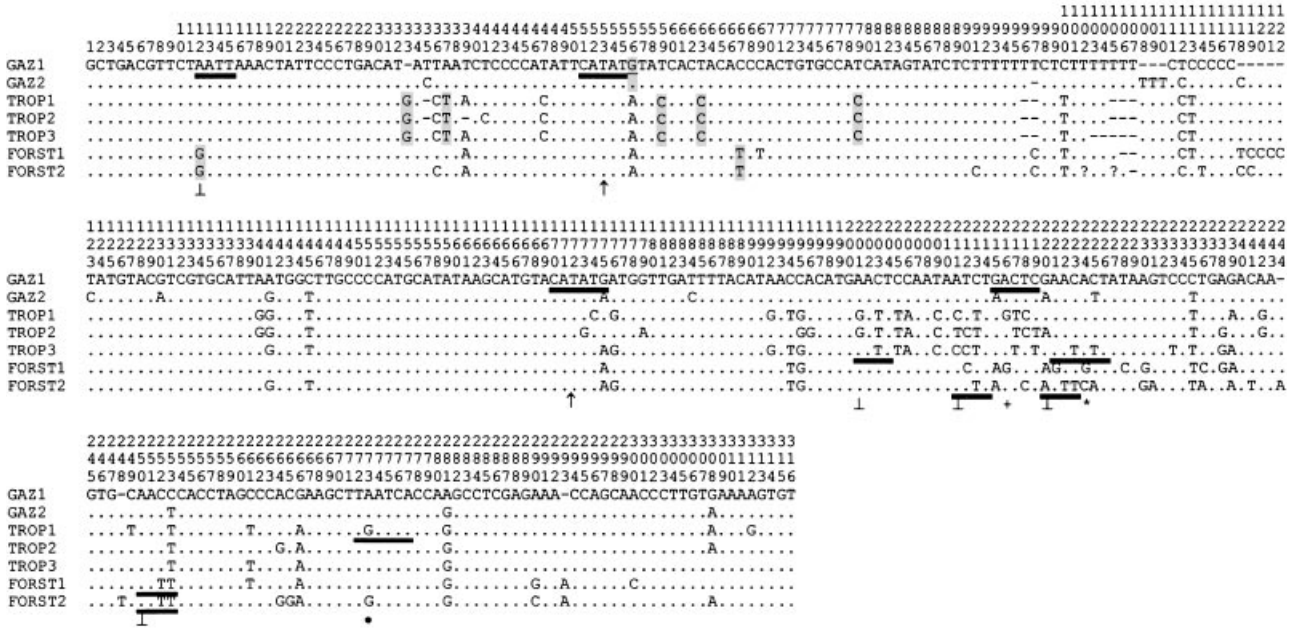

Fig. 2 The 316-bp sequence of the mitochondrial tRNA^{thr} control region from three species of *Arctocephalus*. Identity of sequences as follows: Gaz1 and Gaz2 from *A. gazella*, haplotype numbers 01 and 03; Trop1, Trop2 and Trop3 are haplotypes 01, 18 and 22, respectively, from *A. tropicalis*; and Forst1 and Forst2 from *A. forsteri* are haplotypes A and E as determined by Lento (1995). Shaded regions indicate species-specific nucleotide differences. Underlined sequences indicate recognition sequences of restriction endonucleases, and the markings below each block of sequence indicate the restriction site as follows: ↑ = *Nde*I which distinguishes *A. gazella* from the other species; ⊥ = *Tsp*509I which distinguishes *A. tropicalis* from *A. forsteri*; + = *Hin*fI which distinguishes between two groups of *A. gazella*; * = *Ssp*I which distinguishes clade 3 of *A. tropicalis* from clades 1 and 2; ● = *Bcl*I which distinguishes clade 1 of *A. tropicalis* from clade 2.

(Thompson *et al*. 1994) and resulting alignments were evaluated by eye and corrected where required. All sites containing insertion/deletions and/or missing information were removed prior to further analysis. Data from individuals that were not sampled as part of a breeding population (i.e. the vagrants) were not included in any population analyses.

The program MEGA (Kumar *et al*. 1993) was used for creating NJ trees which were based on distances calculated using Kimura's 2-parameter model (Kimura 1980). The method of tree construction was based on the algorithm of Saitou & Nei (1987). A bootstrap test was performed on each tree and values were obtained after 1000 replications. Data on polymorphic sites, nucleotide diversities and divergences within populations and species were obtained using the program DNASP (Rozas & Rozas 1997). Uncorrected nucleotide diversities (π) were calculated from Nei (1987; equations 10.5 or 10.6). Uncorrected nucleotide divergence data (D_{xy} and D_a) were obtained from respective equations 10.20 and 10.21 (Nei 1987).

To examine within-species population structure based on sequence data, the analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was employed using the program ARLEQUIN (Schneider *et al*. 1997). Population pairwise Φ_{ST} were calculated in ARLEQUIN based on both sequence data and sequence haplotype frequencies (Weir & Cockerham 1984; Weir 1990). Testing for differences between populations was performed by permuting haplotypes between populations and presenting a *P*-value that is the proportion of permutations showing a Φ_{ST} greater than or equal to the observed one. All significance levels were adjusted for multiple comparisons using the Bonferroni test (Rice 1989).

Analysis of heterogeneity was conducted using the MONTE program within REAP (McElroy *et al*. 1992) where significance testing of the estimate of χ^2 was calculated after 1000 replications (Roff & Bentzen 1989) and adjusted for multiple comparisons (Rice 1989). Analysis of isolation by distance was performed by regressing $\Phi_{ST}/1 - \Phi_{ST}$ with the natural logarithm of geographical distance in GENEPOP (version 3.1; Raymond & Rousset 1995; Rousset 1997).

Results

A total of 248 sequences was obtained for analysis, which comprised 103 from *Arctocephalus tropicalis* and 145 from *A. gazella* (Table 1). A further 17 sequences from *A. forsteri* were used for comparison. Significant length variation was observed in all sequences, primarily due to a highly variable TC region from site 91–122 (Fig. 2). This 'TC landmark' (as recognized by Lento (1995)) caused problems with alignment, despite highly conserved flanking

Table 2 (a) Sequence and haplotype statistics for three species of *Arctocephalus* and their populations. Sequences had all insertion/deletions, sites with missing information and the TC landmark removed. (b) Nucleotide diversity (D_{xy}) and divergence (D_a) between populations above and below the diagonal, respectively

(a)	Number of individuals	Total number of haplotypes	Haplotypes $n > 1$	Haplotypes unique to a population	Variable sites	Nucleotide diversity
A. gazella	145	26	16	10	45	0.032
BI	20	9	4	3	37	0.042
CI	20	8	4	1	28	0.029
HI	20	11	4	1	35	0.031
HI-P	7	4	2	0	14	0.025
KI	20	7	5	1	21	0.023
MI	20	9	5	3	23	0.021
MA	20	8	4	0	34	0.034
SG	20	8	4	0	28	0.032
SS	5	4	1	1	11	0.022
A. tropicalis	103	33	13	28	46	0.048
AI	20	9	4	7	28	0.041
CI	20	8	4	5	35	0.044
GI	20	8	5	6	33	0.030
MA	20	9	3	6	31	0.045
MI	17	9	4	4	32	0.046
A. forsteri	17	16	1	15	40	0.051

(b)	*A. gazella*	*A. tropicalis*	*A. forsteri*
A. gazella	—	0.122	0.091
A. tropicalis	0.080	—	0.123
A. forsteri	0.050	0.075	—

HI-P, Heard Island pups only.

regions, due to the length variations and polymorphisms. Sequences varied in length from 294 to 309 bp, but were all aligned to form a 316-bp data matrix (Fig. 2). The TC landmark was removed from all individuals after alignment and prior to analysis.

Interspecific analysis

Species-specific sequence differences were observed through fixed polymorphisms as well as length variation in the TC landmark (Fig. 2). Overall there were eight fixed differences between the three species, all within the first 80 bp. These largely confirm those found by Goldsworthy *et al*. (1999) who identified a total of 11 differences in the same region. One was altered due to alignment differences and the other two were found not to be fixed across species when a larger sample size was examined. There were five fixed differences observed between *A. gazella* and *A. forsteri*, 13 between the latter and *A. tropicalis*, and nine between *A. tropicalis* and *A. gazella*. In *A. gazella* and *A. forsteri* sequences, greater length variation was observed in the TC landmark relative to *A. tropicalis* sequences, which were much more conserved. Summary sequence details of each species are presented in Table 2 and the phylogenetic relationships among the species are shown in Fig. 3. This phylogeny employed the range of lineages for both *A. gazella* and *A. tropicalis* that were obtained in this study. While *A. tropicalis* forms a well-supported monophyletic group, the relationship between *A. gazella* and *A. forsteri* is paraphyletic, with the latter species characterized by two highly divergent clades.

Intraspecific analysis

A. tropicalis. DNA from 103 individuals was sequenced for 316 bp of the $tRNA^{thr}$ control region. Included in these samples were seals from five major breeding populations (Fig. 1, Table 1) plus vagrants from JF (n = 3), AU (n = 2) and SA (n = 1). Three individuals from MI were found to have a control region sequence haplotype of *A. gazella*, despite having phenotypic characteristics of *A. tropicalis*. These putative hybrids were not included in any further analyses.

There were 33 haplotypes, 13 of which were represented in more than one individual. The relationships of

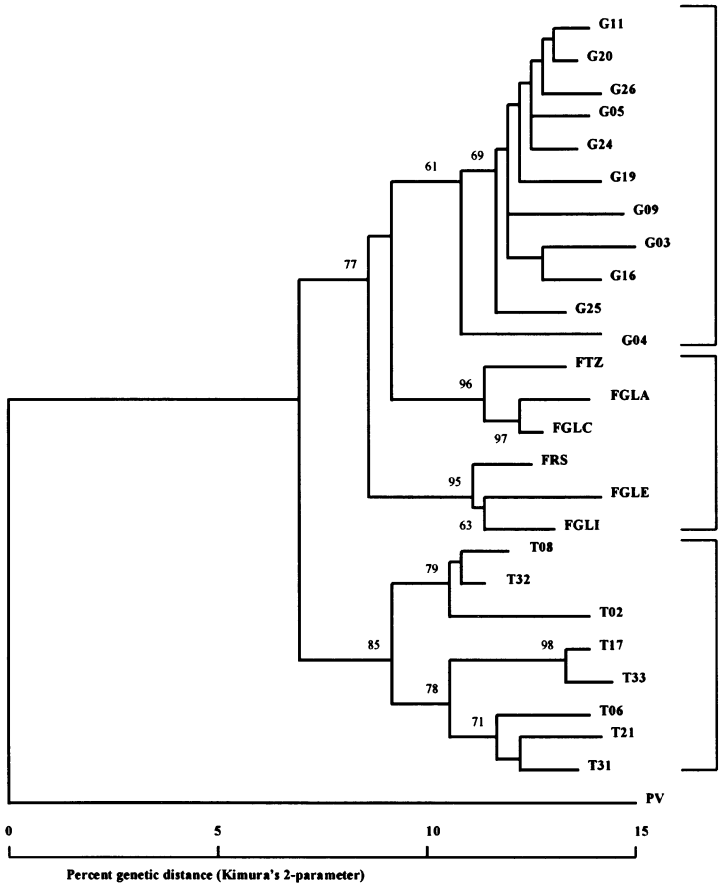

Fig. 3 Neighbour-joining (NJ) tree of representative haplotypes from three species of fur seal with *Phoca vitulina* as the outgroup. The letter of each of the labels denotes the species (G = *Arctocephalus gazella*; T = *A. tropicalis*; F = *A. forsteri*), while the number corresponds to the haplotype. For *A. forsteri*, FRS are from Slade *et al*. (1994), FGL are from Lento (1995: where the last letter corresponds to haplotypes identified therein) and FTZ is from Taronga Zoo, Australia. Bootstrap values are shown only at nodes which were supported in over 60% of the 1000 replications.

these haplotypes to each other, the variable sites that characterize each haplotype and their geographical distribution are displayed in Fig. 4. There was a high degree of lineage structure within the species, with three divergent clades apparent, but no obvious geographical structure in the distribution of lineages. The nucleotide diversities within clades were low (2.1, 0.5 and 1.1% for I, II and III, respectively) relative to the overall value of 4.8% for the species. Sequence statistics for the species and each population are presented in Table 2. Each population had a high level of diversity relative to the low within-clade diversities, reflecting the presence in each population of representatives from more than one clade. This was further reflected in the AMOVA results which indicated that 81% of the variation was distributed within, rather than among populations. An overall Φ_{ST} value of 0.19 was calculated for *A. tropicalis*. To examine the level of population structure within the species, pairwise Φ_{ST}s were calculated and are presented in Table 3. Those calculated based solely on haplotype distribution showed significant structure for eight of the 10 pairwise comparisons. However, when molecular information was also considered, the number dropped to only four. Overall, the latter Φ_{ST} values were higher than those calculated on haplotype frequency alone, which is not unexpected. However, the CI–MI and AI–GI pairwise Φ_{ST} values from haplotype frequency alone were not only greater, but they were also significantly different from zero ($P < 0.01$). The isolation by distance analysis based on sequence haplotype distribution among populations revealed no significant linear relationship between geographical distance and Φ_{ST} ($R^2 = 0.024$). A hierarchical AMOVA was then performed grouping populations by geographical region (Indian, Atlantic and Pacific Ocean groups) and found that little of the variation could be explained by geography (1.35% between groups). However, when the two recolonized populations were grouped together, 21.9% of the variation was among groups and negligible variation within (–1.4%). The between-group variation dropped to 18.1% when MA was included in the group, and to 16.2% when AI replaced MA.

To examine further levels of population structure, 83 additional samples from all populations were screened with a series of restriction enzymes (Table 1) which targeted sites that distinguished between the major clades. The individuals whose DNA had been sequenced were

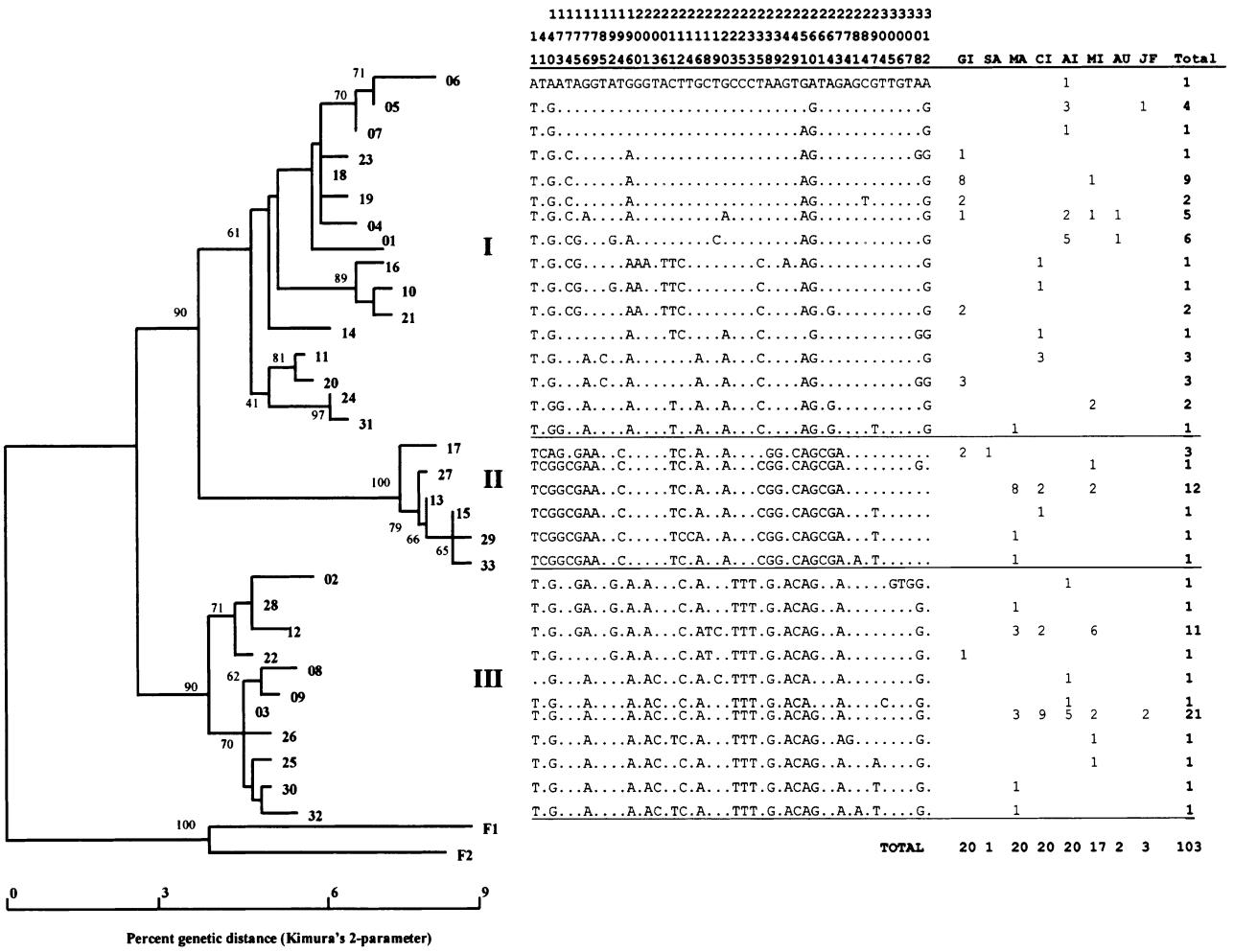

Fig. 4 Neighbour-joining (NJ) tree of 33 sequence haplotypes observed in five populations of *Arctocephalus tropicalis* and vagrants, the variable sites for each haplotype and its geographical distribution. Labels are arbitrarily assigned to haplotypes from 1 to 33. F1 and F2 are *A. forsteri* outgroups corresponding to FORST1 and FORST2 in Fig. 2. Bootstrap values are shown only at nodes which were supported in over 60% of the 1000 replications. The three major clades are labelled as I, II and III. Variable sites are numbered according to their position within the 316-bp aligned sequence. Geographical labels are described in Table 1.

Table 3 Population pairwise Φ_{ST} within *Arctocephalus tropicalis* based on sequence and frequency data (above diagonal) and on haplotype frequency data only (below diagonal). Significance testing of Φ_{ST} performed through 992 permutations and adjusted for multiple comparisons: $P \leq 0.05$* and $P \leq 0.01$**

	AI ($n = 20$)	CI ($n = 20$)	GI ($n = 20$)	MI ($n = 17$)	MA ($n = 20$)
AI	—	0.098	0.123	0.121	0.289**
CI	0.066	—	0.268**	−0.021	0.097
GI	0.148**	0.197**	—	0.303**	0.390**
MI	0.097**	0.083**	0.133**	—	0.062
MA	0.120**	0.085*	0.179**	0.043	—

also included as the RFLP haplotype could be determined from each sequence. A total of seven haplotypes were obtained (Table 4a), and the distribution of these haplotypes showed that the populations are heterogeneous ($\chi^2[24] = 99.96$; $P < 0.001$). In contrast to Φ_{ST} results, all population pairwise comparisons from RFLP data were significant (Table 5).

A. tropicalis — vagrants. Sequence data from six vagrant individuals were compared with that from the breeding populations. The individual from SA was found to have a haplotype unique to the GI population. The haplotype from one of the vagrants from AU was unique to the AI population. The other was shared with AI, MI and

Table 4 Restriction fragment length polymorphism (RFLP) haplotypes for (a) *Arctocephalus tropicalis* and (b) *A. gazella*, where 1 = restriction site and 0 = no restriction site

(a) *A. tropicalis*

No.	Haplotype	Clade	AI	CI	GI	MI	MA	Total
1	0101	I	0	1	1	0	0	2
2	0001	I	25	10	28	10	3	76
3	0000	II	0	6	5	4	15	30
4	0011	III	0	0	0	1	0	1
5	0010	III	12	20	0	7	15	54
6	0110	III	0	0	5	0	1	6
7	1110	III	3	2	0	7	5	17
			40	39	39	29	39	186

(b) *A. gazella*

No.	Haplotype	SS	SG	BI	MA	CI	KI	HI	MI	Total
1	00	13	7	15	8	11	19	12	19	104
2	01	17	24	13	13	17	12	16	21	133
3	11	1	9	12	13	12	9	12	10	78
Total		31	40	40	34	40	40	40	50	315

Table 5 Estimated pairwise population chi-squared values from the restriction fragment length polymorphism (RFLP) data for *Arctocephalus tropicalis* adjusted for multiple comparisons. Significance levels shown are $P \leq 0.05$* and $P \leq 0.01$**

	CI ($n = 39$)	GI ($n = 39$)	MI ($n = 29$)	MA ($n = 39$)
AI	15.6**	26.2**	12.9*	34.1**
CI	—	35.6**	10.2*	11.6*
GI		—	28.8**	48.8*
MI			—	14.2*

GI, indicating a probable origin from one of these sites. However, this untagged juvenile is less likely to have come from MI as all pups from this population have been tagged since the mid-1980s. Two of the JF vagrants had haplotypes shared by all populations except GI, making it difficult to assess from where they originated. The haplotype of the third, however, was unique to AI.

A. gazella. DNA sequences were obtained for a total of 145 individuals from eight populations (Table 1). There were 26 haplotypes found, 16 of which were represented by more than one individual (Table 2). The relationship of these haplotypes (Fig. 5) showed little clade structure in the tree, relative to that observed in *A. tropicalis*, and the nucleotide diversity of 3.2% for this species was also lower. The sequence statistics for *A. gazella* and each of its populations are displayed in Table 2. AMOVA results revealed that 92.6% of the observed genetic variation occurred within the populations. The overall Φ_{ST} for *A. gazella* was 0.074. Pairwise Φ_{ST} calculated for populations using sequence and haplotype frequency data are presented in Table 6. Of the 28 pairwise comparisons, six or seven were significant, depending on the data used for the analysis. The KI and MI populations were found to be significantly different to both BI and SG, which are the supposed source populations for the recolonized populations.

These data, along with haplotype distributions, suggest that *A. gazella* consist of two broad regional groups: region 1 containing SG, SS, BI and MA populations; and region 2 containing KI and MI. The two populations of CI and HI are intermediary, with the former containing haplotypes otherwise found exclusively in each of regions 1 and 2. While HI was not significantly different to the KI/MI region, all the sequence haplotypes found within the known breeding population ($n = 7$) were shared primarily with region 1. However, pairwise Φ_{ST}s calculated between the groups of known and unknown breeding status within the HI population were found to be not significantly different from zero.

Isolation by distance analysis, with the assumption that all islands were recolonized from the SG or BI populations, showed that some variation could be explained by geographical distance ($R^2 = 0.228$). However, if there was an additional population within the KI/MI region that survived sealing, then the distribution of genetic variation within *A. gazella* would not be expected to conform to an isolation by distance model alone. If the data from region 2 are excluded from the analysis (i.e. that from the KI and MI populations), a greater correlation resulted with an R^2 value of 0.600 for region 1. Furthermore, a hierarchical AMOVA was also used to investigate potential geographical structure. Southern elephant seals (*Mirounga leonina*) show strong phylogeographical structure forming three major oceanic populations (Slade 1997). *A. gazella*

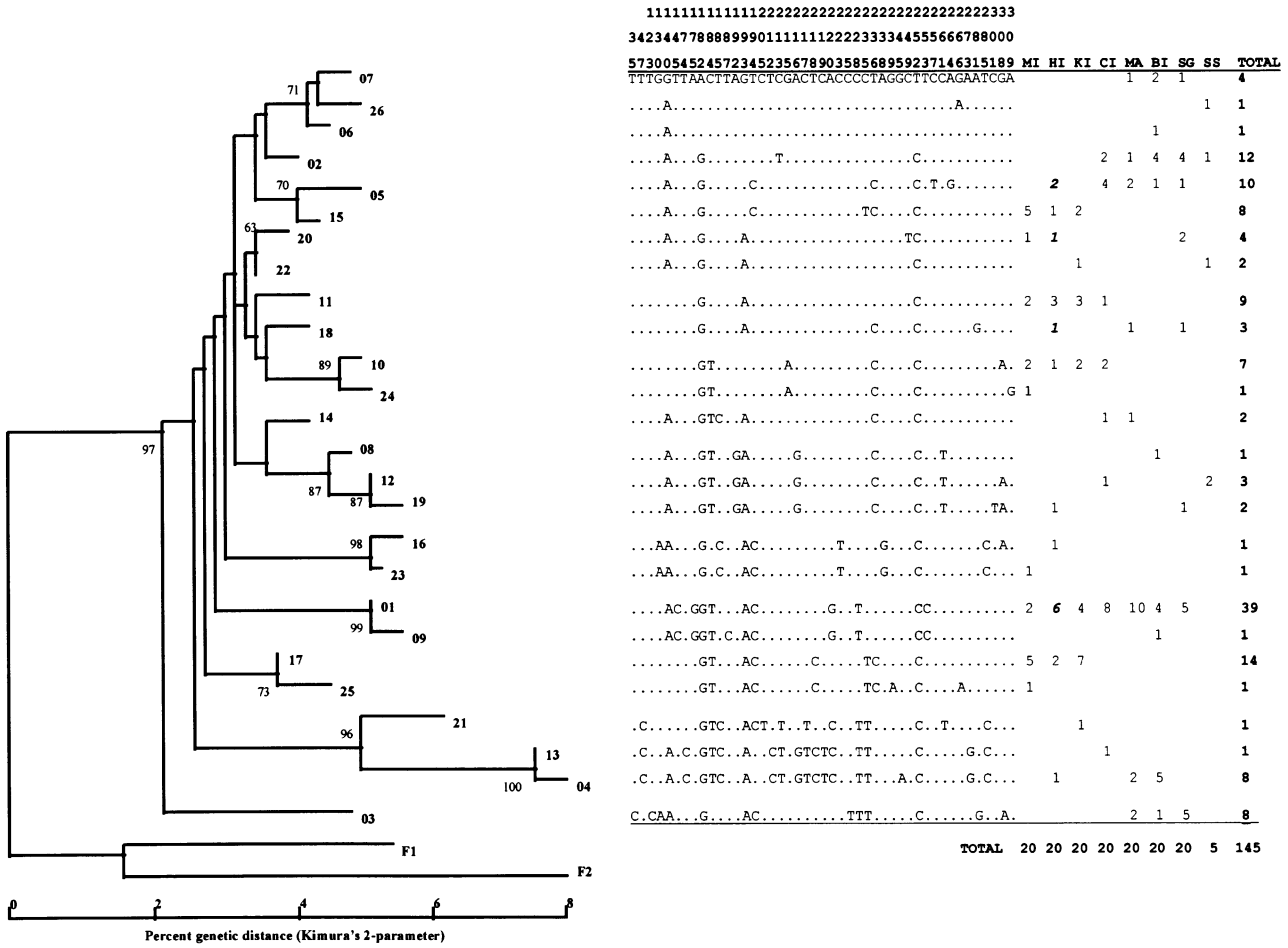

Fig. 5 Neighbour-joining (NJ) tree of 26 sequence haplotypes observed in eight populations of *Arctocephalus gazella*, the variable sites for each haplotype with its geographical distribution. Labels are arbitrarily assigned to haplotypes from 1 to 26. F1 and F2 are *A. forsteri* outgroups corresponding to FORST1 and FORST2 in Fig. 2. Bootstrap values are shown only at nodes which were supported in over 60% of the 1000 replications. Variable sites are numbered according to their position within the 316-bp aligned sequence. Geographical labels are described in Table 1. Heard Island (HI) haplotypes in bold and italic represent those which are represented in pups.

	BI (n = 20)	CI (n = 20)	HI (n = 20)	KI (n = 20)	MI (n = 20)	MA (n = 20)	SG (n = 20)	SS (n = 7)
BI	—	0.050	0.057	0.140*	0.163**	0.032	0.069	0.070
CI	0.051	—	−0.018	0.081	0.111	−0.011	0.045	0.119
HI	0.039	−0.006	—	0.003	0.034	0.012	0.042	0.106
KI	0.109**	0.090	0.011	—	−0.017	0.113	0.133*	0.232*
MI	0.103**	0.106**	0.025	−0.015	—	0.165*	0.138**	0.206
MA	0.043	−0.008	0.014	0.123*	0.144**	—	0.036	0.181
SG	0.024	0.041	0.037	0.111*	0.105**	0.033	—	0.087
SS	0.076	0.119	0.103	0.133	0.113	0.187	0.089	—

Table 6 Population pairwise Φ_{ST} within *Arctocephalus gazella* based on sequence and frequency data (above diagonal) and on haplotype frequency data only (below diagonal). Significance testing of Φ_{ST} performed through 992 permutations and adjusted for multiple comparisons: $P \leq 0.05$* and $P \leq 0.01$**

were also grouped in this fashion: Pacific (MI), Atlantic (SS, SG, BI) and Indian (MA, CI, KI, HI) oceanic populations. This only accounted for 3.8% of the overall variation. Examining populations on a finer scale (SS, SG vs. BI vs. MA, CI vs. KI, HI vs. MI) resulted in 7.1% among groups. However, when HI was removed from the analysis due to the uncertainty of the sampled individuals' origin and KI included with MI, the amount of

Table 7 Estimated pairwise chi-squared values within *Arctocephalus gazella* with and without adjustment for multiple comparisons. Significance levels shown are $P \leq 0.05$* and $P \leq 0.01$**

	CI ($n = 40$)	HI ($n = 40$)	KI ($n = 40$)	MI ($n = 50$)	MA ($n = 34$)	SG ($n = 40$)	SS ($n = 31$)
BI	1.15	0.64	0.94	1.44	1.70	6.61	8.99
CI	—	0.07	3.42	1.65	0.56	2.51	8.47
HI		—	2.58	1.34	0.67	3.34	8.37
KI			—	1.41	4.79	9.54	7.36
MI				—	3.85	4.74	4.71
MA					—	3.60	11.9*
SG						—	8.39

variation among groups was 10.2% with only 0.16% of the variation occurring within groups.

To investigate further structure within *A. gazella*, 315 individuals were screened with two restriction enzymes to produce three RFLP haplotypes (Table 4b). An analysis of heterogeneity on the frequency distribution of these haplotypes showed that the population was heterogeneous, but only at the 5% level ($\chi^2[14] = 25.13$; $P < 0.05$). The population pairwise estimated chi-squared values (Table 7) showed little difference between all populations with respect to the distribution of haplotypes, except for the comparison between SS and MA. This would be due to only one individual in SS with haplotype 3 (Table 4b). A greater number of enzymes could be used to increase the number of RFLP haplotypes observed within *A. gazella*, thereby increasing the resolution of this analysis.

A. forsteri. A. forsteri had high levels of diversity, with 16 haplotypes found from the 17 sequences examined (Table 2). There were two highly divergent clades (Fig. 3) which were also apparent in cytochrome *b* (Lento 1995). The additional samples from Taronga Zoo and Slade *et al.* (1994) showed unique haplotypes from those found by Lento (1995).

Discussion

The phylogenetic relationship between *Arctocephalus tropicalis* and *A. gazella* is characterized by the absence of shared haplotypes, a divergence between the species of 8.0% and the presence of discrete clades in a NJ tree. These points indicate that the reported hybridization between *A. gazella* and *A. tropicalis* is recent, not extensive, and is probably confined to the very small areas of range overlap. The most extensive current hybridization is probably occurring at MI, which has a very small population where both species breed on the same beach. This would explain why three individuals sampled from the island had phenotype characteristics of *A. tropicalis* while having mitochondrial DNA haplotype characteristics of *A. gazella*. The findings of this study and that of Goldsworthy *et al.* (1999) suggest that the results of reciprocal paraphyly of these two species reported by Lento *et al.* (1997) using samples from MI, were probably based on hybrid individuals.

Levels of genetic variation

The levels of genetic variation detected in *A. tropicalis* and *A. gazella* were very high, especially when compared with other vertebrate species for control region I (see Table 2; Slade 1997). The respective nucleotide diversities of 4.8% and 3.2% for these species are among the highest reported for the listed mammalian species. These results might be considered surprising after both species are thought to have experienced recent population bottlenecks. However, the current levels of genetic variation within a species are the result of many factors. These include the amount of pre-existing genetic variation, pre- and postbottleneck population sizes, as well as duration and extent of the bottleneck itself. Nei *et al.* (1975) proposed that if a species with high pre-existing genetic variation was able to recover rapidly from a severe bottleneck, it would be expected to exhibit reduced haplotype variation while retaining prebottleneck nucleotide diversity.

Although the prebottleneck variation for both *A. tropicalis* and *A. gazella* are unknown, they are expected to be high given the large population sizes reported at that time. Despite the lack of specific data from the era, it appears that the population sizes of *A. gazella* were greater than those for *A. tropicalis*. The reported numbers of seal skins removed from *A. gazella* population centres are greater (1.2 million from SG by 1822; approximately 250 000 from SS 1820–1821; Bonner 1958; Bonner & Laws 1964) and it was noted that SS 'revealed what were probably the richest sealing grounds of the nineteenth century in the southern hemisphere' (Bonner & Laws 1964). Specific details pertaining to the duration and extent of the population bottlenecks for both *A. gazella* and *A. tropicalis* are also incomplete. Although rapid population increases have been documented in both species (e.g. Shaughnessy 1982; McCann & Doidge 1987), the estimation of presealing

population sizes is difficult, partly due to the secrecy shrouding a highly competitive sealing industry. Also, many islands in the subantarctic are difficult to search thoroughly due to the length and/or inaccessibility of sections of coast. Thus, reports of population extinctions at islands infrequently visited and/or with inaccessible coastlines may be viewed with scepticism. The more credible reports are from islands with long periods of human habitation, such as MI. Therefore, it is possible that the recent population reductions experienced by both species, particularly *A. gazella*, were not as severe as implied in historic reports. This and the known rapid postbottleneck recoveries suggest that neither species has suffered major reductions in their levels of genetic variation. Nonetheless, the lower level observed in *A. gazella* relative to *A. tropicalis* may be a result of the more intensive sealing efforts waged against this species.

High postbottleneck levels of genetic variation are also found in two other species of fur seal. The Juan Fernandez and Guadelupe fur seals (*A. philippii* and *A. townsendi*, respectively) were both subjected to major reductions in numbers through sealing (Hubbs (1956) as cited in Hubbs & Norris (1971); Fleischer 1987), but have retained high nucleotide diversities in control region I ($\pi = 3.0\%$ and 2.0%, respectively; Goldsworthy *et al*. in press; G. Bernardi, personal communication). These scenarios are in stark contrast to those seen within the northern elephant and Hawaiian monk seals (*Mirounga angustirostris* and *Monachus schauinslandi*, respectively). Both exhibit extremely low levels of variation in the mitochondrial control region, thought to result from severe bottlenecks ($\pi = 0.43\%$ and 0.7%, respectively; Hoelzel *et al*. (1993) derived from Kretzmann *et al*. (1997)). The differences seen between these two phocid species and the four fur seal species may be explained by the latter surviving sealing in greater numbers. Furthermore, the biology of the phocid species suggests that they were more vulnerable to exploitation. The northern elephant seals haul out to breed and/or moult on open beaches (Bonner 1994) relatively close to human civilization, and thus would have been highly accessible to sealers. This may have ensured that low numbers were maintained for prolonged periods. The Hawaiian monk seal population was probably not large to start with. Although this species is solitary, wary and easily disturbed, sealing and other postsealing human activities not only reduced the population markedly, but maintained a sustained pressure on the species (Busch 1987). In contrast, the closely related southern elephant seal breeds on remote subantarctic islands, probably allowing greater numbers to survive sealing (control region I $\pi = 1.95\%$; Slade 1997). This may also be the case for the Antarctic fur seal. On the other hand, the Juan Fernandez, Guadelupe and subantarctic fur seals all prefer a more rugged substrate for breeding, such as rocky sections of coast, often at the base of high cliffs and in caves (King 1983). The nature of this substrate decreases the visibility of the seals, thereby increasing the chance of survival.

Population structure

The observed patterns of genetic variation within *A. tropicalis* and *A. gazella* are a result of a number of processes: mutation, drift, migration, effective population size and selection. The time frame within which the latest recolonization events have taken place (i.e. within the last 100 years), suggests that effects due to mutation and selection would be negligible. Also, genetic drift is probably a consideration only in very small populations, such as at MI and CI. Therefore, the major contributors to the observed distribution of genetic variation probably stem from the associated effects of migration, such as founder effects. Furthermore, as there are large differences in the current sizes of many populations of both species, it would be expected that the strategy employed to sample current genetic variation would also influence the results. All populations are represented by about 40 individuals, regardless of their size, which may lead to under-representation of the number and frequency of haplotypes sampled in the larger populations.

The higher level of population structure and genetic diversity observed within *A. tropicalis* supports historic records that suggest it was subjected to less intensive sealing than *A. gazella*. The sequence data revealed a high proportion of haplotypes within *A. tropicalis* to be unique to certain populations. Each population had between four and seven unique haplotypes, representing 41% of all samples sequenced. Given the expectation that haplotypes in the recolonized populations would also occur in the source population/s, these data suggest that the sampling regime employed may have been insufficient. Although sampling of some populations was conducted across many colonies (e.g. GI and MA), others may be represented only by a single colony (e.g. AI) or a very small colony (MI). The high incidence of singleton sequence haplotypes observed in this species may also be an artefact of sampling, and/or be indicative of a species that has undergone recent rapid population expansion.

There are three highly divergent evolutionary lineages within the *A. tropicalis* tree. These indicate that at some time in the past this species showed phylogeographical structuring. It is impossible to suggest from the data presented here where the three ancient population centres were, although the patterns of distribution may provide some clues. There were no lineages from clade II found at AI, while these are well represented in the MA population. The GI population is dominated by lineages from clade I, as is that at AI, but to a lesser degree. It is possible that the three populations that survived the sealing

era were the centres for each of these divergent clades, and that the current distribution of these lineages within the populations may be a reflection of the rapid postsealing recovery of the species.

Despite the high incidence of singleton sequence haplotypes within *A. tropicalis*, the distribution of shared haplotypes allows some speculation on the pattern of female recolonization. The three surviving populations at GI, AI and MA differ in the distribution and proportion of sequence haplotypes. The population at GI shares only one haplotype with AI, and another with MI. This suggests that MI and CI were most probably recolonized from either AI, MA, or both. This is further supported by the hierarchical AMOVA results as well as both pairwise Φ_{ST} (calculated either way) and chi-squared values suggest that GI is highly significantly different to both CI and MI. MA is the most likely major source population for CI, as all of the shared sequence haplotypes found in the latter are shared with the former. One haplotype is also shared with AI, indicating possible input from this population. The pairwise Φ_{ST} of AI–CI and MA–CI are similar, 0.066/0.098 and 0.085/0.097, respectively. However, the geographical proximity of MA to the west of CI suggests that this population may have a greater influence. The MA population appears also to be a source for MI, with the pairwise Φ_{ST} suggesting panmixia. With the first breeding of *A. tropicalis* on CI recorded in 1976 (Jouventin *et al*. 1982), about 5 years before this species was confirmed on MI (S. D. Goldsworthy *et al*., accepted), it is possible that some immigrants came from here. But as these females would previously have come from MA anyway, it is impossible to distinguish the exact source. Migration from AI to MI is also probable given that the populations share several lineages. The shared haplotype between the latter and GI populations suggests that at least one female made the 12 000 km journey from the South Atlantic ocean. Alternatively, this haplotype may be shared with either AI or MA, but was not sampled. Sampling may also explain the lack of shared haplotypes between GI and either MA or CI. The overall pattern described from the sequence data is supported by the RFLP data, although all populations were found to differ significantly from each other, possibly a function of the increased sample sizes.

In contrast to *A. tropicalis*, there is little structure seen within the *A. gazella* tree, although there were two genetically differentiated regions identified within the species. This was unexpected, as due to its rapid postsealing recovery and current population size, it was thought that SG would be the main source for recolonization across the species' former range (Laws 1973; McCann & Doidge 1987). Although a wide range of the existing lineages is present in this population, 69% of the haplotypes found in *A. gazella* are not represented here. Furthermore, 10 of the sequence haplotypes were found to be unique to some populations, but none of these occurred at SG. This observed haplotype distribution may be a result of drift within the small, recolonized populations and/or the sampling of only a small proportion of the SG population (20 sequences from an estimated 1.5 million individuals; Boyd 1993). Alternatively, it is possible there were other populations that survived sealing that contained additional unique lineages to those found at SG.

Few records exist on the presealing fur seal populations at BI, McDonald Island and KI. Although the two former populations were not considered large (Bonner & Laws 1964; Budd 1972), and the latter was harvested heavily (Budd & Downes 1969), there is nothing to suggest that these populations actually became extinct. In fact, there were 1000–1200 fur seals reported on BI in 1928 (Olstad (1929) as cited in Fevoden & Sømme (1976)), after 800 had been collected by the crew of the *Norvegia* in 1927 (Holdgate *et al*. 1968). This is several years prior to the rediscovery of the small population at SG in 1933, and therefore seems probable that the BI population survived the sealing era. The presence of three unique haplotypes in this population supports this, although there is still evidence of extensive gene flow between the two populations.

The discovery of two genetically differentiated regions within *A. gazella* may give evidence that other populations survived sealing. The first of these regions is represented by populations at SG, BI, MA and SS. The second region is located to the east and is represented by the panmictic populations of KI and MI. Although the two groups are not genetically isolated, there is a greater sharing of haplotypes within rather than between regions. The CI population appears to be an intermediate, sharing haplotypes with both groups, while the HI breeding population shares all haplotypes with SG. This is unexpected given the close proximity of HI to KI, whose populations only share one haplotype.

Two hypotheses can be proposed to account for the observed regional differences. First, the haplotype distribution within the eastern sector may be a result of a marked founder effect, with very few individuals colonizing KI from the western sector. Subsequent colonization of MI predominantly from KI would account for the similarity of these populations. Alternatively, such a distribution may be due to an additional postsealing remnant population at KI. The Kerguelen archipelago is a remote, extensive island group, and it is conceivable that small numbers of fur seals survived here. Each of these hypotheses is equally valid. The former is based on the premise that the current sampling regime was insufficient to detect representative haplotypes within the SG/BI populations. But based on the data currently at hand, it is the latter that is the more plausible.

Because of the geographical proximity of SS to SG, it

was expected that these populations would be more similar. Although there was no significant difference in their pairwise Φ_{ST}, the SS population shares only one of its four haplotypes with SG. The other two are shared with CI and KI. The small sample from the SS population is probably a factor in its difference to SG, but this was not borne out entirely with the RFLP data where the sample size was larger. Although both populations shared similar proportions of haplotype 2 (Table 4b), the SS population was distinguished by an almost complete absence of haplotype 3. It is possible that this distribution is a result of genetic drift and/or a founder effect, but would suggest limited migration of females between the two populations. This is supported by tag resight information, where only one case is documented of a female moving between SG and SS (Bengtson *et al*. 1990). The remaining sightings and additional satellite tracking data show that it is predominantly the males that are dispersing (Laws 1973; Bengtson *et al*. 1990; Boyd *et al*. 1998), a factor that would not be reflected in the mitochondrial DNA data.

Although fur seals are capable of dispersing huge distances, providing the mechanism of rapid recolonization, they are generally regarded as philopatric (Riedman 1990). However, there is little quantitative information pertaining to philopatry (especially in females) in *A. gazella* and *A. tropicalis*, and it is unknown whether any differences between the species may provide some explanation for the differences seen in genetic population structure. Nevertheless, resight information to date of tagged *A. tropicalis* include: three individuals tagged at MA sighted at HI and SA (Bester 1989); one juvenile tagged as a pup at AI and seen at MI in 1998 (S. D. Goldsworthy, unpublished); two AI individuals sighted at KI in early 1999 (C. Guinet, personal communication); and a 1–2-year-old *A. tropicalis* tagged at MI in 1997 sighted at KI in early 1999 (M.-A. Lea, personal communication). This species has also been seen at HI where a pup has been reported (Goldsworthy & Shaughnessy 1989). A juvenile *A. gazella* tagged at MI was later sighted at KI (M.-A. Lea personal communication). Additional sightings of this species have been made on mainland Antarctica (Shaughnessy & Burton 1986) and South America (Payne 1977, 1979).

However, such a dispersal capacity does not automatically presuppose lower levels of population structure, as observed within *A. tropicalis* and *A. gazella*. The southern elephant seal (*M. leonina*) is a species that also breeds on subantarctic islands and has been reported to have a large dispersal capacity (e.g. Hindell & McMahon in press; Slip *et al*. in press). Very high levels of population structure were found to occur in both mitochondrial DNA and nuclear DNA, with three genetically distinct populations being identified (Slade 1997). Gene flow between the most recently diverged populations is estimated to be only three to four females per generation, or if no gene flow, then having a divergence time of about 20 000 years. Such low levels of gene flow between populations is indicative of a species with high female philopatry. Although reported in fur seals, such philopatry was not as strongly evident in *A. tropicalis* and *A. gazella* in this study. Further research and analysis will be conducted to assist in evaluating the patterns of recolonization more fully, employing these data as well as those obtained by screening with bi-parentally inherited microsatellite DNA.

Conclusion

The results from this study indicate high levels of genetic variation and significant population structure within *Arctocephalus tropicalis*, and low but significant structure within *A. gazella*. Overall, *A. tropicalis* exhibited the higher levels of variation and structure, with a high proportion of unique haplotypes and haplotype singletons. The distribution of lineages within *A. tropicalis* suggests the population at MA as the major source for immigrants to MI and CI, although there is evidence for some input from AI. There were two genetically differentiated regions observed within *A. gazella*. One centring on KI and MI, while the second is in the west, with SG and BI, probably the source of immigrants to MA and SS. The populations at CI and HI appear to be intermediates.

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References

Arnason U, Johnsson E (1992) The complete mitochondrial sequence of the harbor seal, *Phoca vitulina*. *Journal of Molecular Evolution*, **34**, 493–505.

Bengtson JL, Ferm LM, Harkonen TJ, Stewart BS (1990) Abundance

of Antarctic fur seals in the South Shetland Islands, Antarctica, during the 1986/87 austral summer. In: (eds Kerry KR, Hempel G) *Antarctic Ecosystems. Ecological Change and Conservation*, pp. 265–270. Springer, Berlin.

Bester MN (1987) Subantarctic fur seal, *Arctocephalus tropicalis*, at Gough Island (Tristan da Cunha group). In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 57–64. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

Bester MN (1989) Movements of southern elephant seals and subantarctic fur seals in relation to Marion Island. *Marine Mammal Science*, **5**, 257–265.

Bonner WN (1958) Notes on the southern fur seal in South Georgia. *Proceedings of the Zoological Society of London*, **130**, 241–252.

Bonner WN (1968) The fur seal of South Georgia. *British Antarctic Survey Scientific Reports*, **56**, 1–82.

Bonner WN (1994) *Seals and Sea Lions of the World*. Blandford, London.

Bonner WN, Laws RM (1964) Seals and sealing. In: (eds Priestley R, Adie RJ, Robin GDQ) *Antarctic Research*, pp. 163–190. Butterworths, London.

Boyd IL (1993) Pup production and distribution of breeding Antarctic fur seals (*Arctocephalus gazella*) at South Georgia. *Antarctic Science*, **5**, 17–24.

Boyd IL, McCafferty DJ, Reid K, Taylor R, Walker TR (1998) Dispersal of male and female Antarctic fur seals (*Arctocephalus gazella*). *Canadian Journal of Fisheries and Aquatic Science*, **55**, 845–852.

Budd GM (1972) Breeding of the fur seal at McDonald Islands and further population growth at Heard Island. *Mammalia*, **36**, 423–427.

Budd GM, Downes MC (1969) Population increase and breeding in the Kerguelen fur seal, *Arctocephalus tropicalis gazella*, at Heard Island. *Mammalia*, **33**, 58–67.

Busch BC (1987) *The War Against the Seals*. McGill-Queen's University Press, Kingston.

Clark JW (1875) On the eared seals of the islands of St. Paul and Amsterdam, with a description of the fur-seal of New Zealand, and an attempt to distinguish and rearrange the New Zealand Otariidae. *Proceedings of the Zoological Society*, **1875**, 650–677.

Condy PR (1978) Distribution, abundance and annual cycle of fur seals (*Arctocephalus* spp.) on the Prince Edward Islands. *South African Journal of Wildlife Research*, **8**, 159–168.

De Villiers RF, Ross GJB (1976) Notes on numbers and distribution of fur seals, *Arctocephalus tropicalis* (Gray) on Marion and Prince Edward Islands, Southern Ocean. *Journal of Mammalogy*, **57**, 595–600.

Excoffier L, Smouse P, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fevoden SE, Sømme L (1976) Observations on birds and seals at Bouvetøya. *Norsk Polarinstitutt Årbok*, **1976**, 367–371.

Fleischer LA (1987) Guadelupe fur seal, *Arctocephalus townsendi*. In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 43–48. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

Goldsworthy SD, Boness DJ, Fleischer RC (1999) Mate choice among sympatric fur seals: female preference for conphenotypic males. *Behavioural Ecology and Sociobiology*, **45**, 253–267.

Goldsworthy SD, Francis J, Boness D, Fleischer R (in press) Variation in the mitochondrial control region in the Juan Fernandez fur seal (*Arctocephalus philippii*). *Journal of Heredity*, in press.

Goldsworthy SD, Shaughnessy PD (1989) Subantarctic fur seals *Arctocephalus tropicalis* at Heard Island. *Polar Biology*, **9**, 337–339.

Goldsworthy SD, Shaughnessy PD, Wynen L, Robinson S (accepted) The population status and hybridisation of three sympatric fur seals (*Arctocephalus* spp.) at Macquarie Island. *Antarctic Science*.

Grewe PM, Krueger CC, Aquadro CF *et al*. (1993) Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. *Canadian Journal of Fisheries and Aquatic Science*, **50**, 2397–2403.

Headland R (1984) *The Island of South Georgia*. Cambridge University Press, Cambridge.

Hindell MA, McMahon CR (in press) A record migration for a southern elephant seal (*Mirounga leonina*): from Macquarie Island to Peter 1øY. *Marine Mammal Science*.

Hoelzel AR, Halley J, O'Brien SJ *et al.* (1993) Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. *Journal of Heredity*, **84**, 443–449.

Hofmeyr GJG, Bester MN, Jonker FC (1997) Changes in population sizes and distribution of fur seals at Marion Island. *Polar Biology*, **17**, 150–158.

Holdgate MW, Tilbrook PJ, Vaughan RW (1968) The biology of Bouvetøya. *British Antarctic Survey Bulletin*, **15**, 1–7.

Hubbs CL (1956) Back from oblivion. Guadelupe fur seal: still a living species. *Pacific Discovery Californian Academy of Science*, **9**, 14–21.

Hubbs CL, Norris KS (1971) Original teeming abundance, supposed extinction, and survival of the Juan Fernandez fur seal. *Antarctic Research Series*, **18**, 35–51.

Isaksen K, Hofmeyr GJG, Dyer BM *et al.* (1997) Preliminary results from CEMP-monitoring of Antarctic fur seals, chinstrap penguins and macaroni penguins at Bouvetøya 1996/1997. Working Group Paper submitted to CCAMLR WG-EMM 1997. Agenda Item Number 2. iii.

Jouventin P, Stahl JC, Weimerskirch H (1982) La recolonisation des Iles Crozet par les otaries (*Arctocephalus tropicalis* et *A. gazella*). *Mammalia*, **46**, 505–514.

Kerley GIH (1983a) Comparison of seasonal haul-out patterns of fur seals *Arctocephalus tropicalis* and *A. gazella* on subantarctic Marion Island. *South African Journal of Wildlife Research*, **13**, 71–77.

Kerley GIH (1983b) Relative population sizes and trends, and hybridisation of fur seals *Arctocephalus tropicalis* and *A. gazella* at the Prince Edward Islands, Southern Ocean. *South African Journal of Zoology*, **18**, 388–392.

Kerley GIH (1987) *Arctocephalus tropicalis* on the Prince Edward Islands. In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 61–64. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

King JE (1983) *Seals of the World*. University of Queensland Press, St Lucia.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science USA*, **86**, 6196–6200.

Kretzmann MB, Gilmartin WG, Meyer A *et al.* (1997) Low genetic variability in the Hawaiian monk seal. *Conservation Biology*, **11**, 482–490.

Kumar S, Tamura K, Nei M (1993) MEGA: *Molecular Evolutionary Genetics Analysis, Version 1.0*. Pennsylvania State University, Pennsylvania.

Laws RM (1973) Population increase of fur seals at South Georgia. *Polar Record*, **16**, 856–858.

Lento GM (1995) Molecular systematics and population genetic studies of Pinnipeds: phylogenies of our fin-footed friends and their surreptitious 'species' status. PhD Thesis, Victoria University of Wellington.

Lento GM, Haddon M, Chambers GK, Baker CS (1997) Genetic variation of southern hemisphere fur seals (*Arctocephalus* spp.): investigation of population structure and species identity. *Journal of Heredity*, **88**, 202–208.

McCann TS, Doidge DW (1987) Antarctic fur seal, *Arctocephalus gazella*. In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 5–8. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

McElroy D, Moran P, Bermingham E, Kornfield I (1992) REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. *Journal of Heredity*, **83**, 157–158.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Olstad O (1929) Trekk fra sydishavets dyreliv. *Norsk Geologisk Tidsskrift*, **2**, 511–534.

Payne MR (1977) Growth of a fur seal population. *Philosophical Transactions of the Royal Society of London B*, **279**, 67–79.

Payne MR (1979) Fur seals *Arctocephalus tropicalis* and *A. gazella* crossing the Antarctic convergence at South Georgia. *Mammalia*, **43**, 93–98.

Rand RW (1956) Notes on the Marion Island fur seal. *Proceedings of the Zoological Society of London*, **126**, 65–82.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2); population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Richards R (1994) 'The upland seal' of the Antipodes and Macquarie Islands: a historian's perspective. *Journal of the Royal Society of New Zealand*, **24**, 289–295.

Riedman M (1990) *The Pinnipeds: Seals, Sea Lions and Walruses*. University of California Press, Berkeley.

Roberts B (1950) Historical notes on Heard and McDonald Islands. *Polar Record*, **5**, 580–584.

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*, **6**, 539–545.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Roux J-P (1987) Subantarctic fur seal, *Arctocephalus tropicalis* in French subantarctic territories. In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 79–81. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

Rozas J, Rozas R (1997) DNASP, Version 2.0: a novel software package for extensive molecular population genetics analysis. *Computer Applications in the Biosciences*, **13**, 307–311.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) ARLEQUIN. Genetics and Biometry Laboratory, University of Geneva.

Shaughnessy PD (1982) The status of the Amsterdam Island fur seal. In: *Mammals in the Seas: FAO Fisheries Series No. 5*, pp. 411–421. FAO, Rome.

Shaughnessy PD, Burton HR (1986) Fur seals *Arctocephalus* spp. at Mawson station, Antarctica, and in the Southern Ocean. *Polar Record*, **23**, 79–81.

Shaughnessy PD, Erb E, Green K (1998) Continued increase in the population of Antarctic fur seals, *Arctocephalus gazella*, at Heard Island, Southern Ocean. *Marine Mammal Science*, **14**, 384–389.

Shaughnessy PD, Fletcher L (1987) Fur seals, *Arctocephalus* spp. at Macquarie Island. In: (eds Croxall JP, Gentry RL) *Status, Biology and Ecology of Fur Seals, Proceedings of an International Symposium and Workshop Cambridge*, pp. 177–188. U.S. Department of Commerce, National Marine Fisheries Service, Seattle.

Slade RW (1997) Genetic studies of the southern elephant seal *Mirounga leonina*. In: (eds Hindell M, Kemper K) *Marine Mammal Research in the Southern Hemisphere*, pp. 11–29. Surrey Beatty & Sons, Sydney.

Slade RW, Moritz C, Heideman A (1994) Multiple nuclear-gene phylogenies: applications to pinnipeds and comparison with a mitochondrial DNA gene phylogeny. *Molecular Biology and Evolution*, **11**, 341–356.

Slip DJ, Hindell MA, van den Hoff J, Burton HR (in press) Movements, foraging areas and diving behaviour of southern elephant seals (*Mirounga leonina*) from Heard Island in relation to bathymetry and water temperature. *Canadian Journal of Zoology*.

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Wace NM, Holdgate MW (1976) *Man and Nature in the Tristan Da Cunha Islands*. IUCN Publications, Morges.

Weir BS (1990) *Genetic Data Analysis*. Sinauer Associates, Sunderland.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wickens P, York AE (1997) Comparative population dynamics of fur seals. *Marine Mammal Science*, **13**, 241–292.

This work was conducted by Louise Wynen as part of a PhD examining the population genetics of Antarctic and subantarctic fur seals. Simon Goldsworthy co-ordinates fur seal research on Australia's subantarctic islands. Marthán Bester, Christophe Guinet and Ian Boyd have all had a long involvement in research in the Southern Ocean, with their many projects encompassing numerous aspects of marine mammal and bird ecology. Ian Gjertz is a zoologist employed at the Norwegian Polar Institute working on marine mammals. Greg Hofmeyr has been involved in fur seal and elephant seal research in the subantarctic and is currently involved with fur seal research in Namibia. Rob Slade is supervising the genetics component of Louise Wynen's PhD.
