Molecular Population Genetics of the Southern Elephant Seal *Mirounga leonina*

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ABSTRACT

Southern elephant seals breed on sub-Antarctic islands and have a circumpolar distribution. We assayed mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) variation in the three main populations in the south Atlantic, south Indian, and south Pacific oceans, and a smaller continental population in South America. Population structure of mtDNA was strong and not consistent with isolation by distance. The nDNA loci, although less informative, were consistent with the mtDNA results. Geographic structure appears to be dominated by historical processes, not contemporary gene flow. Uncorrected levels of nucleotide diversity for mtDNA control region I (2.86%) and nDNA (0.09%) were similar to those in humans and mice. Mutation rates for control region I (75×10^{-9} substitutions per site per year) and nDNA (1.23×10^{-9}) were similar to those in other mammals. Female effective population size and total effective population size were roughly equal at $\sim 4 \times 10^4$, indicating a twofold greater rate of drift for mtDNA. Effective breeding sex ratio of four to five females per male was estimated from nucleotide diversity and mutation rates for mtDNA and nDNA, and was much less than behavioral observations would suggest. There was no evidence for selection at any of the assayed loci.

THE southern elephant seal *Mirounga leonina* has a circumpolar distribution, and breeding colonies are concentrated on sub-Antarctic islands near the Antarctic convergence (Figure 1). The three main populations are centered on South Georgia (SG) in the south Atlantic Ocean, the geographically close Heard (HD) and Kerguelen Islands in the south Indian Ocean, and Macquarie Island (MQ) in the south Pacific Ocean (Ling and Bryden 1992). There are several small breeding populations on other sub-Antarctic islands, and there is a continental breeding population in the south Atlantic ocean at Península Valdés (PV) in Argentina (Campagna and Lewis 1992). On the basis of skull characters, Lydekker (1909) proposed that three subspecies be recognized, *falclandicus*, *crosetensis*, and *macquariensis*, corresponding to type localities in the south Atlantic, south Indian, and south Pacific oceanic regions, respectively. There are different growth patterns between populations, and these probably underlie the skull character differences. Studies during the 1950s and 1960s showed that individuals from MQ and HD grew at a slower rate and had a smaller ultimate size than individuals from SG (Carrick *et al.* 1962a; Bryden 1968). Bryden (1968) suggested that the growth pattern differences were environmentally determined. Alternatively, the growth pattern differences could be genetically determined, in which case we might expect a closer genetic relationship between the MQ and HD populations than either is to SG.

Recent studies on population size change have also indicated similarities between HD and MQ compared with SG. Population size was estimated as 350,000 for SG, 80,000 for HD, 157,000 for Kerguelen, and 136,000 for MQ, and the three main populations account for 96% of total population size (McCann 1985). These figures represent population size during the early 1950s for SG, HD, and MQ, and during the 1970s for Kerguelen. Although the SG population has been stable since that time (McCann and Rothery 1988), populations in the south Indian and south Pacific oceans have declined markedly (Hindell and Burton 1987). The MQ population, for example, has declined by 50% in 30 yr at a rate of −2% per year. One possible explanation for these changes is that they are due to large-scale movement of individuals between populations (Hindell and Burton 1987).

One of the aims of this study was to assess the extent of geographic structure in the southern elephant seal and to determine if the genetic relationship between populations corresponded to the relationship indicated by the morphometric and demographic studies. A previous study analyzed the genetic relationship between only the MQ and HD populations, between which there were significant differences in allozyme frequency

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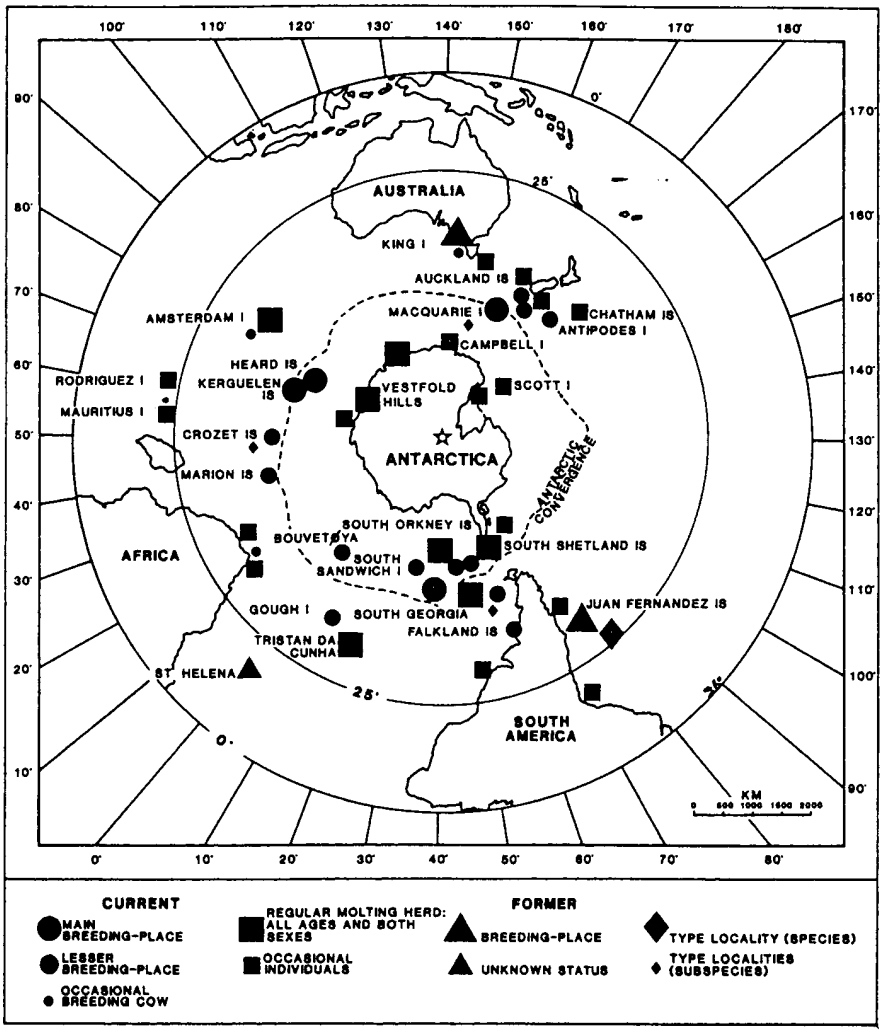

Figure 1.—Past and present distribution of the southern elephant seal (taken from Ling and Bryden 1992 with permission).

(Gales *et al.* 1989). A second aim of this study was to obtain more detailed information on other population genetic parameters for both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). These other parameters include mutation rate, effective population size, and selection for both mtDNA and nDNA. The framework for estimating these parameters relies upon sequence data information on the pattern of variation within and between species. Our previous studies have described the general approach for detecting variation in orthologous nDNA genes within the southern elephant seal and other organisms (Slade *et al.* 1993), and between the southern elephant seal and other pinniped species (Slade *et al.* 1994), and have also compared levels of mtDNA variation in southern and northern elephant seals (Hoelzel *et al.* 1993). In this article, we investigate several areas of the molecular population and evolutionary genetics of the southern elephant seal, including level of genetic diversity, rate of molecular evolution, effective population size and effective breeding ratio, geographic distribution of genetic variation, rate of gene flow, and population divergence time.

MATERIALS AND METHODS

Samples and molecular methods: The southern elephant seal blood tissue samples from Macquarie Island (MQ) in the south Pacific Ocean and Heard Island (HD) in the south Indian Ocean were collected by the Australian Antarctic Division. Samples from South Georgia (SG) in the south Atlantic Ocean were collected by the British Antarctic Survey using ronguers to clip ~1–2 g of skin from the webbing in the rear flippers. The MQ and HD samples were from beaches representing the geographic extremes of each island. The samples from other pinniped species were described in Slade *et al.* (1994). For initial sequencing, to determine levels of variation and to search for diagnostic markers, one individual was sequenced from each of MQ, HD, and SG. Several genes were then selected for further sequencing of a total of five individuals from each population (Table 1). For large-scale screening of diagnostic markers, ~30 individuals from each of MQ, HD, and SG were selected. The gene fragments amplified and sequenced are shown in Table 1. Here we detail only those protocols and primer sequences that have not been described previously (Slade *et al.* 1993, 1994).

For mtDNA, amplification with the THR and TDKD primers from 15 individuals (5 from each major population) resulted in 444 bp of sequence data, and analysis focused on a highly variable 299-bp subset comprising all 264 bp of control region I (CRI) and 35 bp of flanking sequence. This CRI subset

TABLE 1

Genes sequenced

Gene fragment	Region	Accession No.	PCR primers	Sequencing primers	Ref.[a]	Size (bp)	No. haplotypes analyzed	No. alleles	No. variant sites[b]	Mutations and position[c]	Marker	Other spp.[d]
Lysozyme	Exon 1/ intron 1	U91908	LYSO1 LYSO2	LYSO1	1	303	6	1	0	—	—	—
Lysozyme	Intron 1/ exon2	U91909	LYSO1 LYSO2	LYSO2	1	311	6	2	2	C/T @ 239 G/A @ 273	—	—
Corticotropin-releasing factor	5′ UTR	U91906	OSCRFA1 OSCRFA2	OSCRFA1 OSCRFA2	2	209	6	1	0	—	—	—
β-*Globin*	Exon 2/ intron 2	U91910	BETA3 BETA4	BETA3	3	168	6	?[e]	4	G/T @ 72 C/T @ 75 C/T @ 78 T/C @ 109	—	—
β-*Globin*	Intron 2	U91911	BETA6 BETA5	BETA6 BETA5	1	215	30	?[e]	1 (1)	A/T @ 146 $(GGAAA)_n$ @ 123–177	$(GGAAA)_n$	—
β-*Globin*	Intron2/ exon 3	U91911	BETA3 BETA4	BETA4	3	351	30	?[e]	0 (0)	—	—	—
H2AF	Exon 2/ intron 2	L17499	H2A8 H2A5	H2A8	4	270	6	1	0	—	—	—
H2AF	Intron 3/ exon 4	L17500	H2A8 H2A5	H2A5	4	293	6	1	0	—	—	—
H2AF	Exon 4/ intron 4	L17501	H2A8 H2A5	H2A2 H2A5	4	338	6	2	1	C/A @ 259	—	Yes
Ψ*H2AF*	Exon 2–5	L17502	H2A6 H2A4	H2A6 H2A4	4	274	6	1	0	—	—	Yes
ALD-A	Exon 5–6	L17497	ALD1 ALD2	ALD1 ALD2	3	478	30	5	4 (2)	C/T @ 133 G/C @ 202 G/C @ 274 G/C @ 335	*Nsp*I site	Yes
ALD-C	Exon 5–6	L17498	ALD1 ALD2	ALD1 ALD2	3	272	6	1	0	—	—	Yes
Mhc-DQA	Exon 2	U91907	DQA1 DQA2	SQA1 DQA4	1, 4	169	30	2	1 (0)	G/A @ 70	—	Yes
Mhc-DQA	Exon 3	U91907	DQA1 DQA2	DQA2 DQA5	4	230	30	2	1 (1)	C/T @ 828	—	Yes
Mhc-DQA	Intron 2	U91907	DQA1 DQA2	Universal Reverse	4	447	4	2	0	6 bp indel @ 201–206	indel	
mtDNA	CRI	U03591	THR TDKD	THR TDKD	4	299	60[f]	38	38	See Figure 2	*Fok*I, *Rsa*I, *Bsl*I	Yes

[a] (1) This study (see materials and methods); (2) Moore *et al.* (1992); (3) Lessa and Applebaum (1993); (4) Slade *et al.* (1993, 1994).

[b] For those nDNA loci for which 30 genes were sequenced, the values in parentheses are the number of variant sites observed in the initial screen of six genes.

[c] The first mutation given in *N/N* is that in the reference sequence in GenBank. The position of the A/T mutation in the β-*globin* microsatellite at 146 is with respect to the 3′ end of the sequence and may vary between different size repeat units.

[d] See Slade *et al.* (1994) for details of sequence data.

[e] There were two β-*globin* loci amplified simultaneously, and therefore the number of alleles could not be determined (see text).

[f] Includes data from Hoelzel *et al.* (1993).

corresponds to sites 67–365 in the GenBank sequence. (All site numbers refer to the sequences in GenBank; accession numbers in Table 1.) This enabled us to make direct use of published mtDNA sequence data (Hoelzel *et al.* 1993) for the southern elephant seal populations at SG and PV. There were a total of 60 CRI individual sequences available for analysis comprising 28 from SG, 21 from PV, 6 from HD (one individual was heteroplasmic; see results), and 5 from MQ.

For nDNA, an ~800-bp gene fragment of the β-*globin* gene, spanning exons 2–3, was amplified with primers BETA3/BETA4 using cycling parameters of 35 × 94° for 1 min, 65° for 1 min, 72° for 3 min; 168 bp at the 5′ end was sequenced with BETA3, and 351 bp at the 3′ end was sequenced with BETA4. Sequencing with the BETA4 primer revealed a pentameric microsatellite $(GGAAA)_n$, the 3′ end of which was located 297 bp upstream from the beginning of exon 3. Primers BETA6 and BETA5 were designed to regions flanking the microsatellite. The BETA6/BETA5 amplified product consisted of 149 bp of flanking sequence and from 6 to 20 repeats. The cycling parameters for BETA6/BETA5 were 35 × 94° for 1 min, 65° for 1 min, 72° for 1 min. An ~260-bp fragment of the *Corticotropin-releasing factor* gene was amplified and sequenced with primers OSCRFA1/OSCRFA2 using cycling parameters of 35 × 94° for 1 min, 50° for 1 min, and 72° for 1 min. An ~1-kb fragment of the lysozyme gene was amplified with primers LYSO1/LYSO2 using cycling parameters of 35 × 94° for 1 min, 55° for 1 min, 72° for 3 min, and 303 bp at the 5′ end was sequenced using LYSO1, and 311 bp at the 3′ end was sequenced with LYSO2. The PCR primers not described previously are (5′ → 3′) LYSO1 (AGGTCTTTGRACGDTG TGA), LYSO2 (GGGGTTTTGCCATCATTACA), DQA4 (ACA CATACCATTGGTAG), BETA6 (AATTGGGCATGTGATGT ATGAG), and BETA5 (AATTAGTATGATGCTGGGCTGTC).

For microsatellite PCR, the BETA5 primer was end-labeled with [γ-^{33}P]dATP according to a standard protocol (Sambrook *et al.* 1989). This was used in a regular PCR reaction with the BETA6 primer except that the cycling parameters involved denaturation at 94° for 30 sec, variable annealing temperatures (see below), and extension at 72° for 2 min. The annealing temperatures followed a "touchdown" procedure to reduce nonspecific amplification (Don *et al.* 1991) and were 67°, 30 sec for the first 2 cycles, then 66°, 30 sec for 2 cycles, and then 65°, 30 sec for 25 cycles. Formamide stop solution was added to the product, which was heated to 95° for 2 min, and 4 μl was loaded onto a 4.5% sequencing gel. The bands were visualized after autoradiography. Electrophoresis revealed up to four bands per individual, indicating that at least two microsatellite loci were being amplified. Direct sequencing of the BETA6/BETA5 product from several individuals revealed a clean sequence with one variant site, located within the repeat unit. It is likely, therefore, that there are at least two β-*globin* genes in the southern elephant seal and that these are the result of a very recent duplication or possibly a very recent homogenizing event between two preexisting β-*globin* genes. The presence of two β-*globin* genes in the southern elephant seal was previously suggested by isozyme studies (Seal *et al.* 1971). Although it was not possible to allocate alleles to separate microsatellite loci, the clear and reproducible dosage differences did allow scoring of the combined loci allele frequencies. For example, for individuals with three bands there was always one band with increased intensity, indicating the presence of two alleles for that number of repeats.

For each sequence with a variant nucleotide site, a computer search was made using the "MacVector" program (IBI-Kodak) for corresponding variant restriction sites. For mtDNA CRI there were several variant restriction sites, and three enzymes (*Rsa*I, *Fok*I, *Bsl*I) were chosen, on the basis of their geographic distribution, to use in a large-scale survey (Figure 2A). For *ALD-A* (Figure 2B), there was a variant site at position 202 that was part of a *Nsp*I restriction site (Slade *et al.* 1993). The presence/absence of the 6-bp indel in intron 2 of *Mhc-DQA* was assayed by double digestion of the PCR fragment with *Sau*96I and *Nla*IV and subsequent PAGE. The restriction digests were conducted as in Slade *et al.* (1993).

A

Haplotype	Position in sequence																								
	1	1	1	1	**1**	1	1	1	1	**1**	2	2	2	2	2	3	**3**	**3**	3	3	3	3
	2	3	5	5	6	**6**	7	7	8	8	**9**	5	5	8	8	9	0	**0**	2	3	4	4	5
	8	0	2	8	1	**9**	0	1	0	1	8	**6**	7	8	0	5	7	4	1	**5**	7	**6**	8	5	7	5
MQ1	T	C	G	T	C	**T**	A	T	A	C	T	**C**	G	A	G	C	A	T	C	**G**	G	**G**	C	A	T	A
MQ2	T	T	G	C	C	**T**	A	T	A	C	T	**C**	G	A	G	C	A	T	C	**G**	G	**A**	C	A	T	G
MQ3	T	C	G	T	C	**T**	A	T	A	C	T	**C**	G	A	G	C	A	T	C	**G**	G	**A**	C	A	T	G
MQ4	T	T	G	C	C	**T**	A	T	G	T	T	**C**	G	G	G	C	G	T	C	**G**	G	**A**	T	G	T	G
MQ5	T	C	G	**T**	C	**T**	A	T	A	C	C	**C**	G	A	G	C	A	T	C	**G**	G	**A**	C	A	T	G
HD1	C	C	G	T	T	**C**	A	T	A	C	C	**T**	G	A	A	C	A	C	C	**G**	G	**A**	C	A	C	G
HD2	T	C	G	T	T	**T**	A	T	A	C	C	**T**	A	A	G	T	A	C	C	**A**	G	**A**	T	A	C	G
HD3	C	C	A	T	T	**C**	A	C	G	C	C	**T**	G	A	A	C	A	C	C	**G**	A	**A**	C	A	C	G
HD4	C	C	G	T	T	**C**	A	T	A	T	C	**T**	G	A	A	C	A	C	C	**G**	A	**A**	C	A	C	G
HD5	C	C	A	T	T	**C**	A	C	G	C	C	**T**	G	A	A	C	A	C	C	**G**	G	**A**	C	A	C	G
HD6	T	C	G	T	T	**T**	A	T	A	C	C	**C**	G	A	A	C	A	C	C	**A**	G	**G**	C	A	C	G
SG1	T	C	G	T	T	**T**	A	T	A	C	C	**T**	A	A	G	C	A	C	C	**A**	G	**A**	C	A	C	G
SG2	C	C	G	T	T	**T**	G	T	A	T	C	**T**	G	A	G	C	A	C	T	**G**	A	**A**	C	A	C	G
SG3	C	C	G	T	T	**T**	G	T	G	C	C	**T**	G	A	G	C	A	C	T	**G**	G	**G**	C	A	C	G
SG4	C	C	G	T	T	**T**	A	T	G	C	C	**T**	G	A	G	C	A	C	T	**G**	G	**G**	C	A	C	G
SG5	C	C	G	T	T	**T**	A	T	G	C	C	**T**	G	A	G	C	A	C	T	**G**	G	**A**	C	A	C	G

B

Haplotype	Position in sequence	Frequency	Population frequency		
	1 **2** 2 3				
	3 **0** 7 3		MQ	HD	SG
	3 **2** 4 5				
a	C **G** G G	15	5	5	5
b	T **C** G G	5	2	3	0
c	C **C** G G	8	1	2	5
d	C **C** G C	1	1	0	0
e	C **G** C G	1	1	0	0

Figure 2.—(A) The 16 different sequence haplotypes of CRI from 15 individuals. (B) The 5 different sequence haplotypes of *ALD-A* from 15 individuals. The site numbers correspond to the sequences in GenBank, and the reference sequences in GenBank are SG1 and haplotype *a* for CRI and *ALD-A*, respectively. Diagnostic RFLP sites are in bold, and for CRI these are *Fok*I @ 196 and 326, *Rsa*I @ 169, and *Bsl*I @ 305. For CRI, the heteroplasmic individual contained haplotypes HD3 and HD4. See Hoelzel *et al.* (1993) for CRI haplotypes of the PV and other SG sequences.

Data analysis: For those nDNA sequences with multiple variant sites (*i.e.*, *ALD-A* and *Lysozyme*), the haplotypes were determined. For *ALD-A*, the method of phase determination was described in Slade *et al.* (1993). Briefly, the haplotypes of all double heterozygotes were separated by digestion with *Nsp*I, and the separate fragments sequenced. For *Lysozyme*, phase was determined by reference to the unambiguous haplotypes observed after sequencing homozygotes. There was also one individual that was heteroplasmic for mtDNA, and the two haplotypes were determined by reference to the unambiguous haplotypes. Phase can be inferred from the unambiguous haplotypes assuming that there has been no recombination that is reasonable for short PCR fragments (Clark 1990).

The pairwise genetic distances between sequences were calculated with the "MEGA" program (Kumar *et al.* 1993) using pairwise-deletion to account for gaps. The genetic distances were the uncorrected number or proportion of differences, and for some analyses of the CRI data, distances were corrected using the Tamura-Nei gamma model (Tamura and Nei 1993). For the latter, the scale parameter for the negative-binomial distribution was estimated by maximum-likelihood using the macro "kfit" (Crawley 1993) for the program "GLIM" (Re-

lease 3.772; Royal Statistical Society, London). The input data were the number of substitutions that occurred at each site, and this was estimated from a parsimony tree (Wakeley 1993) constructed in "PHYLIP" (Felsenstein 1993). The frequency distribution of the inferred number of substitutions per site was compared with expected poisson (Sokal and Rohlf 1969, p. 86) and negative binomial (Bliss and Fisher 1953) distributions.

Nucleotide diversity within populations was calculated as in equation 10.6 in Nei (1987). For nDNA data, this was based on the uncorrected proportional distance, and for CRI data it was based on both the uncorrected proportional distance and the Tamura-Nei gamma corrected distance. The variance of nucleotide diversity was that because of estimation errors of nucleotide substitutions. For the uncorrected proportional distance, the variances were calculated as in Nei and Jin (1989) using the program "SEND." For the Tamura-Nei corrected distance, the maximum variance was calculated as in Takahata and Tajima (1991) using a BASIC program modified by R. W. Slade from one kindly provided by Y. Satta.

The distribution of genetic variation between populations was estimated in several ways. For CRI data only, we utilized a procedure analogous to analysis of variance with the "AMOVA" program (Excoffier *et al.* 1992). As recommended, the distance matrix used was the number of nucleotide differences between pairs of sequences, and the significance levels were determined after 1000 permutations. Also, for CRI data only, we calculated gross and net nucleotide divergence between populations as in equations 10.20 and 10.21, respectively, in Nei (1987). For CRI haplotype and nDNA allele data, χ^2 tests of independence were conducted using "Monte" (Roff and Bentzen 1989) in the "REAP" suite of programs (McElroy *et al.* 1992), and significance levels were determined after 1000 permutations.

To estimate levels of long-term gene flow, we calculated analogs of G_{ST} between pairs of populations from the CRI sequence data and from the nDNA allele frequency data. For the CRI sequence data, estimates of G_{ST} were calculated between pairs of populations as $K_{ST} = 1 - K_S/K_T$ (Hudson *et al.* 1992), where K_S is the average nucleotide diversity within the two populations, and K_T is the total nucleotide diversity among all sequences from the populations. The degree of maternal gene flow ($N_f m_f$) between each pair of populations was then estimated from $K_{ST} = 1/(2N_f m_f \alpha + 1)$, where $\alpha = (n/(n-1))^2$ and n is the number of populations exchanging migrants (Crow and Aoki 1984), in this case four. For the nDNA data, estimates of G_{ST} between pairs of populations were obtained from allele frequency data from several loci using the program "BIOSYS-1$^{1.7}$" (Swofford and Selander 1989), and total gene flow (Nm) between pairs of populations was estimated from $K_{ST} = 1/(4Nm\alpha + 1)$ (Crow and Aoki 1984).

The CRI sequences were analyzed for any pattern of selection among haplotypes using the methods of Tajima (1989) and Fu and Li (1993). For the latter, a northern elephant seal sequence was used as the outgroup. All sequences were analyzed for selection using 2×2 tables of within-species polymorphism and between-species fixed differences for two loci as in Kreitman and Aguadé (1986). For this test, a significant departure from a neutral model could be because of linkage and stochastic factors rather than selection (Hudson *et al.* 1987). However, the ease of the χ^2 test means that it is an appropriate first choice and that, subsequently, any significant associations can be revisited with the more conservative HKA test. For comparisons between mtDNA and nDNA sequences, the difference in effective population size needs to be accounted for (Nachman *et al.* 1994). All nDNA was assumed to be autosomal, and because N_f was found to be equal to N_e, the polymorphism within the mtDNA was corrected down by one-half.

The total effective population size (N) and the female effective population size (N_f) were estimated from the formulae $\theta = 4Nu_g$ and $\theta = 2N_f u_g$, respectively. The neutral parameter θ was taken to be equivalent to nucleotide diversity (π). The mutation rate per site per generation (u_g) was calculated as the mutation rate per year multiplied by the generation time. The generation time for the southern elephant seal of 8 yr was calculated from female life tables of the stable population at South Georgia (McCann 1985) using the formula $\Sigma x l_x m_x / \Sigma l_x m_x$, where x is age in years, l_x is the proportion of females surviving to age x, and m_x is the number of females produced per female at age x (Begon *et al.* 1986). The effective breeding ratio was estimated from data on mutation rates (μ_n and μ_{mt}) and levels of diversity (π_n and π_{mt}) for nDNA and mtDNA, respectively. The mutation rates do not depend on effective population size, although diversity does, and so for neutrally evolving sequences, and assuming mutation-drift equilibrium, the following should be true:

$$\frac{N_f}{2N} = \frac{\pi_{mt}\mu_n}{\pi_n\mu_{mt}}. \quad (1)$$

N is composed of male and female components such that $N = 4N_m N_f/(N_m + N_f)$ (Wright 1931). Substitution of the right-hand side into Equation 1 above results in the following:

$$\frac{N_f}{N_m} = \left(\frac{8\pi_{mt}\mu_n}{\pi_n\mu_{mt}} - 1\right). \quad (2)$$

RESULTS

From mtDNA, we analyzed 299 bp of CRI from 60 individuals and assayed three diagnostic restriction sites from 115 individuals. From nDNA we sequenced 3594 bp of mostly noncoding regions from seven single-copy loci from 6 haplotypes (three individuals) from the geographic extremes of the range, and 877 bp of this was sequenced from 30 haplotypes. Three nDNA diagnostic markers (restriction site, indel, microsatellite) were assayed from ~186 haplotypes.

Sequence variation: *nDNA:* The pilot analysis of one individual per main population of SG, HD, and MQ (*i.e.*, a total of 6 haplotypes) detected variation in 7 of the 15 nDNA gene segments (Table 1). From this initial screening, several segments were selected to increase the sample size from one individual per population to five individuals per population (*i.e.*, a total of 30 haplotypes). The segments chosen for further analysis were *ALD-A*, exons 2 and 3 of *Mhc-DQA*, and two segments of β-*globin*. Subsequently, it was discovered that two β-*globin* loci were being amplified simultaneously and that it was not possible to distinguish alleles from loci (see materials and methods). Therefore, the 734 bp of β-*globin* sequence was not included in further analyses of sequence variation. The 3594 bp of nDNA analyzed (excluding the 734 bp of β-*globin*) comprised 877 bp sequenced from 30 haplotypes and 2717 bp sequenced from 6 haplotypes. Of the total 3594 bp, there were 2838 bp of silent sites comprised of 467 bp sequenced

from 30 haplotypes and 2371 bp sequenced from 6 haplotypes. The silent sites were assumed to be the synonymous sites in exons, all intron sites except for the AG/GT splice sites, and all of the 5′ UTR and pseudogene sites.

The variants detected included 9-point substitutions, a 6-bp indel, and a pentameric microsatellite. Of the nine variant sites, five were found in introns, two were silent polymorphisms in exons, and there were two nonsynonymous polymorphisms, glycine/arginine in exon 2 of *Mhc-DQA* and glycine/arginine in exon 2 of *Lysozyme*. There were five transitions and four transversions resulting in a roughly twofold bias of transitions over transversions. The total diversity (uncorrected) for nDNA of 0.09 ± 0.03% was calculated from 877 bp from 30 haplotypes and 2717 bp from 6 haplotypes. The level of silent site diversity of 0.08 ± 0.03% was calculated from 467 bp from 30 haplotypes and 2371 bp from 6 haplotypes. The diversity calculated from the samples from the initial screening was the same, and therefore we assume that no systematic bias was introduced by selecting some gene fragments for further sampling. Phase was determined for those nDNA genes with more than one polymorphic site, *ALD-A* and *Lysozyme*. For *ALD-A*, the phase of the variant sites is shown in Figure 2B. for *Lysozyme*, the T/C polymorphism at site 239 was in phase with the A/G polymorphism at site 273.

mtDNA: The level of polymorphism in the 299 bp of mtDNA CRI sequence was considerably higher with 16 different haplotypes observed from 15 individuals, 5 from each of the three main populations (Table 1, Figure 2A). One HD individual was heteroplasmic. All 26 variant sites were transitions. All further analyses of CRI sequence variation combined this data set of 16 sequences with the data set of Hoelzel *et al.* (1993), giving a total of 60 individual sequences with 38 variant sites (five transversions) and 38 different haplotypes. The uncorrected nucleotide diversity in these 60 CRI sequences was 2.86 ± 0.49%. Several studies have shown substitution rate variation among sites in the mammalian control region as evidenced by departure from a Poisson distribution of the number of substitutions per site (*e.g.*, Kocher and Wilson 1991). The mean of the inferred number of substitutions per site from the 60 CRI sequences was less than the variance (0.2843 and 0.8820, respectively), indicating a non-Poisson distribution. The frequency distribution of the inferred number of substitutions per site did not fit a Poisson distribution ($\chi^2 = 161.3$, $P < 0.001$) but did fit a negative-binomial distribution ($\chi^2 = 0.98$, $P > 0.25$). The scale parameter for the negative-binomial distribution was estimated by maximum-likelihood as 0.1042, and therefore we supplied this to the Tamura-Nei gamma model of pairwise distances giving a nucleotide diversity of 4.75 ± 2.67%.

Selection: Using the method of Tajima (1989) on the 60 CRI sequences, the test statistic D was equal to −0.427, which is not significant ($P > 0.1$). Using the method of Fu and Li (1993) on the 60 CRI sequences, with the northern elephant seal as the outgroup, the test statistic D was equal to 0.55, which is not significant ($P > 0.05$). The Kreitman and Aguadé χ^2 test of independence on all the variant nDNA sequences and the 60 CRI sequences showed no significant deviations from the neutral expectation of levels of polymorphism within species, given the number of fixed differences between species.

TABLE 2

Total (N_e) and female (N_f) effective population sizes

Population/species	N_e	N_f (n)
MQ	—	18,000 (5)
HD	—	22,000 (6)
SG	—	30,000 (28)
PV	—	3,000 (21)
Southern elephant seal	40,000[a]	40,000 (60)
Northern elephant seal[b]	—	5,000 (41)

[a] See text for sample size n for N_e.
[b] Sequence data taken from Hoelzel *et al.* (1993).

Rate of molecular evolution, N_e and N_f:N_m: The rate of neutral evolution for ~1200 bp of silent sites in pinniped nDNA (see Table 1 for genes sequenced) was previously estimated with a Kimura two-parameter model (Slade *et al.* 1994) from three fossil-record calibrations (divergence times of 4.5, 14.5, and 23 mya) as being 1.23 ± 0.24 substitutions per nucleotide site per 10^9 yr (*i.e.*, $1.23 \pm 0.24 \times 10^{-9}$). For the 299 bp of CRI, and using the Tamura-Nei gamma model with one fossil-record calibration (the 4.5 mya divergence between *Mirounga* spp. in one clade and *Hydrurga leptonyx* and *Leptonychotes weddelli* in the other clade; Ray 1976; Slade *et al.* 1994), the rate of CRI evolution was estimated as $75 \pm 46 \times 10^{-9}$.

The total effective population size of roughly $N_e = 4 \times 10^4$ (Table 2) was calculated from the silent site nucleotide diversity given above. We considered that there was not enough nDNA variation to properly estimate N_e within populations. The female effective population size calculated from the 60 CRI sequences was roughly $N_f = 4 \times 10^4$, and each of the three main populations has an N_f of roughly 2–3 × 10^4 (Table 2). The PV population is an order of magnitude smaller than the other southern elephant seal populations, and the effective number of northern elephant seals is an order of magnitude smaller than that for the southern elephant seal. The effective breeding ratio was estimated from Equation 2 to be four to five females to one male.

Geographic structure: *Distribution of CRI sequence variation:* The levels of CRI nucleotide diversity and divergence within and between populations are shown in Table 3. Diversity was highest within the SG and HD populations and very low by comparison in the PV population. The greatest divergence was between the PV and

TABLE 3

Percent diversity (uncorrected) within and between populations for CRI

	PV (n = 21)	SG (n = 28)	HD (n = 6)	MQ (n = 5)
PV	*0.297*	3.365	3.071	5.224
SG	2.073	*2.289*	2.394	3.872
HD	1.908	0.235	*2.029*	3.801
MQ	4.273	1.926	1.985	*1.605*

Diversity within populations in italics on diagonal. Gross divergence between populations above diagonal, and net divergence (i.e., gross divergence minus within-population diversity) between populations below diagonal.

MQ populations, and the least was between the SG and HD populations. Although PV and SG are in the same oceanic region, there is almost an order of magnitude of greater divergence between those two populations than between the separate oceanic populations of SG and HD. The AMOVA results showed that 57% of CRI variation was distributed between populations. This distribution of mtDNA variation was significantly different from random ($P < 0.001$).

For a hierarchical AMOVA, we compared oceanic regions, with SG and PV representing the south Atlantic ocean, HD the south Indian ocean, and MQ the south Pacific ocean. This resulted in a decomposition of variation of −5% among the three oceanic regions, 61% among SG and PV within the south Atlantic oceanic region, and 44% within populations. The breakdown into oceanic regions thus explained none of the variation. This is because of heterogeneity within the south Atlantic ocean between SG and PV and the close relationship between haplotypes from SG and HD representing different regions.

Distribution of nDNA allele and CRI haplotype frequencies: Diagnostic markers were screened in ~30 individuals from each of the three main populations. For CRI, the combined RFLP haplotypes and their population frequencies are shown in Figure 3. Most haplotypes were found in more than one population; however, none of the MQ haplotypes were shared with HD, SG, or PV. Haplotype frequency differences were analyzed by χ^2, and significant heterogeneity was found among all populations ($\chi^2 = 224.76$, $P < 0.001$). For each of the pairwise comparisons, there was significant heterogeneity ($P < 0.001$ for each comparison).

For the nDNA loci, the markers analyzed were the presence/absence of the *Mhc-DQA* 6-bp deletion, the *ALD-A Nsp*I restriction site, and the two β-*globin* pentameric microsatellites (Table 4). There were no departures from the Hardy-Weinberg equilibrium (HWE) for genotypes at the *Mhc-DQA* or *ALD-A* loci. The β-*globin* microsatellite data were not tested for HWE because single-locus genotypes could not be determined. There was no significant heterogeneity among the three populations for alleles at the *Mhc-DQA* and *ALD-A* loci. The χ^2 analysis of the two microsatellite loci treated them as a single multiallelic locus in a tetraploid. This approach (Utter *et al.* 1992), or a similar one (Gharrett *et al.* 1987; Bartley and Gall 1990; Gall *et al.* 1992), has been used previously for duplicated loci in salmonid fish (see Waples 1988; Waples and Aebersold 1990 for discussion). This is a conservative approach for detecting population structure because for any one microsatellite allele, the frequency differences between populations could only be the same or greater by partitioning into two loci. The genotype of individuals with fewer than four bands was deduced from the relative band intensitites. Of the 93 individuals assayed, there were 0 single-band, 7 double-band, 31 triple-band, and 55 quadruplex-band genotypes, indicating a high degree of heterozygosity at both loci. That is, 59% (55/93) of individuals were heterozygous at both loci, and a minimum of 92% (55+31/93) were heterozygous for at least one locus. There was significant heterogeneity of allele frequencies for the microsatellite loci among

	Haplotype			Population frequency			
	*Fok*I	*Rsa*I	*Bsl*I	PV	SG	HD	MQ
1)	0 1	1 1	1	0	3	1	0
2)	1 0	1 1	1	0	6	1	0
3)	0 1	0 1	1	0	11	0	0
4)	0 0	0 1	0	0	4	4	0
5)	0 0	0 1	1	15	3	0	0
6)	0 0	1 1	1	6	1	11	0
7)	0 1	0 1	0	0	3	9	0
8)	0 0	0 0	0	0	1	0	0
9)	1 1	0 1	1	0	0	0	11
10)	1 0	0 1	1	0	0	0	21
11)	1 1	1 1	1	0	0	0	1
12)	1 0	0 1	0	0	0	2	0
13)	1 1	0 1	0	0	0	1	0
			Total	21	32	29	33

Figure 3.—The CRI RFLP haplotypes and their frequencies in four populations. Only the polymorphic sites are shown. Presence of a restriction site = 1 and absence = 0. For each of the 21 individuals from PV (Hoelzel *et al.* 1993), the 299 bp of CRI sequence data was converted to a single RFLP haplotype. An extra polymorphic restriction site was observed for the *Rsa*I restriction site @ 251–254 in the large-scale screening that was not observed in the smaller sequence sample shown in Figure 2.

TABLE 4

Frequency of nDNA alleles

Locus	Allele	Frequency SG	Frequency HD	Frequency MQ	Significance test results
Mhc-DQA	6-bp indel				
	+	57	61	60	Overall $\chi^2 = 1.99$
	−	3	5	8	($P = 0.4130 \pm 0.0156$)
	Total	60	66	68	
		($n = 30$)	($n = 33$)	($n = 34$)	
ALD-A	*Nsp*I site				
	+	19	23	30	Overall $\chi^2 = 2.00$
	−	35	39	34	($P = 0.3360 \pm 0.0149$)
	Total	54	62	64	
		($n = 27$)	($n = 31$)	($n = 32$)	
β-*Globin*	No. repeats				
	6	4	15	23	Overall $\chi^2 = 58.87$
	7	0	3	6	($P = 0.0010 \pm 0.0010$)
	8	7	11	7	
	9	8	7	6	SG/HD $\chi^2 = 20.75$
	10	26	15	11	($P = 0.1000 \pm 0.0095$)
	11	32	36	38	
	12	12	15	14	SG/MQ $\chi^2 = 42.82$
	13	5	7	0	($P < 0.001$)
	14	2	2	1	
	15	6	6	4	HD/MQ $\chi^2 = 23.42$
	16	3	6	6	($P = 0.0320 \pm 0.0056$)
	17	1	3	1	
	18	7	2	1	
	19	2	0	10	
	20	1	0	0	
	Total	116	128	128	
		($n = 29$)	($n = 32$)	($n = 32$)	

the three populations. Pairwise comparisons showed significant differences in microsatellite allele frequency between MQ and the other two populations, but not between HD and SG.

Gene flow: For the 60 CRI sequences, the spatial variance of gene frequency (K_{ST}), the number of female migrants exchanged between populations per generation ($N_f m_f$), and the proportion of female migrants exchanged per generation (m_f) were estimated from the uncorrected and Tamura-Nei corrected distances (Table 5). To estimate m_f from $N_f m_f$, N_f was assumed to be the arithmetic mean of the N_f estimates from the two populations (Chakraborty and Nei 1974). There was little difference in the estimates between the uncorrected and Tamura-Nei corrected distances, because the relative levels of diversity and divergence are similar

TABLE 5

Estimates of K_{ST}, $N_f m_f$, and m_f from CRI variation

Comparison (n) between:	Mean N_f ($\times 10^3$)	Distance (km)	Uncorrected diversities K_{ST}	Uncorrected diversities $N_f m_f$	Uncorrected diversities m_f ($\times 10^{-5}$)	Tamura-Nei diversities K_{ST}	Tamura-Nei diversities $N_f m_f$	Tamura-Nei diversities m_f ($\times 10^{-5}$)
SG (28) and PV (21)	16.5	2,400	0.348	0.53	3.21	0.416	0.39	2.36
HD (6) and SG (28)	26	8,400	0.035	7.74	29.77	0.035	7.74	29.77
HD (6) and PV (21)	12.5	10,800	0.354	0.51	4.08	0.404	0.41	3.28
MQ (5) and SG (28)	24	11,500	0.248	0.85	3.54	0.31	0.63	2.63
MQ (5) and HD (6)	20	7,500	0.267	0.77	3.85	0.348	0.53	2.65
MQ (5) and PV (21)	10.5	11,000	0.6	0.19	1.81	0.705	0.12	1.14

TABLE 6

Time of population and species divergence from CRI data

Comparison (*n*) between:	Time (yr)
MQ (5) and HD (6)	270,000
MQ (5) and SG (28)	260,000
HD (6) and SG (28)	23,000
MQ (5) and PV (21)	600,000
HD (6) and PV (21)	215,000
SG (28) and PV (21)	270,000
Southern (60) and northern (41) elephant seals	800,000

despite a large difference in the absolute amounts of estimated variation. There was limited maternal gene flow between all populations, except between South Georgia and Heard Island. For the latter, maternal gene flow was roughly an order of magnitude greater than among other populations. For the nDNA data, the level of sequence variation and the number of loci assayed were too low to use the sequence-based approach. However, it was possible to obtain an estimate of total gene flow between Heard and Macquarie Islands using allele frequency data from three polymorphic allozyme loci, *Acp-1*, *Ada-1*, and *Pgm-1* (Gales *et al.* 1989), and the two polymorphic nDNA loci from this study (*Mhc-DQA*, *ALD-A*). This resulted in an estimate of *Nm* between Heard and Macquarie Islands of 4.11.

Time since divergence: An alternative approach is to assume that there has been no gene flow between populations and that the level of nucleotide divergence between populations reflects only time since population divergence. The time of divergence between southern elephant seal populations and between the southern and northern elephant seal populations was estimated from CRI data using the Tamura-Nei net divergences and the estimated mutation rate of 75×10^{-9} (Table 6). In this historical association model, MQ and PV last shared a common ancestral population some 600,000 yr ago, SG and HD separated as recently as 20,000 yr ago, whereas all other population divergences occurred some 200,000–300,000 yr ago. The northern and southern elephant seals were estimated to have diverged 800,000 yr ago.

DISCUSSION

In this study, we assessed the contribution of mutation rate, effective population size, gene flow, and selection to the amount and distribution of genetic variation in some regions of mtDNA and nDNA of the southern elephant seal.

Selection: Two approaches were used to infer that selection had not played a significant role in the level or distribution of genetic variation in the gene regions analyzed. First, selection can be detected from patterns of DNA sequence variation using several recently developed methods (Kreitman and Aguadé 1986; Tajima 1989; Fu and Li 1993), although the power of at least some of these tests is limited, given the sample size in this study (Braverman *et al.* 1995; Simonsen *et al.* 1995). For all these methods, the distributions of the mtDNA and nDNA variants in the southern elephant seal (with the exception of the β-*globin* microsatellites that were not tested) were compatible with neutrality. Second, it is reasonable to assume that selection is not a factor if there are concordant geographic patterns among several unlinked loci. Although not as informative, the available data from nDNA loci are concordant with the mtDNA data. Three allozyme loci (*Ada*, *Pgam*, *Pgm*) showed significant differences in allele frequency between HD and MQ (Gales *et al.* 1989) as did the β-*globin* microsatellites, and there are nonsignificant indications from the latter of differences between HD and SG.

Mutation rate, genetic diversity, and effective population size: The rate of silent substitution in pinniped nDNA was previously estimated as 1.23 ± 0.24 substitutions per site per 10^9 yr (Slade *et al.* 1994). This was based on three different divergences and independent fossil-record dates of 4.5, 14.5, and 23 mya, each of which gave very similar rate estimates of 1.37, 0.93, and 1.40×10^{-9}, respectively. As discussed previously (Slade *et al.* 1994), this is at the low end of rates among mammals, that is, 6.5×10^{-9} in rodents, 3×10^{-9} in primates and artiodactyls, and 1×10^{-9} in humans (Li *et al.* 1987). A slower rate of nDNA evolution in carnivores compared with other mammals has also been indicated by immunological distances (Sarich 1985). However, these estimates should be treated with caution because they are based on the fossil record, and it is clear that reinterpretation of parts of the mammalian fossil record is necessary (Wilson *et al.* 1987; Easteal 1990).

The rate of mtDNA evolution was estimated for 299 bp of CRI and was calibrated against only the 4.5 mya fossil-record date. Other calibrations were not possible because of alignment difficulties between more divergent sequences. The rate of evolution in CRI was estimated as $75 \pm 46 \times 10^{-9}$. It is clear that the rate is at least an order of magnitude higher than that for nDNA, and it may be close to two orders of magnitude higher. In terms of percent change, the nDNA is evolving at roughly 0.1%/million years (myr) along a lineage, and the mtDNA CRI is evolving at a rate of 5–10%/myr. The rate of substitution averaged over the entire mtDNA molecule in other animals was estimated as roughly 1%/myr along a lineage (Wilson *et al.* 1985), and the silent rate for the ND4 and ND5 mtDNA genes in primates was estimated to be ~5%/myr (Brown *et al.* 1982). Estimates of the rate of control region evolution include 103×10^{-9} and 74×10^{-9} for CRI and CRII, respectively, in great apes (Horai *et al.* 1995), 75×10^{-9} for the entire control region in great apes (Tamura and Nei 1993), 114×10^{-9} for the entire control region in hu-

mans (Stoneking *et al.* 1992), and 20–40 $\times$ 10^{-9} for CRI in the horse (Ishida *et al.* 1995). Therefore, the estimate presented here of 75 $\times$ 10^{-9} for CRI in the southern elephant seal is consistent with estimates for other mammals.

The uncorrected level of nucleotide diversity in the 299 bp of CRI was 2.86 $\pm$ 0.49%. This is among the higher levels so far observed among vertebrate species (see Table 2 in Slade 1998) and is similar to that observed in control regions I and II in African humans of 2.08% (Vigilant *et al.* 1991). Consistent with its lower mutation rate, nucleotide diversity in nDNA was much lower at 0.09%. Less comparative data are available for nDNA compared to mtDNA. Diversity in southern elephant seal nDNA is similar to the 0.11% found for humans (Li and Sadler 1991) and to the 0.08% found for mice (Nachman 1997) but lower than that in *Drosophila pseudoobscura*, *D. simulans*, *D. ananassae*, or *D. melanogaster* (2.2%, 1.4%, 1.0%, and 0.4%, respectively; summarized in Aquadro 1992). The heterozygosity of 3.2% in the protein products of 35 nDNA genes (Gales *et al.* 1989) is similar to the mammalian average of 4.1% (Nevo *et al.* 1984).

Given that the neutral mutation rate and nucleotide diversity in southern elephant seal nDNA is similar to that found in humans, it is not surprising, then, that the total effective population size (N_e) of 4 $\times$ 10^4 is also similiar to that in humans, which is $\sim 10^4$ to 10^5 depending on the time scale over which the estimates are calculated (Takahata 1993). If the worldwide population size of southern elephant seals is 750,000 (McCann 1985), then the ratio of effective to current population size (N_e/N) would be 0.05. N_e/N ratios are highly variable among species; in a summary of ratios, those of nine species of mammal ranged from 0.08 to 0.76 (Frankham 1994). The low ratio for the southern elephant seal would reflect, in part, its highly polygynous breeding system. Such a breeding system would also explain the similarity between N_f and N_e (Birky *et al.* 1983).

We estimated the effective breeding ratio as four to five females to one male. This ratio is perhaps lower than expected considering that, in one study, the average number of cows per bull in a harem varied between 28 and 53 (Carrick *et al.* 1962a). However, not only are estimates of copulatory success often overestimated by behavioral observations (*e.g.*, Pemberton *et al.* 1992; Amos *et al.* 1993; Lambert *et al.* 1994) but also, although males may sire a large number of offspring in the one or two years they are a beachmaster, their lifetime reproductive success would be much lower than observation during beachmaster years would indicate. A female at South Georgia that survives to breeding age produces, on average, five offspring in her lifetime. [This figure was derived from the female life tables in McCann (1985) and from the estimated fecundity rate of 0.391 females born per female per year (Hindell 1991).] Considering this, the above data suggest that the average breeding male would sire 20 offspring in his lifetime, although there would likely be a much larger variance associated with this figure than that for the average female. We should be cautious when interpreting the estimated effective breeding ratio for two reasons. One, there is a large variance associated with each of the mutation rates and diversities that would probably be amplified in the estimated ratio. Second, the ratio may reflect the presence of population structure and sex-biased gene flow, rather than a sex-biased breeding ratio.

Geographic structure: This is the first global survey of genetic variation in the southern elephant seal. For mtDNA, there was significant geographic structure among the four populations of the southern elephant seal for distribution of sequence variation and distribution of haplotype frequencies. A previous phylogeographic analysis had also shown geographic structure for distribution of lineages (Slade 1998). Most of the geographic structure was because of the divergent MQ and PV populations, the lineages of which formed discrete monophyletic groups. The SG and HD populations shared haplotype lineages, but nonetheless there was significant geographic structure for the distribution of those lineages as well as for the distribution of genetic variation and haplotype frequencies. The limited variation at the nDNA loci allowed only an analysis of allele frequency differences, which, among the populations of SG, HD, and MQ, did not exhibit as strong a geographic structure as the mtDNA data. There were no significant differences in allele frequency markers at the *ALD-A* and *Mhc-DQA* loci, whereas for the β-*globin* microsatellites a significant difference was found between MQ and the other two populations. The difference in the microsatellite allele frequencies between SG and HD was significant at the 10%, but not the 5%, level and suggests that further analysis of the distribution of mDNA variation between these two populations is warranted, preferably with highly variable markers such as microsatellites. The genetic discreteness of the HD and MQ populations has also been shown in a previous study of allozyme variation among those two populations (Gales *et al.* 1989). It is clear, therefore, that there is strong geographic structure for the female (*i.e.*, mtDNA) component among the four populations, but for the biparental (*i.e.*, nDNA) component this is clear only between MQ and both HD or SG. The genetic relationship between populations does not correlate with the relationship determined by growth patterns, thus supporting the hypothesis that the growth pattern differences are environmentally determined (Bryden 1968). The degree of genetic structure also did not support the hypothesis that the demographic changes within populations were because of differences in movement of individuals between populations (Slade 1998).

Difference between mtDNA and nDNA: Differences between mtDNA and nDNA in the level of observed geographic structure may reflect either differences in rates of gene flow between males and females, or differences in characteristics of the genes, such as mutation rate and/or rate of genetic drift. If males and females have an equal probability of migrating, then Nm is expected to be twice that of N_fm_f. The observed result showing Nm between HD and MQ to be five to eight times greater than N_fm_f suggests that the rate of male gene flow may be up to four times greater than female gene flow. The result must be viewed cautiously, because the estimate of N_fm_f was derived from one locus, and because the estimate of Nm was derived from only one pairwise population comparison. Nonetheless, male-biased gene flow is consistent with ecological studies on dispersal of male and female southern elephant seals (Slade 1998) and with sex-biased dispersal during the pelagic feeding phase of the annual cycle in which males generally travel farther than females to reach their respective feeding grounds (Hindell *et al.* 1991).

However, we suggest that most of the difference between mtDNA and nDNA in the observed level of geographic structure is accounted for by differences in the characteristics of the genes. The greater sensitivity of mtDNA compared with nDNA is usually explained by two factors: its smaller effective population size and its higher mutation rate (Birky *et al.* 1983; Wilson *et al.* 1985). For the southern elephant seal, N_f is roughly equal to N_e, and so the rate of drift of mtDNA is expected to be roughly twice that of the nDNA, thereby contributing to the increased resolution of the mtDNA variation. However, most of the increased sensitivity for detecting subdivision from mtDNA point mutations compared with nDNA point mutations would come from the 50–100 times greater mutation rate. The higher mutation rate would decrease the frequency of shared alleles when one population separates into two and would increase the chance that isolated populations contain unique alleles that arose within each population after separation (Slade *et al.* 1994). That differences in mutation rate account for a significant part of the difference in detecting subdivision is supported by the results from the highly variable microsatellites, which alone among the nDNA loci in this study showed evidence of geographic structure. Microsatellite loci have mutation rates of the order of 10^{-2}–10^{-4} per locus per generation (Weber and Wong 1993). This is similar to the mutation rate per locus per generation for CRI, which is 1.8×10^{-4} (*i.e.*, $75 \times 10^{-9} \times 299$ bp $\times$ 8 yr). Conversely, the per locus mutation rate of the other 250–450-bp nDNA gene fragments is 2.5–4.5×10^{-6}, some two orders of magnitude lower.

Gene flow vs. historical association: Geographic structure reflects some combination of contemporary levels of gene flow and recent historical association. Considering the former, the dispersal capacity of adult males and females is such that movement between oceanic regions is certainly possible. The longest interisland movement recorded was some 3000 km between Heard and Marion Islands in the south Indian ocean (Carrick *et al.* 1962b), and males and females regularly make long-distance round trips of up to 6000 km from breeding sites to feeding areas (Hindell *et al.* 1991; McConnell and Fedak 1996). However, because only very small amounts of gene flow are required to generate panmixia (Wright 1931), it is reasonable to suggest that geographic structure in the southern elephant seal, especially with respect to females, is not dominated by any substantial amounts of contemporary gene flow. This suggestion is supported by the observation that mtDNA gene flow (or genetic distance) between populations does not correspond very well with geographic distance (Table 5), particularly when comparing SG and PV ($N_fm_f = 0.53$, distance = 2400 km) with SG and HD ($N_fm_f = 7.74$, distance = 8000 km), and that resight records of ~20,000 individuals tagged mostly in the south Indian and south Pacific ocean regions between 1950 and 1980 showed no movement between regions but some movement, mostly by immatures, within regions (Slade 1998).

If historical association, rather than contemporary gene flow, dominates the observed geographic structure of the southern elephant seal, then how do we explain the close genetic relationship between HD and SG, despite their being as geographically separate as HD and MQ? In the historical model, SG and HD separated as recently as 20,000 yr ago, and all other population divergences occurred over 200,000 yr ago. We suggest that the estimated relatively recent separation time between SG and HD is linked with the last ice age 18,000 yr ago (CLIMAP 1976). It is likely that the distribution of southern elephant seals during that period would have been different from the current distribution, because of the unsuitability of current breeding beaches that were iced over at that time (R. W. Slade, unpublished results). Rather than being distributed on sub-Antarctic islands, the southern elephant seal was probably distributed primarily on the southern edge of the continental land masses of Australia, Africa, and South America, and we suggest that at that time HD and SG were a single breeding population on the coast of South Africa. Records of southern elephant seals in South Africa indicate that it is currently used as a haul-out for immature seals to moult, although several births have occurred there also (Oosthuizen *et al.* 1988). It is known that Australia was a former breeding colony (Pemberton and Skira 1989), and PV may be the remnant of the population breeding on the coast of South America.

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