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Genetic and morphometric differentiation between island and mainland southern elephant seal populations

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We compare genetic (both nuclear and mitochondrial) and morphometric measures between two putative populations of southern elephant seal (*Mirounga leonina*), and interpret the results in the context of data from mark-recapture and satellite-telemetric studies. One population is on the Argentine mainland, while the other is 2400 km away on South Georgia island. We found pronounced differentiation at the mitochondrial DNA (mtDNA) control region that was distinct from the pattern of variation seen among island rookeries. Some morphometric characters and seven out of ten nuclear-DNA markers also showed differentiation between the island and mainland sites. Diversity at nuclear markers was high in both populations but mtDNA diversity was low in the mainland population, suggesting a founder event and little subsequent immigration of females. Morphological differences may suggest different selective environments at the two sites.

Keywords: population genetics; morphometrics; dispersal; demography; marine mammals

1. INTRODUCTION

The principal island breeding colonies for the southern elephant seal are located on Macquarie island (MQ) in the South Pacific, Heard (HD) and Kerguelen islands in the south Indian Ocean and South Georgia (SG) in the South Atlantic. The only well-established continental breeding colony is on Peninsula Valdes (PV) in Argentina. There are also a number of smaller breeding colonies on other sub-Antarctic islands. Early studies of morphological and life-history variation suggested some differences between putative populations (represented by breeding colonies) in the South Atlantic, South Pacific and Indian Ocean (see Laws 1994). For example, Lydekker (1909) proposed a subspecific distinction between populations in different ocean basins based on differences in skull morphology. Carrick *et al*. (1962) and Bryden (1968) showed that elephant seals from MQ and HD islands grew more slowly and to a smaller ultimate size than elephant seals on SG. However, Bryden (1968) further suggested that these differences in growth rate are environmentally determined and related to prey resources (especially where this limits the capacity for females to provision pups during suckling). More recently, molecular genetic studies have found significant differentiation between MQ and HD islands at allozyme (Gales *et al*. 1989), microsatellite DNA and mitochondrial control region markers (Slade *et al*. 1998). However, Slade *et al*. (1998) found little differentiation between HD and SG at either microsatellite or mitochondrial DNA (mtDNA) markers, which they suggest may reflect a common origin for these two populations during the last ice age. The PV population was compared using only the mtDNA control region marker and was differentiated from each of the other three populations (HD, MQ and SG; Hoelzel *et al*. 1993; Slade *et al*. 1998). There was, therefore, little correlation between genetic and geographic distance, as PV and SG are only 2400 km apart compared to 6800 km between SG and HD islands.

Historically, there were mainland breeding populations both in South America and Australia, but now mainland sites receive only occasional visitors, with the exception of PV (see Slade *et al*. 1998). Here, we use a diversity of marker systems to assess further the characteristics of genetic differentiation between the mainland site in Argentina and an island site on South Georgia. Our data suggest a founder event, probably involving just one matriline, and no subsequent female recruitment at the Argentine site. Both mark-recapture and genetic analyses based on nuclear markers suggest the possibility of some male-mediated gene flow, though both mtDNA and morphometric characters suggest significant differentiation.

2. METHODS

(a) *Molecular genetic analyses*

Tissue samples were collected from the hind flippers of seals as reported previously (Hoelzel *et al*. 1993) and preserved in the field using a salt–dimethyl-sulfoxide (DMSO) buffer solution (Hoelzel & Dover 1989). Samples were taken from an equivalent number of adult males and females. DNA was extracted by the phenol–chloroform method as described in Hoelzel & Green (1998). The template for sequencing 300 bp from the 5′-end of the mtDNA control region was amplified using the polymerase chain reaction (PCR) under the following conditions: 100 μM dCTP, dTTP, dGTP and dATP, 1.5 mM MgCl, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 1–50 ng genomic DNA and 0.5 μg of each primer (primer sequences as in Hoelzel *et al*. 1993). The template was sequenced directly using the dye-terminator method for the Applied Biosystems (ABI) system. We included mtDNA control region sequences for 32 seals from PV and 28 seals from SG

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(11 of the PV samples were sequenced for this study and the rest were from Hoelzel *et al*. (1993)).

Four dinucleotide microsatellite DNA loci (M2b, M11a, M13.3 and M16.3) were derived from an elephant seal genomic library as described in Hoelzel *et al*. (1999*a*). For these loci, amplified DNA was analysed for length variation on a 6% polyacrylamide denaturing gel following incorporation of ^{33}P α-dATP (PCR reaction conditions: 100 µM dCTP, dTTP and dGTP, 5 µM dATP, 1.5 mM MgCl (except 1.0 mM for M11a), 10 mM Tris-HCl pH 8.4, 50 mM KCl, 250 pM of each primer and 0.1 µCi of ^{33}P α-dATP) or by the automated ABI system. Primer sequences for M2b are 5′-CCGACTGCTGGGGTAAAG and 5′-TCAGTCTCACCCACCTAC; M11a: 5′-TACATTCACAAGGCTCAA and TGTTTCCCAGTTTTACCA; M13.3 5′-GCGCACACGATAGATACC and ACATTTGGTGCAGGGTTC; and M16.3: 5′-CCTAGACTCCCTTGGTAG and AACTCACTGAGCCGTATA. The details of a further three dinucleotide microsatellite loci (Hg6.3, Hg8.9 and Hg8.10) are published elsewhere (Allen *et al*. 1995) and these were analysed on the automated ABI system. Two further loci (β) were simultaneously amplified by the same set of primers from two loci in the β-globin gene family (for details, see Slade *et al*. 1998) and genotyped using the ABI automated method. These are pentameric $(GGAAA)_n$ microsatellite loci. At the two β loci, multiple alleles of a given size could be easily detected by the height of the peak but single-locus genotypes could not be determined (see Slade *et al*. 1998). These closely linked loci were treated as a single locus with four alleles.

An immune-system gene was analysed using single strand conformational polymorphism analysis (SSCP, Orita *et al*. 1989). Primers for the exon 2 region of the DQB gene were designed from a comparison of cow, human and dog sequences from the GEN-EMBL database: sense: 5′-TCGTGTACCAGTTTAAGGGC; antisense: 5′-ACGTCCTTCTGGCTGTTCCA (Hoelzel *et al*. 1999*b*). A 142 bp segment was amplified by PCR from each individual and ^{33}P α-dATP was incorporated into the product under the following assay conditions: 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 100 µM dTTP, dCTP and dGTP, 5 µM dATP, 1 µCi ^{33}P α-dATP and 50 pM of each primer. Labelled PCR product was denatured at 95 °C for 5 min, chilled on ice for 1 min and loaded onto a non-denaturing gel (4.5% acrylamide (37.5/1 acrylamide/bis), 10% glycerol) run at 20 °C to assay for SSCP. Two to four examples of all putative genotypes were cloned and sequenced to confirm the uniqueness of apparent alleles (see Hoelzel *et al*. 1999*b*).

(b) *Morphometric analyses*

Body measurements were collected from sedated (see Baker *et al*. 1990), weaning-age pups. The pups were measured at approximately the same time relative to weaning in each population, though this entailed collecting measurements in October in PV and in November in SG. All measurements were collected by the same researcher (ARH) with the seal or its appendage in a standardized position. For example, standard length was measured with the animal dorsal side up with the neck extended and is the length from the tip of the nose to the tip of the hind flippers. The widths of pectoral and hind flippers were measured at the widest points. Elephant seal hind flippers are each composed of two rigid pads and these were each measured across the maximum width of the pad. The four hind-flipper pad widths were combined to give a comparative measure of hind-flipper width. The two pectoral-flipper widths were also measured at the widest points and combined for comparison. The lengths of the hind flippers were estimated using a measure of the distance from the anus to the tip of the flipper. The total numbers of facial vibrissae were counted from six rows on both sides of the face. Measures were compared between populations using analysis of variance (ANOVA) or analysis of covariance (ANCOVA). As the average standard length was different for the two populations (see § 3(b)), flipper size was compared between populations with an ANCOVA using standard length as a covariate and location and sex as fixed effects. The slopes were tested for uniformity using an *F*-statistic (all comparisons indicated uniform slopes, data not shown).

(c) *Molecular population and phylogenetic analysis*

The standard error of heterozygosity was estimated using the formulations of Weir (1996). Correspondence with Hardy–Weinberg equilibrium was assessed with a Fisher exact test (Guo & Thompson 1992) as implemented by GENEPOP (Raymond & Rousset 1995). Allele frequency differences were also assessed using a Fisher exact test and for both Hardy–Weinberg and allele frequency differentiation an unbiased estimate of the *p* value was determined using a Markov chain method (based on 1000 iterations and implemented using GENEPOP). Allele frequencies at the β loci were compared with published allele frequencies for other populations using a χ^2-test with Williams's correction for discontinuity (see Sokal & Rohlf 1995). An F_{ST} (after Weir & Cockerham 1984) statistic was computed to assess population differentiation at all microsatellite loci with the exception of β (as allelic assignment per locus could not be determined for these two loci). The significance of the differences between zero and small F_{ST} values were tested using the permutation method implemented in FSTAT. Comparisons for the DQB locus were based on allele frequency (from the SSCP patterns). Dinucleotide microsatellite markers were analysed for variation between populations using the F_{ST} statistic as this has been shown to provide good resolution for intraspecific comparisons when the level of differentiation is low (e.g. Forbes *et al*. 1995). Evidence for linkage disequilibrium between loci was tested by permutation analysis using the GENEPOP program. Other measures of diversity (π) and distance (D_{xy}) were computed as described by Nei (1987). An estimate of gene flow (Nm) was based on the seven dinucleotide loci and the private allele method (Slatkin 1995).

Mitochondrial control region sequences were compared phylogenetically using the neighbour-joining (Saitou & Nei 1987) and maximum-likelihood (Felsenstein 1981) methods. For the neighbour-joining tree, a majority rule consensus tree was constructed from 1000 bootstrap replications. The distance matrix was based on the Kimura two-parameter model (Kimura 1980). For maximum likelihood, the starting tree was obtained via stepwise addition. Nucleotide frequencies were set at the empirical levels and mutation rates were assumed to follow a gamma distribution with a shape parameter that was estimated using maximum likelihood. For both methods, the transition–transversion ratio was set at the observed level of 4.8:1.

3. RESULTS

(a) *Molecular genetic comparisons*

Within-population diversity (π) for the control region was 0.003 in PV and nearly an order of magnitude higher at 0.023 in SG. The two populations were also differentiated at this locus with a genetic distance of $D_{xy} = 0.029$. Differentiation as measured by the F_{ST} statistic indicates

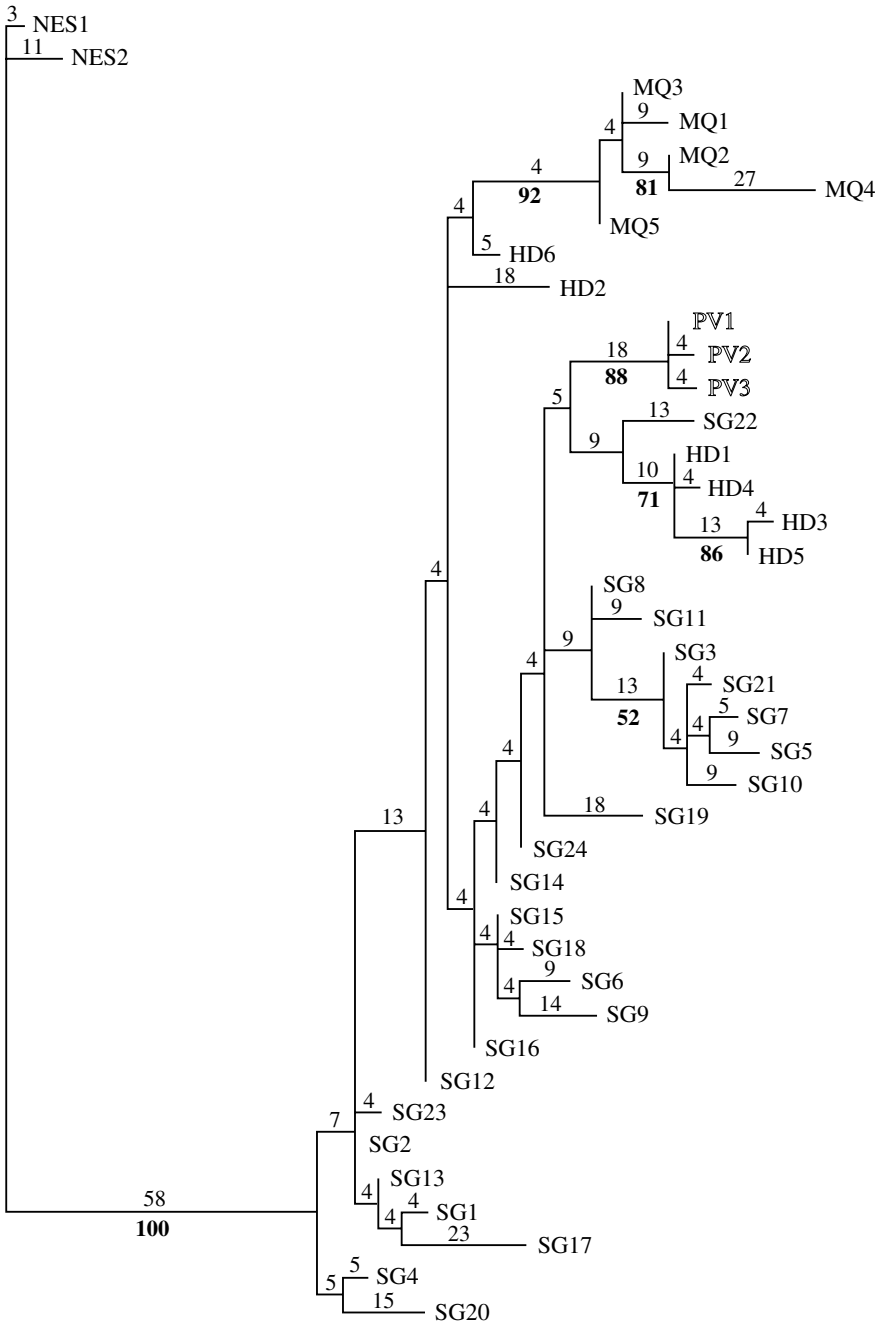

Figure 1. Maximum-likelihood tree of 300 bp of mtDNA control region from four breeding colonies. Branch lengths and bootstrap support (bold) are given. Two northern elephant seal sequences are included as outgroups.

that 56.3% of the variance was due to differences between populations and there are no shared haplotypes between these two populations at this locus. Even though the sample sizes were similar for PV and SG, there were only three haplotypes in PV compared to 24 in SG. Further, the relationship between haplotypes from the PV population suggests one matrilineage since each of two haplotypes can be derived from the third by a single bp change. The frequencies of haplotypes in males as compared to females were not significantly different (data not shown). Phylogenies constructed by the neighbour-joining and maximum-likelihood methods, which also included mtDNA sequence data from MQ and HD (after Slade *et al*. 1998), showed equivalent patterns. The maximum-likelihood tree is shown in figure 1. Four seals in SG had haplotype SG2 and two had SG22, while the remaining seals had unique haplotypes. For the PV population, ten seals had haplotype PV1, 12 had PV2 and ten had PV3. The PV haplotypes are closest to a lineage including haplotype SG22 and a cluster of four out of the six HD haplotypes. Four lineages are well supported by both trees: a lineage including all five MQ haplotypes, the PV lineage, a lineage including four out of the six HD haplotypes and a lineage including five of the SG haplotypes (SG3, SG5, SG7, SG10 and SG21). One of the HD haplotypes (HD6) is associated with the MQ lineage, though the association was not strongly supported.

All nuclear markers showed similar levels of variation in both populations and none showed a significant

Table 1. *Allele frequency and heterozygosity at the dinucleotide microsatellite loci and the DQB locus in the Peninsula Valdes (PV) and South Georgia (SG) populations*

(Sample sizes and estimated standard errors for H_o are given in parentheses. Alleles are given in bold type.)

locus	**alleles**									H_o	H_e
M2b	**233**	**235**	**239**	**241**	**243**	**245**	**247**	**249**	**251**		
SG (40)	4	3	1	6	27	17	4	5	13	0.875 (0.003)	0.798
PV (80)	1	12	20	9	47	13	8	7	43	0.750 (0.002)	0.806
H8.9	**178**	**182**	**184**	**185**	**186**	**187**	**189**	—	—		
SG (36)	2	11	42	5	10	1	1	—	—	0.667 (0.006)	0.611
PV (33)	2	11	44	2	5	0	2	—	—	0.576 (0.007)	0.519
H6.3	**215**	**217**	**219**	**221**	**223**	**225**	**231**	—	—		
SG (30)	3	31	19	3	1	1	2	—	—	0.626 (0.008)	0.567
PV (28)	2	26	27	1	0	0	0	—	—	0.571 (0.009)	0.550
H8.10	**173**	**175**	**177**	**179**	**181**	**183**	—	—	—		
SG (36)	1	19	25	15	6	5	—	—	—	0.806 (0.004)	0.754
PV (36)	4	6	31	4	15	10	—	—	—	0.694 (0.006)	0.739
M11a	**141**	**143**	**145**	**147**	**149**	**151**	—	—	—		
SG (31)	13	19	8	11	11	0	—	—	—	0.783 (0.006)	0.871
PV (24)	8	5	4	21	9	1	—	—	—	0.750 (0.008)	0.746
M16.3	**149**	**152**	**163**	—	—	—	—	—	—		
SG (36)	0	44	28	—	—	—	—	—	—	0.556 (0.007)	0.475
PV (35)	4	49	17	—	—	—	—	—	—	0.371 (0.007)	0.448
M13.3	**133**	**135**	—	—	—	—	—	—	—		
SG (36)	2	70	—	—	—	—	—	—	—	0.056 (0.001)	0.054
PV (32)	12	52	—	—	—	—	—	—	—	0.313 (0.007)	0.305
DQB	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	—		
SG (34)	2	2	16	0	11	18	11	8	—	0.676 (0.080)	0.804
PV (75)	2	7	16	2	16	17	56	34	—	0.760 (0.049)	0.771

Table 2. *Allele frequency at the β-globin microsatellite loci*

(All of the data for Heard island (HD) and Macquarie island (MQ) and part of the data for South Georgia (SG) are from Slade *et al*. (1998). PV, Peninsula Valdes.)

	allele frequency (alleles indicated as number of repeats)															
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	*n*
PV	3	0	3	7	23	48	22	1	0	10	9	12	4	6	0	37
SG	4	0	7	8	28	35	13	5	3	6	4	1	7	2	1	31
HD	15	3	11	7	15	36	15	7	2	6	6	3	2	0	0	32
MQ	23	6	7	6	11	38	14	0	1	4	6	1	1	10	0	32

deviation from expectations for Hardy–Weinberg equilibrium. There is evidence for differentiation, but less than at the mtDNA locus. Table 1 shows allele frequencies and heterozygosity for the dinucleotide microsatellite and DQB loci in each population. Significant differentiation is indicated at the DQB locus for both F_{ST} (0.07, $p = 0.001$) and the exact test comparing allele frequencies ($p = 0.0004$). There was no significant disequilibrium among dinucleotide microsatellite loci. Exact tests showed significant differentiation for four of these loci (M2b, H8.10, M13.3 and M11a) and not the other three. F_{ST} ranged from −0.0019 (H8.9) to 0.116 (M13.3) and was significant at H8.10 (0.047, $p = 0.004$), M13.3 ($p = 0.001$) and M11a (0.052, $p = 0.007$). The combined F_{ST} for all seven dinucleotide microsatellite loci was 0.025 ($p = 0.002$). An estimate of $Nm = 4.9$ was obtained based on the private allele method and these seven loci. Table 2 shows the allele frequencies for the β loci in the PV and SG populations in comparison with published data for HD and MQ. At these loci, the PV population is differentiated from HD ($\chi^2 = 38.2$, d.f. = 13, $p = 0.0003$) and MQ ($\chi^2 = 45.1$, d.f. = 13, $p < 0.0001$) but less so from SG ($\chi^2 = 25.8$, d.f. = 13, $p = 0.018$).

(b) *Morphological characters*

Morphological comparisons between the weaning-age pups in PV and SG are shown in table 3. The standard length of pups in PV is, on average, significantly greater than in SG (males, $F = 222$, d.f. = 194, $p < 0.0001$; females, $F = 221$, d.f. = 200, $p < 0.0001$). The size of appendages is proportionally greater in SG (standardized against standard length, data not shown) and this could be an effect of allometry. However, the uncorrected width of the hind flippers is also greater in SG than in PV for

Table 3. *Comparison of morphometric characters between populations at Peninsula Valdes and South Georgia*

(Data are presented as mean (standard deviation). All measurements are in centimetres on weaning-age pups. SL, standard length; AFL, length from anus to the tip of the hind flippers; HFT, the combined width of the hind flippers (see text); PWT, the combined width of the pectoral flippers; WNT, the total number of facial vibrissae.)

	Peninsula Valdes		South Georgia	
character	males ($n=100$)	females ($n=100$)	males ($n=96$)	females ($n=102$)
SL	167.5 (8.9)	163.6 (9.4)	148.0 (9.2)	145.3 (7.9)
AFL	37.6 (2.8)	38.9 (2.6)	37.3 (2.9)	38.6 (2.7)
HFT	30.6 (2.4)	29.6 (2.4)	31.3 (2.7)	30.4 (2.3)
PWT	19.7 (1.2)	19.1 (1.2)	19.2 (1.2)	18.4 (1.0)
HFT/PWT	1.55 (0.09)	1.55 (0.10)	1.63 (0.11)	1.65 (0.11)
WNT	76.3 (3.8)	75.2 (3.7)	76.7 (3.9)	76.6 (3.1)

both males ($F=4.35$, d.f. $=194$, $p=0.038$) and females ($F=7.06$, d.f. $=200$, $p=0.009$) even though standard length is greater in PV. In comparisons between populations using standard length as a covariant in an ANCOVA (controlling for differences in body size) hind-flipper width (males, $F=85.2$, d.f. $=194$, $p<0.0001$; females, $F=63.3$, d.f. $=200$, $p<0.0001$), pectoral-flipper width (males, $F=16.4$, d.f. $=194$, $p<0.0001$; females, $F=4.33$, d.f. $=200$, $p=0.039$) and hind-flipper length (males, $F=30.9$, d.f. $=194$, $p<0.0001$; females, $F=40.1$, d.f. $=200$, $p<0.0001$) are all greater in SG.

A comparison of the size of hind flippers relative to front flippers (using hind-flipper width as the dependant variable and fitting pectoral-flipper width as a covariant) also showed a significant difference, with hind-flipper width being proportionally larger in SG (males, $F=28.5$, d.f. $=194$, $p<0.0001$; females, $F=33.1$, d.f. $=200$, $p<0.0001$; see table 3). All the above comparisons are for log-transformed data, however, the same comparisons using non-transformed data showed essentially the same F and p values (data not shown). There was little effect of overall size on the ratio of hind-flipper width to pectoral-flipper width (regressions against standard length ranged from $r^2=0.03$ to $r^2=0.07$ for both normal and log-transformed data). A small sample of adults were measured in SG (sedation of adults is risky and therefore this was only done when multiple procedures were to be undertaken, which did not occur in PV). The ratio of hind-flipper width to pectoral-flipper width for adult males was not significantly different from that for male pups (adult males: 1.57 ± 0.08, $n=7$, $t=1.37$, $p=0.17$). However, the ratio for adult females was significantly higher than for female pups (adult females: 1.76 ± 0.13, $n=21$, $t=3.90$, $p=0.0002$). The proportional size of hind-flipper width compared to overall standard length in adult females (0.172 ± 0.011) is significantly greater than for adult males (0.153 ± 0.009, $t=4.03$, $p=0.0004$). These comparisons using non-parametric tests showed the same pattern of significance (Mann–Whitney U-test, data not shown). The total number of whiskers was not significantly different between populations for males, but the distribution for female pups from PV was significantly different from that in SG ($\chi^2=46.9$, d.f. $=19$, $p=0.0004$) with most PV pups having fewer whiskers (PV, mode 72, range 63–85; SG, mode 76, range 75–84; table 3).

4. DISCUSSION

(a) *Genetic differentiation*

Female southern elephant seals in PV are uniquely isolated from other studied populations in the South Atlantic. The data suggest little or no recruitment of females to the PV population since a founding event. This is indicated by the observations that only one mtDNA lineage is represented in PV, this lineage is unique to the PV population and extensive variation is found in the island sites. Any significant immigration of females since the population was founded would have established further matrilines. The nuclear DNA data also show differentiation but may indicate more frequent dispersal by males (especially between PV and SG), since the level and pattern of variation was similar in each population for each nuclear locus and measures of genetic structure suggested relatively low levels of differentiation. Further, a founding event small enough to limit female variation to one matriline should have reduced variation in at least some of the nuclear markers in that population, but there is no evidence of this. A historical bottleneck (perhaps due to hunting pressure) is another possible explanation for the low variation in PV, though available data suggest that most hunting in this region took place in SG (Laws 1994). The genetic data (indicating two haplotypes derived from a single remnant haplotype) also suggest a demographic event that predates the recent exploitation by modern sealers.

Taken together with the mark-recapture data (see below), continuing movement of males seems a more parsimonious interpretation than complete isolation since a founder event (or bottleneck) in PV. While the typical pattern for mammals is female philopatry and male dispersal (Greenwood 1980), the PV population stands out as distinct from the pattern of genetic structure seen between islands (see Slade *et al*. 1998). As seen in the phylogenies, several matrilineages and multiple haplotypes are represented in most island sites, in contrast to the shallow lineage represented in PV. The PV haplotypes are within a loosely defined lineage dominated by HD haplotypes (a breeding colony approximately 9200 km from PV), which may suggest the source of the founding matriline. Slade *et al*. (1998) suggested a possible common origin for the HD and SG populations, based on genetic similarity and the theory that the island habitat would

have been lost during the last glacial maximum (18 000 years before present). They propose that the founding population may have bred on the mainland in southern Africa. However, it is also possible that most island habitats remained habitable for breeding, at least during the summer months (see Crosta *et al*. 1998), or that populations were simply temporarily displaced to islands further north during the ice age (e.g. to the Falkland, Aukland and Kergulan Islands).

Locations of elephant seals at sea during the pelagic phase of their annual cycle, obtained by geolocations (Hill 1994) and satellite relay data loggers, have consistently indicated long-distance excursions and foraging over deep water by females and more restricted excursions by males (Campagna *et al*. 1995, 1998, 1999; McConnell & Fedak 1996). These data show that males often forage in shallower waters near the breeding colony. Mark-recapture studies have indicated a high proportion of seals showing philopatry, and these studies have not produced any matches between PV and SG (see Lewis *et al*. 1996). However, subadult males have been resighted between PV and the Falkland islands during both moulting and breeding seasons (C. Campagna, unpublished data; Galimberti & Boitani 1999), and this geographically intermediate island could serve as a conduit for dispersal between PV and SG. This is supported by the observation that males (but not females) tagged in SG have been resighted at the Falkland islands. Mark-recapture studies in PV (Lewis *et al*. 1996) and in MQ (Nicholls 1970; Hindell & Little 1988) found resightings of about 80% of females within a few kilometres of their place of birth. Out of those animals resighted on haulouts away from PV (50–1000 km), most were males. Therefore, while females may forage over greater distances than males, these data suggest that males may typically disperse greater distances for breeding.

(b) *Morphological differentiation*

Numerous studies describe differences in morphology between natural conspecific populations of vertebrate species (e.g. Molina-Borja *et al*. 1997; Jerry & Cairns 1998), which can be pronounced in spite of relatively low levels of molecular genetic differentiation (e.g. DeWolf *et al*. 1998) and can develop over relatively short periods of time (Kinnison *et al*. 1998). Bryden (1968) proposed that differences in age at puberty and average adult size between elephant seal populations on MQ and the Falkland islands were due to limiting food supplies and a consequent lower rate of pre-weaning growth on MQ. The PV population is the only one of this species known to have increased in size over the last 20 years, while the SG population is currently stable (Campagna & Lewis 1992; Boyd *et al*. 1996; Lewis *et al*. 1998). Further, in our study, weaning-age pups were significantly smaller (as measured for standard length) in SG, which could be a reflection of limiting resources. However, previous studies on weaning mass in PV (Campagna *et al*. 1992) and SG (Arnbom *et al*. 1993) found similar weaning masses for both populations (e.g. male weaning mass of 130 ± 2 kg in SG, 131.5 ± 2.4 kg in PV) and similar durations of lactation and post-weaning fast. Even if differences in population growth could explain differences in overall size at weaning, it is unlikely that environmental factors could explain all of the morphological differences observed in this study. The most striking of these is the relative size of the hind flippers, which were both wider and longer in pups in SG. It was not possible to assess heritability directly due to the difficulty in acquiring a large sample of measurements from the parents of specific pups; however, there was no strong allometric relationship that could account for the differences in the direction and to the degree observed. Further, while adult females showed a more pronounced proportional difference in hind- versus pectoral-flipper size compared to weaners, a small sample of adult males did not.

One possible interpretation of the differences in hind-flipper size is a greater tendency for females with relatively large hind flippers to survive, perhaps in relation to foraging efficiency on long excursions. Eleven females were tracked from PV on foraging excursions, using satellite telemetry or geolocation devices (Campagna *et al*. 1995, 1998). They travelled an average maximum of approximately 1200 km from the breeding/moulting site, while nine females tracked from SG travelled an average maximum of approximately 2600 km (McConnell & Fedak 1996). As elephant seals propel themselves primarily with their hind flippers, there could be selection for greater relative hind-flipper area if there is a need to travel further from the SG breeding site during foraging excursions. The larger relative hind-flipper size of adult females compared to adult males is consistent with this interpretation. However, further details on the relative morphology of adults, behaviour of subadults and the heritability of these characters would be required to test this hypothesis further. The significantly larger numbers of vibrissae on SG females may also be a response to selection, though again we can not assess this in detail with the available data.

Although the molecular data suggest little movement of females, they may also suggest some level of male-mediated dispersal. The persistence of variation (morphometric or otherwise) between these populations would not be contradicted by this finding provided that there is selection for the trait. Selection can maintain differentiation between populations in spite of migration, provided that the variance in fitness is greater than the average selective advantage of different alleles in the different populations and that selection is positive in some populations and negative in others (see discussion in Gillespie (1998)). Thus, even if the migration rate, m, was 1 (all individuals disperse), selection at a locus could, in theory, maintain polymorphism. For the PV and SG populations the migration rate is apparently relatively low but a detailed estimate of the magnitude of selection required to maintain differentiation is not possible due to limitations in our understanding of the genetic component of the morphometric traits.

(c) *Conclusions*

The mainland population at PV is unusual in comparison with all of the island sites that have been studied. The distinction suggests a female founding event, and the apparent lack of further female immigration to this site suggests that female dispersal between island and mainland sites may be rare. Males may infrequently move between island and mainland sites to breed but we

nevertheless find evidence for phenotypic differentiation, possibly consistent with different selective environments in the two habitats.

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REFERENCES

Allen, P. J., Amos, W., Pomeroy, P. P. & Twiss, S. D. 1995 Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between 2 British breeding colonies. *Mol. Ecol.* **4**, 653–662.

Arnbom, T., Fedak, M. A., Boyd, I. L. & McConnell, B. J. 1993 Variation in weaning mass of pups in relation to maternal mass, postweaning fast duration, and weaned pup behaviour in southern elephant seals (*Mirounga leonina*) at South Georgia. *Can. J. Zool.* **71**, 1772–1781.

Baker, J. R., Fedak, M. A., Anderson, S. S., Arnbom, T. & Baker, R. 1990 Use of a tiletamine–zolazepam mixture to immobilise wild grey seals and southern elephant seals. *Vet. Rec.* **126**, 75–77.

Boyd, I. L., Walker, T. R. & Poncet, J. 1996 Status of southern elephant seals in South Georgia. *Antarctic Sci.* **8**, 237–244.

Bryden, M. M. 1968 Control of growth in two populations of elephant seal. *Nature* **217**, 1106–1108.

Campagna, C. & Lewis, M. N. 1992 Distribution and growth of an elephant seal colony. *Mar. Mamm. Sci.* **8**, 387–396.

Campagna, C., LeBoeuf, B. J., Lewis, M. & Bisioli, C. 1992 Equal investment in male and female offspring in southern elephant seals. *J. Zool. (Lond.)* **226**, 551–561.

Campagna, C., LeBoeuf, B. J., Blackwell, S. B., Crocker, D. E. & Quintana, F. 1995 Diving behaviour and foraging location of female southern elephant seals from Patagonia. *J. Zool. (Lond.)* **236**, 55–71.

Campagna, C., Quintana, F., LeBoeuf, B. J., Blackwell, S. & Crocker, D. E. 1998 Diving behaviour and foraging ecology of female southern elephant seals from Patagonia. *Aquat. Mamm.* **24**, 1–11.

Campagna, C., Fedak, M. A. & McConnell, B. J. 1999 Post-breeding distribution and diving behavior of adult male southern elephant seals from Patagonia. *J. Mamm.* **80**, 1341–1352.

Carrick, R., Csordas, S. E. & Ingham, S. E. 1962 Studies on the southern elephant seal, *Mirounga leonina*. IV. Breeding and development. *CSIRO Wildlife Res.* **7**, 161–197.

Crosta, X., Pichon, J. J. & Burckle, L. H. 1998 Reappraisal of Antarctic seasonal sea-ice at the last glacial maximum. *Geophys. Res. Lett.* **25**, 2703–2706.

DeWolf, H., Backeljau, T. & Verhagen, R. 1998 Spatio-temporal genetic structure and gene flow between two distinct shell morphs of the planktonic developing periwinkle *Littorina striata* (Mollusca: Prosobranchia). *Mar. Ecol. Prog. Series* **163**, 155–163.

Felsenstein, J. 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Forbes, S. H., Hogg, J. J., Buchanan, F. L., Crawford, A. M. & Allendorf, F. W. 1995 Microsatellite evolution in congeneric mammals; domestic and bighorn sheep. *Mol. Biol. Evol.* **12**, 1106–1113.

Gales, N. J., Adams, M. & Burton, H. R. 1989 Genetic relatedness of two populations of the southern elephant seal. *Mar. Mamm. Sci.* **5**, 57–67.

Galimberti, F. & Boitani, L. 1999 Demography and breeding biology of a small, localized population of southern elephant seals. *Mar. Mamm. Sci.* **15**, 159–178.

Gillespie, J. H. 1998 *Population genetics: a concise guide*. London: Johns Hopkins University Press.

Greenwood, P. J. 1980 Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.* **28**, 1140–1162.

Guo, S. W. & Thompson, E. A. 1992 Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics* **48**, 361–372.

Hill, R. D. 1994 Theory of geolocation by light levels. In *Elephant seals: population ecology, behavior and physiology* (ed. B. J. LeBoeuf & R. M. Laws), pp. 227–236. Berkeley, CA: University of California Press.

Hindell, M. A. & Little, G. J. 1988 Longevity, fertility and philopatry of two female southern elephant seals at Maquarie Island. *Mar. Mamm. Sci.* **4**, 168–171.

Hoelzel, A. R. & Dover, G. A. 1989 Molecular techniques for examining genetic variation and stock identity in cetacean species. *Int. Whaling Comm. Spec. Issue* **11**, 81–120.

Hoelzel, A. R. & Green, A. 1998 PCR protocols and population analysis by direct DNA sequencing and PCR-based DNA fingerprinting. In *Molecular genetic analysis of populations, a practical approach*, 2nd edn (ed. A. R. Hoelzel), pp. 159–186. Oxford University Press.

Hoelzel, A. R., Halley, J., Campagna, C., Arnbom, T., Le Boeuf, B. J., O'Brien, S. J., Ralls, K. & Dover, G. A. 1993 Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. *J. Hered.* **84**, 443–449.

Hoelzel, A. R., LeBouef, B. J., Campagna, C. & Reiter, J. 1999*a* Alpha male paternity in elephant seals. *Behav. Ecol. Sociobiol.* **46**, 298–306.

Hoelzel, A. R., Stephens, J. C. & O'Brien, S. J. 1999*b* Molecular genetic diversity and evolution at the MHC DQB locus in four species of pinnipeds. *Mol. Biol. Evol.* **16**, 611–618.

Jerry, D. R. & Cairns, S. C. 1998 Morphological variation in the catadromous Australian bass, from seven geographically distinct riverine drainages. *J. Fish Biol.* **52**, 829–843.

Kimura, M. 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.

Kinnison, M., Unwin, M., Boustead, N. & Quinn, T. 1998 Population-specific variation in body dimensions of adult chinook salmon (*Oncorhynchus tshawytscha*) from New Zealand and their source populations, 90 years after introduction. *Can. J. Fish. Aquat. Sci.* **55**, 554–563.

Laws, R. M. 1994 History and present status of southern elephant seal populations. In *Elephant seals: population ecology, behavior and physiology* (ed. B. J. LeBoeuf & R. M. Laws), pp. 49–65. Berkeley, CA: University of California Press.

Lewis, M., Campagna, C. & Quintana, F. 1996 Site fidelity and dispersion of southern elephant seals from Patagonia. *Mar. Mamm. Sci.* **12**, 138–147.

Lewis, M., Campagna, C., Quintana, F. & Falabella, V. 1998 Estado actual y distribución de la población del elefante marino del sur en la Peninsula Valdes, Argentina. *Mastozoología Neotropical* **5**, 29–40.

Lydekker, R. 1909 On the skull characteristics in the southern elephant seal. *Proc. Zool. Soc. Lond.* **1909**, 600–606.

McConnell, B. J. & Fedak, M. A. 1996 Movements of southern elephant seals. *Can. J. Zool.* **74**, 1485–1496.

Molina-Borja, M., Padron-Fumero, M. & Alfonso-Marin, M. T. 1997 Intrapopulation variability in morphology, coloration and body size in two races of lacertid lizard, *Gallotia galloti*. *J. Herpetol.* **31**, 499–507.

Nei, M. 1987 *Molecular evolutionary genetics*. New York: Columbia University Press.

Nicholls, D. G. 1970 Dispersal and dispersion in relation to the birthsite of the southern elephant seal, *Mirounga leonina*, of Macquarie Island. *Mammalia* **34**, 598–616.

Orita, M., Iwahana, H., Kanazawa, H. & Sekiya, T. 1989 Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl Acad. Sci. USA* **86**, 2766–2770.

Raymond, M. & Rousset, F. 1995 GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Slade, R. W., Moritz, C., Hoelzel, A. R. & Burton, H. R. 1998 Molecular population genetics of the southern elephant seal *Mirounga leonina*. *Genetics* **149**, 1945–1957.

Slatkin, M. 1995 A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457–462.

Sokal, R. & Rohlf, F. J. 1995 *Biometry*, 3rd edn. New York: Freeman & Co.

Weir, B. S. 1996 *Genetic data analysis*, vol. 2 Sunderland MA, USA: Sinauer Associates.

Weir, B. S. & Cockerham, C. C. 1984 Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

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