Elephant Seal Genetic Variation and the Use of Simulation Models to Investigate Historical Population Bottlenecks

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Because the northern elephant seal (*Mirounga angustirostrus*) was heavily exploited during the 19th century, it experienced an extreme population bottleneck. Since then, under legislative protection in the United States and Mexico, northern elephant seals have recovered dramatically in number, although their genomic diversity was greatly reduced, apparently as a consequence of the bottleneck. In this study we investigated DNA sequence diversity in two mtDNA regions (the control region and 16S RNA) and found low genetic variation in the northern elephant seal: there were only two control region haplotypes (sequence difference = 1%), which was consistent with an extreme founder event in the recent history of the northern species. We also reaffirmed the lack of allozyme diversity in this species. In contrast, the southern elephant seal (*M. leonina*), which though similarly exploited never fell below 1,000 animals, had 23 control region mtDNA haplotypes (average sequence difference = 2.3%). To investigate the extent of the founder event in the northern elephant seal we devised a simulation model based on extensive demographic data. This allowed a statistical analysis of the likely outcome of bottlenecks of different size and duration. Given these historical data, our results indicate (within 95% confidence) a bottleneck of less than 30 seals and 20-year duration, or, if hunting was the primary pressure on the population, a single-year bottleneck of less than 20 seals.

Elephant seals are the largest of the earless (phocid) seals and are renowned for their highly polygenous social structure (Le Boeuf 1974; Ling and Bryden 1981). Adult males can weigh up to 4 tons, are 11 times larger than adult females, and maintain harems that include over 100 females. Elephant seals return annually to breeding sites on remote coastal beaches and small oceanic islands, dispersing hundreds of miles to feed during the rest of the year. Their diet consists primarily of fish and cephalopods for which they will dive to depths in excess of 1,000 m (Le Boeuf et al. 1988).

Northern and southern elephant seals are geographically isolated. The northern species inhabits a region off the coast of California and Mexico (Figure 1), while the southern species inhabits a region in the Southern hemisphere circumpolar to the Antarctic (Figure 2). Until recently, both species have been hunted for their blubber, although the impact of this was much greater on the northern elephant seal. Before 1810 the northern species spanned 14 degrees latitude along the Pacific coast of North America (Scammon 1874). Beginning in 1810 the northern elephant seal was hunted extensively, until around 1860 when they began to become rare. From 1860 to the 1880s limited numbers were still being taken, especially by collectors, until 1884 when 153 were killed (Townsend 1885). Subsequent expeditions revealed no survivors until 1892, when Townsend (1912) reported eight seals on Guadalupe island; seven of these were killed. The relict population recovered slowly, and in 1922 the population was estimated at 350 (Bartholomew and Hubbs 1960). The species received legislative protection from the United States and Mexico in 1922, and increased to 15,000 seals by 1960 (Bartholomew and Hubbs 1960). In 1980 Le Boeuf and Bonnell estimated a total population of 120,000 northern elephant seals. The southern species has always been more abundant and in 1984 was estimated at 550,000–750,000 individuals (Laws 1984; Riedman 1990).

In a seminal study for conservation genetics, Bonnell and Selander (1974) discovered a remarkable genetic homogeneity in an allozyme survey ($N = 24$ loci)

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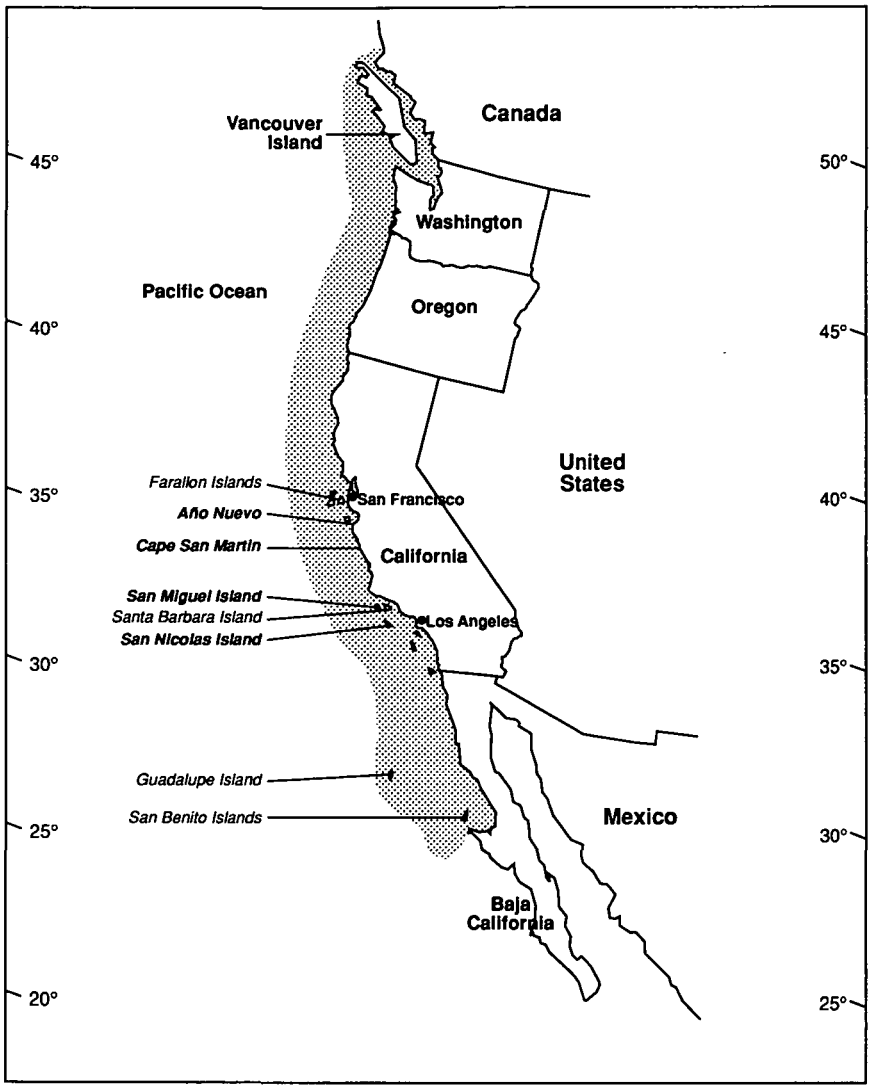

Figure 1. Distribution of northern elephant seals, including sites of sample collection (based on Riedman 1990). Sample sites are labeled in bold italic typeface.

of 159 northern elephant seal blood samples. They interpreted this extreme genetic monomorphism to reflect the species' natural history in passing through an extreme population bottleneck followed by inbreeding in the late 19th century. A recent allozyme survey of southern elephant seals revealed genetic variability similar to that seen in other large mammal populations (Gales et al. 1989). These genetic results were consistent with the history of a large panmictic southern elephant seal population indicated by demographic estimates.

The study reported here presents new molecular estimates of overall genetic variation in natural populations of northern and southern elephant seals using both allozyme and mtDNA sequence variation as measures of genetic diversity. In addition, the pattern of genetic variation within and between an island (South Georgia Island) and mainland (Peninsula Valdez, Argentina) population of southern elephant seals is examined in the context of preliminary tag recovery data that suggest a limited degree of mixing between these populations (Arnbom T, unpublished) and of genetic data that suggest little migration between Heard and Macquarie islands (Gales et al. 1989). Finally, the mtDNA sequence results were combined with accumulated demographic and life history data of northern elephant seals (Le Boeuf 1974; Le Boeuf and Reiter 1988) to simulate demographic recovery while retaining limited mtDNA haplotype variation subsequent to the population bottleneck. The results provide a compelling natural example of demographic recovery associated with genetic drift and their consequences within historic times.

Materials and Methods

Tissue Sample Collections

We collected heparinized blood samples from 67 northern elephant seals from two locales: Cape San Martin and San Miguel Island off southern California (Figure 1). These materials were processed to yield erythrocytes, leukocytes, and plasma suitable for allozyme and DNA analysis. In addition, skin biopsies ($\frac{1}{8}$–$\frac{1}{4}$ inch in diameter) were taken from the rear flippers of 40 northern elephant seals collected at Año Nuevo, California. Since most of the seals had flipper tags indicating place of birth, we selected animals born either at Año Nuevo or on one of two Channel islands near Los Angeles (San Nicolas or San Miguel). Biopsies from southern elephant seals were collected on South Georgia Island ($N = 27$) and on Peninsula Valdez, Chubut, Argentina ($N = 21$).

Allozyme and mtDNA Analysis

We determined allozyme variation for 41 isozyme loci using established methodologies (Newman et al. 1985; O'Brien 1980). A total of 43 loci were tested: (a) from red blood cells, ACP2, ADA, CA2, CPK-B, D1A, ES-α, ESU, G6PD, GLO, GOT1, GP1, GSR, HBB, ITP, LDHA, LDHB, NP, PEPA, PEPB, PEPC, PEPD, PGAM, PGD, PK, PP, SOD2, and TP1; (b) from leukocytes, ACY, AK1, GOT2, GPT, GUSB, HEX, HK, IDH1, IDH2, MDH1, MDH2, MPI, PGM1, and PGM3; and (c) from plasma, ALB and TF.

We extracted DNA from the samples by standard methods and amplified it using the polymerase chain reaction (PCR; Mullis et al. 1986) with oligonucleotide primers homologous to tRNAs on either side of the control region of mitochondrial DNA: sense: 5′-TTCCCCGGTCTTGTAAACC-3′; anti-sense: 5′-ATTTTCAGTGTCTTGCTTT-3′ (Hoelzel and Green 1992). DNA was amplified in a 50-μl reaction volume containing 10–100 ng of template DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 250 pM of each primer, and 1–2 units of *Taq* polymerase. The cycle profile was 2 min at 50°C, 2 min at 70°C, and 45 s at 94°C repeated 30 times. Amplified DNA was sequenced in both directions using the sense primer and an internal control-region primer (5′-CCTGAAGTAAGAACCAGATG-3′), either by boiling and snap-cooling primer and template (Hoelzel and Green 1992) or by separating single strands onto solid phase using biotynilated primers and streptavidin-coated magnetic beads (Schofield et al. 1989), followed by the chain termination method as modified for

T-7 DNA polymerase (Tabor and Richardson 1987). We sequenced a 384-bp segment of the mitochondrial 16S RNA gene in representative samples from each species using PCR amplification and primers described previously (Hoelzel and Green 1992). We compared sequences for pairwise percentage difference and computed genetic distance using the formulations of Nei (1987).

Simulation Model

We developed a computer simulation of population growth following a demographic contraction or bottleneck and applied it to northern elephant seal natural history based on both the extensive life history parameters (such as age-specific mortality and reproductive success) collected from a 24-year study of the Año Nuevo population (Le Boeuf 1974; Le Boeuf and Reiter 1988) and the genetic data reported here. The model is an attempt to simulate the demographic and temporal conditions most likely to have produced the number of northern elephant seals censused in 1960 (Bartholomew and Hubbs 1960) from the survivors of an extreme bottleneck that occurred around 1884 (Townsend 1885, 1912). Further, the simulation tracks the survival versus stochastic loss of mtDNA matriline haplotypes over the course of the recovery period to include both demographic and population genetic aspects of a population recovery.

For efficacious application the simulation model requires the following criteria: (1) availability of accurate data on population age-specific mortality and reproductive success; (2) that the population be in the recovery phase (exhibiting density-independent growth); (3) that the population be reasonably free of environmental stochasticity; and 4) that we have some historical information about the date, duration, or severity of the proposed bottleneck. The northern elephant seal history adequately fulfills each of these requirements.

The objective of the model was to estimate two unknown parameters of the northern elephant seal bottleneck simultaneously: the size of the bottleneck (number of breeding individuals), and the duration of the bottleneck (number of years) based on life history data and the pattern of genetic reduction. Nei et al. (1975) discussed the importance of these factors in determining how much genetic variation is lost during a bottleneck. We know that there were still over 100 northern elephant seals in 1884, because 153 were killed in that year; the species had become extremely rare after 1884, because no elephant seals were found again until 8 years later, despite a concerted effort. Therefore, we used 1884 as an estimate for the initial year of the bottleneck, and we set the duration of the simulation at 76 years (1884–1960). The population was censused at 15,000 in 1960 (Bartholomew and Hubbs 1960).

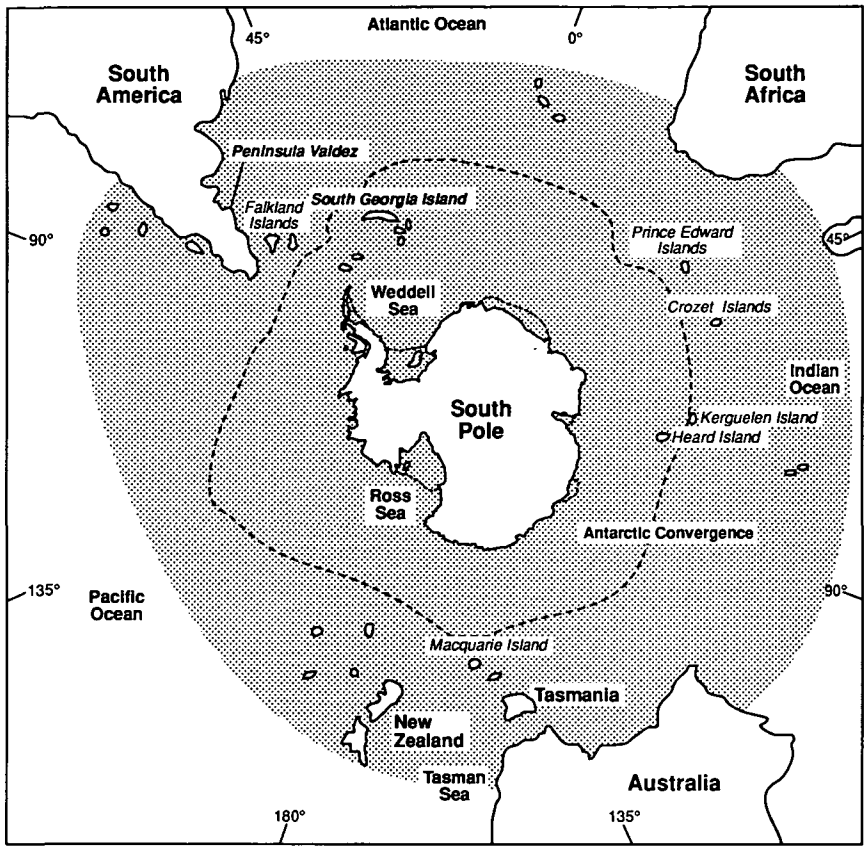

Figure 2. Distribution of southern elephant seals, including sites of sample collection (based on Riedman 1990). Sample sites are labeled in bold italic typeface.

We performed simulations of genetic/demographic recovery using bottleneck sizes varying from 5 to 50 individuals and durations of 1 to 35 years. For the purpose of the model, a "bottleneck" refers only to an imposed limit on population size. All other parameters were held constant and include (1) number of years since the start of the bottleneck = 76 years; (2) male: female birth ratio = 0.5; (3) probability of reproductive success for each sex for each of 14 age classes (see Table 2 below); (4) male and female age-specific mortality (Table 2); (5) initial level of haplotype diversity observed in outbred pre-bottleneck population, based on haplotype diversity reported here in South Georgia Island, southern elephant seal population = 0.85, see below; (6) number of runs per simulation = 500; (7) starting age distribution based on present population data (see Table 2, Le Boeuf and Reiter 1988).

The computer program (written in C and run on a PC-compatible computer with a 486 microprocessor; logic illustrated graphically in Figure 3) simulates a bottleneck by randomly choosing individuals from the age classes of a hypothetical unexploited population (reproductive age females were assumed to be pregnant). These starting age distributions (one for males and one for females) are based on survivorship data for the present-day population of northern elephant seals (see Le Boeuf and Reiter 1988). The simulation model assigns haplotypes to individuals (based on the proportion of unique haplotypes seen in the South Georgia Island southern elephant seal population), and assigns individuals to age/sex classes based on the probabilities given in the starting age distributions. These data are stored by the computer in age class by haplotype number matrices (one for males and one for females). The model then determines the number of survivors each year for each age/sex class, along with the number of reproductively active individuals

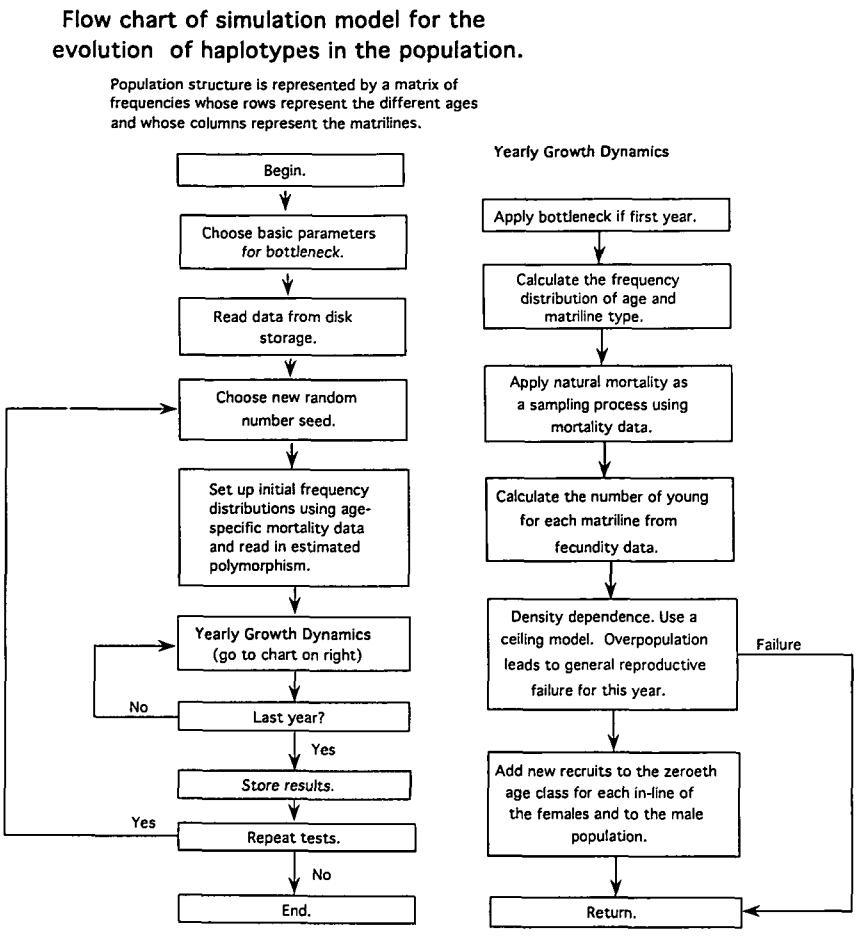

Figure 3. Flow chart of simulation model for the evolution of haplotypes in the population. Population structure is represented by frequency matrices, with rows representing the different ages and columns representing the matrilines.

(based on the age-specific probabilities), and modifies the matrices each year of the simulation. Offspring are given the haplotypes of their mothers. Female reproductive success was estimated from weaning success (Le Boeuf and Reiter 1988). The model assumes no mutation, no selection, and density-independent growth. All runs were repeated 500 times (with different random seeds), providing parametric statistics to account for demographic stochasticity.

Results

Reduced Allozyme Diversity

The severe genetic consequences of the documented 19th-century bottleneck for northern elephant seals (NES) was originally reported by Bonnell and Selander (1974), who detected no genetic variation in an allozyme survey of 159 individuals sampled from five rookeries. This result was particularly dramatic insofar as 16 of the gene enzyme systems tested were among the "polymorphic cluster" loci that are generally polymorphic in most mammalian species (O'Brien et al. 1980). We examined blood samples from 67 individual northern elephant seals collected at two locales (Cape San Martin and San Miguel Island, California, see Figure 1). Our results revealed no detectable allozyme variation at 43 allozyme loci, providing an extended confirmation of the conclusion that modern northern elephant seals have dramatically reduced genetic variability relative to other mammal species (Nevo et al. 1984).

Mitochondrial DNA Sequence Variation

A 300-bp segment of the mitochondrial DNA control region (light strand 5′ portion) was amplified and sequenced from 40 northern elephant seals collected at Año Nuevo, California (ANC) and from two geographically distinct populations of southern elephant seals (SES)—27 from South Georgia Island (SGI) and 21 from Peninsula Valdez Argentina (PVA) (see Figure 2). The complete sequence of this region indicating the pattern of sequence variation is illustrated in Figure 4.

The SGI-SES population had the most variation, with 25 variable sites and 23 haplotypes; most of these detected substitutions were transitional polymorphisms (Figure 4B). The PVA-SES had markedly less mtDNA sequence variability than SGI-SES, with only two polymorphic sites in three haplotypes. As might be expected, the northern elephant seal population also had relatively low variation with three polymorphic sites and two distinct haplotypes. Two of the three polymorphisms in NES and both polymorphisms in PVA-SES represented transitional substitutions.

Pairwise comparisons of aligned sequences within and between populations are summarized in Table 1. The mean pairwise distance between northern elephant seals and SGI-SES, the more outbred population, was 7.0% (after Nei 1987). The two NES haplotypes had the nearest similarity to the most common SGI-SES haplotype (S2 in Figure 4C, $d = 0.05$, 15-bp substitutions and $d = 0.06$, 18-bp substitutions, respectively). The control region results affirm the paucity of genetic variability in northern elephant seals relative to the southern species and suggest that the PVA-SES may have undergone a demographic reduction that reduced mtDNA variability in that population.

We further examined the extent of mtDNA divergence between the two elephant seal species by sequencing a 384-bp segment of the mitochondrial 16S RNA gene. A sequence of three individuals from ANC-NES and from SGI-SES (data not shown) revealed a mean pairwise difference (corrected for intraspecific variation) between the species as 2.4%. This value is approximately one-third of the control region distance (7.0%), reflecting a three-fold slower rate of divergence for this gene than the control region. A relatively rapid rate of sequence divergence in the control region relative to other mtDNA regions is common in other mammal species as well (Aquadro and Greenburg 1983; Wilson et al. 1985).

Simulation Model of the Bottleneck

The collection of extensive behavioral and life history data about the northern elephant seal populations along the California coast has provided a detailed description of the dynamics of demographic processes that affect these populations (Le Boeuf 1974; Le Boeuf and Reiter 1988; Ling and Bryden 1981; Riedman 1990). We have

employed these estimates, including probability of reproductive success and mortality incidence over 14 single-year age classes (Table 2), to simulate the recovery of a population reduced to low numbers by overhunting, as occurred for northern elephant seals late in the 19th century. It is well known that when populations drop to low numbers, there is a high risk for extinction based on demographic factors alone (Goodman 1987; Lande 1988; Lande and Barroclough 1987). Our model combines these demographic considerations with the observed genetic reduction of NES control-region haplotypes relative to a hypothetical "pre-bottleneck" level of variation estimated by the present level of mtDNA variation observed in the SGI-SES population (Table 1).

The model searches for the most probable combination of bottleneck size (5–50 individuals) and duration (1–35 years), based on input parameters described in Table 2 and in Materials and Methods. In general, the model estimated initial bottleneck sizes and duration combinations that would result in a population of two mtDNA haplotypes (as was seen in NES) from a bottleneck sample of a historic population with as much variation as was seen in SGI-SES (Table 1). The bottleneck timing was set at 1884 and the population was seen to grow to approximately 15,000 individuals by 1960, a duration of 76 years. The model predicts an increase from 350 to an average of 15,400 animals over a 38-year period (consistent with the census data, and suggesting density-independent growth over this period).

A summary of the outcome of simulations with varying bottleneck size and duration is presented in Figure 5. For runs of 76 years (1884–1960), we present the number of simulations out of 500 that resulted in an outcome of two haplotypes and 14,000–16,000 seals (an estimated range for the accuracy of the census data) following bottlenecks of varying size and duration. Also presented is the number of simulations (out of 500) that survived—that is, those that did not go extinct due to demographic stochasticity, as well as the mean population size produced by 500 simulations under the constraints of input parameters (Table 2 and Materials and Methods).

The majority of simulations failed to achieve all these outcomes (that were actually observed for the real NES population), but of those that did, 95% are contained within a range of less than 30 individuals surviving bottlenecks of less

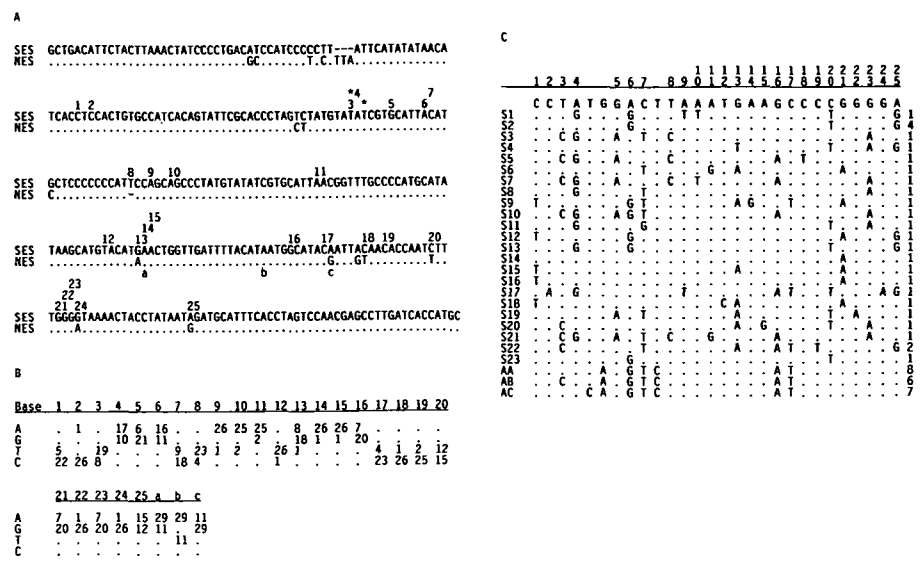

Figure 4. (A) Sequence from the 5′ end of the mitochondrial control region compared between 27 southern elephant seals (SES) from South Georgia Island (SGI) and 40 northern elephant seals (NES) from Año Nuevo. Dots indicate the same base; spaces indicate deletions or insertions relative to the other species. Numbers above the SES sequence show sites of polymorphism in the southern species; letters below the NES sequence show sites of polymorphism in the northern species. Asterisks indicate polymorphisms in the PVA-SES population. (B) Table indicates the frequency of each base pair among haplotypes with respect to 25 polymorphic sites in SGI-SES (1–25) and three sites in NES (a–c). (C) Haplotype variation of variable sites from the control region in SES relative to a consensus for all SES. The number of individuals that express haplotype is listed to the right of the sequence. Dots represent identity to consensus, and letters indicate base substitutions. Numbers along the top correspond to substitution numbers given in A. S1–S23 are haplotypes from the SGI-SES population; AA, AB, and AC are haplotypes from the PVA-SES population.

Table 1. DNA sequence differentiation within and between elephant seal populations for a 300-bp region of the mitochondrial control region. Diagonal is the mean percentage of haplotype difference within each population.

			Sequence difference (%)[b]		Average pairwise distance[c]		
					NES	SES	
Population[a]	*N*	No. of haplotypes	Min.	Max.	ANC	SGI	PVA
ANC-NES	40	2	*1.00*	*1.00*	0.43	*25.00*	*30.00*
SGI-SES	27	23	0.33	4.67	8.35	2.27	9.00
PVA-SES	21	3	0.33	0.67	9.90	2.93	0.30

[a] ANC-NES = Año Nuevo, California, northern elephant seal; SGI-SES = South Georgia Island, southern elephant seal; PVA-SES = Peninsula Valdez Argentina, southern elephant seal.

[b] Minimum and maximum sequence difference is based on the percentage of base-pair differences of all pairwise combinations of haplotypes.

[c] Average pairwise distance equals average percentage sequence difference (lower) and average number of base-pair differences out of 300 aligned base pairs (upper).

Table 2. Male and female life history parameters for northern elephant seals for each of 14 age classes

	Age class													
Parameter[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age distribution of pre-bottleneck population (%)														
Male	23.2	16.9	12.4	9.6	8.1	6.7	5.6	4.7	3.9	3.3	2.0	1.8	1.2	0.6
Female	23.2	16.9	12.4	9.6	8.1	6.7	5.6	4.7	3.9	3.3	2.0	1.8	1.2	0.6
Probability of reproductive success														
Male	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.17	0.30	0.34	0.17	0.0	0.0
Female	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Probability of survival														
Male	0.58	0.73	0.73	0.78	0.83	0.83	0.83	0.83	0.83	0.83	0.78	0.73	0.68	0.48
Female	0.58	0.73	0.73	0.78	0.83	0.83	0.83	0.83	0.83	0.83	0.78	0.73	0.68	0.48

[a] The age distribution for the hypothetical pre-bottleneck population is estimated from the probability of survival data. Survivorship for each age class is shown as a proportion of the previous age class. Data based on tag recoveries from the Año Nuevo, California, population of northern elephant seals (Le Boeuf and Reiter 1988).

Bottleneck Size (population no.)		1	5	10	15	20	25
40					1 500 18,301 (6,108) 4.39 (1.13)	1 500 11,837 (4,096) 3.70 (1.05)	0 500 6,842 (2,509) 3.29 (0.92)
35			0 500 41,382 (15,829) 5.96 (1.66)	0 500 26,777 (9,867) 4.68 (1.25)	1 500 15,843 (6,340) 3.84 (1.10)	2 500 9,816 (3,785) 3.23 (1.00)	1 500 6,250 (2,465) 2.95 (0.91)
30		0 500 61,944 (23,062) 7.99 (2.31)	1 500 35,895 (15,049) 5.05 (1.49)	4 499 21,600 (9,494) 3.96 (1.30)	2 500 13,358 (6,257) 3.30 (1.04)	2 499 8,028 (3,610) 2.83 (0.94)	0 497 5,000 (2,339) 2.53 (0.84)
25		1 500 49,617 (20,995) 6.60 (2.08)	3 492 27,002 (13,727) 4.12 (1.41)	13 494 17,905 (8,882) 3.33 (1.13)	**11** **498** **10,012 (5,374)** **2.72 (0.95)**	4 493 6,272 (3,266) 2.30 (0.83)	0 491 3,894 (2,101) 2.04 (0.80)
20		2 499 41,040 (19,643) 5.34 (1.83)	2 472 21,209 (12,616) 3.29 (1.21)	**15** **472** **12,638 (7,701)** **2.52 (1.01)**	9 465 7,202 (4,743) 2.10 (0.83)	1 454 4,608 (2,865) 1.88 (0.68)	0 439 2,614 (1,728) 1.63 (0.65)
15		4 485 27,867 (16,139) 3.88 (1.54)	**8** **431** **12,853 (10,272)** **2.27 (0.97)**	13 396 7,337 (5,795) 1.90 (0.78)	3 345 4,155 (3,595) 1.56 (0.63)	0 324 2,815 (2,365) 1.40 (0.58)	
10		**8** **408** **14,963 (11,423)** **2.44 (1.09)**	4 259 6,931 (6,236) 1.54 (0.70)	1 204 3,754 (3,869) 1.31 (0.52)	0 146 2,359 (2,318) 1.16 (0.45)		
5	a b c d	2 128 5,354 (5,907) 1.41 (0.58)	0 37 2,758 (3,002) 1.08 (0.43)	0 16 1,686 (1,829) 1.0 (0.0)			

Bottleneck Duration (years)

Figure 5. Genetic and demographic data from simulations of bottlenecks of varying size and duration. Bottleneck durations are given in years. For each bottleneck size and duration, four results from 500 simulation runs are presented: a = the number of simulations out of 500 that produced a result simultaneously showing two haplotypes and 14,000–16,000 individuals after 76 years; b = the number of simulation runs that did not go to extinction; c = the mean (and standard deviation) population size reached after 76 years; and d = the mean (and standard deviation) number of haplotypes remaining. Cells with boldface type indicate the most frequent successful (i.e., surviving) outcome for combinations of bottleneck size and duration.

than 20 years (Figure 5). The restricted resolution or limits on these two variables results from the fact that predictions based on demographic versus genetic data are increasingly incompatible as the duration of the bottleneck increases. Longer bottlenecks reduce genetic variation by genetic drift but also cumulatively increase the probability of population extinction. For example, for a bottleneck of 20 years duration, the model predicts an average survival of two haplotypes following a bottleneck size of 20–25 seals, while an average of 15,000 seals would be observed following a bottleneck of about 60 seals. For the simulation model presented here, the most likely bottleneck size : duration combinations combining both genetic and demographic conditions would be as follows: 10 survivors : 1 year duration; 15 individuals : 5 years; 20 individuals : 10 years; and 25 individuals : 15 years. It is difficult to be more precise; however, historic records tell us that large-scale hunting ended abruptly after 1984, supporting a short-term (possibly 1-year) bottleneck of less than 20 individuals; the most likely estimate for a 1-year bottleneck is 10 survivors.

Although we could not exclude the occurrence of multiple bottlenecks, we believe that this is unlikely because of the persistence of more than one (genetically divergent, with 1% sequence difference) NES haplotype. If a bottleneck had reduced mtDNA variation to two–three haplotypes, then a second bottleneck would likely have eliminated mtDNA variation. At the same time, the model is quite robust to assumptions about the level of pre-bottleneck variation. For example, if only half the haplotype variation seen in South Georgia Island were present in the pre-bottleneck northern elephant seal population, we would still expect two haplotypes 76 years after a 1-year bottleneck of 10 individuals.

Discussion

The implication of the genetic and demographic data for the northern elephant seal reflects the historic events in which the species was hunted to near extinction. The population was down to a level in which the chance of extinction could have been as high as 20% due to demographic stochasticity alone (if the bottleneck restricted the population to 10 individuals for one year). Although the population has recovered dramatically to over 100,000 seals today, the overall genetic diversity of the species has been greatly reduced. Further comparisons with the southern species will give an indication of the possible consequences of the bottleneck on variation in genetically controlled characters such as morphology, reproduction, and sensitivity to infectious agents.

In general, our results indicate an extremely low level of genetic variation in northern elephant seals and a high level of mtDNA variation in the southern elephant seals on South Georgia. The amount and character of mtDNA variation found in the northern species is consistent with an extreme bottleneck event. Comparing present-day genetic diversity with demographic simulation models in the context of the historical data allowed us to define a probable range of bottleneck events. Although the errors associated with demographic stochasticity limit resolution, this approach allows us to assign a probability to each event and so restrict the range of likely events. Therefore, within the limits of the model, the combination of data on recent demographics and molecular variation should prove useful for estimating the size and duration of historical population bottlenecks in other species.

For many of the slow-breeding, mammalian species requiring conservation (such as the northern elephant seal), the reconstruction of historical population bottlenecks will allow previously intractable questions to be addressed. These will include the following: (1) questions related to the interpretation of present-day allozyme variation (when predictions from the model are based on demographics and matriline number); (2) the potential impact of future bottlenecks; and (3) the relationship between bottleneck events of varying severity and variation in quantitative genetic characters.

Another result that deserves mention is the large difference in overall genetic variation observed between the two sampled populations of southern elephant seals (Figure 2 and Table 1). The low amount of variation in the PVA-SEA relative to SGI-SES (Table 1) would be consistent with another demographic contraction in the history of the PVA-SES population. Although we have few demographic data to pinpoint the timing of the proposed bottleneck, aspects of the available data support the hypothesis of an ancient event on the order of 100,000 years before the present. First, the PVA-SES population is rather large (more than 50,000 seals based on recent census data), precluding the possibility of a very recent small stable population size as an explanation. Second, if we hypothesize the existence of a molecular clock for mitochondrial DNA proceeding at a rate similar to other mammals (approximately 2% per million years; see Moritz and Hillis 1990; Wilson et al. 1985), then we can use the ratio of 16s RNA variation and control-region divergence to estimate the time elapsed since a bottleneck that reduced the PVA-SES population to a single ancestral haplotype. The present PVA-SES displays three haplotypes, each related by a single base-pair substitution, suggesting monophyletic origin or, in other words, a single surviving matriline of the historic bottleneck. If we presume that the 16s RNA divergence is representative of mtDNA divergence, then the more rapidly evolving control-region rate would be 6% sequence divergence per 1 million years. The proposed two mutations in the PVA-SES populations would represent 0.67% change and would require approximately 100,000 years to have occurred.

Therefore, a greatly reduced level of variation for PVA-SES compared to SGI-SES suggests that the mainland population could have been founded by as few as only one sub-Antarctic matriline, followed by little or no migration between the two populations. This pattern of population colonization and small-scale subdivision would have important implications for conservation and management. However, we have not tested the possibility that males move between these populations more frequently than females.

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