Genetic Variability in Guadalupe Fur Seals

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The Guadalupe fur seal (*Arctocephalus townsendi*) population underwent one or two severe bottlenecks due to commercial sealing in the late 19th century. Since then the protected population has been growing steadily around their only rookery, Isla de Guadalupe, Mexico. We probed both nuclear and mitochondrial genomes using multilocus nuclear DNA profiling and mitochondrial DNA sequencing to estimate the level of genetic variability of the present population. Unlike other pinniped populations that have experienced similar historical bottlenecks, such as Hawaiian monk seals and northern elephant seals, high levels of genetic variability were found.

The Guadalupe fur seal (*Arctocephalus townsendi*; Merriam 1897) population was nearly exterminated by intensive commercial sealing in the last century (Hubbs 1956; Scammon 1874). The nadir of the population was during the 1890s and the first 2 decades of the present century. In 1892, Townsend (1899) found only seven fur seals on Isla de Guadalupe, Mexico, the last known haven for these animals (Figure 1). Two years later a commercial sealing vessel took 15 on the island, all they could find (Townsend 1899). For the next 3 decades, no further sightings were reported and the species was thought to be extinct until 1926 when a group of 35 to 60 animals was reported on the east side of the island by two fishermen (Huey 1930; Thoburn 1899; Townsend 1931; Wedgeforth 1928). In 1928 one of the fishermen returned to the island and reportedly killed most of the herd (Hubbs 1956). For the next 26 years no fur seals were observed on Guadalupe Island, despite numerous searches for them (Bartholomew 1950), until 1954 when Hubbs (1956) counted at least 14 of them on the eastern shore of the island. The island population has grown slowly since that time; the total population was estimated at 7408 in 1993 (Gallo-Reynoso 1994). Although young and adult males have been observed periodically on southern California islands since 1938 (Bartholomew 1950; Stewart et al. 1987), Isla de Guadalupe remains the only breeding rookery for this species.

From bones in kitchen middens, assumed habitat, and the large numbers taken by 19th century sealing vessels, preexploitation population size has been estimated at from 30,000 (Hamilton 1951) to 200,000 (Hubbs 1979). These sources also indicate that the range of the Guadalupe fur seal was from Isla Socorro on Revillagigedo Archipelago, Mexico, to Monterey Bay, California (Repenning et al. 1971) and the Gulf of the Farallones (Hubbs 1956; Lyon 1937; Peterson and Le Boeuf 1969; Scheffer 1958; Starks 1922; Townsend 1924).

Clearly the Guadalupe fur seal population underwent one and possibly two population bottlenecks during the last 100 years. The severity and duration of the bottlenecks are unknown, but given the historical record it is likely that few fur seals survived in 1894 and again in 1928 (Hubbs 1956). Population bottlenecks reduce genetic variation in a population through genetic drift, inbreeding depression, and founder effects (e.g., Hoelzel et al. 1993). Ostensibly the small number of isolated fur seals that survived the bottlenecks possessed only a fraction of the total genetic variability of the preexploitation population. Effective population size may have been further reduced by the highly polygynous mating system characteristic of the genus (Gallo-Reynoso 1994; Pierson 1978).

The aim of this study was to describe the genetic status of the Guadalupe fur seal and test the hypothesis that they lack genetic variation, relative to other pinnipeds. This goal was accomplished using two independent approaches to quantify-

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 89:301–305

Figure 1. Map of Isla de Guadalupe (Mexico). Sampling localities—Campo Lima, Los Corrales, and Los Arroyitos—are indicated.

ing genetic variation, the combination of multilocus nuclear DNA profiling and mtDNA sequence analysis.

Materials and Methods

Samples

Thirty-six tissue samples were obtained from Guadalupe fur seals at three sites on Isla de Guadalupe, Mexico, during the periods, July 10–13, 1991, and February 9–August 6, 1992 (Figure 1). The sample was composed of 5 adult females and 31 pups (15 males, 12 females, 4 of unknown sex); 1 known mother-pup pair was sampled. Tissue samples weighed approximately 5 g and were taken from the trailing edge of the hindflippers of live seals (except for one stillborn) using rongeurs. The tissue was preserved in saturated salt and DMSO solution and refrigerated. DNA was extracted from 100 mg of each tissue sample (Sambrook et al. 1989). We were successful in obtaining restriction-quality DNA from 29 of the 36 samples: 1 from the Los Arroyitos locale, 4 from Campo Lima, and 24 from Los Corrales (Figure 1). Only three of the five adult samples yielded sufficient DNA, all were from the Los Corrales locality.

Nuclear DNA Fingerprinting Determination

DNA (5 mg) was digested with 40 units of the restriction enzyme *Hae*III after the recommendations of the manufacturer (New England Biolabs). DNA restriction fragments were separated electrophoretically through 1.0% agarose gels (25 cm in length) at 45 V for 24 h with Tris-borate-EDTA buffer (Sambrook et al. 1989). Separated DNA fragments were transferred to nylon filter membranes (Magnagraph, MSI) by vacuum transfer (LKB VacuGene, Pharmacia) followed by cross-linking with 120,000 mJ UV (Stratalinker, Stratagene). Membranes were prehybridized for 30 min at room temperature in blocking buffer: 1× PBS (0.058 M Na_2HPO_4, 0.017 M $Na_2HPO_4 \bullet H_2O$, 0.068 M NaCl) pH 7.3, 0.2% Hammarsten-grade cascin, and 0.1% Tween-20. Hybridization was initiated by transferring the membranes to hybridization buffer preheated to 50°C: 4× PBS (0.23 M Na_2HPO_4, 0.07 M $Na_2HPO_4 \bullet H_2O$, 0.027 M NaCl) pH 7.3, 1% SDS, and 0.5% BSA. An alkaline phosphatase-conjugated oligonucleotide probe (Edman et al. 1988) homologous to a portion of the consensus sequence of the human minisatellite repeat 33.15 (Cellmark) was added to a concentration of 2.0 nM. Hybridization was accomplished by incubating a reaction mixture for 20 min at 50°C. Following hybridization, membranes were washed three times in 1× PBS/1% SDS at 50°C for 10 min and twice in 1× PBS at room temperature for 5 min. To detect hybridized probe, membranes were washed twice in DEA substrate buffer (0.1 M diethanolamine and 1.0 mM $MgCl_2$) pH 10.0, for 5 min at room temperature and then incubated in DEA substrate buffer with chemiluminescent substrate: 0.4 mM AMPPD (1,2-dioxanetane, Tropix) for 5 min (Bronstein and Voyta 1989). Finally, the probed membranes were wrapped in transparent plastic, incubated in the dark for 1 h, and exposed to Kodak XAR-5 film for 15 min to 3 h.

Automated image analysis (Scanmaster 3 + digital scanner, Howtek; Bio-image software, Millipore) was used to characterize and record digitally each DNA profile from the exposed films (not shown). All uniquely sized restriction fragments between 3.0 and 20.1 kb were scored as present or absent in the profiles of the 29 seals. Fragment size was determined by two molecular weight markers (Gene Print Light Equiladder system; Promega).

Genetic variation was quantified by making pairwise comparisons between DNA profiles. This was accomplished with SIM, a DOS-based program (Alberte et al. 1994) that calculates the proportion of restriction fragments shared (S) between two individuals as the number of fragments of equivalent length identified in both DNA profiles divided by the total number of fragments detected (Lynch 1988). The DNA profiles were compared using a match window of 3 SD (empirically derived from the variation in migration distance for fragments of known size) around each band (Galbraith et al. 1991). The proportion of fragment bands shared between two individuals was averaged over the entire sample to obtain an estimate of genetic variation for the population.

Table 1. Comparison of multilocus nuclear DNA fingerprinting results for different species of pinnipeds and Mexican gray wolves

Species	Individuals	Fragments	Frequency of unique fragments[a]	Frequency of common fragments[b]	S
Guadalupe fur seal	29	56	0.14	0.11	0.59
Hawaiian monk seal	11	39	0.05	0.26	0.73
Pacific walrus	19	92	0.27	0.05	0.38
Mexican gray wolf	29	22	0	0.59	0.78

[a] Characters exhibited by only one individual in the sample.

[b] Characters exhibited by more than 70% of the individuals in the sample.

S = mean similarity.

DNA Sequences and Phylogenetic Analysis

The polymerase chain reaction (PCR) (Saiki et al., 1988) was used to amplify a 350 base pair (bp) region of the mitochondrial control region (D-loop). Primers and PCR protocols followed Kocher et al. (1989) and Palumbi et al. (1991). Sequencing and PCR primers used were Pro-L, and D-loop-H (Palumbi et al. 1991). Approximately 100 ng of DNA were used as template for 100 ml PCR reactions containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 mM each dNTP, 2.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus), and 1 mM each amplification primer. PCR products were used for *Taq* dyedeoxy terminator cycle-sequencing reactions (Applied Biosystems Inc.) and loaded on an automated sequencer (Applied Biosystems 373A). Sequences were aligned with the aid of the computer program Sequence Navigator (Applied Biosystems).

Phylogenetic analyses employed maximum parsimony (MP) using the Heuristic option of the PAUP program (phylogenetic analysis using parsimony; Swofford 1993) and neighbor-joining (NJ) method (Nei 1987) using the corresponding option in the PHYLIP package (Felsenstein 1989). The degree of confidence assigned to nodes in phylogenetic trees obtained by MP or NJ was determined by bootstrapping (Felsenstein 1985; but see Hillis 1995 for discussion) with 2000 replicates (Hedges 1992). New Zealand fur seal (*Arctocephalus forsteri*) sequence obtained from the literature (Slade et al. 1994) was used as an outgroup.

Results

DNA Fingerprint Analysis

A total of 56 different DNA fingerprint characters were identified among the 29 Guadalupe fur seal profiles (Table 1). The DNA profiles were of average complexity for pinniped species, ranging from 12 to 22 scorable fragments per individual with a mean complexity of 17.8. Eight fragment bands (14%) were unique to individual seals, all of which were from the Los Corrales locality, while 6 additional bands were found in more than 20 individuals (11%). The similarity (band sharing) between individual profiles ranged from 0.38 to 0.87 for an average of 0.59. There were an insufficient number of samples from the Los Arroyitos and Campo Lima localities to conduct interbreeding site comparisons of DNA fingerprint characters.

DNA Sequence Analysis

A 320 bp portion of the D-loop was analyzed; of 313 aligned positions in Guadalupe fur seal sequences, 18 positions were variable (5.8%) and 16 of them were phylogenetically informative. All substitutions were transitions. Two most parsimonious trees were obtained (tree length = 51 steps, consistency index = 0.49). A consensus tree (50% majority-rule consensus) of 2000 bootstrap replicates is shown in Figure 2 (only bootstrap values higher than 50% are shown). Three main clades (called A, B, and C) strongly supported statistically (68%, 99%, and 100% bootstraps replicates), were found. A and B were found to be sister clades, but with lower statistical support (59% of the bootstrap replicates). Out of 25 individuals, 15 belonged to clade A, 6 to clade B, and 4 to clade C. The average pairwise sequence divergence between clades was 3.6%; the average pairwise sequence divergence within clades was 0.16%.

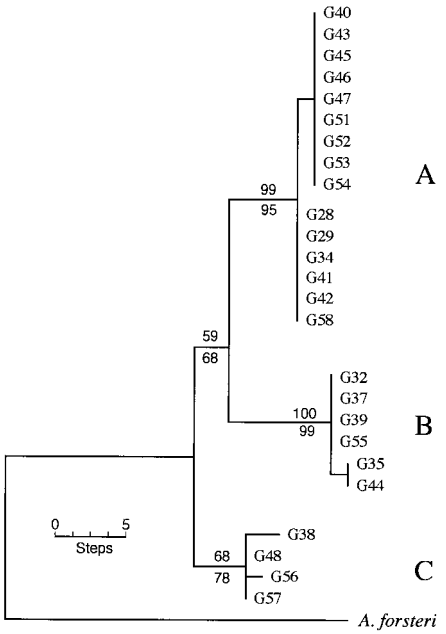

Figure 2. Tree derived from 320 bp of the mitochondrial control region (D-loop) from *Arctocephalus townsendi.* The most parsimonious tree is shown (length = 59 steps). The same tree topology was obtained with neighbor-joining analysis. Numbers on nodes indicate results of bootstrapping (2000 replicates) using maximum parsimony (above) and neighbor-joining (below) methods. Only values grouping the three major clades are shown. Bar corresponds to five steps in maximum parsimony analysis.

Discussion

Genetic Divergence: Nuclear Fingerprints

The average similarity (0.59, range 0.38–0.87) of the DNA profiles obtained from the combined Isla de Guadalupe samples is typical of outbreeding populations of other marine and terrestrial mammals (range of averages = 0.43–0.61; Ruth and Fain 1993). In contrast, we have found low DNA fingerprinting character variability in several taxa that, like the Guadalupe fur seal, have undergone well-documented severe population bottlenecks: sea otters (*Enhydra lutris*) from California (Scribner, Bodkin et al., in press), monk seals (*Monarchus schauinslandi*) from Hawaii (Kretzman et al., in press), and Mexican gray wolves (*Canis lupus baileyi*) from captive lineages (Fain et al., in preparation). The frequency of occurrence of unique characters (i.e., those exhibited by only a single individual in the sample) is also reduced in these species, reflecting the effect of substained inbreeding. Typically, unique characters are either lost or become increasingly common in small populations of interbreeding individuals, the situation that is thought to be the case in the Guadalupe fur seal. The unique character frequency in the Guadalupe fur seal, however, is almost three times as high as in the monk seal (Table 1). For further comparison, the Pacific walrus (*Odobenus rosmarus divergens*) exhibits both high DNA fingerprint character variability and a unique character frequency five times that of the monk seal (Table 1; Scribner, Hills et al., in press). Our DNA fingerprint data reveal moderate genetic variation in

Guadalupe fur seals. They do not support the hypothesis that Guadalupe fur seals lack genetic variation relative to other species, including seals with a similar population history, for example, northern elephant seals (*Mirounga angustirostris*; Hoelzel et al. 1993).

Genetic Divergence: Mitochondrial Sequences

Sequence divergence between individuals and between clades was high. Indeed, with 3.6% average pairwise sequence divergence between clades, Guadalupe fur seals exhibit sequence divergence comparable to the population divergence found between Atlantic and Pacific harbor seal populations (*Phoca vitulina*) (3.28%; Stanley et al. 1996), and definitely higher than the divergence obtained for harbor seals within each of these oceans (0.75%; Stanley et al. 1996). Moreover, it is likely that the divergence found in Guadalupe fur seals was underestimated due to the relatively small number of individuals investigated.

Population Structure and Divergence Time

Population structure in pinnipeds that exhibit breeding site fidelity might be expected (Stanley et al. 1996). In our case, the species is now restricted to a small island which presents separate rookeries. Individuals collected from different locations on the island (Los Corrales, Campo Lima, and Los Arroyitos; see Figure 1) could not be distinguished genetically and belonged to either of the three clades A, B, or C, with no apparent segregation. Furthermore, presumed half-siblings did not partition according to their haplotypes (Table 2), thus indicating randomness of haplotypes in harems.

Guadalupe fur seals, together with northern elephant seals, have undergone a severe bottleneck in the early 19th century due to hunting. An important difference in the behavior of these two species is found at breeding time. When on shore, elephant seals lie on accessible beaches, easily spotted by hunters from boats, while fur seal individuals may hide in nearshore caves, thus concealing themselves from hunters. At the genetic level, northern elephant seals are extremely homogeneous (Hoelzel et al. 1993), possibly indicating an historical bottleneck so severe that only very few haplotypes (possibly as few as 10 individuals) survived. In fur seals, where individuals bred in caves at the time of their smallest population size, it is likely that more haplotypes survived.

Table 2. List of sampling localities, sex, age, and haplotypes of the sampled individuals

Sample	Sex	Age	Location	Haplotype
Nonsibling groups				
28	M	Pup	Los Corrales	A
29	M	Pup	Los Corrales	A
42	F	Adult	Los Corrales	A
43	F	Pup	Los Corrales	A
44	F	Adult	Los Corrales	B
45	F	Adult	Los Corrales	A
46	M	Pup	Los Corrales	A
47	F	Adult	Los Corrales	A
48	M	Pup	Los Corrales	C
37	M	Pup	Los Arroyitos	B
38	M	Pup	Campo Lima	C
39	M	Pup	Campo Lima	B
40	F	Pup	Campo Lima	A
41	M	Pup	Campo Lima	A
Sibling groups				
32	M	Pup	Los Corrales	B
34	F	Pup	Los Corrales	A
35	?	Pup	Los Corrales	B
51	F	Pup	Los Corrales	A
52	F	Pup	Los Corrales	A
53	M	Pup	Los Corrales	A
54	M	Pup	Los Corrales	A
55	M	Pup	Los Corrales	B
56	F	Pup	Los Corrales	C
57	F	Pup	Los Corrales	C
58	M	Pup	Los Corrales	A

Individuals were either isolated (thus no relationship assumed) or grouped (in which case siblinghood was possible). Sibling groups are in order (32, 34, and 35 are presumed siblings, i.e., the same father).

In any case, the major haplotype groups (A, B, and C) are the relict haplotypes of these individuals.

Molecular clocks have been studied in mammalian mitochondrial D-loop sequences and a range of calibrations have been published (discussed in Stewart and Baker 1994). The rate of D-loop sequence divergence in mammals varies from 0.5% per million years in cetaceans to about 14.3% per million years in shrews, with about 6% per million years in elephant seals and an overall average of about 8% per million years. When using a conservative estimate of 6% per million years, the average divergence time between the three major clades—A, B, and C—is 600,000 years ago, with extreme estimates ranging from 7.2 million years to 250,000 years ago. In both cases, these estimates predate the recent historical bottleneck of the early 19th century.

The divergence obtained within clades is too small to give an accurate estimate of its timing. It is important to notice, however, that the divergence observed within clades is comparable to the overall divergence of northern elephant seals or of monk seals, two populations known to have experienced recent severe bottlenecks (Table 3).

Table 3. Intraspecific mtDNA control region sequence variation for *Arctocephalus townsendi* and other pinnipeds

Species	Haplotypes/ Individuals (Populations)	Variable sites/ Total	Percentages of variable sites
Guadalupe fur seal (total)	7/25 (1)	18/313	5.7
Guadalupe fur seal (each clade)	2.3/8.3	1.7/313	0.5
Hawaiian monk seal	3/50 (5)	2/359	0.6
Northern elephant seal	2/40 (1)	3/300	1.0
Southern elephant seal	26/48 (2)	26/300	8.7
California sea lion	11/40 (4)	29/315	9.2

Data for Guadalupe fur seals were either analyzed as a whole, or separated into the three major clades (A, B, and C) and averaged.

Conclusion

Results obtained by two independent methods that probed both nuclear and mitochondrial DNA indicate that Guadalupe fur seals, unlike similar species that have experienced severe bottlenecks, such as northern elephant seals, show a high level of genetic diversity. Several reasons may explain these unexpected findings. One possible explanation is that the species did not in fact experience an extreme bottleneck. Toward the end of exploitation, Guadalupe fur seals reportedly bred in caves (Hubbs 1956), thus making them invisible to observers. The actual number of individuals, at any given time, may therefore have been underestimated. Yet another possible source of genetic diversity is through hybridization with closely related species. The best candidates for this somewhat unlikely possibility are their congeners, the Juan Fernandez fur seals (*A. philippii*). No evidence, however, has yet been found to support this idea.

One important factor in understanding the present level of genetic diversity of the Guadalupe fur seal is the level of genetic diversity of this species before the bottleneck occurred. It is possible that the bottleneck, by its short duration, was not sufficient to significantly depress the level of genetic diversity of the species.

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