

Low Genetic Variability in the Hawaiian Monk Seal

MARIA B. KRETZMANN,*† WILLIAM G. GILMARTIN,‡ AXEL MEYER,†
GERARD P. ZEGERS,* STEVEN R. FAIN,§ BRUCE F. TAYLOR,§ AND
DANIEL P. COSTA*

*Biology Board, University of California, Santa Cruz, CA 95064, U.S.A.

†Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, U.S.A.

‡Hawaii Wildlife Fund, P.O. Box 540, Volcano, HI 96785, U.S.A.

§U.S. Fish and Wildlife Service Forensics Laboratory, 1490 East Main Street, Ashland, OR 97520, U.S.A.

Abstract: *The Hawaiian monk seal (*Monachus schauinslandi*) is a critically endangered species that has failed to recover from human exploitation despite decades of protection and ongoing management efforts designed to increase population growth. The seals breed at five principal locations in the northwestern Hawaiian islands, and inter-island migration is limited. Genetic variation in this species is expected to be low due to a recent population bottleneck and probable inbreeding within small subpopulations. To test the hypothesis that small population size and strong site fidelity has led to low within-island genetic variability and significant between-island differentiation, we used two independent approaches to quantify genetic variation both within and among the principal subpopulations. Mitochondrial control region and tRNA gene sequences (359 base pairs) were obtained from 50 seals and revealed very low genetic diversity (0.6% variable sites), with no evidence of subpopulation differentiation. Multilocus DNA fingerprints from 22 individuals also indicated low genetic variation in at least some subpopulations (band-sharing values for "unrelated" seals from the same island ranged from 49 to 73%). This method also provided preliminary evidence of population subdivision (F_{st} estimates of 0.20 and 0.13 for two adjacent island pairs). Translocations of seals among islands may therefore have the potential to relieve local inbreeding and possibly to reduce the total amount of variation preserved in the population. Genetic variation is only one of many factors that determine the ability of an endangered species to recover. Maintenance of existing genetic diversity, however, remains an important priority for conservation programs because of the possibility of increased disease resistance in more variable populations and the chance that inbreeding depression may only be manifest under adverse environmental conditions.*

Baja Variabilidad Genética en la Foca Hawaiana

Resumen: *La foca hawaiana es una especie en peligro de extinción cuya población nunca se ha restablecido completamente de su explotación humana a pesar de décadas de protección y los sucesivos programas de gestión diseñados para aumentar el crecimiento de la población. La crianza de las focas ocurre en cinco islas del archipiélago hawaiano, y la migración entre las islas es limitada. Se esperaba que la variación en esta especie fuera baja debido a un reciente cuello de botella y al entrecruzamiento probable entre las subpoblaciones pequeñas. Para probar la hipótesis de que el tamaño pequeño de la población y el fuerte arraigo a su lugar natal ha ocasionado baja variabilidad genética dentro de las islas, y una diferenciación significativa entre las islas, usamos dos métodos independientes para medir la variabilidad genética dentro de y entre las subpoblaciones principales. Se obtuvieron secuencias de la región de control del ADN mitocondrial (359 pares de bases) de 50 focas, las cuales revelaron una diversidad genética muy baja (0.6% posiciones variables), sin evidencia de una diferenciación subpoblacional. "Huellas digitales" de ADN de 22 individuos también mostraron baja variabilidad genética, por lo menos en ciertas subpoblaciones (la proporción de fragmentos compartidos por focas "sin parentesco" de la misma isla varió entre el 49 y el 73%). Este método*

Address correspondence to Maria Kretzmann, Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, U.S.A., email kretzman@life.bio.sunysb.edu

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también mostró evidencia preliminar de la subdivisión poblacional (estimaciones del F'_{st} de 0.20 y 0.13 para dos pares de islas adyacentes). Por lo tanto, intercambios de focas entre las islas podrían tener la capacidad de aliviar el entrecruzamiento local, o posiblemente también de disminuir la variabilidad total que se mantiene en la población. La variabilidad genética es un solo factor entre los muchos que determinan la habilidad de recuperación de una especie en peligro de extinción. Sin embargo, el mantenimiento de la diversidad genética existente debería seguir siendo prioritario en los programas de conservación, debido a la posibilidad de una mayor resistencia a enfermedades en poblaciones genéticamente más variables, y a la posibilidad de que los efectos negativos del entrecruzamiento quizás solo se manifiesten en condiciones ambientales adversas.

Introduction

The importance of genetic variation for predicting the vulnerability of an endangered species to extinction is a controversial issue in conservation biology (e.g., Lande 1988; Caro & Laurenson 1994; Merola 1994; O'Brien 1994). Inbreeding and the resulting loss of genetic variability are often inevitable consequences of the severe reductions in population size experienced by endangered species, but the extent to which this results in inbreeding depression is likely to vary tremendously among species and is difficult to document in wild populations. Even more difficult to assess is the importance of the loss of genetic variation for the "adaptive potential" and long-term prospects of populations. Nevertheless, a primary goal of conservation efforts has been to preserve a maximum amount of genetic variation in each species under consideration, based on the perceived link between a lack of genetic variation and an increased extinction risk for small populations (e.g., Beardmore 1983; Gilpin & Soulé 1986; Vrijenhoek 1994; Frankham 1995).

The Hawaiian monk seal (*Monachus schauinslandi*) is an endangered species that currently numbers approximately 1300 individuals (W.G. Gilmartin, unpublished data). This species was hunted to near extinction in the nineteenth century (Kenyon & Rice 1959), but the size and duration of the population bottleneck are not well documented. Following partial recovery, an additional decline of about 50% (from approximately 3000 to 1500 individuals; Kenyon 1972; Johnson et al. 1982; Ragen 1993) occurred between the late 1950s and the 1970s, which has been at least partly attributed to human disturbance at breeding rookeries (Kenyon 1972; Gerrodette & Gilmartin 1990). The seals breed at five main locations in the northwestern Hawaiian Islands (Fig. 1), and they show a high degree of site fidelity (Johnson & Kridler 1983). Although about 10% of adult monk seals have been sighted at a location other than their natal island (Ragen 1993), the degree to which these migrants may contribute to the local gene pool (i.e., their reproductive success) is unknown. Given their history of small population size and relatively isolated subpopulations, monk seals are expected to be relatively low in genetic variability due to the effects of random genetic drift and inbreeding.

The combination of small subpopulations and low inter-island migration rates is also likely to lead to significant genetic differentiation among subpopulations, thereby preserving more genetic variation overall than would exist in a panmictic population (Allendorf 1983; Varvio et al. 1986; Lacy 1987). The importance of a low migration rate among subpopulations in order to ensure the preservation of alleles has been emphasized in recommendations for endangered species management because heterozygosity can be reconstituted by artificially increasing gene flow if inbreeding depression is a serious concern (e.g., Allendorf 1983; Lacy 1987). Recently, however, monk seal population managers have effectively raised migration rates by translocating seals among islands, primarily in order to relocate starving juveniles from French Frigate Shoals to locations where food availability may be greater and survival better (Gerrodette & Gilmartin 1990; Gilmartin & Eberhardt, 1995). Although these relocations have increased reproductive potential for the population (Gilmartin & Eberhardt 1995), the aggregate potential effects of these management actions cannot be properly evaluated in the absence of data on the genetic structure of the monk seal population.

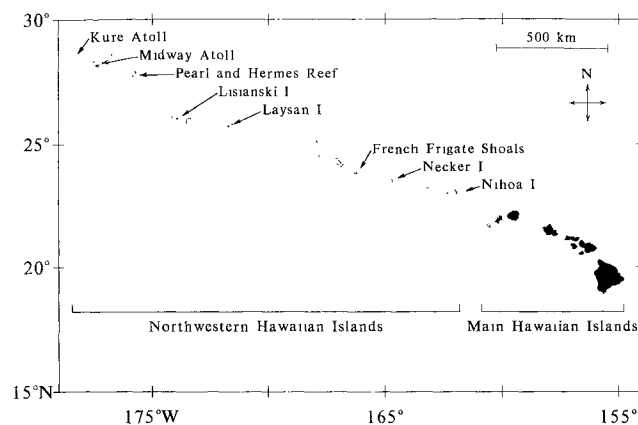


Figure 1. Map of the northwestern Hawaiian islands; arrows indicate islands inhabited by monk seals. Sampling was conducted at the five sites currently used for breeding (French Frigate Shoals, Laysan, Lisianski, Pearl and Hermes Reef, and Kure Atoll). Dotted lines around islands represent the 100-fa isobath. Adapted from Ragen (1993) with permission.

To test the hypothesis that small population size and strong site fidelity has led to low within-island genetic variability and significant between-island differentiation in the Hawaiian monk seal, we used two independent approaches to quantify genetic variation both within and among the principal monk seal subpopulations. Both techniques involve selectively neutral genetic markers; we assume that these provide an index of genome-wide variation, with possible significance for the survival potential of the population. We examined the sequence of the non-coding mitochondrial DNA (mtDNA) control region, the fastest evolving portion of the mitochondrial genome and often a sensitive gauge of intraspecific genetic variation (e.g., Kocher et al. 1989; Meyer 1994). Because many other studies involve sequencing the same segment of DNA, these data also provide a "universal metric," thereby facilitating the interpretation of monk seal genetic variability in the context of what is known about other similar species. Such a comparative approach, including species which have never been threatened with extinction, and those which are recovering to varying degrees, may shed some light on the importance of genetic variation in the conservation of endangered species.

Several authors (e.g., Cronin 1993; Moritz 1994) have stressed the importance of using nuclear DNA markers in conjunction with mtDNA analysis in conservation applications because of the varying modes of inheritance and population dynamics of the two types of genetic material. Maternally-inherited mtDNA is more prone to genetic drift (and therefore more sensitive to population bottlenecks) than are nuclear loci due to an effective population size one-quarter that of nuclear DNA (Birky et al. 1989). Accordingly, we used a multilocus DNA fingerprinting approach, which is based on tandemly repeated nuclear DNA sequences known as minisatellites. Most minisatellites are highly polymorphic due to variation in copy number of the repeat unit, and a single probe based on a shared "core" sequence can detect many variable loci simultaneously (Jeffreys et al. 1985). Although this approach provides a level of resolution often best suited to identify individuals and close relatives, in some species with relatively low genetic variability DNA fingerprinting has been used successfully to examine relationships among subpopulations (Gilbert et al. 1990; Triggs et al. 1992).

Methods

Sampling

Tissue samples were collected from the rear flippers of seals during tagging operations at the five principal breeding sites of the Hawaiian monk seal (French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, and Kure Atoll; see Fig. 1) and were frozen as soon

as possible, although sample preservation was not entirely successful at these remote field sites. Almost all samples were from pups of the year; the exceptions were two juveniles (from Lisianski, Pearl and Hermes) and one adult male (from Laysan). We assumed these samples were representative of the island at which they were collected because no pups born to known immigrant mothers were included, and very few juveniles are sighted at non-natal locations (Ragen 1993). We also assumed these samples were from unrelated animals, although the breeding system in this species is unknown, and if the degree of polygyny is high, some of the pups could be half-siblings. Nevertheless, because monk seal mating occurs in the water, the opportunity for individual males to monopolize access to females is limited, and the degree of polygyny is likely to be much lower than in the closely related terrestrial-breeding elephant seals (Boness et al. 1993). The probability of sampling related animals was also minimized by sampling individuals from different locations within each island (although the extent of geographic separation varies greatly among sites due to island topography).

Mitochondrial DNA Sequencing and DNA Fingerprinting

DNA was extracted from tissue samples from 10 individuals from each of the five islands, following standard protocols (Maniatis et al. 1982). Amplifications via the polymerase chain reaction and generation of single-stranded product for direct sequencing (Sequenase, US Biochemical) were carried out using the mtDNA control region primers described in Kocher et al. (1989; L15915) and Meyer et al. (1990; H16498). Negative controls for contamination were included with each set of samples. A sequence of 359 base pairs was obtained from all 50 seals, including 56 base pairs of the Proline tRNA gene and 303 base pairs of the control region, including all of the "hypervariable" region preceding the "conserved central block" (Slade et al. 1994). An additional 96 base pairs of sequence, for a total length of 455 base pairs, was obtained from some individuals for which both strands were sequenced and/or the sequence was exceptionally clear.

Unlike the mitochondrial sequencing approach, DNA fingerprinting is dependent upon obtaining high quality, non-degraded DNA (see Bruford et al. 1992). Sample quality was examined by running out a small aliquot of DNA on an agarose gel stained with ethidium bromide and visualizing under UV light. Only 22 samples were found to contain sufficient high molecular weight DNA suitable for fingerprinting; these included 4-7 individuals from four of the five main subpopulations. These samples were digested with *Hae* III and run on agarose gels at 19 mAmps for 48 hours. Samples from two different islands were run on each gel, with a lane of molecular weight marker ("Genetic Analysis" ladder, Promega) adjacent to each sample lane. Following transfer of DNA

fragments from gel to nylon membrane, hybridization was accomplished using chemiluminescent alkaline phosphatase-labeled 33.6 probe (Cellmark) and "Genetic Analysis" marker probe (Promega), according to the method of Ruth and Fain (1993).

Fingerprint Analysis

Fragments sized between 2 and 23 kb were scored using an automatic scanner (Scanmaster 3+, Howtek) and Bio-Image computer software, and band sizes were determined by reference to the marker lanes adjacent to each sample lane. The similarity index was defined as the number of fragments shared by individuals x and y , divided by the number of fragments detected in both individuals (Lynch 1990). The computer program SIM (version 1.01; Zimmerman 1993) was used to generate within and between island similarity indices for individuals run on the same gel, using a match window of 3 SD (derived from the variation in migration distance for fragments of known size) around each band (see Galbraith et al. 1991). The SIM program provides variance estimates corrected for the non-independence of data points in multiple pairwise comparisons, as described by Lynch (1990), and estimates the extent of population subdivision according to Lynch (1991):

$$f'_{st} = (1 - s_b)/(2 - s_w - s_b),$$

where s_b is the average between-subpopulation similarity (corrected for within-subpopulation similarity) and s_w is the average within-subpopulation similarity. This provides a downwardly-biased, conservative estimate of population subdivision (Lynch 1991).

Results

Monk seal control region sequence variation was very low; of the 359 sites surveyed for all 50 seals, only 2 were variable. No additional variation was detected among the seals for which longer sequences were obtained. The 2 variable sites defined three haplotypes (Table 1), one of which (III) was found in a single individual

Table 1. Distribution of monk seal mtDNA control region haplotypes by location.

Haplotype	Island*					Total
	FFS	LAY	LIS	PHR	KUR	
I	9	7	9	8	10	43
II	1	2	1	2	0	6
III	0	1	0	0	0	1
Total	10	10	10	10	10	50

*FFS, French Frigate Shoals; LAY, Laysan Island; LIS, Lisianski Island; PHR, Pearl and Hermes Reef; KUR, Kure Atoll (see Fig. 1).

(the adult male from Laysan). The sequence for haplotype I is available in GenBank (accession number U59753); haplotypes II and III were distinguished from I by transitions at positions 58 (in Pro tRNA gene) and 176 (in the control region) respectively. The most common haplotype (I) was found in 43 of 50 individuals, and the remaining haplotype (II) was found in 6 individuals, representing four of the five principal breeding sites (Table 1). Therefore, the control region sequence data provide no evidence for genetic differentiation among monk seal subpopulations.

The combination of the restriction enzyme *Hae* III and alkaline phosphatase-labeled 33.6 probe yielded complex monk seal DNA fingerprints, with 15–31 scorable bands per individual (Fig. 2). Comparisons were limited to two pairs of adjacent islands run on the same gel: Laysan versus Lisianski and Pearl and Hermes versus Kure. Mean within-island similarity estimates ranged from 0.49 to 0.73, and (with one exception) the similarity values for individuals from different islands were lower than the within-island values (Table 2). Although the distributions of band-sharing values within and between islands overlapped, and similarity values between sites were quite high, the differences between these distributions were large enough to yield significant ($p < 0.01$) estimates of population subdivision in both comparisons, with estimated f'_{st} values of 0.20 and 0.13 (Table 2).

Discussion

Evidence for Low Genetic Variability Within Subpopulations

Data from both mtDNA sequencing and multilocus DNA fingerprinting support the hypothesis that monk seal genetic variability is extremely low. Even the cheetah, shown to be depauperate in genetic variation at a variety of nuclear coding loci, exhibited "moderate levels of genetic diversity" in both DNA fingerprint profiles and mitochondrial DNA restriction fragment analysis of the whole genome (Menotti-Raymond & O'Brien 1993). In contrast, even within the most variable region of the mitochondrial genome, we found only three control region haplotypes among 50 individuals representing the entire range of the species, and a very low percentage (0.6) of variable sites in this "hypervariable" region (Table 3).

Monk seal control region sequence variation can be directly compared to homologous sequence data obtained from other pinniped species that have been similarly surveyed (Table 3). This analysis shows that the northern elephant seal is the only other species with a similar paucity of genetic variation in this region (both in terms of haplotypic and nucleotide diversity). The northern elephant seal is a close relative of the monk seal, and has a similar history of human exploitation leading to a well-documented population bottleneck. It has been shown

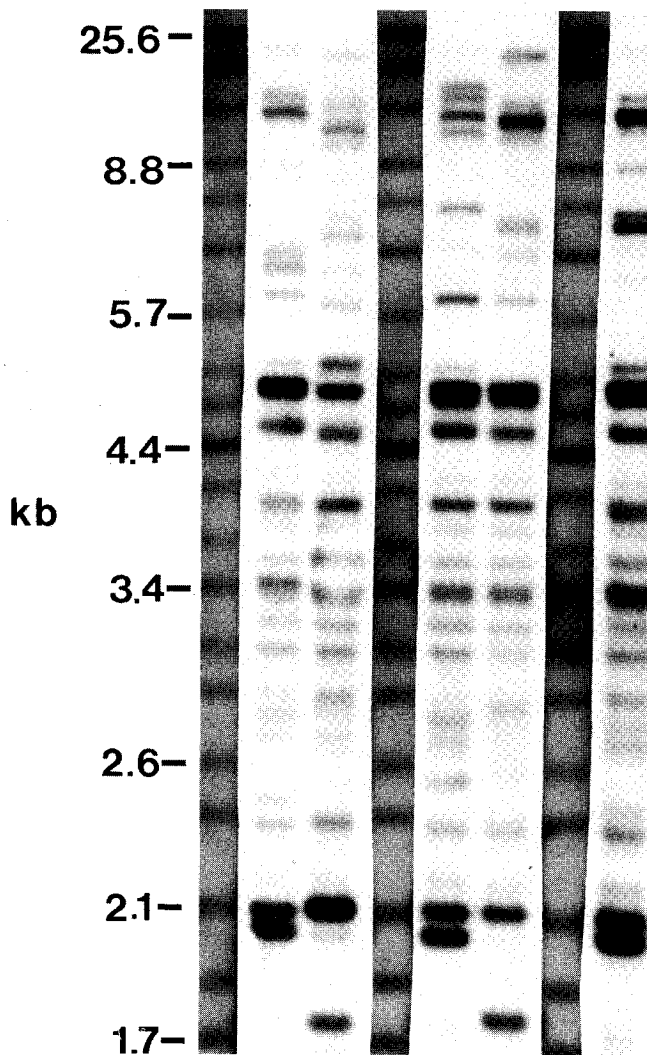


Figure 2. Example of a DNA fingerprint for Hawaiian monk seals from Pearl and Hermes Reef. The darker lanes contain molecular weight marker. Fragment sizes are shown at left. DNA was cut with Hae III and hybridized with alkaline phosphatase-labeled Jeffrey's probe 33.6.

to be depauperate in genetic variation using several approaches (allozyme electrophoresis, Bonnell & Selander 1974; DNA fingerprinting, Lehman et al. 1993; and mtDNA sequencing, Hoelzel et al. 1993).

The fingerprint data must be considered preliminary due to the small number of samples that proved to contain suitable high quality DNA. Nevertheless, for carnivore populations similarly assessed with Jeffrey's multilocus fingerprint probes, band-sharing values above 70% for "unrelated" individuals have been reported only within populations that are small and isolated and/or highly inbred (e.g., Channel Island fox, Gilbert et al. 1990; Isle Royale wolf, Wayne et al. 1991; northern ele-

Table 2. Summary of DNA fingerprint results* for Hawaiian monk seals from four islands.

Result component*	Island			
	Laysan	Lisianski	Pearl and Hermes	Kure
<i>N</i>	7	4	6	5
Mean <i>n</i>	18	16	27	24
Mean <i>s_w</i>	0.65	0.49	0.71	0.73
Mean <i>s_b</i>		0.51		0.64
<i>f'_{st}</i>		0.13		0.20
C.L.		0.07		0.02

**N*, number of individuals; *n*, number of bands scored; *s_w* and *s_b*, similarity indices for individuals within and between islands, respectively. The *f'_{st}* and C.L. (99% confidence limits on *f'_{st}*) were calculated based on Lynch (1991).

phant seal, Lehman et al. 1993). In contrast, outbred mammalian populations typically show similarity values between 20% and 60% (e.g., Ruth & Fain 1993). DNA fingerprint similarity values are well correlated with known inbreeding coefficients in domestic poultry (Kuhnlein et al. 1990), and inbred populations of both laboratory mice (Jeffreys et al. 1987) and humans (Bellamy et al. 1991) show high band-sharing values relative to outbred controls. The similarity estimates obtained in this study are therefore consistent with local inbreeding within at least some monk seal subpopulations.

Problems with DNA Fingerprint Interpretation

Interpretation of our DNA fingerprint results in the context of other studies is somewhat problematic. Band-sharing estimates for the same individuals may vary significantly depending on the particular enzyme/probe combination used (e.g., Georges et al. 1988; Hanotte et al. 1992) and, because there is no standard method for scoring fingerprints, varying match windows may substantially alter band-sharing values. In addition, samples of poor quality are likely to yield misleading results. Degraded DNA will be missing the high molecular weight fragments which tend to show the highest variability (Fig. 2), thereby biasing similarity values upwards. By limiting our analyses to the subset of samples with little or no DNA degradation, we avoided this pitfall.

Inbreeding as a Threat to the Population

In contrast to the monk seal, the northern elephant seal demonstrates ongoing rapid population growth and is now estimated to number over 150,000 individuals (see Stewart et al. 1994), from a post-bottleneck population that probably numbered fewer than 100 (see Hoelzel et al. 1993). These seal species share low genetic variability but have responded very differently following a severe reduction in numbers. Therefore, it might be ar-

Table 3. Intraspecific mtDNA control region sequence variation for pinnipeds.

<i>Species</i>	<i>No. of haplotypes/no. of individuals (no. of "populations")</i>	<i>No. of variable sites/ total no. sequenced</i>	<i>Variable sites (%)</i>
Hawaiian monk seal	3/50 (5)	2/359	0.6
Northern elephant seal ^a	2/40 (1)	3/300	1.0
Southern elephant seal ^a	26/48 (2)	26/300	8.7
Harbor seal ^b	34/227 (24)	40/453	8.8
California sea lion ^c	11/40 (4)	29/315	9.2
Steller sea lion ^d	52/224 (6)	29/238	12.2

^aHoelzel et al. (1993).

^bStanley et al. (1996).

^cMaldonado et al. (1995).

^dBickham et al. (1996).

gued that the two differ markedly in their sensitivity to inbreeding, or, alternatively, that genetic variation per se is not a good predictor of the ability of an endangered species to recover. Inbreeding costs are known to vary widely among mammals (Ralls et al. 1979; 1988), but the extent to which the monk seal may suffer from the deleterious effects of inbreeding is unclear. Inbreeding depression is often manifested as reduced fertility and/or poor juvenile survival in other mammals (Ralls et al. 1979; Brewer et al. 1990; Laikre & Ryman 1991). While female monk seal fecundity is quite low compared to that of other seals (Johanos et al. 1994), juvenile survival in this species can be quite high: 80–90% in the first year and 85–98% for young seals over age one (Gilmartin et al. 1993).

High DNA fingerprint band-sharing values between mated pairs of Puerto Rican Parrots (Brock & White 1992) and Great Reed Warblers (Bensch et al. 1994) were associated with reproductive failure. A similar effect is therefore a plausible explanation for the low reproductive rate of the Hawaiian monk seal. Patenaude et al. (1994) recently concluded that inbreeding depression could be a factor in the failure of an endangered beluga whale population to recover, based on high band-sharing values relative to a healthy reference population of the same species. Inbreeding depression has also been invoked as an explanation for poor reproductive performance in captive cheetah populations (O'Brien et al. 1985), but other studies indicate that non-genetic factors account for most cheetah mortality in the wild (Caro & Laurenson 1994; Laurenson et al. 1995). Similarly, ecological factors such as lack of suitable habitat and a decline in food resources (Polovina et al. 1994) may pose a more important threat to the monk seal population than do genetic factors.

Although a lack of genetic diversity may not always be of foremost concern in conservation efforts, the potential importance of low genetic variability for increasing the vulnerability of a species to infectious disease is often cited (e.g., Bonnell & Selander 1974; O'Brien & Evermann 1988). This effect may be especially important in small populations. Marine mammal epizootics are not

uncommon; a phocine distemper virus killed nearly 18,000 common seals in Europe in 1988 (Harwood & Hall 1990). A similar outbreak among Hawaiian monk seals could potentially drive the species to extinction. Furthermore, recent work by Keller et al. (1994) demonstrated a clear survival bias against inbred individuals following a song sparrow population crash, and Jiménez et al. (1994) reported that inbreeding had a more detrimental effect on mice introduced into a natural habitat than on those maintained in the laboratory. Thus it appears that previously undetected inbreeding depression may be manifested in the face of an environmental challenge. Even an apparently highly successful species like the northern elephant seal might therefore become vulnerable, due to depressed levels of genetic variation, should environmental conditions change markedly.

Evidence for Population Subdivision

The two genetic marker systems used in this study provide conflicting evidence regarding population subdivision. Our extensive mitochondrial control region sequence data set does not indicate genetic differentiation among subpopulations. Indeed, the presence of a relatively rare haplotype on four of five islands (see Table 1) supports the idea that the monk seal population is panmictic. In contrast, the preliminary results of the fingerprinting study indicate that individuals from the same island are genetically more similar than are individuals from adjacent islands. This suggests that some degree of monk seal subpopulation differentiation exists, and that gene flow among the islands has been limited.

Population genetic structure (and local inbreeding) in other species has been inferred from non-overlapping within- and between-subpopulation similarity values (e.g., Hoelzel & Dover 1991; Triggs et al. 1992). Although the ranges of similarity values between monk seal subpopulations overlapped those within subpopulations, the distributions were different enough to allow detection of significant genetic structure in the population as a whole. We concluded that two adjacent pairs of

islands (Kure vs. Pearl and Hermes Reef, and Lisianski vs. Laysan; Fig. 1) were genetically distinguishable from one another, with f'_{st} values of 0.20 and 0.13, respectively (Table 2). These are probably minimum estimates of the proportion of genetic diversity distributed among subpopulations, because more distant islands in the chain are likely to differ even more.

Implications for Conservation

The genetic data presented here have several possible implications for the management practice of translocating seals among islands. Movements of individuals among subpopulations is often opposed due to the risk of disease transmission (e.g., Wilson et al. 1994; Brower et al. 1995). This may be an issue of particular importance for the monk seal, in light of the paucity of genetic variation observed in this species. Also, if monk seal subpopulations are indeed genetically distinct, then increasing the effective migration rate among islands may have the potential to reduce genetic variation retained in the population as a whole. However, the genetic differentiation revealed by DNA fingerprinting was not supported by the mtDNA sequence data and may not reflect biologically meaningful differentiation among islands, because differences in coding DNA will recover much more slowly following a population bottleneck.

The results presented here for the monk seal contrast markedly with the DNA fingerprinting results reported for an endangered Hawaiian bird, in which two subpopulations were each genetically quite diverse but not at all differentiated from one another; in this case proposed translocations from the larger to the smaller subpopulation were clearly favored by the genetic data (Fleischer et al. 1994). For the monk seal the survival benefits associated with moving animals to a location where food resources are more plentiful and the possible advantages of relieving local inbreeding argue in favor of the translocation program. Although inbreeding depression has not been conclusively demonstrated in this species, the evidence for low genetic variability and local inbreeding is strong. The monk seal's low fecundity represents a real threat to its recovery, and a genetic basis for this poor reproductive performance is certainly possible.

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