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Extremely Low Genetic Diversity in the Endangered Hawaiian Monk Seal (*Monachus schauinslandi*)

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Abstract

Hunted to near extinction in the late 19th century, the endangered and endemic Hawaiian monk seal (*Monachus schauinslandi*) exhibits low variation at all molecular markers tested to date. Here we confirm extreme paucity of genetic diversity, finding polymorphisms at only 8 of 154 microsatellite loci tested (143 novel species-specific loci, 10 loci from Antarctic seals, and 1 previously characterized locus). This screening revealed unprecedentedly low levels of allelic diversity and heterozygosity ($A = 1.1$, $H_e = 0.026$). Subsequent analyses of 2409 Hawaiian monk seals at the 8 polymorphic loci provide evidence for a bottleneck ($P = 0.002$), but simulations indicate low genetic diversity ($H_e < 0.09$) prior to recorded human influence. There is little indication of contemporary inbreeding ($F_{IS} = 0.018$) or population structure ($K = 1$ population). Minimal genetic variation did not prevent partial recovery by the late 1950s and may not be driving the current population decline to ~1200 seals. Nonetheless, genotyping nearly every individual living during the past 25 years sets a new benchmark for low genetic diversity in an endangered species.

Genetic variation is the raw material of evolution and a prerequisite for adaptation via natural selection (Fisher 1930). Measures of genetic diversity are often used to estimate individual fitness and the potential for population persistence (Coltman et al. 1999; Hansson and Westerberg 2002; Reed and Frankham 2003). Genetically depauperate species may have a reduced ability to mount an effective defense against pathogens (Hawley et al. 2005) or to adapt to environmental changes (Hoffmann and Parsons 1997), thereby increasing the risk of extinction (Mills and Smouse 1994; Lacy 1997; Frankham et al. 1999, 2002). Although extinction may be a demographic process (Lande 1988; Allendorf and Luikart 2007), meta-analysis of laboratory and wild case studies indicates that inbreeding depression and low genetic diversity cannot be ignored (Frankham 2005) as most species are not driven to extinction prior to being impacted by genetic factors (Spielman et al. 2004).

Measures of genetic diversity, such as proportion of polymorphic microsatellite loci (P), number of alleles per locus or allelic diversity (A), and mean percent heterozygosity (H), reflect previous demographic events. In a meta-analysis of 108 mammalian species, Garner et al. (2005) do not find a consistent trend in the genetic diversity of healthy populations, but there is a pervasive and consistent reduction of genetic variability in populations that have experienced a rapid and severe decline in population size. However, such bottlenecks do not always produce the predicted genetic signature (Busch et al. 2007) and are not the only cause of low genetic diversity. Amos and Harwood (1998) assert that inbreeding, population structure, heterozygote mutation bias, and genome-wide selective sweeps also contribute alone or in synergy to the low variability detected in many endangered species.

The northern elephant seal (*Mirounga angustirostris*) was the first documented case of low genetic diversity in response to near extinction. In a seminal paper, Bonnell and Selander (1974) report unprecedented homogeneity at 24 allozyme loci across 159 individuals. Hoelzel et al. (1993) confirm these results with another 43 allozyme loci and also find low variation at mitochondrial loci, with 2 haplotypes in 40 individuals. At exon 2 of the class II major histocompatibility complex DQB locus, expected to be highly polymorphic in mammals, Hoelzel et al. (1999) found only 2 alleles in 69 individuals. Finally, in a survey of 39 microsatellite loci, Garza (1998) identifies 14 polymorphic loci with 2–4 alleles each. A 19th century population

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bottleneck, the result of overhunting, may account for much of the homogeneity: a simulation model based on extensive demographic data indicates a bottleneck of less than 30 seals and 20-year duration (Hoelzel et al. 1993). High reproductive skew, low reproductive rates, and demographic stochasticity also may have contributed to the low genetic variation (Hoelzel 1999).

The Hawaiian monk seal (*Monachus schauinslandi*) endured a similar history of overexploitation. Originally occurring throughout the Hawaiian archipelago (Figure 1), it was likely extirpated from the main Hawaiian Islands by Polynesian colonizers 1500–1600 years ago (Bellwood 1978; Baker and Johanos 2004). On arrival of the first European sailors in the 19th century, the Hawaiian monk seal was hunted to near extinction at the 6 primary Northwestern Hawaiian Islands subpopulations (French Frigate Shoals, Laysan, Lisianski, Pearl and Hermes Reef, Midway Atoll, and Kure; Ragen 1999). Although historical counts of total population size are not available, records indicate an abundance of seals up to the year 1857 (Hiruki and Ragen 1992), no or few seals at most islands by 1893 (Ragen 1999), and a "large number" at Kur and Pearl and Hermes Reef by 1915 (Hiruki and Ragen 1992). In 1958, mean counts of seals on the beach (an index of abundance) had recovered to 916 individuals, 1 year of age or older (nonpups; Rice 1960). The mean count has since declined to 293 nonpups, and the most recent estimate of total abundance is 1247 seals (Carretta et al. 2007). Extensive monitoring of the Hawaiian monk seal over the past 25 years has yielded over 2400 genetically sampled individuals, which represents a large proportion of the entire species.

Mitochondrial sequencing and multilocus fingerprint analyses indicate low genetic diversity in the Hawaiian monk seal (Kretzmann et al. 1997), and Aldridge et al. (2006) report exceptional uniformity in major histocompatibility class I genes. Of 18 pinniped species tested, the Hawaiian monk seal exhibits the lowest level of genetic diversity when assessed across 20 microsatellite loci isolated from gray (*Halichoerus grypus*), harbour (*Phoca vitulina*), southern elephant (*Mirounga leonina*), and South American fur (*Arctocephalus australis*) seals (Gemmell et al. 1997). Among these 20 loci, 3 are polymorphic in the Hawaiian monk seal (Gemmell et al. 1997), but only 1 locus (Hg6.3; Allen et al. 1995) conforms to expectations of Mendelian inheritance and can be used in population-level analyses (Kretzmann et al. 2001).

Low microsatellite diversity is often attributed to ascertainment bias, that is, loci isolated in one species are comparatively invariant in distantly related species (Ellegren et al. 1995). Reviewing genetic diversity of 108 mammalian species, cross-species amplification results in consistently and significantly reduced mean heterozygosity and number of alleles per locus (Garner et al. 2005). The success of cross-species microsatellite amplification is generally related to the evolutionary distance from the focal species; for species diverging 10–20 million years ago, approximately 25% of primer sets will amplify polymorphic loci (Gemmell et al. 1997; Primmer et al. 1996). Fossil evidence suggests that monachines (southern seals including the monk, elephant, and Antarctic seals) split from phocines (the northern seals including the harbour and gray seals) 15–17 million years ago (Demere et al. 2003; de Muizon 1982). Molecular data indicate a divergence of the monk seals and elephant seals 11–16 million years ago (Fyler et al. 2005; Arnason et al. 2006). Therefore, one would expect approximately 5 of the loci tested above to be polymorphic in monk seals due to ascertainment bias alone, and Gemmell et al. (1997) found 3 to be variable in the Hawaiian monk seal.

The paucity of variable loci has prevented use of genetic data in management initiatives. Population structure remains equivocal, with results based primarily on one microsatellite locus (Kretzmann et al. 2001). Identifying individuals genetically, confirming maternity, and elucidating paternity would provide measures of male and female reproductive

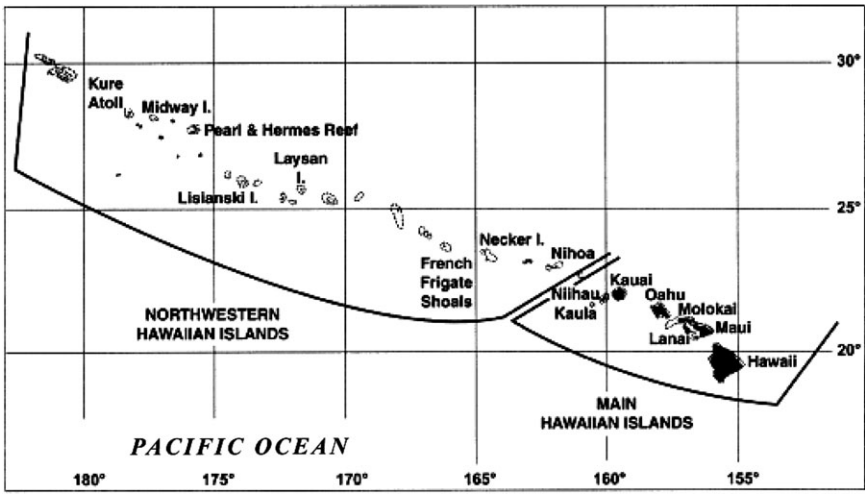

Figure 1. Range of *Monachus schauinslandi* in the Hawaiian archipelago.

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rates, which are priorities to wildlife managers. Finally, it is currently unknown whether inbreeding depression contributes to decline of the species. To address these questions, additional variable loci are needed.

Here we further investigate the genetic diversity of the Hawaiian monk seal. We first test for cross-species amplification using 10 microsatellite loci isolated from Antarctic seals (Davis et al. 2002). We then isolate and test 143 new microsatellite loci from the Hawaiian monk seal and genotype 2409 individuals at 7 novel and 1 previously published (Hg6.3) polymorphic loci. We evaluate possible causes of low genetic diversity, including population bottleneck, inbreeding, and population subdivision. Building on previous studies of nuclear and mitochondrial DNA diversity (Gemmell et al. 1997; Kretzmann et al. 1997, 2001), our survey of 154 loci provides a compelling portrait of depleted genetic diversity across the genome of the Hawaiian monk seal.

Materials and Methods

Tissue samples from Hawaiian monk seals ($N = 2409$) were collected opportunistically and during annual population assessments in the main Hawaiian Islands (2000–2007; $N = 54$) and at 7 sites in the Northwestern Hawaiian Islands (1980–2007): Nihoa (7), French Frigate Shoals ($N = 766$), Laysan (656), Lisianski (310), Pearl and Hermes Reef (260), Midway (134), and Kure (222). Seals were tagged by punching a hole in their flippers; the resulting tissue plugs were preserved in 20% dimethyl sulfoxide salt-saturated solution, 95% ethanol, or frozen in liquid nitrogen (Henderson and Johanos 1988). Genomic DNA was extracted from all samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

To isolate microsatellite loci from the Hawaiian monk seal, we followed the protocol of Glenn and Schable (2005) with modifications. Genomic DNA was extracted from captive seal blood using the DNeasy Tissue Kit. Approximately 5 μg of high–molecular weight DNA was digested using the blunt-end cutting enzyme *Alu*I to avoid methylated CG sequences common to mammals. Resulting fragments containing microsatellite motifs were hybridized to biotinylated oligonucleotides and captured using magnetic beads (Dynal, Oslo, Norway). A T-tailed vector was created by first digesting a pZErO-2 vector (Invitrogen, Carlsbad, CA) with the enzyme *Eco*RV (Promega, Madison, WI) and then adding a T-tail by performing a 2-h extension (72 °C) with 100 mM 2′deoxythymidine 5′-triphosphate and polymerase (Bioline, San Clemente, CA). The fragments were amplified, ligated into the T-tailed vector, and used to transform α-Select Gold Efficiency Competent Cells (Bioline, Randolph, MA). Recombinant clones were selected at random, amplified, and sequenced on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA) by Macrogen, Inc. (Seoul, South Korea). Of 624 positive clones, 470 contained microsatellite sequences. The software PRIMER 3 (Rozen and Skaletsky 2000) was used to develop primers for 143 putative loci that exceeded 10 short tandem repeats. To gain insight into the genomic coverage and possible identity of these 143 novel loci, sequences were blasted to the dog genome in GenBank (http://www.ncbi.nlm.nih.gov/).

To test for polymorphism, we analyzed 8 Hawaiian monk seal DNA samples (1 from each Northwestern Hawaiian Islands subpopulation and 2 from the main Hawaiian Islands) at 143 novel microsatellite loci and a subset of microsatellite loci developed from 3 species of Antarctic seals (Weddell, *Leptonychotes weddellii*; leopard, *Hydrurga leptonyx*; and crabeater, *Lobodon carcinophagus*): Lw8, Lw10, Lw11, Lw16, Lw18, Hl4 Hl15, Hl16, Hl20, and Lc5 (Davis et al. 2002). The polymerase chain reaction (PCR) protocol consisted of 15 min of initial denaturation at 95 °C, followed by 35 cycles of denaturation (94 °C, 30 s), annealing (56–60 °C, 30 s), and extension (72 °C, 30 s), with a final extension (72 °C, 30 min) of the following 10-μl reaction: 2× Biomix Red (Bioline), 5 μM each primer, and 30–50 ng genomic DNA. We ran 5 μl of amplified product on a 3% agarose gel, stained with GelStar (Lonza, Basel, Switzerland) and visualized the bands using a Molecular Image Gel Documentation XR System (Bio-Rad, Hercules, CA).

Primers amplifying the 7 novel polymorphic loci and 1 previously isolated locus (Hg6.3; Allen et al. 1995) were fluorescently labeled and combined in a single multiplex PCR using the following 6-μl reaction: 1× PCR mix (Qiagen), 2 μM primer mix, and 30–50 ng genomic DNA. The PCR protocol consisted of 15 min initial denaturation at 95 °C, followed by 35 cycles of denaturation (94 °C, 30 s), annealing (60 °C, 30 s), and extension (72 °C, 30 s), with a final extension (60 °C, 30min) to ensure the addition of a terminal adenine. Amplified products were run on an ABI 3100 Genetic Analyzer (Applied Biosystems) by the Experimental Program to Stimulate Competitive Research Sequencing Facility at the Hawaii Institute of Marine Biology and scored using the software GENEMAPPER 4.0 (Applied Biosystems).

We scored all products 2 or more times to ensure accuracy. Global tests for Hardy–Weinberg equilibrium and linkage disequilibrium were conducted using FSTAT 2.9.3.2 (Goudet 1995). Mendelian inheritance of the alleles was assessed using known mother–pup relationships. PCR error as a result of null alleles and large allele dropout was tested in MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004). Scoring and human error was estimated by duplicating analyses (from extraction to scoring) for 72 randomly chosen individuals.

The average number of alleles per locus (A) and unbiased heterozygosity (H; Nei 1987) were calculated using EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001). We also used the program to estimate polymorphism information content (PIC; the probability that the parental genotype may be deduced from rare alleles found in its progeny) and evaluated each locus as highly informative ($PIC > 0.5$), reasonably informative ($0.5 > PIC > 0.25$), or slightly informative ($PIC < 0.25$; Botstein et al. 1980).

We assessed the impact of the 19th century population bottleneck on genetic diversity using the software BOTTLENECK 1.2.02 (Piry et al. 1999). In a bottlenecked

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population, rare alleles are the first to be lost, lowering the mean number of alleles per locus. Heterozygosity is less affected, producing a transient excess in heterozygosity relative to that expected given the resulting number of alleles (Cornuet and Luikart 1996). Because we scored less than 20 microsatellite loci, as required by the strict stepwise mutation model (SMM), we performed 10 000 iterations of the Wilcoxon signed-rank test with the infinite allele model (SMM; Kimura and Crow 1964) and two-phase mutation (TPM) model (TPM incorporated 70% stepwise and 30% multistep mutations; DiRienzo et al. 1994). We also applied the *M* ratio method, which measures the number of alleles relative to the overall range in allele size using the software MPVAL (Garza and Williamson 2001). We ran simulation tests (10 000 replicates in CRITICALM) to provide average and lower 95% confidence intervals for *M* ratio values, given $\theta = 4$ and default values for a TPM microsatellite mutation model.

Generally, a bottleneck must persist over several generations to impact heterozygosity significantly. Following Amos and Balmford (2001), we considered various scenarios to describe the loss of heterozygosity relative to time and effective population size using the following formula:

$$H_t = H_0(1 - 1/2N_e)^t,$$

where H_0 is the initial heterozygosity and H_t is the heterozygosity t generations after a decline to size N_e (James 1970). H_t was calculated from the observed genotypes. We estimated H_0 over several generations (t = 1–4 generations or 15–60 years) and for various effective population sizes (N_e = 2–50). Due to the lack of historic total abundance information, we calculated a minimum postbottleneck population size from historic beach counts and records of monk seals available from the early 20th century (Hiruki and Ragen 1992), assuming that following the bottleneck the population grew at the maximum growth rate observed for the species (7% per year; Carretta et al. 2007).

Contemporary inbreeding (calculated as F_{IS}, a measure of within population heterozygote deficit, with 95% confidence intervals by bootstrapping over all loci) was evaluated using FSTAT. Population subdivision was assessed in STRUCTURE 2.0 (Pritchard et al. 2000), which assigns individuals into *K* populations to achieve Hardy–Weinberg and linkage equilibrium. We used the default settings (admixture model, correlated allele frequencies, and $\lambda = 1$) with a 10 000 burn-in length and 100 000 simulations to test K = 1–10 with 3 repetitions each, verifying convergence of alpha and likelihood values. The program POWSIM 4.0 (Ryman and Palm 2006) was used to evaluate the statistical power provided by the 8 polymorphic loci to detect population structure in the Hawaiian monk seal.

Results

Only 7 of the 143 microsatellite loci isolated from the Hawaiian monk seal were polymorphic in the initial survey (GenBank; accession numbers EU913763–EU913769). We followed a standard protocol (Glenn and Schable 2005) with modifications that were unlikely to reduce variability. Though resolution is limited using 3% agarose gels to assess polymorphism, we were able to detect alleles differing by 2 (locus Ms647) and 4 (loci Ms15 and Ms663) base pairs, and subsequent scoring of 2401 individuals on an automated sequencer revealed only 2 additional alleles not observed in the initial screening. All Antarctic seal microsatellite primer pairs yielded amplification products in the Hawaiian monk seal, but none was polymorphic. Of 143 microsatellite loci isolated from the Hawaiian monk seal, 47 could not be matched to sequences within the dog genome, whereas 96 were aligned to sequences on dog chromosomes X, 1–9, 11, 13, 15–18, 20–27, 29–31, and 33–38. No further analysis was performed on the monomorphic markers.

A total of 8 polymorphic loci (7 novel and previously published Hg6.3) were used to genotype 2409 individuals from the main and Northwestern Hawaiian Islands (Table 1). No loci showed significant deviation from Hardy–Weinberg expectations. Due to excessive genome-wide homozygosity, polymorphism at 8 loci may have been maintained by direct or indirect balancing selection. In all, 5 of the 8 polymorphic loci could not be matched to sequences in the dog genome, and the remaining 3, Hg6.3, Ms504, and Ms663, were matched to sequences with no known gene product. Two pairs of loci (Hg6.3 and Ms23, $P = 0.027$; Ms504 and Ms23, $P = 0.033$) exhibited linkage disequilibrium, which was not significant after Bonferroni correction. In the 23 mother–pup comparisons, all shared at least one allele for all loci, supporting Mendelian inheritance. There was no evidence for null alleles, large allele dropout, or scoring error. Seventy-two specimen pairs with identical identification numbers (tissue samples collected independently from the same seal) were independently extracted and genotyped, to test for human error in analysis. Two specimen pairs displayed discrepancies: each contained one mismatched allele at a single locus, an error rate of 0.2% per allele.

Genetic diversity was extremely low. Evaluated across all loci, the average number of alleles per locus was $A = 1.1$ (± 0.79) and the overall unbiased heterozygosity was $H_e = 0.026$ (± 0.12). Excluding 146 monomorphic loci, the number of alleles per locus varied from 2 to 9, with a mean of $A = 3.5$ (± 2.62) and an overall unbiased heterozygosity $H_e = 0.49$ (± 0.061). PIC values indicated 2 highly informative (PIC > 0.5) and 6 moderately informative ($0.5 >$ PIC > 0.25) loci (Table 1).

Analyzing the data following 2 models of microsatellite mutation (SMM and TPM), tests for heterozygosity excess were significant ($P = 0.002$), indicating a population bottleneck (Table 1). We acknowledge that the assumptions of these analyses are not met, namely our population is likely not at mutation-drift equilibrium and our loci do not conform to either model of microsatellite mutation. Therefore, we considered another bottleneck test. Simulation tests of the *M* ratio provided an average ($M = 0.84$) and lower 95% confidence interval ($M_c = 0.74$), describing a critical value above which a bottleneck is indicated. Our data indicate

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Table 1. Microsatellite loci isolated from the Hawaiian monk seal, *Monachus schauinslandi*

Locus	Repeat motif	Primer sequence (5′–3′)	Size (bp)	H_e	H_o	PIC	No. of alleles	$(H_e - H_{eq})$/SD IAM/TPM	*M* ratio	F_{IS}
Ms9	(GAAA)18	F: CCAAAGCCTATTTCTTTCAATCC R: AGCAGAGGCCCTAAGACAGG	340–370	0.68	0.67	0.62	9	1.36/0.88	1.00	0.008
Ms15	(CCTT)6CCCT(CCTT)6	F: CTGAATTCATGCTGTATCTTGG R: GTGCTTGGGACATGATGG	158 162	0.56	0.55	0.48	2	1.86/1.48	1.00	0.043
Ms23	(GAAA)9GGAA(GAAA)8	F: CGCTTAGTGTGGAGTCACTTAGG R: GTGAGATGAATGCCCTTTGG	203–315	0.78	0.75	0.74	3	1.38/0.94	0.60	0.021
Ms265	(GT)13	F: GACTGGTAATTTACGCCCTACC R: AAGTGTTGGGTTGAAAATTGG	297–317	0.49	0.47	0.69	6	2.40/2.16	1.00	-0.020
Ms504	(AAG)24	F: ATCAGCTATCAGGGGTAGGG R: GTCATTCCCTAGTGGTAAAGACTC	308 326	0.29	0.29	0.25	2	1.10/0.98	0.20	0.043
Ms647	(TG)14	F: GAACTCCAAACAGCCATTCC R: CCTGCTCCTTCTTTCTGATCC	115 117	0.46	0.47	0.35	2	2.22/2.00	1.00	0.029
Ms663	(TC)11	F: TCAACTTCTCAATTTAGGATTCACA R: GCAAAAAGGGATGAGCCATA	290 294	0.31	0.29	0.26	2	1.26/1.11	1.00	0.015
Hg6.3	(Allen et al. 1995)	F: CAGGGGACCTGAGTGCTTATG R: GACCCAGCATCAGAACTCAAG	227 237	0.36	0.35	0.30	2	1.54/1.41	0.33	0.010
Overall				**0.49**	**0.48**	**0.46**	**3.5**	***P*= 0.002**	**0.76**	**0.018**

F, forward; R, reverse; IAM, infinite allele model. Locus name is an abbreviation of the genus and species; the number denotes the clone from which the primers were developed. Repeat motif and primer sequence information are reported for a single individual. PCR product size represents the range of allele sizes. Unbiased expected and observed heterozygosity (H_e and H_o; Nei 1987), PIC (Botstein et al. 1980), and number of alleles (overall statistics in bold) are based on 2409 individuals. Excess heterozygosity values are the difference between expected and actual heterozygosity divided by the standard deviation, for 2 models of microsatellite mutation. *M* ratio values and inbreeding coefficients (F_{IS}) are measured at each locus and overall (in bold).

a bottleneck ($M = 0.76$); however, the signal was weak (less than the simulation average), which may indicate rapid population recovery (Garza and Williamson 2001).

We estimated the minimum ($H_0 = 0.030$) and maximum ($H_0 = 0.094$) initial heterozygosity after a bottleneck of different durations (t = 1–4 generations) and effective population sizes (N_e = 2–50). After a single-generation bottleneck (~15 years) in which population decline was minimal (N_e = 50), there is essentially no loss of heterozygosity ($H_0 = 0.030$). An extreme bottleneck (N_e = 2) for 4 generations was required to reduce heterozygosity by 67% (from $H_0 = 0.09$ to $H_t = 0.03$).

The inbreeding coefficient was low over all polymorphic loci (F_{IS} = 0.018; 95% CI −0.001 to 0.023) and at each locus (Table 1). We found evidence for a single population cluster in STRUCTURE. For $K = 1$, estimates of posterior probability approached one, whereas for K = 2–10, the values approached zero. For K = 2–10, there was high variance among α values, and all individuals were roughly symmetrical and admixed over K populations (i.e., no strong population assignments), consistent with a lack of population structure. It is unlikely that such results reflect low resolving power of our markers as a simulation test (POWSIM; Ryman and Palm 2006) revealed a 100% probability of detecting divergence among Hawaiian monk seal populations if the true amount of differentiation corresponds to an F_{ST} value of 0.01 or greater.

Discussion

The Hawaiian monk seal (*M. schauinslandi*) exhibits extremely low genetic diversity. Analyzing 154 microsatellite loci, we find unprecedented levels of allelic diversity ($A = 1.1$) and heterozygosity ($H_e = 0.026$), which in a species with 17 chromosomal pairs, can be considered a relatively thorough sampling of the genome. Only 7 of the 143 novel microsatellites isolated in this study are polymorphic. Four of these, plus the gray seal locus Hg6.3 (Allen et al. 1995), are diallelic, and the average heterozygosity of the 8 variable loci is low ($H_e = 0.49 \pm 0.061$; Table 1).

Our survey confirms the initial reports of low genetic diversity in the Hawaiian monk seal at 20 polymorphic phocid loci (Gemmell et al. 1997) and an additional 7 loci isolated from the harbour seal, found to be monomorphic in the Hawaiian monk seal (Kretzmann et al. 2001). Our low allelic diversity ($A = 1.1$) is similar to the previous studies (A = 1.0–1.3; Table 2). We find slightly higher polymorphism than Kretzmann et al. (2001) but lower than Gemmell et al. (1997). Note that these studies only considered loci previously found to be polymorphic in at least one other seal species, whereas we include 143 novel microsatellite loci, developed specifically for the Hawaiian monk seal. When we consider only polymorphic loci, our average heterozygosity ($H_e = 0.49$) falls within the range of previously reported observed (H_o = 0.1–0.8) and expected (H_e = 0.1–0.53) heterozygosities at 6 Northwestern Hawaiian Islands subpopulations (Kretzmann et al. 2001).

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Table 2. Comparison of measures of microsatellite diversity, including proportion of loci which are polymorphic (*P*), average heterozygosity (H_e which is not significantly different from H_o in examples listed), and average number of alleles per locus (*A*)

	P	H_e	*A*	Sample size	Estimated census size	Reference
Hawaiian monk seal *Monachus schauinslandi*	8/154	0.03	1.1	2409	1247	This study
	3/20	—	1.3	5		Gemmell et al. (1997)
	0/7	—	1.0	5		Kretzmann et al. (2001)
Mediterranean monk seal *Monachus monachus*	15/39	0.16	1.5	41–52	300	Pastor et al. (2004)
Northern elephant seal *Mirounga angustirostris*	14/41	0.13	1.5	80–160	127 000	Garza (1998)
Cheetah *Acinonyx jubatus*	7/10	0.39	3.4	10	15 000	Menotti-Raymond and O'Brien (1995)
North Atlantic right whale *Eubalaena glacialis*	11/27	0.32	3.2	209	300	Waldick et al. (2002)
Northern hairy-nosed wombat *Lasiorhinus krefftii*	9/16	0.27	1.8	28	70	Taylor et al. (1994)
Ethiopian wolf *Canis simensis*	6/9	0.21	2.4	20	500	Gottelli et al. (1994)

Taxa represent the most genetically depauperate species and the lowest values reported for each species based on tables from Pastor et al. (2004) and Frankham et al. (2002). Some studies could not be included in the table because key values, such as number of monomorphic loci or global H_e, were not reported.

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Therefore, we conclude that previously reported low genetic diversity is not a result of ascertainment bias but rather characteristic of the Hawaiian monk seal.

The extremely low diversity reflects a population bottleneck. Using heterozygosity excess and *M* ratio analyses, we find molecular evidence for population depletion, corroborating historical accounts of intense hunting of the Hawaiian monk seal during the 19th century (Ragen 1999).

Records from European exploration of the Northwestern Hawaiian Islands document seals as early as 1805. There are reports of at least 4 shipwrecks from 1842 to 1870 in which the crew consumed seals ($N = 10–60+$) for survival. A vessel visiting all islands in 1859 returned to Honolulu with 1500 skins and 240 barrels of seal oil (Brooks 1860). By the late 1880s and early 1890s, at least 2 colonies appear to have been extirpated (Laysan and Midway) and the others severely depleted (Ragen 1999). Though incomplete, records suggest that the early 1890s marked the nadir of the bottleneck.

Using these accounts as a guide, we infer prebottleneck heterozygosity ($H_0 = 0.03–0.09$) by assessing severe to weak population decline ($N_e = 2–50$) persisting for a range of durations (1–4 generations). At the most extreme bottleneck ($N_e = 2$) for a duration of 4 generations (~60 years), there would have been a ~67% reduction in heterozygosity, which still corresponds to surprisingly low levels prior to exploitation ($H_0 = 0.09$). Furthermore, this estimate is almost certainly an overestimate of heterozygosity because it is unlikely that $N_e = 2$ for 60 years. Based on accounts compiled by Hiruki and Ragen (1992), the species had recovered to at least 184 individuals by the early 1920s (Table 3). Projecting back in time and assuming that the seal population grew at 7% per year (the maximum rate observed for the species), there would have been at least 23 seals that survived in 1893, the last year when a considerable number of seals (60–70 at Laysan Island) were reportedly killed (Hiruki and Ragen 1992). This minimum estimate of 23 bottleneck survivors is quite conservative for a number of reasons. First, we used a conservative minimum estimate of the number extant in the early 1920s, based on counts of seals on land, whereas most seals tend to be at sea at any given time. Second, it is unlikely that the population grew at a sustained rate as high as 7% per year as the seals continued to be impacted by killing, harassment, and human occupation of terrestrial habitats into the 20th century (Ragen 1999). Thus, we think that considerably more than 23 seals survived in the early 1890s. On average, effective population size is a tenth of the total population size (Frankham 1995), and $N_e = 2$ may likewise be underestimated. Finally, it is highly unlikely that the species remained at $N_e = 2$ for 4 generations as there were at least 60 seals in 1893 and "numerous" seals by 1915 (i.e., less than 2 generations). Therefore, heterozygosity

Table 3. Historic records of Hawaiian monk seal counts during or prior to the early 1920s (extracted from Hiruki and Ragen 1992)

Location	Year	Count	Comment
French Frigate Shoals	No data		
Laysan Island	1923	4	2 of 4 were killed
Lisianski Island	1923	10	No census, 10 seal killed
Pearl and Hermes Reef	1923	125	Not full count of atoll
Midway Atoll	1913	5 or 6	No exact count
Kure	1920	40 to 50	No exact count
Total minimum		184	

The minimum count from each site was tallied to calculate minimum abundance during this postbottleneck period.

was likely less than 9% even prior to the 19th century bottleneck.

Extremely low genetic diversity may be due to earlier hunting by indigenous Hawaiians and characteristics intrinsic to the species. Average polymorphism and heterozygosity are lower in vertebrates than invertebrates, with mammals (especially carnivores) exhibiting the lowest genetic diversity (Nevo 1978). There is a pattern of reduced genetic variability with increased body size in mammals (Wooten and Smith 1985). Island populations may have lower genetic diversity than their mainland counterparts as a result of few founding individuals and because small population size increases the rate of genetic drift and decreases the accumulation of new mutations. For example, the Barrow Island population of the black-footed rock wallaby (*Petrogale lateralis*) has extremely low genetic diversity (H_e = 0.053) as compared with mainland populations (H_e = 0.56, 0.62), which is attributed to inbreeding over several generations (Eldridge et al. 1999). Contemporary inbreeding seems an unlikely cause of low genetic diversity in the Hawaiian monk seal because the heterozygosity deficit is low (F_{IS} = 0.018). Likewise, we find no evidence for population structure (K = 1), which has been shown to deplete genetic diversity in small populations (Amos and Harwood 1998).

Other large or carnivorous mammals have similarly undergone recent bottlenecks and have low levels of genetic diversity (Table 2), including those with total census sizes of less than 100 (northern hairy-nosed wombat, *Lasiorhinus krefftii*) and 300 (North Atlantic right whale, *Eubalaena glacialis*) individuals. In such circumstances, some species have reduced fitness: wild and captive cheetah (*Acinonyx jubatus*) populations are often cited as examples of inbreeding depression. Genetic homogeneity in the cheetah, as demonstrated by the acceptance of skin grafts among unrelated individuals, may be correlated to high juvenile mortality, male fertility problems, and viral vulnerability (O'Brien et al. 1985, but see Merola 1994 and O'Brien 1994). Relative to the cheetah (A = 3.4; H_e = 0.39; Menotti-Raymond and O'Brien 1995), estimates of microsatellite diversity in the Hawaiian monk seal (A = 1.1; H_e = 0.026) raise the possibility of a genetic component to low juvenile survival and low reproductive rates.

Perhaps the most appropriate comparisons of genetic diversity are within the family Phocidae, the true seals. Unfortunately, the Caribbean monk seal (*Monachus tropicalis*) went extinct in the mid-20th century (Kenyon 1977; LeBoeuf et al. 1986) before genetic diversity could be assessed. The Mediterranean monk seal (*Monachus monachus*) is the most threatened of pinniped species, with fewer than 400 individuals remaining as a result of disease outbreak (Harwood 1998) and hunting dating back to ancient Greek, Roman, and Byzantine civilizations (Pastor et al. 2004). A single mitochondrial control region haplotype is shared by 18 Mediterranean monk seals (Stanley and Harwood 1997). Microsatellite diversity is low but remains higher than that of the Hawaiian monk seal (Gemmell et al. 1997). Pastor et al. (2004) find 15 polymorphic loci of 39 tested (A = 1.5; H_e = 0.16). Notably, the Hawaiian monk seal also has lower allelic diversity and heterozygosity than the northern elephant seal (*M. angustirostris*), the previous record for depleted genetic diversity among animals.

Low genetic diversity has not prevented the northern elephant seal population from rebounding to more than 175 000 (Weber et al. 2000), but monk seals have not been as resilient. Despite protection of the Hawaiian monk seal (through the US Endangered Species Act and the US Marine Mammal Protection Act) and an isolated, primary habitat in the Papahānaumokuākea Marine National Monument, the species continues to decline at 4% per year (Carretta et al. 2007). The Mediterranean monk seal has endured centuries of exploitation, a recent disease-induced bottleneck (Harwood 1998) and smaller population sizes, yet maintains higher levels of genetic diversity, possibly a result of the preservation of alternate alleles by fragmentation of a once continuous range (Pastor et al. 2007).

The Hawaiian monk seal sets a new standard for low genetic diversity in endangered species, enhanced, but apparently not solely caused, by a bottleneck in the 19th century. Since that time, monk seals have experienced robust population growth, thereby indicating that the species is viable despite minimal genetic variation. Recently, however, the species has declined as a result of low juvenile survival attributed to food limitation, shark predation, and entanglement in marine debris (Antonelis et al. 2006). Although genetic factors may not be driving the current trend, we cannot ignore their potential impact on future population persistence because species with higher genetic diversity have experienced compromised fertility, reduced reproductive rates, high juvenile mortality, and disease epidemics. Despite these hazards, monk seals have stirred the waters of the Hawaiian archipelago for more than 10 million years; given the opportunity, they will continue this ancient relationship.

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References

Aldridge BM, Bowen L, Smith BR, Antonelis GA, Gulland R, Stott JL. 2006. Paucity of class I MHC gene heterogeneity between individuals in the endangered Hawaiian monk seal population. Immunogenetics 58:203–215.

Allen PJ, Amos W, Pomeroy PP, Twiss SD. 1995. Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Mol Ecol. 4:653–662.

Allendorf FW, Luikart G. 2007. Conservation and the genetics of populations. Malden (MA): Blackwell Publishing.

Amos W, Balmford A. 2001. When does conservation genetics matter? Heredity 87:257–265.

Amos W, Harwood J. 1998. Factors affecting levels of genetic diversity in natural populations. Philos Trans R Soc Lond B Biol Sci. 353:177–186.

Antonelis GA, Baker JD, Johanos TC, Braun RC, Harting AL. 2006. Hawaiian monk seal *Monachus schauinslandi*): status and conservation issues. Atoll Res Bull. 543:75–101.

Arnason U, Gullberg A, Janke A, Kullberg M, Lehman N, Petrov EA, Vainola R. 2006. Pinniped phylogeny and a new hypothesis for their origin and dispersal. Mol Phylogenet Evol. 41:345–354.

Baker JD, Johanos TC. 2004. Abundance of the Hawaiian monk seal in the main Hawaiian Islands. Biol Conserv. 116:103–110.

Bellwood P. 1978. The Polynesians: pre-history of an island people. Thames and Hudson: London.

Bonnell ML, Selander RK. 1974. Elephant seals: genetic variation and near extinction. Science. 184:908–909.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 32:314–331.

Brooks NC. 1860. Islands and reefs west-north-west of the Sandwich Islands, Pacific. Naut Mag. 29:499–504.

Busch JD, Waser PM, DeWoody JA. 2007. Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). Mol Ecol. 16:2450–2462.

Carretta JV, Forney KA, Lowry MS, Barlow J, Baker J, Hanson B, Muto MM. 2007. Draft U.S. Pacific marine mammal stock assessments: 2007. US Department of Commerce, NOAA Technical Memorandum

Coltman DW, Pilkington JG, Smith JA, Pemberton JM. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living island population. Evolution 53:1259–1267.

Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Davis CS, Gelatt TS, Strobeck C. 2002. Dinucleotide microsatellite markers from the Antarctic seals and their use in other Pinnipeds. Mol Ecol Notes 2:203–208.

Demere TA, Berta A, Adam PJ. 2003. Pinnipedimorph evolutionary biogeography. Bull Am Mus Nat Hist. 279:32–76.

De Muizon C. 1982. Phocid phylogeny and dispersal. Ann S Afr Mus. 89:175–213.

DiRienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer MB. 1994. Mutational process of simple-sequence repeat loci in human populations. Proc Natl Acad Sci USA. 91:3166–3170.

Eldridge MDB, King JM, Loupis AK, Spencer PBS, Taylor AC, Pope LC, Hall GP. 1999. Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conserv Biol. 13:531–541.

Ellegren H, Primmer CR, Sheldon BC. 1995. Microsatellite 'evolution': directionality or bias? Nat Genet. 11:360–362.

Fisher RA. 1930. The genetical theory of natural selection. Oxford: Oxford University Press.

Frankham R. 1995. Conservation genetics. Annu. Rev Genetics 29:305–327.

Frankham R. 2005. Genetics and extinction. Biol Conserv. 2:131–140.

Frankham R, Ballou JD, Briscoe DA. 2002. Introduction to conservation genetics. Cambridge: Cambridge University Press.

Frankham R, Lees K, Montgomery ME, England PR, Lowe H, Briscoe DA. 1999. Do population size bottlenecks reduce evolutionary potential? Anim Conserv. 2:255–260.

Fyler CA, Reeder TW, Berta A, Antonelis GA, Aguilar A, Androukaki E. 2005. Historical biogeography and phylogeny of monachine seals (Pinnipedia: Phocidae) based on mitochondrial and nuclear DNA data. J Biogeogr. 32:1267–1279.

Garner A, Rachlow JL, Hicks JF. 2005. Patterns of genetic diversity and its loss in mammalian populations. Conserv Biol. 4:1215–1221.

Garza JC. Population genetics of the northern elephant seal [PhD dissertation]. [Berkeley (CA)]: University of California; 1989.

Garza JC, Williamson EG. 2001. Detection in reduction of population size using data from microsatellite loci. Mol Ecol. 10:305–318.

Gemmell NJ, Allen PJ, Goodman SJ, Reed JZ. 1997. Interspecific microsatellite markers for the study of pinniped populations. Mol Ecol. 6:661–666.

Glenn TC, Schable NA. 2005. Isolating microsatellite DNA loci. Methods Enzymol. 295:202–222.

Gottelli D, Sillerozubiri C, Applebaum GD, Roy MS, Girman DJ, Garciamoreno J, Ostrander EA, Wayne RK. 1994. Molecular genetics of the most endangered canid—the Ethiopian wolf (*Canis simensis*). Mol Ecol. 3:301–312.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered. 86:485–486.

Hansson B, Westerberg L. 2002. On the correlation between heterozygosity and fitness in natural populations. Mol Ecol. 11:2467–2474.

Harwood J. 1998. What killed the monk seals? Nature 393:28–29.

Hawley DM, Sydenstricker KV, Kollias GV, Dhondt AA. 2005. Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. Biol Lett. 1:326–329.

Henderson JR, Johanos TC. 1988. Effects of tagging on weaned Hawaiian monk seal pups. Wildl Soc Bull. 16:312–317.

Hiruki LM, Ragen TJ. 1992. A compilation of historical (Monachus schauinslandi) monk seal counts. US Department of Commerce, NOAA Technical Memorandum.

Hoelzel AR. 1999. Impact of population bottlenecks on genetic variation and the importance of life history: a case study of the northern elephant seal. Biol J Linn Soc Lond. 68:23–39.

Hoelzel AR, Halley J, O'Brien SJ, Campagna C, Arnborm T, Le Boeuf B, Dover GA. 1993. Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. J Hered. 84:443–449.

Hoelzel AR, Stephens JC, O'Brien SJ. 1999. Molecular genetic diversity and evolution at the MHC DQB locus in four species of pinnipeds. Mol Biol Evol. 16:611–618.

Hoffmann AA, Parsons PA. 1997. Extreme environmental change and evolution. Cambridge (UK): Cambridge University Press.

James JW. 1970. The founder effect and response to artificial selection. Genet Res. 16:241–250.

Downloaded from https://academic.oup.com/jhered/article/100/1/25/772474 by guest on 16 August 2022

Kenyon KW. 1977. Caribbean monk seal extinct. J Mammal 58:97–98.

Kimura M, Crow JF. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725–738.

Kretzmann MB, Gilmartin WG, Meyer A, Zegers GP, Fain SR, Taylor BF, Costa DP. 1997. Low genetic variability in the Hawaiian monk seal. Conserv Biol. 11:482–490.

Kretzmann MB, Gemmell NJ, Meyer A. 2001. Microsatellite analysis of population structure in the endangered Hawaiian monk seal. Conserv Biol. 15:457–466.

Lacy RC. 1997. Importance of genetic variation to the viability of mammalian populations. J Mammal 78:320–335.

Lande R. 1988. Genetics and demography in biological conservation. Science. 241:1455–1460.

LeBoeuf BJ, Kenyon KW, Villa-Ramirez B. 1986. The Caribbean monk seal is extinct. Mar Mamm Sci. 2:70–72.

Menotti-Raymond M, O'Brien SJ. 1995. Evolutionary conservation of ten microsatellite loci in four species of Felidae. J Hered. 86:319–322.

Merola M. 1994. A reassessment of homozygosity and the case for inbreeding depression in the cheetah, Acinonyx jubatus: implications for conservation. Conserv Biol. 8:961–971.

Mills LS, Smouse PE. 1994. Demographic consequences of inbreeding in remnant populations. Am Nat. 144:412–431.

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

Nevo E. 1978. Genetic variation in natural populations: patterns and theory. Theor Popul Biol. 13:121–177.

O'Brien SJ. 1994. The cheetah's conservation controversy. Conserv Biol. 8:1153–1155.

O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, Meltzer D, Colly L, Evermann JF, Bush M, Wildt DE. 1985. Genetic basis for species vulnerability in the cheetah. Science 227:1428–1434.

Park SDE. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection (PhD dissertation). Dublin (Ireland): University of Dublin.

Pastor T, Garza JC, Allen P, Amos W, Aguilar A. 2004. Low genetic variability in the highly endangered Mediterranean monk seal. J Hered. 95:291–300.

Pastor T, Garza JC, Auilar A, Tounta E, Androukaki E. 2007. Genetic diversity and differentiation between the two remaining populations of the critically endangered Mediterranean monk seal. Anim Conserv. 10: 461–469.

Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered. 90:502–503.

Primmer CR, Moller AP, Ellegren H. 1996. A wide-range survey of cross-species microsatellite amplification in birds. Mol Ecol. 5:365–378.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Ragen TJ. 1999. Human activities affecting the population trends of the Hawaiian monk seal. Am Fish Soc Symp. 23:183–194.

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. Conserv Biol. 17:230–237.

Rice DW. 1960. Population dynamics of the Hawaiian monk seal. J Mammal 41:376–385.

Rozen S, Skaletsky HJ. 2000. PRIMER 3 on the www for general users and for biologist programmers. In: Krawetz S, Misener S, editors. Bioinformatics methods and protocols: methods in molecular biology. Totowa (NJ): Humana Press p. 365–386.

Ryman N, Palm S. 2006. POWSIM—a computer program for assessing statistical power when testing for genetic differentiation. Mol Ecol Notes. 6:600–602.

Spielman D, Brook BW, Frankham R. 2004. Most factors are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA. 101:15261–15264.

Stanley HF, Harwood J. 1997. Genetic differentiation among subpopulations of the highly endangered Mediterranean monk seal. In: Tew TE, Usher MB, Crawford TJ, Stevens D, Warren J, Spencer J, editors. The role of genetics in conserving small populations. Peterborough (UK): Joint Nature Conservation Committee; p. 97–101.

Taylor AC, Sherwin WB, Wayne RK. 1994. Genetic variation of microsatellite loci in a bottlenecked species—the northern hairy-nosed wombat Lasiorhinus krefftii. Mol Ecol. 3:277–290.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538.

Waldick RC, Kraus S, Brown M, White BN. 2002. Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endangered North Atlantic right whale. Mol Ecol. 11:2241–2249.

Weber DS, Stewart BS, Garza JC, Lehman N. 2000. An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. Curr Biol. 10:1287–1290.

Wooten MC, Smith MH. 1985. Large mammals are genetically less variable? Evolution 39:210–212.

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