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Range-Wide Genetic Connectivity of the Hawaiian Monk Seal and Implications for Translocation

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Abstract: *The Hawaiian monk seal (*Monachus schauinslandi*) is one of the most critically endangered marine mammals. Less than 1200 individuals remain, and the species is declining at a rate of approximately 4% per year as a result of juvenile starvation, shark predation, and entanglement in marine debris. Some of these problems may be alleviated by translocation; however, if island breeding aggregates are effectively isolated subpopulations, moving individuals may disrupt local adaptations. In these circumstances, managers must balance the pragmatic need of increasing survival with theoretical concerns about genetic viability. To assess range-wide population structure of the Hawaiian monk seal, we examined an unprecedented, near-complete genetic inventory of the species (*n = 1897 *seals, sampled over 14 years) at 18 microsatellite loci. Genetic variation was not spatially partitioned ($\hat{\theta}_w = -0.03$, $p = 1.0$), and a Bayesian clustering method provided evidence of one panmictic population (*K = 1*). Pairwise* F_{ST} *comparisons (among 7 island aggregates over 14 annual cohorts) did not reveal temporally stable, spatial reproductive isolation. Our results coupled with long-term tag-resight data confirm seal movement and gene flow throughout the Hawaiian Archipelago. Thus, human-mediated translocation of seals among locations is not likely to result in genetic incompatibilities.*

Keywords: pinniped, population connectivity, stock structure, translocation

Conectividad Genética en el Área de Distribución de Monachus schauinslandi e Implicaciones para la Translocación

Resumen: *La foca (*Monachus schauinslandi*) es una de las especies de mamíferos marinos en mayor peligro crítico. Existen menos de 1200 individuos, y la especie está declinando a una tasa de ~4% por año como resultado de la inanición juvenil, depredación por tiburones y enmarañamiento en escombro marino. Algunos de esos problemas se pueden aligerar por translocación; sin embargo, si las colonias reproductivas insulares son subpoblaciones aisladas efectivamente, el movimiento de animales puede alterar adaptaciones locales. En estas circunstancias, los manejadores deben balancear la necesidad pragmática de incrementar la supervivencia con preocupaciones teóricas sobre la viabilidad genética. Para evaluar la estructura de la población de M. schauinslandi en toda su área de distribución, examinamos un inventario genético, casi completo y sin precedentes, de la especie (*n = 1897 *focas, muestreadas a lo largo de 14 años) en 18 loci microsatélite. La variación genética no estaba subdividida espacialmente ($\hat{\theta}_w = -0.03$, $p = 1.0$), y un método de agrupamiento Bayesiano proporcionó evidencia de una población panmíctica (*K = 1*). Comparaciones pareadas* F_{ST} *(entre 7 grupos insulares en 14 cohortes anuales) no reveló aislamiento reproductivo espacial, temporalmente estable. Nuestros resultados, combinados con datos de avistamiento de marcas, confirman el movimiento de individuos y el flujo de genes en el Archipiélago Hawaiano. Por lo*

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tanto, es probable que la translocación de focas por intervención humana no resulte en incompatibilidades genéticas.

Palabras Clave: conectividad poblacional, estructura de la población, pinipedio, translocación

Introduction

During the early 19th century, thousands of Hawaiian monk seals (*Monachus schauinslandi*) were hunted for their meat, skins, and oil (Ragen 1999). From 1893 until 1913, few seals (i.e., 0–2) were observed by hunters, surveyors, or shipwrecked sailors throughout the Northwestern Hawaiian Islands (NWHI), and the species was thought to be near extinction (minimum $N = 23$; Schultz et al. 2009). The first beach-count survey conducted in 1958 indicated a partial recovery ($n_{\min} = 1182$, excluding pups; Hiruki & Ragen 1992). Since then, overall abundance has declined as a result of low juvenile survival, attributed to starvation, shark predation, and entanglement in marine debris (Antonelis et al. 2006; Baker 2008). Fifty percent reduction in the NWHI over the past 45 years and future projections of continued decline have prompted the International Union for Conservation of Nature (IUCN 2009) to categorize the species as critically endangered.

The majority of seals reside in the NWHI ($n \sim 914$), where they give birth, nurse, and rest primarily at six islands/atolls: French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll (Fig. 1). Monk seals also occur at Necker Island ($n \sim 48$) and Nihoa Island ($n \sim 86$) in the NWHI and in the main Hawaiian Islands (MHI) ($n \sim 152$). Demographic trends have been highly variable over time and among sites (Ragen & Lavigne 1999; Antonelis et al. 2006; Baker & Thompson 2007; Harting et al. 2007). Thus, even in the context of the current overall decline in abundance (approximately 4% per year), at any given time some subpopulations experience growth. For example, seal abundance in the MHI is currently increasing by approximately 7% per year (Baker et al. 2010).

This demographic variability presents a potential opportunity for conservation intervention. Translocation has been used as a management tool for Hawaiian monk seal, and its updated recovery plan (NMFS 2007) recommends enhancing survival "by translocating juvenile female seals to areas of higher survival probability." For example, 104 young female seals were translocated between 1984 and 1995 (most after some period of captive care and nutritional support [NMFS, unpublished data]). Animals were taken from French Frigate Shoals, where their survival chances were deemed poor, to small but growing subpopulations at Kure and Midway Atolls, where survival was relatively high. In 1994, 22 adult male monk seals were moved from Laysan Island to the MHI to reduce the incidence of adult female mortality by male aggression (Johanos et al. 2010). Although all but one of these males survived the translocation, it is unknown whether they sired pups in the MHI.

Translocation is often an effective management tool (Van Houtan et al. 2009); however, it could be detrimental in certain circumstances. Individuals adapted to local environments may be less fit in novel habitats; the introduction of their alleles could also dilute local adaptation in the recipient population (Johnson 2000; Tallmon et al. 2004; Edmands 2007). Because mating among highly divergent populations is generally expected to result in reduced fitness (Barton 2001; Edmands 2007), translocation should be avoided if it disrupts existing genetic subdivision and local adaptation (Moritz 1999; Storfer 1999; Robertson et al. 2007).

It is unknown whether Hawaiian monk seals are locally adapted, and previous studies of genetic stock structure are equivocal. Kretzmann et al. (1997) analyzed 359 base pairs of the mitochondrial control region and transfer ribonucleic acid (tRNA) genes of 50 seals (10 individuals each from Kure Atoll, Pearl and Hermes Reef, Lisianski Island, Laysan Island, and French Frigate Shoals) and found no evidence of spatial differentiation among subpopulations. In contrast, preliminary multilocus deoxyribonucleic acid (DNA) fingerprinting analyses (Kretzmann et al. 1997) indicated significant differences between subpopulations from Laysan Island ($n = 7$) and Lisianski Island ($n = 4$; $F_{ST} = 0.13$; $p < 0.01$) and even greater differences between Pearl and Hermes Reef ($n = 6$) and Kure Atoll ($n = 5$; $F_{ST} = 0.20$; $p < 0.01$). Analysis of a single microsatellite locus with two alleles indicated strong genetic subdivision ($R_{ST} = 0.206$; $p = 0.002$) between Kure Atoll ($n = 20$) and French Frigate Shoals ($n = 24$; Kretzmann et al. 2001). Although the latter studies provide evidence of strong reproductive isolation among NWHI subpopulations, small sample sizes and low marker variability prevent definitive conclusions. Furthermore, additional analyses are required to determine whether MHI seals comprise a separate stock (Carretta et al. 2007).

The U.S. Marine Mammal Protection Act (1972) defines a stock as a group of marine mammals in a common spatial arrangement that interbreeds when mature. Although the act does not require that distinct genetic units be managed independently (Wade & Angliss 1997 the revisions to the Guidelines for Assessment of Marine Mammals (NMFS 2005) suggest that reproductively isolated populations should be managed separately. Even small genetic differences among populations may reflect reproductive isolation if these differences are stable over time

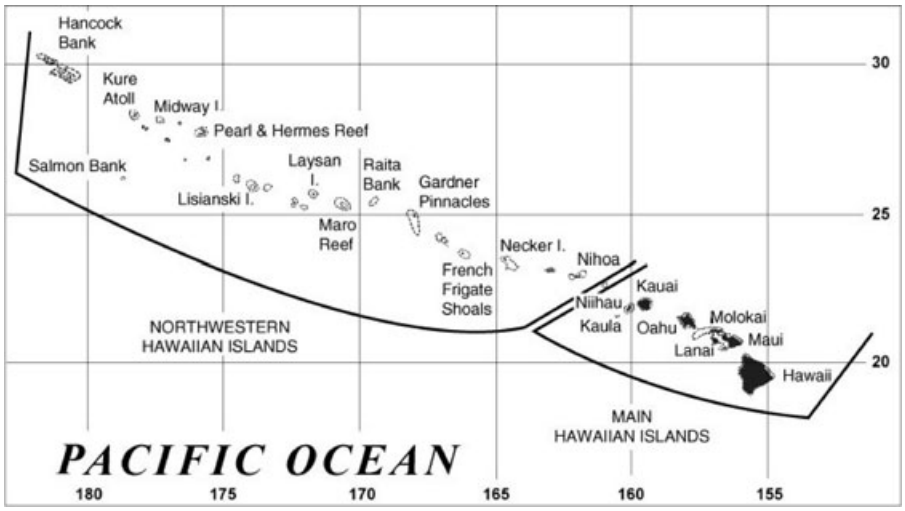

Figure 1. Range of the Hawaiian monk seal (NMFS 2007).

and not an artifact of sampling bias (Waples 1998; Balloux & Lugon-Moulin 2002). This is especially important for marine mammal stocks; precise stock delineation is required to accurately characterize and limit take of each reproductively isolated group (NMFS 2005). The Hawaiian monk seal is protected under the U.S. Endangered Species Act and the Marine Mammal Protection Act, and allowable take is currently zero, regardless of the number of stocks. Extensive monitoring and assessment allows a precise depiction of local trends. Because survival and growth rates vary spatially and temporally (Baker & Thompson 2007), management focuses on increasing overall population size. Therefore, the species likely would be managed much the same whether it was deemed to be one or several stocks, with one exception: translocation would be discouraged among multiple, reproductively isolated stocks.

In the absence of population structure ($F_{ST} = 0$), translocation is not expected to disrupt local adaptations; however, when genetic subdivision is strong (e.g., $F_{ST} > 0.15$ as reported previously in the Hawaiian monk seal), translocation may result in outbreeding depression (Johnson 2000; Edmands 2007). In considering translocation of Hawaiian monk seals, one must weigh existing threats to the species (i.e., starvation, shark predation, and entanglement in marine debris) against the theoretical risk of genetic incompatibilities. Following guidelines proposed by Wright (1978), our criteria for delineating stock structure is designed to maximize management options unless there is compelling evidence for genetic hazards. If $F_{ST} = 0$, there is no stock structure, and genetic considerations should not limit translocations, which would mimic natural movement. If $0.0 < F_{ST} \leq 0.05$, there is little genetic differentiation, and translocations should proceed as if the species is comprised of a single stock. Individual health and reproductive fitness should be monitored. If $0.05 < F_{ST} \leq 0.15$, there is moderate genetic differentiation among stocks; thus, translocation should proceed only if warranted by imminent demographic catastrophe. If $F_{ST} > 0.15$, there is great genetic differentiation among stocks, and translocation among reproductively isolated stocks should be discouraged. These criteria were designed specifically to evaluate genetic differentiation of small, well-sampled Hawaiian monk seal subpopulations through the analysis of microsatellite loci with low variability (i.e., two alleles at most loci) and may not be applicable to other systems.

To address stock structure in the Hawaiian monk seal, we sampled nearly 85% of the pups born in each of 14 annual cohorts (1994–2007), defined as all individuals born within a calendar year at MHI, French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll (Table 1). Necker Island and Nihoa Island pups are not included in our genetic analyses because we were unable to obtain adequate sample sizes from these locations. Genotyping 1897 seals at 18 microsatellite loci (Schultz et al. 2009; 2010), we evaluated overall spatial partitioning of variance ($\hat{\theta}_w$, the weighted average F_{ST} over all loci; Weir & Cockerham 1984), estimated the number of populations (K), assessed genetic subdivision of the population (pairwise exact tests and F_{ST}), and tested for isolation by distance. Finally, we considered our results in the context of long-term movement data to determine whether genetic or demographic isolation should prevent future translocations of Hawaiian monk seals.

Methods

Samples

We collected tissue plugs ($n = 1897$), a byproduct of applying flipper tags, from weaned pups during annual population assessments in the NWHI (1994–2007) and opportunistically in the MHI since 1999 (Table 1). Specimens

Table 1. Hawaiian monk seal annual pup cohorts (number of tissue samples/number of pups) at Kure Atoll (Kur), Midway Atoll (Mid), Pearl and Hermes Reef (PHR), Lisianski Island (Lis), Laysan Island (Lay), French Frigate Shoals (FFS), and the main Hawaiian Islands (MHI).

Cohort	*All*	*Kur*	*Mid*	*PHR*	*Lis*	*Lay*	*FFS*	*MHI*
1994	102/181	0/6*	0/4*	0/0	0/12*	23/49	79/110	0/0
1995	133/185	11/11	4/9	25/27	15/22	36/43	42/73	0/0
1996	111/214	16/17	1/7	4/25	14/24	27/47	49/95	0/0
1997	124/211	12/18	9/11	21/27	19/22	35/47	28/97	0//0
1998	194/248	24/24	12/12	27/32	14/25	44/46	72/109	1/1
1999	192/245	19/21	13/13	26/28	31/33	56/58	46/92	1/3
2000	97/198	13/16	14/14	20/31	9/20	35/43	2/67*	4/7
2001	129/187	16/18	11/12	0/32*	14/17	35/36	46/60	7/12
2002	160/198	18/19	0/13*	19/29	23/25	37/37	59/71	4/4
2003	160/191	18/18	14/15	19/30	28/28	28/33	44/56	9/11
2004	134/218	18/20	13/17	12/34	28/29	22/36	37/71	4/11
2005	84/173	10/23	8/10	1/27*	9/26	22/25	31/52	3/10
2006	145/165	18/21	12/15	12/19	28/28	40/43	23/39	12/12
2007	132/160	19/19	0/12*	14/16	18/21	40/40	31/43	10/10

*Sampling <10% census size of pup cohort.

were preserved in saturated salt (NaCl) solution, 95% ethanol, or liquid nitrogen. We organized specimens by cohort (i.e., calendar year) on the basis of observed birth or nursing of pups and by island of birth (MHI, French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll).

Laboratory Analyses

We extracted genomic DNA from all samples following protocols of the DNeasy Tissue Kit (Qiagen, Valencia, California). We performed amplifications at 17 microsatellite loci isolated from the Hawaiian monk seal genome (Schultz et al. 2009; 2010) and one locus isolated from the grey seal (*Halichoerus grypus*)(Allen et al. 1995) in two multiplex PCR reactions as follows: 1x PCR mix (Qiagen), 2 μM primer mix, and 30–50 ng genomic DNA. The PCR protocol consisted of a 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). A final extension (72 °C, 30 min) was added to ensure the addition of a terminal adenine. The Core Sequencing Facility at the Hawaii Institute of Marine Biology ran amplified products on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). We scored all runs with GeneMapper 4.0 (Applied Biosystems).

Quality Control

We screened our microsatellite results following the quality-control recommendations of Selkoe and Toonen (2006). We scored all genotypes ($n = 1897$) and applied the bin mode in GeneMapper to aid in allele calling. At least 50% of all genotypes were scored two or more times to ensure accuracy. Error was estimated by duplicating analyses (from extraction to scoring) for 78 randomly chosen individuals. Mendelian inheritance of alleles was confirmed by assessing 20 known mother–pup pairs. Within cohorts, we tested each locus for null alleles and large allele dropout with 1000 iterations in MicroChecker 2.2.3 (Van Oosterhout et al. 2004). We performed locus-by-locus tests for Hardy–Weinberg equilibrium (100000 Markov chain steps, 1000 dememorization steps) in Arlequin 3.1 (Excoffier et al. 2005) and assessed genotypic disequilibrium by performing pairwise analyses of all loci for 3060 permutations. Finally, we evaluated the statistical power of these loci to detect genetic differentiation in PowSim 4.0 (Ryman et al. 2006).

Data Analyses

We evaluated spatial and temporal partitioning of variance by performing an analysis of molecular variance (AMOVA) among groups (i.e., annual cohorts; F_{CT}), among subpopulations within groups (i.e., islands; Weir and Cockerham's $\hat{\theta}_w$) and among individuals with 16,000 permutations in Arlequin. For each cohort, we also performed pairwise comparisons of F_{ST} and an exact test of population differentiation among all islands. We ran 16,000 permutations of the data set to test for significance at $\alpha = 0.05$ and corrected for multiple tests (F_{ST} pairwise tests only) by applying a false-discovery-rate method, which is less conservative than Bonferroni correction (Narum 2006). To test for isolation by distance, we calculated corrected average pairwise differences in Arlequin and used geographic distances among all locations to perform a Mantel test.

For each cohort, we used a Bayesian clustering method to estimate the number of genetically distinguishable clusters (K) in Structure 2.3.1, which groups individuals to minimize departures from Hardy–Weinberg and linkage equilibrium (Pritchard et al. 2000). We compared maximum likelihood estimates for $K = 1$–7, where $K = 1$ is the null hypothesis of no structure and $K = 7$ is the total number of breeding aggregates. We used an

admixture model with correlated allele frequencies and prior information regarding the sampling location (birth island) of the individual (Hubisz et al. 2009). We ran five replicates of 1,000,000 MCMC iterations after of burn-in of 100,000. We interpreted the highest mean log likelihood as evidence of the most probable number of subpopulations.

Because a lack of population structure may reflect contemporary migration or very recent divergence, we attempted to determine the relative contribution of each in IMa (Nielsen & Wakely 2001; Hey & Nielsen 2007). We also attempted to estimate contemporary migration rates in BayesAss1.3 (Wilson & Rannala 2003), combining all cohorts into a single data set ($N = 1897$) to provide greater resolution in an analysis that requires a large, but not necessarily unrelated, sample. We set the parameters such that the delta values for allele frequency, migration rate, and level of inbreeding were 0.15. To assess convergence, we compared three independent runs of 120 million simulations with a 40 million burn-in. We compared our results with those of a noninformative data set with seven populations to determine whether our data differed from random expectations.

We summarized the proportion of seals born and tagged at one location that were subsequently observed at another island or atoll to obtain minimum actual movement rates, rather than estimated migration rates. Using the methods of Baker and Thompson (2007), we analyzed tag-resight data on 4151 seals, collected from 1995 to 2008. Movements of seals first tagged at any age, other than recently weaned pups, were omitted from the analyses due to uncertainty in identifying their natal site. Observational effort varied greatly among locations and over time, with typical field seasons ranging from 2 to 5 months. The greatest effort occurred at French Frigate Shoals, Laysan Island, and Lisianski Island. Field seasons were typically shorter at Pearl and Hermes Reef and Kure Atoll, and effort at these locations was highly variable, ranging from a few weeks to year-round at Midway Atoll. Resightings in the MHI occurred year-round, but in contrast to the intensive surveys conducted in the NWHI, MHI sightings were typically opportunistic and reported by volunteers, National Marine Fisheries Service staff, agency partners, or the general public (Baker et al. 2010). Because movement of seals among the MHI is regularly observed and the sample sizes are small, all MHI were treated as a single location. We summarized observed interisland movement as the proportion of seals tagged at their natal location that were observed at other locations, as enumerated over the lifetime of each seal. Multiple movements of a single seal to the same location were only counted once; however, movements of a single seal to multiple locations were each included in the results. Human-mediated movements (translocations or captive care followed by release at a non-natal site) were not included in the analyses.

Results

We found a single discrepancy (a miscalled allele) in our quality assurance assessment of 780 alleles (0.1%/allele error rate). All known mother–pup pairs shared at least one allele, which is consistent with Mendelian inheritance of all loci. We found no evidence for scoring error due to stuttering, large allele dropout, or null alleles. Thirteen of 252 tests (18 loci over 14 cohorts) deviated from Hardy–Weinberg equilibrium (roughly 5% as expected by chance alone), but none remained significant after false-discovery-rate correction ($p = 0.01$; e.g., Table 2). No loci were in linkage disequilibrium across all cohorts or after correcting for multiple tests. Furthermore, none of the polymorphic loci were located on the same chromosome when mapped to the dog genome (Schultz et al. 2010). The power analysis indicated 100% probability of detecting differentiation if $F_{ST} \geq 0.01$ and 98% probability if $F_{ST} \geq 0.007$.

Our AMOVA results revealed that temporal (among cohorts) and spatial (among subpopulations within cohorts) partitions did not explain any of the genetic variance, which was distributed among individuals independent of cohort and location (100%). We found no genetic differentiation among cohorts ($F_{CT} = -0.02, p = 1.0$) or among subpopulations within cohorts ($\hat{\theta}_w = -0.03$; $p = 1.0$). Pairwise exact tests among subpopulations within cohorts were not significant (data not shown). Of 228 pairwise F_{ST} comparisons, only 16 were significantly greater than zero after a false-discovery-rate correction, and none were significant over more than three of the 14 cohorts (Table 3). Furthermore, none exceeded our defined threshold ($F_{ST} > 0.05$) for delineating stocks, and there was no evidence for isolation by distance ($R^2 = 0.0001$; $p = 0.88$).

Analyzing each cohort in Structure with informative priors and an admixture model, we found the highest mean log likelihood for $K = 1$ in all cohorts (i.e., $K > 1$ always resulted in lower likelihood values for all five replicates). Plotting estimates of Q (membership coefficients for each individual) for $K > 1$, the proportion of individuals assigned to each cluster was roughly symmetrical (Fig. 2), further indicating lack of population structure (Pritchard et al. 2009).

We were unable to achieve convergence in our IMa analyses. Similarly, we had little confidence in our BayesAss results because convergence was reached only after 120 million iterations and the signal was weak, with 95% confidence intervals overlapping with confidence intervals of an uninformative data set (data not shown). Thus, we were unable to estimate effective migration rates, as is expected in poorly differentiated systems ($F_{ST} < 0.05$; Faubet et al. 2007).

The proportion of seals observed at a non-natal location ranged from 4% (seals born at French Frigate Shoals) to 18% (seals born at Kure Atoll) (Table 4). Several of

Table 2. Diversity indices of 18 microsatellite loci from the 2007 Hawaiian monk seal cohort.*

Locus	*Genbank number*	A	k	*Size*	H_O/H_E (p)	F_{IS} (p)	F_{ST} (p)	F_{IT} (p)
Msc01	GU206362	3	3.0	161–167	0.41/0.49 (0.06)	0.16 (0.02)	0.02 (0.26)	0.18 (0.008)
Msc03	GU206363	5	5.0	126–136	0.68/0.68 (0.21)	−0.01 (0.63)	−0.008 (0.66)	−0.005 (0.60)
Msc04	GU206364	4	3.3	159–167	0.51/0.50 (0.34)	−0.03 (0.70)	−0.14 (0.35)	−0.02 (0.64)
Msc05	GU206365	2	2.0	198–206	0.25/0.26 (0.52)	0.03 (0.49)	0.03 (0.13)	0.06 (0.38)
Msc09	GU206366	3	3.0	208–216	0.33/0.31 (0.87)	−0.05 (0.84)	−0.003 (0.78)	−0.05 (0.83)
Msc10	GU206367	2	2.0	141–149	0.52/0.49 (0.72)	−0.04 (0.73)	−0.009 (0.96)	−0.05 (0.76)
Msc13	GU206368	3	3.0	194–202	0.38/0.35 (0.72)	−0.10 (0.96)	0.01 (0.23)	−0.08 (0.94)
Msc17	GU206370	3	2.6	115–119	0.45/0.48 (0.36)	0.05 (0.31)	0.01 (0.52)	0.06 (0.27)
Msc19	GU206371	4	3.2	118–138	0.21/0.21 (0.49)	−0.02 (0.65)	0.00 (0.88)	−0.02 (0.67)
Msc23	GU206372	3	3.0	160–168	0.41/0.49 (0.03)	0.17 (0.01)	−0.002 (0.93)	0.16 (0.01)
Ms9	EU913766	5	5.0	297–317	0.80/0.65 (0.14)	−0.24 (0.99)	0.00 (0.93)	−0.24 (0.99)
Ms15	EU913767	3	3.0	203–315	0.57/0.55 (0.96)	−0.07 (0.85)	0.04 (0.08)	−0.03 (0.74)
Ms23	EU913768	6	6.0	340–370	0.81/0.75 (0.75)	0.009 (0.65)	−0.009 (0.81)	0.00 (0.68)
Ms265	EU913769	2	2.0	158, 162	0.47/0.47 (1.00)	0.008 (0.56)	−0.008 (0.94)	0.00 (0.57)
Ms504	EU913763	2	2.0	308, 326	0.24/0.25 (1.00)	−0.05 (0.77)	−0.03 (1.00)	−0.09 (0.84)
Ms647	EU913765	2	2.0	115, 117	0.43/0.42 (1.00)	−0.01 (0.65)	−0.01 (0.99)	−0.03 (0.68)
Ms663	EU913764	2	2.0	290, 294	0.31/0.29 (0.73)	−0.08 (0.87)	0.02 (0.38)	−0.05 (0.83)
Hg6.3	G02092	2	2.0	227, 237	0.35/0.35 (1.00)	−0.01 (0.64)	0.00 (0.78)	−0.009 (0.64)

*Key: A, number of alleles; k, allelic diversity, H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, fixation index of individual relative to subpopulation; F_{ST}, fixation index of subpopulation relative to total population; F_{IT}, fixation index of individual relative to total population.

these seals were observed at more than one non-natal location, especially seals from Pearl and Hermes Reef. Overall, movement was greatest among neighboring islands, especially among the three western atolls, which are geographically clustered relative to the other NWHI sites. These movement values are not estimates; rather, they are observations of actual movements. Thus they represent minimum movement rates. Furthermore, although immature seals were included in these observations, adult movement rates were as high or higher than juvenile movement rates.

Discussion

To assess stock structure in the Hawaiian monk seal, we analyzed approximately 85% of pups born between 1994 and 2007 at 18 microsatellite loci. We did not find spatial ($\hat{\theta}_w = -0.03$) or temporal ($F_{CT} = -0.02$) partitioning of genetic variation. Instead, all variation occurred among individuals independent of cohort and location, and there was no evidence for isolation by distance ($R^2 = 0.0001$, $p = 0.88$). A Bayesian clustering method indicated all seals comprise a single population (Fig. 2). Within each cohort, pairwise exact tests of differentiation were not significant ($p < 0.05$). Only 16 of 228 pairwise F_{ST} comparisons were significant, and none were greater than our threshold criteria for stock delineation ($F_{ST} > 0.05$; Table 3). Furthermore, long-term mark-resight data revealed frequent interisland movement. Our results do not warrant altering current management of the species as a single stock. Given the lack of genetic subdivision and extent of natural movement, translocation of Hawaiian monk seals anywhere within their range is not likely to result in genetic complications, such as local adaptation or outbreeding depression.

The Hawaiian monk seal has extremely low genetic diversity (Gemmell et al. 1997; Kretzmann et al. 1997; Aldridge et al. 2006; Schultz et al. 2009; 2010). With such little genetic variability, it may be argued that there

Table 3. Pairwise comparisons of genetic structure for pup cohorts (by year) among Kure Atoll (Kur), Midway Atoll (Mid), Pearl and Hermes Reef (PHR), Lisianski Island (Lis), Laysan Island (Lay), French Frigate Shoals (FFS), and the main Hawaiian Islands (MHI).*

	Kur	*Mid*	*PHR*	*Lis*	*Lay*	*FFS*	*MHI*
Kur	–			99, 04	98, 04	02	
Mid		–			99, 04		
PHR			–	98, 06	98, 04, 06	95, 97	
Lis	0.02, 0.02		0.03, 0.04	–			07
Lay	0.02, 0.03	0.04, 0.05	0.01, 0.04, 0.03		–		07
FFS	0.03		0.02, 0.02			–	
MHI				0.05	0.04		–

**Above diagonal, annual cohort years (1994 –2007) in which pairwise F_{ST} estimates are significant at $\alpha < 0.01$ after modification for false discovery rate; below diagonal, pairwise F_{ST} values for those years.*

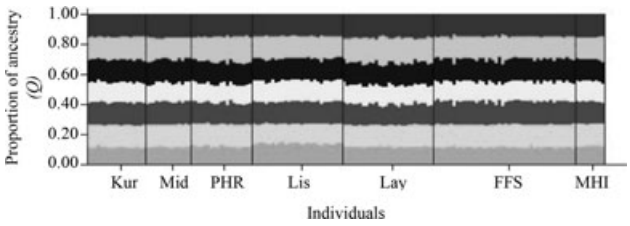

Figure 2. Baysian clustering of individual Hawaiian monk seal genotypes. Seals are represented across the x-axis by vertical bars divided into shaded segments that represent the proportion of ancestry (Q) from Kure Atoll (Kur), Midway Atoll (Mid), Pearl and Hermes Reef (PHR), Lisianski Island (Lis), Laysan Island (Lay), French Frigate Shoals (FFS), and the main Hawaiian Islands (MHI).

is insufficient power to detect differences among subpopulations. To test this hypothesis, we performed a power simulation of our 18 loci. We found 100% probability of detecting differentiation if the true value of genetic subdivision was $F_{ST} \geq 0.01$ or higher and 98% if $F_{ST} \geq 0.007$. Furthermore, our long-term systematic sampling of 14 annual cohorts allowed us to evaluate the temporal stability of small genetic differences (Waples 1998; Johnson 2000; Balloux & Lugon-Moulin 2002). Unlike most studies of natural populations, we sampled the majority of pups born in each cohort and 85% of the entire species. Therefore, small ephemeral genetic differences among subpopulations are likely due to random fluctuations in allele frequencies from year to year rather than anthropogenic sampling bias. For example, a single male could sire multilple offspring in 1 year and none the following year. Similarly, random sampling during meiosis or at the gametic level could lead to temporal variance among allele frequencies. Finally, our methods were comparable to other studies of pinniped genetic differentiation in which only pups were sampled or known relatives were excluded (e.g., Hoffman et al. 2006; Coltman et al. 2007; González-Suárez et al. 2009). Several seal species lack spatial genetic differentiation, including hooded (*Cystophora cristata*), ringed (*Pusa hispida*), leopard (*Hydrurga leptonyx*), Ross (*Ommatophoca rossii*), and crabeater (*Lobodon carcinophagus*) seals (Coltman et al. 2007; Davis et al. 2008), and given our results, the Hawaiian monk seal as well.

Palsboll et al. (2007) suggest interpreting genetic differentiation in a demographic context to address contemporary cohesion. For example, groups connected by migration rates of 10% or higher could be considered to be a single stock (Hastings 1993; Waples & Gaggiotti 2006; Palsboll et al. 2007). Although we were unable to genetically estimate migration in the Hawaiian monk seal, mark-resight data indicate high levels of exchange among the NWHI breeding aggregates (i.e., 10–15% of seals are observed at a location other than their natal island) (Johnson & Kridler 1983; Harting 2002; Carretta et al. 2007). Seals primarily move among neighboring islands (Table 4), especially among islands in close proximity (e.g., Kure Atoll, Midway Atoll, and Pearl and Hermes Reef). Seals from French Frigate Shoals exhibited the lowest movement rate within the NWHI, probably due to geographic isolation. Nevertheless, 36 French Frigate Shoals seals were observed at adjacent Necker Island (data not shown), despite the limited, sporadic effort expended at that site. Seals were seen infrequently in the MHI until the mid-1990s, and although the population is growing, the number of tagged animals is small (Baker & Johanos 2004; Baker et al. 2010). While there are few records of seals tagged at birth eventually moving between the NWHI and the MHI, additional movements of seals whose natal sites were uncertain have been documented (T.C. Johanos, personal communication), indicating that our methods may underestimate the actual rate of movement to and from the MHI.

The movement rates are based on resightings of tagged seals. Actual movement rates are likely to be much higher, especially for those sites with small sample sizes and limited observational effort. Even at those sites that are

Table 4. Observed minimum movement rates of Hawaiian monk seals among Kure Atoll (Kur), Midway Atoll (Mid), Pearl and Hermes Reef (PHR), Lisianski Island (Lis), Laysan Island (Lay), French Frigate Shoals (FFS), and the main Hawaiian Islands (MHI).[a]

Source	*Kur*	*Mid*	*PHR*	*Lis*	*Lay*	*FFS*	*MHI*	n[b]
Kur	0.82	0.15	0.03	0.01	0.00	0.00	0.00	346
Mid	0.10	0.89	0.01	0.00	0.01	0.00	0.01	154
PHR	0.06	0.09	0.86	0.03	0.02	0.00	0.00	504
Lis	0.00	0.00	0.01	0.91	0.07	0.01	0.00	500
Lay	0.00	0.00	0.01	0.06	0.92	0.01	0.00	876
FFS	0.00	0.00	0.00	0.00	0.01	0.96	0.01	1676
MHI	0.00	0.00	0.00	0.00	0.00	0.00	1.00	95

[a] *Values represent the proportion of seals tagged at their natal site that were observed at least once at each of the other sites in 1995–2008. Values along the diagonal represent the proportion of seals never observed at another location, including young that died before or after weaning. The total of values in a single row may exceed 1.0 because some seals were observed at multiple locations throughout their lifetime. Values < 0.005 are recorded as 0.00; values > 0.995 are recorded as 1.00.*

[b] *Total sample size of tagged pups at each site.*

surveyed regularly, certainly some movements go unobserved, especially in the NWHI, where there is no observational effort for most of the year. Also, sighting data are collected almost exclusively on land, whereas seals spend most of their time at sea. Despite these caveats, the available data from tagging efforts demonstrate that seals appear to be moving freely among the islands, and our genetic data confirm interisland mating (i.e., gene flow).

Complementing our movement and genetic data are cases in which colonies of monk seals were extirpated and reestablished through immigration. For example, at least 56 Hawaiian monk seals inhabited Midway Atoll in 1958. By 1968 they had all but disappeared, likely due to human disturbance (Kenyon 1972). Since then, human activity has been greatly reduced, and seals have emigrated from other locations. By 2000 there were 71 seals at Midway Atoll (National Marine Fisheries Service, unpublished data). Earlier in the century, Midway Atoll and the Laysan Island colonies were extirpated and subsequently recolonized (Ragen 1999). Given the level of connectivity demonstrated by the tagging and genetic data and historical recolonizations, we believe the species is properly managed as a single stock. Given our results, genetic factors should not preclude translocation as a means to reverse the current population trend.

Managers of other critically endangered species may not have the luxury of near-exhaustive sampling or temporal replicates. How should they interpret weak, but statistically significant, genetic differentiation derived from a single sample consisting of a small fraction of individuals in the putative populations? Separate management plans for each putative subpopulation is generally regarded as the conservative approach. When dealing with several small, but poorly differentiated subpopulations that are in equal need of protection, independent management may limit options for promoting recovery. If treating such species as a single, large stock allows for use of a broader range of conservation tools, including translocation, perhaps that should be considered the more conservative approach.

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