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# MICROSATELLITE DNA AND MITOCHONDRIAL DNA VARIATION IN REMNANT AND TRANSLOCATED SEA OTTER (*ENHYDRA LUTRIS*) POPULATIONS

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All existing sea otter (*Enhydra lutris*) populations have suffered at least 1, and in some cases 2, population bottlenecks. The 1st occurred during the 18th and 19th centuries as a result of commercial hunting that eliminated sea otters from much their native range and reduced surviving populations to small remnants. The 2nd bottleneck occurred when small numbers of otters were reintroduced, via translocation, to areas where the species had been eliminated. We examined genetic variation at 7 microsatellite loci and the mitochondrial DNA (mtDNA) control region in 3 remnant populations, Amchitka Island (Aleutian Islands, Alaska), central coastal California, and Prince William Sound (Alaska), and in 2 reintroduced populations, southeast Alaska and Washington, that were founded with transplants from Amchitka, and in the case of southeast Alaska, individuals from Prince William Sound as well. We found no evidence of reduced genetic diversity in translocated populations. Average expected microsatellite heterozygosities ($H_E$) were similar in all populations (range, 0.40–0.47), and mtDNA haplotype diversities were higher in reintroduced populations (0.51 for both Washington and southeast Alaska) than in remnant populations ($X$ = 0.35; range, 0.18–0.45). The levels of genetic diversity we observed within sea otter populations were relatively low when compared with other mammals and are thought to be the result of fur trade exploitation.

Key words: *Enhydra lutris*, genetic diversity, marine mammal, microsatellites, mitochondrial DNA, population bottleneck, reintroduction, sea otter, translocation

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Key goals of many wildlife conservation programs are to maintain biodiversity at the ecosystem level and genetic variation at the species level (Frankham 1995; Lynch 1996). One approach to conserving biodiversity is to reintroduce populations into previously occupied habitats. This approach risks loss of genetic variation if founding population sizes are small, as is often the case in reintroductions (O'Brien et al. 1996; Wolf et al. 1996). A potential effect of reduced genetic variation is inbreeding depression that could result in slower growth, and reduced fecundity and survival (Ballou and Ralls 1982; O'Brien et al. 1996; Ralls et al. 1979, 1988). The ability of translocated populations to recover from lost genetic diversity as a result of population bottlenecks has often been questioned, but there are relatively few empirical examples from natural populations (Bodkin et al. 1999; Caro and Laurenson 1994; Wayne

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1996). Consequently, translocation efforts should include monitoring of genetic variation in source and introduced populations because this information may be critical for long-term management of introduced populations and as a strategy for enhancing species survival.

The sea otter (*Enhydra lutris*) has a history of extirpation, population fragmentation, and subsequent recolonization or reintroduction into vacant habitats. Sea otters once ranged throughout coastal regions of the north Pacific rim from the islands of northern Japan to central Baja California, Mexico (Kenyon 1969). They were hunted to near extinction throughout much of their original range during the fur trade of the 18th and 19th centuries, then given protection in 1911 under the International Fur Seal Treaty (Kenyon 1969). Remnant populations persisted in California, south-central Alaska, the Aleutian, Commander and Kuril Islands, and the Kamchatka Peninsula. Sizes of these remnant populations immediately after protection are unknown and likely varied among sites, but they may have ranged from a few tens to hundreds of individuals (Bodkin et al. 1999, 2000; Scribner et al. 1997). By the late 1970s, several otter populations had recovered to preexploitation levels, with the total species numbering approximately 100,000 in 1994 (J. Bodkin et al., in litt.). However, throughout much of their historic range, including southeastern Alaska, the west coast of Canada, Washington, Oregon, and most of California, sea otters remained below preexploitation levels or were absent (Kenyon 1969). In the late 1960s and early 1970s several reintroductions from 1 or 2 remnant populations were made in an effort to reestablish sea otters in areas where they were extinct (Jameson et al. 1982).

Knowledge of the influence of population bottlenecks caused by the fur harvest on genetic variation in remnant sea otter populations is limited, although some post-bottleneck survey data are available for estimating population growth rates (Bodkin et al. 1999). Assumed minimum population sizes of the remnant sea otter populations ranged from 10 to 40 and estimated bottleneck durations ranged from 8 to 44 years (Bodkin et al. 1999). In contrast, the number of animals moved during translocations is known exactly, although there is uncertainty about initial survival rates, and sizes of founding populations can also be back-calculated from posttranslocation surveys (Bodkin et al. 1999; Estes et al. 1998). The southeast Alaska population was established from 1965 to 1969 with 412 sea otters moved from both Amchitka Island in the Aleutians (369 animals) and Prince William Sound (43 animals—Jameson et al. 1982; R. J. Jameson, in litt.). Estimates based on high mortality observed during the translocations indicate about 150 animals formed the founding group for this population (Estes 1990; Riedman and Estes 1990). Another successful reintroduction effort established a population off the northwest coast of Washington. This population descended from 59 individuals captured in 1969 and 1970 from Amchitka Island in the Aleutian Islands (Jameson et al. 1982). Estimates of founding population sizes, using population growth rates and census data, range from as low as 4 to 10 individuals (Bodkin et al. 1999; Estes et al. 1998).

Given this history, it is evident that all extant sea otter populations incurred population bottlenecks of varying severity and duration. The impact of these bottlenecks on genetic variation within surviving sea otter populations remains unclear and will remain so until archival samples predating the impact of the fur trade become available for analysis. However, it is possible to compare levels of genetic variation in translocated (and therefore potentially twice bottlenecked) populations to remnant populations, including those used to found the translocated populations, and therefore to determine whether translocated populations harbor less genetic variation than the remnant and source populations. Such a comparison should ideally be conducted using both nuclear genetic markers and mitochon-

drial DNA (mtDNA), because the former provide the most general measure of genome-wide patterns of genetic variability, whereas the latter provides a particularly sensitive indicator of bottlenecks involving maternal lineages (Avise 1994).

Previous genetic studies of sea otters have revealed low-to-moderate levels of genetic variation within and among populations but have not specifically compared translocated populations with their source populations using microsatellites or other highly polymorphic nuclear markers (Scribner et al. 1997). Bodkin et al. 1999 compared remnant and translocated sea otter populations using restriction-fragment-length polymorphisms of mtDNA and found that mean haplotype diversities were 0.34 in remnant groups and 0.60 in translocated groups, although the differences between the 2 were not significant ($P = 0.12$). Furthermore, the low levels of variability revealed by allozymes, the only single-locus nuclear markers studied to date (W. Z. Lidicker and F. C. McCollum, in litt.; Rotterman 1992; Scribner et al. 1997), limit their potential utility in this context.

Our primary goal in this study was to contrast genetic variation in translocated sea otter populations and their source populations using sequence variation in microsatellites and mtDNA control regions. To this end, we compared 3 remnant sea otter populations from Amchitka, Prince William Sound, and California, and 2 translocated populations from southeast Alaska (founded with individuals from Amchitka Island, 1 of the Rat Islands within the Aleutian Islands, Alaska and Prince William Sound along the west coast of central Alaska east of Kodiak Island) and Washington, derived exclusively from Amchitka. Because highly polymorphic single-locus nuclear DNA markers such as microsatellites have not previously been described for sea otters, we 1st identified a suite of 7 microsatellite markers suitable for assay of genetic variation in sea otters. We then measured genetic variation within and differentiation among the populations using these 7 microsatellite markers as well as mtDNA control region sequences.

## Materials and Methods

In the absence of published microsatellite primers for sea otters, primers developed for mink (*Mustela vison*—Fleming et al. 1999; O'Connell et al. 1996), ermine (*Mustela erminea*—Fleming et al. 1999), and European otters (*Lutra lutra*—Dallas and Piertney 1998) were tested on sea otter nuclear DNA. Of 20 primer sets tested, polymerase chain reaction (PCR) conditions were optimized for 6 loci, and an easily scored microsatellite product was produced for each (Appendix I). In addition, a sea otter genomic library was generated and screened for sea otter–specific microsatellite markers following methods described in Olsen et al. (1998). Primers were developed for 8 novel sea otter microsatellites, but only 3 yielded a viable PCR product, and only 1 of these was variable (locus An 5) and thus included in the analysis.

Samples were taken from Amchitka Island, within the Rat Island group of the Aleutian Island chain in western Alaska (51°25′N, 179°18′E), Prince William Sound off the central west coast of Alaska, east of Kodiak Island (near 61°7′N, 146°20′W), southeast Alaska, the southern coast of Alaska (from approximately 59°14′N, 135°26′W to 55°20′N, 131°38′W), the northwest coast of Washington (near 48°22′N, 124°37′W), and the central coast of California from Monterey Bay (36°36′N, 121°53′W) to Morro Bay (35°21′N, 120°50′W).

Microsatellite genotypes were collected at the following 7 loci: An5 (F: 5′-CTTCTCCCTTTC-CAACTCCC-3′ and R: 5′-TTAACCCACTGA-GCCACACA-3′, present study); Mvi 24, Mvi 57, and Mvi 87 (mink—O'Connell et al. 1996); Mvis 72 and Mvis 75 (mink—Fleming et al. 1999); and Lut 453 (European otter—Dallas and Piertney 1998). A 320-bp stretch of the mtDNA control-region D loop was amplified using the universal primers L15926 (5′-GAATTCCCCGGTCTTGT-AAACC-3′) and H16340 (5′-CCTGAAGTA-GGAACCAGAATG-3′—Cheney 1995).

Pieces of tissue approximately 5 by 5 mm were used for extraction of DNA. These were taken from the hole created during application of rear-flipper tags for individual identification and whole blood samples taken from sea otters during tagging operations. These flipper plugs

were preserved frozen or in 100% ethanol, whereas whole blood was preserved in ethylenediamine-tetraacetic acid (EDTA), and samples were stored at −20 or −40°C before DNA isolation. DNA was extracted from the 5-mm hind-flipper tissue using a standard phenol–chloroform method (Hoelzel and Green 1992), resuspended in 100 μl of Tris–EDTA buffer, and then stored at −20 or −70°C for ≤1 year. DNA from whole blood was extracted using the QIAamp Blood and Tissue Kit (Qiagen, Valencia, California).

Microsatellites and the control region of mtDNA were amplified using a GeneAmp PCR 9600 thermal-cycler (Perkin–Elmer, Wellesley, Massachusetts) in 10 μl containing 100–250 ng purified DNA template, 0.5 μM forward and reverse primer, 0.3 U *Taq* DNA polymerase (Promega, Madison, Wisconsin), 10 mM Tris–HCL (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$, and 0.2 mM each dNTP and $dH_2O$ to make up final volume. The amplification profile was as follows: 1 cycle of 94°C (240 s), 25–35 cycles of 94°C (30 s) + X°C (30 s) + 72°C (30 s), and 1 cycle of 72°C (300 s), where X is the primer-specific annealing temperature (Appendix I). PCR products were stored at 4 or −20°C until analysis for fragment length.

PCR products were analyzed on a 373A-XL Stretch autosequencer–genescanner (Applied BioSystems Inc., ABI, Foster City, California) as described (Olsen et al. 1996) or on an ABI 310 single-capillary system in Genescan mode (Applied BioSystems Inc. 1993). Each run contained at least 1 reference sample as well as several repeated samples to ensure accuracy. Allele scoring for each locus was performed using Genotyper Software, version 2.0 (Applied BioSystems Inc. 1996). Microsatellite sequences and mtDNA sequences were determined using an ABI 373A Stretch and an ABI 377 automated sequencer using ABI prism *Taq* DyeDeoxy® terminator chemistry (ABI). Mitochondrial DNA haplotypes were confirmed by sequence differences in both directions.

The presence of closely related individuals (e.g., mother and offspring or full siblings) within population samples could bias estimates of population genetic parameters. In an effort to eliminate this bias, the program Kinship 1.2 (K. F. Goodnight, in litt.) was used to identify pairs of individuals within population samples that were likely to be closely related. This entailed using microsatellite genotypes to calculate relatedness coefficients ($r$) and simulations to evaluate the likelihood of given values of $r$ occurring in unrelated individuals. As a conservative measure, 1 individual from each pair for which the probability of the null hypothesis (no 1st-order relationship) was less than 5% ($P < 0.05$) was removed from that population before other analyses were performed. This procedure led to the identification of 23 related pairs and subsequent removal of 4 individuals from Amchitka, 10 from California, 1 from southeast Alaska, 2 from Prince William Sound, and 6 from Washington. The following are the final sample sizes included in the statistical analysis: 36 from Amchitka, 54 from California, 24 from southeast Alaska, 33 from Prince William Sound, and 27 from Washington, resulting in a total of 174 otters.

Tests for departures from Hardy–Weinberg equilibrium, genotypic linkage disequilibrium and independence between populations, and allele frequency were performed for all populations using the probability test in GENEPOP 3.1 software (Raymond and Rousset 1995). A Markov chain method was used to provide an unbiased estimate of the exact $P$-value (Guo and Thompson 1992), except for tests of Hardy–Weinberg equilibrium at loci with fewer than 5 alleles. $F$ statistics ($F_{ST}$ and $F_{IS}$) were calculated according to Weir and Cockerham (1984) using GENEPOP software, and the significance of $F_{ST}$ estimates was determined using a permutation test implemented in the GENETIX 4.0 package (K. Belkhir et al., in litt.). Sequential Bonferroni adjustments were used to determine significance levels for all simultaneous tests (Rice 1989). Results are presented as mean ± 1 *SE*.

Population dendrograms were constructed separately for the microsatellite and mtDNA data using the CONTML program in the PHYLIP software package (version 3.5c—J. Felsenstein, in litt.). This program estimates phylogenies from allele frequency data by maximum likelihood under a model that assumes that all divergence is caused by genetic drift in the absence of new mutations (J. Felsenstein, in litt.). The trees were drawn with the aid of the TREEVIEW program (R. Page, in litt.). Assignment tests in which individuals were assigned to populations on the basis of the likelihood of their microsatellite genotypes were conducted

using the Assignment Calculator program (Paetkau et al. 1995, 1997).

## Results

The average number of microsatellite alleles per locus was 4.14 (range, 2–8), and average expected heterozygosity ($H_E$) was 0.426 ± 0.039 (Table 1). Departures from Hardy–Weinberg expectations were not significant for most microsatellite loci (significant $F_{IS}$ values; Table 1). The only exception was locus Mvi57 in California, which exhibited a significant excess of heterozygotes ($F_{IS} = -0.287$, $P = 0.003$, Bonferroni corrected initial $\alpha = 0.007$). In addition, there was no evidence of linkage disequilibrium.

Variability of the mtDNA control region of 320 bp revealed 4 haplotypes, characterized by transitions at 3 sites (Table 2). Average haplotype diversity ($h$) was 0.412 ± 0.061, and average nucleotide diversity ($\pi$) was 0.00098 ± 0.00029 (Table 1).

There was no tendency for translocated populations to show less genetic variation than that of remnant populations; instead, levels of genetic diversity ($H_E$) were similar in all populations (Table 1). Mean $H_E$ in the 2 translocated populations, Washington (0.431) and southeast Alaska (0.467), was not less than $H_E$ in the 2 source populations, Amchitka (0.434) and Prince William Sound (0.414), or in the other remnant population, California (0.401). Although number of alleles at some microsatellite loci varied among populations, the total number of alleles across the 7 loci was similar in all populations (21–23). mtDNA-haplotype diversity ($h$) varied somewhat more among populations than did $H_E$ ($h$, 0.180–0.509) but was similar or higher in the translocated populations (Washington, 0.509 and southeast Alaska, 0.508) than in either of the source populations (Amchitka, 0.451 and Prince William Sound, 0.180) or California (0.414). All populations had 2 mtDNA haplotypes, except for California, which had 3, 1 of which occurred at low frequency (0.03; Appendix I).

Although the 5 sea otter populations exhibited similar levels of genetic diversity, there was marked genetic differentiation among populations. Heterogeneity in allele frequencies among populations was significant for all microsatellite loci taken individually (Bonferroni adjusted $P < 0.007$), except locus An5 ($P = 0.046$), and for all loci taken collectively (Fisher's combined test, $d.f. = 6$, $P = 0.003$). Likewise, heterogeneity in mtDNA haplotype frequencies among populations was highly significant ($d.f. = 4$, $P < 0.001$).

As expected, populations that were related by translocation were less differentiated from each other than were populations not related in this manner (Table 3). Mean microsatellite $F_{ST}$ was 0.049 ± 0.016 for pairs of populations related by translocation and 0.183 ± 0.016 for those that were not. mtDNA showed a similar pattern: mean pairwise $F_{ST}$ for this marker was 0.077 ± 0.083 for related populations and 0.466 ± 0.018 for unrelated populations.

Pairwise $F_{ST}$ estimates between source and translocated groups were consistent with translocation data. Eighty-nine percent of the sea otters translocated to southeast Alaska were from Amchitka, whereas the remaining 11% were from Prince William Sound (Jameson et al. 1982). The microsatellite $F_{ST}$ for Amchitka compared with southeast Alaska was not significantly greater than 0 ($F_{ST} = 0.018$, $P = 0.035$), whereas for Prince William Sound compared with southeast Alaska, $F_{ST}$ was 0.101 ($P < 0.001$). Likewise, the mitochondrial $F_{ST}$ was −0.004 for Amchitka compared with southeast Alaska and 0.321 ($P < 0.001$) for Prince William Sound compared with southeast Alaska (Table 3). The genetic data reflect numerical proportions of translocated population, indicating that Amchitka otters were relatively more important than those from Prince William Sound in founding the population. By comparison, although Washington was entirely derived from Amchitka founders, it was rather more differentiated from Amchitka than south-

TABLE 1.—Summary of microsatellite and mtDNA data for sea otters from 5 locations: number of alleles or haplotypes (A), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), departure from Hardy–Weinberg equilibrium ($F_{IS}$), and nucleotide diversity ($\pi$).

| Population | Parameters | $n$ | Locus | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | An5 | Mvi24 | Mvi57 | Mvi87 | Mvis72 | Mvis75 | Lut453 | All loci | mtDNA |
| Amchitka | | 36 | | | | | | | | | |
| | A | | 2 | 1 | 6 | 3 | 2 | 5 | 4 | 23 | 2 |
| | $H_O$ | | 0.277 | 0 | 0.812 | 0.294 | 0.288 | 0.571 | 0.618 | 0.409 | |
| | $H_E$ | | 0.430 | 0 | 0.776 | 0.427 | 0.285 | 0.579 | 0.539 | 0.434 | 0.451[a] |
| | $F_{IS}$ | | 0.358 | | −0.048 | 0.314[b] | 0.009 | 0.013 | −0.149 | | |
| | $\pi$ | | | | | | | | | | 0.001 |
| California | | 54 | | | | | | | | | |
| | A | | 3 | 2 | 4 | 3 | 2 | 7 | 2 | 23 | 3 |
| | $H_O$ | | 0.488 | 0.200 | 0.820 | 0.588 | 0.038 | 0.796 | 0.348 | 0.468 | |
| | $H_E$ | | 0.384 | 0.182 | 0.640 | 0.474 | 0.037 | 0.775 | 0.318 | 0.401 | 0.414[a] |
| | $F_{IS}$ | | −0.274 | −0.101 | −0.287[c] | −0.243 | −0.010 | −0.028 | −0.094 | | |
| | $\pi$ | | | | | | | | | | 0.0007 |
| Southeast Alaska | | 24 | | | | | | | | | |
| | A | | 2 | 1 | 6 | 3 | 2 | 5 | 3 | 22 | 2 |
| | $H_O$ | | 0.353 | 0 | 0.913 | 0.609 | 0.478 | 0.750 | 0.364 | 0.495 | |
| | $H_E$ | | 0.428 | 0 | 0.726 | 0.505 | 0.414 | 0.765 | 0.434 | 0.467 | 0.508[a] |
| | $F_{IS}$ | | 0.179 | | −0.264 | −0.210 | −0.158 | −0.020 | 0.166 | | |
| | $\pi$ | | | | | | | | | | 0.001 |
| Prince William Sound | | 33 | | | | | | | | | |
| | A | | 3 | 1 | 4 | 3 | 2 | 5 | 3 | 21 | 2 |
| | $H_O$ | | 0.438 | 0 | 0.545 | 0.454 | 0.370 | 0.467 | 0.332 | 0.372 | |
| | $H_E$ | | 0.520 | 0 | 0.684 | 0.503 | 0.391 | 0.482 | 0.322 | 0.414 | 0.180[a] |
| | $F_{IS}$ | | 0.161 | | 0.206 | 0.098 | 0.055 | 0.032 | 0.028 | | |
| | $\pi$ | | | | | | | | | | 0.0002 |
| Washington | | 27 | | | | | | | | | |
| | A | | 3 | 1 | 5 | 3 | 2 | 7 | 2 | 23 | 2 |
| | $H_O$ | | 0.428 | 0 | 0.778 | 0.364 | 0.320 | 0.741 | 0.304 | 0.442 | |
| | $H_E$ | | 0.533 | 0 | 0.657 | 0.305 | 0.372 | 0.781 | 0.372 | 0.431 | 0.509[a] |
| | $F_{IS}$ | | −0.107 | | −0.187 | −0.143 | 0.143 | 0.053 | 0.185 | | |
| | $\pi$ | | | | | | | | | | 0.002 |

TABLE 1.—Continued.

| Population | Parameters | *n* | Locus An5 | Mvi24 | Mvi57 | Mvi87 | Mvis72 | Mvis75 | Lut453 | All loci | mtDNA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Over all populations | | | | | | | | | | | |
| | Total A | | 3 | 2 | 6 | 4 | 2 | 8 | 4 | 4 | |
| | Mean $H_O$ | | 0.397 | 0.040 | 0.774 | 0.462 | 0.299 | 0.665 | 0.393 | 0.433 | |
| | Mean $H_E$ | | 0.459 | 0.036 | 0.697 | 0.443 | 0.300 | 0.676 | 0.369 | 0.426 | 0.412 |

[a] Haplotype diversity within mtDNA is equivalent to $H_E$ for microsatellites.
[b] Significant *P* following Bonferroni correction for multiple comparisons across all populations ($n = 5$, $d.f. = 4$, initial $\alpha = 0.01$).
[c] Significant *P* following Bonferroni correction for multiple comparisons across loci ($n = 7$, $d.f. = 6$, initial $\alpha = 0.007$).

TABLE 2.—Mitochondrial haplotypes for control regions identified for sea otters.

| Haplotype | Nucleotide position 191 | 192 | 208 | GenBank accession number |
|---|---|---|---|---|
| A | G | T | C | Bankit378170 |
| B | A | T | T | Bankit378172 |
| C | G | T | T | Bankit378176 |
| D | G | C | C | Bankit378177 |

east Alaska was from Amchitka. Microsatellite $F_{ST}$ for Amchitka compared with Washington was 0.049 ($P < 0.001$), whereas the mtDNA $F_{ST}$ for that pairwise comparison was 0.036 ($P < 0.001$). This suggests that the Washington population has experienced some combination of a founder effect and genetic drift that caused it to diverge from Amchitka.

Among remnant populations, California appeared most divergent. This was apparent in pairwise $F_{ST}$ values for both microsatellites and mtDNA (Table 3) and was also evident in maximum-likelihood trees based on allele or haplotype frequencies (Figs. 1 and 2). In both the microsatellite and mtDNA dendrograms, specimens from California and Prince William Sound appeared divergent from the remaining populations relative to Amchitka. The divergence of California was especially pronounced on the mtDNA tree (Fig. 2), reflecting the fact that 2 haplotypes, D and E, which had a combined frequency of 94% in California, were rare or absent in the other populations (Appendix I), similar to the pattern of mtDNA haplotype frequency reported by Cronin et al. (1996).

Assignment tests confirmed the general pattern of differentiation described previously (Table 4). Overall, 71% of individuals were assigned to the correct populations. Correct assignment rates were highest for California (91%) and Prince William Sound (79%) and ranged from 29% to 67% among the remaining populations. Most incorrectly assigned otters were assigned to populations that were related to the correct population by translocation. For example, some

TABLE 3.—Tests for genetic differentiation between population pairs of sea otters from 5 locations. Above the diagonal: pairwise $F_{ST}$ values based on microsatellite loci. Below the diagonal: pairwise $F_{ST}$ values for mtDNA. Bonferroni adjusted $P = 0.01$ across 5 populations. Asterisks indicate nonsignificant differences between groups.

| $F_{ST}$ | Amchitka | California | Southeast Alaska | Prince William Sound | Washington |
|---|---|---|---|---|---|
| Amchitka | — | 0.167 | 0.018* | 0.193 | 0.049 |
| California | 0.264 | — | 0.142 | 0.257 | 0.165 |
| Southeast Alaska | −0.004* | 0.518 | — | 0.101 | 0.029* |
| Prince William Sound | 0.185 | 0.656 | 0.321 | — | 0.176 |
| Washington | 0.036 | 0.517 | −0.036* | 0.381 | — |

Washington otters were misassigned to Amchitka (11%) and southeast Alaska (22%) but not to either Prince William Sound or California. Similarly, some Prince William Sound otters were misassigned to southeast Alaska (21%) but not to other populations. In total, among 50 otters assigned incorrectly, only 10 (20%) were assigned to populations not related to the correct population by translocation, as opposed to the approximately 25 otters (50%) that would have been expected to have been assigned to unrelated populations by chance.

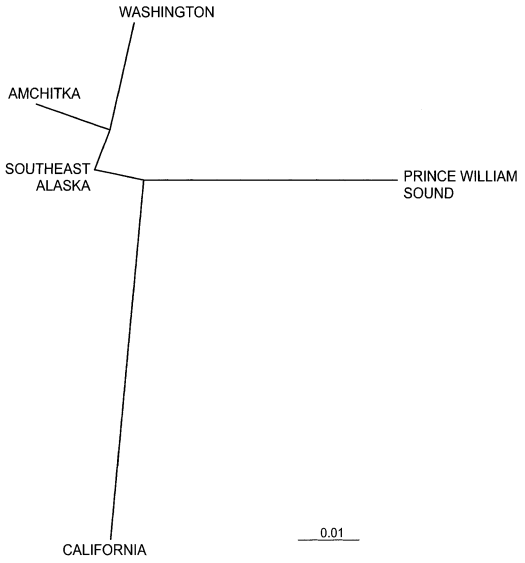


FIG. 1.—Maximum-likelihood tree of sea otter populations based on microsatellite allele frequencies. Scale indicates Nei's genetic distance.

## DISCUSSION

The primary goal of this study was to compare genetic variability in translocated sea otter populations relative to populations that were not translocated. To facilitate this we compared levels of genetic variation at 7 microsatellite loci that are variable in sea otters, as well as a portion of the mitochondrial control region in translocated and remnant populations. We found that neither the microsatellites nor the mtDNA sequence revealed any evidence of reduced genetic variation in translocated populations relative to source populations. In another study that compared genetic diversity within remnant and translocated sea otter populations, Bodkin et al. (1999) found mtDNA diversity of restriction fragment length polymorphism haplotypes was lower, but not significantly, in remnant populations (Amchitka = 0.569 and Prince William Sound = 0.178) than in

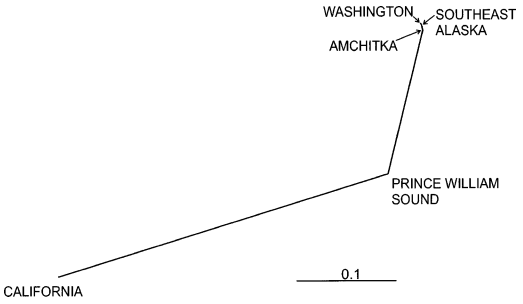


FIG. 2.—Maximum-likelihood tree of sea otter populations based on mtDNA haplotype frequencies. Scale indicates Nei's genetic distance.

TABLE 4.—Results of assignment tests using multilocus microsatellite genotypes to diagnose source population for 5 populations of sea otters. The diagonal represents correct classifications.

| | Amchitka | California | Southeast Alaska | Prince William Sound | Washington |
|---|---|---|---|---|---|
| Amchitka | 24 | 2 | 6 | 2 | 2 |
| California | 0 | 49 | 1 | 1 | 3 |
| Southeast Alaska | 10 | 1 | 7 | 3 | 3 |
| Prince William Sound | 0 | 0 | 7 | 26 | 0 |
| Washington | 3 | 0 | 6 | 0 | 18 |

most translocated populations (Washington = 0.469, southeast Alaska = 0.744, and British Columbia = 0.591). Hence, it appears that founding-population sizes in translocated populations have been sufficient to preserve measurable molecular genetic variation that remains in the species. It is noteworthy, however, that the Washington population has diverged significantly in frequencies of microsatellite alleles and mtDNA haplotypes from the Amchitka source population, whereas the southeast Alaska population, founded with approximately six times as many individuals from the same population, has not differentiated significantly from Amchitka (Table 3). This suggests that the lesser number of individuals (as many as 43—Jameson et al. 1982; R. J. Jameson, in litt.) used to found the Washington population, although sufficient to preserve overall genetic variability, was small enough that drift during the years after translocation altered allelic frequencies to account for the relative divergence from Amchitka.

Even though translocated populations apparently have not suffered a loss of genetic diversity, most studies of genetic variation in sea otters have revealed relatively low levels of diversity compared with other taxa. In the 1 exception, Lidicker and McCollum (1997) reported mean allozyme heterozygosity ($H_E$ = 0.074) in sea otters comparable with values in terrestrial animals. In contrast, Rotterman (1992) found a mean allozyme heterozygosity of only 0.021 in sea otters. All other studies of genetic variation in sea otters have focused on variation in mtDNA. Cronin et al. (1996) found that mtDNA diversity of restriction fragment length polymorphism haplotypes ranged from 0.180 to 0.590, with the lowest in Prince William Sound and the highest within Adak Island followed closely by Amchitka (0.570). Bodkin et al. (1999) found, using the same method, mtDNA haplotype diversities ranged from 0.178 (Prince William Sound) to 0.744 (southeast Alaska).

Comparison of results of this study with those from studies of mtDNA and microsatellite diversity in other mammals suggests that sea otters have low levels of genetic variation in comparison with other mammalian taxa (Table 5). Moreover, levels of diversity in sea otters are most similar to those seen in other species that have undergone known bottlenecks or persistent population declines. For example, haplotype diversity in mtDNA control regions in sea otters is comparable with that in northern elephant seal (*Mirounga anguistirostris*). Northern elephant seals were reduced to approximately 20–30 animals in 1884 after exploitation in the 19th century (Hoelzel 1997). Similarly, both control region haplotype and nucleotide diversity in sea otters are lower than the corresponding values in the European wolf, *Canis lupus*, another species that has suffered extreme population decline and fragmentation. In the case of microsatellites, mean $H_E$ in sea otters (0.42) is less than that in the endangered red wolf (*Canis rufus*; Table 5). Mammalian species with lower mean heterozygosity for microsatellites than what we ob-

TABLE 5.—Comparison of genetic variation in sea otters and other marine and terrestrial mammals, including species known to have undergone population bottlenecks.

| Species | Number of alleles or haplotypes | Mean $H_E$ or haplotype diversity (h) | % mean nucleotide diversity ($\pi$) | Reference |
|---|---|---|---|---|
| mtDNA control region | | | | |
| No known bottleneck | | | | |
| *Leopardus wiedii* | 21 | 0.98 | 18.3 | Eizirik et al. 1998 |
| *Leopardus pardalis* | 24 | 0.96 | 6.8 | Eizirik et al. 1998 |
| *Panthera onca* | 22 | 0.94 | 0.77 | Eizirik et al. 2001 |
| *Dasypus novemcinctus* | 12 | 0.86 | 3.04 | Huchon et al. 1999 |
| *Eumetopias jubatus* | 13 | 0.85 | 1.7 | Bickham et al. 1996 |
| *Canis familiarus* | 43 | 0.76 | 1.30 | Randi et al. 2000 |
| *Mirounga leonina* | 23 | 0.66 | | Hoelzel 1997 |
| Bottlenecks | | | | |
| *Bettongia tropica* | 11 | 0.50 | 0.55 | Pope et al. 2000 |
| *Cervus nippon* | 10 | | 2.8 | Tamate & Tsuchiya 1995 |
| *Canis lupus* | 20 | 0.50 | 0–1.45 | Randi et al. 2000 |
| *Mirounga angustirostris* | 2 | 0.42 | | Hoelzel et al. 1997 |
| *Enhydra lutris* | 4 | 0.40 | 0.098 | This study |
| Microsatellites | | | | |
| No known bottleneck | | | | |
| *Apodemus sylvaticus* | 6 | 0.86 | | Harr et al. 2000 |
| *Panthera pardus* | 18 | 0.84 | | Spong et al. 2000 |
| *Taxidea taxus* | 4 | 0.83 | | Davis and Strobeck 1998 |
| *Syncerus caffer* | 14 | 0.76 | | Van Hooft et al. 2000 |
| *Panthera onca* | 29 | 0.74 | | Eizirik et al. 2001 |
| *Canis familiarus* | 10 | 0.73 | | Wayne 1996 |
| *Orcinus orca* | 3 | 0.73[a]<br>0.49[b] | | Hoelzel et al. 1998 |
| *Bettongia tropica* | 7 | 0.71 | | Pope et al. 2000 |
| *Delphinapterus leucas* | 5 | 0.70 | | Brown et al. 1999 |
| *Canis latrans* | 10 | 0.68 | | Wayne 1996 |
| *Ursus maritimus* | 16 | 0.68 | | Paetkau et al. 1999 |
| *Martes americana* | 14 | 0.67 | | Davis and Strobeck 1998 |
| *Marmota marmota* | 6 | 0.66 | | Goossens et al. 2001 |
| *Canis lupus* | 10 | 0.62 | | Wayne 1996 |
| *Mustela vison* | 12 | 0.61 | | O'Connell et al. 1996 |
| *Lutra lutra* | 13 | 0.54 | | Dallas and Piertney 1998 |
| Bottlenecks | | | | |
| *Enhydra lutris* | 7 | 0.42 | | This study |
| *Canis rufus* | 10 | 0.55 | | Wayne 1996 |
| *Ursus thibetanus* | 8 | 0.39 | | Kitahara et al. 2000 |
| *Gulo gulo* | 15 | 0.39 | | Walker et al. 2001 |
| *Urocyon littoralis* | 10 | 0.36 | | Wayne 1996 |
| *Alces alces* | 5 | 0.26[c]<br>0.33[d] | | Broders et al. 1999 |
| *Canis simensis* | 10 | 0.24 | | Wayne 1996 |

[a] Transient population.
[b] Resident population.
[c] Translocated.
[d] Not translocated.

served in sea otters include Asiatic black bear (*Ursus thibetanus*), Scandinavian wolverine (*Gulo gulo*), Channel Island foxes (*Urocyon littoralis*), and moose (*Alces alces*), all species that have experienced extreme population declines or bottlenecks (in the case of moose, through translocation to Newfoundland; Table 5).

It is also noteworthy that mean microsatellite heterozygosity in sea otter is lower than that in 4 of 5 other mustelid species, the exception being Scandinavian wolverine (Table 5). Because most of the microsatellites used in this study were originally developed for other mustelid species, the low microsatellite diversity in sea otter could be caused, in part, by ''ascertainment bias,'' the suggested tendency for microsatellites to be less variable in species other than those from which they were originally selected (Ellegren et al. 1995). Although this possibility cannot be ruled out with our data, comparison of $H_E$ for the 1 sea otter–derived locus used in this study, An5, and $H_E$ values for the remaining loci, reveals little evidence for ascertainment bias in this study. Mean $H_E$ for locus An5 was 0.46, whereas for the remaining loci not derived from sea otters it averaged 0.42 or 0.50, depending on whether or not the nearly fixed Mvi24 locus was included (Table 1). Hence, it seems likely that the low diversity of microsatellites (and mtDNA) in sea otters is caused, at least in part, by the severe population decline in this species that predated recent translocation efforts.

Among populations that we studied, Prince William Sound had the lowest diversity of mtDNA and microsatellites (Table 1). Other genetic studies have also found low levels of variation in Prince William Sound relative to other populations (Bodkin et al. 1999; Cronin et al. 1996). Bodkin et al. (1999) hypothesized that the relative lack of genetic variation in Prince William Sound is a consequence of the fact that it suffered one of the longest population bottlenecks after the fur trade and continues to have a lower growth rate than most populations. Apart from its low diversity, $F_{ST}$ estimates and neighbor-joining analyses based on both microsatellite and mtDNA data indicate that Prince William Sound is relatively divergent from the other Alaska populations (Table 3; Figs. 1 and 2). In addition, Prince William Sound shares a haplotype with California that is not found in any other Alaskan population. The presence of this ''California'' haplotype in Prince William Sound supports the hypothesis that there was gene flow between these 2 remnant groups and that the northern limit of the southern sea otter before fragmentation by the fur trade may have been Prince William Sound (Estes 1990; Riedman 1997; Riedman and Estes 1990). A similar divergence of the Prince William Sound population from nearby Alaskan populations is also found in another marine mammal that has similar geographic range, the Steller sea lion (*Eumetopias jubatus*—Bickham et al. 1998).

Our data on microsatellites and mtDNA provide several useful perspectives for conservation biology of sea otters. First, they suggest that translocations of relatively small numbers of animals (20–30) are sufficient to avoid further loss of genetic diversity if the introduced population expands relatively quickly and the population bottleneck is of short duration; hence, they lend support to this management approach. Second, they provide baseline data with which to evaluate any future changes in genetic diversity within these populations. Third, by comparison with data from other species they provide some suggestion that population decline induced by the fur trade in the 19th century may have caused a loss of genetic variation in this species. Verification of this suggestion, and quantification of any loss of genetic variation that has occurred, must await study of sea otter tissues that predate the 19th century population reduction.

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## Literature Cited


Applied Biosystems Inc. 1993. Genescan 672 users manual revision A. Perkin–Elmer Corp., Foster City, California.

Applied Biosystems Inc. 1996. Genotyper 2.0 users manual. Perkin–Elmer Corp., Foster City, California.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Ballou, J. D., and K. Ralls. 1982. Inbreeding and juvenile mortality in small populations of ungulates: a detailed analysis. Biological Conservation 24:239–272.

Bickham, J. W., T. R. Loughlin, J. K. Wickliffe, and V. N. Burkanov. 1998. Geographic variation in the mitochondrial DNA of steller sea lions: haplotype diversity and endemism in the Kuril Islands. Biosphere Conservation 1:107–117.

Bickham, J. W., J. C. Patton, and T. R. Loughlin. 1996. High variability for control-region sequences in a marine mammal: implications for conservation and biogeography of Steller sea lions (*Eumetopias jubatus*). Journal of Mammalogy 77:95–108.

Bodkin, J. L., B. E. Ballachey, M. A. Cronin, and K. T. Scribner. 1999. Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conservation Biology 13:1378–1385.

Bodkin, J. L., A. M. Burdin, and D. A. Ryzanov. 2000. Age and sex specific mortality and population structure in sea otters. Marine Mammal Science 16: 201–219.

Broders, H. G., S. P. Mahoney, W. A. Montevecchi, and W. S. Davidson. 1999. Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. Molecular Ecology 8:1309–1315.

Brown Gladden, J. G., M. M. Ferguson, M. K. Friesen, and J. W. Clayton. 1999. Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with population structure revealed by mtDNA variation. Molecular Ecology 8: 347–363.

Caro, T. M., and K. M. Laurenson. 1994. Ecological and genetic factors in conservation: a cautionary tale. Science 263:386–485.

Cheney, L. C. 1995. An assessment of genetic variation within and between sea otter (*Enhydra lutris*) populations off Alaska and California. M.S. thesis, California State University, San Jose.

Cronin, M. A., J. L. Bodkin, B. E. Ballachey, J. A. Estes, and J. C. Patton. 1996. Mitochondrial DNA variation among subspecies and populations of sea otters (*Enhydra lutris*). Journal of Mammalogy 77: 546–557.

Dallas, J. F., and S. B. Piertney. 1998. Microsatellite primers for the Eurasian otter. Molecular Ecology 7: 1248–1251.

Davis, C. S., and C. Strobeck. 1998. Isolation, variability, and cross species amplification of polymorphic microsatellite loci in the family mustelidae. Molecular Ecology 7:1776–1778.

Eizirik, E., et al. 1998. Phylogeographic patterns and evolution of the mitochondrial DNA control region in two Neotropical cats (Mammalia, Felidae). Journal of Molecular Evolution 47:613–624.

Eizirik, E., K. Jae-Heup, M. Menotti-Raymond, P. G. Crawshaw, S. J. O'Brien, and W. E. Johnson. 2001. Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). Molecular Ecology 10:65–79.

Ellegren, H., C. R. Primmer, and B. C. Sheldon. 1995. Microsatellite evolution: directionality or bias in locus selection. Nature Genetics 11:360–362.

Estes, J. A. 1990. Growth and equilibrium in sea otter populations. Journal of Animal Ecology 59:385–401.

Estes, J. A., M. T. Tinker, T. M. Williams, and D. F. Doak. 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. Science 282:473–476.

Fleming, M. A., E. A. Ostrander, and J. A. Cook. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology 8:1352–1354.

Frankham, R. 1995. Conservation genetics. Annual Review of Genetics 29:305–327.

Goossens, B., L. Chikhi, P. Taberlet, L. P. Waits, and D. Allaine. 2001. Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. Molecular Ecology 10:41–52.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Harr, B., K. Musolf, and G. Gerlach. 2000. Characterization and isolation of DNA microsatellite primers in wood mice (*Apodemus sylvaticus*, Rodentia). Molecular Ecology 9:1664–1665.

Hoelzel, A. R. 1997. Molecular ecology of pinnipeds. Pp. 147–157 in Molecular genetics of marine mammals (A. E. Dizon, S. J. Chivers, and W. F. Perrin, eds.). Special Publication, Society for Marine Mammalogy 3:1–388.

Hoelzel, A. R., M. Dahlheim, and S. J. Stern. 1998. Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and the genetic differentiation between foraging specialists. Journal of Heredity 89:121–128.

Hoelzel, A. R., and A. Green. 1992. Analysis of population-level variation by sequencing PCR-amplified DNA. Pp. 159–187 in Molecular genetic analysis of populations: a practical approach (A. R. Hoelzel, ed.). IRL Press, Oxford, United Kingdom.

Huchon, D., F. Delsuc, F. M. Catzeflis, and E. J. P. Douzery. 1999. Armadillos exhibit less genetic polymorphism in North America than in South America: nuclear and mitochondrial data confirm a founder effect in *Dasypus novemcinctus* (Xenarthra). Molecular Ecology 8:1743–1748.

Jameson, R. J., K. W. Kenyon, A. M. Johnson, and

H. M. WRIGHT. 1982. History and status of translocated sea otter populations in North America. Wildlife Society Bulletin 10:100–107.

KENYON, K. W. 1969. The sea otter in the eastern Pacific Ocean. North American Fauna 68:1–352.

KITAHARA, E., Y. ISAGI, Y. ISHIBASHIS, AND T. SAITOH. 2000. Polymorphic microsatellite DNA markers in the Asiatic black bear *Ursus thibetanus*. Molecular Ecology 9:1661–1662.

LIDICKER, W. Z., AND F. C. MCCOLLUM. 1997. Genetic variation in California sea otters. Journal of Mammalogy 78:417–425.

LYNCH, M. 1996. A quantitative-genetic perspective on conservation issues. Pp. 471–501 in Conservation genetics: case histories from nature (J. C. Avise and J. L. Hamrick, eds.). Chapman and Hall, New York.

O'BRIEN, S. J., et al. 1996. Conservation genetics of the felidae. Pp. 50–74 in Conservation genetics: case histories from nature (J. C. Avise and J. L. Hamrick, eds.). Chapman and Hall, New York.

O'CONNELL, M., J. M. WRIGHT, AND A. FARID. 1996. Development of PCR primers for nine polymorphic American mink, *Musela vison*, microsatellite loci. Molecular Ecology 5:311–312.

OLSEN, J. B., P. BENTZEN, AND J. E. SEEB. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1083–1090.

OLSEN, J. B., J. K. WENBERG, AND P. BENTZEN. 1996. Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Molecular Marine Biology and Biotechnology 5: 259–272.

PAETKAU, D., W. CALVERT, I. STERLING, AND C. STROBECK. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347–354.

PAETKAU, D., L. P. WAITS, P. L. CLARKSON, L. CRAIGHEAD, AND C. STROBECK. 1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics 147:1943–1957.

PAETKAU, D., et al. 1999. Genetic structure of the world's polar bear populations. Molecular Ecology 8:1571–1584.

POPE, L. C., A. EST OUP, AND C. MORITZ. 2000. Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. Molecular Ecology 9: 2041–2053.

RALLS, K., J. D. BALLOU, AND A. TEMPLETON. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2:185–193.

RALLS, K., K. BRUGGER, AND J. D. BALLOU. 1979. Inbreeding and juvenile mortality in small populations of ungulates. Science 206:1101–1103.

RANDI, E., et al. 2000. Mitochondrial DNA variability in Italian and east European wolves: detecting the consequences of small population size and hybridization. Conservation Biology 14:464–473.

RAYMOND, M., AND F. ROUSSET. 1995. Genepop (Version 3.1): population genetics software for exact tests and ecumenicism. Journal of Heredity. 86:248–249.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

RIEDMAN, M. L. 1997. Sea otters. Monterey Bay Aquarium Foundation, Monterey, California.

RIEDMAN, M. L., AND J. A. ESTES. 1990. The sea otter (*Enhydra lutris*): behavior, ecology and natural history. United States Fish and Wildlife Service, Washington D.C., Biological report 90(14).

ROTTERMAN, L. M. 1992. Patterns of genetic variability in sea otters after severe population subdivision and reduction. Ph.D. dissertation, University of Minnesota, Minneapolis.

SCRIBNER, K. T., J. BODKIN, B. BALLACHEY, S. R. FAIN, M. A. CRONIN, AND M. SANCHEZ. 1997. Population genetic studies of the sea otter (*Enhydra lutris*): a review and interpretation of available data. Pp. 197–208 in Molecular genetics of marine mammals (A. E. Dizon, S. J. Chivers, and W. F. Perrin, eds.). Special publication, Society for Marine Mammalogy 3: 1–388.

SPONG, G., M. JOHANSSON, AND M. BJORKLUND. 2000. High genetic variation in leopards indicates large and long-term stable effective population size. Molecular Ecology 9:1773–1782.

TAMATE, H. B., AND T. TSUCHIYA. 1995. Mitochondrial DNA polymorphism in subspecies of Japanese Sitka deer (*Cervus nippon*). Journal of Heredity 86:211–215.

VAN HOOFT, W. F., A. F. GROEN, AND H. H. T. PRINS. 2000. Microsatellite analysis of genetic diversity in African buffalo (*Synceru scaffer*) populations throughout Africa. Molecular Ecology 9:2017–2025.

WALKER, C. W., C. VILA, A. LANDA, M. LINDEN, AND H. ELLEGREN. 2001. Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. Molecular Ecology 10:53–63.

WAYNE, R. K. 1996. Conservation genetics in the canidae. Pp. 75–118 in Conservation genetics: case histories from nature (J. C. Avise and J. L. Hamrick, eds.). Chapman and Hall, New York.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

WOLF, C. M., B. GRIFFITH, C. REED, AND S. A. TEMPLE. 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology 10:1142–1154.

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APPENDIX I
Frequency of alleles for loci used from 5 sea otter populations.

| Locus | Annealing temperature (°C) | Number of PCR cycles | Allele | | Populations | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. | Size (bp) | Amchitka | California | Southeast Alaska | Prince William Sound | Washington |
| An5 | 66 | 35 | 1 | 146 | 0.69 | 0.76 | 0.71 | 0.53 | 0.56 |
| | | | 2 | 150 | 0 | 0.02 | 0 | 0.02 | 0.03 |
| | | | 3 | 152 | 0.31 | 0.22 | 0.29 | 0.45 | 0.41 |
| Mvi24 | 57 | 35 | 1 | 92 | 0 | 0.10 | 0 | 0 | 0 |
| | | | 2 | 96 | 1.0 | 0.90 | 1.0 | 1.0 | 1.0 |
| Mvi57 | 57 | 35 | 1 | 118 | 0.17 | 0.01 | 0.11 | 0 | 0.31 |
| | | | 2 | 120 | 0.08 | 0 | 0.02 | 0.17 | 0.02 |
| | | | 3 | 122 | 0.38 | 0.33 | 0.41 | 0.47 | 0.48 |
| | | | 4 | 124 | 0.17 | 0.47 | 0.30 | 0.10 | 0.15 |
| | | | 5 | 126 | 0.03 | 0.18 | 0.04 | 0.26 | 0 |
| | | | 6 | 128 | 0.17 | 0 | 0.11 | 0 | 0.04 |
| Mvi87 | 57 | 35 | 1 | 85 | 0 | 0.02 | 0 | 0.08 | 0 |
| | | | 2 | 87 | 0.09 | 0.33 | 0.33 | 0.65 | 0.13 |
| | | | 3 | 89 | 0.74 | 0.65 | 0.63 | 0.27 | 0.83 |
| | | | 4 | 91 | 0.17 | 0 | 0.04 | 0 | 0.04 |
| Mvis72 | 57 | 35 | 1 | 270 | 0.83 | 0.98 | 0.72 | 0.74 | 0.76 |
| | | | 2 | 272 | 0.17 | 0.02 | 0.28 | 0.26 | 0.24 |
| Mvis75 | 57 | 35 | 1 | 160 | 0 | 0.02 | 0 | 0 | 0 |
| | | | 2 | 162 | 0 | 0.14 | 0 | 0 | 0.02 |
| | | | 3 | 164 | 0.10 | 0.02 | 0.12 | 0.08 | 0.18 |
| | | | 4 | 166 | 0.09 | 0.34 | 0.12 | 0.02 | 0.33 |
| | | | 5 | 168 | 0.19 | 0.27 | 0.19 | 0.03 | 0.24 |
| | | | 6 | 172 | 0.01 | 0 | 0 | 0 | 0.02 |
| | | | 7 | 176 | 0 | 0.09 | 0.17 | 0.70 | 0.04 |
| | | | 8 | 178 | 0.61 | 0.12 | 0.40 | 0.17 | 0.17 |
| Lut453 | 57 | 35 | 1 | 103 | 0.12 | 0 | 0.07 | 0.05 | 0 |
| | | | 2 | 107 | 0.63 | 0.20 | 0.73 | 0.81 | 0.76 |
| | | | 3 | 109 | 0.01 | 0 | 0 | 0 | 0 |
| | | | 4 | 111 | 0.24 | 0.80 | 0.20 | 0.14 | 0.24 |
| Mt | 55 | 30 | A | | 0.67 | 0.07 | 0.55 | 0.93 | 0.48 |
| DNA | | | B | | 0.33 | 0 | 0.45 | 0 | 0.52 |
| L15926 | | | C | | 0 | 0.74 | 0 | 0.07 | 0 |
| H16340 | | | D | | 0 | 0.19 | 0 | 0 | 0 |