MITOCHONDRIAL-DNA VARIATION AMONG SUBSPECIES AND POPULATIONS OF SEA OTTERS (*ENHYDRA LUTRIS*)

MATTHEW A. CRONIN, JAMES BODKIN, BRENDA BALLACHEY, JAMES ESTES, AND JOHN C. PATTON

United States Fish and Wildlife Service, Alaska Fish and Wildlife Research Center, 1011 East Tudor Road, Anchorage, AK 99503 (MAC, JB, BB)
National Biological Service, University of California, Santa Cruz, CA 95064 (JE)
LGL Ecological Genetics, Inc., 1410 Cavitt Street, Bryan, TX 77801 (JCP)
Present address of MAC: LGL Alaska Research Associates, Inc., 4175 Tudor Centre Drive, Suite 202, Anchorage, AK 99508

We used restriction-enzyme analysis of polymerase-chain reaction-amplified, mitochondrial DNA (mtDNA) to assess genetic differentiation of subspecies and populations of sea otters, *Enhydra lutris,* throughout the range of the species. There were several haplotypes of mtDNA in each subspecies and geographically separate populations. MtDNA sequence divergence of haplotypes of sea otters was 0.0004–0.0041 base substitutions per nucleotide. *E. l. nereis* appears to have monophyletic mitochondrial DNA, while *E. l. lutris* and *E. l. kenyoni* do not. Different frequencies of haplotypes of mtDNA among populations reflect current restriction of gene flow and the unique histories of different populations. There are two or three haplotypes of mtDNA and diversity of haplotypes is 0.1376–0.5854 in each population of otters. This is consistent with theoretical work, which suggests that population bottlenecks of sea otters probably did not result in major losses of genetic variation for individual populations, or the species as a whole.

Key words: *Enhydra lutris,* sea otter, mitochondrial DNA, subspecies, genetic variation

Sea otters (*Enhydra lutris*) occur in coastal habitats along the shores of the northern Pacific Ocean. Historically, sea otters occurred along coastal habitats from northern Japan, northeast through the Kuril Islands, along the Kamchatka Peninsula, across the Aleutian archipelago and Alaska Peninsula, and south along the coast of North America to central Baja California (Fig. 1). Sea otters have been the subject of studies of range expansion (Lubina and Levin, 1988), community ecology (Estes et al., 1982, 1989; Kvitek et al., 1992), and population genetics (Ralls et al., 1983; Rotterman, 1992; Sanchez, 1992). There have been several attempts to classify subspecies of sea otters based on morphology (including external characters such as color of pelage) and geographic distribution (Barabash-Nikiforov, 1947; Davis and Lidicker, 1975; Grinnell et al., 1937; Kenyon, 1969; Roest, 1971; Scheffer and Wilke, 1950; Stroganov, 1962), but the number or quality of specimens has been inadequate for a thorough systematic assessment. Wilson et al. (1991) reviewed these assessments and conducted a cranial-morphometric analysis. They concluded there are three subspecies, *E. l. lutris* from the northwestern Pacific Ocean, *E. l. kenyoni* from the Aleutian Islands east and south to Oregon, and *E. l. nereis* from California (Fig. 1).

Several hundred thousand sea otters may have occupied historical ranges, but commercial hunting in the 18th and 19th centuries resulted in local extermination or drastic reductions in populations across the range of the species. Eleven populations survived, although the total size of these populations was probably <2,000. Following legal protection, many populations have recovered, and continue to grow through re-

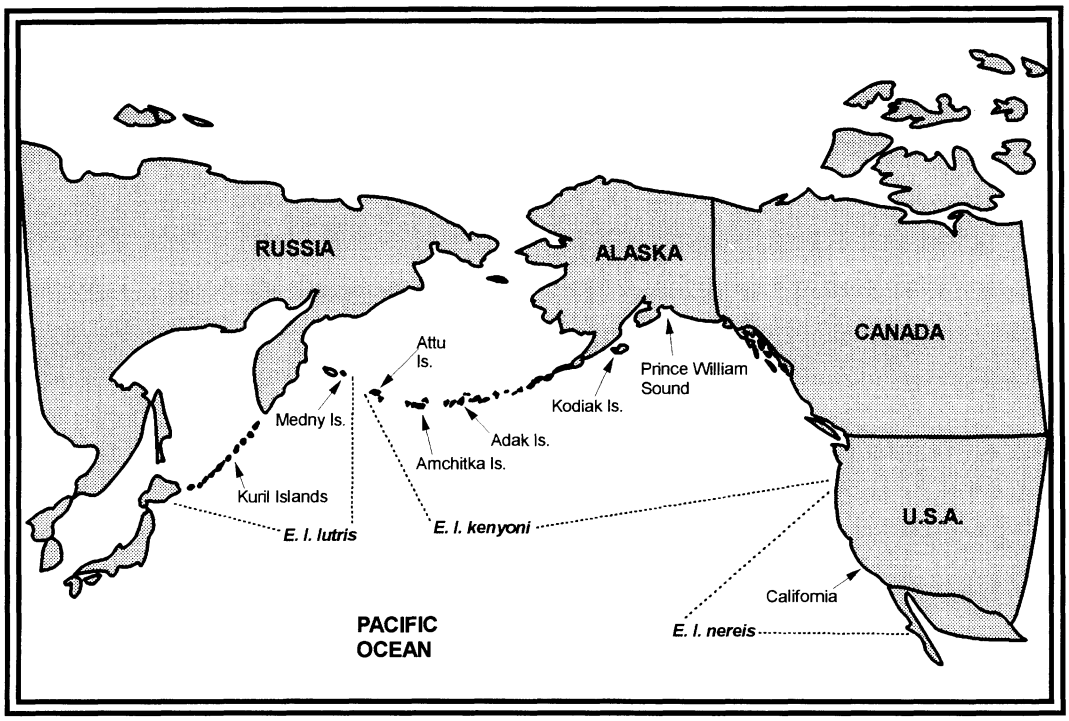

FIG. 1.—Geographic distribution of sampling locations and ranges of subspecies of sea otters.

cruitment, recolonization of vacant habitat, or reintroductions by humans (Bodkin et al., 1994). The drastic reduction of sea otters has caused concern about potential loss of genetic variation (Rotterman, 1992; Sanchez, 1992). However, studies of theoretical population genetics on sea otters in California indicate that the amount of genetic variation lost may be relatively small, ca. 20% of the original variation (Ralls et al., 1983). In addition, analyses of allozymes and mitochondrial DNA (mtDNA) have shown substantial amounts of genetic variation in some populations of sea otters (Rotterman, 1992; Sanchez, 1992; Scribner et al., in press).

Additional genetic data may be useful for assessing systematic relationships and levels of variation among populations of sea otters. We analyzed mtDNA with the specific objective of quantifying genetic variation within and among subspecies and populations of sea otters. Analyses of mtDNA have been particularly common for studies of subspecies and closely related species because of the relatively rapid rate of evolution and ease of analysis relative to nuclear (chromosomal) DNA (Avise et al., 1987; Wilson et al., 1985). There are limitations to the utility of mtDNA in systematic assessments below the species level because it is maternally inherited and represents only matriarchal phylogeny. In addition, mtDNA is inherited as one, linked, DNA segment, and thus behaves as a single locus in the context of population genetics. Single-locus phylogenies may not represent accurately phylogenies of species or populations (Pamilo and Nei, 1988). However mtDNA analyses can be useful for assessing phylogeny, population differentiation, and gene flow, particularly if used in conjunction with other genetic markers (Cronin, 1993; Cronin et al., 1991*a*; DeSalle et al., 1987).

MATERIALS AND METHODS

Samples of blood or muscle were collected from sea otters from several locations as fol-

lows: *E. l. lutris*—Kuril Islands, $n = 17$; Medny Island, $n = 24$. *E. l. kenyoni*—Attu Island, $n = 20$; Amchitka Island, $n = 20$; Adak Island, $n = 21$; Kodiak Island, $n = 14$; Prince William Sound, $n = 31$. *E. l. nereis*—California, $n = 20$ (Fig. 1). Live sea otters were captured with tangle nets, dip nets, or diver-operated underwater capture traps (Ames et al., 1986; Bodkin and Weltz, 1990). Blood was collected by venipuncture with EDTA as anticoagulant, and separated into blood cells and plasma by centrifugation. Samples of muscle were collected during necropsy of beach-cast carcasses or from otters that were shot (samples from Kuril and Medny). Blood was obtained from all samples from Kodiak, Attu, Amchitka, and Adak islands. Samples from Prince William Sound and California were either blood or muscle. All samples from Kuril and Medny islands were muscle. Samples were frozen (−20–−15°C) until analyzed in the laboratory.

We extracted genomic DNA from muscle or blood with standard methods involving SDS proteinase-k digestion and phenol-chloroform (muscle) or phenol-chloroform-methelyene chloride (blood) extractions (Cronin et al., 1994). Using the polymerase chain reaction (PCR—Mullis and Faloona, 1987), four segments of mtDNA were amplified with the following primers: NADH dehydrogenase 1 (ND-1) 5′-ACCCCGCCTGTTTACCAAAAACAT (LGL primer 381), 5′-GGTATGAGCCCGATAGCTTA (LGL primer 563); ND-3/4 5′TAA(C/T)TAGTACAG(C/T)TGACTTCCAA (LGL primer 772), 5′TTTTGGTTCCTAAGACCAA(C/T)GGAT (LGL primer 773); ND-5/6 5′ AATAGTTTATCC(G/A)TTGGTCTTAGG (LGL primer 763), 5′TTACAACGATGGTTTTTCAT(G/A)TCA (LGL primer 764); 12S-16S ribosomal RNA 5′TGGGATTAGATACCCCACTAT (LGL primer 284), 5′TGATTATGCTACCTTTGCAC(A/G)GT (LGL primer 384). These primers were designed by comparing sequences of mtDNA of *Bos* (Anderson et al., 1982), *Homo* (Anderson et al., 1981; Kocher et al., 1989), *Mus* (Bibb et al., 1981), and *Xenopus* (Roe et al., 1985). Positions of primers relative to numbered nucleotide positions of the human-mtDNA sequence (Anderson et al., 1982) are: ND-1, nucleotides 2487–2510 and nucleotides 4337–4418; ND-3/4, nucleotides 10003–10025 and nucleotides 12309–12286; ND-5/6, nucleotides 12278–12301 and nucleotides 14727–14704; 12S–16S rDNA, nucleotides 1071–1091 and 2604–2582.

Each PCR reaction was composed of: 0.1–0.5 μg genomic DNA; 5 μL 10× buffer (0.1 M tris-HCl pH 8.7, 0.025 M $MgCl_2$, 0.5 M KCl, 1 μg/μL bovine serum albumin); 5 μL dNTP mix (2 mM each of dATP, dTTP, dCTP, dGTP, in 10 mM tris-HCl, pH 7.9); 5 μL of a 2 μM solution of each of the two primers; 1.25–2.50 units of *Taq* polymerase; deionized water to a final volume of 50 μL. The amplification cycle was 95°C for 45 s, 50°C for 30 s, and 70°C for 2 min, 30 s. Reactions were run for 32 cycles.

To assess variation in mtDNA and identify haplotypes, restriction enzymes were used to digest each of the four segments of mtDNA (ND-1, 19 enzymes; ND-3/4, 18 enzymes; ND-5/6, 13 enzymes; 12s–16s, 10 enzymes) of sea otters from across the range of the species (Appendix I). These included 6 otters from California, 6 otters from the Kuril Islands, and 10 otters from Prince William Sound. One hundred forty-five additional otters from these and the other sampling locations, and outgroups (mink, *Mustela vison,* and river otter, *Lontra canadensis*), were analyzed with only the enzymes resulting in variable fragment patterns. After restriction-enzyme digestion, DNA was run on 2–3% agarose gels with TEA buffer (Sambrook et al., 1989), stained with ethidium bromide, and photographed under ultraviolet light. Sizes of restriction fragments were estimated by comparison with a standard (Lambda-phage DNA digested with *Hind* III or *Bst*E II). Haplotypes were defined from the composite restriction-fragment patterns for enzymes showing variable fragment patterns (Lansman et al., 1981).

We calculated the proportion of restriction fragments shared among haplotypes (F) and nucleotide sequence divergence (p, base substitutions per nucleotide) with the fragment method using the computer program RESTSITE (Miller, 1991). For each haplotype we combined fragments for all four amplified segments of mtDNA. F and p were calculated separately for 4-, 5-, and 6-base enzymes and an average p was calculated weighted by the number of restriction fragments for each type of enzyme. We calculated p for the five haplotypes (A, B, C, H, I) identified in the initial screening of 22 otters with 10–19 restriction enzymes for each segment of mtDNA. All fragment patterns, variant and invariant, were used in these calculations.

TABLE 1.—*Haplotypes of mitochondrial DNA of sea otters. Haplotypes are defined by the fragment patterns for the restriction enzymes listed across the top of the table; ND-3/4, ND-1, ND-5/6, and 12S–16S are the four segments of mtDNA analyzed. Haplotypes M and R are for the mink and river otter, respectively.*

	Restriction-fragment patterns									
	ND-3/4				ND-1			ND-5/6		12S–16S
Haplotype	*Xba* I	*Hinf* I	*Hha* I	*Bsp* 1286	*Hinf* I	*Bsp* 1286	*Hph* I	*Hinf* I	*Dde* I	*Ase* I
A*	A	A	A	A	A	A	A	A	A	A
B*	A	A	A	B	A	A	A	A	A	A
C*	B	B	B	B	B	B	B	B	A	A
D	B	B	B	B	B	B	B	C	A	A
E	B	B	B	B	B	B	B	B	A	B
F	B	A	A	B	A	A	A	A	A	A
H*	A	A	A	B	A	A	A	B	A	A
I*	B	B	B	B	A	A	B	B	B	B
J	A	A	A	B	A	A	A	B	B	A
M	B	D	D	B	D	D	D	E	D	C
R	B	C	C	C	C	C	C	D	C	B

* Haplotypes for which all 10–19 enzymes were used on each segment.

During subsequent analysis of 145 additional sea otters, 2 river otters, and 1 mink with only the enzymes revealing variable fragment patterns, additional variation, which defined new haplotypes, was detected. For an interspecies perspective, we calculated p among all haplotypes of sea otters, minks, and river otters using only the fragment patterns for the enzymes revealing variation.

Relationships of all nine haplotypes of sea otters and the haplotypes of minks and river otters were further assessed with the computer program Phylogenetic Analysis Using Parsimony (PAUP—Swofford, 1993). We used the branch-and-bound and bootstrap (100 replicates) options of the program, individual restriction fragments (Table 1, Appendix I) as characters, and minks and river otters as outgroups to generate cladograms.

We used the computer program BIOSYS (Swofford and Selander, 1981) to assess frequencies of haplotypes among populations. This included calculation of Nei's (1978) identity and generation of a dendrogram with the UPGMA (unweighted pair-group method using arithmetic averages) method (Sneath and Sokal, 1973). To assess variation of mtDNA within populations, we calculated diversity of haplotypes (Nei, 1987) within each sampling location.

RESULTS

Sizes in kilobases (kb) of amplified segments of mtDNA for the sea otter, mink, and river otter were ND-1 = 2.0 kb, ND-3/4 = 2.4 kb, ND-5/6 = 2.4 kb, 12S-16S rDNA = 1.7 kb. Each segment of mtDNA had variable restriction-fragment patterns for one or more enzymes, which occurred as nine haplotypes in sea otters, and one each in minks and river otters (Table 1, Appendix I).

Divergence of sequences, p, of the five haplotypes of mtDNA (A, B, C, H, I) were: 0.0006, A versus B; 0.0038, A versus C; 0.0010, A versus H; 0.0041, A versus I; 0.0032, B versus C; 0.0004, B versus H; 0.0034, B versus I; 0.0028, C versus H; 0.0018, C versus I; 0.0030, H versus I. There was no clear pattern of divergence of sequences and geographic proximity. For example, haplotypes that occurred at the geographic extremes of the species' range (haplotype C in California and haplotype I in the Kuril Islands) have an intermediate level of divergence, and haplotypes I and H occurred together in the Kuril Islands and had a relatively high level of divergence.

Using the limited set of enzymes that resulted in variable fragment patterns in sea otters (Table 1), we compared divergences of sequences of mtDNA among the sea otter, river otter, and mink. These divergences

of sequences were considerably higher than intraspecific divergences in sea otters. Divergence of mtDNA of river otters and sea otters (p = 0.0771–0.1012, $\bar{X}$ = 0.0912, SD = 0.0076) is higher than the divergence of mtDNA of mink and sea otter (p = 0.0688–0.0881, $\bar{X}$ = 0.0774, SD = 0.0067). The divergence of mtDNA of the mink and river otter is intermediate (p = 0.0887).

We also identified additional haplotypes of sea otters (D, E, F, J) using only the enzymes resulting in variable fragment patterns in sea otters (Table 1). These haplotypes differed from the other five haplotypes by only one or two fragment patterns. Relationships of all nine haplotypes of sea otters and haplotypes of minks and river otters were assessed by using 79 restriction fragments as characters in a phylogenetic analysis using parsimony. For a given haplotype, fragment patterns can be identified in Table 1 and the fragments for that pattern can be identified in Appendix I. Each haplotype is characterized by at least one unique fragment pattern and all haplotypes share several patterns (Table 1). For example, the close relationships of haplotypes C, D, and E (Fig. 2) result from sharing fragment pattern B for *Hinf* I, *Bsp*1286, and *Hph* I for the ND1 segment, and pattern B for *Hinf* I and *Hha* I for the ND-3/4 segment.

Two equally parsimonious trees (92 steps, consistency index = 0.859) were found and are summarized in a strict consensus tree (Fig. 2a). One of the two parsimonious trees is identical to the consensus tree. The other parsimonious tree differs from the consensus tree only in placement of haplotype A in a clade with haplotype B. A bootstrap analysis of 100 replicates resulted in one tree, identical to the consensus tree (Fig. 2a). In addition to the trees of 92-steps, we also considered trees of ≤95 steps. There were three trees with 93 steps (consistency index = 0.849), 14 trees with 94 steps (consistency index = 0.840), and 27 trees with 95 steps (consistency index = 0.832). A strict consensus tree of all 46 trees of 92–95 steps was constructed (Fig. 2b).

Phylogenetic analysis resulted in two major clades of haplotypes of sea otters, which are separate from clades of the mink and river otter. Haplotypes B, H, J, A, and F of sea otters and haplotypes C, D, I, and E occur in two separate clades. As was shown earlier with estimates of divergence of sequences, there is little concordance of phylogenetic relationship of haplotypes and geographic distribution (Fig. 2). Haplotypes from clades of different mtDNA of sea otters occur in the same population (e.g., H and I in the Kuril Islands). Conversely, haplotypes within a clade occur across the entire range of sea otters (e.g., haplotypes I in the Kuril Islands, E in Amchitka and Adak islands, and C and D in California). An exception to the lack of concordance of phylogeny and geography is the clustering in the most-parsimonious tree of two haplotypes, C and D, which occur only in California and may represent a monophyletic lineage (Fig. 2a).

Considerable population structure was apparent from distributions of haplotypes (Table 2, Fig. 3). Haplotypes vary across the range of the species from exclusively C and D in California, to primarily A in Prince William Sound, to mostly B and H in the Kodiak, Adak, Amchitka, Attu, and Medny islands, to primarily I in the Kuril Islands. Nei's (1978) identity values, derived from frequencies of haplotypes, ranged from zero to one. The population in California had unique haplotypes and, thus, had identities of zero with other populations. Populations from Kuril Island and Prince William Sound had relatively low identity values with the other populations and with each other (0.000–0.196). The populations from Medny, Attu, Amchitka, Adak, and Kodiak islands had relatively high identities with each other (0.285–1.000). Hence, four major groups are apparent in the dendrogram resulting from the UPGMA analysis of the genetic-identity values; California, Prince William Sound,

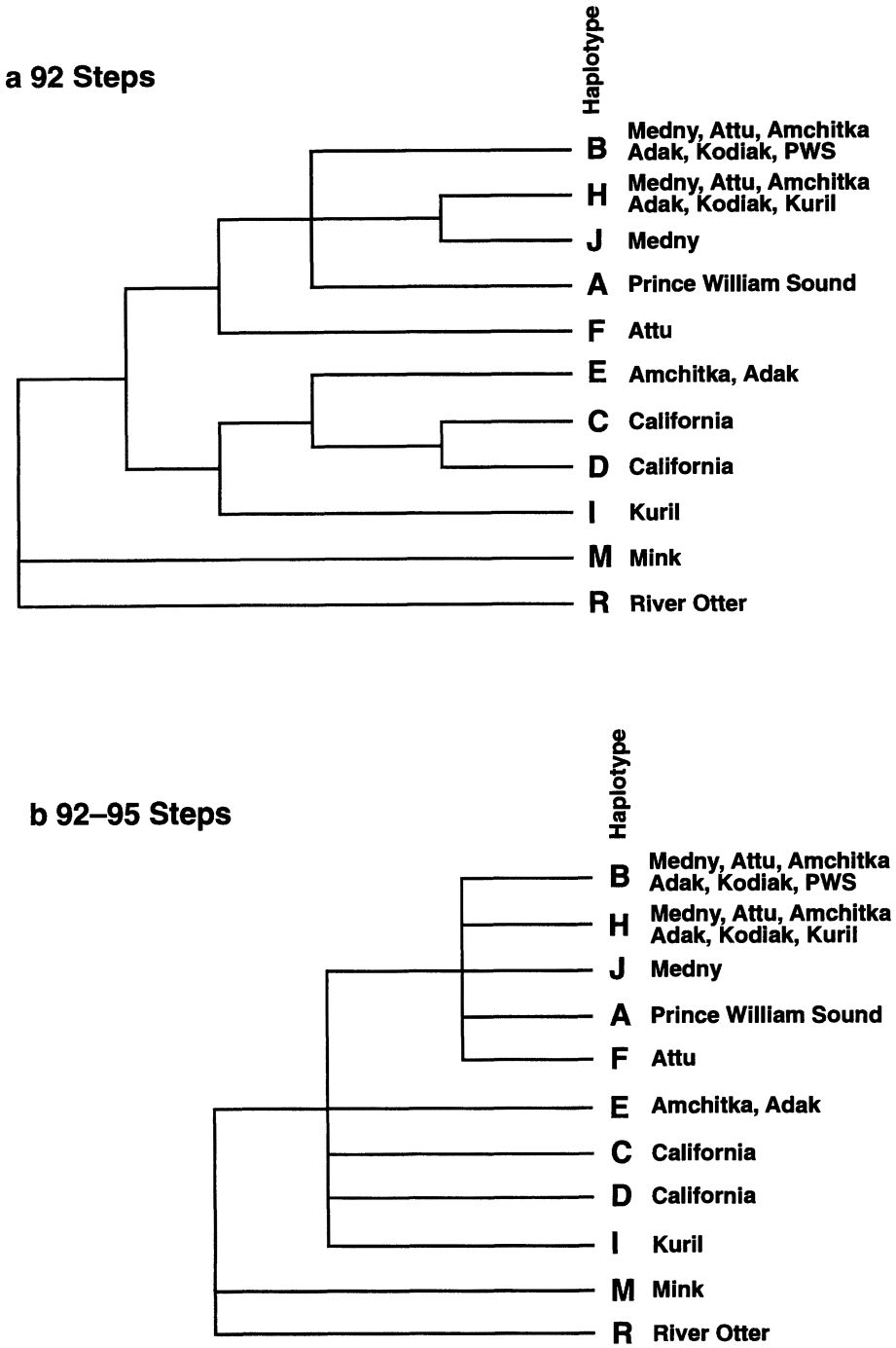

FIG. 2.—Strict consensus cladograms (a, length is 92 steps; b, length is 92–95 steps) of mtDNA haplotypes of sea otters made with PAUP using restriction fragments as characters (PWS = Prince William Sound).

TABLE 2.—*Numbers of haplotypes of mtDNA and diversity of haplotypes within populations of the sea otter, mink, and river otter.*

Population	(*n*)	Haplotypes											Haplotype diversity
		C	D	I	E	A	B	H	F	J	M	R	
Kuril Islands, Russia	(17)	0	0	14	0	0	0	3	0	0	0	0	0.2995
Medny Island, Russia	(24)	0	0	0	0	0	4	19	0	1	0	0	0.3511
Attu Island, Alaska	(20)	0	0	0	0	0	14	5	1	0	0	0	0.4564
Amchitka Island, Alaska	(20)	0	0	0	3	0	12	5	0	0	0	0	0.5692
Adak Island, Alaska	(21)	0	0	0	2	0	8	11	0	0	0	0	0.5854
Kodiak Island, Alaska	(14)	0	0	0	0	0	13	1	0	0	0	0	0.1376
Prince William Sound, Alaska	(31)	0	0	0	0	28	3	0	0	0	0	0	0.1777
California	(20)	13	7	0	0	0	0	0	0	0	0	0	0.4667
Mink	(1)	0	0	0	0	0	0	0	0	0	1	0	
River otter	(2)	0	0	0	0	0	0	0	0	0	0	2	
Totals	(170)	13	7	14	5	28	54	44	1	1	1	2	

Kodiak-Adak-Amchitka-Attu-Medny islands, and the Kuril Islands (Fig. 3).

Despite this population structure, distributions of haplotypes do not vary in a consistent manner among subspecies. *E. l. nereis* from California have unique haplotypes of mtDNA, which we already have shown may be phylogenetically distinct (Fig. 2). However, populations of each of the other two subspecies have considerably different frequencies of haplotypes. Populations from Kuril and Medny islands are considered *E. l. lutris,* but differ considerably in frequencies of haplotypes. Prince William Sound and the other populations in Alaska are considered *E. l. kenyoni,* but have different frequencies of haplotypes. At the intrapopulation level, each population has two or

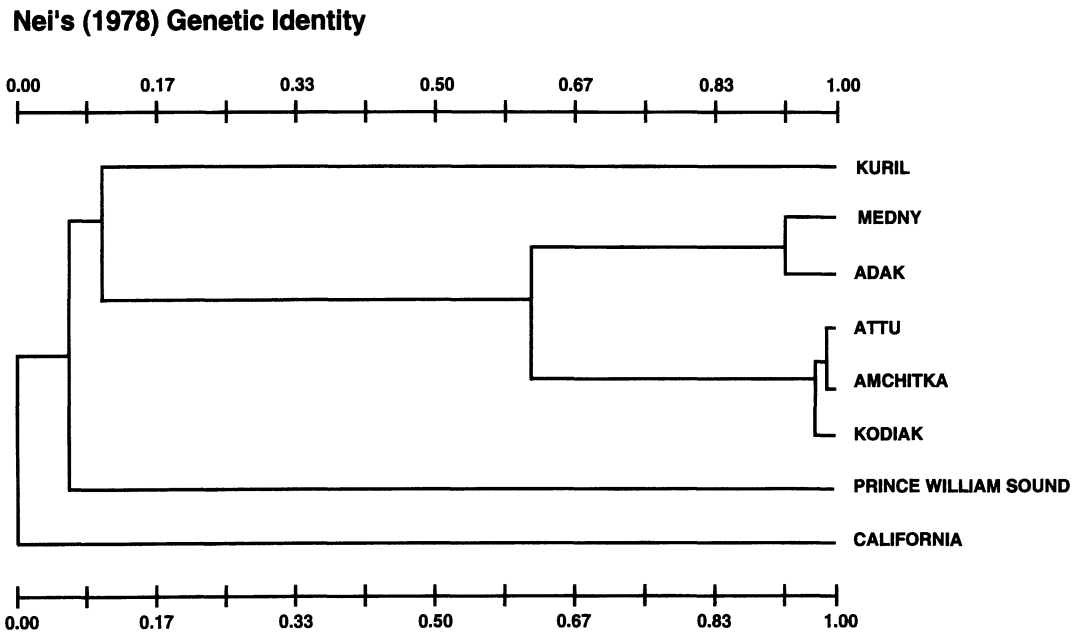

FIG. 3.—UPGMA dendrogram reflecting distributions of mtDNA haplotypes among populations of sea otters.

three haplotypes, and diversity of haplotypes is 0.1376–0.5854 (Table 2).

Discussion

Our estimates of divergence of sequences of mtDNA in sea otters (p = 0.0004–0.0041) are of the same order of magnitude as those reported by Sanchez (1992), who analyzed the entire mtDNA molecule with Southern blots (p = 0.0008–0.0060). This is a relatively low level of divergence of sequences considering these haplotypes were distributed across the entire range of sea otters. Our estimates of p should be considered approximations of divergences of mtDNA because restriction fragments were generated from four separate linear fragments from different parts of the mtDNA molecule and we did not map restriction sites.

Assuming the commonly used rate of divergence for humans of ca. 2% divergence of sequences of mtDNA per 10^6 years (Brown et al., 1979; Wilson et al., 1985), haplotypes of sea otters probably diverged over the past 20,000–200,000 years, during the late Pleistocene. The interspecies p-values (p = 0.0688–0.1012) were an order of magnitude higher than the intra-sea-otter values and suggest divergence of mtDNA of minks, river otters, and sea otters 3–5 $\times$ 10^6 years ago. However, use of mtDNA to estimate times of divergence of taxa must be done with caution because single-locus phylogenies may not reflect species phylogenies (Pamilo and Nei, 1988). In addition, interspecies estimates of p were made with only the enzymes found to be variant in sea otters and could be considered overestimates of actual divergence. However, it could be argued that there is no reason these enzymes are more likely to show variation among these species, so these estimates may be as good as any using the limited number of enzymes and segments of mtDNA in our analysis. Further assessment of interspecies relationships of mtDNA in mustelids is needed.

Enhydra l. nereis appears to have monophyletic haplotypes of mtDNA, which are not shared with other populations; whereas, *E. l. lutris* and *E. l. kenyoni* do not. For example, the population at Medny Island, which is considered *E. l. lutris,* based on morphology (Wilson et al., 1991), has frequencies of haplotypes of mtDNA similar to populations of *E. l. kenyoni* (Fig. 3). These results are consistent with those of Sanchez (1992) who identified four monophyletic haplotypes of mtDNA in sea otters from California, and one haplotype shared by otters from the Kuril Islands and Prince William Sound. However, it is important to note that monophyly of the haplotypes in California is not unequivocal (Fig. 2b), and other gene phylogenies should be integrated with morphology and other traits for taxonomic designations (Cronin, 1993).

Regardless of subspecies designations, the distribution of haplotypes of mtDNA suggests little or no female-mediated gene flow among several of the sampling locations at present. This may reflect population structure predating exploitation by humans during the 18th and 19th centuries. Alternatively, severe population bottlenecks may have resulted in genetic drift and changes in frequencies of haplotypes, so current genetic patterns may not reflect pre-exploitation conditions. Although we cannot be certain, frequencies of haplotypes are distinct enough between California, Prince William Sound, the Kodiak-Adak-Amchitka-Attu-Medny islands, and the Kuril Islands to suggest these four groups were somewhat differentiated before exploitation by humans. Regardless of the causes of distributions of haplotypes, frequencies of haplotypes of mtDNA could be used in conjunction with other data to identify populations that could be managed as independent units.

Despite different frequencies of haplotypes among locations, the low level of divergence of sequences of haplotypes of mtDNA in sea otters across their range suggests there are no major phylogenetic breaks or long-term barriers to gene flow

(Avise et al., 1987). Habitat and distributional changes of sea otters during Pleistocene glacial periods probably resulted in episodic population fluctuations, extinctions, recolonization, and high levels of gene flow, as in many other species (Cronin, 1992; Cronin et al., 1991*b*; Sage and Wolff, 1986; Slatkin, 1987). On a shorter time scale, the relatively rapid recovery of decimated populations indicates sea otters are capable of rapid dispersal and recolonization (Lubina and Levin, 1988). As populations continue to expand, mixing of populations may occur, which may change the genetic patterns reported here.

Given the lack of pre-bottleneck, genetic data, it is difficult to assess the extent of loss of genetic variation in sea otters, but a few comments are warranted. First, there is apparently low allozymic variation in some populations of sea otters. Three of our study populations (Adak Island, Attu Island, Prince William Sound) were monomorphic at 24 allozymic loci (M. Cronin and B. May, in litt.). Rotterman (1992) also found a small proportion of polymorphic loci in sea otters (three of 41 loci). However, A. Burdin (pers. comm.) found 17 of 46 protein loci to be polymorphic in sea otters from the Kuril Islands. Other marine mammals with few, or no, polymorphic allozymic loci include elephant seals (*Mirounga angustirostris*), which have undergone severe, human-induced, population bottlenecks (Bonnell and Selander, 1974), and polar bears (*Ursus maritimus*), which have not (Allendorf et al., 1979; Larsen et al., 1983). Other mustelids may have considerable allozymic variation (Mitton and Raphael, 1990) or none at all (Simonsen, 1982). Low allozymic variation is not restricted to mustelids or populations that have undergone bottlenecks. Allozymes may be subject to selection, and their use to infer gene flow, overall levels of genetic variation, or genetic drift must be done with caution (Karl and Avise, 1992).

Second, numbers of haplotypes of mtDNA within populations of sea otters, or the species as a whole, are not particularly low compared to other mammals (Cronin, 1992; Cronin et al., 1991*b*). Sanchez (1992) found four haplotypes of mtDNA in sea otters from California, which indicates our analysis gives an underestimate of variation of mtDNA in this population. Our results, and those of Sanchez (1992), suggest many haplotypes were maintained in the population in California, and other populations as well. Diversities of haplotypes in populations of sea otters (0.1376–0.5854; Table 2) fall within the range observed for polar bears (0.4478–0.5111—Cronin et al., 1991*b*) and walruses (0.2001–0.8889—Cronin et al., 1994).

These data and the predictions of Ralls et al. (1983) suggest that a considerable amount of the genetic variation of the species as a whole may have been maintained among the multiple population isolates during the period of reduced populations and recovery. The relationship between genetic variation and fitness is complex, and patterns vary among species (Avise, 1994; Caro and Laurenson, 1994; Mitton and Grant, 1984). However, presence of considerable variation in mtDNA and rapid recovery of populations of sea otters suggests that there are no genetic manifestations of population bottlenecks apparent at this time.

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APPENDIX I.

Variable-mtDNA-restriction-fragment patterns for four segments (ND-3/4, ND-1, ND-5/6, 12S–16S) of mtDNA of the sea otter, river otter, and mink. Fragment sizes are in base pairs. For example, fragment-pattern A, for the ND-3/4 segment and restriction enzyme Xba *I, consists of fragments 1,850 and 550 base pairs in length.*

Restriction enzyme	Base pairs	Fragment pattern A	B	C	D
ND-3/4[a]					
Xba I	2,400		+		
	1,850	+			
	550	+			
Hinf I	860	+	+	+	+
	560			+	+
	530	+	+	+	
	500	+	+	+	
	450		+		
	410	+			
	380				+
	150	+	+	+	+
Hha I	2,400				+
	1,620	+			
	1,440			+	
	1,200		+		
	980			+	
	780	+	+		
	650		+		
*Bsp*1286	2,300	+			
	1,370		+		
	1,300			+	
	1,100			+	
	1,000		+		

APPENDIX I.—*Continued.*

Restriction enzyme	Base pairs	Fragment pattern A	B	C	D
ND-1[b]					
Hinf I	690		+		
	620	+		+	+
	540				+
	495			+	
	450			+	+
	370	+	+		
	335				+
	300	+	+		
	230	+	+		+
	185	+	+	+	
*Bsp*1286	860			+	+
	811	+	+		+
	690	+	+		
	580			+	
	300	+		+	+
	180	+	+		
	170		+		
	100	+	+	+	
Hph I	980	+			
	935		+		
	810				+
	620			+	
	615				+
	400	+	+	+	+
	265			+	
	255	+	+		+

Restriction enzyme	Base pairs	Fragment pattern A	B	C	D	E
ND-5/6[c]						
Hinf I	800				+	
	780				+	
	620					+
	550					+
	520	+	+	+		+
	470	+	+	+		
	450	+	+	+	+	
	440	+		+		
	400			+		
	266					+
	240		+			
	220	+	+	+		+
	190				+	
	140	+	+	+		+
	100	+	+	+		+

APPENDIX I.—*Continued.*

Restriction enzyme	Base pairs	Fragment pattern A	B	C	D	E
ND-5/6[c]						
Dde I	1,000			+		
	850				+	
	750				+	
	600	+	+			
	590			+		
	400	+	+			
	385			+	+	
	375	+	+			
	360		+			
	350	+	+	+		
	300	+				
	275	+	+			
	200	+	+			

Restriction enzyme	Base pairs	Fragment pattern A	B	C
12S–16S[d]				
Ase I	1,550		+	
	1,400	+		
	980			+
	600			+
	190	+		

[a] Restriction enzymes that resulted in invariant patterns for ND-3/4 (and number of fragments) were *Ase* I(5), *Ava* II(2), *Bsa*J I(3), *Bsl* I(3), *Bsr* I(3), *Bst*N I(3), *Bst*U I(3), *Dde* I(4), *Dpn* II(3), *Hae* III(5), *Hin*d III(3), *Hph* I(5), *Rsa* I(2), and *Ssp* I(2).

[b] Restriction enzymes that resulted in invariant patterns for ND-1 (and number of fragments) were *Ase* I(3), *Ava* II(3), *Bsa*J I(3), *Bsr* I(4), *Bst*N I(2), *Bst*U I(2), *Dde* I(5), *Dpn* II(3), *Hae* III(4), *Hha* I(3), *Hin*c II(2), *Hin*d III(2), *Msp* I(3), *Rsa* I(3), *Ssp* I(3), and *Tag* I(5).

[c] Restriction enzymes that resulted in invariant patterns for ND-5/6 (and number of fragments) were *Ava* II(2), *Bsa*J I(5), *Bsl* I(2), *Bsp*1286(2), *Bsr* I(2), *Eco*R I(2), *Hae* III(3), *Msp* I(3), *Rsa* I(4), *Ssp* I(2), and *Tag* I(4).

[d] Restriction enzymes that resulted in invariant patterns for 12S–16S (and number of fragments) were *Aci* I(3), *Bsa*J I(2), *Bsr* I(2), *Dpn* II(2), *Dra* I(3), *Hae* III(2), *Hin*c II(2), *Rsa* I(3), and *Tag* I(3).

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