HIGH VARIABILITY FOR CONTROL-REGION SEQUENCES IN A MARINE MAMMAL: IMPLICATIONS FOR CONSERVATION AND BIOGEOGRAPHY OF STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

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The Steller sea lion (*Eumetopias jubatus*) is a threatened species that has experienced significant population declines over the past 3 decades. Previous genetic studies indicated low allozymic variability in this monotypic species. However, high levels of variation exist in the mitochondrial control-region, as revealed by a 238 base-pair sequence from 224 specimens taken over most of the range of the species. Patterns of macrogeographic variation indicate the presence of two genetically differentiated populations of Steller sea lions. A western population included rookeries from the Commander Islands in Russia and the Aleutian Islands and Gulf of Alaska in Alaska. An eastern population included rookeries from southeastern Alaska and Oregon. Phenetic analysis of the mitochondrial-DNA (mtDNA) haplotypes indicates that certain haplotype lineages are specific to one or the other populations. Thus, these populations have been separated for a sufficient amount of time to allow diversification of lineages. However, the two populations are paraphyletic with respect to mtDNA, which indicates that they do not trace their ancestries back to a single maternal ancestor in either case. The populations likely diverged as a result of separation in different glacial refugia.

Key words: marine mammals, Steller sea lions, *Eumetopias jubatus,* mitochondrial DNA, phylogeography

The Steller sea lion (*Eumetopias jubatus*) is the largest species of the family Otariidae and is distributed along the North Pacific Ocean rim from Hokkaido, Japan, north to the Commander Islands, east across the Aleutian Island chain to mainland Alaska, and south to southern California (Loughlin et al., 1987). Centers of distribution and abundance encompass the Aleutian Islands and the Gulf of Alaska, which include most of the largest rookeries (Calkins and Pitcher, 1983; Kenyon and Rice, 1961; Loughlin et al., 1984, 1992). This species has experienced a precipitous decline in numbers from ca. 240,000–300,000 in the 1960s (Kenyon and Rice, 1961) to ca. 116,000 in 1989 (Loughlin et al., 1992). This represents a decline of 39–48% in the worldwide population and led to this species being listed as threatened under the United States Endangered Species Act in 1991. This decline, which amounts to a reduction of ca. 18% per year, has not occurred at the same rate in all populations (Merrick et al., 1987). Therefore, some populations are experiencing drastic reductions in numbers (such as in the Aleutian Islands, where populations have declined by 81%), whereas populations in other areas have either not changed (British Columbia) or have increased slightly (southeastern Alaska) since the early 1960s (Loughlin et al., 1992; Merrick et al., 1991).

Because different populations are threatened to varying degrees, it is desirable to have the ability to identify the natal rookery or geographic area of origin for individuals, such as when individuals are killed during commercial fishing activities (Loughlin et al., 1983). In this way, conservation and management of Steller sea lions could be conducted on a local or regional level rather than being based on species-level patterns of abundance. The Steller sea lion is a monotypic species (Hall, 1981; Loughlin et al., 1987) and morphological characters are not known to be sufficiently variable for this purpose. Protein electrophoresis also has proven of little use in this regard because of a near absence of allozymic variation in this species (Lidicker et al., 1981).

Many species of marine mammals are known to possess low genetic variability based on allozymic studies (Bonnell and Selander, 1974; Lidicker et al., 1981; Shaughnessy, 1974; Testa, 1986) and restriction-enzyme analyses of MHC (major histocompatibility complex) genes (Slade, 1992; Trowsdale et al., 1989). Bonnell and Selander (1974) explained the low level of genetic variation in the northern elephant seal (*Mirounga angustirostris*) as the result of a severe population bottleneck in historical times. Although a hypothesis based on historical bottlenecks is plausible for the elephant seal, it does not likely explain the low level of allozymic variation observed in Steller sea lions (Lidicker et al., 1981) or the low level of MHC polymorphism observed in the southern elephant seal and two species of whales (Slade, 1992). Alternatively, it is possible that something about the marine environment or the biology of marine mammals has resulted in reduced genetic variation through some selective process. With regard to the MHC loci, Slade (1992) suggested that low variability could be due to decreased exposure to parasite diversity. Whatever the reason, from a theoretical standpoint low genetic variability is believed to be potentially harmful because it increases the probability of extinction and reduces the ability of a population to cope with environmental variability (Beardmore, 1983). Thus, the accurate determination of genetic variability in natural populations is a critical concern for population management. This is particularly true for endangered or threatened species wherein genetic drift or inbreeding in small populations can result in the erosion of genetic variability and potentially increase the probability of extinction.

The purpose of this study was to investigate genetic variability and the evolution of maternal lineages in Steller sea lions using a nucleotide sequence analysis of the mitochondrial control region. Mitochondrial DNA (mtDNA) was used because it is inherited in a clonal fashion through the maternal lineage and evolves at a rate 5–10× faster than nuclear genes (Wilson et al., 1985). The control region was selected for analysis because of the presence of a hypervariable region, which results in the overall rate of evolution of this portion of the molecule being 5× greater than the mtDNA in general (Cann et al., 1987; Greenberg et al., 1983). Although the Steller sea lion has been shown to have low levels of genetic variability in nuclear genes (Lidicker et al., 1981), the rapidly evolving mtDNA control-region could provide sufficient genetic variation to discern any existing pattern of population subdivision (which was not demonstrated by Lidicker et al., 1981). Subdivision for the maternally inherited mtDNA could be expected because of the presumed high degree of philopatry of females in this species (Gentry, 1970). Finally, it was our goal to determine if a molecular approach to population genetics would be useful in identification of management units and to provide genetic markers that could be used to identify the population of origin of individuals collected away from their rookeries.

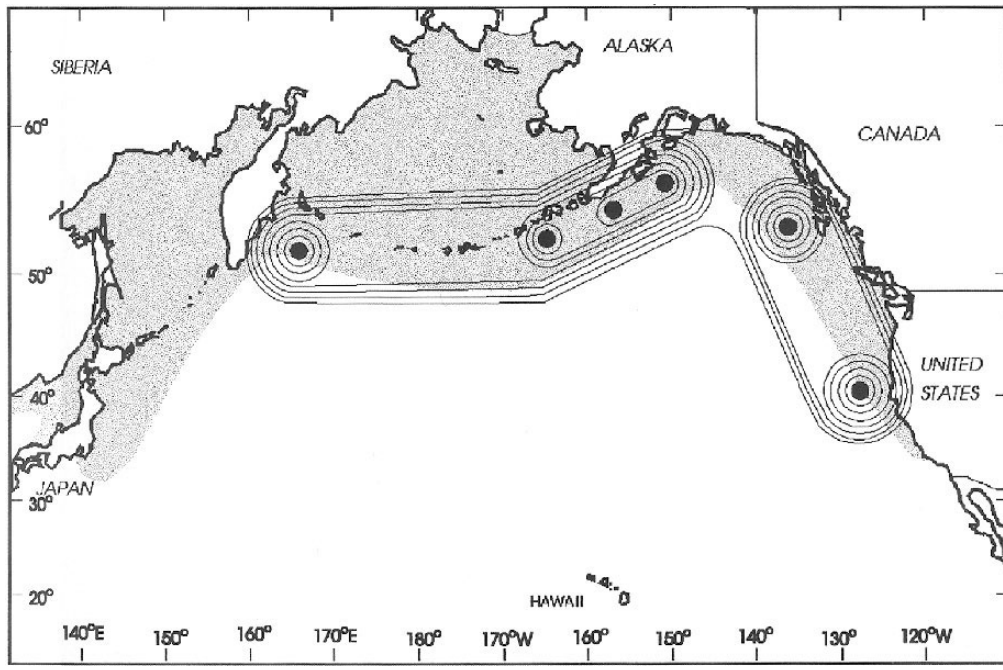

FIG. 1.—Genetic identity among six grouped localities of Steller sea lions. The grouped localities from west to east are: Russia, eastern Aleutians, western Gulf of Alaska, central Gulf of Alaska, southeastern Alaska, and Oregon. The contour lines are drawn at intervals of 10% genetic identity and were computed by a UPGMA cluster of Nei's unbiased genetic identity. All samples are joined at a contour level of 30% identity. Shaded area of the map is the approximate oceanic distribution of the Steller sea lion.

MATERIALS AND METHODS

Samples (n = 226; including 224 Steller sea lions and 2 California sea lions; *Zalophus californianus*), which were collected in 1991–1993, included frozen white-blood cells (n = 40) and skin samples (n = 186) from punches of the flipper. The latter samples were preserved in saturated NaCl with 20% dimethylsulphoxide (DMSO) and stored at room temperature (Amos and Hoelzel, 1991). Six populations (grouped localities) were sampled (Fig. 1) from Russia to Oregon, which spans most of the range of distribution of the species (Loughlin et al., 1987). Samples (n = 201) collected from rookeries during the breeding season and used in the population analyses were as follows: RUSSIA; Zheleznaya Bay (on the Kamchatka Peninsula) n = 12; Kozlova Cape (on the Kamchatka Peninsula), n = 10; Medny Island, n = 22. EASTERN ALEUTIANS; Ugamak Island, n = 17; Akun Island, n = 2; Bogoslof Island, n = 10; Akutan Island, n = 11. WESTERN GULF OF ALASKA; Pinnacle Rocks, n = 11; Whaleback Islets, n = 2; Chernabura Island, n = 5; Atkins Island, n = 17; CENTRAL GULF OF ALASKA; Marmot Island, n = 20; Sugarloaf Island, n = 7; Chirikof Island, n = 11. SOUTHEASTERN ALASKA; White Sisters Islands, n = 12; Hazy Islands, n = 11; Forester Island, n = 6. OREGON; Rogue Reef, n = 15. Additional samples (n = 23) were taken from specimens outside of the breeding season, and thus are of uncertain population affinity.

Genomic DNA was extracted by established protocols (Maniatis et al., 1982). A segment of the control-region ca. 450 base pairs (bp) in length was amplified using the polymerase chain reaction (PCR). We used 50 µl reactions, which consisted of the following: 0.1–0.5 µg genomic DNA; 5 µl 10× buffer (0.1 M Tris-HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl), 5 µl dNTP mix (2 mM dATP, dTTP, dCTP, dGTP, in 0.1 M Tris-HCl, pH 7.9), 5 µl of a 10 µM solution of each primer, 0.025-0.5 µl Taq DNA polymerase, and

brought the volume to 50 μl with deionized water. Primers LGL 283 (5′-TACACTGGTCTTGT AAACC-3′), which attaches to a site within the $tRNA^{Thr}$ gene, and LGL 1115 (5′-ATGACCCT-GAAGAA(A/G)GAACCAG-3′), which recognizes a site within the control-region, were used in the PCR reaction. Amplifications were done by 32 cycles of 95°C for 45 s of denaturing, 50°C for 40 s of annealing, 70°C for 2.5 min of extension, and 4 s of autoextension. Sequencing was performed using automated, DNA-sequence analysis employing dye-labelled terminators (Carr and Marshall, 1991; Ferl et al., 1991). The PCR-amplified products were sequenced from the LGL 1115 primer end with an-ABI 373A automated DNA sequencer. A segment of the control-region of 238 bp was analyzed for each specimen. Haplotypes were identified by nucleotide differences and were assigned letter designations.

A phenetic analysis was performed to assess the relationships among haplotypes using the Phylogenetic Inference Package (PHYLIP) of Felsenstein (1993). A distance matrix was produced using the Jukes and Cantor (1969) model, which assumes independant change at all sites and equal probability of a site being changed to any of the three alternative nucleotides. The tree was constructed using the FITCH option, which does not assume a molecular clock and, thus, branch lengths are not constrained.

Genotypic diversity was estimated using the nucleon diversity index (h) of Nei and Tajima (1981; equation 7). Nucleon diversity was estimated for each locality and for the total population. Estimates of population subdivision were performed by calculating F_{ST} values for each haplotype using the program of Weir (1990). Estimates of gene flow, calculated as N_em or the number of effective female migrants per generation, were calculated using the equation

$$F_{st} = 1/2N_em + 1,$$

which assumes an island model of distribution (Wright, 1943). Genetic differentiation among populations was investigated by estimating Nei's (1978) unbiased genetic identity among populations using frequencies of haplotypes. A cluster analysis was performed on the genetic identities using the unweighted-pair-group method using arithmetic averages (UPGMA) in the program BIOSYS-1 (Swofford and Selander, 1981). Data were entered in BIOSYS-1 as a single locus with multiple alleles.

Results

Sequence analysis of a 238 bp segment of the control-region of the mtDNA (Fig. 2) revealed 29 variable sites among the 224 Steller sea lions (Table 1). Only two nucleotides were observed at any of the variable positions, and these substitutions included 25 transitions (including 16 T ↔ C and 9 A ↔ G) and 4 transversions (including 2 T ↔ G at positions 63 and 118, an A ↔ T at position 131, and an A ↔ C at position 224). The high transition-to-transversion ratio (25:4) observed in this study is usual for control-region sequence analyses involving conspecific populations (Greenberg et al., 1983; Thomas et al., 1990).

The 29 variable sites defined 52 haplotypes, designated A–Z and AA–ZZ (Table 1). The average percent sequence divergence among the 52 haplotypes was 1.7% with a range of 0.4–3.8%. Percent sequence divergence between haplotype A of the Steller sea lion and haplotype 1 of the California sea lion was 9.6% (Fig. 2). Of the 52 haplotypes, 49 were found among the 201 individuals taken from rookeries during the breeding season (Table 2). The remaining three haplotypes (KK, LL, and XX) were each found only in animals taken outside of the breeding season. Individuals taken outside the breeding season, and their respective haplotypes, are as follows: EASTERN ALEUTIANS; Akun Island, n = 1 (haplotype W). CENTRAL GULF OF ALASKA; Latax Rocks, n = 1 (A); Long Island, n = 2 (G and S); Kodiak Island, n = 1 (BB); Sea Otter Island, n = 1 (XX). EASTERN GULF OF ALASKA; Cape St. Elias, n = 3 (all S). PRINCE WILLIAM SOUND; Sea Lion Rocks, n = 3 (K, L, LL). SOUTHEASTERN ALASKA; Cape Horn Rocks, n = 3 (two H, one BB); North Rocks, N = 3 (A, H, N). CENTRAL COAST OF WASHINGTON STATE; no exact locality, n = 5 (A, L, P, AA, KK).

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                        10          20          30          40          50
Eumetopias (A)    CCCCCCATGT  ATATCGTGCA  TTAATGGCTT  GCCCCATGCA  TATAAGCATG
Zalophus 1        .....T....  .C........  ..G....T..  ..........  ..........

                        60          70          80          90         100
Eumetopias (A)    TACATATTAT  GATTGATTTT  ACATAATGAC  ATGAACTCCA  ATACCTTGAT
Zalophus 1        .......C..  ..........  ....G.....  ..A.CT..A.  ...AT....C

                       110         120         130         140         150
Eumetopias (A)    CTAAACACTA  TGACTTCTTG  GAGCGGATGT  AACTCACTTA  GTCCACGAAG
Zalophus 1        TC...T....  .A........  AT..AA....  ..T.......  ..........

                       160         170         180         190         200
Eumetopias (A)    CTTGATCACC  AGGCCTCGAG  AAACCAGCAA  CCCTTGTGAA  AAGTGTACCT
Zalophus 1        ..........  ..........  ..........  ..........  ..........

                       210         220         230         238
Eumetopias (A)    CTTCTCGCTC  CGGGCCCATC  TTAACGTGGG  GGTAGCTA
Zalophus 1        ..........  ..........  .C........  ........
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FIG. 2.—Aligned control-region sequences for mtDNA of Steller sea lions (*Eumetopias jubatus*), haplotype A, and California sea lions (*Zalophus californianus*) haplotype 1.

Phenetic analysis was performed on the dataset to estimate the evolutionary relationships of maternal lineages. Because of the large number of haplotypes represented by only one or a few individuals (Table 2), we used the FITCH analysis to identify groups of related haplotypes. Eight groups of haplotypes, referred to as lineages 1–8, are recognized and consist of 2–16 haplotypes (Fig. 3).

The distribution of haplotypes (Table 2) shows evidence of population subdivision for maternal lineages. The eight lineages, composed of multiple haplotypes, include four (lineages 1, 2, 7, and 8) that are restricted to southeastern Alaska and Oregon, two that are restricted to Russia, eastern Aleutians, central Gulf of Alaska, and western Gulf of Alaska (lineages 3 and 6), and two that are restricted to Russia, eastern Aleutians, western Gulf of Alaska, central Gulf of Alaska, and southeastern Alaska (lineages 4 and 5).

Cluster analysis (Fig. 1) using Nei's (1978) unbiased genetic identity (Table 3) indicates that the six grouped localities are subdivided into two genetically differentiated populations including an eastern population (Oregon and southeastern Alaska) and a western population (Russia, eastern Aleutians, western Gulf of Alaska, and central Gulf of Alaska). The eastern and western populations cluster at an identity of 0.299. Among the four localities in the western population, Russia is the most divergent, clustering at an identity of 0.62. The remaining three populations have high levels of genetic identity (all >0.8). Considering all localities, the highest genetic identity was between the two populations from the Gulf of Alaska (0.968) and the lowest between Oregon and the western Gulf of Alaska (0.103).

Nucleon diversity estimates (h; Nei and Tajima, 1981) for each population were as follows: Oregon, 0.936; southeastern Alaska, 0.948; central Gulf of Alaska, 0.814; western Gulf of Alaska, 0.884; eastern Aleutians, 0.919; Russia, 0.892. Nucleon diversity for all populations combined was $h = 0.927$. The maximum value of h is 1.0 if all members of a population possess dif-

TABLE 1.—*MtDNA haplotypes represented among samples of Steller sea lions as defined by nucleotide sequence at 29 variable nucleotide positions.*

Haplo-types	Nucleotide position																												
	6	12	24	28	57	58	59	63	67	76	94	102	106	108	112	114	118	119	121	125	126	131	133	134	163	181	207	222	224
A	C	T	A	C	T	T	A	T	T	A	C	T	C	C	G	C	T	T	G	G	G	A	C	T	G	C	G	T	A
B					C									T															
C																					A								
D														T													A		
E																													C
F												C																	C
G												C																	
H														T															
I	T												T	T									T						
J	T													T									T					C	
K	T													T															
L	T													T	A									C					
M	T													T										C					
N														T										C					
O	T													T					A										
P		C								G									A										
Q										G									A										
R					C					G									A										
S										G										A									
T	T									G										A			T						
U	T									G										A									
V							G													A			T						
W							G													A									
X							G			G										A									
Y										G										A		T							
Z										G																		C	
AA										G																	A		
BB										G																			
CC										G	T																		
DD		C								G	T																		
EE	T										T																		
FF	T									G																			

TABLE 1.—*Continued.*

Haplotypes	Nucleotide position																												
	6	12	24	28	57	58	59	63	67	76	94	102	106	108	112	114	118	119	121	125	126	131	133	134	163	181	207	222	224
GG							G			G										A					A		A		
HH							G		C	G										A									
II									C	G										A									
JJ										G										A							A		
KK						C				G							G			A									
LL														T									T						
MM								G		G										A					A				
NN										G										A			T						
OO										G															A				
PP										G																	A	C	
QQ			G					G		G																		C	
RR		C								G																			
SS						C								T		T										T			
TT										G										A					A		A		
UU				T	C					G																		C	
VV																									A				C
WW																				A									
XX											T																		C
YY													T	T									T						
ZZ	T													T				C						C			C		
Zalophus 1	T	C		T		C					A	C	T		A				A	A	A		T				C		
Zalophus 2		C	G	T		C						C	T		A				A	A	A						C		

TABLE 2.—*Numbers of individuals and frequencies in parentheses per haplotype are given for each of six grouped localities of Steller sea lions* (Eumetopias jubatus). *All individuals were taken from rookeries during breeding season. Three haplotypes (KK, LL, and XX) identified only from individuals taken outside the breeding season are not included in this table. Grouped localities are listed from west to east and include Russia (RUS), eastern Aleutians (EAL), western Gulf of Alaska (WGA), central Gulf of Alaska (CGA), southeastern Alaska (SEA), and Oregon (ORE) as illustrated in Fig. 1.*

	Localities (*n*)						
Haplotypes	RUS (44)	EAL (40)	WGA (35)	CGA (38)	SEA (29)	ORE (15)	TOTAL (201)
A	7 (0.16)	1 (0.03)	4 (0.11)		4 (0.14)		16
B						1 (0.07)	1
C		1 (0.03)					1
D					1 (0.03)		1
E	2 (0.05)	3 (0.08)	6 (0.16)	3 (0.08)			14
F		1 (0.03)					1
G		1 (0.03)	1 (0.03)	1 (0.03)			3
H					4 (0.14)	2 (0.14)	6
I						1 (0.07)	1
J						1 (0.07)	1
K					3 (0.10)		3
L					2 (0.07)		2
M						1 (0.07)	1
N						3 (0.20)	3
O					1 (0.03)		1
P						1 (0.07)	1
Q					2 (0.07)	1 (0.07)	3
R					1 (0.03)	1 (0.07)	2
S	3 (0.07)	9 (0.23)	8 (0.22)	13 (0.35)	1 (0.03)		34
T			1 (0.03)				1
U	1 (0.02)	2 (0.05)		1 (0.03)			4
V		1 (0.03)					1
W		2 (0.05)		1 (0.03)			3
X	1 (0.02)						1
Y				1 (0.03)			1
Z	3 (0.07)	2 (0.5)	8 (0.22)	8 (0.22)			21
AA	2 (0.05)						2
BB	12 (0.27)	4 (0.10)	2 (0.05)	4 (0.11)	3 (0.10)	3 (0.20)	28
CC	2 (0.05)	4 (0.10)	2 (0.05)	2 (0.05)			10
DD	1 (0.02)	3 (0.08)		1 (0.03)			5
EE	1 (0.02)	1 (0.03)		1 (0.03)			3
FF	2 (0.05)				1 (0.03)		3
GG	1 (0.02)						1
HH	2 (0.05)						2
II		1 (0.03)					1
JJ	2 (0.05)	2 (0.05)					4
KK							
LL							
MM			1 (0.03)				1
NN			1 (0.03)				1
OO		1 (0.03)					1
PP	1 (0.02)						1
QQ			1 (0.03)				1
RR				1 (0.03)			1
SS					1 (0.03)		1
TT		1 (0.03)					1
UU					1 (0.03)		1
VV	1 (0.02)						1
WW				1 (0.03)			1
XX							
YY					2 (0.07)		2
ZZ					2 (0.07)		2

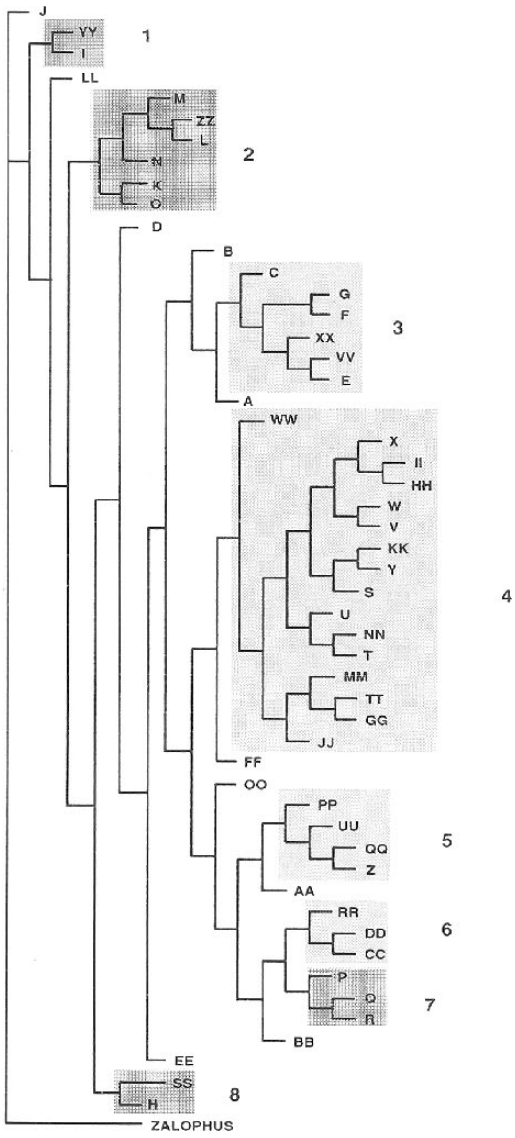

FIG. 3.—Dendrogram of the 52 haplotypes of Steller sea lions generated from distance estimates by use of the Fitch-Margoliash method (program FITCH). Eight lineages (shaded areas of the tree) are identified that include two to 16 related haplotypes that show restricted distributions. Lineages 1, 2, 7, and 8 (dark shading) are composed of haplotypes found only in southeastern Alaska and Oregon. Lineages 3 and 6 (pale shading) are composed of haplotypes found only in the four western localities (Russia, eastern Aleutians, western Gulf of Alaska, and central Gulf of Alaska). Lineages 4 and 5 (pale shading) comprise haplotypes restricted to the four western localities, but also include a single haplotype in each lineage that is found in southeastern Alaska.

ferent mtDNA haplotypes, and the minimum value is 0 if all members have the same haplotype. Thus, populations of Steller sea lions have high levels of genotypic diversity for mtDNA.

Estimate of the overall F_{st} for mtDNA of the Steller sea lion was 0.05 (95% confidence limits = 0.02-0.08). This yields an estimate of the effective number of female migrants per generation to be $N_em = 9.5$.

DISCUSSION

Steller sea lions possess a high level of genetic diversity as measured by nucleotide sequence of the mitochondrial control region. We identified a total of 52 haplotypes in the 224 individuals analyzed and the maximum number of individuals that shared a haplotype was 34 for haplotype S. Thus, the pattern that is evident from these data is that there is no common haplotype (or haplotypes) that predominates throughout the range of this species. Rather, there are many haplotypes of relatively low frequency.

There is a distinct break in distribution of haplotypes between the four western localities and the two eastern localities, with the southeastern Alaska locality being somewhat intermediate between these two major groups, both in position and composition of haplotypes. The FITCH analysis indicates that related haplotypes tend to be geographically sequestered. The most hierarchical structure occurs between the two eastern populations (southeastern Alaska and Oregon). Maternal lineages 1, 2, 7, and 8 (Fig. 3) include phylogenetically related haplotypes that are restricted in distribution to these two localities (Table 2). Moreover, six of the 10 haplotypes present in the samples from Oregon are found only at that locality and nine of the 15 haplotypes from the southeastern Alaska samples are found only at that locality. Three haplotypes (H, Q, and R) are restricted to the localities in southeastern Alaska and Oregon, and nine haplotypes are restricted to two or more of the four western localities. The southeast-

TABLE 3.—*Nei's (1978) unbiased genetic identity estimated for all pairwise comparisons of the frequencies of mitochondrial-DNA haplotypes from six populations of Steller sea lions* (Eumetopias jubatus).

	Russia	Eastern Aleutians	Western Gulf of Alaska	Central Gulf of Alaska	Southeastern Alaska	Oregon
Russia		0.633	0.647	0.582	0.595	0.488
Eastern Aleutians			0.821	0.935	0.285	0.169
Western Gulf of Alaska				0.968	0.352	0.103
Central Gulf of Alaska					0.240	0.161
Southeastern Alaska						0.536
Oregon						

ern Alaska locality shares only four haplotypes with any of the four western localities and there is a single haplotype (BB) found among all populations. The western population (central Gulf of Alaska, western Gulf of Alaska, eastern Aleutians, and Russia) is characterized by lineages 3–6 (groups of phylogenetically related haplotypes; Fig. 3). Two of these lineages (4 and 5) have haplotypes also found in southeastern Alaska.

The recognition of two genetically divergent populations of Steller sea lions also is supported by the cluster analysis based upon pairwise comparisons of Nei's (1978) genetic identity for all localities (Fig. 1). Although the estimate of the number of effective female migrants per generation was high ($N_em = 9.5$), we conclude that sufficient genetic differentiation has occurred between the eastern and western populations to allow recognition of these two distinct stocks. This recognition is based upon the distribution of haplotypes, reflected in the UPGMA analysis (Fig. 1), as well as the phenetic relationships among the haplotypes (Fig. 3). It is apparent that these two stocks were separated for sufficient time to allow the evolutionary diversification of haplotypes to occur within each, but apparently insufficient time has occurred since the populations have achieved their present distributions to allow gene flow to bring the distributions of all haplotypes to equilibrium (Neigel and Avise, 1993). For example, haplotype lineage 2 (Fig. 3) includes six related haplotypes representing 10 individuals, all of which are found in the eastern population. Although we have sampled 157 individuals from the western population, we have not yet found a single individual representing this lineage. The same can be said of the other lineages unique to the eastern population (lineages 1, 7, and 8), and vice versa for the lineages unique to the western population (3 and 6). Lineage 4 is particularly exemplary of the apparent low level of gene flow between the eastern and western populations of Steller sea lions. This lineage includes 16 haplotypes representing a total of 57 individuals. Of these, only a single haplotype (S) was found in the eastern population ($n = 1$), whereas all of the haplotypes were found in the western population. Considering that lineage 4 represents 31% of the observed diversity of haplotypes and 28% of the number of individuals sampled from rookeries, the observation of only a single ostensible migrant individual indicates that effective gene flow in females from the western to the eastern population has been extremely limited.

The relatively high percentage of sequence divergence observed between the most divergent haplotypes of *E. jubatus* (3.8%) compared with that observed between *E. jubatus* and *Z. californianus* (9.6%) suggests that haplotypes of Steller sea lions have persisted for a long time. That is, assuming a constant evolutionary

rate, the time since divergence of the two most divergent haplotypes of the Steller sea lion is ca. 40% that of the time since divergence of the haplotypes of the two species. The mtDNA molecule as a whole evolves at a rate of 2%/ 1×10^6 years (Wilson et al., 1985), but the hypervariable area of the control region evolves ca. 5× faster than the molecule as a whole (Cann et al., 1987; Greenberg et al., 1983). This results in an expected rate of ca. 10%/ 1×10^6 years for the region examined in this study, or an estimated divergence time of 9.6×10^5 years for the two species of sea lions. If these estimates are correct, the mtDNA haplotypes of the Steller sea lion have diverged within the past 3.84×10^5 years. This is significant because it indicates that this species has not undergone a recent population bottleneck. The absolute dates of divergence estimated from molecular data should be viewed with caution in this instance. We do not know that the control-region sequences in sea lions evolve at a constant rate, nor do we have any independant estimates of rates of mtDNA evolution in sea lions. Moreover, the fossil record indicates that the genus *Eumetopias* may be $2–4 \times 10^6$ years old (Loughlin et al., 1987). Nonetheless, as uncertain as these estimates are, they are useful to emphasize the point that Steller sea lions have maintained significant diversity of mtDNA through evolutionary time.

The low level of genetic variability observed in an allozymic study of Steller sea lions (Lidicker et al., 1981) contrasts with the high genotypic diversity of mtDNA estimated in this study ($h = 0.937$). Lidicker et al. (1981) examined populations from a limited portion of the distributional range of the Steller sea lion (from ca. 200 km E of Prince William Sound to ca. 200 km W of Kodiak Island in the Gulf of Alaska). Although a larger study might reveal more allozymic variation, our samples from this region are highly variable for mtDNA ($h =$ 0.814, 0.884, and 0.919). If low allozyme variability in Steller sea lions is the result of a population bottleneck, it must have occurred prior to the diversification of the haplotypes observed in this study—ca. 4×10^5 years ago. Alternatively, selection could be acting on the nuclear loci to keep variability low (Slade, 1992).

Lidicker et al. (1981) reported an absence of geographic variation except for the weakly differentiated population at Prince William Sound. Although we did not examine any specimens taken from rookeries in the Sound, we did examine three individuals taken outside the breeding season from Seal Rocks (a rookery within Prince William Sound). Two of those individuals had haplotypes found only in southeastern Alaska (K and L) and the third had a unique haplotype (LL) phylogenctically near the base of the tree (Figs. 3 and 4). All the other basal haplotypes are from the eastern population. Therefore, it appears that at least some sea lions from Prince William Sound might be related to the eastern population, not to the adjacent Gulf of Alaska localities, which belong to the western population. However, this needs to be confirmed by analysis of specimens taken from rookeries during the breeding season because Steller sea lions, especially young animals 1-4 years of age, are known to disperse over long distances outside the breeding season (Calkins and Pitcher, 1983). This observation is intriguing because the allozymic study of Lidicker et al. (1981) might have included animals representative of the eastern population within their samples from Prince William Sound. If this is true, there could be some degree of allozymic differentiation between eastern and western populations.

Lidicker et al. (1981) suggested that the populations of Steller sea lions encompassed in their study could be managed as a single unit. We conclude that there are at least two genetically differentiated stocks of sea lions that likely should be managed separately. The western stock has declined dramatically since the 1960s, whereas the eastern stock has not. Remarkably, the popu-

lation in Prince William Sound has the only rookery (Seal Rocks) in the Gulf of Alaska area that has not declined substantially. Thus, recognition of what environmental stresses are unique to the western stock could help identify the causes of decline. Moreover, our data might be indicative of a genetic contribution to the success of the eastern stock.

The recognition of two genetically differentiated populations of Steller sea lions seems to be inconsistent with the high level of estimated effective migration. Moreover, it could be argued that the recognition of two distinct stocks is not warranted because there is no evidence for monophyly of the mtDNA haplotypes that characterize the two populations. However, it should be noted that the estimated rate of effective migration is likely heavily influenced by gene exchange among populations within the stocks, and that recognition of stocks is not dependant upon monophyly. The paraphyletic nature of the mtDNA haplotypes in the two populations of Steller sea lions simply indicates that these two populations do not trace their ancestry back to a single maternal ancestor in either case. It is likely that the two populations of Steller sea lions are descended from populations isolated in glacial refugia in Beringia and the Pacific Northwest. A similar pattern of population differentiation was observed in sockeye salmon (Bickham et al., 1995; Wood et al., 1994) and in chinook salmon (Cronin et al., 1993) and may be indicative of a generalized phylogeographic track (Avise et al., 1987). A similar study recently was performed on California sea lions (Maldonado et al., 1995). Two distinct populations were identified based upon mtDNA control-region sequences. Animals obtained at rookeries within the Gulf of California belonged to a distinct mitochondrial lineage that was on average 4.3% divergent from the lineage represented by animals from rookeries on the Pacific coast. Thus, genetically differentiated stocks that are reflective of general phylogeographic patterns also are found in this close relative of the Steller sea lion.

We have shown in this study that detailed information on genetic subdivision in marine mammals is readily obtained using PCR and automated-sequencing technology combined with nondestructive tissue sampling. By use of this technology, we have demonstrated that a threatened species with presumed low genetic variability and little apparent geographic subdivision, in fact, has high diversity of mtDNA haplotypes and a clear pattern of macrogeographic variation. Knowledge of the patterns of genetic variation in marine mammals, such as Steller sea lions, will contribute to conservation efforts in several ways, including allowing more accurate determination of management units, better estimates of dispersal and gene flow, identification of genetic stocks, and a better understanding of biodiversity.

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