Demographic independence along ecosystem boundaries in Steller sea lions revealed by mtDNA analysis: implications for management of an endangered species

G. O'Corry-Crowe, B.L. Taylor, T. Gelatt, T.R. Loughlin, J. Bickham, M. Basterretche, K.W. Pitcher, and D.P. DeMaster

Abstract: Previous genetic studies indicate Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) comprise three phylogeographically distinct populations. However, differences in population trends and ecology and the limited extent of recorded dispersal suggest structure may be present at smaller scales. We examined sequence variation within a longer segment (531 bp) of the mtDNA control region in greater numbers (n = 1654) of sea lions from across Alaska than earlier investigations to investigate fine-scale dispersal patterns in Steller sea lions. We detected high levels of haplotypic diversity (h = 0.934) and confirmed phylogeographic differentiation between southeastern and western Alaska (Φ_{st} = 0.23, P < 0.0001), but also found significant differentiation at regional and local scales. Rookeries in the Gulf of Alaska, eastern Bering Sea, and eastern Aleutians were distinct from rookeries in the central and western Aleutians (F_{st} = 0.021, P < 0.0001; Φ_{st} = 0.017, P < 0.0001). The location of this split coincides with an oceanographic divergence between continental shelf and ocean basin waters and with differences in sea lion foraging ecology and population trends. A number of rookeries were also significantly differentiated from nearby rookeries (F_{st} = 0.02–0.025, P < 0.05), signifying substantial female-mediated philopatry, in some cases, at local scales. These findings have important implications for understanding the ecology of Steller sea lions in relation to marine ecosystems and the causes of population declines, and they provide guidance for management, including the identification of management stocks.

Résumé : Des études génétiques antérieures ont montré que les lions de mer de Steller (*Eumetopias jubatus* (Schreber, 1776)) forment trois populations phylogéographiquement distinctes. Cependant, des différences dans les tendances démographiques et l'écologie ainsi que des données limitées disponibles sur la dispersion indiquent qu'il peut exister une structure à des échelles plus restreintes. Nous avons examiné la variation des séquences dans un segment plus long (531 pb) de la région de contrôle de l'ADNmt chez un plus grand nombre de lions de mer (n = 1654) des différentes régions de l'Alaska que dans les études antérieures afin de déterminer les patrons de dispersion à échelle fine des lions de mer de Steller. Nous avons trouvé des taux élevés de diversité des haplotypes (h = 0,934) et confirmé la différentiation phylogéographique entre le sud-est et l'ouest de l'Alaska (Φ_{st} = 0,23, P < 0,0001); nous avons aussi découvert une différentiation importante aux échelles régionales et locales. Les roqueries du golfe de l'Alaska, de l'est de la mer de Béring et de l'est des Aléoutiennes sont différentes des roqueries du centre et de l'ouest des Aléoutiennes (F_{st} = 0,021, P < 0,0001; Φ_{st} = 0,017; P < 0,0001). L'emplacement de cette division coïncide avec une divergence océanique entre les eaux du plateau continental et celles du basin océanique et avec des différences dans l'écologie de l'alimentation et les tendances démographiques chez les lions de mer. Plusieurs roqueries se distinguent aussi de façon significative des roqueries adjacentes (F_{st} = 0,02–0,025, P < 0,05), ce qui indique une philopatrie substantielle déterminée par les femelles, en certains cas, à l'échelle locale. Ces observations ont des conséquences importantes sur la compréhension de l'écologie des lions de mer de Steller et elles fournissent des informations importantes pour la gestion; en particulier, elles permettent l'identification des stocks de gestion.

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Introduction

Steller sea lions, *Eumetopias jubatus* (Schreber, 1776), are distributed throughout the North Pacific Ocean rim from Japan to California. They range across continental shelf and ocean basin waters and return annually to breed on a series of remote islands termed rookeries (Loughlin 2002; Fig. 1). Historically, Alaska was the stronghold for this species, with some rookeries exceeding 10 000 individuals in the 1960s (Kenyon and Rice 1961; York et al. 1996). In recent decades, sea lion numbers from Prince William Sound westward throughout the Aleutian Islands declined by approximately 85% from an estimated 177 000 in 1960 to 26 600 in 2002, an average decrease of ~5%/year (Merrick et al. 1987; Loughlin et al. 1992; Trites and Larkin 1996; Sease and Gudmundson 2002). By contrast, abundance in southeastern Alaska, and farther south in British Columbia and Oregon, has increased at an average annual rate of 3.1% (Pitcher et al. 2007). The cause or causes of the declines are, as yet, unresolved. Hypotheses center on the potential role of commercial fisheries and environmental change in sea lion foraging ecology, predation, and the effects of changing prey composition and availability on body condition, reproduction, and survival in sea lions (Pascual and Adkison 1994; Loughlin 1998; National Research Council 2003; Springer et al. 2003; Trites and Donnelly 2003). Other potential factors include human take incidental to commercial fishing and poaching (for a review see National Research Council 2003).

Phylogeographic partitioning of mitochondrial DNA (mtDNA) lineages revealed an ancient divergence of two populations of Steller sea lions (Bickham et al. 1996, 1998) and formed the basis of the designation of two distinct population segments (DPSs) or stocks of this species as defined under the United States Endangered Species Act (ESA): an eastern and a western DPS with the boundary near Cape Suckling (longitude 144°W) in the Gulf of Alaska (Loughlin 1997; Fig. 1). In 1997, the status of the western DPS was changed from threatened to endangered, while the status of the eastern DPS remained as threatened. Subsequent studies of this genetic marker and several microsatellite (i.e., nuclear) markers have generally supported and expanded on these findings (Trujillo et al. 2004; Baker et al. 2005; Harlin-Cognato et al. 2006; Hoffman et al. 2006), one of the most recent recommending that rookeries in Asia be managed as a separate stock (Baker et al. 2005). All these investigators assessed population subdivision and approached stock identity primarily from an evolutionary perspective. Specifically, they invoked long-term isolation of populations, likely dating back to the Pleistocene, to explain macro-geographic patterns of heterogeneity in nuclear markers and the phylogeographic pattern observed within mtDNA on regional scales. Using this inferred ancient divergence, they argued that regional clusters of rookeries were demographically discrete populations that likely possessed separate evolutionary trajectories and potential, and as such should be managed separately. Low sample sizes for many rookeries, the a priori grouping of rookeries, and in some cases short fragment length limited the resolution of subdivision on smaller scales.

Much, however, remains to be resolved regarding the population structure and dispersal patterns of the Steller sea lion. Differences in trends in abundance and ecology exist within these large, evolutionarily distinct populations (York et al. 1996; Sease and Gudmundson 2002; Sinclair and Zeppelin 2002; Pitcher et al. 2007). Marking studies indicate that dispersal occurs over much smaller spatial scales (Raum-Suryan et al. 2002), while major climatic and oceanographic oscillations and marine ecosystem regime shifts operate on much shorter time frames and smaller spatial scales (Anderson and Piatt 1999; National Research Council 2003) than the glacial oscillations that shaped macro-geographic patterns of population subdivision in this species.

Further elucidating population subdivision also has management implications. ESA objectives center on recognizing the biological and ecological importance of discrete populations and taking action when necessary to preserve them (United States Fish and Wildlife Service – United States National Oceanic and Atmospheric Administration 1996). While ancient isolation of populations is usually taken as strong evidence for DPS discreteness, it is not a prerequisite, nor is the presumed evolutionary uniqueness that such isolation may represent. Rather, marked differences in ecology, behavior, or other aspects of the biology of populations can also be used to support the designation of distinct populations. In addition, the significance of a population to the species can be assessed by the uniqueness of its ecological setting or by whether its loss would result in a significant gap in the species range.

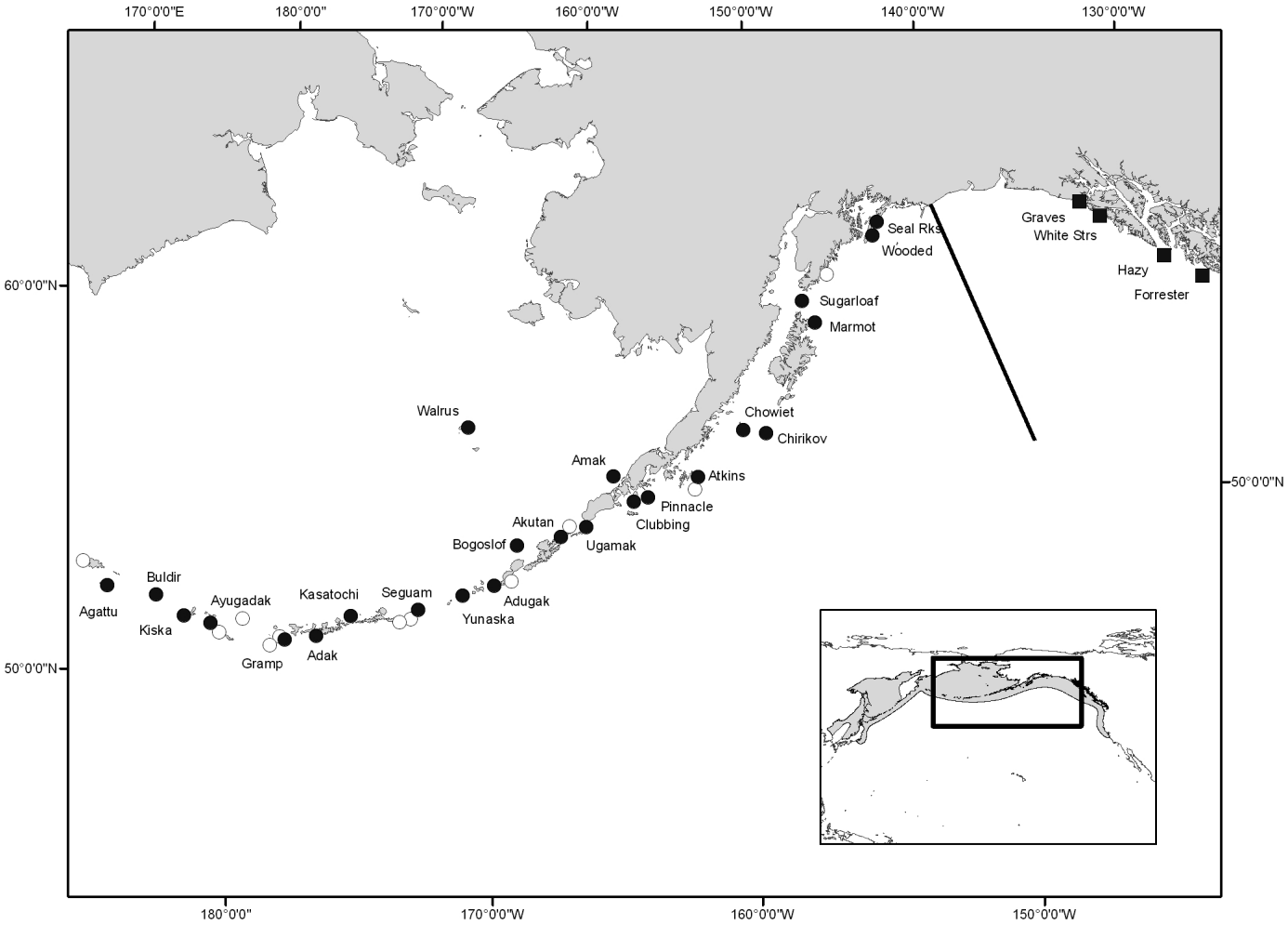
We used a molecular genetic approach to investigate detailed population subdivision, dispersal patterns, and rookery fidelity in Steller sea lions throughout Alaska. Because females, as the limiting sex, are primarily responsible for a population's ability to recover, we examined variation within the maternally inherited mitochondrial genome (mtDNA) to resolve patterns of female dispersal and philopatry at a number of spatial and temporal scales. Our study builds on earlier studies by increasing sample size and screening for variation in a longer segment of the genome. We sequenced an extended region of the mtDNA control region (531 bp) in 1654 pups from 28 rookeries within Alaska. We assessed our findings from an ecological as well as an evolutionary perspective and integrated our results with other data relevant to DPS definition criteria. The eastern DPS is considered in this paper only to evaluate status relative to its neighbor to the west. Further analysis of the genetic data for the eastern stock appears in a separate paper. We also cropped our data to the shorter fragment (238 bp) used by others (Bickham et al. 1996, 1998; Trujillo et al. 2004; Baker et al. 2005) to assess the effect of fragment length and sample size on estimates of population subdivision and dispersal. This also facilitated a comparison with previously published sequence data from a further 581 samples from California, Oregon, British Columbia, and Russia to evaluate our findings in relation to the entire species range.

Materials and methods

Sample collection and DNA extraction

Skin plugs were collected in accordance with US permit guidelines during branding operations from 1654 Steller sea lion pups at 28 rookeries in Alaska from 1994 to 2003

Fig. 1. The locations of the major Steller sea lion (*Eumetopias jubatus*) rookeries in Alaska and the position of the current stock (distinct population segment) boundary. Sampled rookeries are highlighted and the approximate world distribution of Steller sea lions is inset (modified from Loughlin 2002).



(Table 1, Fig. 1). All pups were sampled within 3 weeks of birth, ensuring that sampling occurred at the rookery of birth. Tissues were preserved in 20% dimethyl sulfoxide saturated with NaCl (National Marine Mammal Laboratory samples) or in 90% ethanol (Alaska Department of Fish and Game samples). Total DNA was isolated using standard cell lysis – protein digestion methods followed by silica-based DNA extraction and recovery protocols. Tissue lysis and digestion steps were automated using the FastDNA™ kit and the FastPrep™ instrument (BIO 101, Carlsbad, California, USA), and DNA was recovered using the DNeasy™ Blood & Tissue Kit (QIAGEN, Valencia, California, USA). The concentration and quality of the purified DNA from all samples were estimated by spectrophotometry. For a number of individuals, total DNA was already available (Texas A&M University samples).

Amplification and sequencing of mtDNA

PCR amplification of target DNA (Saiki et al. 1988) was performed in 25 or 50 μL reactions in a 9600 thermal cycler (Perkin-Elmer, Norwalk, Connecticut, USA), a GeneAmp 2700 PCR system (Applied Biosystems, Foster City, California, USA), or a PTC-100 Thermal Controller (MJ Research, Inc., Watertown, Massachusetts, USA). Reactions contained approximately 0.1 μg of template DNA, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.01% gelatin, 150 μmol/L of each dNTP, 0.3 μmol/L of each primer, and 2.5 units (1 U ≈ 16.67 nkat) of *Taq* DNA polymerase. Following denaturation at 90 °C for 2 min, DNA was amplified by 35 cycles consisting of denaturation at 94 °C for 45 s, annealing at 48 °C for 1 min, and extension at 72 °C for 1.5 min. A final extension period of 5 min at 72 °C was followed by cooling of the PCR product to 4 °C. A series of species-specific primers were designed to maximize the length of sequence analyzed. Part of the threonine tRNA gene, the entire proline tRNA gene, and about 600 bp of the control region of the mitochondrial genome were amplified using primers H00034 (5′-TACCAAATGTATGAAACCTCAG-3′, Rosel et al. 1994) and Ejub-R (5′-ACCATTGACTGAAATACACC-3′, this study) (primer names refer to the position on the type species mtDNA corresponding to the 3′ end of the primer). The amplified PCR products were purified by membrane-based filtration using QIAquick (QIAGEN) columns.

PCR products were sequenced by the direct dideoxy sequencing method of Sanger et al. (1977) using the four-dye

fluorescent technology of Applied Biosystems. Sequencing was performed in 12 or 20 μL reactions containing 20–200 ng of purified PCR product, 0.10 μmol/L primer, and 2.5 or 7.0 μL of BigDye® Terminator mix (v. 3.1, Applied Biosystems). Cycle sequencing was carried out in a Perkin-Elmer 9600 thermal cycler or a GeneAmp 2700 PCR system, with the following profile: denaturation at 96 °C for 4 min followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s, and extension at 60 °C for 4 min. Both strands were sequenced, the heavy strand with an internal species-specific primer, Ejub-F (5′-GCCCCATGCATATAAGCATG-3′, this study), and the light strand with Ejub-R. Excess dye-labeled terminators were removed from sequencing reactions by ethanol precipitation. Sequences were run on an ABI 377 gel or 3100 capillary automated sequencer (Applied Biosystems) and edited and aligned with the Sequencher™ 4.1 multiple-sequence editor program (Gene Codes Corp., Ann Arbor, Michigan, USA).

Analysis of mtDNA data

Genetic diversity

The amount and nature of variation within the sequenced region were assessed by determining the number of variable sites and the number of unique haplotypes using MEGA 2.1 (Kumar et al. 2001) and MacClade 3.02 (Maddison and Maddison 1992) software, respectively. Haplotypic (*h*; Nei and Tajima 1981) and nucleotide (π; Nei 1987) diversity estimates of genetic variation were calculated using ARLEQUIN 3.01 software (Excoffier et al. 2006).

Genetic differentiation

Genetic relationships among rookeries were assessed using statistical hypothesis testing, where the null hypothesis was random mixing and the significance criterion was $\alpha = 0.05$. Both frequency-based and distance-based statistics were used. The frequency-based methods analyzed genetic structure using haplotype frequencies only. Conventional *F*-statistics (Wright 1951) were estimated by the analysis of variance method of Weir and Cockerham (1984) using ARLEQUIN. The distance-based method involved estimating *F*-statistic analogues (Φ-statistics; Excoffier et al. 1992) in ARLEQUIN that incorporate information on the number of mutational steps among individual haplotypes as well as differences in haplotype frequency. The statistical significance of parameter estimates (F_{st} and Φ_{st}) under a hypothesis-testing framework, at both individual pairwise comparison and table-wide levels, was estimated by 10 000 randomizations of the original data, in ARLEQUIN.

Genetic differentiation was also assessed by agglomerative hierarchical phylogeny reconstruction and clustering analyses, which combined rookeries into nested, mutually exclusive groupings based on estimated genetic distances among rookeries. We used two traditional distance-based phylogeny reconstruction and clustering methods: neighbor joining (NJ; Saitou and Nei 1987) and the unweighted pair group method with arithmetic averages (UPGMA; Sokal and Michener 1958; Sneath and Sokal 1973). NJ and UPGMA both group strata solely on the basis of estimated genetic distance, regardless of the geographic locations of the strata, but differ in their assumptions regarding the ultrametric properties of the data. Wright's (1951) F_{st} and Excoffier et al.'s (1992) Φ_{st} were used as the measure of genetic distance in MEGA.

To test for departure from randomness in geographic patterns of genetic variation, regressions of pairwise genetic distances among rookeries on geographic distances and correlations between the two distance matrices were computed, and their significance was tested in Genepop v. 1.2 (Raymond and Rousset 1995). The test statistic was based on the *Z* statistic of Mantel (1967), and its significance was determined by 10 000 permutations of the data under the null hypothesis of independence between genetic and geographic distances. Dispersal was considered to occur primarily in continental shelf waters and so geographic distances were measured as the minimal "swim distances" in shelf waters between rookeries in ArcGIS® 9.1 (Environmental Systems Research Institute, Inc., Redlands, California, USA). Genetic distances were estimates of the frequency-based parameter F_{st} or the distance-based parameter Φ_{st}.

We used the maximum-likelihood coalescence theory based method of Beerli and Felsenstein (1999, 2001) to estimate migration rates from the mtDNA sequence data. The maximum-likelihood estimate (MLE) of N_m, the number of females that disperse between populations per generation, was approximated using a Metropolis–Hastings Markov chain Monte Carlo approach in the Migrate program (v. 2.1.3, Beerli 1997–2004). This approach has an advantage over conventional F_{st}-based approaches in that it estimates migration rates among populations of unequal size that experience asymmetric migration rates (Beerli and Felsenstein 1999, 2001). We compared the MLEs with those based on a modification of Wright's indirect method of estimating gene flow among populations from the extent of genetic differentiation, $N_m = (1/F_{st} - 1)/2$ (Takahata and Palumbi 1985).

A concern with assessments of population structure in species that exhibit an isolation-by-distance pattern of genetic heterogeneity (e.g., linearly distributed, with limited individual dispersal distance) is that statistical power is greatest when the range is split evenly in two. This is because sample size as well as genetic divergence is often maximized near midrange (Martien and Taylor 2003). Furthermore, under these conditions agglomerative hierarchical clustering and tree reconstruction methods will tend to place the deepest node midrange. To test for these types of potential artifacts, we developed a "sliding window" analysis of genetic differentiation, where sets of contiguous rookeries spanning ~1000 km were split into two groups and tested for genetic differentiation. Starting at the western end of the range, we compared two contiguous groups of rookeries: (1) Agattu, Buldir, Kiska, and Ayugadak and (2) Gramp Rock, Adak, Kasatochi, and Seguam. Moving the 1000 km window eastward one rookery at a time, we estimated F_{st} and Φ_{st} among groups while keeping sample size and spatial extent approximately constant.

DPS assessment

We used the following sequential approach for evaluating whether strata are "distinct": (*i*) do clustering results produce consistent patterns between NJ and UPGMA?; if so, then (*ii*) are there far more differences between than within clusters in the large pairwise tables of *P* values?; if so, then

Table 1. Summary of the Steller sea lion (*Eumetopias jubatus*) rookeries sampled and the number of samples, mtDNA nucleotide diversity, and haplotypic diversity by rookery.

	Rookery	Sample size (n)	Nucleotide diversity (π)	Haplotypic diversity (h)
1	Forrester	140	0.0063	0.935
2	Hazy	129	0.0063	0.944
3	White Sisters[a]	180	0.0065	0.962
4	Graves Rocks[a]	50	0.0067	0.944
5	Seal Rocks	80	0.0055	0.857
6	Wooded Island	56	0.0053	0.874
7	Sugarloaf	64	0.0049	0.876
8	Marmot	67	0.0049	0.843
9	Chirikov	65	0.0049	0.873
10	Chowiet	24	0.0047	0.826
11	Atkins	56	0.0050	0.898
12	Pinnacle	51	0.0055	0.895
13	Clubbing Rocks	25	0.0043	0.753
14	Ugamak	93	0.0057	0.913
15	Akutan	78	0.0058	0.906
16	Amak	53	0.0048	0.840
17	Walrus[b]	16	0.0063	0.917
18	Bogoslof[b]	11	0.0062	0.855
19	Adugak[b]	10	0.0058	0.933
20	Yunaska	44	0.0053	0.893
21	Seguam	57	0.0047	0.902
22	Kasatochi	55	0.0045	0.867
23	Adak	65	0.0047	0.912
24	Gramp Rock	56	0.0043	0.844
25	Ayugadak	20	0.0041	0.895
26	Kiska	39	0.0057	0.931
27	Buldir	30	0.0053	0.920
28	Agattu	40	0.0057	0.921
	Alaska	1654	0.0061	0.934

[a]White Sisters and Graves Rocks are recently colonized rookeries and are analyzed in more detail in a companion paper.

[b]Walrus, Bogoslof, and Adugak were excluded from the analysis of population differentiation because of small sample size.

(*iii*) can we exclude an isolation-by-distance pattern of genetic differentiation as the sole contributor to genetic differences among clusters?; if so, then (*iv*) are P values significantly different and numbers of migrants per generation low when rookeries within a cluster are pooled and compared?

Results

A total of 531 bp of the mtDNA control region were analyzed for sequence variation in 1654 Steller sea lions sampled at 28 rookeries throughout Alaska (Table 1). Sixty-two variable sites were identified, 60 with substitutions (55 transitions and 7 transversions) and 2 with indels. A total of 130 unique haplotypes were identified, with over one third (48/130) represented by a single individual. Overall haplotypic diversity was very high (h = 0.934) owing to the large number of rare haplotypes, while overall nucleotide diversity was moderate (π = 0.6%), indicating that the majority of haplotypes were phyletically closely related.

Only long-established rookeries with ≥20 samples were used in the analysis of population subdivision. Newly colonized rookeries in the eastern stock are the subject of a companion paper. We found substantial levels of subdivision among rookeries both within and between the current Steller sea lion stocks in Alaska (Table 2). Many pairwise comparisons among 23 well-sampled ($n \geq 20$) rookeries were statistically significant under a null hypothesis of random mixing (Table 2). F_{st} values among rookeries from different stocks were, on average, significantly larger (F_{st} = 0.084, range = 0.057 to 0.142) than values estimated among rookeries within the eastern (F_{st} = 0.004) and western (F_{st} = 0.016, range = –0.015 to 0.072) stocks (t test, $P < 0.0001$). Patterns of differentiation for the distance-based statistic (Table 2B), Φ_{st}, were similar except that differences among the eastern and western rookeries were substantially larger (Φ_{st} = 0.225, range = 0.176 to 0.275) than those found using frequency-based statistics (Table 2A).

Virtually no significant pairwise differences were found among rookeries from Yunaska in the central Aleutians west through Agattu (Table 2A, lower right-hand box). Similarly, with the exception of a number of comparisons involving Akutan, Amak, and Wooded Islands, there were relatively few significant differences among rookeries within the area from Seal Rocks in the Gulf of Alaska to Amak, the largest rookery in the eastern Bering Sea (Table 2A, middle box). By comparison, nearly all of the pairwise comparisons between the former set of rookeries (referred to subsequently as the "oceanic" rookeries) and the latter set of rookeries (referred to subsequently as the "shelf" rookeries because they are all well within the continental shelf break (i.e., 200 m isobath)) were larger and significantly different (Table 2A, middle lower region). Pairwise F_{st} values among rookeries from the two different groups in the western stock were, on average, 5 to 14 times larger (F_{st} = 0.028, range = 0.005 to 0.072) than values estimated among rookeries within the western shelf group (F_{st} = 0.005, range = –0.012 to 0.045) and within the western oceanic group (F_{st} = 0.002, range = –0.015 to 0.022). This difference was found to be statistically significant (t test, $P < 0.0001$).

The results of the NJ and UPGMA analyses are presented in Figs. 2 and 3. Low levels of genetic differentiation among several rookeries resulted in several poorly resolved polytomies within the Gulf of Alaska, the Aleutian Islands, and the Bering Sea. The NJ tree based on F_{st} as the estimate of genetic distance has a deep node separating the southeast Alaska rookeries from all the western stock rookeries (Fig. 2A). This method also revealed a deep division within the western stock. Rookeries in the Gulf of Alaska, the Bering Sea, and the eastern Aleutian Islands formed one distinct "shelf" group, while rookeries in the central and western Aleutian Islands formed a second distinct "oceanic" group (Fig. 2A). Similar groupings were found when distance-based (Φ_{st}) statistics were used as the measure of genetic distance (Fig. 3A). The cluster (UPGMA) analysis yielded very similar results to the NJ analysis (Figs. 2B, 3B). The southeast Alaska (eastern DPS) rookeries were quite divergent from all other (western DPS) rookeries. As with the NJ analysis, the western stock rookeries were divided into a shelf and an oceanic group.

These patterns of differentiation were also evident in the Mantel test. A significant isolation-by-distance pattern was found across the entire western stock (r^2 = 0.359, P =

Table 2. Population genetic subdivision among Steller sea lion rookeries in Alaska: (A) genetic differentiation based on the frequency-based statistic F_{st}; (B) genetic differentiation based on the distance-based statistic Φ_{st}.

	Eastern DPS		Western DPS												Ocean group								
			Shelf group																				
	Forrester	Hazy	S. Rocks	Wooded	Sugarloaf	Marmot	Chirikov	Chowiet	Atkins	Pinnacle	Clubbing	Ugamak	Akutan	Amak	Yunaska	Seguam	Kasatochi	Adak	Gramp R.	Ayugadak	Kiska	Buldir	
n	140	129	80	56	64	67	65	24	56	51	25	93	78	53	44	57	55	65	56	20	39	30	1347
(A) F_{st}																							
Forrester																							
Hazy	0.004																						
Seal Rocks	0.102	0.096																					
Wooded	0.084	0.077	0.004																				
Sugarloaf	0.087	0.080	-0.001	0.005																			
Marmot	0.109	0.104	-0.003	0.010	-0.007																		
Chirikov	0.091	0.085	0.003	0.020	-0.003	-0.005																	
Chowiet	0.112	0.106	-0.006	0.007	-0.010	-0.011	-0.004																
Atkins	0.074	0.067	0.002	0.010	-0.005	0.002	-0.001	0.001															
Pinnacle	0.080	0.074	-0.002	0.008	0.000	0.003	0.002	-0.002	-0.010														
Clubbing	0.142	0.138	0.017	0.045	0.006	-0.005	-0.004	-0.006	0.012	0.020													
Ugamak	0.076	0.071	0.003	0.012	0.004	0.006	0.003	0.002	-0.002	-0.005	0.025												
Akutan	0.071	0.064	0.014	0.019	0.013	0.015	0.011	0.030	0.000	0.005	0.031	0.005											
Amak	0.104	0.097	0.002	0.014	-0.001	-0.001	0.000	-0.011	0.007	0.011	0.002	0.010	0.023										
Yunaska	0.087	0.079	0.022	0.043	0.025	0.023	0.016	0.033	0.019	0.014	0.054	0.013	0.015	0.045									
Seguam	0.074	0.067	0.020	0.027	0.013	0.017	0.005	0.015	0.014	0.015	0.035	0.007	0.018	0.022	0.006								
Kasatochi	0.094	0.085	0.022	0.044	0.023	0.026	0.017	0.034	0.024	0.020	0.057	0.024	0.026	0.045	-0.011	0.013							
Adak	0.069	0.059	0.019	0.030	0.017	0.025	0.013	0.025	0.011	0.008	0.054	0.008	0.015	0.036	-0.009	0.001	-0.006						
Gramp Rocks	0.102	0.092	0.019	0.046	0.018	0.023	0.017	0.029	0.023	0.025	0.047	0.023	0.025	0.032	0.000	0.012	-0.007	0.003					
Ayugadak	0.081	0.073	0.029	0.043	0.024	0.036	0.026	0.026	0.026	0.024	0.071	0.024	0.046	0.039	0.014	0.006	0.006	0.010	0.006				
Kiska	0.064	0.057	0.021	0.031	0.017	0.024	0.017	0.034	0.010	0.010	0.056	0.011	0.009	0.038	-0.004	0.000	-0.001	-0.004	0.002	0.001			
Buldir	0.075	0.068	0.040	0.052	0.034	0.040	0.023	0.044	0.022	0.024	0.061	0.020	0.019	0.056	-0.004	-0.001	0.011	0.000	0.018	0.022	0.001		
Agattu	0.070	0.061	0.031	0.047	0.029	0.039	0.028	0.050	0.018	0.022	0.072	0.023	0.017	0.051	-0.004	0.008	0.000	0.001	0.002	0.009	-0.015	0.000	
(B) Φ_{st}																							
Forrester																							
Hazy	-0.002																						
Seal Rocks	0.239	0.216																					
Wooded	0.216	0.194	-0.008																				
Sugarloaf	0.232	0.211	-0.001	0.003																			
Marmot	0.262	0.240	0.003	0.007	-0.007																		
Chirikov	0.245	0.222	0.011	0.019	-0.004	-0.004																	
Chowiet	0.238	0.215	-0.012	-0.006	-0.019	-0.018	-0.019																
Atkins	0.197	0.176	0.002	0.004	-0.002	0.011	0.005	-0.005															
Pinnacle	0.213	0.189	0.001	0.004	0.012	0.019	0.013	-0.006	-0.007														
Clubbing	0.255	0.232	0.013	0.023	-0.006	-0.017	-0.012	-0.016	0.009	0.021													
Ugamak	0.220	0.201	0.012	0.016	0.006	0.008	0.002	-0.007	0.002	0.004	0.008												
Akutan	0.209	0.188	0.000	-0.001	0.001	0.006	0.011	0.000	-0.004	0.003	0.009	0.005											
Amak	0.234	0.211	-0.003	0.003	-0.005	-0.005	-0.003	-0.019	-0.005	0.000	-0.010	0.005	0.002										
Yunaska	0.244	0.223	0.006	0.013	0.002	0.001	-0.002	-0.011	0.011	0.013	0.012	-0.006	0.002	0.009									
Seguam	0.274	0.253	0.040	0.044	0.025	0.015	0.008	0.008	0.046	0.053	0.019	0.018	0.033	0.030	0.000								
Kasatochi	0.256	0.233	0.014	0.020	0.008	0.014	0.008	0.000	0.023	0.028	0.032	0.014	0.013	0.024	-0.013	0.012							
Adak	0.250	0.227	0.022	0.026	0.018	0.023	0.011	0.002	0.024	0.022	0.040	0.005	0.016	0.027	-0.013	0.010	-0.009						
Gramp Rocks	0.270	0.248	0.020	0.026	0.010	0.013	0.016	0.009	0.034	0.047	0.032	0.024	0.014	0.030	-0.006	0.011	-0.012	0.002					
Ayugadak	0.221	0.195	0.026	0.030	0.028	0.042	0.011	0.007	0.023	0.017	0.062	0.015	0.029	0.031	0.006	0.019	0.001	-0.004	0.029				
Kiska	0.229	0.212	0.019	0.021	0.006	0.011	0.013	0.011	0.017	0.034	0.022	0.007	0.001	0.023	-0.010	0.006	-0.004	0.002	-0.009	0.025			
Buldir	0.236	0.215	0.040	0.038	0.024	0.024	0.016	0.020	0.031	0.038	0.030	0.010	0.011	0.038	-0.007	0.004	0.001	-0.002	0.002	0.025	-0.010		
Agattu	0.219	0.203	0.022	0.026	0.014	0.028	0.031	0.026	0.022	0.043	0.046	0.024	0.006	0.034	0.005	0.032	0.006	0.017	0.000	0.038	-0.018	0.010	

Note: Significance (P) values are divided into three broad categories for ease of interpretation. The dark shaded cells are comparisons where $P \leq 0.05$; the light shaded cells are comparisons where $0.05 \leq P \leq 0.1$; and the unshaded cells are comparisons where $P > 0.1$. Comparisons among sets of contiguous rookeries within the three regional groupings are outlined.

Fig. 2. Tree of the outcome of the neighbor-joining analysis (A) and dendrogram from the UPGMA cluster analysis (B) based on F_{st} of 23 well-sampled Steller sea lion rookeries in Alaska: southeast Alaska rookeries (●), western shelf rookeries (○), and western oceanic rookeries (■). Negative values are estimates of $F_{st} = 0$ and were thus converted to 0.0.

(A) Neighbor Joining - F_{st} (B) UPGMA - F_{st}

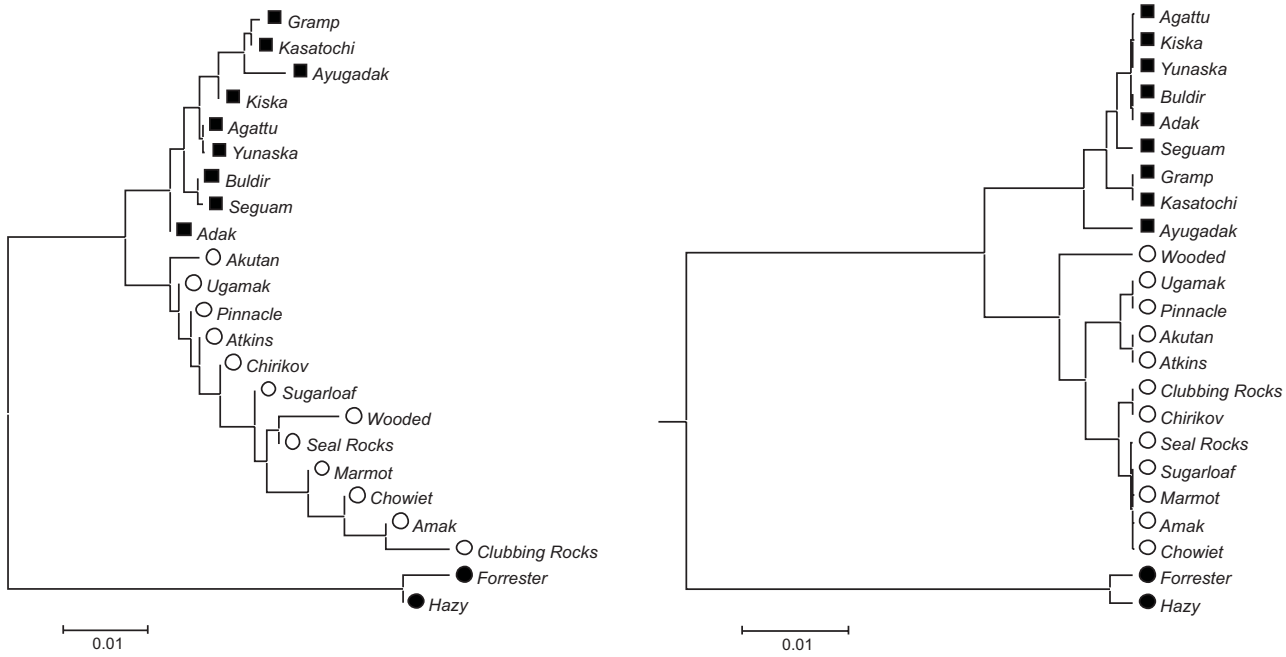
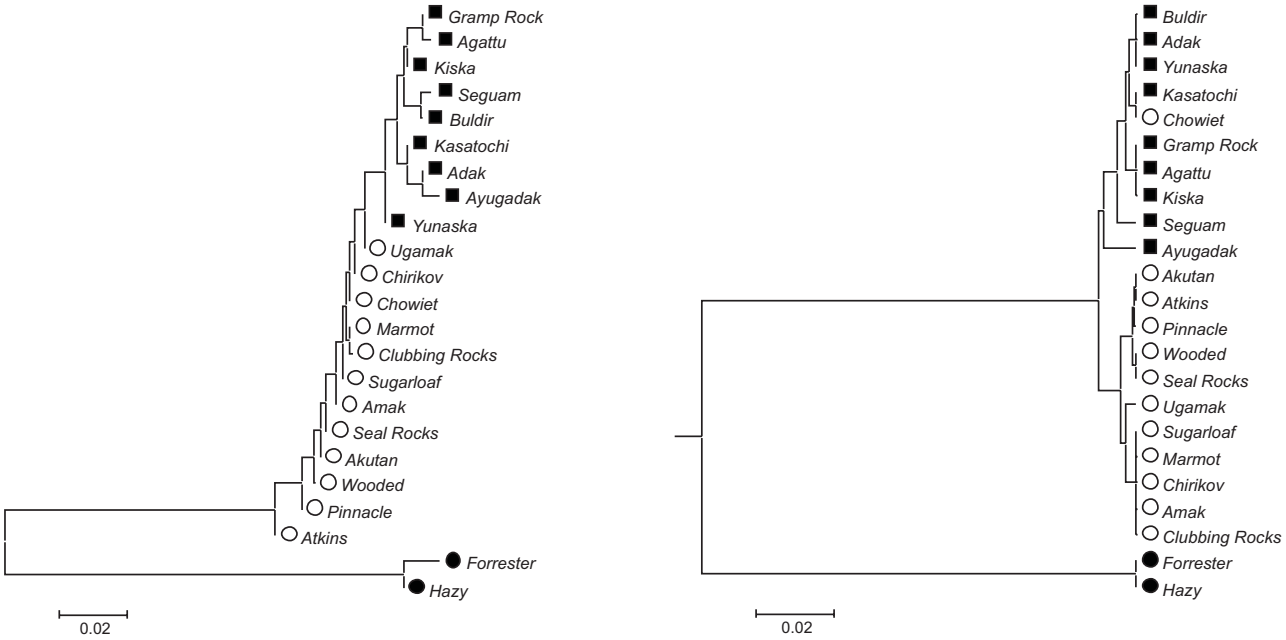


Fig. 3. Tree of the outcome of neighbor-joining analysis (A) and dendrogram from the UPGMA cluster analysis (B) based on Φ_{st} of 23 well-sampled Steller sea lion rookeries in Alaska: southeast Alaska rookeries (●), western shelf rookeries (○), and western oceanic rookeries (■). Negative values are estimates of $\Phi_{st} = 0$ and were thus converted to 0.0.

(A) Neighbor Joining - Φ_{st} (B) UPGMA - Φ_{st}



0.0001). However, we found little evidence of a correlation between genetic and geographic distance within the oceanic and shelf groups (12 shelf rookeries: $r^2 = 0.048$, $P = 0.06$; 9 oceanic rookeries: $r^2 = 0.004$, $P = 0.69$) or in comparisons of rookeries between groups ($r^2 = 0.035$, Fig. 4).

When rookeries were combined within the groups suggested by the clustering analysis, highly significant differences were found for both frequency and distance statistics (Table 3). Consistent maximum-likelihood estimates of N_m across multiple runs and a variety of search strategies were

Fig. 4. The relationship between genetic distance (F_{st}) and geographic distance (minimum swim distance in km) in Steller sea lions in western Alaska. The relationship involving rookeries from across the entire western stock ($r^2 = 0.36$, $P < 0.0001$) is presented as a heavy black line. Relationships within the oceanic group ($r^2 = 0.004$, $P = 0.69$) and the shelf group ($r^2 = 0.05$, $P = 0.06$) of rookeries are indicated as light black lines and ♦. The regression of genetic to geographic distance among rookeries from different groups ($r^2 = 0.035$) is indicated by a heavy gray line and ○.

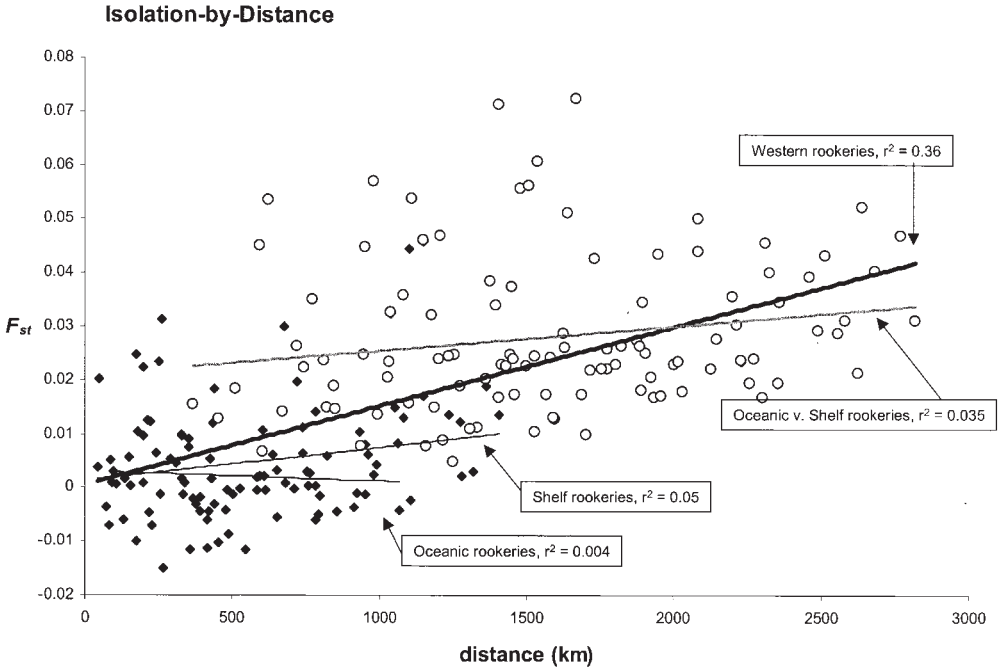


Table 3. Population subdivision among three groups of Steller sea lion rookeries in Alaska.

	Eastern: SE Alaska (n = 269)	Western: shelf (n = 714)	Western: oceanic (n = 406)
Eastern: SE Alaska		*** (***)	*** (***)
Western: shelf	0.087 (0.232)		*** (***)
Western: oceanic	0.076 (0.255)	0.021 (0.017)	

Note: Frequency-based and distance-based statistics are presented below the diagonal: F_{st} values are followed by Φ_{st} values in parentheses. P values from 10 000 permutations are presented above the diagonal: ***, $P < 0.0001$.

Table 4. Maximum-likelihood estimates and 90% confidence intervals (in parentheses) of the number of female migrants (N_m) between three groups of Steller sea lion rookeries in Alaska.

	Eastern: SE Alaska (n = 269)	Western: shelf (n = 714)	Western: oceanic (n = 406)
Eastern: SE Alaska	—	4.40 (3.03–6.12)	2.60 (1.59–3.97)
Western: shelf	5.34 (3.68–7.44)	—	45.64 (40.39–51.34)
Western: oceanic	4.50 (2.8–6.78)	67.84 (60.44–75.82)	—

Note: 4×10^6 trees were sampled. The receiving populations are in the rows.

found among the regional groups of rookeries suggested by the clustering analysis. In all runs the numbers of female migrants per generation were highest between the shelf and oceanic groups (Table 4). Estimates of N_m based on Wright's island model were similar to MLEs (data not shown) except that the female migration rate between the two groups within the Western stock was roughly half the MLE ($N_{mFst} \approx 25$ vs. $N_{mMLE} \approx 45$–67). Despite extensive simulations, MLEs at the rookery level were highly variable among runs.

The sliding window analysis of genetic differentiation indicated that subdivision was greatest between Yunaska and Akutan, further supporting a break in the eastern Aleutian Islands (Fig. 5). We also tested whether the size of the sampling gap between southeast Alaskan and western rookeries (895 km) and between shelf and oceanic rookeries within the western stock (365 km) affected the clustering and phylogenetic analyses by comparing only rookeries separated on similar scales. In all cases, the first-order split was between eastern and western rookeries and the second divergence was between shelf and ocean basin rookeries (not shown).

Cropping the 531-bp sequence data to the 238-bp fragment used in earlier studies reduced the number of unique haplotypes from 130 to 85 and revealed a similar pattern of relatively large differences between shelf and ocean basin rookeries compared with small or no differences among many rookeries within these regions (data not shown). However, fewer pairwise differences were found to be significantly different ($\alpha = 0.05$) between the two groups for the

Fig. 5. A "sliding window" analysis of population subdivision within the western stock of Steller sea lions. Shown are sequential Φ_{ct} values between pairs of contiguous groups of rookeries that, combined, span ~1000 km of the Steller sea lion's range. Comparison labels reflect the nearest rookeries from the two groups; for example, Ayugadak/Gramp represents the comparison between the following two contiguous groups of rookeries: Agattu, Buldir, Kiska, and Ayugadak and Gramp Rock, Adak, Kasatochi, and Seguam. Negative values are estimates of $\Phi_{ct} = 0$.

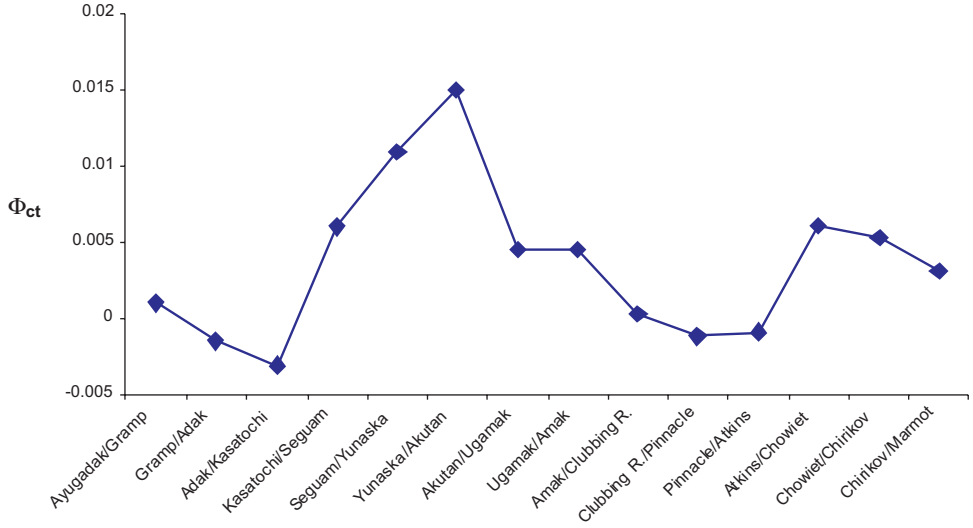


Table 5. Population subdivision and migration rates among the eastern stock, the Asian stock (as recommended by Baker et al. 2005), and the two groups within the western stock of Steller sea lions, based on 238 bp of the mtDNA control region: (A) F_{st} and Φ_{st} (in parentheses) estimates of differentiation (below the diagonal) and *P* values from 10 000 permutations (above the diagonal); (B) maximum likelihood estimates and 90% confidence intervals (in parentheses) of the number of female migrants (N_m) between four groups of Steller sea lion rookeries in Alaska.

	Eastern stock (n = 317)	Western shelf (n = 714)	Western oceanic (n = 512)	Asian stock (n = 427)
(A) F_{st} and Φ_{st} estimates and *P* values				
Eastern stock		*** (***)	*** (***)	*** (***)
Western shelf	0.110 (0.281)		*** (***)	*** (***)
Western oceanic	0.088 (0.295)	0.022 (0 0.016)		*** (***)
Asian stock	0.052 (0.217)	0.099 (0.076)	0.073 (0.080)	
(B) Maximum likelihood estimates and 90% confidence intervals				
Eastern stock		3.58 (2.56–4.83)	1.85 (1.16–2.8)	5.84 (4.51–7.41)
Western shelf	5.04 (3.51–6.98)		41.01 (36.27–46.14)	15.57 (12.72–18.81)
Western oceanic	2.20 (1.16–3.74)	55.58 (49.4–62.27)		23.11 (19.21–27.51)
Asian stock	5.63 (4.33–7.16)	10.61 (8.79–12.67)	9.56 (7.84–11.52)	

Note: 4×10^6 trees were sampled. The receiving populations are in the rows. California, Oregon, and British Columbia are included in the eastern stock, and the Commander Islands (Russia) are included in the western oceanic group. ***, $P < 0.0001$.

shorter fragment (70/108 comparisons) than for the longer fragment (100/108 comparisons). An analysis of the 238-bp data set with published data on 48 samples from California, Oregon, and British Columbia and 533 samples from Russia (Bickham et al. 1996, 1998; Baker et al. 2005) confirmed the close relationship between the Commander Islands and rookeries to the east reported earlier (Baker et al. 2005) and revealed that the level of differentiation observed between the oceanic and shelf groups within the western stock, while significant, was less than the differentiation observed between these groups and the proposed Asian and existing eastern stocks (Table 5).

Discussion

As well as confirming a phylogeographic-level divergence in mtDNA between eastern (southeast Alaska) and western (Prince William Sound west) Steller sea lion rookeries in Alaska, this study revealed a clear separation between "oceanic" and "shelf" rookeries within the western DPS and identified a number of rookeries that were significantly differentiated from neighboring rookeries. The regional break was evident in the phylogeny reconstruction and cluster analyses for both frequency- and distance-based statistics and in tests for correlations between genetic and geographic distance. The hypothesis testing results were consistent with this split by having larger and many more statistically significant inter-rookery differences between the two groups than within either group (Table 2). Pooling the rookeries within these groups resulted in large, highly significant differences (Table 3) and estimates of female dispersal that are low from a demographic, if not evolutionary, perspective (Table 4). Combined, these results indicate that the oceanic and shelf groups represent two demographically independent

populations of Steller sea lion. The two closest rookeries from the groups in our analysis, Akutan and Yunaska, lie 365 km apart. Current sampling limitations preclude a more precise estimate of the location of the boundary between these two subpopulations, if indeed a hard boundary exists.

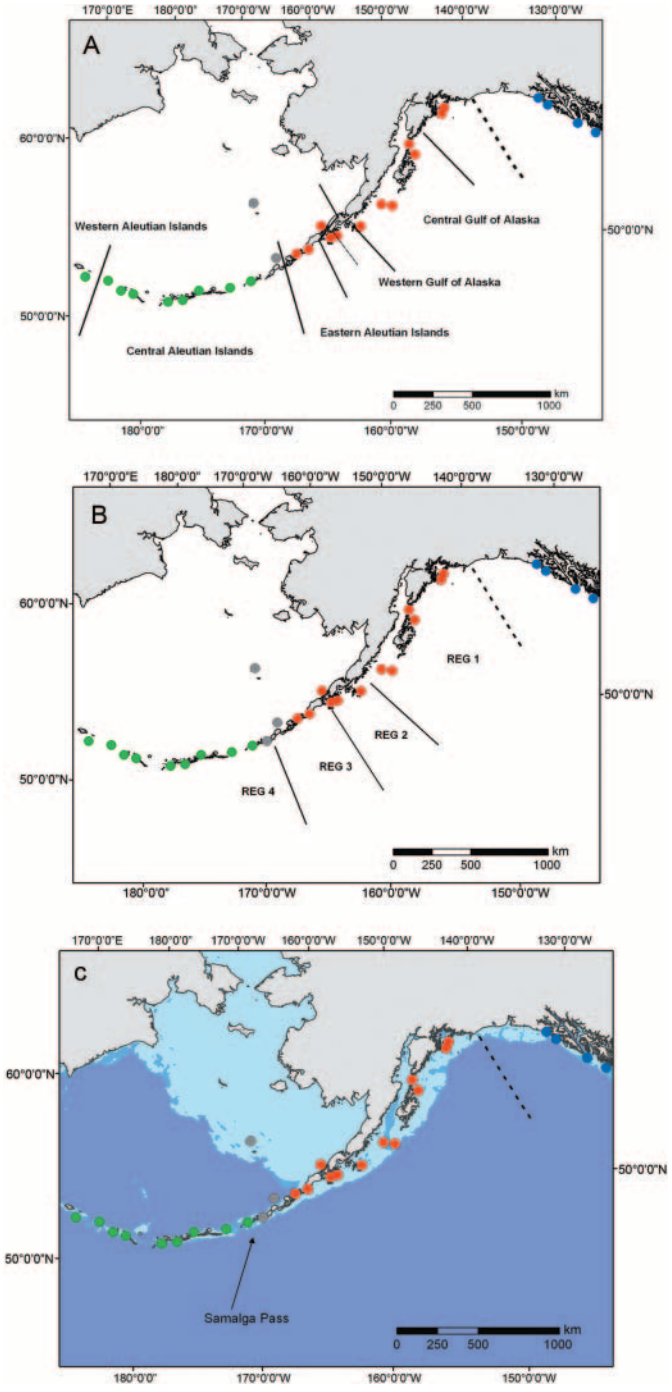
The longer fragment length examined and the increased sample size per rookery in this study compared with earlier investigations dramatically improved the resolution of underlying patterns of female dispersal and population subdivision. Screening an extended segment of the mtDNA control region revealed 45 new haplotypes, several restricted in their geographic distribution. The larger sample sizes increased statistical power and eliminated the need to group rookeries prior to the analyses of differentiation, as was done in earlier studies, thereby reducing the risk of missing subdivision. However, the resultant estimates of female dispersal, while informative as to the relative scales and direction of migration among populations, should be treated with caution, as all existing methods for estimating migration rates from genetic data make several simplifying assumptions about population histories and migration patterns and the origin and loss of genetic variation over time (Wright 1951; Slatkin 1985; Takahata and Palumbi 1985; Beerli and Felsenstein 1999, 2001) that are not always applicable to natural populations.

The spatial scales at which we observed genetic subdivision are consistent with what we know about movement and dispersal patterns of Steller sea lions from telemetry and marking studies. While individual sea lions, particularly juveniles, may undertake movements in excess of 1000 km and adult males may disperse over 500 km from their natal site (Merrick and Loughlin 1997; Raum-Suryan et al. 2002, 2004), marked adult females in both the eastern and the western stock were found to return to their natal rookery or to another rookery within 500 km to breed (Raum-Suryan et al. 2002; National Marine Fisheries Service and Alaska Department of Fish and Game, unpublished data). These genetic findings also provide insight into the spatial characteristics of Steller sea lion trends in abundance and ecology and the relationship between spatial variation in the physical environment and sea lion population structure.

Genetic differentiation and population trend

Regional differences in population trend exist within the western stock (Trites and Larkin 1996; York et al. 1996; Sease and Gudmundson 2002). Between 1991 and 2002, the change in counts of non-pups varied significantly: Gulf of Alaska, decline of 6%–39% depending on subregion; eastern Aleutians, increase of 1.4%; central Aleutians, decline of 21%; and western Aleutians, decline of 75% (Sease and Gudmundson 2002). York et al. (1996), using a geographically constrained cluster analysis, identified five groupings of rookeries in the western stock based on geographic proximity and similarities in rates of decline over various periods of time from 1976 to 1994. Similar to our genetic findings, a distinct difference in trends in abundance was observed between populations in the eastern and central Aleutian Islands (Fig. 6A). As in our genetic analysis, the two closest rookeries from the island groups were Akutan and Yunaska.

Fig. 6. Subdivision within the western population of Steller sea lions in relation to (A) differences in population trend (York et al. 1996; Sease and Gudmundson 2002), (B) sea lion diet (Sinclair and Zeppelin 2002), and (C) the physical environment (Ladd et al. 2005). Shelf rookeries are highlighted in red, oceanic rookeries in green, under-sampled rookeries in grey, and eastern rookeries in blue. The limit of the continental shelf (200 m isobath) is indicated by a transition from light to dark blue shading.



Thus, at least some of these regional differences in population trend are occurring in demographically distinct subpopulations.

Differences in population trajectories have also been re-

corded among neighboring rookeries. For example, over the past decade or so the overall trend in abundance at Amak Island in the Bering Sea has been positive, in marked contrast to the variable trends of most other rookeries in the western stock (York et al. 1996; Sease and Gudmundson 2002). The genetic distinctness of this rookery from neighboring rookeries suggests that the recent population growth on Amak might be largely independent of female immigration from outside. It should be noted, however, that even substantial levels of exchange among local populations that are not in equilibrium might not diminish, and may even generate, heterogeneity.

Genetic differentiation and sea lion ecology

In a recent analysis of seasonal and spatial variation in the diet of the western stock of Steller sea lions, Sinclair and Zeppelin (2002) identified four discrete dietary regions (Fig. 6B). Region 4, encompassing the central and western Aleutians, was the most distinct. It was dominated by Atka mackerel (*Pleurogrammus monopterygius* (Pallas, 1810)) and cephalopods, while the other three regions to the east were characterized by walleye pollock (*Theragra chalcogramma* (Pallas, 1814)) and high frequencies of salmon (*Oncorhynchus* sp.) in summer. The division between regions 3 and 4 was east of Adugak Island in the Aleutian chain (Fig. 6B). This primary break in sea lion diet coincides with regional differences in trends in abundance (York et al. 1996; Sease and Gudmundson 2002; Fig. 6A) and with the major genetic break in the western stock discovered in the present study. Furthermore, Amak Island in the Bering Sea was identified as an outlier in the dietary analysis. This matches well with its unique recent population history and its genetic distinctness reported here.

Sinclair and Zeppelin concluded that prey are targeted by Steller sea lions when they are near shore and densely schooled in spawning or migratory aggregations. Further, the regional differences in diet were taken as evidence of site fidelity by females year-round. The authors went on to suggest that this reflects long-term fidelity to breeding sites and surmised that if the spatial variation in diet and population trend indicates that female dispersal from natal rookeries is low, then the region of female birth may dictate foraging behavior and future reproductive success. Such dietary studies, however, do not directly address questions of female site tenacity and dispersal, as they cannot distinguish between geographic variation due to long-term site fidelity and philopatry and variation due to food availability and the limits of individual foraging ranges at the time of the study. However, our genetic findings indicate that female dispersal is indeed low between the central and western Aleutian Islands (dietary region 4) and sites to the east (dietary regions 1, 2, and 3) and between Amak Island and some other rookeries. By contrast, we found no indications (as yet) of similarly low dispersal among the other dietary regions in the western stock.

Genetic differentiation and the physical environment

Oceanographic features and bathymetry have been shown to influence prey distribution (Logerwell et al. 2005) and diving behavior in Steller sea lions (Fadely et al. 2005) and have recently been used along with geographic differences in sea lion diet and trends to classify rookeries into discrete ecological regions (Call and Loughlin 2005). Spatial variation in the physical environment may also provide insight into the ultimate causes of some of the patterns of population structure we observed. Ladd et al. (2005) recently determined that island pass bathymetry, land mass topography, and the location of the continental shelf in relation to island passes regulates the influence of ocean currents, tides, and mixing on water properties in the eastern and central Aleutian Islands. They recorded a fundamental change in the marine environment along the Aleutian chain at Samalga Pass (~170°W). Surface waters were significantly warmer and fresher, and nitrate concentrations were lower, east of Samalga Pass compared with west of this pass (Fig. 6C). This step change in water properties in the transition zone from shallow continental shelf waters to deep ocean waters is coincident with the primary genetic break we found within the western stock of Steller sea lions and with regional differences in primary productivity (Mordy et al. 2005), zooplankton species composition and abundance (Coyle 2005), and fish communities (Logerwell et al. 2005), as well as the sea lion diet and population trends mentioned earlier, suggesting a link between spatial variation in the physical environment and variation in the ecology and population dynamics of, and the pattern of population subdivision in, Steller sea lions.

The following hypothesis is proposed to explain these congruent spatial patterns. The Steller sea lion's reliance on terrestrial sites for breeding, molting, and rest necessitates that the distribution of the western stock is defined by the east–west axis of the Alaska Peninsula and the Aleutian–Commander Island chain. Physical oceanographic differences along this axis underpin spatial variation in biological oceanography (nutrients, primary productivity, and zooplankton), which is an important factor in determining the prey base for apex predators such as Steller sea lions. Differences in prey in turn influence the foraging ecology, movement, and dispersal patterns, and thus population subdivision, of sea lions on regional and local scales.

Management implications

By tracking the dispersal patterns of females over time, patterns of variation within mtDNA provide unique insights into the evolutionary and demographic relationships among groups of organisms. As such, this marker has a unique application to the identification of units of conservation (Avise 1995). Phylogeographic-level divergence in this marker is typically taken as evidence of ancient divergence of populations and can be used to identify evolutionarily distinct stocks (Dizon et al. 1992; Moritz 1994). Frequency-level divergence is more indicative of restricted dispersal over ecological time scales and can help identify demographically distinct management units (Moritz 1994). Ultimately, stock designations typically come down to whether a subpopulation or population satisfies a series of criteria codified in legislation or official policy (Taylor and Dizon 1999). Under the US Endangered Species Act, distinct population segments (DPSs) are determined based on three sequential considerations: (1) the discreteness of the population relative to the rest of the species; (2) the significance of the population segment to the species; and (3) the population segment's

conservation status in relation to the ESA's standards for listing (i.e., is the population segment endangered or threatened when treated as if it were a species?) (United States Fish and Wildlife Service – United States National Oceanic and Atmospheric Administration 1996). Under the US Marine Mammal Protection Act, stocks are defined as demographically isolated biological populations (Wade and Angliss 1997). The phylogeographic partitioning between eastern and western mtDNA lineages reported here agrees with earlier studies (e.g., Bickham et al. 1996) and supports the current DPS designations within Alaska. The assessment of our genetic findings in combination with information on ecology, habitat, and population trend argues that both the oceanic group and the shelf group of rookeries within the western DPS represent demographically discrete, and biologically and ecologically significant, populations of Steller sea lions. As such, these findings should inform future decisions about DPS and stock designations.

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References

Anderson, P.J., and Piatt, J.F. 1999. Community reorganization in the Gulf of Alaska following ocean climate regime change. Mar. Ecol. Prog. Ser. **189**: 117–123.

Avise, J.C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conserv. Biol. **9**: 686–690. doi:10.1046/j.1523-1739.1995.09030686.x.

Baker, A.R., Loughlin, T.R., Burkanov, V., Matson, C.W., Trujillo, R.G., Calkins, D.G., Wickliffe, J.K., and Bickham, J.W. 2005. Variation of mitochondrial control region sequences of Steller sea lions: the three-stock hypothesis. J. Mammal. **86**: 1075–1084. doi:10.1644/04-MAMM-A-113R1.1.

Beerli, P. 1997–2004. Migrate: documentation and program, part of LAMARC. Version 2.1.3. Revised 23 December 2004. Available from http://evolution.gs.washington.edu/lamarc/.

Beerli, P., and Felsenstein, J. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics, **151**: 763–773.

Beerli, P., and Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* populations by using a coalescent approach. Proc. Natl. Acad. Sci. U.S.A. **98**: 4563–4568. doi:10.1073/pnas.081068098. PMID:11287657.

Bickham, J.W., Patton, J.C., and Loughlin, T.R. 1996. High variability for control region sequences in a marine mammal: implications for conservation and biogeography of the Steller sea lion (*Eumetopias jubatus*). J. Mammal. **77**: 95–108. doi:10.2307/1382712.

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

Call, K.A., and Loughlin, T.A. 2005. An ecological classification of Alaska Steller sea lion (*Eumetopias jubatus*) rookeries: a tool for conservation/management. Fish. Oceanogr. **14**(Suppl. 1): 212–222. doi:10.1111/j.1365-2419.2005.00370.x.

Coyle, K.O. 2005. Zooplankton distribution, abundance and biomass relative to water masses in eastern and central Aleutian Island passes. Fish. Oceanogr. **14**(Suppl. 1): 77–92. doi:10.1111/j.1365-2419.2005.00367.x.

Dizon, A.E., Lockyer, C., Perrin, W.F., DeMaster, D.P., and Sisson, J. 1992. Rethinking the stock concept: a phylogeographic approach. Conserv. Biol. **6**: 24–36. doi:10.1046/j.1523-1739.1992.610024.x.

Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491. PMID:1644282.

Excoffier, L., Laval, G., and Schneider, S. 2006. ARLEQUIN Version 3.01. An integrated software package for population genetics data analysis. Computational and Molecular Population Genetics Lab, Institute of Zoology, University of Berne.

Fadely, B.S., Robson, B.W., Sterling, J.T., Greig, A., and Call, K.A. 2005. Immature Steller sea lion (*Eumetopias jubatus*) dive activity in relation to habitat features of the eastern Aleutian Islands. Fish. Oceanogr. **14**(Suppl. 1): 243–258. doi:10.1111/j.1365-2419.2005.00379.x.

Harlin-Cognato, A., Bickham, J.W., Loughlin, T.R., and Honeycutt, R.L. 2006. Glacial refugia and the phylogeography of Steller's sea lion (*Eumetopias jubatus*) in the North Pacific. J. Evol. Biol. **19**: 955–969. doi:10.1111/j.1420-9101.2005.01052.x. PMID:16674591.

Hoffman, J.I., Matson, C., Amos, W., Loughlin, T.R., and Bickham, J.W. 2006. Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lion *Eumetopias jubatus*. Mol. Ecol. **15**: 2821–2822. PMID:16911203.

Kenyon, K.W., and Rice, D.W. 1961. Abundance and distribution of the Steller sea lion. J. Mammal. **42**: 223–234. doi:10.2307/1376832.

Kumar, S., Tamura, K., Jakobsen, I.B., and Nei, M. 2001. MEGA2.1: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe, Ariz.

Ladd, C., Hunt, G.L., Jr., Mordy, C.W., Salo, S.A., and Stabeno, P.J. 2005. Marine environment of the eastern and central Aleutian Islands. Fish. Oceanogr. **14**(Suppl. 1): 22–38. doi:10.1111/j.1365-2419.2005.00373.x.

Logerwell, E.A., Aydin, K., Barbeaux, S., Brown, E., Conners, M.E., Lowe, S., Orr, J.W., Ortiz, I., Reuter, R., and Spencer, P. 2005. Geographic patterns in the demersal ichthyofauna of the Aleutian Islands shelf. Fish. Oceanogr. **14**(Suppl. 1): 93–112. doi:10.1111/j.1365-2419.2005.00366.x.

Loughlin, T.R. 1997. Using the phylogeographic method to identify Steller sea lion stocks. *In* Molecular genetics of marine mammals. *Edited by* A. Dizon, S.J. Chivers, and W.F. Perrin. Special Publication No. 3, Society for Marine Mammalogy, Orlando, Fla. pp. 159–171.

Loughlin, T.R. 1998. The Steller sea lion: a declining species. Biosphere Conserv. **1**: 91–98.

Loughlin, T.R. 2002. Steller's sea lion. *In* Encyclopedia of marine mammals. *Edited by* W.F. Perrin, B. Wursig, and J.G.M. Thewissen. Academic Press, San Diego. pp. 1181–1185.

Loughlin, T.R., Perlov, A.S., and Vladimirov, V.A. 1992. Range-wide survey and estimation of total number of Steller sea lions in 1989. Mar. Mamm. Sci. **8**: 220–239.

Maddison, W.P., and Maddison, D.R. 1992. MacClade Version 3. Analysis of phylogeny and character evolution. Sinauer Associates, Inc., Publishers, Sunderland, Mass.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. **27**: 209–220. PMID:6018555.

Martien, K.K., and Taylor, B.L. 2003. Limitations of hypothesis-testing in defining management units for continuously distributed species. J. Cetacean Res. Manag. **5**: 213–218.

Merrick, R.L., and Loughlin, T.R. 1997. Foraging behavior of adult females and young-of-the-year Steller sea lions in Alaskan waters. Can. J. Zool. **75**: 776–786.

Merrick, R.L., Loughlin, T.R., and Calkins, D.G. 1987. Decline in abundance of the northern sea lion, *Eumetopias jubatus*, in Alaska, 1956–86. Fish. Bull. (Washington, D.C.), **85**: 351–365.

Mordy, C.W., Stabeno, P.J., Ladd, C., Zeeman, S., Wisegarver, D.P., Salo, S.A., and Hunt, G.L., Jr. 2005. Nutrients and primary production along the eastern Aleutian Island Archipelago. Fish. Oceanogr. **14**(Suppl. 1): 55–76. doi:10.1111/j.1365-2419.2005.00364.x.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. **3**: 401–411.

National Research Council. 2003. Decline of the Steller sea lion in Alaskan waters: untangling food webs and fishing nets. The National Academies Press, Washington, D.C.

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

Nei, M., and Tajima, T. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, **97**: 145–163. PMID:6266912.

Pascual, M.A., and Adkison, M.D. 1994. The decline of the Steller sea lion in the Northeast pacific: demography, harvest or environment? Ecol. Appl. **4**: 393–403.

Pitcher, K.W., Olesiuk, P.F., Brown, R.F., Lowry, M.S., Jeffries, S.J., Sease, J.L., Perryman, W.L., Stinchcomb, C.E., and Lowry, L.F. 2007. Status and trends in abundance and distribution of the eastern population of Steller sea lions (*Eumetopias jubatus*). Fish. Bull. (Washington, D.C.). In press.

Raum-Suryan, K.L., Pitcher, K.W., Calkins, D.G., Sease, J.L., and Loughlin, T.R. 2002. Dispersal, rookery fidelity, and metapopulation structure of Steller sea lions (*Eumetopias jubatus*) in an increasing and a decreasing population in Alaska. Mar. Mamm. Sci. **18**: 746–764.

Raum-Suryan, K.L., Rehberg, M.J., Pendleton, G.W., Pitcher, K.W., and Gelatt, T.S. 2004. Development of dispersal, movement patterns, and haul-out use by pup and juvenile Steller sea lions. Mar. Mamm. Sci. **18**: 746–764.

Raymond, M., and Rousset, F. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Rosel, P.E., Dizon, A.E., and Heyning, J.E. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). Mar. Biol. **119**: 159–167. doi:10.1007/BF00349552.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science (Washington, D.C.), **239**: 487–491. PMID:2448875.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425. PMID:3447015.

Sanger, F., Nicklen, S., and Coulsen, A.R. 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**: 5463–5467. doi:10.1073/pnas.74.12.5463. PMID:271968.

Sease, J.L., and Gudmundson, C.J. 2002. Aerial and land-based surveys of Steller sea lions (*Eumetopias jubatus*) from the western stock in Alaska, June and July 2001 and 2002. US Department of Commerce, NOAA Tech. Memo. NMFS-AFSC-131.

Sinclair, E.H., and Zeppelin, T.K. 2002. Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). J. Mammal. **83**: 973–990. doi:10.1644/1545-1542(2002)083<0973:SASDID>2.0.CO;2.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution, **39**: 53–65. doi:10.2307/2408516.

Sneath, P.H.A., and Sokal, R.R. 1973. Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman and Company, San Francisco.

Sokal, R.R., and Michener, C.D. 1958. A statistical method for evaluating systematic relationships. Univ. Kans. Sci. Bull. **38**: 1409–1438.

Springer, A.M., Estes, J.A., van Viliet, G.B., Williams, T.M., Doak, D.F., Danner, E.M., Forney, K.A., and Pfister, B. 2003. Sequential megafaunal collapse in the North Pacific Ocean: an ongoing legacy of industrial whaling? Proc. Natl. Acad. Sci. U.S.A. **100**: 12223–12228. doi:10.1073/pnas.1635156100. PMID:14526101.

Takahata, N., and Palumbi, S.R. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics, **109**: 441–457.

Taylor, B.L., and Dizon, A.E. 1999. First policy then science: why a management unit based solely on genetic criteria cannot work. Mol. Ecol. **8**: S11–S16. doi:10.1046/j.1365-294X.1999.00797.x. PMID:10703548.

Trites, A.W., and Donnelly, C.P. 2003. The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis. Mammal Rev. **33**: 3–28. doi:10.1046/j.1365-2907.2003.00009.x.

Trites, A.W., and Larkin, P.A. 1996. Changes in the abundance of Steller sea lions (*Eumetopias jubatus*) in Alaska from 1956 to 1992: how many were there? Aquat. Mamm. **22**: 153–166.

Trujillo, R.G., Loughlin, T.R., Gemmell, N.J., Patton, J.C., and Bickham, J.W. 2004. Variation in microsatellites and mtDNA across the range of the Steller sea lion, *Eumetopias jubatus*. J. Mammal. **85**: 338–346. doi:10.1644/1545-1542(2004)085<0338:VIMAMA>2.0.CO;2.

United States Fish and Wildlife Service – United States National Oceanic and Atmospheric Administration. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. U.S. Fed. Regist. **61**: 4722–4725.

Wade, P.R., and Angliss, R.A. 1997. Guidelines for assessing marine mammal stocks: report of the GAMMS Workshop, April 3–5, 1996, Seattle, Washington. US Department of Commerce, NOAA Tech. Memo. NMFS-OPR-12.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for

the analysis of population structure. Evolution, **38**: 1358–1370. doi:10.2307/2408641.

Wright, S. 1951. The genetical structure of populations. Annals in Eugenics, **15**: 323–354.

York, A.E., Merrick, R.L., and Loughlin, T.R. 1996. An analysis of the Steller sea lion metapopulation in Alaska. *In* Metapopulation and wildlife conservation. *Edited by* D.R. McCullough. Island Press, Washington, D.C. pp. 259–292.