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MACROGEOGRAPHIC STRUCTURE AND PATTERNS OF GENETIC DIVERSITY IN HARBOR SEALS (*PHOCA VITULINA*) FROM ALASKA TO JAPAN

ROBIN L. WESTLAKE AND GREGORY M. O'CORRY-CROWE*

Southwest Fisheries Science Center, National Marine Fisheries Service, P.O. Box 271, La Jolla, CA 92038

We examined sequence variation in the control region of the mitochondrial genome from 778 seals sampled at 161 locations from northern Japan to southeastern Alaska to learn more about the evolutionary history and population structure of, and effects of recent declines on genetic diversity in, harbor seals (*Phoca vitulina*) in the northern Pacific Ocean. High haplotypic diversity ($H = 0.975$) and a poorly resolved mitochondrial genome (mtDNA) phylogeny suggest that harbor seals in the Pacific underwent a rapid expansion in population size in their recent evolutionary past, possibly after the retreat of Pleistocene ice sheets. Weak phylogeographic partitioning of lineages attests to a complex evolutionary and demographic history of contemporary Pacific populations. Extensive macrogeographic subdivision was evident among a subset of grouped localities that represent centers of abundance along the distributional continuum. Heterogeneity was influenced by population size and correlated with geographic distance, suggesting that dispersal occurs primarily among neighboring subpopulations. The 2 currently recognized subspecies of harbor seal in the Pacific, *P. v. richardii* of North America and *P. v. stejnegeri* of Asia, do not represent phylogenetically discrete mtDNA assemblages. The greatest differentiation detected was along the Commander–Aleutian Island chain, the region of the presumed subspecies boundary and a likely contact zone for expanding refugial populations of a number of marine mammal species after retreat of ice sheets. Differentiation between the Kodiak Archipelago and Prince William Sound, and between Bristol Bay and the Pribilof Islands, indicates that current management stocks are inappropriate and highlights the need for a detailed analysis of population and stock structure in Alaska. A decline in population size in Prince William Sound over the past few decades was accompanied by a discernible reduction in mtDNA diversity, manifested as a loss of rare haplotypes through random drift. A continued population decline will erode genetic diversity further, with potentially adverse effects on evolutionary potential and individual fitness.

Key words: genetic diversity, genetic drift, harbor seal, isolation-by-distance, mitochondrial DNA

Harbor seals (*Phoca vitulina*) in the Pacific Ocean are distributed along 16,000 km of the coastline across the North Pacific rim from Hokkaido, Japan, in the west to Baja California, Mexico, in the east and form hundreds of breeding groups that occupy a diverse array of habitats (Hoover-Miller 1994; Rice 1998; Shaughnessy and Fay 1977; Fig. 1). Limited population structuring in this species is suggested by a continuous distribution over much of the geographic range, by records of long-distance movements (>320–500 km—Lowry et al. 2001), and by a paucity of physical barriers to movement. Paradoxically, population

* *Correspondent: greg.o'corry-crowe@noaa.gov*

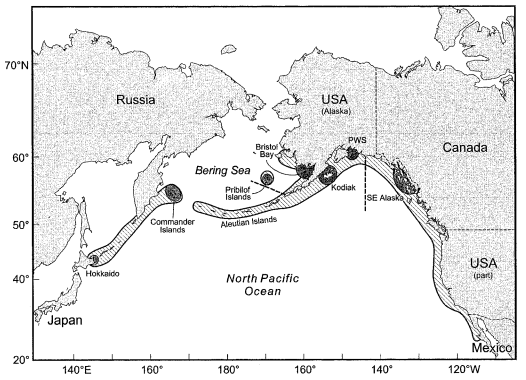

FIG. 1.—Distribution of harbor seals in the Pacific Ocean. Centers of abundance are shaded, and boundaries of the currently recognized management stocks within Alaska are indicated by dashed lines. PWS, Prince William Sound.

structuring is suggested by geographic variation in body size, coat color, annual timing of births, and cranial morphology (Bigg 1969; Burns and Gol'tsev 1984; Burns et al. 1984; Kelly 1981; Shaughnessy and Fay 1977; Temte et al. 1991).

Morphological differences between eastern and western forms are considered extensive enough by some authors for each to qualify as a separate subspecies: *P. v. stejnegeri* of Asia is predominantly dark phase and larger in body size with a more massive skull than the predominantly light phase *P. v. richardii* of North America (Burns et al. 1984; Shaughnessy and Fay 1977). There is still some dispute, however, as to where the boundary between these 2 subspecies lies or whether, in fact, *P. vitulina* in the Pacific comprises a single polytypic taxon (Shaughnessy and Fay 1977). Despite occasional long-distance movements (Bonner and Witthames 1974; Lowry et al. 2001; Thompson et al. 1994; U. Swain et al., in litt.), harbor seals in both the Atlantic and Pacific oceans rarely forage more than 50 km away from haul-out sites (Lowry et al. 2001; Thompson 1993; R. J. Small and J. M. Ver Hoef, in litt.). This, combined with the high rate of recorded returns to haul-out sites (Lowry et al. 2001; U. Swain and R. J. Small, in litt.), has helped characterize this species as relatively sedentary, exhibiting long-term fidelity to particular areas. Regional differences in habitat and prey (Hoover-Miller 1994) and in trends in abundance (K. J. Frost et al., in litt.; R. J. Small et al., in litt.) may be further evidence of population subdivision.

Knowledge of population structure and dispersal in Pacific harbor seals is important for the conservation of the species in parts of its range. Harbor seals have declined sharply over the past 2 decades in some areas of Alaska, whereas in other areas numbers of seals have remained stable or have even increased during this period (Pitcher 1990; K. J. Frost et al., in litt.; E. A. Matthews and G. W. Pendleton, in litt.; R. J. Small et al., in litt.). The United States National Marine Fisheries Service currently recognizes 3 large provisional management stocks defined primarily on the basis of regional differences in trends in abundance: Southeast Alaska where numbers are stable or increasing; the Gulf of Alaska (including the Aleutian Islands) where numbers have declined dramatically; and the Bering Sea where numbers are thought to have declined (Ferrero et al. 2000; Fig. 1). Detailed information on population structure and dispersal patterns, however, is required to identify more meaningful management stocks of this species in Alaska.

Population subdivision is often influenced by evolutionary relationships among natural groupings as well as by contemporary patterns of gene flow and dispersal (Avise 1994). On an evolutionary time scale, much of the range of the Pacific harbor seal is geologically and climatically dynamic (Mann and Hamilton 1995; Mann and Peteet 1994). Successive glaciations and local tectonic activity are constantly changing the coastal and insular landscape. One might expect that the periodic changes in sea level and in coastal geography and topography would result in a complex history of range expansions and contractions, relict populations, and founder events in a coastal species such as the harbor seal, a

history not immediately apparent from its present-day distribution. Reconstructing this evolutionary past may create a greater understanding of current patterns of geographic variation and the demographic history and biogeography of this species in the Pacific.

Although recent telemetry studies have revealed much about movement patterns in Alaskan harbor seals, the studies have typically been of short duration (<1 year) and tell us little, as yet, about the rate and form of dispersal. Similarly, although studies of environmental heterogeneity and regional differences in ecology, trends, morphology, and physiology may reflect or contribute to population subdivision, the resolution of the evolutionary history and population structure of Pacific harbor seals requires the examination of markers that directly reflect patterns of dispersal or breeding (or both) and record evolutionary relationships among natural groupings.

Examination of variation within selectively neutral, highly variable molecular genetic markers can provide insight into the evolutionary affinities of natural groupings and document patterns of dispersal and interbreeding (Avise 1994; Avise et al. 1987). We examined sequence variation within the control region of the mitochondrial genome (mtDNA) to investigate the evolutionary history and resolve the population structure of harbor seals across much of their Pacific range and to provide a framework for the identification of management stocks of this species in Alaska. The relatively rapid rate of evolution and predominantly maternal mode of inheritance (Brown et al. 1979; Denver et al. 2000; Hutchison et al. 1974; Ingman et al. 2000) make mtDNA a particularly informative marker in the study of dispersal patterns and in the identification of demographically discrete management units as well as in the resolution of evolutionary relationships among natural groupings below the species level (Avise 1995; Avise et al. 1987; Moritz 1994; Moritz et al. 1987). In this article, we present our findings on macrogeographic patterns of variation within mtDNA of harbor seals across the North Pacific, including the phylogeography of this marker and the general pattern and extent of population subdivision across the range studied.

Materials and Methods

Sample collection and DNA extraction.—Harbor seal tissue samples were collected at 161 locations from southeastern Alaska to northern Japan from 1975 to 1999 (Table 1). The sequences of 9 of the 14 Japanese seal samples were from a previous study by Stanley et al. (1996) and were accessed from GenBank (4 unique haplotypes, accession numbers U36372–U36375). All age classes and both sexes were represented, and samples were collected in all seasons (details of which are available at http://swfsc.nmfs.noaa.gov). Only individuals from localities within geographically distinct centers of abundance across the distributional continuum were used in the analysis of population subdivision (Table 1; Fig. 1).

Several tissue types were used. Skin biopsies were taken from seals during tagging and rehabilitation operations; shed hair was collected at molting sites; and skin, muscle, and liver samples were collected from subsistence hunts, strandings, and scientific collections. Most tissues were preserved in 20% dimethyl sulfoxide saturated in NaCl (Amos and Hoelzel 1991). Molted hair samples and some tissues were stored at −80°C; several skin samples were fixed in formalin and then stored in ethanol. Total DNA was isolated from most samples using standard sodium dodecyl sulfate (SDS) lysis-proteinase K digestion methods followed by phenol : chloroform extraction and ethanol precipitation protocols (Sambrook et al. 1989). For some samples, a similar method used a cethyltrimethylammonium bromide (CTAB) buffer (55 mM CTAB, 0.5 M NaCl/20 mM ethylenediaminetetraacetic acid [EDTA], 100 mM Tris, pH 8.5–9) in the initial stages (Winnepenninckx et al. 1993). Subsequent extraction and recovery of DNA were similar to the 1st protocol. More recently, the tissue lysis and digestion steps were automated by using the FastDNA® kit and the FastPrep® (BIO 101, Carlsbad, California) instrument. DNA from molted hair was successfully extracted using the lysis buffer of Tikel et

TABLE 1.—Summary of the number of sampling sites and samples of harbor seals by region in Alaska, the Commander Islands, and northern Japan. The number of unique haplotypes and haplotypic and nucleotide diversities are also given for centers of abundance (Fig. 1) that were compared in the analysis of population structure. The values in italics represent subsets of the Prince William Sound data set that were used in the analysis of temporal changes in genetic variation and the contribution to the Japan data set from this and the Stanley et al. (1996) studies.

Location	Number of sampling sites	Sample size	Number of haplotypes	Haplotypic diversity, *H*	Nucleotide diversity, π (%)
Southeast Alaska	42	210	83	0.968	1.36
Central Gulf of Alaska coast	8	59			
Prince William Sound	37	153	63	0.965	1.41
Prince William Sound, 1970s	*18*	*42*[a]	*31*	*0.983*	*1.47*
Prince William Sound, 1990s	*22*	*116*	*50*	*0.960*	*1.40*
Kenai Peninsula—Cook Inlet	17	91			
Kodiak Archipelago	31	142	67	0.971	1.52
Southern Alaska Peninsula	4	24			
Bristol Bay	9	33	18	0.936	1.33
Aleutian Islands	4	14			
Pribilof Islands	2	16	10	0.892	1.35
Commander Islands	4	22	10	0.849	0.96
Hokkaido	3	14	5	0.813	0.90
Hokkaido, this study	*2*	*5*			
Hokkaido, Stanley et al. (1996)	*1*[b]	*9*			
All locations	161	778	225	0.975	1.47

[a] Includes 5 samples from Middleton Island.

[b] No information on the number of sampling sites is available in Stanley et al. (1996). The number was conservatively estimated as 1.

al. (1996—40 mM Tris, 2 mM EDTA, 0.2 M NaCl, 10% SDS, pH 9.2) followed by several phenol : chloroform extractions (De Angelis 2000). The salting-out protocol of Miller et al. (1988) was used to extract DNA from 20-year-old formalin-preserved tissues. Concentration and quality of the purified DNA from all samples were estimated by spectrophotometry and visualized on 1% agarose gels stained with ethidium bromide.

Amplification and sequencing of mtDNA.—Polymerase chain reaction (PCR) amplification of target DNA (Saiki et al. 1988) was performed in 25-, 50-, or 100-μl reactions in a 480 or 9600 thermal cycler (Perkin–Elmer, Norwalk, Connecticut). Reactions contained approximately 0.1 μg template DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 150 μM of each deoxynucleoside triphosphate, 0.3 μM of each primer, and 2.5 units of *Taq* DNA polymerase. After denaturation at 90°C for 2.5 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. Part of the threonine transfer RNA (tRNA) gene, the entire proline tRNA gene, and over 500 base pairs of the control region of mtDNA were amplified using primers H00034 (5′-TACCAAATGTATGAAACCTCAG-3′, Rosel et al. 1994) and L15926 (5′-ACACCAGTCTTGTAAACC-3′), modified from Kocher et al. (1989). A species-specific variant of the former primer, PvH00034 (5′-TACCAAATGCATGACACCAC-AG-3′—the underlined bases denote the changes), was later designed to improve the fit to the sequence of harbor seal control region (primer names refer to position on the type species mtDNA corresponding to the 3′ end of the primer). For those samples where amplification was difficult, such as formalin-fixed tissues, a shorter region was amplified with the internal primers used in sequencing (see below). The amplified PCR products were purified by membrane-based filtration using Microcon® (Millipore, Bedford, Massachusetts) or QIAquick® (Qiagen, Valencia, California) columns.

PCR products were sequenced by the direct dideoxy sequencing method of Sanger et al. (1977) using 4-dye fluorescent technology of Applied Biosystems (ABI 1992). Sequencing was performed in 12- or 20-μl reactions containing 20–200 ng of purified PCR product, 0.10 μM primer, and 2.5 or 7.0 μl of PRISM™ or PRISM™ dRhodamine dye-terminator mix (PE-Applied Biosystems, Foster City, California). Cycle sequencing was carried out in a PE 9600 thermal cycler, 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 primer H16498 (5′-CCTGAAGTAAGAACCAGATG-3′—Rosel et al. 1994) and the light strand with L15926 or an internal primer L15829 (5′-CCTCCCTAAGACTCAAGG-3′, Southwest Fisheries Science Center). Excess dye-labeled terminators were removed from sequencing reactions using Centri-Sep™ spin columns (empBiotech GmbH, Berlin, Germany) or by ethanol precipitation. Sequences were run on an ABI 373A or ABI 377 automated sequencer and were edited and aligned with the SeqEd™ multiple-sequence editor program (ABI 1992).

Analysis of mtDNA data.—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 1.0 (Kumar et al. 1993) and MacClade 3.02 (Maddison and Maddison 1992) softwares, respectively, and by estimating haplotypic (Nei and Tajima 1981) and nucleotide (Nei 1987) diversity with ARLEQUIN 1.1 software (Schneider et al. 1997).

Evolutionary relationships among haplotypes were estimated with discrete character and distance methods. Maximum parsimony and neighbor-joining (Saitou and Nei 1987) analyses were conducted in PAUP* 4.0b8 (Swofford 1998). In the former analysis, the heuristic search algorithm was used; all characters were included, the COLLAPSE and MULTREES options were in effect, and simple taxon addition and tree bisection–reconnection branch-swapping options were used. Three weighting schemes for transition (TS) versus transversion (TV) subsitutions were tried: equal weighting, 1:5, and 1:10. Insertions and deletions (indels) were weighted the same as transversions. A homologous sequence from an east Atlantic harbor seal *P. v. vitulina* (Árnason and Johnsson 1992) was used as an out-group. Absolute distances among haplotypes were used in the neighbor-joining analysis. Bootstrap analysis (Felsenstein 1985—1,000 replicates) using the heuristic or fast stepwise search algorithms was used to place confidence on interior branches in the most parsimonious trees and the neighbor-joining tree.

Phylogenetic relationships among mtDNA sequences were also inferred from a minimum-spanning network of all unique haplotypes (Bandelt et al. 1995; Excoffier and Smouse 1994; Excoffier et al. 1992). The number of nucleotide differences between haplotypes was calculated in MEGA, and haplotypes were connected by a series of mutational events to all other haplotypes through a set of equally parsimonious pathways with the aid of the MINSPNET software (Excoffier and Smouse 1994). In these networks, haplotypes serve both as nodes and branch tips, and all character conflicts are included in the form of reticulations. To clarify relationships among the major lineages, we pruned the network by removing terminal haplotypes. Most of these were assumed to be evolutionarily recent (Donnelly and Tavaré 1986), hence the least informative phylogenetically. Because most of these haplotypes were rare (1 or 2 individuals), the pruning process involved simply removing all haplotypes with a frequency ≤ 2.

The recent demographic history of this species in the Pacific was inferred from a comparison of the frequency distribution of pairwise genetic distances (i.e., mismatch distribution) among all individuals with the model of sudden population expansion (Rogers 1995; Rogers and Harpending 1992) using ARLEQUIN 2.0 software (Schneider et al. 2000).

Two main approaches were used to estimate genetic differentiation. One involved estimating *F*-statistic analogues (Φ-statistics) that incorporate information on the number of mutational steps among individual haplotypes and differences in haplotype frequency, using an analysis of variance (ANOVA) framework (AMOVA; Excoffier et al. 1992). The variance components and indices of genetic structure were estimated in ARLEQUIN. The 2nd approach analyzed the genetic structure using haplotype frequencies only. Conventional *F*-statistics were estimated by ANOVA of haplotype frequencies within and among strata using the same software for Φ-sta-

tistics. Subdivision was also assessed using a chi-square contingency test of independence (Roff and Bentzen 1989). Statistical significance of parameter estimates (F_{ST} and Φ_{ST}) and the chi-square statistic under a hypothesis-testing framework were estimated by 10,000 randomizations of the original data in ARLEQUIN and Chi-Square software, respectively. Genetic relationships among strata were also assessed by constructing a neighbor-joining tree in MEGA based on estimates of Φ_{ST} among strata.

To determine if a change in the observed proportion of rare haplotypes over time in Prince William Sound was due to a loss of rare haplotypes or was an artifact of unequal sample size, we estimated the probability of observing the same number of unique haplotypes in the 1990s that was observed in the 1970s if we had sampled a similar number of individuals (sample sizes were 42 in the 1970s, 116 in the 1990s). We used a program (by B.L. Taylor written in True BASIC v. 3.0) that randomly assigned a haplotype, based on the observed frequencies of the unique haplotypes recorded in the 1990 sample, to each of 42 individuals (i.e., a number equivalent to the 1970 sample) and totaled the number of unique haplotypes assigned at the end of each trial. We ran 10,000 trials and created a distribution of the number of haplotypes expected within a sample of 42 individuals taken from Prince William Sound in the 1990s. The probability of observing the same number or greater in the 1990s equaled the proportion of trials with a total number of haplotypes greater than or equal to that observed in the 1970s.

To test for departure from randomness in geographic patterns of genetic variation, regressions of pairwise genetic distances among strata on geographic distances and correlations between the 2 distance matrices were computed and their significance tested using ISOLDE software (in Genepop v. 3.1d—Raymond and Rousset 1999). The test statistic was based on the *Z*-statistic of Mantel (1967) and its significance determined by multiple 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 the centers of each stratum. Genetic distances were estimates of the frequency-based parameter F_{ST} or of the distance-based parameter Φ_{ST}.

Results

A total of 435 base pairs of the mtDNA control region and adjacent proline tRNA gene were analyzed for sequence variation in 778 harbor seals sampled from across the North Pacific rim (Table 1). Eighty variable sites were identified, 75 with substitutions (73 transitions and 3 transversions) and 5 with indels. Altogether, 225 different haplotypes were identified, with more than half (126) represented by single individuals. Overall haplotypic diversity was high (H = 0.975) due to the large number of rare haplotypes, and overall nucleotide diversity was moderate (π = 1.47%), suggesting that most haplotypes were closely related phylogenetically. All unique haplotypes have been submitted to GenBank (accession numbers AF522643–AF522866).

Phylogenetic analysis.—In the maximum parsimony analysis, 57 out of 80 variable sites were phylogenetically informative. The intraspecific phylogeny of the mtDNA of the North Pacific harbor seal was poorly resolved using this approach. Several thousand equally most parsimonious trees were identified, and the majority-rule consensus tree was highly polytomous (data not shown). Few nodes were supported by more than 60% in the bootstrap analysis, and no geographic concordance was found within the best supported clades. The different TS/TV weighting schemes resulted in almost identical tree topologies and bootstrap values. The neighbor-joining analysis yielded similar findings (data not shown).

The mtDNA phylogeny was more clearly represented in a minimum-spanning network of all unique haplotypes. Haplotypic diversity was high relative to the low-to-moderate nucleotide diversity; so, many haplotypes were 1 or 2 mutational events from several other haplotypes. Therefore, many evolutionary relationships among unique haplotypes were unresolved, resulting in a highly reticulated network with

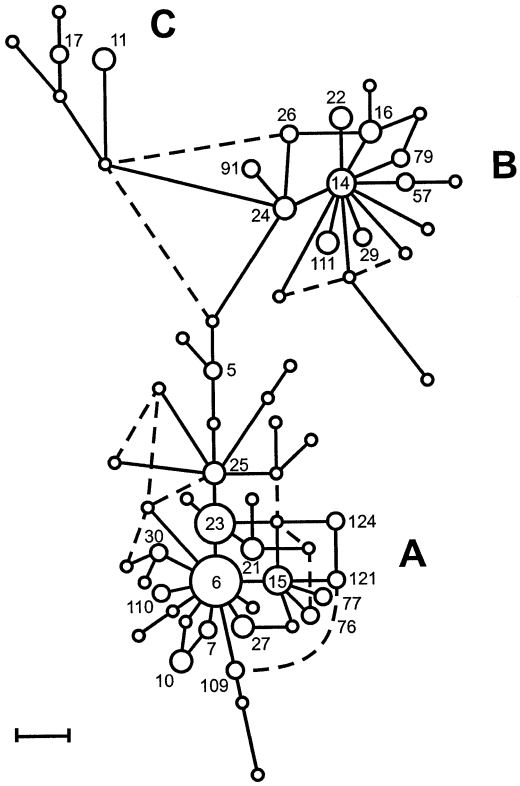

FIG. 2.—Pruned minimum-spanning network of the 64 core mtDNA haplotypes in harbor seals in the northern section of their Pacific range. Branch lengths are equivalent to the minimum number of steps between haplotypes. Haplotype size reflects the abundance of the haplotype in the total sample, and all alternative connections are included. The 3 primary lineages are indicated, and haplotype labels are included for the more common haplotypes.

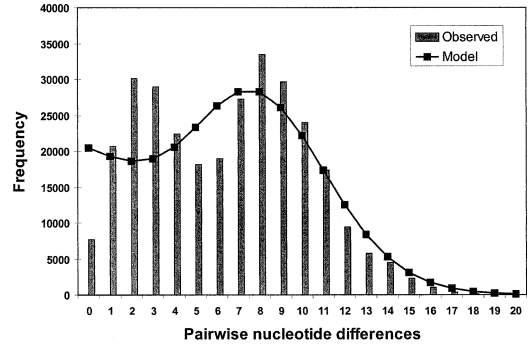

FIG. 3.—Observed distribution of pairwise genetic differences between individual harbor seals in the Pacific compared with the distribution under the model of sudden expansion in population size.

many alternative minimum-spanning trees. Although pruning the network resulted in the loss of some internal haplotypes, the original 225 unique haplotypes were reduced to the 64 core, or common, haplotypes. The resulting minimum-spanning network was composed of 3 distinct lineages (A, B, and C in Fig. 2). Two are characterized by a ''star-like'' phylogeny with several rarer haplotypes radiating from a more abundant central haplotype. A comparison of haplotypic diversity among the major areas of abundance in Alaska, the Commander Islands, and Japan revealed limited phylogeographic structuring. Most core haplotypes from A, B, and C occurred throughout Southeast Alaska and the Gulf of Alaska. Representatives of all 3 were recorded in Bristol Bay, Alaska. Haplotypes from A and B were sampled in the Pribilof Islands, but only haplotypes from lineage A were found in the Commander Islands and Japan. The mismatch distribution of the entire data set was somewhat bimodal but did not differ significantly from the simulated mismatch distribution based on the sudden expansion model (sum of square deviations, $SSD = 0.006$, $P = 0.650$; Fig. 3).

Population subdivision.—Substantial levels of population subdivision were detected and found to be significant in most comparisons using an $\alpha = 0.05$ under a null hypothesis of random mixing, for both frequency-based (F_{ST}, χ^2) and distance-based (Φ_{ST}) statistics over spatial scales on the order of 600–800 km and more (Table 2). Evidence of population subdivision within Alaska is also shown by strong genetic differentiation among the 3 currently recognized management stocks (Table 3).

No differentiation was found between samples from Prince William Sound in the 1970s and animals sampled in the 1990s ($\Phi_{ST} = -0.012$, $P = 0.997$; $F_{ST} = -0.004$, $P = 0.870$). The level of genetic diversity

TABLE 2.—Estimates of genetic differentiation (below diagonal) and significance (*P*) values from 10,000 permutations (above diagonal) among major centers of harbor seal abundance in Alaska, the Commander Islands, and northern Japan. The chi-square analysis gave results similar to that of F_{ST}.[a]

	Centers of harbor seal abundance						
	I	II	III	IV	V	VI	VII
Φ_{ST}							
I	—	0.009	0.000	0.008	0.009	0.000	0.000
II	0.015	—	0.140	0.056	0.026	0.000	0.000
III	0.035	0.004	—	0.110	0.056	0.000	0.000
IV	0.048	0.020	0.012	—	0.297	0.000	0.000
V	0.085	0.049	0.028	0.006	—	0.000	0.000
VI	0.235	0.206	0.205	0.190	0.226	—	0.000
VII	0.225	0.168	0.140	0.181	0.195	0.405	—
F_{ST}							
I	—	0.011	0.002	0.000	0.000	0.000	0.000
II	0.004	—	0.006	0.000	0.000	0.000	0.000
III	0.007	0.006	—	0.000	0.001	0.000	0.000
IV	0.031	0.031	0.027	—	0.000	0.000	0.000
V	0.065	0.065	0.044	0.085	—	0.000	0.000
VI	0.080	0.083	0.079	0.104	0.131	—	0.000
VII	0.095	0.098	0.097	0.120	0.147	0.168	—

[a] I, Southeast Alaska; II, Prince William Sound; III, Kodiak Archipelago; IV, Bristol Bay; V, Pribilof Islands; VI, Commander Islands; VII, Hokkaido.

TABLE 3.—Genetic differentiation among the 3 management units currently recognized by the United States National Marine Fisheries Service. Both distance-based (Φ_{ST}) and frequency-based (F_{ST} and χ^2) statistics were used. Significance values (*P*) are based on 10,000 randomizations of the data set. Estimates of genetic differentiation are below the diagonal, and significance values are above. Results from chi-square analysis (not shown) were also highly significant.

	Bering Sea	Gulf of Alaska	Southeast Alaska
n	49	444	249
Φ_{ST}			
Bering Sea	—	0.0100	0.0005
Gulf of Alaska	0.024	—	0.0006
Southeast Alaska	0.053	0.147	—
F_{ST}			
Bering Sea	—	0.0001	0.0000
Gulf of Alaska	0.018	—	0.0019
Southeast Alaska	0.024	0.003	—

in Prince William Sound in the 1970s (H = 0.983, π = 1.47%), however, was slightly higher than that of the 1990s (H = 0.960, π = 1.40%), and there was a significantly greater proportion of rare haplotypes (i.e., frequency = 1) in the 1970 sample (77.4% versus 58%; $\chi^2 = 10.758$, $P < 0.005$). This reduction in the proportion of rare haplotypes was not an artifact of the more extensive sampling in the 1990s but signaled a loss of rare haplotypes in Prince William Sound. The average number of haplotypes expected within a sample of 42 individuals taken from Prince William Sound in the 1990s was 23, a 26% reduction on the 31 observed in the 1970s with the same sample size, and the probability of recording 31 or more haplotypes in the 1990s was P = 0.0008.

The proportion of genetic distances (both F_{ST} and Φ_{ST}) explained by geographic distance was small when Japan was included in the analysis, probably because of the small Japanese sample (n = 14). Although the possibility of a real effect cannot be ex-

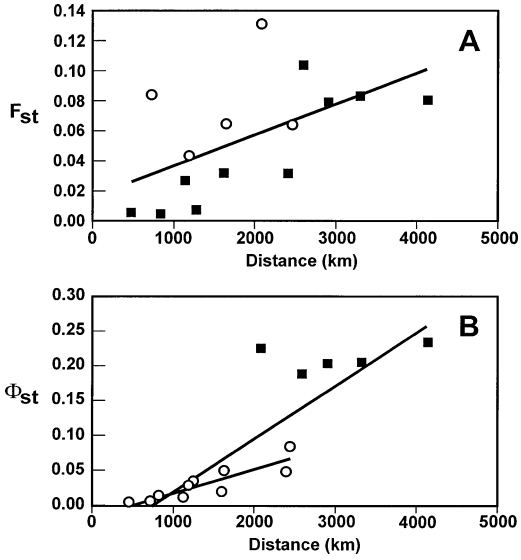

FIG. 4.—The relationship between genetic and geographic distance in harbor seals across the North Pacific Ocean from the Commander Islands to Southeast Alaska. A) Relationship between frequency-based F_{ST} and minimum swim distance ($r^2 = 0.317$, $P = 0.069$). Pairwise comparisons involving the Pribilof Islands are represented by open circles. B) Relationship between distance-based Φ_{ST} and minimum swim distance ($r^2 = 0.729$, $P = 0.00001$). A regression of Φ_{ST} on swim distance for the Alaska regions only (represented as open circles) is also presented ($r^2 = 0.775$).

cluded, we decided to remove the Japanese sample from further analysis. The relationship of F_{ST} to geographic distance for the other 6 strata was poor ($r^2 = 0.317$; Fig. 4A), and the Mantel test was nonsignificant at $\alpha = 0.05$ ($P = 0.069$). In contrast, the relationship of Φ_{ST} to geographic distance for these areas was strong ($r^2 = 0.729$) and highly significant ($P = 0.00001$; Fig. 4B). Finally, the neighbor-joining tree of grouped localities based on Φ_{ST} tends to cluster strata according to geographic proximity (Fig. 5).

DISCUSSION

Mitochondrial DNA phylogeny and diversity.—Because no reliable estimates of substitution rate exist for harbor seal mtDNA, we are unable to date the divergence of separate lineages or to estimate the timing of past demographic events using this marker. Patterns of mtDNA diversity nonetheless provide insight into the evolutionary and demographic history of this species in the North Pacific.

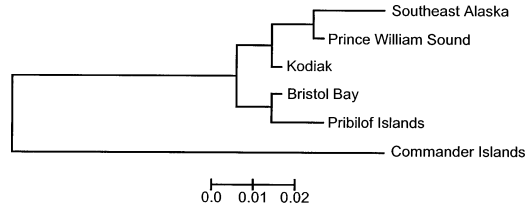

FIG. 5.—A neighbor-joining tree based on estimates of Φ_{ST} among sets of grouped localities that represent 6 centers of abundance of harbor seals across the North Pacific. The scale represents units of genetic distance (Φ_{ST}).

Haplotypic diversity in Pacific harbor seals is among the highest recorded for any marine mammal (e.g., northern elephant seals, *Mirounga angustirostris*, $H < 0.5$—Hoelzel et al. 1993; beluga whale, *Delphinapterus leucas*, $H = 0.85$—O'Corry-Crowe et al. 1997; Steller sea lion, *Eumetopias jubatus*, $H = 0.85$—Bickham et al. 1998a; Dall's porpoise, *Phocoenoides dalli*, $H = 0.96$—Escorza-Treviño and Dizon 2000). By contrast, estimates of nucleotide diversity are moderate relative to other species (e.g., beluga whale, $\pi = 0.51\%$—O'Corry-Crowe et al. 1997; Dall's porpoise, $\pi = 1.42\%$—Escorza-Treviño and Dizon 2000; northern right whale dolphin, *Lissodelphis borealis*, $\pi = 2.1\%$—Dizon et al. 1994). This is because many haplotypes are part of shallow star-like phylogenies, a form of gene tree that suggests a rapid population expansion in recent evolutionary times (Ingman et al. 2000; Slatkin and Hudson 1991). The existence of a number of deeper branches linking these stellate phylogenies is evidence of more ancient divergences.

The combination of these 2 features of the mtDNA phylogeny is evident in the bimodal mismatch distribution. The 1st peak

is due to pairwise comparisons within the separate star-like phylogenies, whereas the 2nd peak is due to comparisons between these clades (Fig. 3). The model of sudden population expansion (Rogers and Harpending 1992) fits the observed mismatch distribution quite well. But it should be noted that similar wavelike distributions are also generated by other models of population growth (Rogers and Harpending 1992; Slatkin and Hudson 1991).

Thus, both the haplotypic diversity and phylogeny of mtDNA suggest that harbor seals in the North Pacific underwent an increase in population size in their recent evolutionary history. This may reflect a period of expansion and recolonization after the retreat of the Pleistocene ice sheets and may be a feature of the mtDNA of many other high latitude species whose evolutionary and demographic history has been shaped by similar paleoclimatic changes.

Phylogeography.—Our findings confirm and expand on those from an earlier study that the 2 currently recognized subspecies of harbor seals in the Pacific do not represent phylogenetically distinct mtDNA assemblages (O'Corry-Crowe and Westlake 1997). This lack of reciprocal monophyly is not unexpected at this taxonomic level. The pruned minimum-spanning network, however, did provide some evidence of phylogeographic structuring across the northern Pacific Ocean. Comparison with the findings of a number of other studies on mtDNA variation in Pacific harbor seals may further resolve the mtDNA phylogeny and tell us something about the origins of the Pacific populations. Eastern Pacific (Washington and California) haplotypes in a study by Stanley et al. (1996) are intermediate to lineages A and C, whereas western Pacific (Japan, Commander Isles, and Alaska) haplotypes are within lineage A, most of which (6/9) were also identified by us. From an assessment of 2 other studies, lineages A and C also occur in British Columbia, Canada, and Puget Sound, Washington (Burg et al. 1999; Lamont et al. 1996).

The presence of lineage A across much of the Pacific, from Japan to Washington, suggests that A may be the ancestral lineage in this ocean. This is further supported by the high haplotypic and nucleotide diversity observed in this lineage compared with lineages B and C, which have only been found in the eastern Pacific to date. Stanley et al. (1996) analyzed 50 samples from Japan to California and inferred, from a neighbor-joining tree, that 2 of the 4 Japanese haplotypes were basal to all others in the Pacific. They conclude that the direction of colonization within this ocean basin was from west to east. We also found weak support for a basal position for some of the Japanese haplotypes in our neighbor-joining tree on a much expanded data set (data not shown). But considering the greater sequence divergence observed in the eastern Pacific (3 lineages) relative to that in the west (1 lineage), an ancient radiation of lineages from east to west is perhaps a more parsimonious interpretation of the current data. Sample size, however, is uneven, and resolution of these questions awaits a more thorough analysis across the entire species range.

The pruning of the minimum-spanning network aided in interpreting the evolutionary relationships among mtDNA lineages and in determining the extent of the phylogeographic signal. Some of the assumptions of the pruning approach may have been violated. For instance, rare haplotypes are not always phylogenetically the most derived or the result of mutations at hypermutable sites (Donnelly and Tavaré 1986). Nevertheless, exclusion of all rare haplotypes did not alter the major branching patterns of the network.

Evidence and form of population subdivision.—The analysis of sample sets from primary centers of abundance revealed population subdivision at least on a scale of 600–800 km, whereas an examination of relationships of genetic to geographic dis-

tance suggested that harbor seal population structure in Alaska, and probably across the entire North Pacific, adheres to a general isolation-by-distance pattern, where genetic differences increase with geographic distance because dispersal distances are short relative to the species range (Slatkin 1993; Wright 1943). This relationship is also evident in the neighbor-joining tree based on Φ_{ST} estimates among centers of abundance, where neighboring areas clustered together (Fig. 5). These findings suggest that dispersal distances are a fraction of the entire range, and dispersal occurs primarily along an axis coincident with the coastline and continental shelf. The findings also suggest that there may be a gene flow component to the clinal patterns of variation and regional differences observed in the morphology and pupping phenology of this species (Bigg 1969; Burns and Gol'tsev 1984; Burns et al. 1984; Kelly 1981; Shaughnessy and Fay 1977; Temte 1991; Temte et al. 1991). A number of recent molecular genetic studies on macrogeographic structure in this species in both the Atlantic and Pacific Oceans also determined that harbor seal populations are structured on a scale of a few to several hundred kilometers and that the extent of heterogeneity is correlated with geographic distance (Burg et al. 1999; Goodman 1998; Lamont et al. 1996; Stanley et al. 1996).

A cursory assessment of harbor seal distribution in the Pacific might suggest that this intraspecific structure adheres to a continuous model of population differentiation (Fig. 1). The limited movements and nonuniform abundance of harbor seals along the distributional continuum, however, suggest regions of high and low dispersal that are more indicative of a stepping-stone model of population structure, where dispersal occurs primarily among neighboring subpopulations (Kimura and Weiss 1964). Such a model is further suggested by distinct and sometimes abrupt changes in habitat. This stepping-stone nature of differentiation may also reflect the evolutionary history of these populations. Throughout much of the Wisconsin glaciation, the most recent cold period from about 10,000 to 70,000 years ago (Péwé 1975), much of the present-day harbor seal habitat in the North Pacific was unavailable to, or unsuitable for, this species. It was either covered by ice, submerged beneath the sea, or too inhospitable to marine mammals that bear their young on land or ice floes (Mann and Hamilton 1995; Mann and Peteet 1994; Péwé 1975). Despite this harsh environment, numerous ice-free areas were dotted along the shore and offshore islands and acted as refugia for entire communities of plant and animal species (Heaton et al. 1996; Pielou 1991; R. P. Goldthwait, in litt.). These coastal areas may very well have been refugia for harbor seals too and may have functioned as a chain of stepping stones for dispersal and range expansion once the ice sheets finally retreated. Part of the mtDNA signal observed today may reflect a degree of matrilineal philopatry to these ancient sites.

Under this isolation-by-distance pattern of population subdivision, the greatest level of statistically significant differentiation will be found when the range is divided into a few large strata, even if the true structure consists of several discrete subpopulations (K. K. Martien and B. L. Taylor, in litt.). There are 2 reasons for this: sample size is maximized, so statistical power to detect differentiation is optimized, and neighboring strata contain individuals from the extremes of the range and are therefore likely to be the most distantly related. Thus, the finding that the 3 currently recognized management stocks in Alaska (Fig. 1) are significantly differentiated is not surprising and sheds little light on the true scale and configuration of population structure or on the location of biologically meaningful management stock boundaries. This is further exemplified by the significant differentiation found between Kodiak and Prince William Sound, both placed within the currently defined Gulf of Alaska stock, and be-

tween Bristol Bay and the Pribilof Islands, both within the Bering Sea stock (Ferrero et al. 2000).

The range of factors that influence genetic differentiation.—Two forces other than dispersal and interbreeding can influence patterns of variation in neutral genetic markers in populations at equilibrium, i.e., mutation and random genetic drift (Hartl and Clark 1989; Wright 1969). The weak relationship of F_{ST} to geographic distance in this study is due, at least in part, to the influence of population size, and therefore drift, on F_{ST}. This is demonstrated most clearly in comparisons involving the Pribilof Islands population, which may never have been very large ($n \leq 1{,}500$), and is currently estimated at only 202 individuals (L. A. Jemison, in litt.). Most comparisons involving this population have F_{ST} values that are higher than values that would be expected from a uniform isolation-by-distance model (i.e., most values are on or above the regression line in Fig. 4A). These relatively large genetic differences can be explained by greater effects of genetic drift acting on haplotype frequencies of the small Pribilof population, resulting in a haplotypic makeup that is quite different from all other areas, including nearest neighbors. By contrast, the relationship of Φ_{ST} to geographic distance is stronger (Fig. 4B). This parameter is influenced by mutation rate and evolutionary relationships among haplotypes as well as by haplotype frequencies (Excoffier et al. 1992). The haplotypes in the Pribilofs are phyletically representative of the region and thus contribute to a uniform cline in evolutionary relationships among populations.

An interesting finding of the relationship between Φ_{ST} and geographic distance is the distinct break between all pairwise comparisons involving the Commander Islands sample and all other comparisons. In fact, excluding the Russian comparisons improves the correlation between Φ_{ST} and geographic distance from $r = 0.85$ to $r = 0.88$ (Fig. 4B). This distinction, which is also evident in the neighbor-joining tree, is consistent with the findings of numerous morphological analyses of a subspecific boundary somewhere along the Commander–Aleutian chain (Burns et al. 1984; Shaughnessy and Fay 1977). This break, where the Commander Islands are substantially more different than would be predicted by the regression across Alaska, coupled with the lower nucleotide diversity recorded at this location relative to others (Table 1), could represent a genetic bottleneck or founder event that occurred on the Commander Islands some time in the past. An alternative interpretation is that this distinction is evidence of an ancient divergence between eastern and western Pacific harbor seals, possibly reflecting 2 refugia or sets of refugial populations to the east and west of the Cordilleran ice sheet, which during the last glacial maximum (22,000 to ca. 17,000 years ago) covered most of Alaska's southern coastline and many of the Aleutian Islands (Mann and Hamilton 1995; Mann and Peteet 1994).

Similar patterns of mtDNA variation have been found in 2 other species of marine mammals that range across the perimeter of the North Pacific, the sea otter (*Enhydra lutris*) and the Steller sea lion. A morphological study of sea otters placed the boundary between the 2 most northerly subspecies along the Commander–Aleutian Ridge, whereas a recent mtDNA study detected a major break to the west of the Commander Islands (Cronin et al. 1996; Wilson et al. 1991). In the case of the Steller sea lion, a study of mtDNA variation distinguished sea lions from the Commander Islands, Kamchatka, and the Kuril Islands in the west from sea lions on the Aleutian Islands, the Pribilof Islands, and in the Gulf of Alaska to the east, prompting the authors to suggest that these populations originated from 2 distinct Pleistocene refugia, in Asia and Beringia, respectively (Bickham et al. 1998b).

Regardless of the timing and the sequence of the historical events that have

contributed to the patterns of mtDNA variation observed in harbor seals, our findings attest to a complex evolutionary history of this species in the Pacific Ocean, in general, and across the Commander–Aleutian Ridge in particular. Although caution is required when making inferences about the evolutionary history of taxa from a single genetic locus (Cronin 1993; Hewitt 2001), the striking similarities observed in the pattern of variation within mtDNA in harbor seals, sea otters, and Steller sea lions suggest that certain geological and climatic events that have shaped marine and coastal habitats in the North Pacific over time have influenced the evolution and biogeography of a number of marine mammal species in this region in similar ways.

Genetic consequences of population decline.—The loss of important genetic diversity through major population declines may lead to a reduction in fitness and may inhibit population recovery or even accelerate extinction (Lacy 1997; Soulé 1985). Two harbor seal populations in the eastern Atlantic (eastern Baltic Sea and Dutch Wadden Sea) have low levels of variation at nuclear and mtDNA sites, possibly due to historical population bottlenecks and founder effects (Goodman 1998; Kappe et al. 1995; Stanley et al. 1996; Swart et al. 1996). Both these populations are small in size (Helander and Bignert 1992; Reijnders and Lankester 1990) and have little or no gene flow with larger neighboring populations. Consequently, genetic drift and inbreeding may cause further loss of genetic diversity. The low level of variation in the Wadden Sea has been suggested to be a possible factor in the slow recovery of this population after the cessation of hunting and high mortality during the 1988 phocine distemper virus epizootic (Swart et al. 1996).

Harbor seal numbers in Prince William Sound have declined dramatically over the past few decades. K. J. Frost et al. (in litt.) estimated a 58% decrease since counts began in the early 1980s, but the recent decline may be even greater because numbers are likely to have been falling since the 1970s (Pitcher 1990). Despite this reduction in population size, mtDNA diversity, at first glance, does not appear to have been substantially reduced. Unlike the above-mentioned European populations, seals in Prince William Sound are estimated to still number in ''the several thousand'' (T. R. Loughlin, cited in K. J. Frost et al., in litt.). Thus, the effects of genetic drift have been moderate to date, and current levels of genetic variation still largely reflect predecline levels. But we found that the number of unique haplotypes observed had declined by 26% between the 1970s and 1990s due primarily to the loss of rare haplotypes. We believe that the influence that any differences in age, sex, or seasonal composition of samples between the 2 temporal strata may have had on this finding is limited by the large spatial scale of this comparison relative to the range of seal movements in this region (Lowry et al. 2001). The change in the level of mtDNA diversity likely reflects changes in heterozygosity at other loci (Taylor and Rojas-Bracho 1999), where the loss of even rare alleles is cause for concern because it may reduce the evolutionary potential of populations and compromise the ability of individuals to deal with environmental challenges and disease (Lacy 1997). Continued population decline in harbor seals could further erode genetic diversity with potentially adverse consequences for individual fitness and population viability. The assessment of diversity at several nuclear loci would be informative and is currently underway.

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Literature Cited

ABI. 1992. SeqEd™: DNA sequence editor. Version 1.0.3. Applied Biosystems Incorporated, Foster City, California.

Amos, W., and A. R. Hoelzel. 1991. Long-term preservation of whale skin for DNA analysis. Reports of the International Whaling Commission Special Issue 13:99–103.

Árnason, Ú., and E. Johnsson. 1992. The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina*. Journal of Molecular Evolution 34: 493–505.

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

Avise, J. C. 1995. Mitochondrial DNA polymorphism and the connection between genetics and demography of relevance to conservation. Conservation Biology 9:686–690.

Avise, J. C., et al. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Bandelt, H. J., P. Forster, B. C. Sykes, and M. B. Richards. 1995. Mitochondrial portraits of human populations using median networks. Genetics 141: 743–753.

Bickham, J. W., T. R. Loughlin, D. G. Calkins, J. K. Wickliffe, and J. C. Patton. 1998a. Genetic variability and population decline in Steller sea lions from the Gulf of Alaska. Journal of Mammalogy 79: 1390–1395.

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

Bigg, M. A. 1969. Clines in the pupping season of the harbor seal, *Phoca vitulina*. Journal of the Fisheries Research Board of Canada 26:449–455.

Bonner, W. N., and S. R. Witthames. 1974. Dispersal of common seals (*Phoca vitulina*), tagged in the Wash, East Anglia. Journal of Zoology 174:528–531.

Brown, W. M., M. George Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences 76:1967–1971.

Burg, T. M., A. W. Trites, and M. J. Smith. 1999. Mitochondrial and microsatellite DNA analyses of harbor seal population structure in the northeast Pacific Ocean. Canadian Journal of Zoology 77:930–943.

Burns, J. J., F. H. Fay, and G. A. Fedoseev. 1984. Craniological analysis of harbor and spotted seals of the North Pacific region. Pp. 5–16 in Soviet-American cooperative research on marine mammals. Pinnipeds (F. H. Fay and G. A. Fedoseev, eds.). National Oceanic and Atmospheric Administration, Technical Report National Marine Fisheries Service 12.

Burns, J. J., and V. N. Gol'tsev. 1984. Comparative biology of harbor seals *Phoca vitulina* Linnaeus 1758, of the Commander, Aleutian, and Pribilof islands. Pp. 17–24 in Soviet-American cooperative research on marine mammals. Pinnipeds (F. H. Fay and G. A. Fedoseev, eds.). National Oceanic and Atmospheric Administration, Technical Report National Marine Fisheries Service 12.

Cronin, M. A. 1993. Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. Wildlife Society Bulletin 21:339–348.

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

De Angelis, M. L. 2000. The mating system of Pacific harbor seals (*Phoca vitulina richardsi*) and its influence on estimates of effective population size. M.S. thesis. San Diego State University, San Diego, California.

Denver, D. R., K. Morris, M. Lynch, L. L. Vassilieva, and W. K. Thomas. 2000. High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. Science 289:2342–2344.

Dizon, A. E., C. A. LeDuc, and R. G. LeDuc. 1994. Intraspecific structure of the northern rightwhale dolphin (*Lissodelphis borealis*): the power of an analysis of molecular variation for differentiating genetic stocks. California Cooperative Fishing Investigation Report 35:61–67.

Donnelly, P., and S. Tavaré. 1986. The ages of alleles and a coalescent. Advances in Applied Probability 18:1–19.

Escorza-Treviño, S., and A. E. Dizon. 2000. Phylogeography, intraspecific structure and sex-biased dispersal of Dall's porpoise, *Phocoenoides dalli*, revealed by mitochondrial and microsatellite DNA analyses. Molecular Ecology 9:1049–1060.

Excoffier, L., and P. E. Smouse. 1994. Using allele frequencies and geographic subdivision to recon-

struct gene trees within a species: molecular variance parsimony. Genetics 136:343–359.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

FERRERO, R. C., D. P. DEMASTER, P. S. HILL, AND M. MUTO. 2000. Alaska marine mammal stock assessments, 2000. Annual Report. National Marine Mammal Laboratory, Seattle, Washington.

GOODMAN, S. J. 1998. Patterns of extensive genetic differentiation and variation among European harbour seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. Molecular Biology and Evolution 15:104–118.

HARTL, D. L., AND A. G. CLARK. 1989. Principles of population genetics. 2nd ed. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

HEATON, T. H., S. L. TALBOT, AND G. F. SHIELDS. 1996. An ice age refugium from large mammals in the Alexander Archipelago, southeastern Alaska. Quaternary Research 46:186–192.

HELANDER, B., AND A. BIGNERT. 1992. Harbor seal (*Phoca vitulina*) on the Swedish coast: population trends and reproduction. Ambio 21:504–510.

HEWITT, G. M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. Molecular Ecology 10:537–549.

HOELZEL, A. R., ET AL. 1993. Elephant seal genetic variation and the use of simulation models to investigate historical population bottlenecks. Journal of Heredity 84:443–449.

HOOVER-MILLER, A. A. 1994. Harbor seal (*Phoca vitulina*) biology and management in Alaska. Marine Mammal Commission, Washington, D.C., Contract #T75134749:1–45.

HUTCHISON, C. A., III, J. C. NEWBOLD, S. S. POTTER, AND M. H. EDGELL. 1974. Maternal inheritance of mammalian mitochondrial DNA. Nature 251:536–538.

INGMAN, M., H. KAESSMANN, S. PÄÄBO, AND U. GYLLENSTEN. 2000. Mitochondrial genome variation and the origin of modern humans. Nature 408:708–713.

KAPPE, A. L., L. VAN DE ZANDE, E. J. VEDDER, R. BIJLSMA, AND W. VAN DELDEN. 1995. Genetic variation in *Phoca vitulina* (the harbor seal) revealed by DNA fingerprinting and RAPDs. Heredity 74:647–653.

KELLY, B. P. 1981. Pelage polymorphism in Pacific harbor seals. Canadian Journal of Zoology 59:1212–1219.

KIMURA, M., AND G. H. WEISS. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561–576.

KOCHER, T. D., ET AL. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences 86:6196–6200.

KUMAR, S., K. TAMURA, AND M. NEI. 1993. MEGA: molecular evolutionary genetics analysis. Version 1.01. The Pennsylvania State University, University Park, Pennsylvania.

LACY, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy 78:320–335.

LAMONT, M. M., ET AL. 1996. Genetic substructure of the Pacific harbor seal (*Phoca vitulina richardsi*) off Washington, Oregon, and California. Marine Mammal Science 12:402–413.

LOWRY, L. F., K. J. FROST, J. M. VER HOEF, AND R. A. DELONG. 2001. Movements of satellite-tagged subadult and adult harbor seals in Prince William Sound, Alaska. Marine Mammal Science 17:835–861.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade version 3, analysis of phylogeny and character evolution. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

MANN, D. H., AND T. D. HAMILTON. 1995. Late Pleistocene and Holocene paleoenvironments of the north Pacific coast. Quaternary Science Reviews 14:449–471.

MANN, D. H., AND D. M. PETEET. 1994. Extent and timing of the last glacial maximum in southeastern Alaska. Quaternary Research 42:136–148.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

MILLER, S. A., D. D. DYKES, AND H. F. POLESKY. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16:1215.

MORITZ, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology 3:401–411.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics 18:269–292.

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

NEI, M., AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97: 145–163.

O'CORRY-CROWE, G. M., R. S. SUYDAM, A. ROSENBERG, K. J. FROST, AND A. E. DIZON. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Molecular Ecology 6:955–970.

O'CORRY-CROWE, G. M., AND R. L. WESTLAKE. 1997. Molecular investigations of spotted seals (*Phoca largha*) and harbor seals (*P. vitulina*), and their relationship in areas of sympatry. Marine Mammal Science, Special Publication 325–338.

PÉWÉ, T. L. 1975. Quaternary geology of Alaska. Geological Survey Professional Paper 835, United States Printing Office, Washington, D.C.

PIELOU, E. C. 1991. After the Ice Age: the return of life to glaciated North America. University of Chicago Press, Chicago, Illinois.

PITCHER, K. W. 1990. Major decline in the number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Marine Mammal Science 6: 121–134.

RAYMOND, M., AND F. ROUSSET. 1999. GENEPOP (version 3.1d): population genetics software for exact tests and ecumenicism. An updated version of GENPOP (v 1.2). Journal of Heredity 86:248–249.

REIJNDERS, P. J. H., AND K. LANKESTER. 1990. Status of marine mammals in the North Sea. Netherlands Journal of Sea Research 26:427–435.

RICE, D. W. 1998. Marine mammals of the world: systematics and distribution. Special Publication, The Society for Marine Mammalogy 4:1–231.

ROFF, D. A., AND P. BENTZEN. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Molecular Biology and Evolution 6:539–545.

ROGERS, A. A. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

ROSEL, P. E., A. E. DIZON, AND J. E. HEYNING. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). Marine Biology 119:159–167.

SAIKI, R. K., ET AL. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SANGER, F., S. NICKLEN, AND A. R. COULSEN. 1977. DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Sciences 74:5463–5467.

SCHNEIDER, S., J. M. KUEFFER, D. ROESSLI, AND L. EXCOFFIER. 1997. ARLEQUIN. Version 1.1. A software for population genetic data analysis. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER. 2000. ARLEQUIN. Version 2.0. A software for population genetic data analysis. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

SHAUGHNESSY, P. D., AND F. H. FAY. 1977. A review of the taxonomy and nomenclature of North Pacific harbor seals. Journal of Zoology 182:385–419.

SLATKIN, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

SLATKIN, M., AND R. R. HUDSON. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

SOULÉ, M. E. 1985. What is conservation biology? Bioscience 35:727–734.

STANLEY, H. F., S. CASEY, J. M. CARNAHAN, S. GOODMAN, J. HARWOOD, AND R. K. WAYNE. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). Molecular Biology and Evolution 13:369–382.

SWART, J. A. A., P. J. H. REIJNDERS, AND W. VAN DELDEN. 1996. Absence of genetic variation in harbor seals (*Phoca vitulina*) in the Dutch Wadden Sea and the British Wash. Conservation Biology 10:289–293.

SWOFFORD, D. L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

TAYLOR, B. L., AND L. ROJAS-BRACHO. 1999. Examining the risk of inbreeding depression in a naturally rare cetacean, the vaquita (*Phocoena sinus*). Marine Mammal Science 15:1004–1028.

TEMTE, J. L. 1991. Precise birth timing in captive harbor seals (*Phoca vitulina*) and California sea lions (*Zalophus californianus*). Marine Mammal Science 7:145–156.

TEMTE, J. L., M. A. BIGG, AND O. WIIG. 1991. Clines revisited: the timing of pupping in the harbor seal (*Phoca vitulina*). Journal of Zoology 224:616–632.

THOMPSON, P. M. 1993. Harbour seal movement patterns. Symposia of the Zoological Society of London 66:225–239.

THOMPSON, P. M., K. M. KOVACS, AND B. J. MCCONNELL. 1994. Natal dispersal of harbour seals (*Phoca vitulina*) from breeding sites in Orkney, Scotland. Journal of Zoology (London) 234:668–673.

TIKEL, D., D. BLAIR, AND D. MARSH. 1996. Marine mammal faeces as a source of DNA. Molecular Ecology 5:456–457.

WILSON, D. E., M. A. BOGAN, R. L. BROWNELL JR., A. M. BURDIN, AND M. K. MAMINOV. 1991. Geographic variation in sea otters, *Enhydra lutris*. Journal of Mammalogy 72:22–36.

WINNEPENNINCKX, B., T. BACKELJAU, AND R. DEWACHTER. 1993. Extraction of high molecular weight DNA from molluscs. Trends in Genetics 12: 407.

WRIGHT, S. 1943. Isolation by distance. Genetics 28: 114–138.

WRIGHT, S. 1969. Evolution and the genetics of populations: the theory of gene frequencies. The University of Chicago Press, Chicago, Illinois 2:1–511.

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