Worldwide Patterns of Mitochondrial DNA Differentiation in the Harbor Seal (*Phoca vitulina*)

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The harbor seal (*Phoca vitulina*) has one of the broadest geographic distributions of any pinniped, stretching from the east Baltic, west across the Atlantic and Pacific Oceans to southern Japan. Although individuals may travel several hundred kilometers on annual feeding migrations, harbor seals are generally believed to be philopatric, returning to the same areas each year to breed. Consequently, seals from different areas are likely to be genetically differentiated, with levels of genetic divergence increasing with distance. Differentiation may also be caused by long-standing topographic barriers such as the polar sea ice. We analyzed samples of 227 harbor seals from 24 localities and defined 34 genotypes based on 435 bp of control region sequence. Phylogenetic analysis and analysis of molecular variance showed that populations in the Atlantic and Pacific Oceans and east and west coast populations of these oceans are significantly differentiated. Within these four regions, populations that are geographically farthest apart generally are the most differentiated and often do not share genotypes or differ in genotype frequency. The average corrected sequence divergence between populations in the Atlantic and Pacific Oceans is 3.28% ± 0.38% and those among populations within each of these oceans are 0.75% ± 0.69% and 1.19% ± 0.65%, respectively. Our results suggest that harbor seals are regionally philopatric, on the scale of several hundred kilometers. However, genetic discontinuities may exist, even between neighboring populations such as those on the Scottish and east English coasts or the east and west Baltic. The mitochondrial data are consistent with an ancient isolation of populations in both oceans, due to the development of polar sea ice. In the Atlantic and Pacific, populations appear to have been colonized from west to east with the European populations showing the most recent common ancestry. We suggest the recent ancestry of European seal populations may reflect recolonization from Ice Age refugia after the last glaciation.

Introduction

The causes of speciation and genetic differentiation in marine organisms are poorly understood (Palumbi 1992; Knowlton and Jackson 1993), and even well-separated populations may be genetically similar (e.g., Graves and Dizon 1989; Palumbi and Wilson 1990; Lacson 1992). A primary reason for this uncertainty is that marine organisms are often transported, actively or passively, long distances (e.g., Baker et al. 1990; Bowen et al. 1995). Consequently, gene flow may prevent differentiation, even between populations separated by long distances or topographic barriers. In contrast, dispersal in terrestrial animals is often limited by their locomotory capabilities and by barriers such as mountains, rivers, or inhospitable habitats (see Avise 1994). Moreover, for many terrestrial species, the recent Ice Ages have greatly influenced the geographic pattern and levels of genetic differentiation (e.g., Ferris et al. 1993; Taberlet and Bouvet 1994; Cooper, Ibrahim, and Hewitt 1995). Therefore, patterns of differentiation among terrestrial organisms can often be understood as the result of interactions between known historical, topographic, and environmental factors. In contrast, life history characteristics may be the primary influence on the amount of genetic differentiation among populations of marine organisms rather than barriers to dispersal or geographic distance (Jackson 1974; Knowlton and Jackson 1993).

The harbor seal (*Phoca vitulina*) has the most extensive breeding distribution of any seal, with colonies distributed over 16,000 kilometers from the east Baltic to Japan (fig. 1). Tagging studies have shown that harbor seals can have extensive migrations of several hundred kilometers (Bonner and Witthames 1974; Wigg and Oien 1988), but telemetry data suggest that most individuals return to their natal area (Thompson 1993). Thus, maternally inherited genetic markers, such as mitochondrial DNA, would be predicted to show a segregated pattern among breeding grounds (e.g., Bowen et al. 1992, 1995). Moreover, although harbor seals can disperse over long distances, a primary geographic division within the species should exist between Atlantic and Pacific populations because harbor seals are unlikely to traverse the pack ice in the high Arctic. This barrier may have existed since the beginning of the Ice Ages

Key words: control region sequences, harbor seal, phylogeography, AMOVA.

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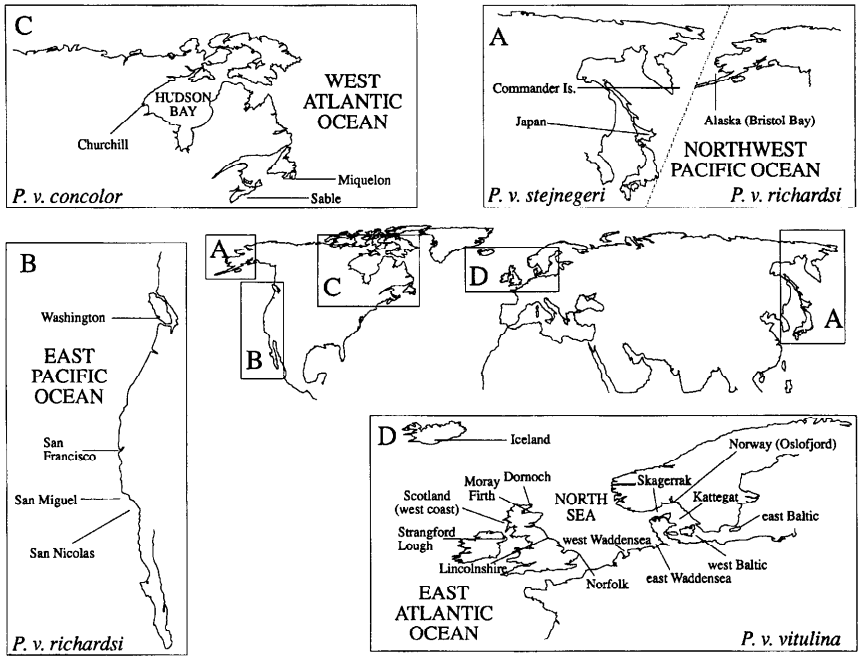

FIG. 1.—Sampling localities and geographic range of the harbor seal.

and the first appearance of Arctic sea ice about 2–3 Mya (Stanley 1986; Harland et al. 1990). A second division might exist within each ocean basin between east and west coastal breeding populations because they are isolated by long distances (fig. 1). Consequently, on geographic grounds, the following hierarchy of geographic partitioning is predicted: first, between Atlantic and Pacific populations; second, between east and west coast populations of each ocean; and finally, within each regional unit, a pattern of genetic differentiation increasing with distance. Where no barriers to dispersal exist, differentiation with distance is a predicted consequence of the finite dispersal distance of individuals (Slatkin 1993).

Support for interocean and coast-to-coast differentiation comes from morphologic and taxonomic studies that have described four primary subspecies (Doutt 1942; Scheffer 1958; McLaren 1966; Shaughnessy and Fay 1977; Smith, Lavigne, and Leonard 1994; fig. 1). These taxonomic schemes have recognized the division in the Pacific occurring between the Kuril Islands, Hokkaido, and the North American coast. In the Atlantic Ocean, the division occurs between Greenland and North America, and Europe. In this study, we assessed the validity of geographic subdivisions suggested by past taxonomic research and assessed gene flow and genetic differentiation within regions as a function of distance between breeding colonies. Moreover, we attempted to determine the degree to which harbor seals are philopatric. To do this, we analyzed control region sequences of 227 seals from 24 localities (fig. 1). We examined clustering and minimum spanning trees of genotypes found in each population and population trees based on pairwise sequence divergence values. These analyses identified the primary regional population units. We then used an AMOVA (analysis of molecular sequence variance) approach to determine subdivision within regions, assess the degree of philopatry, and estimate rates of gene flow.

Materials and Methods

Populations Sampled

Blood or tissue samples were collected from 227 harbor seals from 24 locations, representing all four subspecies (fig. 1). Tissues samples were stored either dry or in 10 mM Tris-HCl (pH 8.0)/100 mM EDTA, at ambient temperature or frozen. Whole blood drawn in EDTA was frozen as soon after collection as possible. Specimens obtained were *Phoca vitulina vitulina*: Iceland (8); Strangford Lough, Northern Ireland (7); west coast of Scotland (7); Dornoch, east coast of Scotland (16); Moray Firth, east coast of Scotland (15); Norfolk, England (17); Lincolnshire, England (4); Oslofjord, Norway (15); east Waddensea, Germany (17); west Waddensea, Holland (18); west Baltic (9); Island of Öland, east Baltic (8); Kattegat Strait (8); and Skagerrak Strait (9); *Phoca vitulina concolor*: Sable Island, Nova Scotia, Canada (11); Miquelon Island, Nova Scotia, Canada (7); and Churchill, Manitoba, Canada (1); *Phoca*

vitulina richardsi: Bristol Bay, Alaska, USA (12); Puget Sound, Washington, USA (4); San Francisco, California, USA (5); San Nicolas Island, California, USA (12); and San Miguel Island, California, USA (5); *Phoca vitulina stejnegeri*: Hokkaido, Japan (9); and Bering Island, Commander Islands, Russia (3).

Mitochondrial DNA Amplification and Sequencing

Total genomic DNA was extracted using standard procedures (Sambrook, Fritsch, and Maniatis 1989). Amplification conditions for the polymerase chain reaction (PCR) (Mullis and Faloona 1987; Saiki et al. 1988) were 35 cycles of denaturation at 95°C for 20 s, annealing at 45°C for 20 s, and extension at 72°C for 30 s. The PCR was performed in a 25-μl reaction volume containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl_2, 1.5 mM $MgCl_2$, 0.1% non-ionic detergent, 100 μM each of dGTP, dATP, dTTP, and dCTP, and 0.3 Units of *Taq* DNA polymerase in a programmable thermal cycler (Perkin Elmer-Cetus, Model 9600). Direct sequencing of double-stranded DNA (Sanger, Nicklen, and Coulson 1977) was carried out using modifications of DMSO-based protocols (Green et al. 1989; Winship 1989) and a Sequenase Version 2.0 kit (US Biochemicals).

We initially amplified the control region from five individuals from four geographically distant localities using the primers ThrL 16272 (5′-CCCGGTCTTGTAAACC-3′) and PheH1 H957 (5′-ATTTTCAGTGTCTTGCTT-3′), both modified from Hoelzel, Hancock, and Dover (1991), or ThrL 16272 and H189 (5′-CTATGTCCCGCTACCATTGAC-3′, designed by H.F.S.). In the primer nomenclature, L and H refer to the light and heavy strands of mitochondrial DNA, respectively, and the numbers refer to the position of the 3′ base in the harbor seal reference sequence (Árnason and Johnsson 1992). In the sequencing with DLH 16750 (5′-CCTGAAGTAGGAACCAGATG-3′; Wilkinson and Chapman 1991), we identified a 200-bp region that was highly variable. This segment was sequenced in all 227 samples, and 28 genotypes were identified, defined by 19 variable sites. A representative of each of these genotypes was sequenced for 453 bp by extending the sequencing in the 5′ direction to position 16370 at the start of the control region using H16539 (5′-CAACCACTTCATGTACATGC-3′; H.F.S.) and in the 3′ direction using H34 (5′-CCAAATGCATGACACCACAG-3′, H.F.S.). This analysis generated a total of 34 genotypes, defined by 40 variable sites (fig. 2). All genotypes were sequenced at least twice using heavy strand primers and representative genotypes were confirmed using the ThrL 16272. For 21 of the genotypes, we sequenced a second individual to assess within-genotype variation. None was found. Sequences have been deposited in GenBank (accession numbers U36342–U36375).

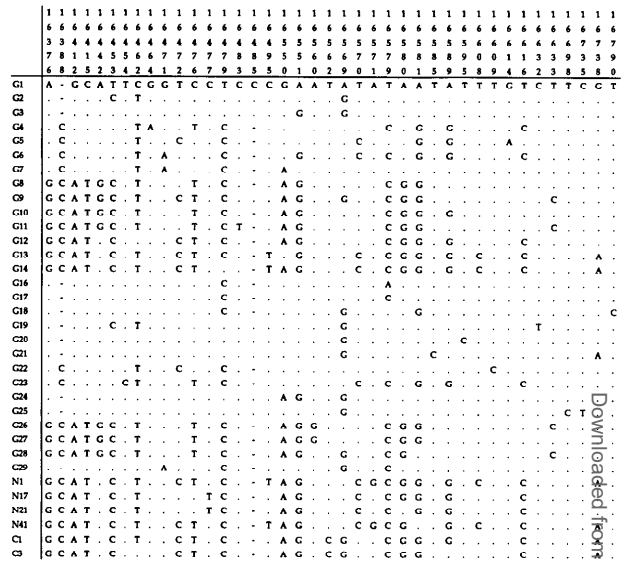

FIG. 2.—Substitutions and deletions (indicated by a dash) observed in 453 bp of control region sequence. Numbered sites at the head of each column are with reference to Árnason and Johnsson (1992), where 16370 is the first position in the control region.

Sequence Alignment and Phylogeny

We used several approaches to analyze the relationships among control region sequences and populations. First, sequences were aligned using the program Clustal V (Higgins and Sharp 1989) and the relationships of genotypes determined by maximum-parsimony analysis using PAUP version 3.1.1 for the Apple Macintosh (Swofford 1990). Because of its close relationship to the harbor seal (Árnason et al. 1995), sequence from the spotted seal (*Phoca largha*) was used as an outgroup for the analysis. However, the resolving power of maximum-parsimony analysis was of limited value here because of the combination of a large number of taxa, limited number of informative sites, and many most parsimonious trees (>100). Consequently, we report results from three other approaches. First, we computed genetic distances between genotypes assuming a gamma distribution of substitution rates across nucleotide sites (Tamura and Nei 1993; Wakeley 1993). A value of a = 0.5 for the gamma distribution parameter in the Tamura and Nei model (1993) was used. This value is appropriate for sequences in control region I (Kumar, Tamura, and Nei 1993; Wakeley 1993). Gamma distances were then used to construct a neighbor-joining (NJ) tree, and bootstrap values were calculated using the computer program MEGA (Saitou and Nei 1987; Kumar, Tamura, and Nei 1993). Values from 0.2 to 0.9 of the gamma distance parameter were also tried and found to have only minor effects on the NJ tree.

Second, the discovery of so many most parsimonious trees suggested that a "star" phylogeny better

characterized the evolution of many sequences. Thus, we used an alternative portrayal of sequence evolution, a minimum spanning network, in which sequences are the nodes of the network rather than terminal tips of a tree. Networks may more effectively portray the relationships among sequences for populations in which many sequences may be derived from the same ancestral genotype (see examples in Excoffier, Smouse, and Quattro 1992). Using the pairwise gamma distance matrix as input to the program NTSYS (Numerical Taxonomy and Multivariate Analysis System; Rohlf 1990), we calculate a minimum spanning tree and scaled the size of nodes in proportion to genotype frequency, as in Excoffier, Smouse, and Quattro (1992). Alternative minimum spanning networks were uncovered using a program supplied by Laurent Excoffier (Department of Anthropology, University of Geneva). Finally, we computed average sequence divergence between populations (Nei 1987) and used the resulting divergence matrix, with a neighbor-joining algorithm, to create a clustering tree of populations. By using these three approaches, we hoped to identify possible geographic population units to be used in the analysis of molecular variance (see below).

Regional Patterns of Geographic Subdivision, Gene Flow, and Philopatry

We used an analysis of variance format specified for molecular sequence data to deduce the significance of geographic divisions among local and regional population groupings (Excoffier, Smouse, and Quattro 1992). This approach, termed analysis of molecular variance (AMOVA), is a hierarchical approach analogous to analysis of variance (ANOVA) in which the correlations among genotype distances at various hierarchical levels are used as F-statistic analogs, designated as Φ statistics; Φ_{st} is the correlation of random genotypes within a population relative to that from the whole species and is analogous to F_{st} of Wright (1951); Φ_{ct} is the correlation of random genotypes within a group of populations relative to that drawn from the entire species and measures the proportion of genetic variation among groupings of populations; and lastly, Φ_{sc} is the correlation of random genotypes within populations relative to that within a regional grouping of populations and measures the proportion of variation among populations within a region. The significance of these F-statistic analogs is evaluated by random permutations of sequences among populations. We experimented with various groupings of populations suggested by the analysis of DNA sequence and population trees (see above) and those suggested by taxonomy and geographic isolation (fig. 1). The groupings that maximize values of Φ_{ct} and are significantly different from random distributions of individuals are assumed to be the most probable geographic subdivisions. Other F_{st} analogs have been utilized in the study of population structure based on mitochondrial data (c.g., Takahata and Palumbi 1985; Lynch and Crease 1990; Hudson, Slatkin, and Maddison 1992; Georgiadis et al. 1994). However, the Excoffier, Smouse, and Quattro (1992) method is not sensitive to deviations from normality and can be used conveniently in a hierarchical framework through the program AMOVA supplied by the authors.

Gene flow within and among regions is approximated as Nm, the number of female migrants occurring between population units per generation, and is approximated by the expression $F_{st} = 1/(1 + 2Nm)$, where N is the female effective population size and m is the female migration rate (Slatkin 1987, 1993; Baker et al. 1994). We used pairwise estimates of Φ_{st} as surrogates for F_{st} among regional groupings of populations and calculated migration rates. Following Slatkin (1993), we assessed differentiation by distance by plotting pairwise log(Nm) values against log(geographic distance). The significance of the association was determined by applying a Mantel's permutation test (Mantel 1967). A significant association between Nm and distance indicates genetic structuring in populations and that dispersal of individuals is limited (Slatkin 1993). Finally, we measured the degree of philopatry as the smallest grouping of populations among which the observed value of Φ_{st} was less than that found in 5% or more of random population groupings. These population groupings are assumed to represent areas in which females do not appear to discriminate among breeding sites frequently enough to cause differentiation by genetic drift.

Results

Geographic Distribution of Control Region Sequences

We found 34 control region genotypes in our sample of 227 individuals from 24 localities (table 1 and fig. 2). Regional segregation of genotypes was suggested by the restriction of some genotypes to a single locality and the observation that some localities had no genotypes in common with other populations. In the Pacific Ocean, genotypes are not shared among localities in Japan, the Commander Islands, Alaska, and the east Pacific coast. In the Atlantic Ocean, populations from Miquelon and Sable Islands, Churchill, Europe, and east Baltic do not share control region sequences (fig. 1 and table 1). The difference between control region genotypes of the east and west Baltic was surprising considering that they are separated by only about 150 kilometers and other European populations separated by a similar distance extensively share genotypes (table 1).

Table 1
Absolute Frequency of Harbor Seal Control Region Genotypes at 24 Locations. Unit Refers to Population Clusters Grouped According to Shared Genotypes and/or Absence of Subdivision (See Text)

Region	Unit	Population	Genotype																																	Sample Size
			N1	N-17	N-21	N-41	C1	G-C3	G-12	G-13	G-14	G8	G9	G-10	G-11	G-26	G-27	G-28	G5	G-G6	G-23	G4	G7	G1	G2	G3	G-24	G-22	G-29	G-18	G-20	G-21	G-25	G-16	G-17	G-19	
Northwest Pacific	1	Japan	4	3	1	1																															9
	2	Commander Islands					2	1																													3
	3	Alaska							4	6	2																										12
East Pacific	4	Washington													2			2																			4
	5	San Francisco										3	2																								5
	6	San Nicolas										6		2	1	2	1																				12
	6	San Miguel										2			3																						5
West Atlantic	7	Miquelon Island																		3	4																7
	7	Sable Island																	3	3		5															11
	8	Churchill																					1														1
East Atlantic	9	Iceland																						3	3			2									8
Scotland and Northern Ireland	10	Moray Firth																							5	10											15
	10	Dornoch																							7	8	1										16
	10	Scotland (west coast)																							5	2											7
	10	Strangford Lough																							2	5											7
North Sea	11	Norfolk																						5	12												17
	11	Lincolnshire																						1	3												4
	11	Norway																						2	13												15
	11	West Waddensea																							18												18
	11	East Waddensea																						1	16												17
West Baltic	12	West Baltic																						1	5				1	2							9
	12	Kattegat																							6					1	1						8
	12	Skagerrak																							6							2	1				9
East Baltic	13	East Baltic																																5	1	2	8
		Total Frequency	4	3	1	1	2	1	4	6	2	11	2	2	6	2	1	2	3	6	4	5	1	13	101	25	1	2	1	3	1	2	1	5	1	2	227

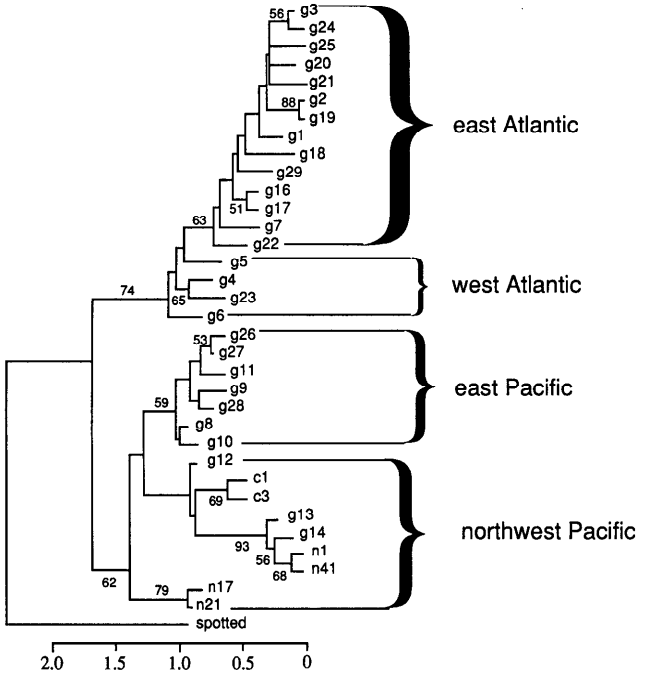

FIG. 3.—Neighbor-joining tree based on gamma distances among control region genotypes. Nodes supported in over 50% of 1,000 bootstrap replications are indicated below nodes.

Within regions, some populations had both unique and ubiquitous control region genotypes. For instance, Iceland has three genotypes, two of which, G1 and G2, have wide distributions in Europe, whereas the third, G22, is found only in Iceland (table 1). Genotypes that had a ubiquitous distribution within regions include G11 that is found in the east Pacific, G3 found in Scotland and Northern Ireland, and G1 found in populations from Iceland, the North Sea, and west Baltic.

Sequence Divergence and Relationships of Control Region Genotypes

Forty variable sites, including two indels, were identified in 453 bp of control region sequence (fig. 2). The number of substitutions between harbor seal genotypes varied from 1 to 23, corresponding to gamma distance values of 0.02% and 6.02%, respectively. Generally, genotypes within populations and within regions were genetically the most similar. Genotypes found in Iceland, the British Isles, or the North Sea (G1, G2, G3, G24) had very low levels of divergence (range: 0.2%–0.9%), as did those from the east Pacific (range: 0.2%–0.7%).

The neighbor-joining tree suggests two primary divisions of genotypes: one group containing Atlantic and the other, Pacific Ocean sequences (fig. 3). The bootstrap supports for these two groupings are 74% and 62%, respectively. Within the Pacific grouping, two clusters of sequences were more weakly supported: a northwest Pacific grouping contained genotypes from Alaska, the Commander Islands, and Japan; and an east Pacific grouping contained genotypes from the Washington and California coasts. No clear divisions were evident among Atlantic Ocean sequences. However, all genotypes from Miquelon and Sable Islands (G6, G4, G23, G5) were basal to the group containing genotypes from Iceland (G22), Churchill (G7), and Europe.

Population frequency information and the relationships among sequences can be portrayed simultaneously on a minimum spanning tree (fig. 4). East Atlantic ge-

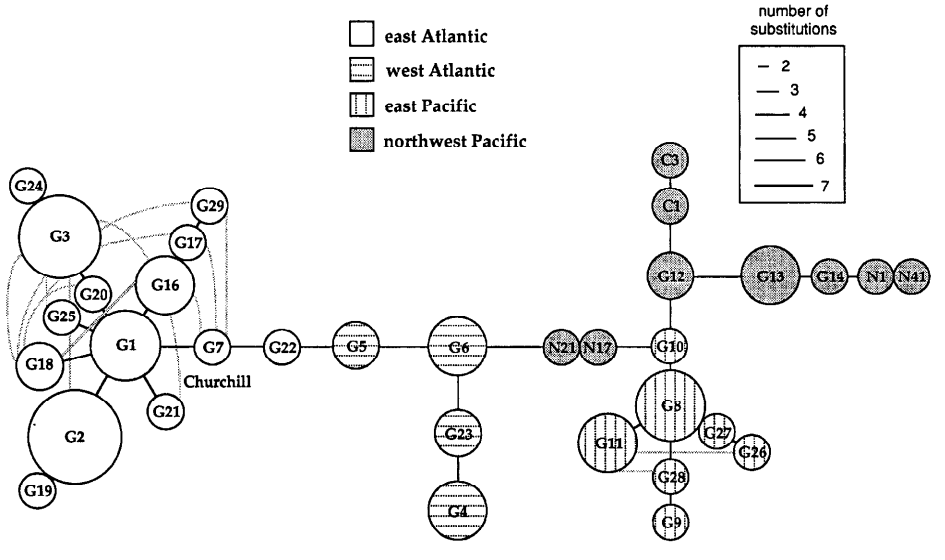

FIG. 4.—Minimum spanning tree based on gamma distances among control region genotypes. Shadow lines indicate groupings in alternative minimum spanning trees.

notypes form a star-like cluster, with the widely distributed genotype G1 as a hub. G1 is three or fewer substitutions different from 7 of 11 east Atlantic genotypes (fig. 2). Genotypes G1 and G2 are the only ones found throughout the Baltic and North Sea (table 1) and are directly linked to all genotypes restricted to the east Baltic (G16, G17, G19). The Churchill genotype (G7) and the one genotype restricted to Iceland (G22) connect the cluster of the four east Atlantic genotypes from Miquelon and Sable Islands (G5, G6, G23, G4). The east Atlantic genotypes are then connected to the group containing all Pacific Ocean genotypes. All California and Washington genotypes have the common and widespread G8 as a hub. This pattern of common, widespread genotypes connecting to several other region-specific genotypes suggests recurrent evolution of sequences from the same ancestral sequences that are still extant within the population. In general, the minimum spanning tree supports the inter-ocean division of genotypes and the east and west divisions within each ocean. Alternative minimum spanning trees, as indicated by shadow lines in figure 4, show that these large-scale relationships are well supported, but many possible trees exist within regional groupings. Consequently, a simple bifurcating tree is an inadequate description of population-level sequence evolution.

Population-Level Divergence

Gamma distances between genotypes were used to calculate the average sequence divergence between populations (Nei 1987). These distances ranged from about 0.2% between closely spaced populations (e.g., Scotland and Northern Ireland, Lincolnshire and Norfolk, and the California Channel Islands) to about 3%–3.6% between widely separated populations (e.g., between populations in the Atlantic and Pacific Oceans). The average sequence divergence between populations in the Atlantic and Pacific Oceans was 3.28% ± 0.38% and those between populations within each of these oceans were 0.75% ± 0.69% and 1.19% ± 0.65%, respectively.

The neighbor-joining population phenogram clearly identifies four regional units: west Atlantic (Miquelon and Sable Islands); north and east Atlantic (Churchill through east Baltic); northwest Pacific (Alaska, Commander Islands, and Japan); and east Pacific (Channel Islands, San Francisco, and Washington) (fig. 5). In the north Pacific, Alaska and Japan are genetically similar (average sequence divergence = 0.72%), as are all populations on the U.S. Pacific coast (average sequence divergence = 0.29% ± 0.08%). West Atlantic populations are distinct from those in the north and northeast Atlantic (average sequence divergence = 1.95% ± 0.23%). Populations from the British Isles, Waddensea, Norway, and west Baltic are genetically similar (average sequence divergence = 0.23% ± 0.10%), whereas those in the east Baltic, Iceland, and Churchill have higher values of average sequence divergence and are located in a basal position (fig. 5). In sum, the population tree highlights the similarity of populations within the four regions but identifies additional distinct population units, such as east and west Baltic, Iceland, and Churchill, which have unique genotypes that are relatively divergent from those found elsewhere in Europe.

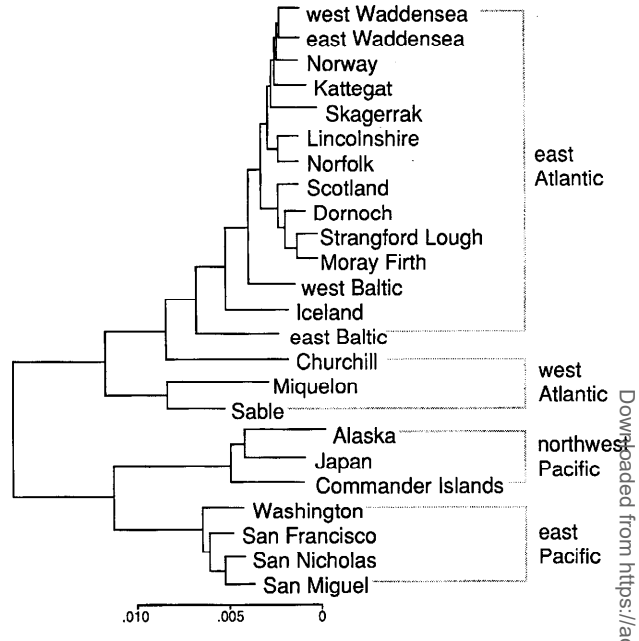

FIG. 5.—Neighbor-joining tree based on average sequence divergence between populations.

Analysis of Molecular Variance (AMOVA)

We first used AMOVA to contrast the four primary regional divisions suggested by the clustering analysis with those suggested by taxonomic studies (fig. 1) and with other geographically conceivable population units. The division of populations into four groups that consistently showed the highest degree of among-population variation was that suggested by the previous sequence and population-level cluster analysis. In this partition, 82% of the total variance was due to that among regions compared with 5% within regions (table 2). The Φ_{ct} value of 0.82 is far above that of four random divisions containing the same number of populations (fig. 6).

Within these large regional groupings, additional subdivisions are statistically significant but less dramatic. In the Pacific Ocean, 35% of the genetic variation is distributed among the populations from Alaska, Japan, and the Commander Islands (northwest Pacific), a value that is significantly different from random (table 2). Even among populations on the U.S. Pacific coast (east

Table 2
AMOVA Results for Global and Pacific Subdivisions of Harbor Seal Populations[a]

Groupings	Permutations	Divisions	Variance Component	% Total Variance	Φ-Statistics	*P* (more extreme value)
Global Φ_{st}	9,999	None	AP	77.19	Φ_{st} = 0.772	<0.0001
		All populations	WP	22.81		
Oceanic divisions	5,000	Atlantic	AG	72.58	Φ_{ct} = 0.726	<0.0002
		Pacific	AP/WG	15.59	Φ_{sc} = 0.568	<0.0002
			WP	11.84	Φ_{st} = 0.882	<0.0002
4 Global divisions	5,000	Northwest Pacific	AG	81.85	Φ_{ct} = 0.819	<0.0002
		East Pacific	AP/WG	5.03	Φ_{sc} = 0.277	<0.0002
		West Atlantic	WP	13.12	Φ_{st} = 0.869	<0.0002
		East Atlantic & Churchill				
4 Global divisions subspecies	5,000	*P. v. richardsi*	AG	77.22	Φ_{ct} = 0.772	<0.0002
		P. v. stejnegeri	AP/WG	9.23	Φ_{sc} = 0.405	<0.0002
		P. v. concolor	WP	13.54	Φ_{st} = 0.865	<0.0002
		P. v. vitulina				
4 Global divisions counter example	5,000	Channel Islands	AG	59.39	Φ_{ct} = 0.594	<0.0002
		Other Pacific	AP/WG	21.93	Φ_{sc} = 0.540	<0.0002
		West Atlantic & British Isles	WP	18.68	Φ_{st} = 0.813	<0.0002
		Norway, Baltics & Waddensea				
Pacific divisions	1,500	California & Washington	AG	60.36	Φ_{ct} = 0.604	<0.0007
		Japan, Alaska & Commander Islands	AP/WG	11.73	Φ_{sc} = 0.296	<0.0007
			WP	27.91	Φ_{st} = 0.721	<0.0007
Pacific divisions counter example	1,500	California, Alaska & Washington	AG	7.9	Φ_{ct} = 0.079	0.3338
		Japan & Commander Islands	AP/WG	57.34	Φ_{sc} = 0.623	<0.0007
			WP	34.76	Φ_{st} = 0.652	<0.0007
Indivisible Groups						
Northwest Pacific	500	Japan	AP	35.21	Φ_{st} = 0.352	<0.002
		Commander Islands	WP	64.79		
		Alaska				
East Pacific	500	Washington	AP	25.28	Φ_{st} = 0.253	<0.002
		San Francisco	WP	74.72		
		San Nicolas				
		San Miguel				
California	500	San Francisco	AP	17.81	Φ_{st} = 0.178	0.0299
		San Nicolas	WP	82.19		
		San Miguel				
Channel Islands	500	San Nicolas	AP	19.38	Φ_{st} = 0.194	0.0619
		San Miguel	WP	80.62		

[a] AG is the among-groups component of variance, AP/WG is the among-populations/within-group component of variance, WP is the within-population component of variance. The best groupings have maximal values of AG and minimal values of AP/WG. See table 1 for grouping definition.

Pacific), subdivision is significant and 25% of the variance is distributed between populations. This division reflects the presence of unique genotypes G28, found in Washington, and G9, found in San Francisco. However, partitioning appears nonsignificant between the two Channel Island populations: in over 6% of random permutations, a value of Φ_{st} higher than that actually observed was found.

We also explored various subdivisions of Atlantic populations (table 3). Populations in the west and east Atlantic are significantly subdivided, but the precise delimitations of the population groupings are difficult to place. Marginally, the groupings of Sable and Miquelon Islands (Nova Scotia); Churchill; Northern Ireland and Scotland; North Sea, Iceland, and west Baltic; and east Baltic provide the highest value of variance distributed

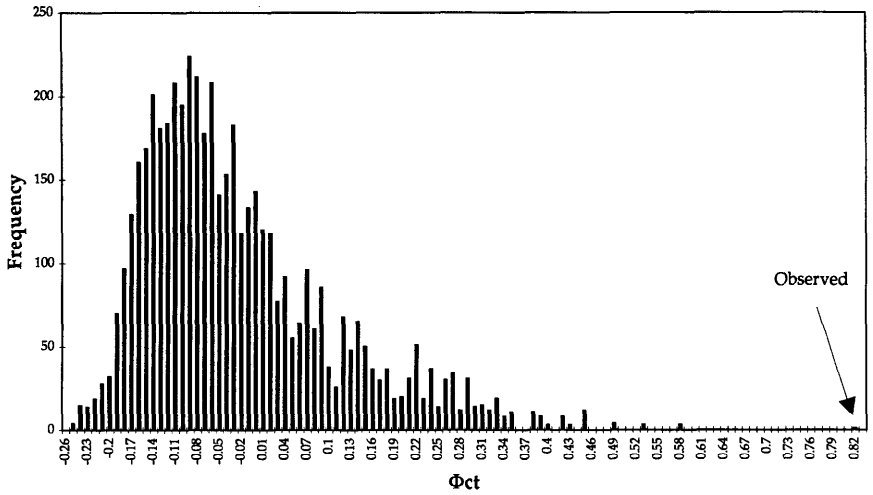

FIG. 6.—Histogram of Φ_{ct} values among four randomly constructed groupings of harbor seals. The actual observed value among the four global groupings is 0.82.

among populations (62%). Unique genotypes are evident in each of these population groupings (table 1).

In the east Atlantic, a division of localities into groups including Scotland and Northern Ireland; the North Sea; west Baltic; and east Baltic is significant and has the highest Φ_{ct} value of 0.31 (table 3). West Baltic could also be grouped with the North Sea localities without changing Φ_{ct} appreciably. However, other explored divisions are either not significantly different from random or have much lower values of Φ_{ct} (e.g., table 3). Only three regional groupings appear not to be subdivided relative to a random assortment of individuals: (1) the North Sea; (2) Northern Ireland and Scotland; and (3) west Baltic, Skagerrak, and Kattegat. Surprisingly, east and west Baltic appear highly differentiated, with 60% of the variation distributed between regions and no genotypes shared between the two regions.

Gene Flow and Differentiation by Distance

Populations separated by less than a few hundred kilometers often are not significantly subdivided relative to a random distribution of individuals (tables 2 and 3). Clusters of populations from Northern Ireland and Scotland, from the North Sea, from Miquelon and Sable Islands, and from southern California had the smallest Φ_{st} values, less than about 0.20, and greater than 5% of random population groupings had higher values of Φ_{st} than the values of Φ_{st} actually observed. Moreover, populations in these clusters shared genotypes. These results indicate high rates of gene flow, in excess of one migrant per generation, that are large enough to prevent genetic differentiation (Slatkin 1987). Apparently, harbor seal females are only regionally philopatric, suggesting population or management units on the scale of a few hundred kilometers (table 1).

If dispersal of females is limited, a large-scale pattern of genetic differentiation with distance should be apparent (e.g., Slatkin 1993). To assess differentiation with distance, we grouped the 24 populations into 13 clusters that included localities that were not significantly subdivided relative to randomized populations (tables 2 and 3) and/or extensively shared genotypes (table 1). In each ocean, the degree of differentiation among these population clusters, as measured by Φ_{ct}, was significantly correlated with geographic distance ($P \leq 0.05$, Mantel's test; fig. 7). A significant correlation was also found between average sequence divergence and geographic distance ($P \leq 0.05$, Mantel's test).

Discussion

Our analysis shows that populations of harbor seals in the Pacific and Atlantic Oceans and on east and northwest coasts of these oceans are highly differentiated. Division into these four groupings accounts for more of the between-region component of genetic differentiation (82%) than other possible groupings (table 2 and fig. 6). The amounts of control region diversity as measured by the number of genotypes (Atlantic, 18; Pacific, 16; table 1) and the average sequence divergence among populations within each of the oceans are similar (Atlantic, 0.75% ± 0.69%; Pacific, 1.19% ± 0.65%). These results suggest that both oceans have been occupied by harbor seals for an equivalent duration and have had similarly large population sizes.

The average sequence divergence between Pacific and Atlantic Ocean populations is 3.28% ± 0.384% and

Table 3
AMOVA Results for Atlantic Subdivisions of Harbor Seal Populations

Groupings	Permutations	Divisions	Variance Component	% Total Variance	Φ-Statistics	*P* (more extreme value)
All Atlantic main divisions	1,000	Miquelon I. & Sable I.	AG	61.53	$\Phi_{ct} = 0.615$	<0.001
		Churchill	AP/WG	3.65	$\Phi_{sc} = 0.095$	0.003
		Northern Ireland & Scotland	WP	34.82		<0.001
		North Sea, Iceland & west Baltic				
		East Baltic				
All Atlantic main divisions	1,000	Miquelon I. & Sable I.	AG	57.85	$\Phi_{ct} = 0.578$	<0.001
		Churchill	AP/WG	1.93	$\Phi_{sc} = 0.046$	0.1357
		Iceland	WP	40.22	$\Phi_{st} = 0.598$	<0.001
		Northern Ireland & Scotland				
		North Sea				
		West Baltic				
		East Baltic				
All Atlantic counter example	1,000	West Atlantic	AG	55.42	$\Phi_{ct} = 0.554$	<0.001
		Scotland & Northern Ireland,	AP/WG	15.23	$\Phi_{sc} = 0.342$	<0.001
		Iceland, North Sea & west Baltic	WP	29.35	$\Phi_{st} = 0.706$	<0.001
		East Baltic				
All Atlantic counter example	1,000	West Atlantic	AG	54.41	$\Phi_{ct} = 0.544$	<0.001
		Iceland, Northern Ireland & Scotland, & England	AP/WG	8.02	$\Phi_{sc} = 0.176$	<0.001
			WP	37.57	$\Phi_{st} = 0.624$	<0.001
		Norway, Waddensea & west Baltic				
		East Baltic				
West Atlantic division	500	Miquelon I. & Sable I.	AG	46.75	$\Phi_{ct} = 0.467$	<0.002
		Churchill	AP/WG	8.65	$\Phi_{sc} = 0.162$	0.0399
			WP	44.68	$\Phi_{st} = 0.554$	<0.002
East Atlantic divisions	1,000	Northern Ireland & Scotland	AG	35.15	$\Phi_{ct} = 0.351$	<0.001
		North Sea	AP/WG	1.62	$\Phi_{sc} = 0.025$	0.2468
		West Baltic	WP	63.23	$\Phi_{st} = 0.368$	<0.001
		East Baltic				
East Atlantic divisions counter example	1,000	Northern Ireland & Scotland	AG	24.75	$\Phi_{ct} = 0.248$	0.002
		North Sea	AP/WG	10.3	$\Phi_{sc} = 0.137$	0.01
		West Baltic & East Baltic	WP	64.94	$\Phi_{st} = 0.351$	<0.001
East Atlantic divisions counter example	1,000	Northern Ireland & Scotland (west coast)	AG	24.61	$\Phi_{ct} = 0.246$	0.005
			AP/WG	10.86	$\Phi_{sc} = 0.144$	<0.001
		East coast of Scotland & England	WP	64.54	$\Phi_{st} = 0.355$	<0.001
		Norway, Waddensea & west Baltic				
		East Baltic				
East Atlantic divisions counter example	1,000	British Isles	AG	18.11	$\Phi_{ct} = 0.181$	0.025
		Norway, Waddensea, & west Baltic	AP/WG	11.72	$\Phi_{sc} = 0.143$	<0.001
		East Baltic	WP	70.17	$\Phi_{st} = 0.298$	<0.001
Indivisible Groups						
Nova Scotia	500	Miquelon I.	AP	16.25	$\Phi_{st} = 0.162$	0.0898
		Sable I.	WP	83.75		
Northern Ireland & Scotland	500	Moray Firth	AP	1.19	$\Phi_{st} = 0.012$	0.3234
		Dornoch	WP	98.81		
		Scotland				
		Strangford Lough				
North Sea	500	Norfolk	AP	7.72	$\Phi_{st} = 0.077$	0.0599
		Lincolnshire	WP	92.28		
		Norway				
		West Waddensea				
		East Waddensea				
West Baltic	500	West Baltic	AP	−0.34	$\Phi_{st} = -0.003$	0.487
		Kattegat	WP	100.34		
		Skagerrat				
West Baltic & North Sea	500	West Baltic	AG	10.5	$\Phi_{ct} = 0.105$	<0.002
		North Sea	AP/WG	3.85	$\Phi_{sc} = 0.043$	0.1936
			WP	85.64	$\Phi_{st} = 0.143$	<0.002

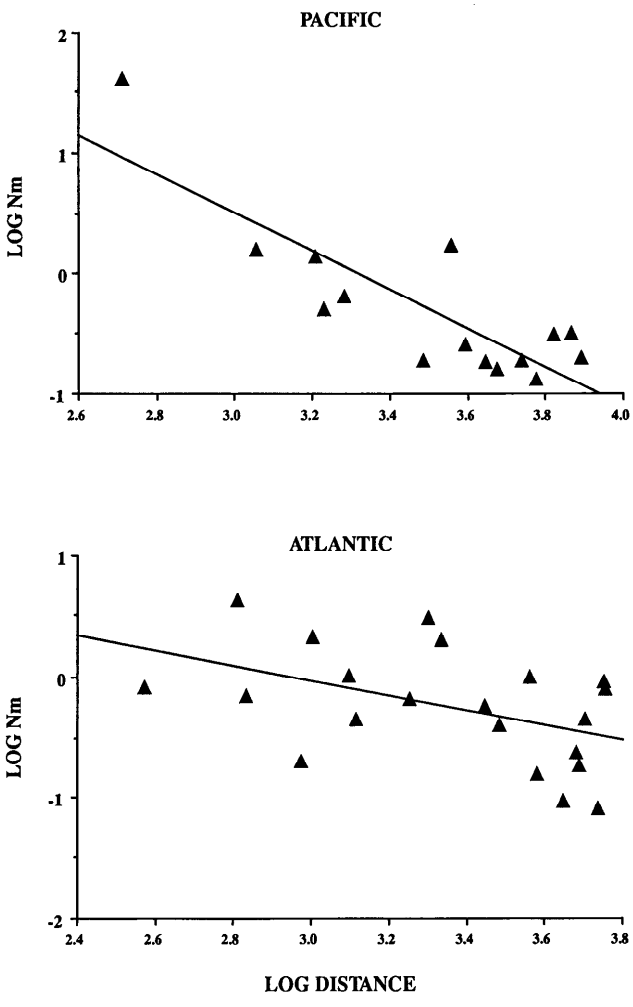

FIG. 7.—Scatterplots of log(*Nm*) and geographic distance between Pacific (top) and Atlantic (bottom) population clusters as defined in table 1. Regression statistics: Pacific: log(*Nm*) = 5.2905 − 1.5953(log distance), $r = 0.84$; Atlantic: log(*Nm*) = 1.8201 − 0.61679(log distance), $r = 0.47$.

potentially might be used to estimate divergence time. However, the reported divergence rates for control region sequences vary considerably. The rates in humans and snow geese have been estimated as 8.4% and 20.8% per million years, respectively (Vigilant et al. 1989, 1991; Quinn 1992). In contrast, the divergence rate in rodents, primates, and whales appears to be slower, nearer to 1%–2% per million years (see review in Hoelzel, Hancock, and Dover 1991). The apparent variation in molecular evolutionary rates among groups suggests that the calibrations for harbor seals, given the absence of within-species divergence times, should be based on known divergence times in closely related phocid seals. The weddell seal (*Leptonychotes weddelli*) and leopard seal (*Hydrurga leptonyx*) are two phocid seals that diverged about 4.5 Mya and have a control region sequence divergence of 9.93% (Slade, Moritz, and Heideman 1994). Consequently, the divergence rate based on these data is about 2.2% per million years. Southern California and Gulf of Mexico populations of the California sea lion (*Zalophus californianus*) are isolated from each other by the Baja Peninsula and have a sequence divergence of 4.4% (Maldonado et al. 1995). These two populations were likely isolated for at least 3 Myr, suggesting a divergence rate of about 1.5% per million years. If these divergence rates are used, they predict a divergence time of Pacific and Atlantic harbor seal populations of about 1.7 and 2.2 Mya. This divergence time is close to the first record of sea ice and continental glaciation about 2–3 Mya (Stanley 1986; Harland et al. 1990). Thereafter, movement around the Northwest Passage through the Bering Strait was probably constrained. Thus, the molecular data are consistent with the development of extensive sea ice as being the cause of restricted inter-oceanic gene flow.

In the Atlantic Ocean, the pattern of divergence in sequence and population trees suggests that the colonization of the Atlantic Ocean proceeded from the west Atlantic coast of North America to the north and then east to Europe. This conclusion follows from the basal position of sequences from Miquelon and Sable Islands that is followed by divergence of sequences from Churchill and Iceland. Sequences from Europe are most closely related to each other and are the last to diverge (fig. 3). The average sequence divergence between genotypes from west Atlantic populations, 1.28% ± 0.65%, was much greater than the 0.31% ± 0.17% between European populations. This pattern is reflected in the population tree that has Miquelon and Sable Islands populations as the most divergent, followed by Churchill and Iceland and, lastly, the closely related European populations (fig. 5). The degree of divergence between European and west Atlantic populations, about 2%, suggests this colonization may have begun from 0.9 to 1.1 Mya (see above). However, with the exception of the east Baltic, the average divergence among European populations, 0.23%, is nearly an order of magnitude smaller than that between east and west Atlantic populations, and the DNA sequence and minimum spanning trees suggest a star phylogeny best characterizes the evolution of European genotypes. Therefore, these results suggest a more recent colonization of Europe by harbor seals. An evolutionary scenario consistent with the close relationship of European genotypes is that these populations were eliminated during the last Ice Age, about 18,000 years ago, when ice sheets covered the entire present-day European range, and have recently been recolonized from a southern ice-free refugium, such as the southern French or northern Spanish coast (Pielou 1979; Peres 1985).

Within and among regions in both oceans, the degree of gene flow between populations often varies with distance (fig. 7). For example, the extent of gene flow as measured by *Nm*, the number of female migrants per generation, is highest between San Francisco and southern California (*Nm* = 20.5, distance = 600 km) and between the west Baltic and North Sea populations (*Nm* = 2.1, distance = 500 km). Values of *Nm* greater than one would be expected to largely counteract differentiation due to drift. However, in the Atlantic Ocean, several closely spaced populations have low values of migration, confounding the statistical association of *Nm* and distance (fig. 7). For example, Scottish and Northern Ireland populations are separated by about 300–800 km of coastline from those on the east coast of Britain, but the two population groupings are significantly subdivided and have values of *Nm* of less than 0.1 between them. Moreover, these two population groupings differ not only in genotype frequency; populations on the Scottish and Northern Ireland coasts have genotype G3 at high frequency that is not found elsewhere. Similarly, the North Sea and Iceland populations have genotype G1 that is not found in localities off the Scottish or Northern Ireland coasts (table 1). These results indicate that females are regionally philopatric despite the absence of apparent barriers to dispersal. In contrast, among North Sea and Iceland populations, values of *Nm* generally approach one, suggesting weak philopatry, if any, over this region. An analysis of hypervariable simple repeat loci has identified similar patterns of divergence in European harbor seal populations and suggests that males may be regionally philopatric there as well (Goodman et al., unpublished).

Another surprising genetic division occurs between east Baltic and other European harbor seal populations. The east Baltic population does not share any genotypes with other east Atlantic populations and has an average divergence value of 0.65% ± 0.07% from them, twice that observed among other European populations. Moreover, the east Baltic population is only about 150 kilometers from sites in the west Baltic having different genotypes (fig. 1). Geological data indicate that the southern portion of the Baltic was ice free about 9,000 years ago (Lundqvist 1980). However, about 6,000–8,000 years ago, a land bridge across the mouth of the Baltic, west of Öland Island, joined Denmark and Germany to Sweden and isolated populations in the east Baltic until about 4,000 years ago (Bjorck and Digerfeldt 1991; Lykke-Andersen, Knudsen, and Christiansen 1993). Therefore, the presence of unique genotypes in the east Baltic may reflect drift in past isolated populations rather than a current barrier to immigration between the west and east Baltic populations. However, there are no intervening populations between west and east Baltic localities, and an alternative explanation is that movement between the two populations may be infrequent. Larger samples of each of these populations are needed to detect the possible presence of rare shared genotypes indicating limited gene flow.

In the Pacific Ocean, genotypes in the northwest are basal to those in the east, suggesting a west to east colonization pattern similar to that found in the Atlantic (fig. 3). Moreover, as in the Atlantic Ocean, genetic divergence does not always correspond with geographic distance. The Alaskan harbor seal population is genetically more similar to populations in Japan and the Commander Islands than to geographically closer populations in Washington and California (fig. 5). For instance, the sequence divergence between Alaska and Japan, about 3,600 km distant, is 0.72% and between Alaska and Washington, about 3,100 km distant, is 2.13%. During the last glaciation from about 21,000 to 10,000 years ago, most of the Aleutian Island chain was covered with ice (Peltier 1994), and this might have prevented southward dispersal of Alaskan harbor seals. In contrast, northern Alaska and the Bering Sea were not glaciated. Conceivably, the lack of southward dispersal might have become an established behavior during this last glaciation or earlier during similar glacial epochs (Dawson 1992). The retention of this behavior, despite altered climatic conditions, might have resulted in an absence of genetic exchange between Alaskan and southern seal populations.

Our results can be used to define population units for conservation (e.g., Baker et al. 1994; Maldonado et al. 1995). However, at least two important qualifications need to be considered before defining stocks for conservation. The first qualification concerns the power of our statistical analyses (Taylor and Gerrodette 1993). Sample sizes for many of our populations are small; thus, with larger samples sizes, we might be able to show significant differentiation in genotype frequencies within some of the population groupings. A second concern is the diversity of definitions for population units within a species, such as stock or management units, evolutionarily significant units, and subspecies (Ryder 1986; Avise and Ball 1990; Waples 1991; Dizon et al. 1992). Recent discussion has concluded that a hierarchy of population units should be defined according to the degree of evolutionary divergence (Moritz 1994). It has been suggested that the designation of evolutionarily significant units should be reserved for populations that are highly differentiated and in which sufficient time has elapsed such that they are reciprocally monophyletic (Moritz 1994). In contrast, management units are more recently diverged populations between which there may be some gene flow and that differ primarily in genotype frequencies. Additional subdivisions might be made ac-

cording to the degree to which populations share genotypes and the levels of genetic differentiation among populations (Avise and Ball 1990; Waples 1991; Dizon et al. 1992).

Considering these criteria, populations in the Atlantic and Pacific are the most fundamental division because sequences within each basin are reciprocally monophyletic. Within each ocean, only the Commander Island genotypes and the three west Atlantic genotypes are monophyletic. Population groupings having closely related genotypes but not sharing genotypes with other populations merit a second level of distinction, analogous to category one of Dizon et al. (1992), and include (1) Japan, the Commander Islands, and Alaska; (2) east Pacific; (3) Churchill, Iceland, and Europe; and (4) west Atlantic. These divisions roughly correspond with previous subspecies limits except that Churchill shows a greater affinity to Icelandic and European populations and Alaska and the Commander Islands show greater affinity to west Pacific populations than to those from Washington and California (fig. 5). However, only a single seal was obtained from Churchill, and the former conclusion needs confirmation with a more extensive sample. Less significant units for conservation may be defined as those characterized by unique genotypes (although this might change if sample size is increased) and include (1) Japan; (2) Commander Islands; (3) Alaska; (4) Washington, San Francisco, and the Channel Islands; (5) Nova Scotia; (6) Churchill; (7) Iceland, British Isles, and the North Sea; (8) west Baltic, Kattegat, and Skagerrak; and (9) east Baltic. Finally, the most reduced groupings are those 13 listed in table 1 that have unique genotypes or are subdivided with respect to genotype frequency.

In conclusion, we have shown that the harbor seal is regionally philopatric on the scale of several hundred kilometers. With some exceptions, differentiation among regional units reflects increasing geographic distance and suggests limitations on female dispersal (fig. 7). Historical events have greatly influenced the large-scale pattern of genetic differentiation in harbor seals. The mitochondrial data are consistent with an ancient isolation of populations in both oceans coincident with the development of continental glaciers and extensive sea ice. In the Atlantic and Pacific Oceans, populations appear to have been established from west to east, with the European populations showing the most recent common ancestry. We suggest this may reflect recolonization from an Ice Age refugium after the last glaciation. Finally, a hierarchy of population units can be defined that can be used as a basis for ranking conservation priorities.

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