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Gene flow among Pacific harbor seals in Northern California

Michele Maria Conrad
San Jose State University

GENE FLOW AMONG PACIFIC HARBOR SEALS IN NORTHERN CALIFORNIA

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Michele Maria Conrad

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APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Leslee Parr	Department of Biological Science	08/09
Dr. Jim Harvey	Moss Landing Marine Laboratories	08/09
Dr. Michael Sneary	Department of Biological Science	08/09

APPROVED FOR THE UNIVERSITY

Associate Dean	8/31/09 08/09

ABSTRACT

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A fragment of the mitochondrial DNA control region was sequenced in 96 harbor seals (*Phoca vitulina richardii)* from Northern California. This sequence was combined with previously published sequence data from Oregon for analysis in order to investigate female gene flow and detect the presence of genetic population substructure in harbor seal populations throughout this region. Geographic partitioning was not detected; however, phylogenetic structure was revealed in the form of two lineages. Twenty-nine variable sites among 27 haplotypes were identified, nine of which were unique to Oregon. The greatest frequency haplotypes were all weakly detected in Oregon, with the single most highly represented haplotype from Northern California (48%) absent from Oregon. Genetic distances between California and Oregon were significantly different, lending support to the current stock delimitation between Oregon and California. Genetic structure within California indicates more than one California stock designation may be necessary.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

INTRODUCTION

Two sub-species of Pacific harbor seal (*Phoca vitulina*) are recognized in the North Pacific. This paper focuses on the sub-species *Phoca vitulina richardii*. It's range extends along the eastern part of the Bering Sea, east along the Aleutian Islands and Alaskan coast, along the coasts of British Colombia and the Western U.S., and terminates around Cedros Island in Baja California, Mexico (King 1983). Its wide distribution along the coastal mainland, off-shore islands, bays, estuaries, and rivers make the harbor seal an integral part of ecosystems both terrestrial and aquatic, which inevitably has brought them into close contact with humans. The exploitation of shared resources and human encroachment made this relationship one of conflict and competition. Adequate population management is essential to ensure this species remains sustainable and to protect the integrity of the various ecosystems in which harbor seals have a vital role. Properly defined population structure is necessary for determining meaningful conservation strategies. A failure to do so will lead to inaccurate population estimates with potentially negative consequences.

Genetic studies are useful in examining population substructure and detecting structural relationships that may otherwise go undetected by morphological, ecological, and behavioral data (Lamont et al. 1996, Stanley et al. 1996, Burg et al. 1999, O'Corry-Crowe et al. 2003). For most of this sub-species' range, genetic data does exist; however, a paucity of data and a lack of synthesis remain regarding harbor seals south of Washington State. In an attempt to partially bridge the discontinuity of available genetic data, I studied genetic substructure in harbor seals from Northern California and Oregon

using mtDNA analysis. Throughout California, harbor seals are distributed along a coastal continuum without any apparent oceanic barriers imposing movement restrictions. More than 400 haul-out sites can be identified along their coastal range, none of which are isolated sufficiently from neighboring haul-out sites to prevent genetic exchange (Hanan 1996). Any genetic structure detected must be ascribed to ecological and or behavioral factors limiting harbor seal dispersal or to historical isolation events.

Female harbor seals remain close to their natal ground to give birth. This behavior becomes more pronounced with age (Härkönen and Harding 2001), indicating that restricted movement may be a product of maternal experience. The survivability of a pup is dependant upon its ability to persist through its first winter which is highly dependant upon, among other factors, food availability and the amount of time it has to feed after its post-weaning fast (Harding et al. 2005). Furthermore, a female's birth timing and ability to rear her pup at or near the site where she was successfully reared may increase the pup's ability to make it through its first winter season. Although restricted movement by females does seem to have its advantages, the ability of harbor seals to seek new breeding and or haul-out sites also must have its advantages.

Adults, juveniles, and pups move readily among closely spaced haul-out sites (Slater and Markowitz 1983, Lowry et al. 2001, Härkönen and Harding 2001). It has been speculated that predation and changes in rearing site conditions, such as overcrowding and fluctuations in food availability (Brown and Mate 1983, Ainley et al. 1985, Nickel 2003), may cause movement of these animals. Alternative sites also have been sought in response to certain human-caused disturbances (Allen et al. 1984).

If female harbor seals move minimally in relation to their natal site to give birth, genetic structure should be detectable via mtDNA d-loop analysis despite a

female's necessity to occasionally visit different haul-out sites or seek new rearing sites.

Studies of the mtDNA control region have been useful in tracking female gene flow and identifying population substructure (Lamont et al. 1996, Stanley et al. 1996, Burg et al. 1999, O'Corry-Crowe et al. 2003). The control region of mtDNA is a non-protein coding region that is greatly variable, making it a good marker for detecting differences below the species level such as for populations and subpopulations (Brown et al. 1978).

Currently, populations along the California and Oregon-Washington coasts are considered separate management stocks (Carretta et al. 2007). A human-delineated political boundary, however, is not a realistic stock boundary for wild populations. The latest California stock revision, concluded in 2007, supports this important point. It states, "Although the need for stock boundaries for management is real and is supported by biological information, the exact placement of a boundary between California and Oregon was largely a political/ jurisdictional convenience." (Carretta et al. 2007). This revision did not refer to Scribner's (2005) extensive genetic work covering Southern and Central California. It was based solely on genetic data from 15 harbor seals from one location in California, Monterey Bay (Lamont et al. 1996). Harbor seals in California were a discrete subpopulation in the analysis by Lamont et al. (1996); however, the substructure within California could not be determined given the small and limited range of samples. A pupping cline exists along the Eastern Pacific, indicating that pupping occurs later in the season with increasing latitude (Temte et al. 1991). One sample site, therefore, is not an adequate representation of harbor seal genetic substructure along California's wide latitudinal range.

This study presents new data on the mitochondrial control region of harbor seals from Northern and Central California in combination with previously published data from

Oregon obtained from Lamont et al. (1996). The objectives of this paper were to 1) demonstrate haplotypes and frequencies present in Northern California, 2) assess the level of female gene flow in this part of the harbor seal's range, 3) detect the presence of genetic substructure from San Francisco Bay, California, to Umpqua River in Oregon, and 4) address whether the data support the current MMPA-designated stock boundary between California and Oregon-Washington.

MATERIALS AND METHODS

Harbor seal tissue samples were taken from live animals. Seals were captured with nets, restrained, measured, sex determined, and sampled. A small piece of tissue consisting of connective tissue and skin, approximately 1.5 cm^3, was taken from the hind flipper of each animal. These samples were collected by Dr. Jim Harvey and crew under IACUC protocol #828 at eight locations in Northern and Central California (Figure 1). All samples were stored in 20% dimethyl sulfoxide in a saturated salt solution at -20°C. The data derived from 96 harbor seal samples from eight locations in Northern California were analyzed in combination with the data derived from 20 harbor seal samples from the Umpqua River in Oregon gathered by Lamont et al. (1996).

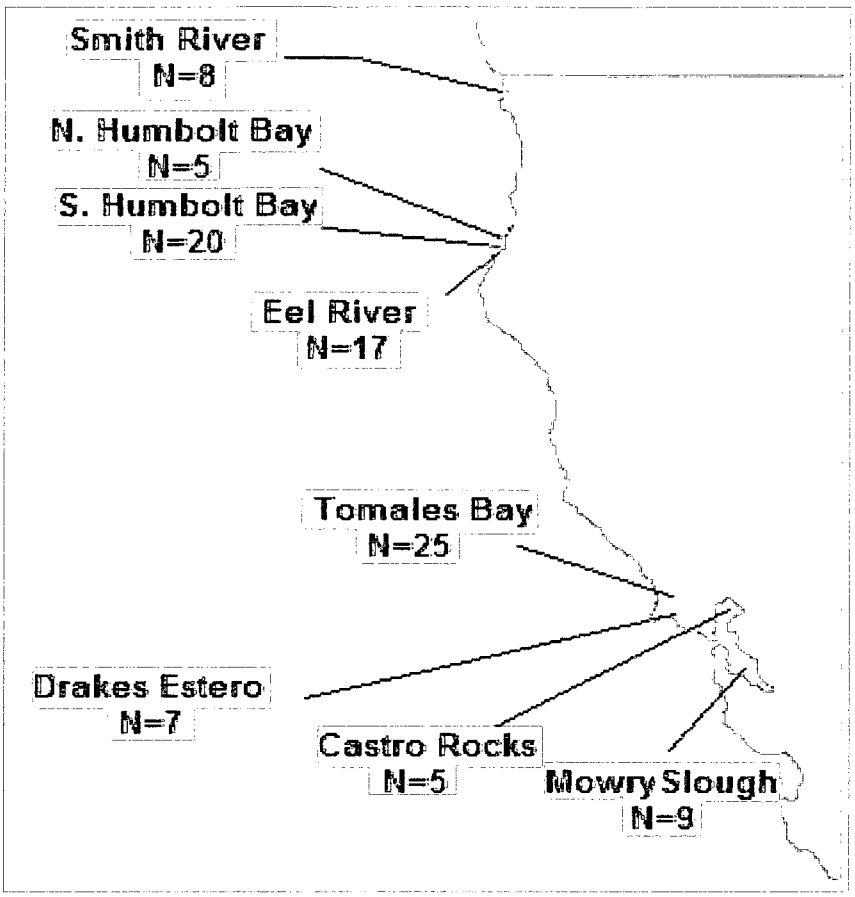

Figure 1. Locations and numbers of new harbor seal samples used for mtDNA analyses.

Total genomic DNA was extracted by adding 620 µl of an EDTA nuclei lyses solution to ~0.5 cm^3 of tissue. A 17.5 µl volume of Proteinase K (Promega) was then added followed by an overnight rotating incubation at 55°C. To separate protein from genomic DNA, 200 µl of Protein Precipitation solution (Promega) was added. The digest was then chilled and spun. Samples were treated with RNAase solution (Promega) following the manufacturer's protocol. The supernatant was then added to 600 µl of isopropanol at room temperature to precipitate the DNA. The pellet was then washed with 70% ethanol. DNA was resuspended with 50 µl of rehydration solution, followed by an incubation period of an hour at 60°C to aid in the resuspension process.

DNA extracts were run on a 1.5% agarose gel to assess the quality of genomic DNA. The remaining extract was then stored at -20°C. Approximately 1500 bps of the mitochondrial genome were amplified. This amplification product included the entire control region and a portion of the conserved tRNA genes flanking it.

Polymerase chain reactions were conducted based on a total volume of 30 µl per reaction. The following reagents with the following final concentrations were used: 10 mM Tris-HCL (PH 8.4), 50 mM KCL, 2 mM MgCL, 0.25 mM per dNTP, 0.06 units/ mL of Taq polymerase, 0.5 µM of each primer, and 0.5 M betaine. The forward primer, L15926Pvit, located in the transfer RNA threonine and proline region and the reverse primer, Tphe-Phoca, located in the conserved tRNA phenylalanine region, were used for PCR amplification (Table 1). All other primers were used as internal primers for sequencing (Table 1).

Table 1. A summary of all primers used for amplification and sequencing.

Primer Name	Sequence 5' to 3'	Citation
L15926Pvit	TATTACCTTGGTCTTGTAAACC	Scribner 2005, adapted from Kocher et al. 1989
Tphe-Phoca	CCTTGCTTTGGTTTATTAAGC	Scribner 2005
L-ctrl-Pvla	CCGGGCCCATAACATGTG	Scribner 2005
H16498	CCTGAAGTAAGAACCAGATG	Scribner 2005, adapted from Rosel et al. 1994
L-ctrl-Pv2	CCATGAGGCGCATTTTAGTC	Scribner 2005
H-ctrl-Pv3	TGTACGTGTACGTAACGTAAC	Scribner 2005

PCR products were purified using exonuclease-shrimp alkaline phosphatase solution and cycle sequenced by the direct dideoxy sequencing method of Sanger et al. (1977). Sequencing services were provided by the Conservation Genetics Lab at San Francisco State University. The addition of 0.5 M Betaine was recommended by Scribner (2005) for those reactions with primers tPhe-phoca and L-ctrl-Pv2. This addition was intended to improve sequencing of the 3' end repeat region. However in most cases it seemed to have no effect.

Sequence bases were identified using the Phred algorithm and assembled using the Phrap algorithm from MacVector/ Assembler version 10.0. The final contigs were visually scanned for what may have been artifact substitutions and manually edited. They were also cropped to eliminate poor quality sequence on both ends. Unreliable, poor quality contigs were not used in the final analysis.

Good quality contigs both edited and cropped were then aligned using the ClustalW multiple alignment algorithm (Hall 1999). All base substitutions were once again visually inspected; if the substitution appeared only once among all sequence data, it was considered a potential artifact. In such a case, the original sequence chromatogram would once again be referred to and carefully inspected to ensure that the single substitution was in fact real. Once aligned, contigs were then cropped to the same length. The final cropped alignment yielded 578 nucleotides (nts). This file was then input to MEGA4 to generate a rooted neighbor-joining phylogenetic tree using Kimura 2-parameter distances with 1000 bootstrap replicates for statistical support (Kumar et al. 1993). To root the tree, the published sequence of an Eastern Atlantic harbor seal from Arnason and Johnsson (1992) was downloaded from GenBank and used as an outgroup. A minimum spanning tree was constructed based upon the Steiner maximum parsimony

algorithm by Polzin and Daneshmand, using NETWORK v.4.5.1.0. The resulting tree depicted the relationship of haplotypic frequencies and mutational distances among haplotypes.

The phylogenetic clustering method mentioned above along with an analysis of molecular variance (AMOVA) (Wier and Cockerham 1984) were used to detect the presence of group partitioning. All possible structural combinations were attempted. Genetic diversity within and among sample groups was analyzed, and pairwise Fst (Wright 1951) was calculated using 10,000 permutations. The most likely structural scenario was selected based upon the nodal statistics of the phylogenetic tree generated, along with the AMOVA schematic that demonstrated the greatest among population percentage variation. Genetic variation was analyzed by calculating haplotypic diversity (H) and nucleotide diversity (n) (Nei 1987), using ARLEQUIN 3.11 (Excoffier et al. 2005). To provide insight into female gene flow, a female migration rate was estimated using the equation Fst = 1/ (1+Nm) (Wright 1969).

Lastly, isolation by distance was analyzed using the Isolation by Distance web service v.3.15 from San Diego State University (Jenson et al. 2005). The measuring tool of ArcGIS Explorer was used to measure distances between localities by closely contouring the coastline except in bays. The web service first carries out a Mantel test by assessing whether the pairwise genetic distance matrix is correlated with the pairwise geographic distance matrix. Then a null distribution is generated by randomizing rows and columns of one matrix while holding the other constant. Furthermore, the slope and intercept of this relationship are calculated using reduced major axis regression.

RESULTS

Although more than 1000 nts were successfully sequenced for 8 individuals, only 578 nts were used in the final alignment due to highly repetitive motifs found in the latter portion of the control region making sequencing problematic. These repetitive motifs were first recognized in the completion of the entire mtDNA genome of an Eastern Atlantic harbor seal by Arnason and Johnsson (1992) and were also detected by Scribner (2005). Informative sites were limited to the first ~420 nts of the control region with the remainder of the sequence being highly conserved. Nineteen variable sites were found in Northern California samples. By including 20 individuals from Oregon (Lamont et al. 1996), a total of 27 variable sites were detected (Table 2). Eighteen haplotypes were recognized in Northern California, and nine were unique to Oregon.

Table 2. Aligned haplotypes and frequencies of haplotypes for each sampling location, including the Oregon data from Lamont et al. (1996). Variable sites are numbered according to positions indicated in the complete mtDNA genome of the Eastern Atlantic harbor seal, spanning positions 16388-16745 (Arnason and Johnsson 1992).
MS = Mowry Slough, CR = Castro Rocks, DE = Drakes Estero, TB = Tomales Bay, ER = Eel River, SH = South Humbolt Bay, NH = North Humbolt Bay, SR = Smith River, OR = Oregon. Haps. = haplotypes. pub seq = haplotype of an Eastern Atlantic harbor seal taken from Arnason and Johnsson (1992).

Haps.	Variable sites	MS	CR	DE	TB	ER	SH	NH	SR	OR
	82524732126934190289890477925									
	81144456777788945566778990134									
	34444444444444455555555556667									
	66666666666666666666666666666									
	11111111111111111111111111111									
H01	TATAGACTGCTCC-AAGGATCGGTTTATC	2	5	4	13	7	9	5	1	
H02	C..G.-...T...CG.......A....C.	1				1	8		1	
H03	C..G.-........G...G........C.	4		1	2	2	1		1	1
H04	C..G.-.....T..G...G....C...C.			1	2		1			3
H05	C..G.-........G...G....C...C.			1	2				1	2
H06	................A.....	1			1					
H07	C..G.-.C......G...G....CC.GC.					2	1			
H08	C..G.-...T....G.......A....C.					1	1			
H09	..................C.....				3					
H10	C..G.-...T...CG...........C.					1			1	
H11	.............G.......A..C...								1	
H12	C..G -.....T..G...G....C...CT	1								
H13	C..G -..A..T..G...G....C...C.				1					
H14	................G.....				1					
H15	....T.G..........								1	5
H16	C..G.-..AT....G.......A....C.					1				
H17	........A.....					1				
H18	C..G.-........G...G....CC.GC.					1				
H19	.............G.....									1
H20	C...A.........G.....									1
H21	.....-......T.G......A.....									1
H22	C..G.-..A.....G...G........C.									1
H23	C...........T.G...G........C.									1
H24	C..G.-...T....G...G...A.....									1
H25	C.............G.....									1
H26	C..G.-...T....G.....									1
H27	....A...A.....G.....									1
pub seq	CGC..-T...C...GGA...TAA.....									

Overall, 48% of all haplotypes consisted of Haplotype no. 1 (H1), which was detected in all locations except Oregon. Oregon did however have a closely related haplotype that differed by only one substitution. The second most common haplotype, which was Haplotype no. 3 (H3), was found in 13% of all individuals. The third most common haplotype, Haplotype no. 2 (H2), was found in 11.5% of all samples of which 8% were found in South Humbolt Bay.

All haplotypic diversity values are listed in Table 3. The overall haplotypic diversity value for all locations was low (H = 0.6289). Haplotypic diversity values for the various sampling locations spanned from complete fixation (H = 0), which was identified at the Castro Rocks and at the North Humbolt Bay location, to a high haplotypic diversity value of H = 0.8309 +/- 0.085, which was identified at the Eel River location. A haplotypic diversity value of H = 0.9263 +/- 0.0431 was identified in Oregon. An overall nucleotide diversity of $\pi = 0.124$ was detected also reflecting a low level of genetic differentiation. The neighbor-joining tree was not included in the results due to a lack of statistical support for all nodes. This apparent lack of structure was also supported by the AMOVA results. All structural possibilities were attempted, yet they all yielded lesser among population values than the values generated for all localities assigned to a single group.

Table 3. Harbor seal genetic diversity values for all locations sampled in Northern California, including the Oregon data from Lamont et al. (1996). The sample sizes, number of haplotypes, and number of polymorphic sites found for each location are listed along with haplotypic diversity and nucleotide diversity values with standard deviations. These values are the percent likelihood that two haplotypes or nucleotides selected at random will be different. The mean pair wise difference is the average number of haplotypes that were different when compared in a pair wise manner. MS = Mowry Slough, CR = Castro Rocks, DE = Drakes Estero, TB = Tomales Bay, ER = Eel River, SH = South Humbolt Bay, NH = North Humbolt Bay, SR = Smith River, OR = Oregon.

Sample Location	Sample Size	Number of haplotypes	Number of polymorphic sites	Haplotype diversity, H	Nucleotide diversity (%)	Mean pairwise difference
MS	9	5	13	0.806 +/- 0.120	0.159 +/- 0.098	4.611 +/- 2.500
CR	5	1	0	0.000 +/- 0.000	0.000 +/- 0.000	0.000 +/- 0.000
DE	7	4	8	0.714 +/- 0.181	0.150 +/- 0.097	4.190 +/- 2.366
TB	25	8	11	0.720 +/- 0.090	0.125 +/- 0.073	3.480 +/- 1.839
ER	17	9	14	0.831 +/- 0.085	0.178 +/- 0.178	5.176 +/- 2.636
SH	20	5	12	0.663 +/- 0.069	0.160 +/- 0.091	4.642 +/- 2.376
NH	5	1	0	0.000 +/- 0.000	0.000 +/- 0.000	0.000 +/- 0.000
SR	8	8	13	1.000 +/- 0.063	0.186 +/- 0.114	5.393 +/- 2.907
OR	20	13	13	0.926 +/- 0.043	0.160 +/- 0.092	4.490 +/- 2.307

The frequencies of all haplotypes and the locations in which those haplotypes were found, in relation to how many mutational steps away they are from each other, were assessed using a minimum spanning network (Figure 2). Two separate lineages, both descendant from Oregon, are apparent and indicated as A and B.

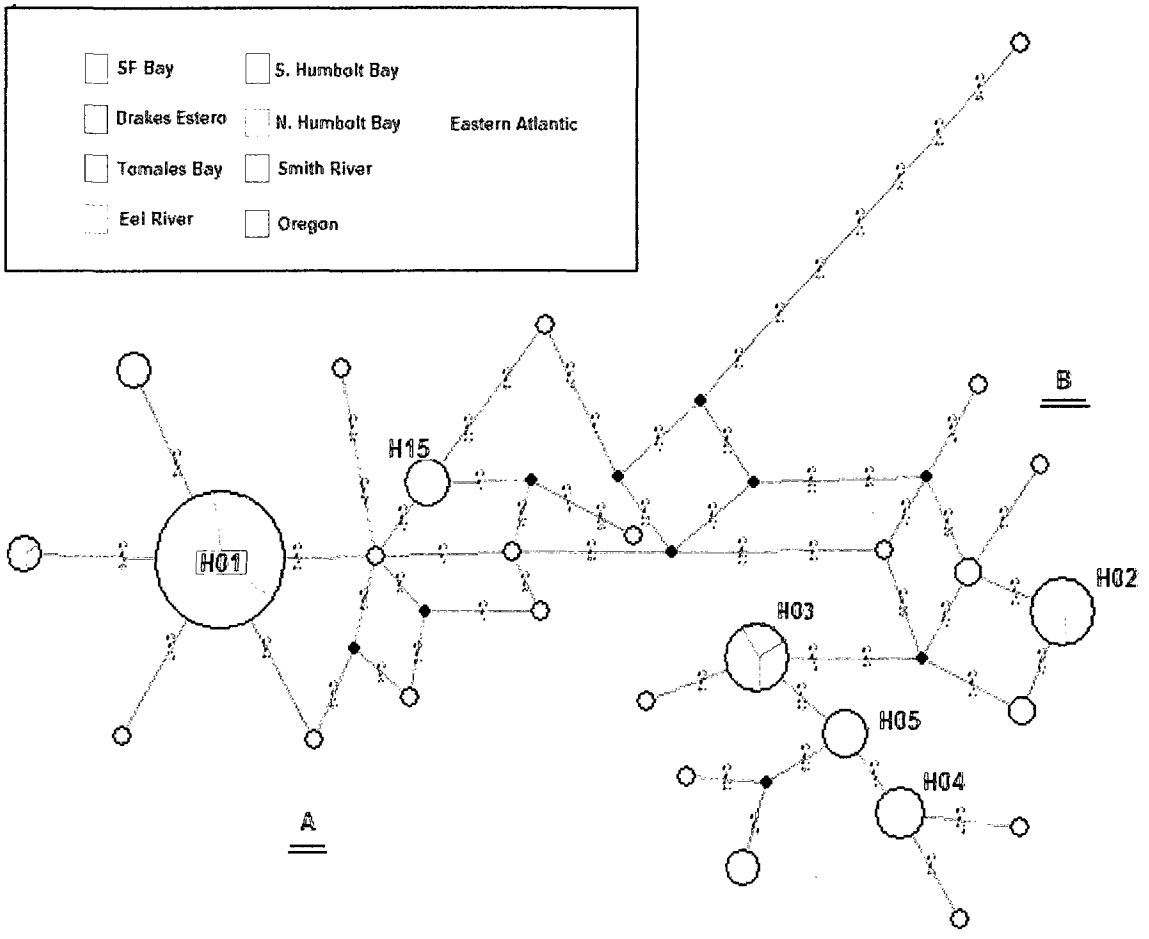

Figure 2. Minimum spanning network of Northern California partial control region haplotypes, including the Oregon data from Lamont et al. (1996), and an Eastern Atlantic species from Arnason and Johnsson (1992). Character number (CH#) indicates the number of mutational steps among haplotypes. Size of spheres are proportional to the haplotype frequency. The size of the sphere slices are proportional to the haplotype frequency found for each sampling location. A and B identifies two distinct lineages.

Because Castro Rocks and North Humbolt Bay were fixed for Haplotype 1 (H1), which is not present in Oregon, pairwise Fst values differed significantly between them (Table 4). And although this haplotype was present in Mowry Slough, the pairwise Fst value also differed significantly due to a wide variety of other haplotypes present in Mowry Slough but not present in Castro Rocks. Similarly, although some haplotypes were shared, significant differences were estimated between Tomales Bay and South Humbolt Bay; Eel River and Oregon; South Humbolt Bay, Eel River, and Oregon; and Mowry Slough and South Humbolt Bay (Table 4).

Table 4. Significant pairwise genetic distance matrix for all sample locations, including the Oregon data from Lamont et al. (1996). Fst values demonstrating P values < 0.05 are given. MS = Mowry Slough, CR = Castro Rocks, DE = Drakes Estero, TB = Tomales Bay, ER = Eel River, SH = South Humbolt Bay, NH = North Humbolt Bay, SR = Smith River, OR = Oregon.

	MS	CR	DE	TB	ER	SH	NH	SR	OR
MS									
CR	0.410								
TB	0.137	-							
DE	-	-	-						
ER	-	-	-	0.066					
SH	0.054	-	-	0.177	0.006				
NH	0.410	-	-	-	-				
SR	-	-	-	-	-	-			
OR	-	0.345	-	0.155	0.054	0.147	0.280	-	-

The overall Fst was 0.112, for which 11.15% of its variation is attributable to variation among locations and 88.85% to variation within location (Table 5). This fixation index was used to estimate female gene flow based on Wright's corrected equation estimating 7.91 female migrants per generation or about 1 female per year.

Table 5. Analysis of molecular variance among and within all locations, including the Oregon data from Lamont et al. (1996).

Source of Variation	Variance	% Total Variation	F-Statistic	P-value
Among all sample locations	0.258	11.15		
			Fst = 0.112	<0.001
Within each sample location	2.055	88.85		

DISCUSSION

The ability of harbor seals to travel long distances and use multiple haul-out sites makes identifying population substructure difficult for this species (Thompson et al. 1994). In addition, most analytical programs expect the user to define structure *a priori*, which can lead to biases based on preconceptions while constructing structural scenarios. After attempting all structural scenarios, the AMOVA indicated that the percent total variation among sample locations was greatest when all locations were treated as part of the same group with each location serving as its own subgroup. In some cases this may not have been the best approach for those locations with low sample sizes. It may be better to pool adjacent sites with low sample sizes. For example, pooling Castro Rocks with Mowry Slough is reasonable, since both locations have low sample sizes, and are

found within the San Francisco Bay. Because Castro Rocks is represented by such a small sample size, the diversity value of Castro Rocks may be severely biasing the overall diversity value.

According to O'Corry-Crowe et al. (2003), an adjusted sample size is recommended for a better understanding of whether divergence estimates among two groups will be affected by low sample size. The adjusted sample size is determined by subtracting the number of unique haplotypes in one group from the total number of individuals sampled. In the case of Castro Rocks (5-1), the adjusted sample size value is four. Although there were nine samples originally in the Mowry Slough region, the adjusted sample size (9-5) is also four. Based on these modified estimates, Mowry Slough is as well represented as Castro Rocks.

Westlake and O'Corry-Crowe (2002) concluded that harbor seals throughout Alaska and Japan have a high overall haplotypic diversity value (H= 0.98) compared with other marine mammals. For example, the northern elephant seal (*Mirounga angustirostris*) has undergone an extreme bottleneck in the recent past and is considered to have a low haplotypic diversity value ($H < 0.5$) (Hoelzel et al. 1993). The Dall's porpoise (*Phocoenoides dalli*), populations of which are numerous and robust, have a high haplotypic diversity value ($H = 0.96$) (Escorza-Trevino and Dizon 2000). Scribner's (2005) harbor seal data from Southern California also yielded high haplotype diversity ($H = 0.84$). She recognized that her diversity values (although not as high as those found in Alaska) demonstrate a geographically restricted sample range. My analyses also show a lower haplotypic diversity value ($H = 0.63$). This lower diversity value can be explained through the high occurrence, almost 50%, of a single haplotype throughout Northern California. Overall nucleotide diversity was also low ($\pi = 0.12$) compared with

Alaska and Japan data ($\pi = 1.47$) and Southern California data ($\pi = 0.53$). This indicates that haplotypes must be closely related and may vary by only one nucleotide.

The samples included in my study were gathered over a 789 km range from the Umpqua River in Oregon south to Mowry Slough in the San Francisco Bay. My results did not support an isolation-by-distance pattern, which may have been missed due to a geographically limited sample range. However, a 789 km range falls within sample ranges from previous studies (150-800 km) that did detect isolation-by-distance (O'Corry-Crowe et al. 2003 and Westlake and O'Corry-Crowe 2002).

The results of my haplotype divergence analyses suggest that Eastern Pacific harbor seals dispersed in a manner that follows the stepping-stone model of dispersal (Kimura 1953) and lends support to Burg's (1999) theory of southward colonization by seals of the Eastern Pacific. Seal populations from Oregon, possess haplotypes that appear to have given rise to Northern California haplotypes, indicating a southward step-wise colonization. There is a marked divergence among the two sets of haplotypes found in harbor seals throughout the Central and Northern California range. The co-existence of two lineages (lineage A and lineage B) along the sample range make it difficult for geographic partitioning to be detected. Although I did not attempt to do so, it may have been informative to run a separate isolation by distance analysis for individuals within each lineage, as geographic partitioning may have been missed.

Lineage A contains both the most common haplotype (H1) and several rare haplotypes that vary only by a few single base-changes. This phenomenon results in a 'star-like' phylogeny that is recognized as diagnostic of a recent and sudden population expansion (Westlake and O'Corry-Crowe 2002). Recent expansion is also supported by the low nucleotide diversity values observed in this study.

The most common haplotype identified in this study (H1) dominates most of the seal populations within the sample range except in Oregon. The present analyses, in combination with data derived from Scribner (2005) and Lamont et al. (1996), confirm the full range of H1. Haplotype H1 was identified along a ~920 km range and occurs in all locations from Smith River to San Luis Obispo. H1 was identified in lower frequencies at the outer limits of this range and was found in higher frequencies within a ~420 km central range: Humbolt Bay 56%, Tomales Bay 52%, Drakes Estero-Point Reyes at 44% (present study's data combined with those of Scribner 2005) and San Francisco Bay at 45% (present study's data combined with those of Scribner 2005).

Although the two lineages identified in this study are found throughout the Eastern Pacific, the rare haplotypes reported in Vancouver Island and Puget Sound by Burg et al. (1999) and Lamont et al. (1996) were not observed in Northern California. This is likely the result of the dispersal of harbor seals that originally colonized Southern British Columbia and were subsequently isolated during the last glaciation ~10,000 years before present (Burg et al. 1999).

Lineage B appears to have had more time to establish itself in Central and Northern California compared with lineage A. This is based upon the presence of a network of several haplotypes of greater frequency, which has been reported as evidence of more ancient divergences (Westlake and O'Corry-Crowe 2002). There is also evidence of a third lineage, possibly recently derived from lineage B, in Oregon, which appears to be expanding southward. The haplotypes belonging to the lineage give the impression of being restricted to Northern California locations close to the Oregon border. Only one individual representing this lineage was found in San Francisco. Surprisingly, this one individual, is a female harbor seal which could suggest that females travel greater

distances and play a greater role in gene flow than previously expected.

The existence of three lineages indicate genetic subdivision. However, the degree of gene flow among them cannot be properly addressed using only mtDNA analysis, which tracks maternal heritage exclusively. Therefore, microsatellite analysis is needed to test for non gender biased patterns of dispersal.

The purpose of stock identification under the Marine Mammal Protection Act (MMPA) is to make sure populations remain sustainable and are maintained as an integral part of the ecosystem. Currently Oregon and California harbor seals are being managed as separate stocks by the National Marine Fisheries Service (NOAA-NMFS). Because Northern California and Oregon share more than 50% of their haplotypes, the Oregon/ California border may not be an accurate dividing line for stock delimitation. However there is a risk in misguidedly merging two potential subpopulations and designating them as a single stock. This could endanger the integrity of harbor seal populations (O'Corry-Crowe et al. 2003) and fail to uphold the objectives of the MMPA.

It has been proposed that although the two designated stocks have few haplotypes in common, it is the similarity of the frequencies of those that are shared that make the two groups more closely related (O'Corry-Crowe et al. 2003). The common H1 haplotype has yet to be detected north of California. Although Oregon and California populations share haplotypes, they do not share similar haplotype frequencies. Oregon populations also display significant pairwise genetic distances with Eel River, South and North Humbolt Bay, Tomales Bay, and Castro Rocks. Therefore, I recommend placing more focus on reevaluating stock designations within California. Although a thorough analysis of all California mtDNA data must be made before forming solid conclusions, there is evidence of the presence of at least two stocks within California: (1) a single stock from

Smith River to north of Point Conception and (2) a second stock from Point Conception to Southern California possibly extending down into Baja (present study, Scribner 2005).

Lesser genetic variation among sampled locations (11%) and greater variation within locations (88%) indicates a high degree of homogeneity spanning the entire study range (~780 km). The relatively low Fst value of 0.112 is also an indicator of a low level of genetic differentiation within the sample range. Although some haplotypes are more heavily represented than others, the low Fst indicates that the frequencies of haplotypes are similar throughout this region. Homogeneity is diagnostic of a population with a large degree of gene flow. The female migration estimate of approximately one individual per year is relatively high for a sample range of ~780 km as compared with 1-4 individuals per year detected among smaller ranges of 150-500 km by O'Corry-Crow et al. (2003). Lower female migration values (0.4-1.0 female migrants per year) were detected for a sample range similar in size to that of the present study (Herreman et al. 2009).

Although microsatellite studies have demonstrated that most migration is attributable to males in certain areas of Alaska (Herreman et al. 2009), only female geneflow can be properly addressed in the present study which uses only mtDNA analysis. Microsatellite analysis is needed to assess male migration patterns. Scribner's (2005) mtDNA and microsatellite data provide some insight on the influence of male migration on gene flow in Southern and Central California. Interestingly, the number of migrants per generation, although slightly larger for males, was not substantial. This, along with high levels of female gene flow detected herein, may be an indication that female philopatry may not be as pronounced along Oregon and California as previously detected further north. Female harbor seals are therefore migrating only slightly less than male harbor seals in this part

of their range. A microsatellite analysis for Northern California and Oregon harbor seals is essential to understand the influence of male mediated migration on gene flow.

In conclusion, this study demonstrates the presence of two harbor seal lineages in Northern California, one of which is comprised predominantly of a single haplotype. This haplotype has evolved recently and is restricted to Central and Northern California. A low level of genetic diversity and a high degree of gene flow was detected and supports previous findings which indicate that female harbor seal migration may be comparable to that of male harbor seals. The difference in haplotype frequencies among Oregon and Northern/ Central California confirm the current MMPA-designated stock boundary between them. This study in combination with the results reported by Scribner (2005) support the presence of at least two harbor seal stocks within California. In order to address these stock designations more precisely microsatellite data from Northern California as well as mtDNA and microsatellite data from Oregon locations south of Umpqua River are necessary. A reanalysis of mtDNA and microsatellite data from California and Oregon is advised.

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