

Phylogeography of California and Galápagos sea lions and population structure within the California sea lion

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Abstract We investigate the phylogeography of California (*Zalophus californianus*) and Galápagos (*Z. wolfebaeki*) sea lions and describe within-population structure for the California sea lion based on mitochondrial DNA. Fifty control-region haplotypes were found, 41 from *Z. californianus* and 9 from *Z. wolfebaeki*, with three fixed differences between the two species. Ranked population boundaries along the range of *Z. californianus* were defined based on the Monmonier Maximum Difference Algorithm, resulting in five genetically distinct populations, two in the Pacific Ocean and three inside the Gulf of California. A Minimum Spanning Network showed a strong phylogeographic signal

with two well-defined clusters, *Z. californianus* and *Z. wolfebaeki*, separated by six base-pair differences, supporting the existence of two genetically distinct species with an estimated divergence time of ~0.8 Ma. Results are discussed in the context of the historical geologic and paleoceanographic events of the last 1 Ma in the eastern Pacific.

Introduction

California sea lions (*Zalophus californianus*, Lesson 1828) are distributed along the mainland and offshore islands of the eastern North Pacific Ocean from British Columbia, Canada, to central México, including the Gulf of California (King 1983). The species is occasionally recorded outside

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of its normal range, as far as Alaska to the north (Maniscalco et al. 2004) and southern México to the south (Gallo-Reynoso and Solórzano-Velasco 1991). There are four main breeding rookeries in the United States, on San Miguel, Santa Barbara, San Nicolas, and San Clemente islands (Lowry et al. 1992). In México, there are 19 main breeding rookeries, from the Coronado Islands to Margarita Island along the Pacific coast (Le Boeuf et al. 1983) and from Rocas Consag to Los Islotos in the Gulf of California (Aurioles-Gamboa and Zavala-González 1994; Fig. 1). For management purposes there are three currently recognized stocks defined by the geographic location of their reproductive core areas (Lowry et al. 1992). The “United States” stock extends northward of the México–United States border, including Canada and Alaska, with a reproductive center at the Channel Islands in Southern California and an estimated population size of 238,000–241,000 (Lowry and Maravilla-Chávez 2002). The “Western Baja California” stock extends southward from the México–United States border to the tip of the Baja California peninsula, with its reproductive center at islands near Punta Eugenia and at Santa Margarita Island and an estimated population size of 75,000–85,000 (Lowry and Maravilla-Chávez 2002). The “Gulf of California” stock has its reproductive center at islands located within the central and northern portions of the Gulf of California and has an estimated population size of 31,393 (Aurioles-Gamboa and Zavala-González 1994).

Despite being one of the most common marine mammals in the eastern North Pacific, little is known about the genetic relationships among California sea lion rookeries. While there is clear evidence of genetic differentiation

between geographically isolated rookeries in the Pacific Ocean and the Gulf of California (Maldonado et al. 1995; Bowen et al. 2006), the picture is complicated by the fact that males (at least within Pacific populations) undertake extensive seasonal migrations and individuals of both sexes are capable of moving between rookeries (Bartholomew 1967; Aurioles-Gamboa et al. 1983; M. S. Lowry unpublished data), although the rate of exchange among them is unknown.

Galápagos sea lions (*Zalophus wolfebaeki*, Sivertsen 1953) are endemic and common throughout the Galápagos Archipelago, although major rookeries in the central and southern islands (Florea, Santa Cruz, San Cristóbal, Isabela, Santiago, Española, Mosquera, Santa Fé, and Fernandina) represent almost 90% of the population, which is currently estimated at 16,000–18,000 animals (Salazar 2002; Salazar and Michuy 2008). Despite their smaller geographic range and lack of an established migration, Galápagos sea lions are capable of long-range movements. Vagrant individuals are occasionally reported off the Central and South American coasts as far as 1,570 km from their population center (Palacios et al. 1997; Capella et al. 2002).

After the Galápagos sea lion was described, a close relationship with the California sea lion was assumed. However, the taxonomic designation has been controversial. While most researchers support a species-level separation based on differences in cranial morphometrics (Sivertsen 1953, 1954), social behavior (Eibl-Eibesfeldt 1984), vocalizations (Cenami Spada et al. 1991) and molecular genetics (Wolf et al. 2007), a recent taxonomic review of the family Otariidae based on cranial morphometry supported division of the two taxa only at the subspecific level (Brunner 2004). In this study, we investigate variation in mitochondrial DNA (mtDNA) throughout the entire breeding range of both California and Galápagos sea lions and make phylogeographic inferences to help explain their current distribution and degree of taxonomic differentiation. Further, we examine the level of genetic structuring among California sea lion rookeries to identify distinct population units that can lead to improved management practices.

Materials and methods

Samples

A total of 299 tissue samples were collected in California, USA ($n = 82$ from 2 islands, from 1996 to 1998), México ($n = 170$ from 5 islands along the Pacific coast and 6 islands in the Gulf of California, during 1997) and the Galápagos Islands, Ecuador ($n = 47$ from 8 islands, in 1998 and 1999) (see Figs. 1, 2 for sampling sites). Animals were sampled

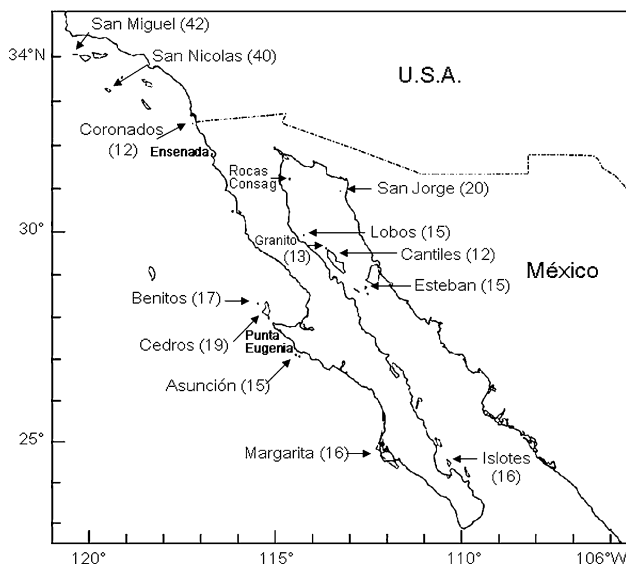


Fig. 1 Geographic location and number in parenthesis of *Z. californianus* samples collected from California and México. Rocas Consag (no samples) is shown because it is the northernmost rookery in the Gulf of California

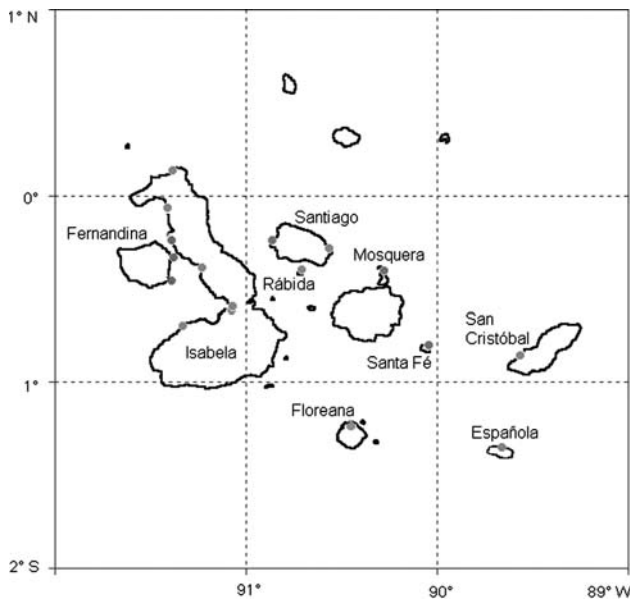


Fig. 2 Geographic location of 47 *Z. wollebaeki* samples collected from 17 different sites on nine islands of the Galápagos Archipelago

by clipping a small piece of skin from the hind limbs. Live pups were sampled in México, while stranded dead pups or yearlings were sampled in California, and dead individuals from all age categories were sampled in Galápagos. Samples of California sea lions were collected from a single rookery on each island except for San Miguel (several sites along a 3-km stretch of coastline) and San Nicolas (two sites). In total, we defined 13 sampling strata for California sea lions. Galápagos samples were obtained from 17 sites on 9 different islands (Fig. 2). Due to the low sample size per site, we combined these samples into one stratum for all Galápagos rookeries.

Samples were either stored in liquid nitrogen in the field, later transferred to an ultrafreezer, and kept at -70°C or in an aqueous solution of 20% (v/v) DMSO saturated with NaCl (Amos and Hoelzel 1991) and kept at -40°C until DNA extraction.

DNA extraction

Samples (40 mg) were digested for 20 h at room temperature in 40 μL of Proteinase K (10 mg/mL, SIGMA or Gibco BRL), 400 μL extraction buffer (0.1 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA), and 40 μL 10% SDS. The DNA was purified by standard phenol-chloroform-isoamyl alcohol (25:24:1) extractions (modified from Sambrook et al. 1989). The precipitate was resuspended in Tris-EDTA buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) to an average concentration of 150 ng/ μL . The quality of the DNA was examined via electrophoresis on 1% agarose gels using ~ 150 ng of DNA.

Amplification and sequencing

A mtDNA fragment of ~ 550 base pairs (bp) from the hypervariable region I of the control region was amplified from the 299 individuals using the polymerase chain reaction (PCR). Two primers developed by the Marine Mammal Genetic Group at the Southwest Fisheries Science Center (SWFSC), La Jolla, California, were used. The Tro primer (5'-CCTCCCTAAGACTCAAGG-3') anneals between the tRNA threonine gene and the tRNA proline gene (L-strand) and the Dx primer (5'-CCTGAAGTAAGAAACCAGATG 3') anneals within the conserved domain of the control region (H-strand). Reactions were performed in 25 μL volumes, containing: ~ 20 ng of genomic DNA, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 3 mM MgCl_2 , 200 μM of each dNTP, 0.3 μM of each primer and 1 U of *Taq* DNA polymerase (Gibco BRL). The thermal cycling profile was as follows: an initial hot-start of 5 min at 94°C ; 35 amplification cycles of denaturation for 1 min at 94°C , annealing for 1 min at 50°C and extension for 1.5 min, with a 2 s increase per cycle, at 70°C , and a final 5 min incubation at 70°C to ensure complete extension of the PCR products.

Successful amplification products were then cleaned by purification columns (ConcertTM Rapid PCR Purification System, Gibco BRL) according to the manufacturers' specifications. Both heavy and light strands were cycle-sequenced using the BigDye[®] Terminator Sequencing Standard (Applied Biosystems Inc.). Reactions were performed in 12 μL volumes, containing: 60–100 ng of double-stranded cleaned PCR product, 0.25 μM of one primer and 2 μL of terminator ready-reaction mix. The thermal cycling profile included an initial hot-start of 5 min at 95°C , followed by 25 cycles of denaturation for 30 s at 95°C , annealing for 15 s at 50°C , extension for 4 min at 60°C and a final 2 min incubation at 60°C to ensure complete extension of the PCR products. Sequenced products were purified by ethanol precipitation and then run on an ABI 377 DNA automated sequencer.

Data analyses

Editing of opposite strands was performed simultaneously using SequencherTM version 4.1 software to produce 383 bp-long sequences. Initial sequence comparisons and measures of variability were performed using MEGA version 2.1 (Kumar et al. 2001). Final sequences were compared with the complete mitochondrial genome of the harbor seal (*Phoca vitulina*) from GenBank accession number NC 001325 (Arnason and Johnsson 1992) as a reference. Haplotype (h) and nucleotide (π) diversity was estimated, and Tajima's test of neutrality was performed on both groups of samples (California and Galápagos), and on each putative

population within California samples using Arlequin, version 2.0 (Schneider et al. 2001).

The phylogeographic structure was analyzed by comparing phylogenetic relationships among unique haplotypes and the geographic location of each haplotype. Phylogenetic relationships were inferred from a Minimum Spanning Network (MSN) of all haplotypes. The number of pairwise nucleotide differences among haplotypes was used in Minspnet (Excoffier and Smouse 1994) to derive the MSN. Because there were no shared haplotypes between California and Galápagos samples (see “Results”), and because of the number and type of mutations separating these clusters in the MSN were both indicative of species-level differentiation, the Galápagos samples were excluded from analyses of population genetic structure within California sea lions.

Steller sea lion (*Eumetopias jubatus*), the sister taxon to *Zalophus* and its closest extant relative in the North Pacific (Wynen et al. 2001; Deméré et al. 2003), was used as a calibration point to estimate the minimum time of genetic divergence between *Z. californianus* and *Z. wolfebaeki*. First, the three most divergent sequences in the *E. jubatus* were chosen from GenBank and compared to three of the most divergent *Z. californianus* sequences from the present study. The mean divergence time between *Z. californianus* and *E. jubatus* was then estimated based on the mean D-loop sequence divergence rate for marine mammals (3.25% per million years (My); i.e., the mean between the divergence rate in cetaceans (0.5% per My) and that in elephant seals (~6% per My); Stewart and Baker 1994). The procedure was then repeated for the three most divergent sequences of *Z. californianus* and *Z. wolfebaeki* to estimate the minimum mean genetic divergence time between the two.

To assess the extent of genetic structure within California sea lions, the Monmonier Maximum Difference Algorithm (MMDA; Manel et al. 2003) was used to determine, in a ranked order, potential boundaries separating putative populations. First, the 13 sampling strata were placed on a Delaunay network (Brassel and Reif 1979) connecting adjacent sampling strata. The MMDA was then implemented in the Barrier version 2.2 program (Manni et al. 2004), using Nei's D_a genetic distance between mtDNA haplotypes as a measure of genetic distance among the 13 sampling strata. The resultant putative populations defined by these potential and ranked boundaries were then tested using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin version 2.0 (Schneider et al. 2001). AMOVA was performed to estimate F -statistics and their analogue Φ -statistics. For Φ_{ST} , the genetic distance between pairs of haplotypes was estimated as the proportion of the nucleotide differences between them. The null distribution of pairwise F_{ST} and

Φ_{ST} values under the hypothesis of panmixia was obtained by 16,000 permutations of haplotypes between populations, guaranteeing less than 1% difference with the exact probability in 99% of the cases (Guo and Thomson 1992). The final number of populations was determined as that which resulted in statistically significant differentiation between all pairs of adjacent populations when using the largest number, in their ranked order, of potential boundaries.

Results

Genetic diversity and neutrality

Fifty haplotypes were found; forty-one specific to *Z. californianus* and nine specific to *Z. wolfebaeki* (Table 1). Twenty-nine sites were variable, with 28 transitions and a single transversion. The 50 different haplotypes were deposited in the GenBank database under accession numbers EF512168 to EF512217. Overall haplotype diversity for California sea lions was $h = 0.8860 \pm 0.0123$. The lowest values were found in the “Pacific Temperate” ($h = 0.6712 \pm 0.0404$) and in the Galápagos ($h = 0.7604 \pm 0.521$) populations (Table 2; see “Population Structure” section for population definitions). Overall nucleotide diversity from California sea lions was $\pi = 0.0088 \pm 0.0050$. The lowest value was found in Galápagos ($\pi = 0.0037 \pm 0.0026$); this value was almost half that of the nearest lowest values (“Pacific Temperate” and “Southern Gulf”; Table 2). The null hypothesis of neutrality was not rejected; in all cases, Tajima's D -statistics were statistically non-significant ($P > 0.3$, Table 2). No shared haplotypes were found between samples collected from *Z. californianus* and *Z. wolfebaeki* (Table 1). A unique transversion (site number 214, Table 1) and two transitions (sites 233 and 234, Table 1) represented fixed differences that distinguished Galápagos from California samples.

Phylogeographic structure and divergence time

The MSN showed a strong phylogeographic signal, with two distinct clusters corresponding to California (haplotypes H1–H41) and Galápagos (haplotypes H42–H50) sea lions (upper and lower clusters in Fig. 3, respectively). Haplotype H1 was the most common one, with the highest number of connections, followed by H29 and H34. Galápagos haplotypes (H42–H50) were grouped together and separated from California haplotypes by six mutations (Fig. 3). The Galápagos haplotype cluster had fewer reticulations than the California cluster (Fig. 3). In the latter, common haplotypes and haplotypes with high numbers of connections occurred more frequently in the Pacific populations (“Pacific Temperate” and “Pacific Subtropical”; Fig. 4). Peripheral and private

Table 1 List of 50 haplotypes defined by 29 variable sites on the basis of 383 bp of the hypervariable region I of mitochondrial DNA of *Z. californianus* (H01–H41) and *Z. wollebaeki* (H42–H50)

Haplotype	Site Number																													
	4	7	9	1	1	2	3	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	
H01	C	T	T	G	T	T	A	G	A	T	T	A	T	C	C	T	A	A	C	T	T	T	C	A	A	T	A	G	T	
H02	T
H03	C
H04	C
H05	G
H06	G	.	G
H07	G
H08	A	C
H09	C
H10	G	C
H11	C
H12	A	C
H13	A
H14	G	A	.	C
H15	G	A
H16	T	A	G	.	C
H17	T	C	.	A	C	C
H18	T	C	.	A	C	.	.	G	C
H19	T	.	C	.	.	C	.	A	C	.	C	C
H20	T	A	C	C
H21	T	G	A	C	C
H22	T	G	A	.	C	.	.	C
H23	T	G	A	A	.
H24	T	G	A	A	.
H25	A	G
H26	C	.	A	G
H27	T	C	.	A	G
H28	T	C	.	A
H29	T	A	G	C
H30	T	.	.	C	.	.	.	A	G	C
H31	T	G	A	G	C
H32	T	C	G	A	G	C
H33	T	C	.	A	G	G	.	C
H34	T	C	.	A	G	C
H35	G	A	G	.	.	.	C	C
H36	G	A	G	.	.	.	C	C	C
H37	G	A	G	G	C	G	.	.	.
H38	G	A	G	G	C	G	.	.	.
H39	G	A	G	.	.	.	C	.	C	C	G
H40	.	.	.	A	.	.	G	A	G	C	.	.	C	.	.	.
H41	.	.	.	A	.	.	G	A	G	C	.	.	C	.	.	C
H42	G	A	G	.	.	C	.	.	.	G	T	C	C	.	G	C	.	.	C
H43	G	A	G	.	.	C	.	.	.	G	T	C	C	C	.	G	C	.	.
H44	G	.	G	.	.	C	.	.	.	G	T	C	C	.	G	C	G	.
H45	G	.	G	.	.	C	.	.	.	G	T	C	C	.	G	C	.	.	.
H46	G	.	G	.	.	C	.	.	T	.	G	T	C	C	.	G	C	.	.	.
H47	G	A	G	.	.	C	.	.	T	.	G	T	C	C	.	G	C	.	.	.
H48	.	C	G	A	G	.	.	C	.	.	T	.	G	T	C	C	.	G	C	.	.	.
H49	G	A	G	.	.	C	C	.	T	.	G	T	C	C	T	.	G	C	.	.
H50	G	A	G	.	.	C	C	.	T	T	.	G	T	C	.	.	.	C	T	.	G	C	.	.

Site No.1 of the complete sequence is equivalent to site No.16304 of the harbor seal (*Phoca vitulina*) sequence by Arnason and Johnsson (1992); GenBank accession number: NC 001325. A transversion in site number 214 and two transitions (233, 234) are fixed differences that distinguished *Z. wollebaeki* from *Z. californianus* haplotypes

haplotypes occurred most frequently in “Northern Gulf” and “Central Gulf” populations, with whole haplotype clusters present only in the Gulf of California (Fig. 4). Despite their lower sample sizes, Gulf populations showed higher haplotype diversities (Table 2). Very few haplotypes were shared between all populations (Fig. 4).

The three most divergent sequences for *E. jubatus* (GenBank accession numbers AY340888, AY340917 and

AY340937; Baker et al. 2005) and for *Z. californianus* (haplotypes 1, 29 and 35 from the present study) yielded a mean sequence divergence of 9.9% between the two genera and a mean genetic divergence time of 3.05 million years ago (Ma) (using the 3.25% per My mean D-loop sequence divergence rate for marine mammals). Based on this estimate, the three most divergent *Z. californianus* and *Z. wollebaeki* sequences (haplotypes 42, 47 and 50) yielded a

Table 2 Measures of genetic diversity (\pm SD) and the results of Tajima's neutrality test by population, and totals for *Z. californianus* and *Z. wollebaeki*

Population	No. of samples	No. of haplotypes (PS)	Haplotype diversity (h) (%)	Nucleotide diversity (π) (%)	Tajima's D statistic	
Pacific Temperate	94	10 (9)	67.12 \pm 4.04	0.64 \pm 0.39	0.9647	$P = 0.854$
Pacific Subtropical	67	11 (10)	81.73 \pm 2.47	0.81 \pm 0.47	1.3157	$P = 0.910$
Southern Gulf	16	9 (6)	88.33 \pm 6.12	0.62 \pm 0.40	1.0730	$P = 0.873$
Central Gulf	27	12 (12)	87.46 \pm 5.13	0.82 \pm 0.49	0.0099	$P = 0.556$
Northern Gulf	48	19 (16)	93.09 \pm 1.68	1.09 \pm 0.61	0.4770	$P = 0.725$
Total (<i>Z. californianus</i>)	252	41 (21)	88.60 \pm 1.23	0.88 \pm 0.50	-0.0621	$P = 0.543$
<i>Z. wollebaeki</i>	47	9 (8)	76.04 \pm 5.21	0.37 \pm 0.26	-0.6008	$P = 0.314$

"Pacific Temperate" includes San Miguel, San Nicolas, and Coronados islands. "Pacific Subtropical" includes Benito, Cedros, Asunción, and Margarita islands. "Southern Gulf" includes Islotes. "Central Gulf" includes Esteban Island and Cantiles rookery on Ángel de la Guarda Island. "Northern Gulf" includes Granito, Lobos, and San Jorge islands

PS = Polymorphic sites

mean divergence of 2.6% and a minimum mean divergence time of 0.8 Ma.

Population structure

When the first four potential boundaries produced by the MMDA were used, overall AMOVA results for the resulting five putative populations were statistically significant for both fixation indexes ($F_{ST} = 0.135$, $P < 0.001$; $\Phi_{ST} = 0.135$, $P < 0.001$). Additionally, all pairwise comparisons showed statistically significant differences among the five populations for both F_{ST} and Φ_{ST} values (Table 3). Based on the approximate geographic range of each of these populations, we name them: "Pacific Temperate" (comprising San Miguel, San Nicolas and Coronados islands), "Pacific Subtropical" (including Benito, Cedros, Asunción, and Margarita islands), "Southern Gulf" (Los Islotes), "Central Gulf" (comprising San Esteban Island and Los Cantiles rookery on Ángel de la Guarda Island), and "Northern Gulf" (including Granito, Lobos and San Jorge islands) (see Fig. 1).

Discussion

Genetic diversity and neutrality

All substitutions in California sea lion haplotypes were transitions, in agreement with values reported for control region sequences from nine other species of Otariidae, all of which showed values for transitions $>90\%$ (Wynen et al. 2001), but higher than in a previous California sea lion study that reported 71% transitions (Maldonado et al. 1995).

The overall haplotype diversity among *Z. californianus* samples ($h = 0.8860$) fell among the values reported for

E. jubatus ($h = 0.927$, Bickham et al. 1996), *Callorhinus ursinus* ($h = 0.994$, Dickerson et al. 2008) and the Guadalupe fur seal (*Arctocephalus townsendi*) ($h = 0.798$, Weber et al. 2004). Similarly, nucleotide diversity for *Z. californianus* ($\pi = 0.0088$) was comparable to that reported for other species of sea lions, such as *E. jubatus* ($\pi = 0.004$), *Otaria byronia* ($\pi = 0.008$), and *Phocartos hookeri* ($\pi = 0.004$) (Wynen et al. 2001). The lowest haplotype and nucleotide diversity values were found in the "Pacific Temperate" and in the Galápagos samples (Table 2), despite the fact that these were the only strata where samples were collected from more than one site per island and in more than 1 year.

This low genetic diversity may be related to a possible founder effect or to historical events that could have reduced the populations, such as strong El Niño events, disease epidemics or commercial harvesting. For instance, there is archeological evidence that the San Miguel Island ("Pacific Temperate") population was considerably smaller ("rare") between 1425 and 1500 AD than it is today (Walker et al. 1999), suggesting this rookery may have been colonized only in the last few centuries. Faunal remains from archaeological sites on San Miguel Island are of particular importance in this respect since they are the only source of information currently available concerning the recent history. However, since faunal remains are a product of human subsistence activity, they cannot be considered an unbiased sample of the sea mammal populations living prehistorically in the vicinity of San Miguel Island (Walker and Craig 1979). In more recent times, commercial harvesting in Southern California and the Mexican Pacific reduced these populations to only about 1,500 animals by the 1920s (Heath 2002), while harvesting in the Gulf of California was not as intensive (Lluch-Belda 1969; Zavala-González and Mellink 2000). In addition, all Pacific coast rookeries are exposed to dramatic population fluctuations associated with recurring El Niño events (DeLong et al.

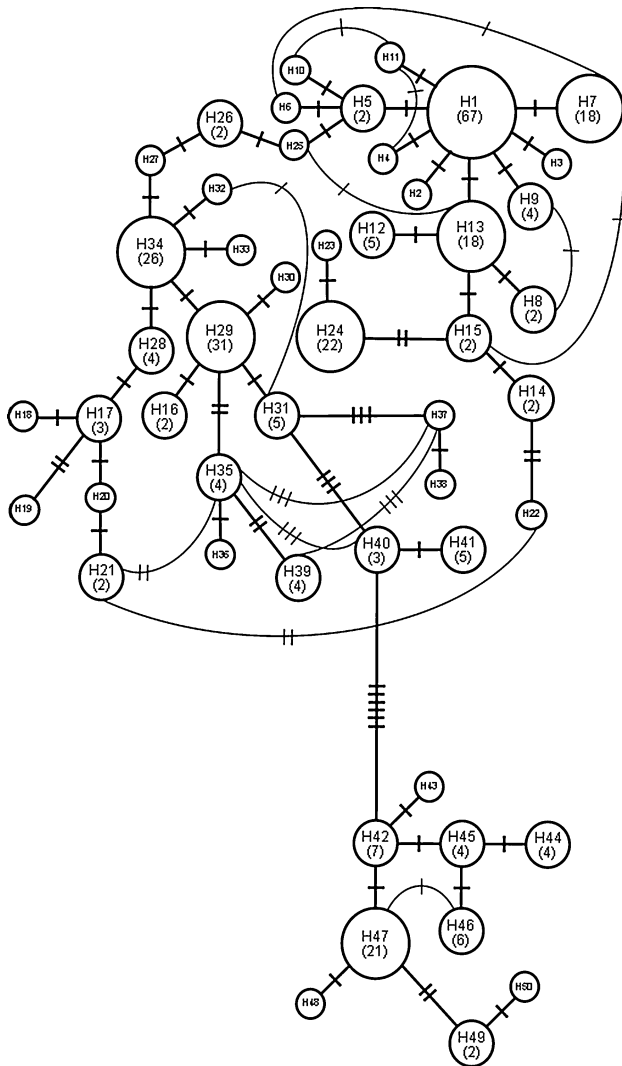


Fig. 3 Minimum Spanning Network for 41 haplotypes of *Z. californianus* (252 samples) and 9 haplotypes (H42–H50) of *Z. wollebaeki* (47 samples). Each circle represents a haplotype; inside are its number and frequency (in parenthesis). The small circles represent one individual. The transverse marks between haplotypes indicate the number of mutations between them. Straight lines represent direct relations and curved lines are alternative relations

1991; Boness et al. 1991; Francis and Heath 1991; Morris et al. 1999), in contrast to the Gulf of California, where populations are relatively protected (Aurióles-Gamboa and Le Boeuf 1991; Hernández-Camacho et al. 2008a). For example, in the islands of the Southern California Bight during the 1982–1983 El Niño, births decreased 30% at San Miguel Island, 43% at San Nicolas Island, 62% at San Clemente Island, and 71% at Santa Barbara Island (DeLong et al. 1991). The effects of El Niño are also pervasive in Galápagos, where population declines >30% and pup mortalities >90% have been documented during the 1982–1983 (Trillmich and Limberger 1985; Trillmich and Dellinger 1991) and 1997–1998 (Salazar and Bustamante 2003) strong events.

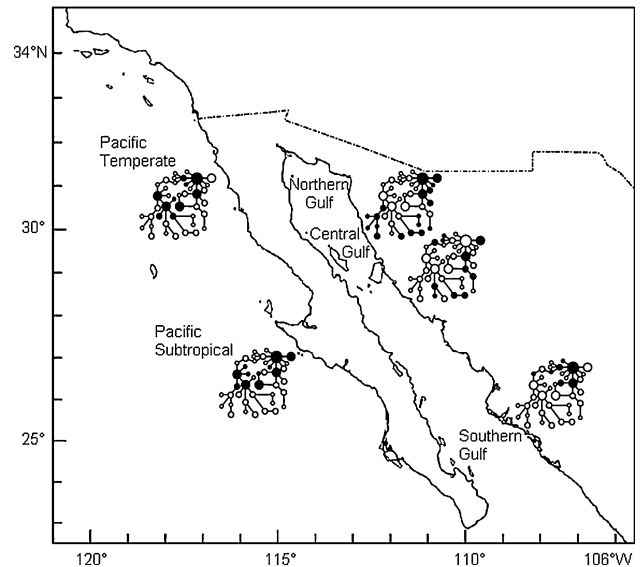


Fig. 4 Geographic representation of the Minimum Spanning Networks for the five populations of *Z. californianus*. The “Pacific Temperate” population includes San Miguel, San Nicolas, and Coronados islands; “Pacific Subtropical” comprises Benito, Cedros, Asunción, and Santa Margarita islands; “Southern Gulf” is represented by Los Islotes; “Central Gulf” includes San Esteban Island and Los Cantiles rookery on Ángel de la Guarda Island; “Northern Gulf” comprises Granito, Lobos, and San Jorge islands. The arrangement of haplotypes corresponds to that in Fig. 3, with filled circles representing the haplotypes found in that population

Phylogeography of *Zalophus*

A strong phylogeographic signal with two well-defined clusters separated by six mutations in the MSN supported the existence of two species, *Z. californianus* and *Z. wollebaeki*. Our estimated mean genetic divergence time between *Eumetopias* and *Zalophus* of 3.05 Ma fell within the 95% confidence interval (1.57–3.06 Ma) for the minimum divergence time of 2.25 Ma reported by Harlin-Cognato et al. (2006) for these two taxa. Based on this value, the estimated time of genetic divergence between *Z. californianus* and *Z. wollebaeki* is 0.8 Ma, which is three times lower than the 2.3 ± 0.5 Ma recently estimated by Wolf et al. (2007). This discrepancy may be due to differences in the calibration value used or to the different number of fixed differences detected in each study (nine in Wolf et al. 2007 versus three in the present study; see Hey 1991), which, in turn, may be due to the larger and more geographically representative sample size evaluated in our study. In particular, the closest haplotypes to *Z. wollebaeki* (H40 and H41) were found only in samples from the “Northern Gulf” and “Central Gulf” populations, which were not represented in Wolf et al. (2007). We emphasize, however, that our estimate is only an approximation that suggests a more recent time of divergence; the inexact mutation rate used in our study and

Table 3 Pairwise F_{ST} and Φ_{ST} values (lower matrix) for comparisons among five populations: “Pacific Temperate” includes San Miguel, San Nicolas, and Coronados islands

	Pacific Temperate	Pacific Subtropical	Southern Gulf	Central Gulf	Northern Gulf
Pacific Temperate	F_{ST}	<0.001	<0.001	<0.001	<0.001
	Φ_{ST}	<0.001	0.015	<0.001	<0.001
Pacific Subtropical	0.1329		0.002	<0.001	<0.001
	0.1063		0.003	<0.001	<0.001
Southern Gulf	0.1956	0.0912		<0.001	0.001
	0.1072	0.1443		<0.001	0.003
Central Gulf	0.2418	0.1475	0.1089		0.032
	0.2228	0.2462	0.2191		0.024
Northern Gulf	0.1500	0.0954	0.0619	0.0243	
	0.1020	0.1398	0.1002	0.0446	

“Pacific Subtropical” includes Benito, Cedros, Asunción, and Margarita islands. “Southern Gulf” includes Islotes. “Central Gulf” includes Esteban Island and Cantiles rookery on Ángel de la Guarda Island. “Northern Gulf” includes Granito, Lobos, and San Jorge islands

Corresponding P values (upper matrix) were calculated from 16,000 random permutation tests. The null hypothesis of panmixia (no structure) was rejected in all cases, at $P < 0.05$

the very limited representation of *Zalophus* in the fossil record (see Deméré et al. 2003; Ho et al. 2005) do not allow a more constrained value. Additionally, the need for a good fossil dating will be necessary to more accurately trace the evolutionary history of pinnipeds.

The historical process by which *Zalophus* sea lions arrived in the Galápagos Islands and became a distinct species remains highly conjectural. The basal otariinae evolved in the temperate eastern North Pacific in the middle Miocene (before 11 Ma) and it is generally assumed that they dispersed into the Southern Hemisphere (in one or multiple waves) in the late Pliocene/early Pleistocene (~2–3 Ma), via a cool-water pathway known as the East Pacific Corridor connecting the California and Perú currents (Davies 1958; Repenning et al. 1979; Deméré et al. 2003). This dispersal event, which is consistent with cooling and very high levels of biological productivity in the tropics at the time (Lawrence et al. 2006), likely culminated in the origin of the three genera of present-day Southern Hemisphere otariinae (*Otaria*, *Phocarctos*, *Neophoca*). In an analogous manner, we suggest that the establishment of *Z. wollebaeki* as a distinct species in Galápagos occurred later in the Pleistocene.

A determining factor for the colonization of the Galápagos by a large predator like *Zalophus* must have been the availability of suitable foraging habitat and adequate prey base. Unlike the extensive and relatively more stable continental shelves, which support ample fish and cephalopod biomasses, the Galápagos are the product of hotspot volcanism with a complex history of emergence and submergence. The age of the present-day Galápagos has been estimated at between 0.3 Ma for Fernandina Island in the west and 6.3 Ma for San Cristóbal Island in the east (Geist 1996), although now-drowned but once-emergent volcanoes have been dated at 5–14 Ma (Christie et al. 1992;

Werner et al. 1999). This process would have provided the stepping stones for the persistence and evolution of the unique Galápagos terrestrial biota (Rassmann 1997; Grehan 2001; Beheregaray et al. 2004), but would not have been conducive to the development of a marine ecosystem capable of supporting a large biomass of epipelagic and demersal prey until a configuration similar to the present-day Archipelago was reached. Further, while cool upwelling and high oceanic productivity characterized the glacial periods of the Pleistocene (1.8–0.01 Ma) (Lawrence et al. 2006; Lea et al. 2006; Koutavas and Sachs 2008), sea level was 100–125 m below present, such that most of the shallow shelves connecting the islands, where most foraging by sea lions takes place today (Villegas-Amtmann et al. 2008), were exposed (see Fig. 2 in Geist 1996). For these reasons, the successful colonization and establishment of a distinct *Zalophus* form in Galápagos may have only occurred in the middle–late Pleistocene. Indeed, speciation in other central-place foraging marine predators of Galápagos appears to have occurred very recently (~0.5 Ma) (Browne et al. 1997; Akst et al. 2002; Friesen et al. 2002).

The phylogeographic signal from the presence/absence of haplotypes in the MSN (Fig. 4) also reveals information about the origin and history of *Z. californianus* populations. All regions presented haplotype H1 as well as haplotypes derived from it, indicating that this haplotype was present in the ancestral population (Crandall and Templeton 1993; Excoffier and Smouse 1994). In contrast, haplotypes H29 and H34 (second and third most common haplotypes) only occurred in the “Pacific Temperate” and “Pacific Subtropical” populations, revealing a strong separation between populations in the Pacific Ocean and in the Gulf of California. However, the “Northern Gulf” and “Central Gulf” populations were characterized by whole clusters of private

haplotypes derived from H29 and H34, including haplotypes H40 and H41, which link *Z. californianus* with *Z. wolfebaeki*. Furthermore, haplotype diversity was highest in all three Gulf populations.

The Gulf of California in its current configuration has been in existence since ~3.7 Ma (Jacobs et al. 2004), and therefore a possible interpretation for the patterns of haplotype diversity in our MSN is that the Gulf was initially colonized by eastern North Pacific animals, which later dispersed to Galápagos. An alternative and more plausible interpretation involves a more complex scenario in which North Pacific marine fauna colonized and evolved in isolation inside the Gulf during times of inhospitable conditions in the outer Pacific coast (e.g. Bernardi et al. 2003; Jacobs et al. 2004) resulting from strong oscillations in upwelling and sea level such as occurred around the middle Pleistocene transition peaking ~0.9 Ma (Clark et al. 2006; Lawrence et al. 2006). Once conditions became favorable again in the late Pleistocene, animals from the Gulf recolonized the Pacific, giving rise to the present-day populations (Jacobs et al. 2004). Under this “refugium” hypothesis for North Pacific marine fauna, *Zalophus* populations from the Gulf of California would have been the source for both the Galápagos and Pacific populations. An analogous vicariant process occurring in insular and continental refugia south of the North American and Eurasian ice sheets during the Plio-Pleistocene has been invoked by Harlin-Cognato et al. (2006) to explain the phylogeography of Steller sea lions.

Haplotypes in the clusters derived from H29 and H34 would have evolved in the Gulf of California during the time when the species was restricted to this area. The absence of H29 and H34 in the Gulf could be explained by lower frequencies in the Gulf or by their disappearance after migration had occurred back to the Pacific. The lower haplotype diversity found in the Pacific would further support a more recent origin of Pacific populations. This “refugium” hypothesis is also supported by a recent morphological study across the breeding range of *Zalophus*, reporting a cline in the presence of double or triple roots in the postcanines and/or the presence of a sixth postcanine, such that the highest frequencies occur in Galápagos animals, followed by those in the Gulf of California and, finally, those in the Pacific (Aurioles-Gamboa et al. 2000).

Population structure within California sea lions

The pattern of genetic variation found in this study not only confirms previous results regarding the genetic isolation of sea lions in the Gulf of California (Maldonado et al. 1995; Bowen et al. 2006), but it also provides evidence for latitudinal structuring in the Pacific populations. Further, the high degree of genetic differentiation among the five putative populations identified ($F_{ST} = 0.024\text{--}0.242$, $\Phi_{ST} =$

$0.045\text{--}0.246$; Table 3) is up to five times higher than that reported for Steller sea lions ($F_{ST} = 0.05$, Bickham et al. 1996) in most pairwise comparisons. This population-level differentiation is in general agreement with the studies of population structure among California sea lions based on cranial morphometrics (Zavaleta-Lizárraga 2003), feeding habits and trophic level (García-Rodríguez and Aurioles-Gamboa 2004; Porras-Peters et al. 2008), heavy metal concentrations (Elorriaga-Verplancken and Aurioles-Gamboa 2008), diseases (Szteren 2006), and population trends (González-Suárez et al. 2006; Szteren et al. 2006), although some of the boundaries vary among the various studies. Despite the well-known capability of individual California sea lions for long-distance travel, our population structure results are consistent with the strong philopatric behavior displayed by the species, not only in reproductive females (Riedman 1990) but also in males (Hernández-Camacho et al. 2008a). Additional evidence for a low reproductive exchange among rookeries is the large difference in chlorinated hydrocarbon contents in the blubber of California sea lions from Southern California, USA, and Ensenada, Baja California, México (Kannan et al. 2004; Del Toro et al. 2006), separated by only 350 km.

Ecological studies of *Z. californianus* (García-Rodríguez and Aurioles-Gamboa 2004; Espinosa de los Reyes 2007; Porras-Peters et al. 2008) and *Z. wolfebaeki* (Wolf et al. 2008) suggest that inter-population differentiation may be related to differences in feeding habits. “Northern Gulf”, “Central Gulf”, and Galápagos populations have foraging distances from the rookeries of ≤ 20 km (García-Rodríguez and Aurioles-Gamboa 2004; Kooyman and Trillmich 1986; Villegas-Amtmann et al. 2008). In contrast, foraging distances in San Miguel Island (“Pacific Temperate” population) have been reported at 70–100 km (Antonelis et al. 1990; Melin and DeLong 1999). It is likely that these differences are driven by prey distribution and abundance in different oceanographic regimes.

The boundaries among *Z. californianus* populations are consistent with the major oceanographic patterns in the region. The “Pacific Temperate” population is contained within a recirculation cell of the California Current known as the Southern California Eddy, and is separated from the more open, upwelling-dominated coast of northern Baja California by the Ensenada Front (Hickey 1998; Santamaría del Ángel et al. 2002). About halfway down the peninsula, and within the range of the “Pacific Subtropical” population, the prominent headlands of Punta Eugenia and Cape San Lázaro induce flow instabilities that result in dynamic eddies and jets forming at these locations (Hickey 1998; Espinosa-Carreón et al. 2004). Inside the Gulf of California, four oceanographic regimes can be distinguished: (1) the shallow northern Gulf, (2) the tidally energetic islands and sills region, (3) the central deeper gulf, and (4) the mouth region

(Kahru et al. 2004). While we did not sample the small breeding rookeries found in their third region (San Pedro Mártir, San Pedro Nolasco and Farallón de San Ignacio), our “Northern Gulf”, “Central Gulf”, and “Southern Gulf” populations correspond well with the first, second, and fourth oceanographic regions of Kahru et al. (2004), respectively.

The behavior of adult females is a strong force in the process of population isolation. In California sea lions, females return to their natal beach to give birth and nurse their young. Lactation may last for a year or more (Peterson and Bartholomew 1967; Newsome et al. 2006), and a female may give birth to a pup each year almost without interruption from 4 to 12 years of age (Hernández-Camacho et al. 2008b). This long and nearly continuous period of maternal investment is the driver for the observed pattern of philopatry in adult females, who, needing to forage and return expeditiously to nurse their pups, develop specific feeding habits and strategies adapted to local conditions, as many recent ecological studies have shown (García-Rodríguez and Aurióles-Gamboa 2004; Espinosa de los Reyes 2007; Porras-Peters et al. 2008; Wolf et al. 2008). The variation in the oceanographic regimes noted above correlate well with the sea lion population clusters identified in this study. Thus, it is likely that the oceanographic regions within the eastern North Pacific support different feeding grounds to which females, with dispersal constrained by the need to return to the rookery, have adapted for a period long enough to create the present genetic structure.

Management implications

It is important to consider the vulnerability of the different *Zalophus* population for management purposes. Special attention is warranted for sea lions in the Gulf of California because of their lower abundance (31,393, Aurióles-Gamboa and Zavala-González 1994) and greater population structure when compared to Pacific populations (75,000–85,000 in México and 238,000–241,000 in the United States, Lowry and Maravilla-Chávez 2002). Also, “Northern Gulf” and “Central Gulf” populations may be especially vulnerable because of higher reported frequencies of tempo-oro-mandibular osteoarthritis and mandibular osteomyelitis compared to colonies along the western Baja California coast (Aurióles-Gamboa et al. 2009). The high frequency of tooth erosion in sea lions of the Gulf of California (Labrada et al. 2007) may be linked to these diseases. Leptospirosis, a sea lion disease caused by the bacteria *Leptospira interrogans*, results in early births, abortions and kidney problems, and occurs in highest frequencies in the northern Gulf of California (Acevedo-Whitehouse et al. 2003). Along the Pacific coast, the highest susceptibility to hookworm (*Uncinaria* spp.) infection has been shown to be an important cause of pup mortality at San Miguel Island (“Pacific

Temperate” population) (Acevedo-Whitehouse et al. 2006), which is one of the two largest California sea lion breeding rookeries (the other being San Nicolas Island) and produces nearly 42% of pups in the United States population (Lowry and Maravilla-Chávez 2002). These results indicate that more studies about diseases in natural populations are needed in order to ascertain the extent to which selective mortality occurs and what the consequences are with respect to the maintenance of genetic variation of the population (Acevedo-Whitehouse et al. 2006).

The strong intraspecific structure within the California sea lion, with five distinct populations in the eastern North Pacific, probably reflects the adaptability of the species to local and regional environmental conditions. These populations may be considered different “Management Units,” a fundamental concept for proper short-term management and the logical unit for population monitoring and demographic studies (Moritz 1994). The support for *Z. wolfebaeki* as a separate species is also timely, given the current concerns over the impacts of climate variability, widespread disease and human interactions on a rapidly declining population (Salazar 2002; Wolf et al. 2007; Salazar and Michuy 2008). Efforts toward strengthened conservation strategies for this species should be a priority within local and regional plans.

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