Molecular Ecology (2009) **18**, 1088–1099 doi: 10.1111/j.1365-294X.2009.04093.x

Isolation by distance among California sea lion populations in Mexico: redefining management stocks

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Abstract

Understanding the spatial structure of a population is critical for effective assessment and management. However, direct observation of spatial dynamics is generally difficult, particularly for marine mammals. California sea lions (*Zalophus californianus*) are polygynous pinnipeds distributed along the Pacific coast of North America. The species' range has been subdivided into three management stocks based on differences in mitochondrial DNA, but to date no studies have considered nuclear genetic variation, and thus we lack a comprehensive understanding of gene flow patterns among sea lion colonies. In light of recent population declines in the Gulf of California, Mexico, it is important to understand spatial structure to determine if declining sea lion colonies are genetically isolated from others. To define population subdivision and identify sex biases in gene flow, we analysed a 355-bp sequence of the mitochondrial DNA control region and 10 polymorphic microsatellite loci from 355 tissue samples collected from six colonies distributed along Mexican waters. Using a novel approach to estimate sex biases in gene flow, we found that male sea lions disperse on average 6.75 times more frequently than females. Analyses of population subdivision strongly suggest a pattern of isolation by distance among colonies and challenge current stock definitions. Based on these results, we propose an alternative classification that identifies three Mexican management units: Upper Gulf of California, Southern Baja Peninsula, and Upper Pacific Coast of Baja. This revised classification should be considered in future assessment and management of California sea lion populations in Mexican waters.

Keywords: Gulf of California, microsatellites, mitochondrial DNA, pinniped, population structure, sex-biased dispersal, *Zalophus californianus*

Received 31 August 2008; revision received 25 November 2008; accepted 8 December 2008

Introduction

Effective assessment and management of a population requires understanding spatial structure (i.e. how individuals are distributed and disperse over geographical space). Assumptions about spatial structure can significantly affect estimates of extinction risk (Gonzalez-Suarez *et al*. 2006; González-Suárez & Gerber 2008), and management units are often defined based on the spatial structure of populations (Dizon *et al*. 1992; Fraser & Bernatchez 2001). Unfortunately, in many cases spatial structure may be difficult to infer from observable dynamics. For example, many pinniped species (seals and sea lions) use seemingly continuous and extensive foraging habitats (oceans) and have the capacity to travel over large distances (Mate 1975; Campagna *et al*. 2001). However, pinnipeds also aggregate and mate in highly distinct terrestrial colonies (Riedman 1990) and often exhibit high levels of philopatry (Pomeroy *et al*. 2000). Therefore, one could predict little spatial subdivision across the continuous foraging habitat or expect strong clustering around the distinct breeding colonies. In these cases, direct observation of dispersal events could help identify true spatial structure. However, direct observation of dispersal is generally difficult (e.g. Baguette *et al*. 2000; Lebreton *et al*. 2003), particularly for long-lived

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marine species. Research on marine mammals is specially challenging because they have very long lifespans, which makes detection of infrequent dispersal events unlikely. Moreover, marine mammals disperse in a large three-dimensional environment (ocean) in which tracking individuals can be very complicated. In these situations, spatial structure may be inferred from analyses of genetic subdivision because genetic differences in neutral markers are evidence of limited, or nonrandom, dispersal between subpopulations (Hedrick 2005a).

In many mammalian species, dispersal is sex-biased (Greenwood 1980; Hedrick 2007; Lawson Handley & Perrin 2007) and this bias should be taken into account when making inferences about spatial dynamics based on genetic population structure. For example, most mammals, including pinnipeds are able to disperse over very large distances, but movement is often male-biased because females are generally philopatric while males disperse (Burg *et al*. 1999; Hoffman *et al*. 2006). In these populations, inferences about spatial structure based on mitochondrial DNA data (female-inherited) are likely to differ from those based on nuclear (biparentally inherited) or Y-chromosome DNA (male-inherited) data (e.g. Burg *et al*. 1999; Chappell *et al*. 2004). Therefore, in species with sex-biased movement patterns, a greater understanding of spatial structure will be achieved by using data from both female-inherited and male- or biparentally inherited markers.

California sea lions (*Zalophus californianus*) are polygynous, sexually dimorphic pinnipeds that breed on land (Peterson & Bartholomew 1967). They are distributed along the Pacific coast of North America from British Columbia to the Baja California Peninsula and into the Gulf of California, although their breeding range is restricted to areas south of the Channel Islands, California (Peterson & Bartholomew 1967; Carretta *et al*. 2007). Based on mitochondrial DNA (mtDNA) genetic differences (Maldonado *et al*. 1995), the species has been subdivided into three management stocks: the US stock, the Western Baja California stock (Baja), and the Gulf of California stock (Gulf). However, it is currently unclear if this stock classification reflects true population subdivision or only matrilineal structure. Understanding spatial structure is essential for effective conservation in these populations. While the overall California sea lion population appears to be increasing (Carretta *et al*. 2007), a recent study found that total abundance in the Gulf colonies had declined > 20% in the last decade (Szteren *et al*. 2006). However, this decline has not been uniform across all colonies; instead, some areas show an increasing population while others exhibit declines (Gonzalez-Suarez *et al*. 2006; Szteren *et al*. 2006). This variability in trends complicates the overall assessment of the population status in the Gulf as varying rates could be due to migration or reflect true disparity in population growth rates. Moreover, a recent attempt to predict extinction risk for the entire Gulf sea lion population found that assumptions about spatial structure in the area influence predictions (Gonzalez-Suarez *et al*. 2006). Therefore, an accurate viability assessment of the California sea lion population in the Gulf will require a better understanding of spatial dynamics. In addition, current management strategies are based on the overall protection of the species by the Mexican government (listed as under 'Protección Especial', NOM-059-ECOL-1994) but with limited spatial considerations. An understanding of sea lion spatial structure in Mexican waters will provide a valuable tool for management of this species by providing information needed to focus efforts on areas where isolation may be highest and population declines greatest.

In this study, we used a 355-bp sequence of the mtDNA control region and the allele frequencies of 10 polymorphic nuclear microsatellite loci to explore population structure and subdivision among six Mexican colonies of the California sea lion. Based on these results, we identified sex-biases in movement patterns and defined appropriate management units for the Mexican population of California sea lions. This is the first study of population structure for this species that considers nuclear DNA, and the first to consider large sample sizes from multiple Mexican colonies. Our results provide a better understanding of the spatial dynamics in this species and also have significant implications for the conservation of the California sea lion population in Mexico. In addition, we present a novel approach to estimate sex-bias in gene flow, which may be applied to other species and populations.

Materials and methods

Sample collection and DNA extraction

We collected tissue samples from California sea lion pups (young of the year) at six breeding colonies: five selected among the 13 known breeding colonies in the Gulf stock (Szteren *et al*. 2006) and one from the six known colonies in the Baja stock (Carretta *et al*. 2007) (Fig. 1). Colonies represent the latitudinal range of the study area and have diverse characteristics, such as varying distances to the nearest colony (i.e., range in degree of geographical isolation), population abundance and trend (Zavala-Gonzalez 1990; Szteren 2006).

During the months of June and July 2004 (the Gulf colonies) and July 2007 (the Baja colony), we obtained tissue samples from at least 50 individuals at each colony. Individuals were sampled at 2–5 spatially distinct areas within each colony (i.e. coves), which were generally distributed along the perimeter of the island. In total, we sampled 18 locations. Tissue was collected from restrained pups by removing a small biopsy sample (< 5 mm) from the end of a posterior digit using a sterilized surgical scalpel. After

Table 1 Summary of 10 microsatellite loci amplified in 355 California sea lions including polymorphism, mean (± SD) observed (H_O) and expected (H_E) heterozygosity estimates, and F_{IS}, F_{ST}, and F_{IT} values calculated following Weir & Cockerham (1984)

Locus	GenBank Accession no.	Alleles	H_O	H_E	F_{IS}	F_{ST}	F_{IT}
SGPv9*	G02091	9	0.485 ± 0.120	0.518 ± 0.078	0.056	0.013	0.069
SGPv11†	U65444	6	0.568 ± 0.100	0.575 ± 0.087	0.012	0.035	0.047
Pvc29‡	L40987	20	0.868 ± 0.049	0.852 ± 0.029	–0.017	0.023	0.007
OrrFCB24§	G34932	14	0.834 ± 0.054	0.839 ± 0.021	0.004	0.021	0.025
ZcCgDh1.8¶	AY676475	7	0.702 ± 0.103	0.701 ± 0.045	–0.003	0.014	0.011
ZcCgDh3.6¶	AY676476	7	0.647 ± 0.114	0.632 ± 0.091	–0.025	0.021	–0.004
ZcCgDh4.7¶	AY676478	4	0.494 ± 0.070	0.488 ± 0.064	–0.013	0.032	0.019
ZcCgDh5.16¶	AY676477	11	0.711 ± 0.140	0.730 ± 0.086	0.031	0.038	0.068
ZcCgDh5.8¶	AY676474	13	0.834 ± 0.054	0.833 ± 0.028	–0.002	0.026	0.024
ZcCgDh48¶	AY676467	6	0.604 ± 0.055	0.607 ± 0.036	0.010	0.009	0.018

*Allen *et al.* (1995).
†Goodman (1997).
‡Coltman *et al.* (1996).
§Buchanan *et al.* (1998).
¶Hernandez-Velazquez *et al.* (2005).

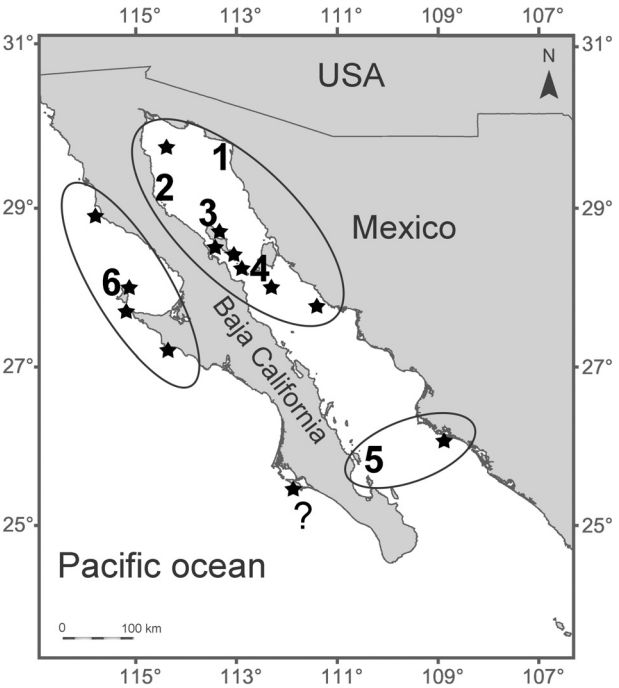

Fig. 1 Location of the six sampled California sea lion colonies in the Gulf of California and the Pacific coast of Baja California, Mexico. Numbers identify sampled localities (1. San Jorge; 2. Los Lobos; 3. Granito; 4. San Esteban; 5. Los Islotes; and 6. Benitos); stars indicate all other sea lion breeding colonies found in the study area but not sampled for this study. Lines indicate the boundaries of proposed management units.

collection, samples were preserved in 2-mL tubes filled with 95% ethanol and placed in a freezer at –20 °C until analysed. DNA was extracted from 50–63 samples per island (San Jorge, $N = 63$; Los Lobos, $N = 59$; Granito, $N = 63$; San Esteban, $N = 60$; Los Islotes, $N = 60$; Benitos, $N = 50$; Total $N = 355$, Fig. 1) using the Puregene DNA purification kit (Gentra).

DNA amplification

We considered 10 polymorphic microsatellite loci (Table 1) which had been previously amplified in California sea lions (Acevedo-Whitehouse *et al.* 2003, 2006; Hernandez-Velazquez *et al.* 2005). A review of the literature suggests that 10 polymorphic markers is an adequate sample to determine population structure in pinnipeds (e.g. Hoelzel *et al.* 2001; Hoffman *et al.* 2006). This range also yielded strong results in a study that tested the accuracy of genetic subdivision estimates using a data set with previously known population structure (Rosenberg *et al.* 2001). Microsatellite length polymorphisms for these loci were detected by polymerase chain reaction (PCR). Negative controls were included in each manipulation. All loci were amplified using an Eppendorf Mastercycler gradient 5331 under four different protocols (see Table S1, Supporting Information).

Genotypes for each locus were detected using an ABI 377 or ABI 3730 DNA sequencer (Applied Biosystems, Inc.), and were visualized and scored using GeneScan 3.1.2 and GeneMapper 4.0. Microsatellite genotyping errors might greatly bias results (Hoffman & Amos 2005), and thus, the rate of these errors should always be assessed.

All data were cross-read and double-checked in order to eliminate errors that may have occurred during data entry. In addition, we re-genotyped a minimum of 66 (> 18.6%) randomly selected samples at each locus. Allelic mismatches were counted by comparing the retyped genotypes to the previous ones, and reported as error rates by allele and by reaction (Hoffman & Amos 2005). Mistyped samples were re-genotyped at least three times to establish the correct genotype.

We amplified a ~360 bp section of the mtDNA control region using primers described by Schramm-Urrutia (2002). The primers were located between the $tRNA^{Pro}$ and $tRNA^{Thr}$ (Tro: 5′-CCTCCCTAAGACTCAAGG-3′) and within the conserved region of the D-loop (Dxx: 5′-CCTGAAGTAA-GAAACCAGATG-3′). We used the QIAGEN Multiplex PCR Kit and combined 1 μL DNA template, 5 μL QIAGEN Multiplex PCR Master Mix, 12 μL of 2 μM primer, and 1 μL sterile water. PCR conditions were: 15 min of denaturation at 95 °C, followed by 36 cycles of 30 s at 94 °C, 90 s at 50 °C, and 60 s at 72 °C. Final extension was 30 min at 60 °C. PCR clean-up was performed by adding 1 μL ExoSAP-IT (USB) to 2.5 μL of the PCR product and incubating for 15 min at 37 °C followed by 15 min at 80 °C. Sequencing reactions were executed using the standard protocol for Big Dye version 3.1 (Applied Biosystems, Inc.) and purified using solid phase reversible immobilization (SPRI) technology following the manufacturer's recommended protocol (Agencourt Corp.). Sequences were resolved on an ABI 3730 DNA sequencer (Applied Biosystems, Inc.) and are deposited in GenBank with Accession nos FJ026895–FJ026960.

Microsatellite data analysis

We used ARLEQUIN 3.11 (Excoffier *et al*. 2005) and FSTAT 2.9.3.2 (Goudet 1995; Goudet *et al*. 1996) to test whether loci were in Hardy–Weinberg equilibrium (HWE) and linkage equilibrium, as well as to calculate expected heterozygosities and the estimators of Wright's (1931) *F*-statistics (as defined by Weir & Cockerham 1984) at each locus.

We made an initial comparison of genetic structure among the six islands using F_{ST}, the standardized metric G'_{ST} (Hedrick 2005b), and a locus-by-locus analysis of molecular variance, AMOVA, with 10 000 data permutations (Excoffier 2003). For F_{ST}, we report the 95% bootstrapped CI provided by FSTAT. In addition, we explored genetic subdivision using two approaches that do not require defining putative populations a priori. We compared results from the programs STRUCTURE 2.1 (Pritchard *et al*. 2000), and BAPS 5.1 (Corander *et al*. 2003, 2006). STRUCTURE is a model-based clustering approach that constructs genetic clusters from a collection of individual multilocus genotypes, estimating for each individual the fraction of its genome that belongs to each cluster. We used a burn-in period of 20 000 and 75 000 Markov chain Monte Carlo (MCMC) steps for different values of *K* ranging from 1 to 8. Using longer MCMC runs did not modify the results. We assumed correlated allele frequencies among putative subpopulations and allowed population mixture (default options). We repeated the runs 20 times in order to check the stability of the results. Because clustering algorithms incorporate stochastic simulation, independent analysis of the same data may result in different outcomes, even if the same initial conditions are used. We used the program CLUMPP (Jakobsson & Rosenberg 2007) to align these multiple outcomes and determine the optimal clustering, which was graphically displayed by DISTRUCT (Rosenberg 2004). Inference on population structure was made by comparing the posterior probabilities of each *K* [Pr(*K*)], the values of the log-likelihood [Ln(*X* | *K*)], the proportion of the sample assigned to each population, and the values of Δ*K* as suggested by Evanno *et al*. (2005).

BAPS also detects clusters of genetically similar populations but uses a stochastic optimization algorithm for analysing Bayesian models of population structure which greatly improves the speed of the analysis compared to STRUCTURE (Latch *et al*. 2006). We used the option 'Clustering of groups of individuals' with a predefined maximum number of $K = 6$. We repeated the run 10 times in order to check the stability of the results. The number of detected groups was inferred from the optimal number of clusters and the probability associated with each cluster size estimated by BAPS.

We used the test of Mantel (1967) to search for correlation between genetic and geographical distance using ARLEQUIN 3.11. Significance value was determined based on 100 000 permutations. Genetic distance was defined as $F_{ST}/(1 - F_{ST})$ (Rousset 1997). Geographical distance was calculated as shortest sea-lion-travel distance between colonies in kilometres. Sea-lion-travel distance was defined as the shortest path between two sites, considering sea lions must travel by water.

mtDNA data analysis

Alignments were optimized manually using BioEdit 7.0.9.0 (Hall 1999). A single continuous gap was introduced in some cases to maximize sequence similarity. We used ARLEQUIN to estimate the number of haplotypes (*n*), haplotype diversity (*h*), and nucleotide diversity (π) incorporating Jukes & Cantor's (1969) model of sequence evolution as recommended by Nei & Kumar (2000). We used Tajima's (1989) *D* and Fu's (1997) F_S tests to detect departures from neutrality. Population subdivision and structure were estimated using an analysis of molecular variance (AMOVA; Excoffier 2003), and population pairwise Φ_{ST} estimates implemented in ARLEQUIN. Significance of Φ_{ST} values was determined via 10 000 data permutations. For AMOVA tests,

Table 2 F_{ST} and Φ_{ST} estimates for pairwise comparisons among six California sea lion colonies. F_{ST} values for microsatellite loci are given below the diagonal line, and Φ_{ST} estimates for mtDNA above the diagonal line. Φ_{ST} was calculated using Jukes & Cantor (1969) distance estimates

Population	San Jorge	Los Lobos	Granito	San Esteban	Los Islotes	Benitos
San Jorge	—	0.000	0.003	0.008	0.171*	0.140*
Los Lobos	0.017*	—	0.000	0.009	0.212*	0.181*
Granito	0.014*	0.004	—	–0.003	0.141*	0.173*
San Esteban	0.012*	0.013*	0.010*	—	0.157*	0.168*
Los Islotes	0.033*	0.032*	0.019*	0.018*	—	0.285*
Benitos	0.043*	0.050*	0.037*	0.029*	0.030*	—

*significant at nominal $P < 0.05$ after B-Y FDR correction for multiple comparisons (Benjamini & Hochberg 1995; Narum 2006).

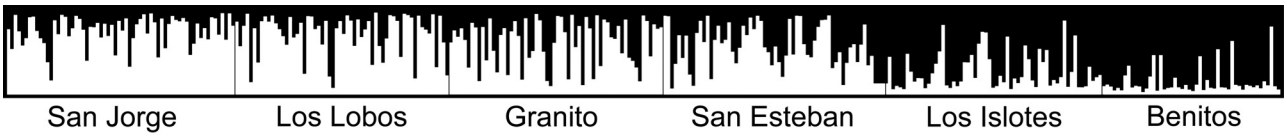

Fig. 2 Optimal membership coefficients for data on 355 California sea lions sampled from six breeding colonies (Fig. 1) and assigned to two clusters (identified by the white and black colours). The optimal clustering was generated by CLUMPP and DISTRUCT based on data from 20 runs completed in Structure with $K = 2$.

we also used Jukes & Cantor's (1969) model of sequence evolution. As with the microsatellite data, we used the test of Mantel (1967) to search for correlation between genetic $[\Phi_{ST}/(1 - \Phi_{ST})]$ and geographical distance using ARLEQUIN (significance value was determined based on 100 000 permutations).

A statistical parsimony network was constructed using TCS 1.21 (Clement *et al*. 2000) which identifies unrooted cladograms that have a high probability (> 95%) of being true based on a finite-site model of DNA evolution (Templeton *et al*. 1992). To resolve loops (ambiguities), we applied the criteria identified by Crandall & Templeton (1993): rare haplotypes occur preferentially at the tips of the cladogram, and singletons are more likely to be connected to haplotypes from the same population.

Results

Microsatellite data analysis

All samples amplified successfully at all loci, except for one sample at locus ZcCgDh1.8. We observed low genotyping error rates of 0.003 per allele and 0.009 per reaction based on the re-genotyped data. Studied loci were highly polymorphic, ranging from 4 (ZcCgDh4.7) to 20 (Pvc29) observed alleles with high observed heterozygosity (Table 1). All loci were in concordance with HWE and there was no evidence of linkage disequilibrium (at nominal level of $P < 0.05$ after Bonferroni correction for multiple tests). Estimates of F_{IS}, F_{ST}, and F_{IT} for each locus are provided in Table 1.

Observed mean (± standard deviation, SD) heterozygosity was generally high in all six sea lion colonies (San Jorge = 0.687 ± 0.173; Los Lobos = 0.675 ± 0.170; Granito = 0.683 ± 0.119; San Esteban = 0.698 ± 0.160; Los Islotes = 0.616 ± 0.166; Benitos = 0.688 ± 0.182). Pairwise F_{ST} values among the colonies were low, but significantly different from zero after B-Y FDR correction for multiple tests (Benjamini & Hochberg 1995; Narum 2006) in all but one case (Table 2). The overall F_{ST} value was 0.023 (95% bootstrapped CI = 0.018–0.029), while the standardized G'_{ST} was 0.104.

Using STRUCTURE, we identified two groups ($G1_{STR}$: San Jorge, Los Lobos, Granito, and San Esteban, and $G2_{STR}$: Los Islotes and Benitos) based on $\Pr(K = 2) = 1$ and the largest mean value of log-likelihood observed for $K = 2$ ($\mathrm{Ln}(X \mid K) = -10761$). Evanno *et al*. (2005) approach also indicated two groups with $\Delta K(2)$ being much larger than all other ΔK-values. There was strong evidence of admixture between the two groups based on the assignment of individuals, possibly suggesting a pattern of isolation by distance (Fig. 2). The analysis from CLUMPP indicates a very high agreement among the 20 STRUCTURE runs for $K = 2$ ($H' = 0.989$). Results from BAPS also revealed an optimal number of $K = 2$ ($\Pr(K = 2) = 1$). However, the groups formed by BAPS were different from those identified by STRUCTURE ($G1_{BAPS}$: San Jorge, Los Lobos, Granito, San Esteban, and Los Islotes; and $G2_{BAPS}$: Benitos). We ran independent AMOVA tests for the optimal clusters identified by STRUCTURE and BAPS (Table S3, Supporting Information). Among-group differences were small but significant under both clustering

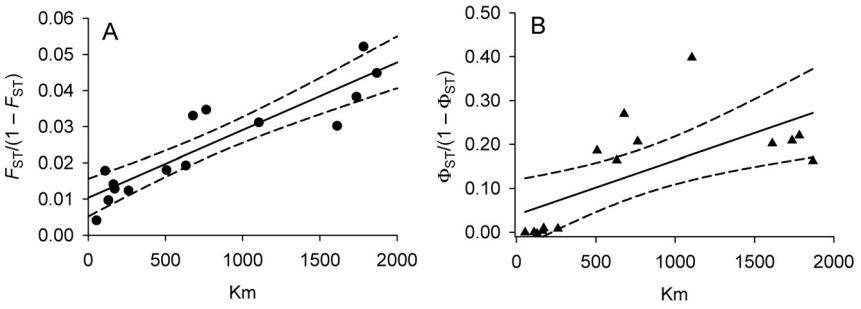

Fig. 3 Pairwise genetic distances plotted against geographical distance (km) between six California sea lion breeding colonies. The linear regression slopes (solid lines) and their 95% CI (broken lines) are given. Panel A represents nuclear $[F_{ST}/(1-F_{ST})]$ genetic distance, while panel B represents mtDNA $[\Phi_{ST}/(1-\Phi_{ST})]$ genetic distance. Note the difference on the *y*-axis scales.

Table 3 Number of individuals (*N*) and haplotypes (*n*), and haplotype (*h*) and nucleotide (π) diversity mean (± SD) estimates from six California sea lion colonies

Island	*N*	*n*	*h*	π
San Jorge	63	16	0.915 ± 0.015	0.012 ± 0.007
Los Lobos	59	21	0.926 ± 0.017	0.011 ± 0.006
Granito	63	16	0.916 ± 0.015	0.012 ± 0.006
San Esteban	60	24	0.894 ± 0.031	0.011 ± 0.006
Los Islotes	60	11	0.841 ± 0.031	0.005 ± 0.003
Benitos	50	8	0.755 ± 0.032	0.010 ± 0.006
Total	355	46	0.937 ± 0.005	0.014 ± 0.008

approaches (STRUCTURE: 1.43%, $P = 0.0002$, BAPS: 2.13%, $P = 0.0003$), variance among populations within groups was low but significant in both cases (STRUCTURE: 1.57%, $P < 0.0001$, BAPS: 1.67%, $P < 0.0001$), and variance within populations was large and highly significant in both cases (STRUCTURE: 96.99%, $P < 0.0001$, BAPS: 96.20%, $P < 0.0001$).

Genetic distance $F_{ST}/(1 - F_{ST})$ had a strong positive correlation to geographical distance (Fig. 3A). A Mantel test found this relationship to be highly significant ($r = 0.902$, $P = 0.001$).

mtDNA data analysis

The amplified segment of the mtDNA control region from 355 individuals ranged from 355 to 364 bp. The variation in size was due to an indel section composed of a repeated sequence, which varied in length among individuals. Gaps were initially inserted to maximize sequence similarity but for the analysis, we deleted the entire 9-bp section that contained the gap.

We found 46 haplotypes representing single site transitions, 25 were unique to one colony, whereas two were found in all colonies (Table S2, Supporting Information). The maximum number of individuals sharing a haplotype was 51, but we identify as many as 13 singletons (Table S2). Haplotype diversity ($h = 0.755$ to 0.926) and nucleotide diversity ($\pi = 0.005$ to 0.012) were relatively high in all sea lion colonies (Table 3). We found no evidence of departure from neutrality using Tajima's *D* test ($D = -0.044$–1.819, $P > 0.54$). However, Fu's F_S statistic, which is considered a more powerful test to detect population expansion (Ramos-Onsins & Rozas 2002), was significantly negative at two colonies: Los Lobos ($F_S = -7.3$, $P = 0.010$) and San Esteban ($F_S = -11.083$, $P = 0.001$; other colonies $F_S = -3.073$–1.901, $P > 0.077$). Recent analyses of > 20 years of abundance estimates from these two colonies also suggest a trend of demographic expansion, particularly in San Esteban (Gonzalez-Suarez *et al*. 2006; Szteren *et al*. 2006).

The statistical parsimony network analysis suggested weak phylogeographical clustering of haplotypes (Fig. 4). However, there was statistically significant structure among colonies based on Φ_{ST} values (overall $\Phi_{ST} = 0.163$, $P < 0.0001$; Table S3). In particular, pairwise Φ_{ST} values revealed two colonies (Los Islotes and Benitos) were significantly different from all others, while the remaining four colonies were grouped together (Table 2). Based on these results, we considered three groups for the AMOVA test ($G1_{mt}$: San Jorge, Los Lobos, Granito, and San Esteban, $G2_{mt}$: Los Islotes, and $G3_{mt}$: Benitos). Population variance among groups was relatively large but only marginally significant (15.91%, $P = 0.0642$), variance among populations within groups was small and not significant (0.43%, $P = 0.2758$), and variance within populations was highly significant (83.66%, $P < 0.0001$, Table S3).

mtDNA genetic distance $\Phi_{ST}/(1 - \Phi_{ST})$ had a significant positive correlation (Mantel test $r = 0.672$, $P = 0.048$; Fig. 3B) to geographical distance, although the linear relationship was not as clear as that observed for the microsatellite data (Fig. 3A). In particular, there seems to be little change in genetic distance among colonies located farther than ~500 km away. Moreover, genetic distances were higher than expected from each colony to Los Islotes, and lower than expected from all colonies (except Los Islotes) to Benitos.

Discussion

We used 10 polymorphic microsatellite loci and 355 bp of the mtDNA control region to explore population subdivision among Mexican sea lion colonies. Overall, we found high genetic diversity in all studied colonies for both nuclear and mitochondrial DNA (Tables 1 and 4). In particular, we

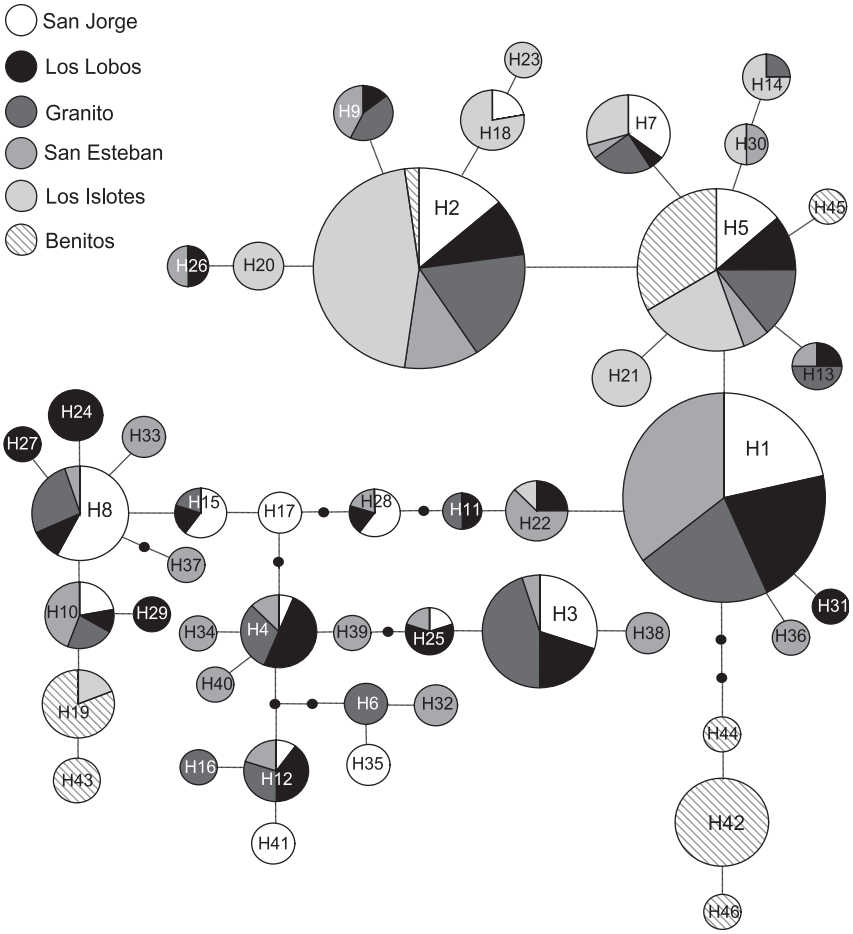

Fig. 4 Statistical parsimony network of haplotypes observed in California sea lion from six breeding colonies. All haplotypes are separated by one mutation with solid black circles representing hypothetical haplotypes not observed in this study. The size of the circle is proportional to the frequency of the haplotypes. Each colour or pattern represents a sampled colony as indicated on the legend.

Table 4 The expected level of differentiation for paternally inherited genes ($F_{ST(m)}$) and the ratio of male to female gene flow (m_m/m_f) observed among six California sea lion colonies

	Colonies	San Jorge	Los Lobos	Granito	San Esteban	Los Islotes
$F_{ST(m)}$	Los Islotes	0.036	0.034	0.021	0.019	
	Benitos	0.052	0.057	0.041	0.032	0.030
m_m/m_f	Los Islotes	5.47	7.70	7.80	9.52	
	Benitos	2.94	3.63	4.86	6.18	12.66

detected up to 20 microsatellite alleles per locus and 46 mtDNA haplotypes in our sample. This variability was similar to that reported in previous studies (Hernandez-Velazquez *et al*. 2005; Acevedo-Whitehouse *et al*. 2006), although we found a greater diversity of haplotypes than Maldonado *et al*. (1995) and Schramm-Urrutia (2002). Unlike Maldonado *et al*. (1995), we identified several haplotypes shared between the two currently defined Mexican stocks, possibly because of our larger sample size and more diverse geographical sampling.

Our results suggest that the main factors determining population structure in California sea lions are isolation by distance and the geographical distribution of the breeding colonies. We found a pattern of isolation by distance in both microsatellite and mtDNA data (Fig. 3). Mantel tests revealed a positive correlation between genetic and geographical distance, this correlation was particularly strong for microsatellite loci. For mtDNA data, we actually observed only small changes in genetic distance with increased geographical distance among colonies farther than 500 km from each other. This suggests female dispersal may be more limited and rarely occurs among distant colonies, whereas males apparently disperse over greater distances.

The geographical distribution of the breeding colonies in the studied area also influenced patterns of population subdivision and revealed geographically defined clusters (i.e. clusters that minimize distance between colonies within a cluster while maximizing distance among clusters).

In particular, Φ_{ST} values indicated substantial genetic differences among three groups: upper Gulf colonies, Benitos Island, and Los Islotes Island (Table 2). AMOVA test revealed percentage of variation among groups represented nearly 16% of the total variation although this value was only marginally significant (Table S3). Similarly, STRUCTURE and BAPS analyses of microsatellite data also recognized the upper Gulf and Benitos Island as distinct, and AMOVA tests suggested variance among these groups was small but significant (Table S3). Interestingly, STRUCTURE and BAPS identified different optimal solutions for the clustering of Los Islotes Island. This colony was not classified as a separated group by either program and instead was clustered with Benitos Island in one approach (STRUCTURE) and with the upper Gulf colonies in the other (BAPS). The fact that each approach linked this colony to a different cluster possibly suggests Los Islotes Island may in fact have an intermediate position between both groups, which is consistent with the mtDNA results. This was further supported by an additional analysis of our microsatellite data in BAPS using the 'Fixed *K*' option with $K = 3$. This analysis grouped the six colonies into the three identified groups: upper Gulf colonies, Benitos Island, and Los Islotes Island, and had only a slightly worse fit [log(mL) = –11026.68] than the optimal $K = 2$ [log(mL) = –11012.77] supporting the separation of the colonies into three clusters.

One potential caveat with our study is the fact that we considered genetic structure based on samples from newborn individuals. California sea lions are generally assumed to have a polygynous mating system, but it is currently unclear what proportion of adult males actually achieves paternity in a colony. If mating is dominated by very few males within a colony (i.e., extreme polygyny), pups in a cohort could be related to each other more than the average population. In this case, it could be possible that different patterns of genetic structure would be found if samples from adult individuals were analysed. We collected samples from pups for practical and ethical reasons. First, pups were already being captured for a separate study (L.R. Gerber, unpublished), and thus, the collection of these samples did not represent an additional disturbance to breeding colonies. Second, pups are easiest to capture which facilitated obtaining a relatively large sample size per island. Finally, young pups very rarely travel between islands (Young *et al*. 2007), and hence, by using samples from pups, we had a very high certainty that individuals were sampled at their natal colony. To explore the possibility that different patterns of genetic structure could have been found if samples from adult individuals had been analysed, we considered data from 196 samples obtained from adults (96 males and 100 females) at San Jorge Island (Fig. 1; R. Flatz, M. González-Suárez, J. Young, P.W. Hedrick & L.R. Gerber, unpublished). We compared allele frequencies from our San Jorge pup sample with the adult samples using a chi-square test and Fisher's exact test. We found no significant differences in allele frequencies between pups and adults for these islands ($\chi^2 = 26.75$, d.f. = 20, $P = 0.14$; Fisher's exact test $P = 0.29$), suggesting pup samples adequately represent the population in this colony. Because we have no reason to expect different patterns in other colonies, pup samples from the remaining six colonies should also be a good representation of allele frequencies at each site. Therefore, we think our results are unlikely to be affected by our sampling design.

Sex-biased movement

Overall, we found population structure among California sea lion colonies in Mexico is determined by isolation by distance with greater genetic subdivision among females (mtDNA) than in the entire population (microsatellite data). A general estimate of the relative amount of male and female gene flow can be obtained in the following way assuming the population is at, or near, migration–drift equilibrium. First, assume that the amount of differentiation for nuclear genes (microsatellite loci here) is

$$F_{ST} = \frac{F_{ST(f)}F_{ST(m)}}{F_{ST(f)} + F_{ST(m)} - 3F_{ST(f)}F_{ST(m)}}, \qquad \text{(eqn 1a)}$$

where $F_{ST(f)}$ and $F_{ST(m)}$ are the amounts of female and male differentiation for maternally and paternally inherited genes (Ennos 1994). This equation can be solved for the amount of male differentiation as

$$F_{ST(m)} = \frac{F_{ST}F_{ST(f)}}{F_{ST(f)} - F_{ST} + 3F_{ST}F_{ST(f)}}. \qquad \text{(eqn 1b)}$$

Second, the expected number of female migrants at equilibrium under the island model for maternally inherited mtDNA is

$$Nm_f = \frac{1 - F_{ST(f)}}{2F_{ST(f)}} \qquad \text{(eqn 2a)}$$

and the expected number of male migrants for paternally inherited Y chromosomes is

$$Nm_m = \frac{1 - F_{ST(m)}}{2F_{ST(m)}}, \qquad \text{(eqn 2b)}$$

where m_m and m_f are the amounts of male and female gene flow, respectively. From the ratio of these equations, the ratio of male and female gene flow rates is

$$\frac{m_m}{m_f} = \frac{F_{ST(f)}(1 - F_{ST(m)})}{F_{ST(m)}(1 - F_{ST(f)})}. \qquad \text{(eqn 2c)}$$

Table 4 gives the expected level of divergence among paternally inherited genes $F_{ST(m)}$ using equation 1b, given the estimated overall nuclear and maternal divergence, and the ratio of male to female gene flow using equation 2c

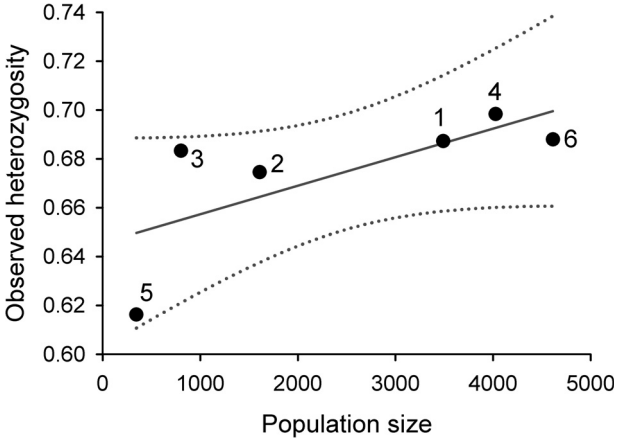

Fig. 5 Relationship between observed heterozygosity from 10 nuclear microsatellite loci and estimated population size for six California sea lion colonies. Population size was estimated as an average of yearly censuses completed from 2004 to 2007 for the Gulf colonies and as the 2008 census estimate for Benitos (see Wielgus *et al*. 2008 for details on census methodology). The fitted curve (continuous line) and its 95% confidence intervals (dotted line) are shown. Adjusted $R^2 = 0.501$. The colonies are: 1. San Jorge; 2. Los Lobos; 3. Granito; 4. San Esteban; 5. Los Islotes; and 6. Benitos.

for the populations with higher levels of divergence. $F_{ST(m)}$ is only slightly larger than the overall estimated F_{ST} for all the pairs of populations (Table 2), indicating the relatively high rates of male gene flow. As a result, the ratio of male to female gene flow is much greater than unity for all the comparisons. The average male to female gene flow ratio for the four comparisons of Los Islotes and the four upper Gulf populations was 7.62, for the four comparisons of Benitos and the four upper Gulf populations was 4.40, and for Los Islotes–Benitos comparison was 12.66. Overall, the male to female gene flow ratio for these nine comparisons was 6.75.

Stronger genetic differentiation based on analyses of mtDNA is a common pattern in mammalian species (e.g. Chappell *et al*. 2004; Hoffman *et al*. 2006) and reflects sex biases in dispersal (Lawson Handley & Perrin 2007). In our case, this pattern suggests male sea lions disperse much more often, nearly seven times as much between groups, and likely over larger distances, than females. In general, females are likely to remain within their natal cluster, although they may occasionally move between clusters (Young *et al*. 2007), whereas males appear to disperse more often between groups. This pattern of male-biased dispersal has also been described in other pinniped species (e.g. Burg *et al*. 1999; Hoelzel *et al*. 2001; Hoffman *et al*. 2006).

Implications for conservation

Although all sampled sea lions colonies had generally high genetic variability, the average observed heterozygosity generally increased with population size (Fig. 5, $R^2 = 0.501$). Population size was estimated as an average of yearly censuses completed from 2004 to 2007 for the Gulf colonies and as the 2008 census estimate for Benitos (see Wielgus *et al*. 2008 for details on census methodology). Reduced genetic variability in smaller colonies could affect demographic processes (Lande 1988). However, we found no clear positive relationship between observed heterozygosity and population growth rate (λ) for the five studied colonies for which an estimate of λ is available (Gonzalez-Suarez *et al*. 2006). In fact, the lowest genetic variability was found in Los Islotes Island, which has the highest λ (= 1.060) in the area (Gonzalez-Suarez *et al*. 2006). Therefore, although smaller populations are likely to have reduced genetic variability, at least within the range of genetic variability observed here, there appears to be no negative demographic effects of lower diversity. In fact, genetic variability may be only weakly affected by population size as a result of a storage effect of genotypes which helps maintain genetic variability even at smaller populations sizes (Gaggiotti & Vetter 1999). This effect occurs in species with long and overlapping generations, such as sea lions which have an approximate generation time of 12 years and a lifespan of > 20 years (Hernandez-Camacho *et al*. 2008).

Previous studies (Maldonado *et al*. 1995; Bowen *et al*. 2006) described strong genetic differences between colonies in the Pacific coast of Baja California and colonies in the Gulf of California which were used to define two distinct management stocks in Mexico (Carretta *et al*. 2007). However, these studies only sampled sea lion colonies located in the central region of the Gulf. We sampled colonies distributed throughout the entire Gulf, and our results offer little support to the current stock definitions. Instead, analysis of microsatellite data revealed a strong pattern of isolation by distance with relatively high admixture among all colonies, whereas analysis of mtDNA indicated a pattern of isolation by distance with three fairly distinct regions. Based on these results, we propose an alternative stock classification consisting of three management units: upper Gulf of California, southern Baja Peninsula, and upper Pacific Coast of Baja (Fig. 1). These units not only represent mostly matrilineal structure but also aggregate nearby colonies within which greater migration of both females and males is expected. Therefore, these units are not strictly genetically isolated areas or genetic stocks, but instead are intended to provide a conservative clustering for management purposes which reflects overall spatial dynamics. We suggest that these units should also be considered in a reassessment of California sea lion population status in Mexican waters.

Finally, we recommend further sampling from intermediate colonies not considered in this study to accurately determine their classification. In particular, we could not readily group the southernmost colony on the Pacific coast

of Baja (identified with a question mark in Fig. 1) because of its intermediate position between two clusters. This colony is nearly equidistant to the nearest colony on each of the neighbouring clusters, and thus based on the observed pattern of isolation by distance, we could not clearly associate it with a given stock. Future research should explore whether this southernmost colony belongs to the upper Pacific Coast of Baja stock, the Southern Baja Peninsula stock, or a new still- undefined stock. Future studies should also consider sea lion colonies from the USA to explore the complete distribution of the species and gain a comprehensive understanding of population structure in the California sea lion.

Acknowledgements

The 2004 Lobos crew and F. Elorriaga helped collect tissue samples. T. Dowling provided generous access to his laboratory, and offered excellent advice and suggestions. R. Chapman and K. Robertson provided assistance with laboratory procedures. S. Bingham at the ASU DNA laboratory provided valuable advice and assisted with laboratory procedures. J. Alcock, C.D. Anderson, and two anonymous reviewers provided helpful comments to improve earlier versions of this manuscript. J. Corander helped us implement the analysis on BAPS. This work was supported by the National Science Foundation (Grant no. 0347960 to LRG), the Graduate and Professional Student Association of Arizona State University (research grant to M.G.S.), and the 2007 Lisa Dent Memorial Fund (to M.G.S.). All procedures were approved by the Arizona State University Animal Care and Use Committee (07-918R). Data collection was authorized by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT, Oficio num/SGPA/DGVS/04160, and/02709). Samples were imported from Mexico under permits by SEMARNAT (no. 19198, and no. 23010) and the US National Marine Fisheries Service (no. 782-1694-02).

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This work is part of a collaborative research project on behaviour and demography of California sea lions in Mexico. Manuela González-Suárez is a PhD student interested in exploring the interface of behavioural ecology and biodiversity conservation. Ramona Flatz is a Master's student using genetic tools to address questions about paternity and population dynamics. Dr David Aurioles-Gamboa uses diverse approaches to explore how pinnipeds adapt to environmental changes. Dr Phil Hedrick's research focuses on conservation and population genetics. Dr Leah Gerber is broadly interested in applied population biology, particularly in marine systems.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Summary of conditions and PCR protocols for 10 microsatellite loci amplified in 355 samples from California sea lions. All reactions included 1 ng/μL of DNA

Table S2 Geographical distribution of 46 mtDNA haplotypes found in 355 California sea lions from six breeding colonies in Mexico

Table S3 Analysis of molecular variance in California sea lions for microsatellite and mtDNA data. Microsatellite data were explored using two different cluster scenarios identified by the programs BAPS and STRUCTURE

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