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ORIGINAL RESEARCH

Population genetic structure and historical population dynamics of the South American sea lion, *Otaria flavescens*, in north-central Patagonia

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Abstract The north-central Patagonian coast is the sea lions most abundant area in Argentina. As occurs along the entire Atlantic coast, the distribution of breeding colonies at this smaller geographical scale is also patchy, showing at least three areas with breeding activity. We study the genetic structure and historical population dynamics of the species in five colonies in this area, analysing a 508 base-pair segment of the D-loop control region. *Otaria flavescens* showed 10 haplotypes with 12 polymorphic sites. The genealogical relationship between haplotypes revealed a shallow pattern of phylogeographic structure. The analysis of molecular variance showed significant differences between colonies, however, pairwise comparisons only indicate significant differences between a pair of colonies belonging to different breeding areas. The pattern of haplotype differentiation and the mismatch distribution analysis suggest a possible bottleneck that would have occurred 64,000 years ago, followed by a demographic expansion of the three southernmost colonies. Thus, the historical population dynamics of *O. flavescens* in north-central Patagonia appears to be closely related with the dynamics of the Late Pleistocene glaciations.

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Introduction

Historical, environmental and anthropogenic factors, such as glaciations, topographic characteristics of the habitat, or harvesting, can affect the spatial structure of populations and act on the genetic variation patterns of a species. The knowledge of these patterns of geographical differentiation at the molecular level may reveal valuable information on the underlying evolutionary processes and past demographic events (Milá et al. 2000). Particularly, historical events and gene flow/genetic drift are the main evolutionary factors explaining the geographical distribution of neutral genetic diversity within species (Dutech et al. 2004). Under constant gene flow and demographic equilibrium, spatial genetic structure among populations can be predicted by several theoretical models (e.g. Wright 1931; Slatkin 1993). However, historical events, such as fragmentation or rapid expansion of a species distribution, can modify these genetic patterns. In some instances, genetic drift and reduction of gene flow during periods of contraction can create high genetic divergence between isolated populations (Latta and Mitton 1997). Furthermore, recolonization by a few founders during periods of expansion can produce population bottlenecks and loss of genetic diversity (Nei et al. 1975; Cornuet and Luikart 1996). These historical events can be detected by comparing genetic diversity among individuals and populations (Frankham et al. 2002; Avise 2004).

Quaternary climatic fluctuations have been widely recognized as one of the main historical process influencing

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the genetic diversity of natural populations both in the northern and southern hemispheres (e.g. Vuilleumier 1971; Frenzel 1973; Hewitt 1996, 2004; Siegel-Causey 1997). The last glacial maximum occurred in Patagonia around 56,000 years before present (Mercer 1976) and was characterized by an extensive decrease of the average temperatures that led to the formation of an ice sheet all over the Patagonia. The term 'glacial refugia' is used to describe the only suitable localities where temperate fauna and flora could have existed during full-glacial conditions. Based on geological and fossil evidence, coastal refugia existed in Malvinas Islands (Falkland), the Atlantic coast of northern Argentine Patagonia, and in regions north of Isla Chiloe, Chile (Vuilleumier 1971).

The north-central Patagonian coast is the South American sea lion, *Otaria flavescens* (Shaw 1800) most abundant area in Argentina, with approximately 40 breeding colonies and 80,000 individuals (Reyes et al. 1999; Dans et al. 2004; Schiavini et al. 2004). As occurs along the entire Atlantic coast (Fig. 1a; Túnez et al. 2008), the distribution of breeding colonies at this smaller geographical scale is also patchy, showing at least three areas with breeding activity: (1) northern Chubut, from Punta Bermeja to Punta León (43°40′S, 64°28′W), (2) central-southern Chubut, from Isla Escondida (43°43′S, 65°17′W) to Isla Quintano (45°14′S, 66°42′W), and (3) northern Santa Cruz, from Monte Loayza (47°05′S, 66°16′W) to Cerro Ordoñez (Fig. 1b).

In a previous study (Túnez et al. 2007), we analysed the genetic structure of the South America sea lion, in two (Uruguay and north-central Patagonia) of the three breeding areas of the species on the Atlantic coast (Fig. 1a). We compared 445 bp of the mitochondrial cytochrome *b* gene in 60 individuals from 6 colonies. We found 4 haplotypes with three polymorphic sites. Haplotype A was the most common ($n = 42$) and the unique present in the Uruguayan colonies. Results also showed a genetic differentiation between the Uruguayan and Patagonian colonies, which suggest that, in the Atlantic coast, sea lions show two conservation units that correspond with the two breeding areas. Additionally, we found preliminary results which suggest that the Patagonian population suffered a genetic bottleneck that would have occurred approximately 110,000 years ago.

The study of genetic variability and population structure, especially from the mtDNA coding regions, is limited by the availability of a relatively small number of polymorphisms in the sequences (Torroni et al. 1996; Wallace et al. 1999). Alternatively, sequences from the first hypervariable segment of the rapidly evolving noncoding control region have been extensively used in pinnipeds in the last decade for the study of these topics (Burg et al. 1999; Wynen et al. 2000; Hoelzel et al. 2001; Mizuno et al. 2003; Trujillo et al. 2004; Weber et al. 2004, Hoffman et al. 2006; Matthee et al. 2006; Túnez et al. 2007). However, reliance on hypervariable control-region sequences is not without controversy, particularly because of the effects that homoplasy has at sites with high mutation rates (e.g., see Howell et al. 1996).

The present study complements our previous study using cytochrome *b* gene. Here, we analyzed a more variable molecular marker (the mitochondrial D-loop region) in a smaller geographical area with the aim to study the phylogeography of the South America sea lion in north-central Patagonia. Specifically, these data were used to: (1) examine whether the Pleistocene glaciations helped shape the genetic structure currently found in sea lions populations, and (2) determine if, in north-central Patagonia, the different breeding areas previously described correspond to different conservation units as occurred at a higher geographical scale with the cytochrome *b* gene. We predict that populations that experienced genetic bottlenecks as a result of Pleistocene habitat contractions and a subsequent postglacial expansion over the present range should show: (1) a star-like phylogeny of haplotypes with few high-frequency ancestral haplotypes and numerous low-frequency alleles separated from the ancestral ones by a few mutational steps (Slatkin and Hudson 1991), (2) low levels of genetic subdivision among populations, and (3) a distribution of pairwise nucleotide differences among haplotypes indicating a sudden increase in effective population size (Rogers and Harpending 1992).

Materials and methods

Sample collection

Samples were collected from 49 South American sea lions in five colonies located along the coast of north-central Patagonia (Fig. 1b; Table 1). Samples were collected from dead animals found in beaches near the colonies in Puerto Pirámide (PP), Isla Vernacci Oeste (IVO) and Monte Loayza (ML), and from live animals in Punta Norte (PN) and Isla Arce (IA). In the case of dead animals, skin, muscle and liver samples were taken and stored in preservation buffer containing 20% DMSO, EDTA 0.25 N (pH = 8.0) saturated with sodium chloride. For live animals, blood and tissue samples were taken following the methodology described in Cappozzo et al. (1991) and stored at −20°C. Mitochondrial cytochrome *b* sequences from 24 of these 49 individuals were obtained in a previous work (Túnez et al. 2007) (GenBank accession numbers AY712975 to AY713034) (Table 1).

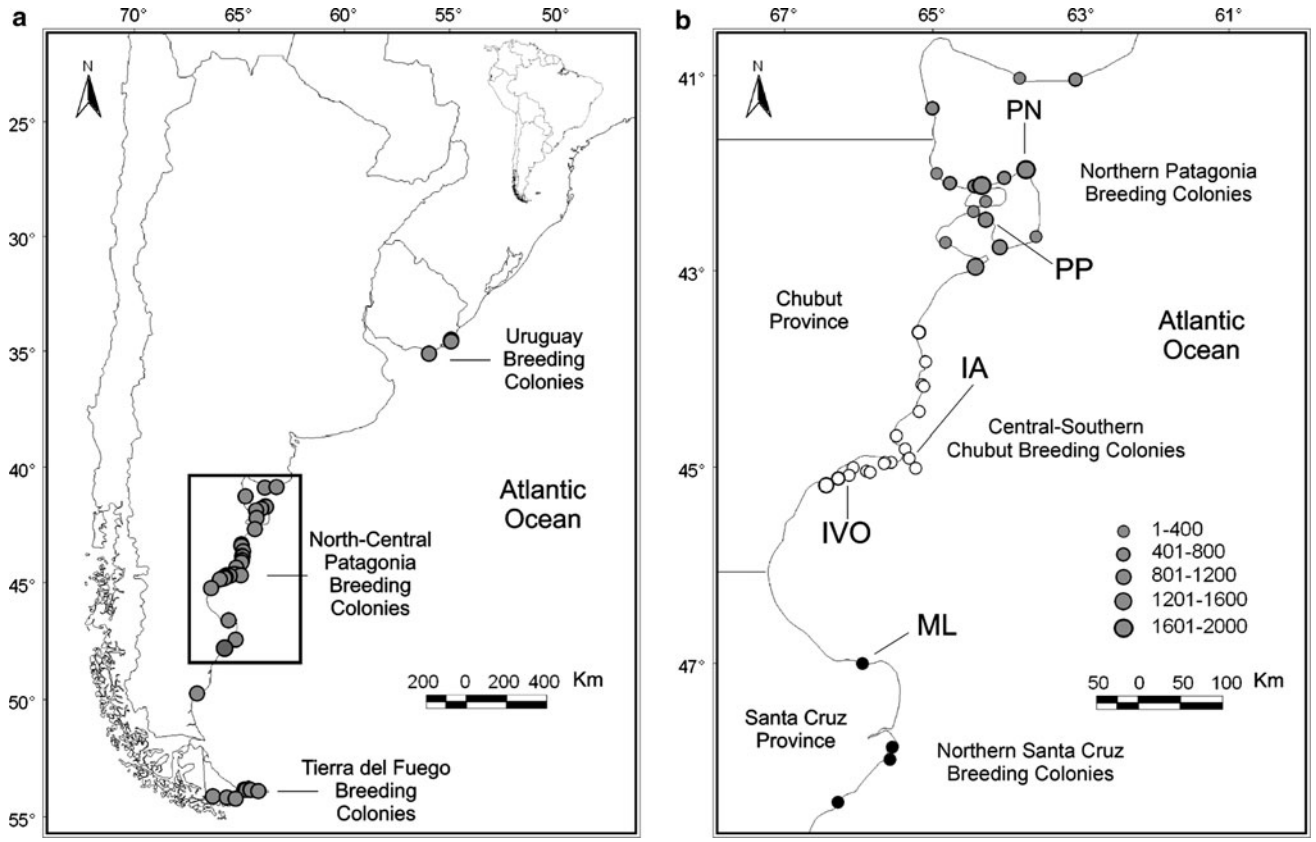

Fig. 1 Distribution of *O. flavescens* breeding colonies and location of colonies sampled. **a** Atlantic coast of Uruguay and Argentina, **b** north-central Patagonia breeding area. Colours of the *circles* correspond to the different breeding areas: *Grey*: Northern Patagonia, *White*: Central-Southern Chubut, *Black*: Northern Santa Cruz. Size of the *circles* is proportional to the number of individuals in each colony, grouped in five categories (1–400 to 1,601–2,000 individuals). Colonies names correspond to those in Table 1

Table 1 Origin of South American sea lions samples used in this study

Colony	Breeding area	Location	n^a	Date
Punta Norte (PN)	Northern Chubut	42°04′S, 63°45′O	12	Jan 1992
Puerto Pirámide (PP)	Northern Chubut	42°35′S, 64°17′O	7 (4)	Jan/may 2003
Isla Arce (IA)	Central-southern Chubut	45°00′S, 65°29′O	10 (10)	Feb 1998
Isla Vernacci Oeste (IVO)	Central-southern Chubut	45°10′S, 66°31′O	10	Oct 2003
Monte Loayza (ML)	Northern Santa Cruz	47°05′S, 66°16′O	10 (10)	Jan 2003

[a] Number of cytochrome *b* sequences previously obtained between brackets

Mitochondrial DNA extraction and PCR amplification

Depending on the sample source, different DNA extraction protocols were used. Tissue samples were incubated overnight at 37°C in extraction buffer containing 10 µl of proteinase K, 10 mg/ml; 5 µl of RNase, 20 mg/ml and 10% SDS. DNA was isolated from the sample by phenol–chloroform extraction and alcohol precipitation. For blood samples, white corpuscles were isolated by centrifugation and lysed by incubation in lysis buffer I (0.3 M sucrose; 1 mM TrisHCl, pH 7.5; 5 mM $MgCl_2$; 1% Triton X-100), followed by incubation in lysis buffer II (75 mM NaCl; 24 mM EDTA); SDS 2.5% and 10 µl of proteinase K, 10 mg/ml. DNA was precipitated in NaCl 6 M and ethanol. DNA obtained from the different sources was dried at room temperature, re-suspended in buffer TE, pH 8.0 and stored at −20°C.

Aliquots of total DNA were used as templates in polymerase chain reaction (PCR) to amplify double-stranded DNA products from the 3′ end of the mitochondrial tRNA-Pro gene (63 bp) and the adjacent 5′ end of the mitochondrial control region (341 bp of the hypervariable region I and 104 bp of the variable region I). Each PCR had a reaction volume of 100 µl and contained 20 µl of

5 ng/ml DNA, 20 µl of 5× Green GoTaq Reaction Buffer (7.5 mM $MgCl_2$, pH 8.5), 0.5 µl of 20 mM premixed deoxynucleotide triphosphates, 5 µl of 10 mg/ml bovine serum albumin, 1.25 units of GoTaq polymerase (Promega), 4 µl of 5 mM oligonucleotide primers and water to reach the final volume reaction. The primer pairs used were: L16274, 5′-TACACTGGTCTTGTAAACC-3′ y H34, 5′-CCAAATGCATGACACCACAG-3′ (Lamont et al. 1996). Amplification protocol consisted in 35 cycles of PCR, each one involving denaturation at 94°C for 1 min, annealing at 46°C for 30 s and extension at 72°C for 1 min, and was carried out in a MyCycler thermal cycler (BIO-RAD). Twenty microlitres of the PCR products were resolved in 1.5% agarose gel electrophoresis, visualised and photographed under UV light. The rest of the amplification products (80 µl) were purified using a Wizard SV Gel and PCR Clean-Up System (Promega) and sent to an external laboratory (Macrogen Inc.) where sequencing was performed with the same oligonucleotide primers used in PCR reactions using an ABI 377 Automated DNA PrismTM Sequencer (Applied Biosystems, Inc). Three features of our data suggest that the obtained sequences corresponded to mitochondrial DNA copies and not to nuclear-mitochondrial ones (numts) (Lopez et al. 1994; Triant and DeWoody 2007): (1) most of the samples used were tissues, which are rich in mtDNA relative to nuclear DNA, (2) we do not obtain multiple bands during electrophoresis or ambiguities in chromatograms, and (3) the first 63 bp of our sequences, that correspond to the coding region of the tRNA-Pro gene, do not present stop codons, insertions-deletions, or frameshift mutations.

Data analyses

Phylogeographic structure

Sequences were aligned and analysed for polymorphic sites using ClustalX (1.83). Aligned sequences were manually edited using Chromas, version 2.23. Absolute and relative frequencies of haplotypes within colonies and pairwise comparisons of percent sequence divergence were computed using ARLEQUIN software, version 3.11 (Excoffier et al. 2005). An Analyses of Molecular Variance (AMOVA; Excoffier et al. 1992) was performed using ARLEQUIN 3.11 in order to estimate the partitioning of genetic variation. Genetic differences were calculated using the method of pairwise differences. Population pairwise F_{ST} values were calculated for every pair of colonies. Sequential Bonferroni corrections were applied to adjust the statistical significance levels for multiple simultaneous comparisons (Rice 1989). F_{ST} estimates were then used to test for isolation by distance using the Mantel procedure (Manly 1986). Correlation was examined between $F_{ST}/(1 - F_{ST})$ and the logarithm of Euclidean geographical distance between colonies in kilometres.

The median-joining network method (Bandelt et al. 1999) implemented in the Network 4.5 program (Fluxus Technology Inc.) was applied to our dataset in order to estimate the phylogeographic structure and divergence time between haplotypes. This method, using a parsimony criterion, combines the minimum-spanning trees (MSTs) with a single network, allowing more detailed population information than do strictly bifurcating trees (Posada and Crandall 1998).

MDIV (Nielsen and Wakeley 2001) was used to estimate divergence time between colonies. This program uses a Bayesian approach to estimate this parameter between pairs of populations that are assumed to have diverged from a common ancestral population. MDIV was run multiple times with different random seeds and lengths of the Markov chain to assess the stability of the results. Final results were obtained using the following parameters: Finite sites (HKY) model (Hasegawa et al. 1985); Markov chain simulation for 5,000,000 steps, where the first 500,000 were discarded as burn-in; and a uniform prior distribution between 0 and 10 for T (divergence time/(2 × effective population size)). MDIV measures divergence time in units of effective population time (N_e) that can be calibrated into generations and hence years when a specific mutation rate and generation time are assumed. The modes of the posterior distribution for both population divergence time and θ (where $\theta = 2\ N_e\mu$, and μ is the mutation rate per sequence per generation) were used to estimate divergence times between colonies, and to explore the probability that the signatures of population segregation and range expansion were congruent with late Pleistocene glaciations. As the rate of substitution for the mitochondrial control region in *O. flavescens* is not known, the rate of substitution at the hypervariable region I (HVRI) in Steller sea lions, *Eumetopias jubatus*, (27.45% per million years; Phillips et al. 2009) was used to convert mutational time (τ) into real time according to equation: $\tau = 2\mu t$, where t is the time in years. The generation time used for the calculations was 3 years (Vaz-Ferreira 1982). The election of this divergence rate for the calculations was based on the phylogenetic proximity between *O. flavescens* and *E. jubatus*, both members of the subfamily Otariinae. However, as the control region evolves different from species to species, the parameters estimated using this mutation rate should be treated with caution.

Historical population dynamics

The demographic history of each population was examined using a mismatch distribution analysis. This method, based on an assumed stepwise growth model (Rogers and

Harpending 1992), was used to evaluate: (1) whether there was signature of population expansion, and (2) the timing of demographic expansion measured in units of mutational time. Typically, a population with a constant size in the past has a multimodal and ragged mismatch distribution, while a population that has undergone expansion usually shows a unimodal and smooth distribution (Rogers and Harpending 1992; Harpending 1994; Rogers 1995). Approximate confidence intervals for growth parameters are obtained by a parametric bootstrap approach (1,000 replicates). The validity of the estimated stepwise expansion model is tested using the same parametric bootstrap approach by a goodness-of-fit test between the observed distribution of the pairwise differences pattern and the simulated one based on the estimated model parameters. To quantify the smoothness of the observed haplotype frequency distribution, Harpending's raggedness index (Harpending 1994) was applied. A non-significant index would indicate a good fit of the data to a population expansion model. All these computations were also performed in ARLEQUIN 3.11 (Excoffier et al. 2005). Mutational time was converted into real time as described above.

Nucleotide sequences

Mitochondrial sequences described in this article have been deposited in the EMBL/GenBank data libraries (accession numbers: HM347787-96).

Results

From the 49 samples analyzed we found a total of 10 haplotypes with a length of 508 bp and 12 polymorphic sites (Table 2). The genealogical relationship between haplotypes was estimated in the median-joining network illustrated in Fig. 2a, which revealed a shallow pattern of phylogeographic structure. The most parsimonious explanation for the genealogical relationship observed, is the evolution of haplotypes II-X from haplotype I, which had the highest probability of representing an ancient form because of their central position. This haplotype was present in all colonies, was the most common ($n = 15$ individuals), and differed from most of the other haplotypes by only one to three nucleotide changes (Table 2). Using the divergence rate at the HVRI in Steller sea lions (27.45% per million years; Phillips et al. 2009) and the percentage of divergence between the ancestral haplotype (haplotype I) and the most divergent one (haplotype VIII, 1.76% for the HVRI) we estimated the time elapsed since a possible bottleneck could have reduced the variability of the population on the Atlantic coast to a single haplotype. According to our values, this bottleneck would have occurred approximately 64,000 years ago. From the 24 of the 60 samples analyzed for the cytochrome *b* that were included here, we found four haplotypes with a length of 445 bp and three polymorphic sites (Table 2). No other haplotypes were identified in the samples not included. The genealogical relationship between haplotypes is shown in Fig. 2b. Haplotypes B, C, and D were related by a single base-pair substitution to haplotype A. The star-like phylogeny of haplotypes suggests that haplotype A represents the ancient form. This haplotype was the most common (42 of the 60 individuals previously analyzed in Túnez et al. 2007) and the unique haplotype in the Uruguayan breeding colonies located northern to the Patagonian ones (data not shown). The relationship between cytochrome *b* and control region haplotypes is shown in Fig. 2. Individuals with control region haplotypes I, II, III and VIII possessed the cytochrome *b* haplotypes A and B, which suggests some level of homoplasy in the control region.

The results of the AMOVA analysis showed significant differences between colonies ($F_{ST} = 0.093$, $P = 0.041$; Table 3). However, the greatest source of variation (90.7%) was found at the intrapopulation level. Pairwise comparisons of F_{ST} values indicate no significant differences between colonies belonging to the same breeding area (PN/PP, IA/IVO, $P > 0.54$; Table 3). However, significant differences were found between colonies belonging to different breeding areas. ML, the southernmost colony, showed significant differences with PN and PP, the northernmost colonies in northern Chubut breeding area, and IVO, one of the colonies in central-southern Chubut breeding area (Table 3). Only ML/PN comparison was significant after Bonferroni correction ($P = 0.009$; Table 3). Differences were not significant between the other pairs of colonies ($P > 0.11$; Table 3). Correlation between genetic and geographical distances was not significant (Mantel test; $P > 0.28$).

MDIV was used to estimate divergence time between all pairs of colonies. For nine of the 10 comparisons carried out, the results indicate that there is no enough information about T in the data, which suggest that colonies are genetically indistinguishable and there is no evidence for population subdivision. As an example of the results obtained for these nine comparisons, Fig. 3a shows the posterior distribution for population divergence time between Punta Norte and Monte Loayza, the northernmost and southernmost colonies. Population divergence time could only be estimated between Puerto Pirámide and Monte Loayza. The posterior distribution for population divergence time between these colonies is shown in Fig. 3b. The estimated population divergence time calculated from a $T_{\max} = 0.440$, and a $\theta_{\max} = 1.847$ was

Table 2 Percent of divergence from the most common haplotype, relative position of variable nucleotides and absolute [relative] frequency of mitochondrial DNA control region and cytochrome *b* (reported in Túnez et al. 2007) haplotypes in colonies of *Otaria flavescens* in North-central Patagonia. Colonies names correspond to those in Table 1

Control region haplotypes	*N*	Divergence from I (%)[a]	Relative position of variable nucleotides												Distribution				
			197	207	209	235	236	238	250	265	268	280	331	358	PN	PP	IA	IVO	ML
I	15	–	T	A	G	C	A	A	T	C	C	G	T	A	2 (0.17)	3 (0.43)	3 (0.30)	4 (0.40)	3 (0.30)
II	11	0.98 (1.47)	·	·	·	T	·	G	·	T	T	A		·	2 (0.17)		3 (0.30)	2 (0.20)	4 (0.40)
III	9	0.20 (0.29)	·	·	·	·	·	·	·	·	·	·	·	G	3 (0.25)	2 (0.29)	2 (0.20)	2 (0.20)	
IV	4	0.59 (0.88)	·	·	A	·	G	·	·	·	·	·	·	G	2 (0.17)		1 (0.10)	1 (0.10)	
V	2	0.39 (0.59)	·	·	·	·	·	·	·	·	·	·	A	G	1 (0.08)	1 (0.14)			
VI	2	0.98 (1.47)	·	G	·	A	·	·	C	·	·	A	·	G	1 (0.08)			1 (0.10)	
VII	2	0.20 (0.29)	·	·	·	·	·	·	·	·	·	A	·	·	1 (0.08)	1 (0.14)			
VIII	2	1.18 (1.76)	G	·	·	T	·	G	·	T	T	A	·	·					2 (0.20)
IX	1	0.79 (1.17)	·	·	·	·	·	G	·	T	T	A	·	·			1 (0.10)		
X	1	0.20 (0.29)	G	·	·	·	·	·	·	·	·	·	·	·					1 (0.10)

Cytochrome *b* haplotypes	*N*	Divergence from A (%)	Relative position of variable nucleotides			Distribution				
			155	379	392	PN	PP	IA	IVO	ML
A	11	–	A	C	T	nd	2 (0.50)	4 (0.40)	nd	5 (0.50)
B	11	0.22	G	·	·	nd	2 (0.50)	5 (0.50)	nd	4 (0.40)
C	1	0.22	·	·	C	nd		1 (0.10)	nd	
D	1	0.22	·	G	·	nd			nd	1 (0.10)

nd not determined

[a] Percent sequence divergence from the most common haplotype in the 341 bp of the HVRI between square brackets

17,500 years, suggesting that the divergence between these two colonies occurred after the last glacial maximum.

The observed distribution of pairwise differences did not differ significantly from the simulated pattern based on a sudden expansion model in four of the five colonies analyzed ($P \geq 0.10$), supporting the demographic expansion hypothesis. Harpending's raggedness index also provided statistical support for these results. Table 4 shows the parameters estimated under the model. The tau value (τ), which reflects the location of the mismatch distribution crest, provides a rough estimate of the time when rapid population expansion started (Rogers and Harpending 1992; Rogers 1995). The tau values for IA, IVO, ML were all larger than 6.0, while those of PP and PN were smaller than 1.4 (Table 4). In the case of PN, this figure should be regarded with caution, since this population did not show clear evidence of any expansion at all. Tau values larger than 6.0 correspond to estimated expansion times of more than 43,000 years before present (YBP), while the value of PP (1.313) correspond to an estimated expansion time of 9,415 YBP, assuming a divergence rate of 27.45% per million years (Phillips et al. 2009) (Table 4).

Discussion

In north-central Patagonia, *O. flavescens* shows a patchy distribution of breeding activity with 40 breeding colonies separated in three different clumps: northern Chubut, central-southern Chubut and northern Santa Cruz. Populations of sea lions from the same breeding area do not show significant differences in haplotype frequencies, neither in northern Chubut, nor in central-southern Chubut. However, in spite of the greatest source of variation was found at the intrapopulation level, population pairwise F_{ST} values showed significant differences between one of the northernmost colonies and the southernmost one, Monte Loayza, which indicate some degree of genetic differentiation. This significant difference between breeding areas was not detected in a previous study conducted with cytochrome *b* gene, a less variable marker (Túnez et al. 2007). Genetic differentiation between relatively close breeding areas is not expected considering the potentially high mobility of animals and the historical demographic events that could have shaped the current phylogeographical pattern of the species in north-central Patagonia (see below). Moreover,

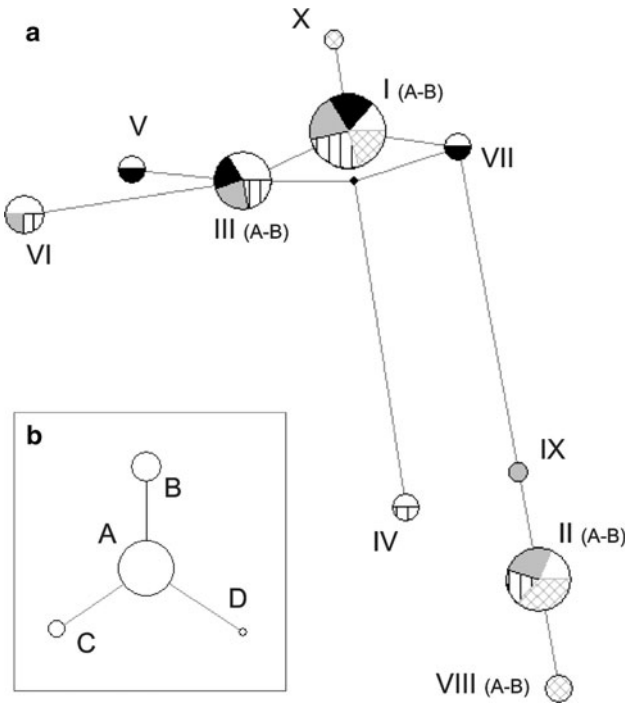

Fig. 2 Genealogical relationships between *O. flavescens* haplotypes obtained from the median-joining network method. **a** Control region, **b** Cytochrome *b* (data reported in Túnez et al. 2007). *Circle* areas are proportional to haplotype frequencies and length of the branches to the number of changes from one haplotype to the following. Haplotype numbers correspond to those in Table 2. References: **a** *white*—Punta Norte, *black*—Puerto Pirámide, *grey*—Isla Arce, *vertical lines*—Isla Vernacci Oeste, *diagonal crosses*—Monte Loayza. Cytochrome *b* haplotypes found for the same control region haplotype between brackets

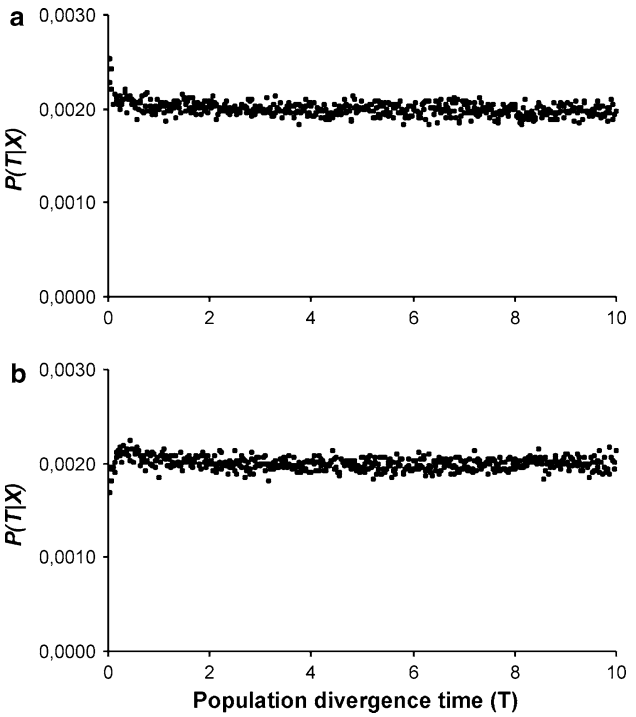

Fig. 3 MDIV results for the posterior distribution of T (population divergence time) for the following *O. flavescens* colonies pairs: **a** Punta Norte-Monte Loayza, **b** Puerto Pirámide-Monte Loayza

Table 3 Analysis of Molecular Variance (AMOVA) between colonies of South American sea lion, *Otaria flavescens*, in north-central Patagonia

Comparison	Fixation index	*P*
Among colonies	$\Phi_{ST} = 0.09$	0.041[a]
Between pairs of colonies		
Punta Norte/Puerto Pirámide	$\Phi_{ST} = -0.01$	0.549
Punta Norte/Isla Arce	$\Phi_{ST} = -0.01$	0.387
Punta Norte/Isla Vernacci Oeste	$\Phi_{ST} = -0.08$	0.937
Punta Norte/Monte Loayza	$\Phi_{ST} = 0.23$	0.009*
Puerto Pirámide/Isla Arce	$\Phi_{ST} = 0.13$	0.117
Puerto Pirámide/Isla Vernacci Oeste	$\Phi_{ST} = -0.01$	0.631
Puerto Pirámide/Monte Loayza	$\Phi_{ST} = 0.42$	0.018**
Isla Arce/Isla Vernacci Oeste	$\Phi_{ST} = -0.05$	0.685
Isla Arce/Monte Loayza	$\Phi_{ST} = 0.04$	0.144
Isla Vernacci Oeste/Monte Loayza	$\Phi_{ST} = 0.17$	0.045**

* Significant after Bonferroni correction ($0.05 < P < 0.01$)

** Significant at $P < 0.05$

the low level of genetic differentiation in north-central Patagonia could also be caused by the homoplasy detected in our control region data. Homoplasy at hotspots for substitutions in the mitochondrial control region has been described in several species of mammals (e.g., see Tamura and Nei 1993; Wakeley 1993; Fernando et al. 2000; Herrnstadt et al. 2002). In north-central Patagonia, distribution of colonies is associated with islands availability and negatively correlated with places were anthropogenic disturbance is high (Túnez et al. 2008). Other natural or anthropogenic variables, as marine productivity, interaction with fisheries or harvesting appears to be less important. The genetic discontinuity between Monte Loayza and the other sample locations could be due to the characteristics of the 400 km of coast that separate this colony with those in central-southern Chubut. This segment of coast is characterized by the absence of suitable islands for sea lions reproduction and a high anthropogenic disturbance because of the settlement of several petroleum industries. Moreover, extremely high philopatry in female pinnipeds is a well-known phenomenon that can accelerate the process of genetic differentiation between colonies (Trujillo et al. 2004; Matthiopoulos et al. 2005; Hoffman et al. 2006; Campbell et al. 2008). A more detailed study of the genetic structure of sea lions in north-central Patagonia, comparing the results obtained for mtDNA with nuclear markers as microsatellites, would help to understand the causes of the

Table 4 Parameters estimated under the Stepwise expansion model of population expansion. Colonies names correspond to those in Table 1

	Parameters	PN	PP	IA	IVO	ML
Stepwise model[a]	$\theta_0 = 2uN_0$	0.000 [0.000–0.004]	0.004 [0.000–0.028]	0.002 [0.000–3.008]	0.000 [0.000–8.868]	0.000 [0.000–7.656]
	$\theta_1 = 2uN_1$	99,999 [1.434–99,999]	99,999 [1.107–99,999]	6.346 [1.866–99,999]	6.040 [2.556–99,999]	6.174 [1.969–99,999]
	$\tau = 2ut$	0.977 [0.342–2.910]	1.313 [0.223–2.377]	6.178 [0.143–11.631]	6.098 [0.664–9.646]	6.637 [0.135–11.549]
	P (obs vs. sim)	0.00	0.30	0.50	0.55	0.10
Raggedness index	r	0.01745	0.23129	0.08938	0.10173	0.21432
	P	1.00	0.35	0.45	0.35	0.25
Expansion time (YBP)	t	nd	9,415	44,303	43,730	47,595

[a] Parameters are given as estimates [95% confidence limits], *nd* not determined

genetic discontinuity observed and the possible implications that this discontinuity would have for microevolution in the species.

O. flavescens showed for the 508 bp of the mitochondrial control region analyzed, a pattern of haplotype differentiation with nine haplotypes (II-X) that appeared having evolved from a founding haplotype (haplotype I) via single base-pair substitutions. The same occurred in our previous study using the cytochrome *b* gene (Túnez et al. 2007). The star-like phylogeny of cytochrome *b* haplotypes suggests that haplotype A represents the ancient form. Our estimates for the time since a possible bottleneck would have reduced the variability of the population to a single haplotype were approximately 64,000 and 110,000 YBP for control region and cytochrome *b* haplotypes, respectively. Hoelzel et al. (1993) also proposed that South American elephant seal, *Mirounga leonina*, located in Peninsula Valdés, Argentina, suffered a drastic decrease in population numbers that derived in a population bottleneck that occurred 100,000 years ago, suggesting a common process of population reduction in Patagonian pinnipeds.

The mismatch distribution analysis supported the demographic expansion hypothesis in four of the five colonies analyzed. Three of these four colonies (the southernmost ones) showed similar τ values, which correspond to an estimated expansion time of approximately 45,000 YBP. Several glacial episodes have occurred during the Late Pleistocene, being the most extensive those before 56.000 YBP (Mercer 1976). These glaciations covered the southern Andes from approximately 40°S to the Fuegian archipelago, and east to the Atlantic coast to about 52°S (Siegel-Causey 1997). During this period, the coasts of southern Argentina and Tierra del Fuego were probably uninhabitable by coastal breeding animals, because formerly used shoreline habitats were subsequently covered by ice or drastically altered by changing sea levels (Mercer 1976; Vuilleumier 1985). Based on geological and fossil evidence, coastal refugia existed in the Malvinas (Falkland) Islands and the Atlantic coast of northern Patagonia (Vuilleumier 1971). Evidence to date indicates that the Patagonian fauna experienced changes as profound as those studied in the northern hemisphere (Vuilleumier 1971; Vuilleumier 1985; Báez and Scillato Yane 1979; Rasmussen 1987, 1991). Thus, the historical population dynamics of *O. flavescens* in north-central Patagonia appears to be closely related with the dynamics of the Late Pleistocene glaciations. Our genetic analyses suggest that the sea lions population in Patagonia suffered a demographic contraction during the glacial period, followed by a population expansion when the glaciers retracted. Northern colonies, as Punta Norte or Puerto Pirámide, which did not shown clear evidence of any expansion at that moment, could have acted as refugia during glaciations. Is spite of

MDIV results indicate that there is no enough information in our data to estimate T in nine of the 10 comparisons carried out, the estimate obtained for Puerto Pirámide-Monte Loayza (17,500 YBP) is in accordance with the hypothesis that Puerto Pirámide would have acted as a glacial refuge and Monte Loayza was colonized after the glaciers retracted.

Habitat contractions caused by Pleistocene glacial cycles across temperate regions have been proposed as a major process in reducing genetic diversity in populations from the northern hemisphere (Rising and Avise 1993; Hewitt 1996; Merilä et al. 1997; Zink 1997; Milá et al. 2000). According to this hypothesis, loss of allelic diversity due to genetic drift in bottlenecked refugial populations would have been followed by rapid range expansions northward as ice sheets receded. If such expansions occurred too recently for mtDNA to accumulate point mutations, low genetic diversity and weak phylogeographical patterns would be observed today, as appears to occur in *O. flavescens* in north-central Patagonia. Other evidences in favour of habitat contractions and reduced genetic diversity caused by Pleistocene glacial cycles came from studies in different species that inhabits the southern hemisphere, which include several species of plants (Allnutt et al. 1999, 2003; Premoli et al. 2002; Muellner et al. 2005) and fishes (Zattara and Premoli 2005; Ruzzante et al. 2006); the rock shags, *Stictocarbo magellanicus*, (Siegel-Causey 1997) and the saxicolous mice, *Phyllotis xanthopygus* (Kim et al. 2002). Another member of the Family Otariidae, *Arctocephalus pusillus pusillus*, also show a population dynamics associated with the dynamics of glaciations, but in a inverse way. Colonies of the species in northern Namibia and south-eastern South Africa experienced a historical population expansion probably between c. 37,000–18,000 YBP (Matthee et al. 2006). This date coincides with the height of the last glacial maximum in the area. Authors suggested that the population expansion could be attributable to the high ocean productivity that characterizes maxima glacial periods that made resources abundant in the South Atlantic.

In summary, we investigate the genetic structure and the historical population dynamics of the South American sea lions in north-central Patagonia. We found (1) low levels of genetic subdivision among populations, (2) a star-like phylogeny of haplotypes with few high-frequency ancestral haplotypes and more low-frequency alleles separated from the ancestral ones by a few mutational steps, and (3) a distribution of pairwise nucleotide differences among haplotypes indicating a sudden increase in effective population size. Our results suggest that the historical population dynamics of the species in Patagonia appears to be closely related with the dynamics of the Late Pleistocene glaciations. This preliminary conclusion must be taken as working hypotheses for future studies of potential factors that must have shaped the genetic structure of sea lions and other species in Patagonia.

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