Mammalian Biology
Zeitschrift für Säugetierkunde
www.elsevier.de/mambio

Original investigation

Geographic distribution and diversity of mitochondrial DNA haplotypes in South American sea lions (*Otaria flavescens*) and fur seals (*Arctocephalus australis*)

By J.I. Túnez, Daniela Centrón, H.L. Cappozzo and M.H. Cassini

Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Argentina; Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina; Estación Hidrobiológica de Puerto Quequén, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina; Fundación Organización Profauna, Buenos Aires, Argentina

Receipt of Ms. 3.1.2006
Acceptance of Ms. 2.8.2006

Abstract

Genetic diversity and population structure of two species of South American pinnipeds, *Otaria flavescens* and *Arctocephalus australis*, from colonies located along the south-eastern coast of South America, were analysed using mitochondrial DNA haplotypes and compared with two populations of these species from the Pacific coast. A 445 base-pair segment, that included the tRNA-Glu gene (31 bp) and the adjacent cytochrome *b* gene (414 bp), was amplified using the polymerase chain reaction and sequenced directly. *O. flavescens* and *A. australis* showed six and seven haplotypes with 12 and 20 polymorphic sites, respectively. In the Atlantic Ocean there was an individual of *A. australis* that showed an haplotype that was highly divergent from the others. If this haplotype is excluded, the pattern of haplotype differentiation obtained for both species indicated a possible bottleneck that would have occurred 110,000 years ago, which also affected other pinnipeds. Colonies of the Atlantic and the Pacific did not share haplotypes. This result, based on a limited number of samples for the comparisons between oceans, suggests that populations from both oceans correspond to different evolutionarily significant units. *O. flavescens* on the Atlantic coast shows two clusters of breeding colonies in Uruguay and Patagonia, separated by a thousand kilometres. Colonies within clusters did not show significant differences in haplotype frequencies, but the difference between the clusters was significant, suggesting that they correspond to different conservation stocks.
© 2006 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Key words: *Otaria flavescens*, *Arctocephalus australis*, genetic diversity, mtDNA

Introduction

The South American sea lion, *Otaria flavescens* (Shaw, 1800), is distributed along approximately 10,000 km of the coast of South America, from Recifes das Torres (29°20′S, 49°43′W) in southern Brazil to Cape Horn in the extreme south of the Atlantic coast, and from Cape Horn to Zorritos (3°40′S, 80°34′W) in northern Peru in the Pacific Ocean (Cappozzo 2002).

On the Atlantic coast, there are two distinct areas with breeding activity: (1) along the coast of Uruguay at Isla de Lobos (35°02′S,

1616-5047/$ - see front matter © 2006 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.
doi:10.1016/j.mambio.2006.08.002

52°55′W), Cabo Polonio (34°24′S, 53°46′W), and La Coronilla (33°55′, 53°30′) (Vaz-Ferreira 1982), with a total of 15,000 individuals (Cappozzo and Rosas 1991); and (2) along the Patagonian coast, from Punta Bermeja (41°08′S, 63°04′W) to Tierra del Fuego (54°48′S, 65°15′W), in 31 colonies with a total of approximately 80,000 individuals (Szapkievich 1992; Reyes et al. 1999). Fifty-two non-breeding colonies are found also in this Patagonian area. Between Uruguay and Patagonia, there are only two colonies in Buenos Aires province at Mar del Plata (38°00′S, 57°33′W) and Quequén (38°32′S, 58°42′W) harbours (Szapkievich et al. 1999). These colonies were recently formed and are composed of approximately 800 and 100 males, respectively (Rodríguez 1996; F. H. Pérez and H. L. Cappozzo, unpublished).

The South American fur seal, *Arctocephalus australis* (Zimmermann, 1783) has almost the same geographic range as *O. flavescens* (King 1983; Riedman 1990). On the Atlantic coast, more than 80% of the population and all breeding colonies are located along the coast of Uruguay at Isla de Lobos, Cabo Polonio and La Coronilla Islands (Vaz-Ferreira 1982; Vaz-Ferreira and Ponce de León 1984), with an estimated population of 252,000 individuals (Vaz-Ferreira 1982). In Argentina, there are 17 colonies located in Patagonia (southern to the 43°S) with a total of 20,000 individuals, and with low reproductive activity (Crespo et al. 1999). In summary, *O. flavescens* and *A. australis* on the Atlantic coast show a patchy distribution of breeding activity at two scales. Not only do they aggregate in colonies but breeding colonies aggregate in certain areas of the coast, and can be separated by hundreds and even thousands of kilometres from other clumps of colonies. These large distances do not necessarily mean isolation between colonies because marine mammals show a large capacity of dispersal (Renouf 1990). Our aim was to investigate the genetic consequences of this patched distribution of breeding activity, using mitochondrial DNA (mtDNA) markers.

Comparing the sequence information from DNA target regions between individuals and populations has become a powerful tool in the investigation of genetic variability and structure (Frankham et al. 2002; Avise 2004), and has been extensively used in pinnipeds in the last decade (Lento et al. 1994, 1997; Maldonado et al. 1995; Lamont et al. 1996; Burg et al. 1999; Mizuno et al. 2003; Trujillo et al. 2004; Weber et al. 2004). There are two previous studies that analyse genetic data of South American otariids. Szapkievich et al. (1999) compared nine serum protein systems from two *O. flavescens* rookeries from Uruguay and Patagonia. Wynen et al. (2001) analysed the phylogenetic relationships and the historical biography of the Family Otariidae for what they sampled 16 species of eared seals.

Materials and methods

Sample collection

Tissue samples were collected from 70 South American sea lions, *O. flavescens* in six colonies located along the Atlantic coast of Uruguay and Argentina and from 19 South American fur seals, *A. australis* in a colony located in Cabo Polonio, Uruguay (Fig. 1, Tab. 1). Samples were collected from dead animals found in beaches near the colonies in Cabo Polonio (CP), Puerto Pirámide (PP) and Monte Loayza (ML), and from alive animals in Isla de Lobos (IL), Puerto Quequén (PQ) and Isla Arce (IA). In the case of alive animals, approximately 1 cm^3 of tissue was clipped from the tip of a digit on a hind flipper. For pups, we followed the methodology described in Cappozzo et al. (1991) and for adult individuals samples were taken when the animal was sleeping using a punch. In the case of dead animals, skin, muscle and liver samples were taken. All samples were stored in preservation buffer containing 20% DMSO, EDTA 0.25 N (pH = 8.0) saturated with sodium chloride.

Mitochondrial DNA extraction and PCR amplification

DNA was isolated from tissue samples by digestion with proteinase K, RNase and 10% SDS and incubation at 37 °C overnight, followed by extraction with phenol–cloroform and alcohol precipitation. Precipitated DNA samples were re-suspended in 10 mM Tris, 1 mM EDTA, pH = 8.0 and stored at −20 °C. Aliquots of total DNA were used as

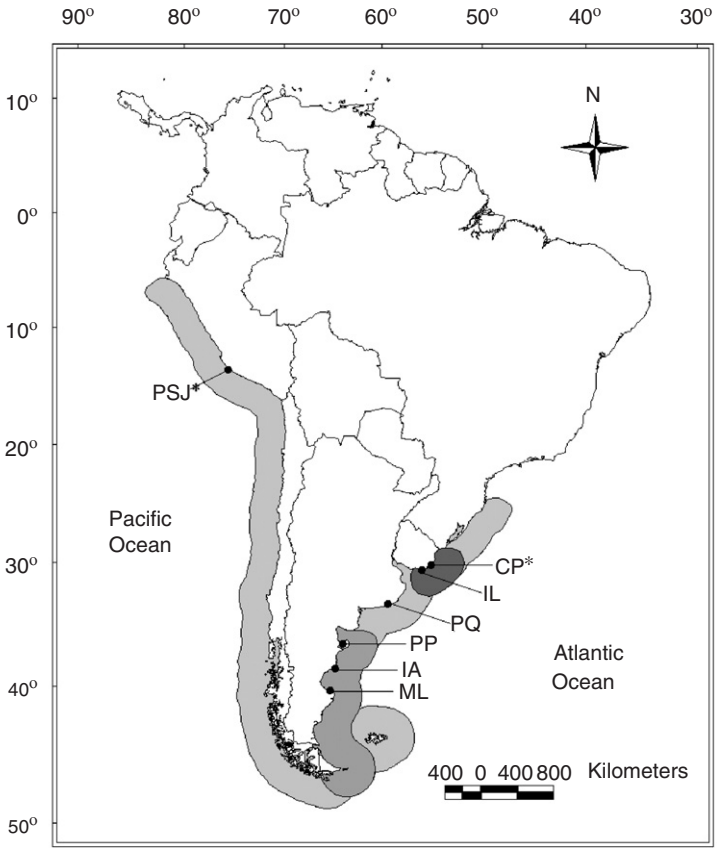

Fig. 1. Map showing the geographic distribution (light grey), the areas with breeding activity at Patagonia (grey) and Uruguay (dark grey), the location of the colonies sampled and the breeding colony of Punta San Juan for the South American sea lions and fur seals (*).

Table 1. Origin of South American sea lion and fur seals samples used in this study.

Colony	Country	Location	*n*	Date
South American sea lion, *Otaria flavescens*				
Cabo Polonio (CP)	Uruguay	34°24′S, 53°46′W	8	Nov 2002/Jan 2003
Isla de Lobos (IL)	Uruguay	35°02′S, 52°55′W	13	Jan 1993
Puerto Quequén (PQ)	Argentina	38°32′S, 58°42′W	25	Jan 2001
Puerto Pirámide (PP)	Argentina	42°35′S, 64°17′W	4	Jan/May 2003
Isla Arce (IA)	Argentina	45°00′S, 65°29′W	10	Feb 1998
Monte Loayza (ML)	Argentina	47°05′S, 66°16′W	10	Jan 2003
Punta San Juan (PSJ)	Perú	15°22′S, 75°12′W	5	GenBank sequences[a]
South American fur seal, *Arctocephalus australis*				
Cabo Polonio (CP)	Uruguay	34°24′S, 53°46′W	19	Nov 2002/Jan 2003
Punta San Juan (PSJ)	Perú	15°22′S, 75°12′W	5	GenBank sequences[a]

[a]Accession numbers: AF380901-10 (Wynen et al. 2001).

templates in polymerase chain reaction (PCR) to amplify double-stranded DNA products from the 3′ end of the mitochondrial tRNA-Glu gene (31 bp) and the adjacent 5′ end of the mitochondrial cytochrome *b* gene (414 bp). The election of the molecular marker used in this study was based in our aim to detect shallow (between populations in the same ocean) and relatively deep (between oceans) genetic structure in the populations studied. Cytochrome b shows an appropriated resolution capability to assess these two levels of genetic structure (Avise 2004).

Each PCR had a reaction volume of 100 µl and contained 20 µl of 5 ng/µl DNA, 5 µl of 5 mM $MgCl_2$, 5 µl of 10X reaction buffer, 0.5 µl of 20 mM premixed deoxynucleotide triphosphates, 5 µl of 10 mg/ml bovine serum albumin, 1.25 units of Taq polymerase (Invitrogen, Life technologies), 4 µl of 5 µM oligonucleotide primers and water to reach the final volume reaction.

The primer pairs were: GLUDG-L, 5′-TGACTT-GAARAACCAYCGTTG-3′ and CB2-H, 5′-CCC-TCAGAATGATATTTGTCCTCA-3′ (Palumbi and Kessing 1991). Amplification protocol consisted in 35 cycles of PCR, each one involving denaturation at 94 °C for 1 min, annealing at 50 °C for 30 s and extension at 72 °C for 1 min, and was carried out in an Eppendorf Mastercycler. Twenty microlitre 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 were sequencing was performed in both directions with the same oligonucleotide primers used in PCR reactions using an ABI 377 Automated DNA Prism™ Sequencer (Applied Biosystems, Inc).

Analysis of DNA sequences

Sequences were aligned and analysed for polymorphic sites using ClustalX (1.83). Aligned sequences were manually edited using Chromas, version 2.23. Of the 70 *O. flavescens* DNA samples used in PCR amplifications, 10 were not included in the sequence analysis because of the lack of PCR product during amplification or poor resolution during sequencing. These 10 samples were from IL ($n = 9$) and PQ ($n = 1$). IL samples were collected in 1993. We think that PCR products were not obtained because DNA was degraded. The 19 *A. australis* DNA samples used in PCR amplification were successfully amplified. Sequences for *O. flavescens* and *A. australis* from Punta San Juan, Perú (PSJ) were obtained from the GenBank (accession numbers: AF380906-10, AF380901-5, respectively) (Wynen et al. 2001) and consisted in a 360 bp segment of the 5′ end of the mitochondrial cytochrome *b* gene. In the statistical comparisons between oceans, we reduced the size of Atlantic sequences from 445 to 360 bp taking into account that position 1 of the 360 bp fragments from GenBank corresponds to position 32 in the 445 bp sequences.

Absolute and relative frequencies of haplotypes within colonies and pairwise comparisons of per cent sequence divergence were computed using ARLEQUIN software, version 2000 (Schneider et al. 2000).

Molecular variance between colonies (for *A. australis* and *O. flavescens*) or groups of colonies (for *O. flavescens* only) was calculated using AMOVA (Excoffier et al. 1992), which is part of the ARLEQUIN software. For *O. flavescens* we defined the groups in the AMOVA analysis joining colonies located in the same ocean basin. Genetic differences were calculated using the method of haplotype frequency. Estimates of Φ_{CT}, Φ_{SC}, and Φ_{ST}, analogues of Wright's fixation indexes F_{CT}, F_{SC} and F_{ST}, were computed. Significance of these multiple pairwise comparisons was corrected using a more stringent *P*-value (0.01) and performing an Exact Test of population differentiation based in a Markov-Chain procedure (Raymond and Rousset 1995).

Geographic distances were calculated from Argentina and South America maps of scales 1:120,000 and 1:337,500, respectively. Distances were measured in the maps following the shape of the coast.

Nucleotide sequences

Mitochondrial DNA sequences described in this article for *O. flavescens* and *A. australis* have been deposited in the EMBL/GenBank data libraries (accession numbers: AY > 12956-3034). First base of the *O. flavescens* and *A. australis* sequences corresponds to position 14,153 in the *Eumetopias jubatus* mtDNA sequences (GenBank accession number: NC004030).

Results

For *O. flavescens*, we found four haplotypes with a length of 445 bp and three polymorphic sites (Tab. 2). Haplotypes B, C, and D were related by a single base-pair substitution to haplotype A. Wynen et al. (2001) found two haplotypes with a length of 360 bp and two polymorphic sites between them (Tab. 2). For *A. australis*, we found five

Table 2. Relative position of variable nucleotides and absolute [relative] frequency of mitochondrial DNA cytochrome *b* haplotypes in seven colonies of *Otaria flavescens* and two colonies of *Arctocephalus australis*.

South American sea lion, *Otaria flavescens*

Haplotype	*n*	Changes from A[a]	Sequence divergence from A (%)[b]	Relative position of variable nucleotide: 0 8 1	1 5 5	1 7 5	1 8 2	1 9 6	2 0 8	2 2 6	3 4 6	3 5 5	3 7 9	3 8 9	3 9 2	Distribution: Uruguay CP	Uruguay IL	Argentina PQ	Argentina PP	Argentina IA	Peru ML	Peru PSJ
A	42	—	—	C	A	C	C	T	C	A	G	G	C	T	T	8 [1]	4 [1]	19 [0.8]	2 [0.5]	4 [0.4]	5 [0.5]	
B	13	1	0.22 (0.28)	.	G	.	.	.	.	.	.	.		.	.			2 [0.1]	2 [0.5]	5 [0.5]	4 [0.4]	
C	4	1	0.22 (0.28)	.	.	.	.	.	.	.	.	.	.	.	C			3 [0.1]		1 [0.1]		
D	1	1	0.22 (0.28)	.	.	.	.	.	.	.	.	.	G	.	.						1 [0.1]	
E	4	9	(2.50)	T	G	T	T	C	T	.	A	A	.	C	—							4 [0.8]
F	1	9	(2.50)	T	G	.	T	C	T	G	A	A	.	C	—							1 [0.2]

South American fur seal, *Arctocephalus australis*

Haplotype	*n*	Changes from G[a]	Sequence divergence from G (%)[b]	Relative position of variable nucleotide: 0 2 8	0 7 0	0 9 1	1 1 2	1 7 8	2 1 4	2 6 5	2 7 4	2 7 7	2 8 9	3 1 0	3 2 5	3 4 3	3 4 9	3 5 8	3 8 2	3 9 1	3 9 8	4 0 0	4 3 3	Distribution: Uruguay CP	Peru PSJ
G	13	—	—	T	T	C	C	T	G	C	T	G	A	T	G	C	C	A	C	A	A	C	G	13 [0.7]	
H	3	1	0.22 (0.28)	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	3 [0.2]	
I	1	1	0.22 (0.28)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	1 [0.05]	
J	1	1	0.22 (0.28)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	1 [0.05]	
K	1	10	2.24 (1.94)	C	.	T	T	A	.	T	C	.	.	.	A	.	.	T	.	.	.	T	A	1 [0.05]	
L	3	1	(0.27)	—	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	—	—	—		3 [0.6]
M	2	10	(2.77)	—	C	.	.	.	A	.	C	A	.	C	A	T	T	.	T	G	—	—	—		2 [0.4]

[a]Number of nucleotide changes in comparison to the most common haplotype.
[b]Per cent sequence divergence from the most common haplotype for 445 (and 360) bp sequences.

Table 3. Hierarchical Analysis of Molecular Variance (AMOVA) between colonies of South American sea lion *Otaria flavescens*, using 360 and 445 bp sequences.

Geographical arrangement	Fixation index	*P*
Among groups (Atlantic vs. Pacific)	Φ_{CT}=0.95	0.00001[b]
Among populations within groups (Atlantic colonies)	Φ_{SC}=0.20	0.007[b]
Uruguay/Puerto Quequén	Φ_{ST}=0.001	0.11
Uruguay/Puerto Pirámide	Φ_{ST}=0.61	0.001[b]
Uruguay/Isla Arce	Φ_{ST}=0.49	0.002[b]
Uruguay/Monte Loayza	Φ_{ST}=0.39	0.005[b]
Puerto Quequén/Puerto Pirámide	Φ_{ST}=0.39	0.09
Puerto Quequén/Isla Arce	Φ_{ST}=0.36	0.013[a]
Puerto Quequén/Monte Loayza	Φ_{ST}=0.22	0.004[b]
Puerto Pimámide/Isla Arce	Φ_{ST}=−0.21	0.55
Puerto Pirámide/Monte Loayza	Φ_{ST}=−0.17	0.99
Isla Arce/Monte Loayza	Φ_{ST}=−0.08	0.99
Within populations	Φ_{ST}=0.96	—

[a]Significant at $P<0.05$.
[b]Significant at $P<0.01$.

haplotypes with a length of 445 bp and 12 polymorphic sites. Haplotype K showed large variation with 10 changes from haplotype G. As in *O. flavescens*, haplotypes H, I, and J were related by a single base-pair substitution to haplotype G. Wynen et al. (2001) found two haplotypes with a length of 360 bp and nine polymorphic sites between them (Tab. 2).

An AMOVA analysis conducted with the 360 bp sequences of *A. australis* indicated highly significant differences between Uruguayan and Peruvian colonies ($\Phi_{ST}=0.55$, $P<0.0001$). Another AMOVA analysis conducted with the 360 bp sequences of *O. flavescens* indicated highly significant geographic differentiation between the two oceans ($\Phi_{CT}=0.95$, $P<0.00001$) and between the six breeding colonies of the Atlantic Ocean ($\Phi_{SC}=0.20$, $P<0.007$). There were no significant differences between colonies of each breeding cluster (the two Uruguayan colonies were joined in the AMOVA because they showed the same unique haplotype; in Patagonia, IA/ML: $\Phi_{ST}=-0.08$, $P=0.99$, IA/PP: $\Phi_{ST}=-0.21$, $P=0.55$, ML/PP: $\Phi_{ST}=-0.17$, $P=0.99$). The non-breeding colony of Puerto Quequén showed an intermediate genotype: it shows non-significant differences with Uruguay ($\Phi_{ST}=0.001$, $P=0.11$) and with the nearest Patagonian colony PP ($\Phi_{ST}=0.39$, $P=0.09$), and it shows significant differences with IA ($\Phi_{ST}=0.36$, $P=0.013$) and ML ($\Phi_{ST}=0.22$, $P=0.004$). We conducted an additional AMOVA only with data from the Atlantic coast, using the sequence of 445 bp and comparing the Uruguayan colonies and the three Patagonian colonies of *O. flavescens* ($\Phi_{ST}=0.21$, $P=0.015$). Population pairwise Φ_{ST} value comparisons indicated significant differences between CP/IL (Uruguay) and the three Patagonian colonies ($\Phi_{ST}=0.61$, 0.49 and 0.39, for PP, IA and ML, $P\leqslant0.01$), and non significant differences between colonies from Patagonia ($P>0.67$) (Tab. 3).

Significant differences between oceans for both species and between breeding clusters for *O. flavescens* in the Atlantic coast were confirmed by the Exact Test of population differentiation (for all comparisons, Exact *P*-value<0.001) after 6000 Markov steps done.

Geographic distribution of frequencies of the most frequent haplotype of *O. flavescens* showed North–South gradients (Fig. 2). Haplotype A had the highest frequency in Uruguayan colonies (frequency = 1, IL and CP), and the lowest in Patagonian ones (frequency ranging from 0.4 and 0.5 in PP, IA and ML). PQ colony, a non-breeding colony located between these two breeding

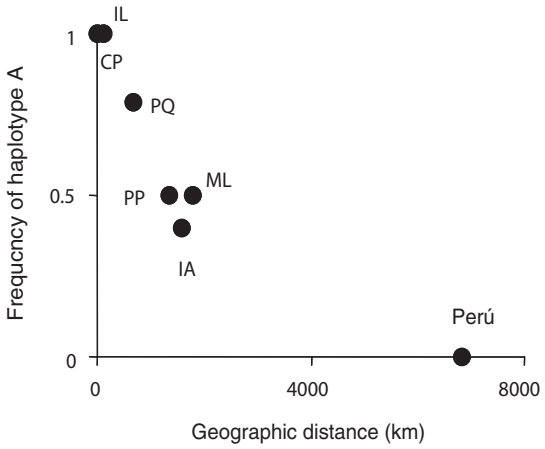

Fig. 2. Relationship between haplotype frequencies and geographic distance for the most common haplotype (haplotype A) found in the sampled colonies of *O. flavescens*. The analysis included data from the six rookeries sampled during this study and data calculated from 5 sequences from a Peruvian colony that were published in the GenBank.

clusters, showed and intermediate value (frequency = 0.79) (Fig. 2). This haplotype is absent on the Pacific coast (Fig. 2). Regression analysis between frequency of haplotype A in each colony and geographic distance was highly significant (Simple regression, $r^2 = 0.80$, $F_{1,5} = 20.72$, $P < 0.006$).

Discussion

Overall genetic variability

There was an individual of *A. australis* that contained a haplotype that was highly divergent from other haplotypes found in Uruguay. The occurrence of outliers has been previously described in other fur seal species and it is probably due to hybridisation (Lento et al. 1994; Goldsworthy et al. 2000). If the haplotype K is not considered, then both species showed the same pattern of haplotype differentiation: three new haplotypes (B, C, and D in sea lions, H, I, and J in fur seals) that appeared having evolved from a founding haplotype (A in sea lions and G in fur seals) via single base-pair substitutions. Taking into account that cytochrome *b* gene shows a divergence rate of approximately 2% per million years (Moritz and Hillis 1990), we can use the percentage of mtDNA divergence between the ancestral haplotype and the other three to estimate the time elapsed since a bottleneck had reduced the variability of the population on the Atlantic coast to a single haplotype. According to our values, this bottleneck would have occurred approximately 110,000 years ago. Hoelzel et al. (1993) also proposed that South American elephant seals (*Mirounga leonina*) located in Peninsula Valdés suffered a drastic decrease in population numbers that derived in a population bottleneck that occurred 100,000 years ago, in coincidence with our estimations and suggesting a common process of population reduction between species. Several glacial episodes have occurred during the Pleistocene that made the southern coast of the Atlantic uninhabitable (Hoelzel et al. 1993; Siegel-Causey 1997).

In Uruguay, South American sea lions did not show haplotype variation while South American fur seals showed 12 polymorphic sites over 445 bp that defined five haplotypes. Because there is no evidence of a recent bottleneck due to severe hunting, we suggest that the lack of variance in sea lions is because they colonised Uruguay in recent historical times. This is supported by well established historical patterns of dispersal that indicate sea lions arrived later than fur

seals at the Atlantic coast from the north-east Pacific region, which is considered the centre of origin of the family Otariidae (Wynen et al. 2001).

Evolutionary significant units

Our preliminary results suggest that populations from both oceans correspond to different evolutionarily significant units. Populations of both species from the Atlantic Ocean do not share any haplotype with populations in the Pacific. In other words, mtDNA haplotypes exhibit reciprocal monophyly, which is consistent with a phylogenetic distinction between otariids of both oceans (Moritz 1994). There are at least two other requisites for the definition of evolutionary significant units, i.e., to show significant differences of allele frequencies at nuclear loci, and of phenotypic traits, mainly variation in skull metrics, but also other characteristics such as body weight, life history and behaviour (Moritz 1994; Frankham et al. 2002). There are no published studies using nuclear markers in either species. Oliveira et al. (1999) and Brunner (2004) described significant differences in skull metrics of *A. australis* from Perú and Uruguay (Uruguayan fur seals were collected on the southern coasts of Brazil) and C. Drehmer (personal communication) also found significant differences in skull measures of *O. flavescens* between oceans. Other differences have been described, such as different female body size, mating system and some life history traits for *A. australis* (Oliveira et al. 1999) and behaviour for *O. flavescens* (Cassini 1998).

Spatial pattern of haplotype distribution in *O. flavescens*

Distribution of breeding sea lions shows heterogeneity at three geographic scales. They congregate in 37 breeding colonies, these colonies clumped in two sectors of the Atlantic coast (Uruguay and Patagonia) separated by hundred kilometres, and these otariids are separated from those breeding in the Pacific Ocean by thousands of kilometres. We found a strong correlation between this spatial pattern of distribution of individuals and the distribution of haplotypes. Populations of sea lions from the same cluster do not show significant differences in haplotype frequencies, neither in Uruguay, nor in Patagonia, while there are significant differences between the two clusters. This pattern of haplotype frequency distribution resembles a stepping-stone model of gene flow among colonies, where most gene flow occurs between colonies within a cluster and flow between clusters is absent or very low (Kimura and Weiss 1964). This pattern has been proposed previously for *Phoca vitulina richardsi* (Lehman et al. 1993; Lamont et al. 1996).

'Females stocks' and sexual patterns of dispersal in *O. flavescens*

Stocks are defined as a group of individuals, different from other groups, in which a parental pattern of ancestors and descendants exists (Wang 2002). Determining how a species is divided into stocks is fundamental to the conservation of marine mammals because the inadequate definition of stocks can lead to unnecessary regulations or inadequate management that would result in the disappearance of a stock (Wang 2002). The definition of a Uruguayan stock of *O. flavescens* is of critical conservation relevance because sea lion populations in this country have been continuously declining in the last decades (E. Páez, unpublished), and there is a trend for future reduction in population numbers due to human impact (Szteren and Páez 2002). Previous studies using capture–recapture and protein markers techniques, indicated dispersion of males (Vaz-Ferreira 1982; J. Lorenzani and J. Lorenzani, unpublished) and protein marker homogeneity (Szapkievich et al. 1999) between Uruguay and Patagonia. In other words, these studies indicated that Uruguayan populations did not form a separated stock from Argentinean ones. However, our values of pairwise Φ_{ST} statistics between colonies have shown that there are significant genetic differences between the Uruguayan and the Patagonian colonies suggesting that Uruguayan and

Argentinean colonies correspond to different stocks.

This apparent contradiction between studies can be explained when taking into account that males and females differ in their dispersal pattern and that mtDNA markers are maternally inherited. Because mtDNA is transmitted exclusively via females, gene flow between colonies estimated with these markers can only be detected when females disperse (Trujillo et al. 2004). Previous information indicates that males can maintain gene flow between colonies (all marked individuals that were found to disperse were males), but our results suggest that female dispersion between Uruguay and Patagonia is rare or non-existent. These two breeding clusters are separated by approximately 1000 km, and several non-breeding colonies were located between them. As expected by our results, females are not observed in these colonies, which are formed exclusively by males. Dispersion is biased towards males in most mammals (Chepko-Sade and Halpin 1987). In pinnipeds, recent studies that compared male and female rates of migration based on the comparison between microsatellites and mtDNA data, showed the same trend (Burg et al. 1999; Trujillo et al. 2004). In summary, *O. flavescens* from Uruguay appears to represent a separate 'female stock' that, due to the population decrease rate of the last years, requires special conservation considerations.

Acknowledgements

We thank Ezequiel A. Saavedra for his help in the sequencing process, Sofía Piekar Centrón, Enrique Crespo, Fabián Pérez, Javier Corcuera, Jorge O. Owen, Captain Hugo Gómez (Yoshua), Luis M. Batallés, Mauricio Lima, Alberto Ponce de León and Diana Szteren for assisting with sample collection and Gerhard Krumschnabel for his help in the abstract translation from English to German. Juan Ignacio Túnez was supported by postgraduate scholarships from the Agencia Nacional de Promoción Científica y Tecnológica and CONICET. This research was founded by grants from Universidad Nacional de Luján, Agencia Nacional de Promoción Científica y Tecnológica, Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 02193-CONICET) and Fundación Antorchas (A-13672/1-3).

Zusammenfassung

Geographische Verteilung und Diversität von mitochondrialen DNA-Haplotypen von Südamerikanischen Seelöwen *(Otaria flavescens)* und Pelzrobben (*Arctocephalus australis*)

Die genetische Diversität und Populationsstruktur zweier südamerikanischer Robbenarten von Kolonien entlang der Südostküste Südamerikas, von *Otaria flavescens* und *Arctocephalus australis*, wurde in Bezug auf mitochondriale Haplotypen analysiert und mit jener von Populationen der gleichen Arten an der Pazifikküste verglichen. Ein Segment von 445 Basenpaaren (bp), in dem das tRNA-Glu Gen (31 bp) und das anschließende Cytochrome *b* Gen (414 bp) enthalten ist, wurde mittels Polymerase-Ketten-Reaktion amplifiziert und direkt sequenziert. *O. flavescens* und *A. australis* zeigten sechs bzw. sieben Haplotypen mit 12 bzw. 20 polymorphen Stellen. Im atlantischen Ozean fanden wir ein Individuum der Art *A. australis*, dessen Haplotyp äußerst stark von dem der anderen abwich. Wurde dieser Haplotyp aus der Analyse ausgeschlossen, so weist das Muster der Haplotyp-Differenzierung, welches für beide Arten gefunden wurde, auf einen möglichen genetischen Engpass hin, der vor 110,000 Jahren aufgetreten ist und sich auch auf andere Robbenarten auswirkte. Kolonien des Atlantik und des Pazifik teilten keine gemeinsamen Haplotypen. Basierend auf einer begrenzten Anzahl von Vergleichsproben zwischen beiden Ozeanen deutet dieses Resultat darauf hin, dass die Populationen dieser Ozeane evolutionär signifikant

unterschiedlichen Einheiten angehören. *O. flavescens* an der atlantischen Küste weist zwei Cluster von Brutkolonien in Uruguay und Patagonien auf, die durch tausend Kilometer getrennt sind. Die Kolonien innerhalb eines Clusters zeigten keine signifikanten Unterschiede in der Häufigkeit des Auftretens der Haplotypen, während der Unterschied zwischen den Clustern signifikant war. Dies deutet darauf hin, dass sie unterschiedlichen Konservierungsbeständen entsprechen.
© 2006 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

References

Avise, J. C. (2004): Molecular Markers, Natural History and Evolution. 2nd ed. Massachusetts: Sinauer Associates, Inc. Publishers.

Brunner, S. (2004): Fur seals and sea lions (Otariidae): identification of species and taxonomic review. Syst Biodiversity **1,** 339–439.

Burg, T. M.; Trites, A. W.; Smith, M. J. (1999): Mitochondrial and microsatellite DNA analysis of harbour seal population structure in the northeast Pacific Ocean. Can. J. Zool. **77,** 930–943.

Cappozzo, H. L. (2002): South American sea lion (*Otaria flavescens*). In: Encyclopaedia of Marine Mammals. Ed. by W. F. Perrin, B. Wursig and J. G. M. Thewissen. San Diego: Academic Press. Pp. 1143–1146.

Cappozzo, H. L.; Campagna, C.; Monserrat, J. (1991): Sexual dimorphism in newborn southern sea lions. Mar. Mammal Sci. **7,** 385–394.

Cappozzo, H. L.; Rosas, F. C. W. (1991): León Marino Sudamericano, *Otaria flavescens* (Shaw, 1800). In: Estado de Conservación de los Mamíferos Marinos del Atlántico Sudoccidental. Ed. by H. L. Cappozzo and M. Junin. California: PNUMA (Programa de Naciones Unidas para el Medio Ambiente). Pp. 166–170.

Cassini, M. H. (1998): Inter-specific infanticide in Otariids. Behaviour **135,** 1005–1012.

Chepko-Sade, B.; Halpin, Z. T. (1987): Mammalian Dispersal Patterns: The Effect of Social Structure on Population Genetics. Chicago and London: The University of Chicago Press.

Crespo, E. A.; Schiavini, A. C. M.; Pérez, F. H.; Cappozzo, H. L.; (1999): Distribution, abundance and seasonal changes of South American fur seals, *Arctocephalus australis*, along the coasts of Argentina. In: Proceedings of the 13th Annual Conference of the European Cetacean Society. Ed. by: European Cetacean Society. Valencia: European Cetacean Society. Pp. 36.

Excoffier, L.; Smouse, P.; Quattro, J. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131,** 479–491.

Frankham, R.; Ballou, J. D.; Briscoe, D. A. (2002): Introduction to Conservation Genetics. Cambridge: Cambridge University Press.

Goldsworthy, S.; Francis, J.; Boness, D.; Fleischer, R. (2000): Variation in the mitochondrial control region in the Juan Fernandez fur seal (*Arctocephalus philippii*). J. Hered. **91,** 371–377.

Hoelzel, A. R.; Halley, J.; O'Brien, J.; Campagna, C.; Arnbom, T.; Le Boeuf, B.; Ralls, K.; Dover, G. A. (1993): Elephant seals genetic variation and the use of simulation models to investigate historical population bottlenecks. J. Hered. **84,** 443–449.

Kimura, M.; Weiss, G. H. (1964): The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics **49,** 561–574.

King, J. E. (1983): Seals of the World. St. Lucia: University of Queensland Press.

Lamont, M. M.; Vida, J. T.; Harvey, J. T; Jeffries, S.; Brown, R.; Huber, H. H.; DeLong, R.; Thomas, W. K. (1996): Genetic substructure of the pacific harbor seal (*Phoca vitulina richardsi*) off Washington, Oregon, and California. Mar. Mammal Sci. **12,** 402–413.

Lehman, N.; Wayne, R. K.; Stewart, B. S. (1993): Comparative levels of genetic variability in harbour seals and northern elephant as determined by genetic fingerprints. Symp. Zool. Soc. London **66,** 49–60.

Lento, G. M.; Mattlin, R. H.; Chambers, G. K.; Baker, C. S. (1994): Geographic distribution of mitochondrial cytochrome *b* DNA haplotypes in New Zealand fur seals (*Arctocephalus forsteri*). Can. J. Zool. **72,** 293–299.

Lento, G. M.; Haddon, M.; Chambers, G. K.; Baker, C. S. (1997): Genetic variation of southern hemisphere fur seals (*Arctocephalus spp.*). Investigation of population structure and species identity. J. Hered. **88,** 202–208.

Maldonado, J. E.; Orta Davila, F.; Stewart, B. S.; Geffen, E. (1995): Intraspecific genetic differentiation in California sea lions (*Zalophus californianus*) from southern California and the Gulf of California. Mar. Mammal Sci. **11,** 46–58.

Mizuno, A. W.; Onuma, M.; Takahashi, M.; Ohtaishi, N. (2003): Population genetic structure of the spotted seal *Phoca largha* along the coast of Hokkaido, based on mitochondrial DNA sequences. Zool. Sci. **20,** 783–788.

Moritz, C. (1994): Defining evolutionary significant units for conservation. Trends Ecol. Evol. **9,** 373–375.

Moritz, C.; Hillis, D. M. (1990): Molecular Systematics. Massachusetts: Sinauer Associates, Inc. Publishers.

Oliveira, L. R.; de, Malabarba, L. R.; Majluf, P. (1999): Variação geográfica em crânios do lobo-marinho sul-americano *Arctocephalus australis* (Zimmerman, 1783) das populações do Brasil e Peru. Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia, Porto Alegre **12,** 179–192.

Palumbi, S. R.; Kessing, B. D. (1991): Population biology of the trans-Arctic exchange: mtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution **45,** 1790–1805.

Raymond, M.; Rousset, F. (1995): An exact test for population differentiation. Evolution **49,** 1280–1283.

Renouf, D. (1990): The Behaviour of Pinnipeds. London: Chapman & Hall.

Reyes, L. M.; Crespo, E. A.; Szapkievich, V. (1999): Distribution and population size of the southern sea lion (*Otaria flavescens*) in central and southern Chubut, Patagonia, Argentina. Mar. Mammal Sci. **15,** 478–493.

Riedman, M. L. (1990): The Pinnipeds. Seals, Sea Lions and Walruses. Los Angeles and Oxford: University of California Press and Oxford University Press.

Rodríguez, D. H. (1996): Biología y ecología de los pinnípedos del sector bonaerense. Diss. thesis, Universidad de Mar del Plata.

Schneider, S.; Roessli, D.; Excoffier, L. (2000): Arlequin, Version 2000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Siegel-Causey, D. (1997): Molecular variation and biogeography of rock shags. Condor **99,** 139–150.

Szapkievich, V. B. (1992): Estimación de la población del lobo marino de un pelo Otaria flavescens (Shaw, 1800) en el litoral patagónico mediante fotografías obtenidas en relevamientos aéreos. Diss. thesis, Universidad de Buenos Aires.

Szapkievich, V. B.; Cappozzo, H. L.; Crespo, E. A.; Bernabeu, R. O.; Comas, C.; Mudry, M. D. (1999): Genetic relatedness in two Southern sea lion (*Otaria flavescens*) rookeries in the southwestern Atlantic. Z. Säugetierkunde **64,** 246–250.

Szteren, D.; Páez, E. (2002): Predation by southern sea lions (*Otaria flavescens*) on artisanal fishing catches in Uruguay. Mar. Fresh. Res **53,** 1161–1167.

Trujillo, R. G.; Loughlin, T. R.; Gemmell, N. J.; Patton, J. C.; Bickham, J. W. (2004): Variation in microsatellites and mtDNA across the range of the Steller sea lion, *Eumetopias jubatus*. J. Mammalogy **85,** 338–346.

Vaz-Ferreira, R. (1982). *Otaria flavescens* (Shaw), South American sea lion. In: Mammals in the Seas. Small cetaceans, seals, sirenians and otters. Ed. FAO and UNEP. Rome: FAO Fisheries Series. Pp. 477–495.

Vaz-Ferreira, R.; Ponce de León, A. (1984): Estudios sobre *Arctocephalus australis* (Zimmermann, 1783) lobo de dos pelos sudamericano en Uruguay. Contribuciones **1,** 1–19.

Wang, J. Y. (2002): Stock identity, In: Encyclopaedia of Marine Mammals. Ed. by W. F. Perrin; B. Wursig and J.G.M. Thewissen. San Diego: Academic Press. Pp. 1189–1192.

Weber, D. S.; Stewart, B. S.; Lehman, N. (2004): Genetic consequences of a severe population bottleneck in the Guadalupe fur seal (*Arctocephalus townsendi*). J. Hered. **95,** 144–153.

Wynen, L. P.; Goldsworthy, S. D.; Insley, S. J.; Adams, M.; Bickham, J. W.; Francis, J.; Gallo, J. P.; Hoelzel, A. R.; Majluf, P.; White, R. W. G.; Slade, R. (2001): Phylogenetic relationships within the eared seals (Otariidae: Carnivora): implications for the historical biogeography of the family. Mol. Phylog. Evol. **21,** 270–284.

Authors' addresses:
Juan Ignacio Túnez, Departamento de Ciencias Básicas, Universidad Nacional de Luján, Rutas 5 y 7 (6700), Luján, Provincia de Buenos Aires, Argentina (e-mail: jitunez@datafull.com)
Daniela Centrón, Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 (1121), Buenos Aires, Argentina Humberto Luis Cappozzo, Estación Hidrobiológica de Puerto Quequén, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-CONICET), Av. Ángel Gallardo 470 (C1405DJR), Buenos Aires, Argentina
Marcelo Hernán Cassini, Departamento de Ciencias Básicas, Universidad Nacional de Luján, Rutas 5 y 7 (6700), Luján, Provincia de Buenos Aires, Argentina and Fundación Organización PROFAUNA, Corrientes 1145, 4° oficina 47 (1043), Buenos Aires, Argentina