Mitochondrial DNA sequence data of the Cape fur seal (Arctocephalus pusillus pusillus) suggest that population numbers may be affected by climatic shifts

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RESEARCH ARTICLE

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Mitochondrial DNA sequence data of the Cape fur seal (*Arctocephalus pusillus pusillus*) suggest that population numbers may be affected by climatic shifts

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Abstract The Cape fur seal, *Arctocephalus pusillus pusillus* is distributed along the southern African coastline from northern Namibia to the south-east coast of South Africa. The species has been impacted by sealing operations since the 1600s, and historical records suggest that the taxon experienced a bottleneck prior to the 20th century. Mitochondrial DNA control region sequences were generated for 106 individuals belonging to six breeding colonies. Haplotype diversity was found to be high (0.975 ± 0.014) whereas levels of nucleotide diversity were much lower compared to other seal species (0.011 ± 0.006). An analysis of molecular variance indicated that the largest percentage of haplotype diversity is distributed within colonies rather than among them. This could be attributed to either extensive gene flow among colonies, a lack of substantial female site philopatry, or incomplete lineage sorting of haplotypes. Mismatch distribution and Fu's F_S test indicated that the population has experienced a historical population expansion probably between *c*. 37,000–18,000 YBP and this date coincides very well with the height of the last glacial maximum when food resources were abundant in the South Atlantic. These results also suggest that the recent sealing-induced bottleneck did not have a profound influence on the haplotype diversity and a historical bottleneck prior to a demographic expansion may have been severe enough to reduce nucleotide diversity substantially.

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Introduction

The fur seal, *Arctocephalus pusillus*, has a disjunct distribution in the southern hemisphere and two subspecies have been recognized. The Cape fur seal, *A. p. pusillus*, occurs along the south-west African coastline while the Australian fur seal, *A. p. doriferus* occupies a section of southern and south-eastern Australian coastline. These two taxa are phylogenetically closely related and Lento et al. (1997), after investigating mtDNA cytochrome b sequence variation, argued that the current sub-specific status of the two taxa should remain based on geographic isolation and minor differences in skull morphology (Repenning et al. 1971). Although, the Cape fur seal is a highly mobile species with individuals observed up to 2,000 kilometres from their breeding sites (Kerley 1983; Thibault 1999), the species keep predominantly to the continental shelf (Oosthuizen 1991). It thus seems reasonable to suggest that long distance dispersal between the two subspecies is very limited (Rand 1959; King 1983; Brunner et al. 2002).

At present, approximately 1.7 million Cape fur seals aggregate in 34 colonies around the southern African coastline (also see Oosthuizen 1991). Twenty-five of these colonies are permanent breeding settlements (Butterworth et al. 1995) and it has been proposed that the species might show some level of female site philopatry (King 1983). Mark-recapture data indicate that some females do not return to the breeding colonies at which they were tagged as newly born pups (Oosthuizen 1991). Importantly, molecular studies on related species (*A. gazella* and *A. tropicalis*) suggest mainly male-mediated dispersal with no strong support for female philopatry (Wynen et al. 2000).

Historical records suggest that Cape fur seals experienced an anthropogenically induced bottleneck in the recent past through sealing activities, leading to substantial eradication of breeding colonies prior to the 20th century (Shaughnessy 1984). In 1893 the South African Fish Protection Act was implemented to allow only permit holders to harvest seals, resulting in rapid recovery with the number of breeding colonies increasing from 17 (Rand 1959) to 25 (Butterworth et al. 1995). In addition, this species has been impacted in the northern part of their distribution in Namibia through natural mass mortality and this coincides well with El Niño events that caused food scarcity and mass mortality in other seal species (Gerber and Hillborn 2001). This recent reduction in seal numbers could potentially affect the genetic variability in *A. pusillus pusillus* (for example human induced mortalities caused a reduction in the genetic heterozygosity found in the Guadalupe fur seal, *A. townsendi*—Hoelzel 1999; Weber et al. 2004).

By making use of mtDNA control region sequences we examined the partitioning of genetic variability across breeding colonies in *A. p. pusillus*. Substantial differences among colonies could provide evidence for female philopatry. In addition, the effect of recent sealing activities on the genetic variability within this species is unknown and therefore investigated.

Material and methods

Samples

Between 1998–2004 flipper clippings were collected from 106 pups younger than six months of age from six breeding colonies in Namibia and South Africa (Fig. 1). Pups were targeted in order to exclude individuals that were not born on the colony sampled and to exclude the inclusion of siblings (a single pup is born per female per annum, Wickens and York 1997). Six colonies were selected to represent a range of geographic localities. Two colonies in South Africa (Kleinzee and Black Rocks) were comprehensively sampled (N = 30 individuals each) in order to minimize the effects of low statistical power due to small sample sizes. The four Namibian colonies (Atlas Bay, Cape Cross, Cape Frio and Van Reenen Bay) were less intensively sampled (9–11 individuals each) due to logistical constraints.

Laboratory analyses

Total genomic DNA was isolated by a standard proteinase-K digestion and phenol/chloroform extraction (Sambrook et al. 1989). A segment of the hypervariable region I in the mtDNA control region was amplified by the polymerase chain reaction with primers L15926 (Kocher et al. 1989) and TDKD (Slade et al. 1994) following standard PCR procedures (94°C for 30 s for initial denaturation, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 30 s). Amplified PCR products were agarose gel-purified and cycle sequencing was performed using an ABI 3100 Genetic Analyser.

Data analyses

Sequences were deposited in Genbank (DQ176872–DQ176977) and aligned by eye. Haplotype (h) and nucleotide diversity (π) were estimated for each colony and an analysis of molecular variance (AMOVA) was run to estimate the amount of variation among and within colonies using Arlequin 2.0 (Schneider et al. 2000). Genetic differentiation was estimated using F_{ST} and ϕ_{ST} with the Tamura-Nei model of evolution with gamma correction (α = 0.80) estimated using maximum likelihood in PAUP 4.0b10 (Swofford 2002).

A mismatch distribution (Rogers and Harpending 1992) was constructed to examine the recent demographic history of the population (combined data for all the colonies). To quantify the smoothness of the observed haplotype frequency distribution, and thus to distinguish between populations recently expanded and populations at equilibrium Harpending's raggedness index (Harpending 1994) and Fu's F_S test of selective neutrality (Fu 1997) were applied (Harpending et al. 1998). The model proposed by Rogers and Harpending (1992) was used to determine a rough estimate of time since coalescence. For this estimate, generation time was set at four years (time to sexual maturity for *A. p. pusillus* females, Wickens and York 1997) and three potential mutation rates 5, 8 and 10% per lineage per million years following Slade et al. (1994).

To examine geographic structure among the colonies, a median-joining network was constructed taking into account the pairwise differences between haplotypes using Network 4.1.0.0 (Flexus Technology Inc.). To investigate whether *A. p. pusillus* is monophyletic with respect to the Australian subspecies, *A. p. doriferus*, four mtDNA control region sequences from the latter were downloaded from GenBank (accession numbers AF384392–AF384395; Wynen et al. 2001). Three *A. australis* (AF384402, AF384401, AF384400; Wynen et al. 2001) and three *A. tropicalis* (AF384385, AF384384, AF384383; Wynen et al. 2001) sequences were used as outgroup. The 5′ side of the alignment contained a C/T repetitive region and 19 base pairs were excluded from the alignment. The phylogeny was thus based on the remaining 342 characters and a neighbor-joining tree was constructed using uncorrected sequence distances in PAUP* 4.0b10. Confidence in nodes were determined by 1,000 bootstrap replicates.

Results

For the consensus region of 361 bases, there were 57 different haplotypes for the 106 *A. p. pusillus* sequences (h = 0.975 ± 0.006). Nucleotide diversity (π = 0.011 ±

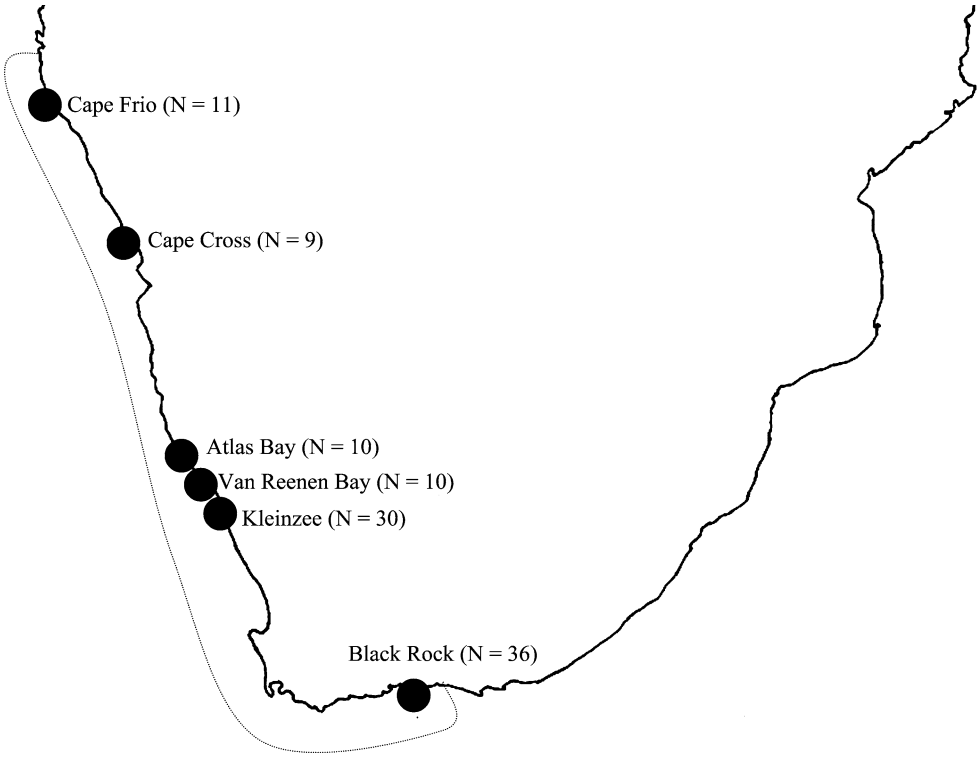

Fig. 1 *Arctocephalus pusillus pusillus* sampling localities and number of individuals (*N*) used in the present study. The distribution of the species along the southern African coast is indicated by the *dotted line*

0.006) was fairly low and haplotype and nucleotide diversity values within colonies were similar to that for all the colonies combined. Analysis of molecular variation (AMOVA) indicates that all of the variation is partitioned within colonies ($F_{ST} = 0$, $P > 0.05$). Pair wise comparisons between colonies revealed a maximum F_{ST} value of 0.025 for the Cape Frio and Black Rocks (Algoa Bay) comparison, which are the two breeding colonies on the extreme opposite of its range, but this was not significant ($P > 0.05$). All other pairwise comparisons between colonies were also not significant. The ϕ_{ST} comparisons also failed to show any significant difference among colonies.

The haplotype network had multiple single step reticulations among haplotypes and to improve visual interpretation we present one haplotype tree out of 12 equally parsimonious solutions (Fig. 2a). No single haplotype was found to be dominant and the average number of mutational steps separating haplotypes was 4.04 ± 2.03.

Fu's F_S test of selective neutrality yielded a significant negative result ($F_S = -25.92171$, $P < 0.01$), which indicates that the population is not in equilibrium. The observed mismatch distribution closely matches that expected given a recent population expansion (Fig. 2b). Harpending's raggedness index also provided statistical support for these results ($r = 0.023$, $P = 0.220$). The τ value estimated from the mismatch distribution ($\tau = 5.321$) provides an estimate of the time since expansion given in mutational units. Hence, it can be useful in determining the absolute time since the demographic expansion, provided the mutation rate is known. Given that the mutation rate for this species has not been estimated, we utilised 5, 8, and 10% rates as previously reported for pinnipeds (Slade et al. 1994, 1998) and estimate the times since divergence as 36,849, 23,030 and 18,424 years, respectively. The neighbor-joining tree supports the monophyly of *A. pusillus* (100% bootstrap) and the *A. p. doriferus* individuals were nested among the southern African subspecies (Fig. 3).

Discussion

The subspecies dilemma

The mtDNA control region sequences support previous suggestions that the two *A. pusillus* subspecies are not genetically distinct from each other (Fig. 3; Lento et al. 1997; Wynen et al. 2001). In fact, there is more mtDNA control region divergence within *A. p. pusillus* than between the two subspecies (*A. p. pusillus* vs *A. p. doriferus*). These results confirm that *doriferus* and *pusillus* share a recent common ancestor and gives credence to long-range dispersal between the continents (Lento et al. 1997). The frequency and timing of dispersal events can only be resolved through more comprehensive sampling, which will also allow for a better taxonomic assessment regarding the sub-specific status of the two taxa.

Population structure and female site philopatry

It is clear from the haplotype tree (Fig. 2a) that there is no geographical partitioning of haplotypes, and indeed statistical support was given by the AMOVA for a lack

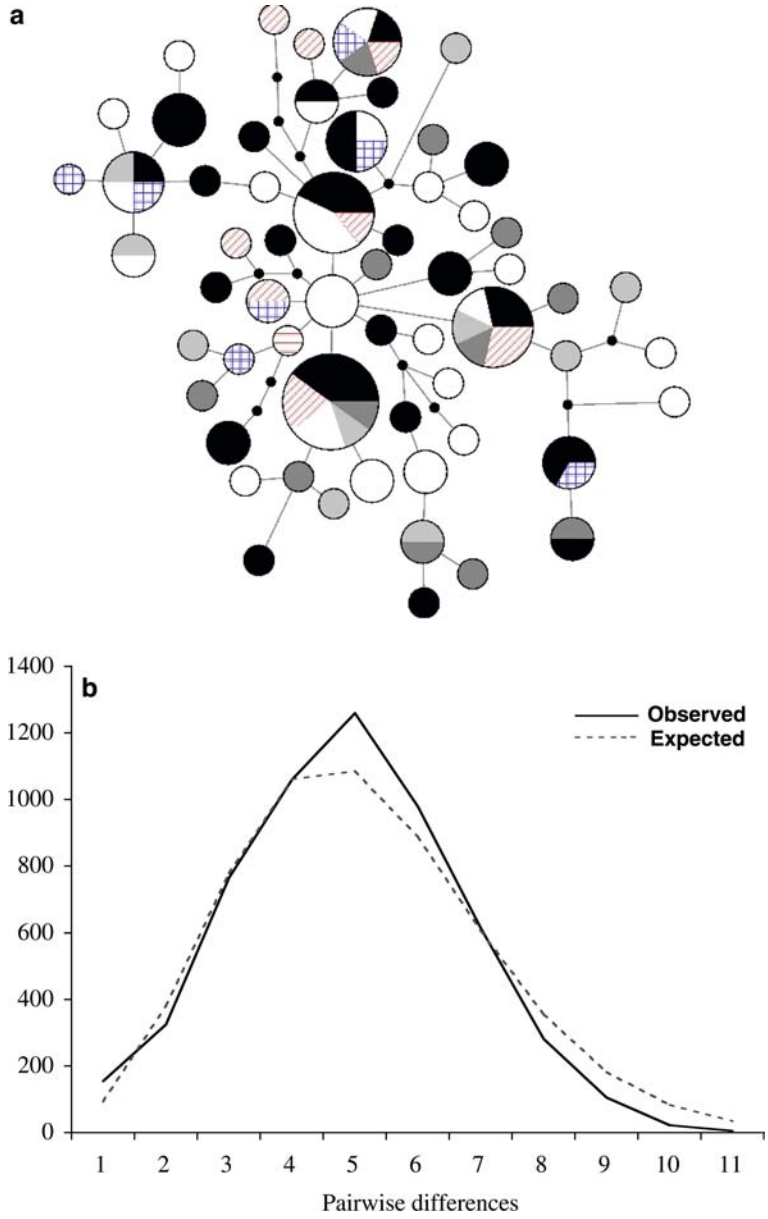

Fig. 2 **a** Minimum spanning tree for the 106 *Arctocephalus pusillus pusillus* individuals in the present study. Six colonies are represented (Cape Cross *cross hatching*; Cape Frio *solid dark grey*; Van Reenen Bay *diagonal grey*; Kleinzee *solid white*; Black Rocks *solid black* and Atlas Bay *solid light grey*). The size of the circles represents the frequency of haplotypes and the line length corresponds to the distances among haplotypes. **b** Mismatch distribution for 106 *Arctocephalus pusillus pusillus* control region sequences, where the number of pairwise differences among individuals is plotted against frequency of occurrence. Both the observed (*solid line*) and expected (*dotted*) distributions are shown

of significant variation among colonies. The apparent lack of significant geographically orientated mtDNA population structure could be because *A. p. pusillus* females are not philopatric, and the results from this study would support extensive female gene flow among colonies. Considering other fur seal species, no strong molecular support for female philopatry was found in either the Antarctic (*A. gazella*) or subantarctic (*A. tropicalis*) fur seals (Wynen et al. 2000) and Cape fur seals also seem to fit this pattern. Furthermore, the current lack of differences among colonies may be a consequence of the recent extirpation of some colonies during sealing activities, with the subsequent recolonisation from a number of different source colonies. For example, unpublished counts done at Cape Cross and Black Rocks suggest that they are very old colonies going back hundreds of years. In contrast, the Kleinzee, Van Reenen Bay and Atlas Bay assemblages are relatively new, most likely younger than 100 years. Cape Frio is a new breeding colony less than 20 years old. Recolonisation into areas where populations have gone extinct is common when recent bottlenecks or local extinctions have occurred (Slatkin 1985). Thus, the lack of genetic structure could be a combination of high-gene flow among colonies, and recent migrations into formerly extirpated breeding colonies.

Variation within the species

Haplotype diversity in *A. p. pusillus* ($h = 0.975 \pm 0.006$) is similar to the pre-bottleneck values detected in the Guadalupe fur seal, *A. townsendi* ($h = 0.997 \pm 0.012$; Weber et al. 2004) and significantly higher than that

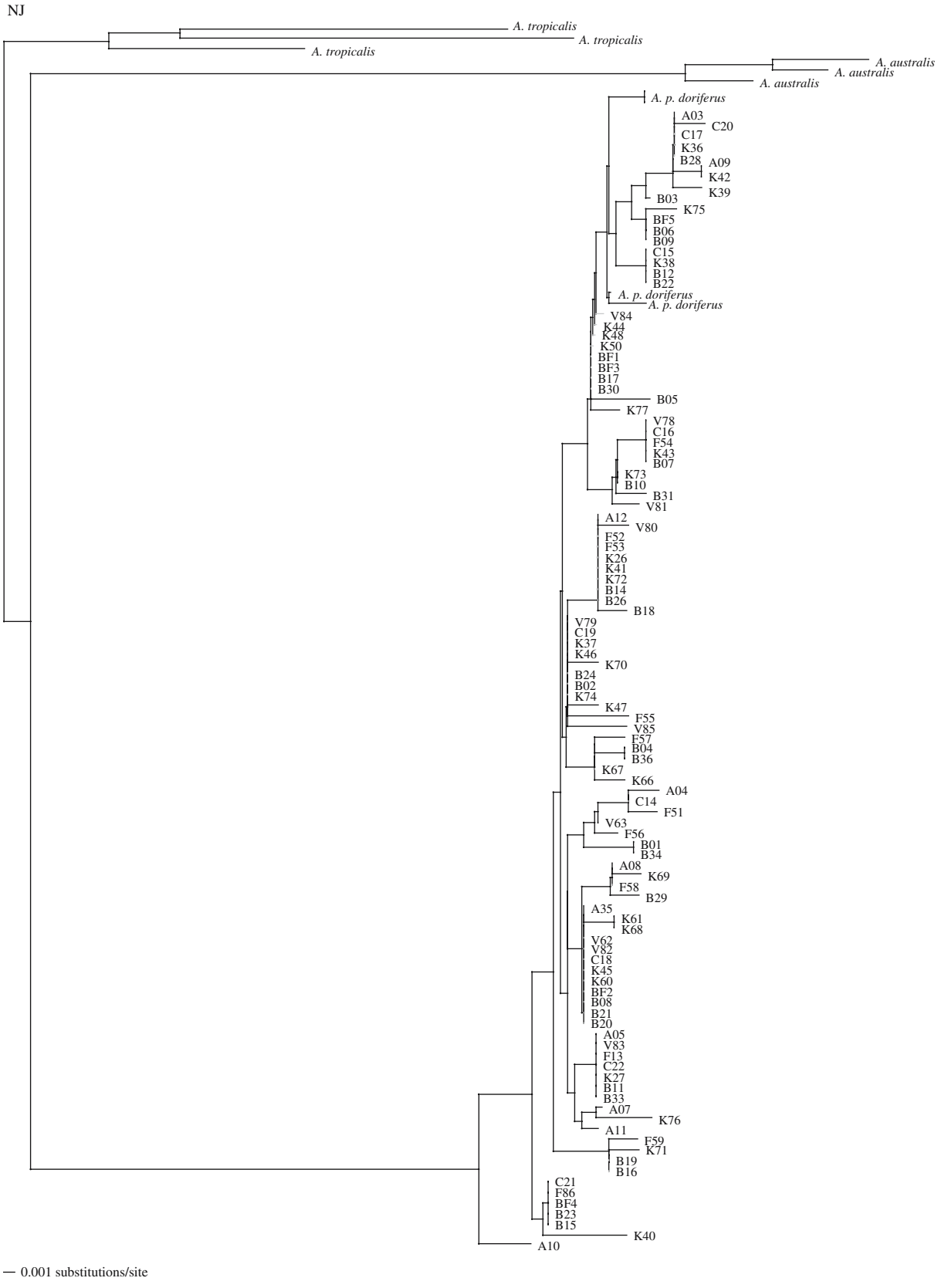

Fig. 3 Neighbour-joining tree of mtDNA control region haplotypes of *A. pusillus pusillus* from South Africa and *A. pusillus doriferus* from Australia. *A. p. doriferus* and the outgroups are indicated by name and the sampling localities for *A. pusillus pusillus* are indicated as follows: *C* Cape Cross; *F* Cape Frio; *V* Van Reenen Bay; *K* Kleinzee; *B* Black Rocks; *A* Atlas Bay

reported for the Juan Fernández fur seal (*A. phillippii*, $h = 0.905$ (± 0.034)). The latter species was heavily impacted by sealing and thought nearly extinct by the late 1800s (Goldsworthy et al. 2000). In contrast, however, the nucleotide diversity of *A. p. pusillus* ($\pi = 0.011 \pm 0.006$) is lower than that reported for *A. phillippii* ($\pi = 0.030 \pm 0.006$) and *A. townsendi* (pre-bottleneck $\pi = 0.055 \pm 0.004$ and post-bottleneck $\pi = 0.025 \pm 0.003$; Bernardi et al. 1998 and Wynen et al. 2001). This indicates that although the Cape fur seal, *A. p. pusillus* is characterised by a relatively large number of unique mtDNA haplotypes, the diversity among the haplotypes is low compared to the other two species of fur seal.

One of the main reasons for low-genetic diversity could be a recent bottleneck as was the case for the Jan Fernández fur seal (Weber et al. 2004). The high levels of haplotypic diversity, but low-nucleotide diversity, in the Cape fur seal could be attributed to rapid population growth that followed an initial bottleneck. In other words, low-nucleotide diversity during a bottleneck, followed by a rapid increase in population size accompanied by many new but closely related haplotypes would produce the observed pattern of haplotype and nucleotide diversity in the Cape fur seal. Quick population recoveries have been reported in other species belonging to the genus *Arctocephalus* (Goldsworthy et al. 2000; Wynen et al. 2000; Weber et al. 2004) and may have been possible for the Cape fur seal.

A population expansion for *A. p. pusillus* is supported by the mismatch distribution (Fig 2a), low Harpending's raggedness index values and Fu's F_S test showing that the population is not in mutation-drift equilibrium. From the mtDNA analyses it appears that such a population expansion would date back to at least 18,000 years before present and could possibly be as old as 38,000 years. If this is the case, then it seems reasonable that the reduction in *A. p. pusillus* numbers caused by sealing and El Niño events during the last four centuries did not cause a severe genetic bottleneck that influenced the mitochondrial DNA diversity of this species, but that older events have had a more profound effect on this species.

It has been suggested in the past that climate change linked to El Niño events can cause mass reductions in the survival of seal pups (Gerber and Hillborn 2001). Our study tends to support the hypothesis that Cape fur seal numbers is positively correlated with primary oceanic productivity. Glacial periods, especially glacial maxima, are characterised by high-ocean productivity (Martin 1990), and the timing of the population expansion in the Cape fur seal, (37,000 and 18,000 years ago), overlaps very well with the last glacial maximum which peaked approximately 22,000 to 19,000 years before present (Yokoyama et al. 2000). Importantly, however, the nutrient content of oceanic waters is thought to fluctuate substantially during glacial/interglacial periods and particularly in the southern oceans between 22°S and 41°S (Martin 1990; Oppo and Horowitz 2000) and the LGM was terminated by an abrupt warming event approximately 15,000 YBP (Severinghaus and Brook 1999). If this holds, it would mean that Cape fur seal numbers fluctuate in sync with climatic changes that affect food availability and that Cape fur seal numbers are probably much higher during glacial than during interglacial periods.

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