

Effects of Quaternary climatic changes on the phylogeography and historical demography of the subterranean rodent *Ctenomys porteousi*

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Abstract

The contemporary distribution of organisms cannot be understood without knowing how species have responded to the geologic and climatic history of their environments. Genetic studies related to the demographic history of wildlife species can help us to elucidate the role of climate changes and other environmental forces in shaping patterns of distribution and population structure of the species. In this work we investigated the phylogeographic pattern and historical demography of the subterranean rodent *Ctenomys porteousi*. We examined mitochondrial DNA control region sequences of 101 individuals collected from 7 localities that cover the complete distributional range of this species. Haplotype frequencies showed a significant population differentiation whereas the spatial distribution of haplotypes suggests moderate geographical structure. Genetic differentiation was not consistent with a simple model of isolation by distance and several independent estimates suggest that the observed phylogeographical pattern is the consequence of a complex demographic scenario. Our data suggest both reduction and population expansion events. Both kinds of demographic events were associated to major climatic changes that affected the study area during the Late Pleistocene and the Holocene. In particular, a relationship between historical changes in the degree of vegetation cover and population size for this rodent was inferred. We propose that the decrease in aridity of the Pampean region that started in the Pleistocene–Holocene boundary could have promoted a major decline in the effective population size of this species.

Introduction

Inferring past demographic changes in wildlife populations is an essential step to understand the zoogeographical history of regions (Storz, Beaumont & Alberts, 2002), allowing the identification of factors that shaped the distribution of species over space and time (Drummond *et al.*, 2005; Goossens *et al.*, 2006). If reliable estimates of the time when demographic changes occurred can be achieved, this information can be matched to the time of occurrence of environmental events, providing a powerful tool for identifying the environmental factors that could have been involved in shaping the population dynamics of a given species (Heller *et al.*, 2008).

Historical demographic processes such as population subdivision, range reductions or expansions or, alternatively, long periods of demographic stability, have contrasting effects on the patterns of spatial distribution of genetic variation (Hewitt, 1996; Matocq, Patton & Da Silva, 2000; Mora *et al.*, 2007). The phylogenetic signals of these

processes can be used to reconstruct population demographic histories (Avice, 2000; Hewitt, 2000; Ruzzante *et al.*, 2008).

Environmental changes associated with Quaternary glacial cycles are known to have altered the distribution and genetic structure of species in the Northern Hemisphere (Avice, 2000; Hewitt, 2000). In South America, the extent of glaciation was notably lower than in North America and Europe (Clapperton, 1993). Although environmental conditions changed notoriously also in nonglaciated regions (Quattrocchio *et al.*, 2008), their effects on the South American fauna and flora are poorly known (Lessa, Cook & Patton, 2003; Ruzzante *et al.*, 2008).

South American subterranean rodents of the genus *Ctenomys* (tuco-tucos) are a good model to study the demographic responses of populations to Quaternary climate change. Being highly restricted to sandy and friable soils, these rodents show specialized habitat requirements (Busch *et al.*, 2000; Antinuchi *et al.*, 2006). Tuco-tucos show important restrictions in habitat use associated mainly to

the amount of plant cover (Mapelli & Kittlein, 2009) and exhibit low rates of migration and gene flow among populations (Wlasiuk, Garza & Lessa, 2003; Fernández-Stolz, Stolz & De Freitas, 2007; Mora *et al.*, 2010). These characteristics suggest that changes in the extent and degree of fragmentation of their characteristic habitats (and the demographic processes associated with them) might have produced conspicuous signals in the geographic distribution of genetic variation.

Ctenomys porteousi is a highly endemic species, occupying a small habitat extension in west-central Buenos Aires province, Argentina. Its habitat comprises a paleodune system originated and modified during the Quaternary. The total area of suitable habitat has been estimated in only 509 km² (Mapelli & Kittlein, 2009). This habitat is naturally fragmented, but in recent years the degree of fragmentation has been notably increased because the expansion of soybean cultivation in the region (Mapelli & Kittlein, 2009), imposing a great threat to the viability of the species, which is declining because of habitat loss along much of its range (Díaz & Ojeda, 2000). Experts consider it as close to qualifying for vulnerable (IUCN Red List: <http://www.iucnredlist.org/apps/redlist/details/5823/0>).

Here we assessed the pattern of genetic differentiation in *C. porteousi* using mitochondrial DNA (mtDNA) control region sequences from a phylogeographic and population genetic perspective and established associations between historical changes in effective population size and major climatic changes occurred during the late Quaternary.

Materials and methods

Sample collections

We obtained tissue samples from a total of 101 individuals of *C. porteousi* (see Mora *et al.*, 2006 for details on the capture and handling of animals). Samples comprised between 12 and 16 individuals for each one of seven localities along the entire distributional range of this species (Fig. 1, Table 1). All sampling localities consisted of a discrete habitat patch located in areas with sandy and friable soils

with little vegetation cover (for a better description of the habitat see Mapelli & Kittlein, 2009).

DNA extraction, PCR amplification and sequencing

Total DNA extractions were performed following Mora *et al.* (2006). A fragment of 420 bp of the 5' hypervariable portion of the mitochondrial DNA control region was amplified by polymerase chain reaction (PCR) from all specimens using the primers TucoPro (5'-TTC TAA TTA AAC TAT TTC TTG-3', Tomasco & Lessa, 2007) and TDKD (5'-CCT GAA GTA GGA ACC AGA TG-3', Kocher *et al.*, 1989). Amplification was carried out following Mora *et al.* (2007). Amplicons were purified with shrimp alkaline phosphatase and exonuclease I (Amersham Biosciences). These final PCR products were sequenced with a capillary sequencer ABI3100 (Macrogen Inc., Korea), with the primers used in PCR.

Electropherograms were scored and analyzed using Chromas 2.01 (Technelysium, Helensvale, Qld, Australia) and aligned using CLUSTAL X (Thompson *et al.*, 1997).

Phylogenetic, population genetic and demographic analyses

Haplotype and nucleotide diversity were calculated to estimate DNA polymorphisms in each locality using DNAsp 4.0 (Rozas *et al.*, 2003) and ARLEQUIN 3.0 (Excoffier, Laval & Schneider, 2005).

For examining the overall level of genetic divergence among localities we used the F_{ST} statistics based on sequence data (Φ_{ST} , Hudson, Slatkin & Maddison, 1992). Significance of these values was evaluated using 1000 permutations in ARLEQUIN 3.0 (Excoffier *et al.*, 2005). Pairwise estimates of Φ_{ST} and linear geographic distances among localities were used to examine the fit of the data to an isolation by distance pattern (Slatkin, 1993). A Mantel (1967) nonparametric test was used to assess the significance of the correlation between estimates of population divergence and geographic distances using 1000 permutations of the original matrices.



Figure 1 Map of the geographic distribution of *Ctenomys porteousi* in central Argentina. The names of the seven localities sampled are indicated. Geographic coordinates of these localities are: San Fermín (36°48'S, 62°36'W), Bonifacio (36°47'S, 62°18'W), La Calma (36°37'S, 62°13'W), El Inca (36°50'S, 62°09'W), La Manuela (36°48'S, 62°02'W), La Larga (36°44'S, 61°39'W) and Daireaux (36°34'S, 61°39'W).

Table 1 Number of sampled individuals, polymorphic sites, number of haplotypes and haplotypic and nucleotidic diversity for the sampled localities of *Ctenomys porteousi*

Locality	Sampled individuals	Number of polymorphic sites	Number of haplotypes	Haplotypic diversity	Nucleotidic diversity
San Fermin	15	7	2	0.47	0.0081
Bonifacio	13	8	4	0.72	0.0053
El Inca	12	10	5	0.74	0.0086
La Calma	16	13	4	0.67	0.0101
La Manuela	14	7	3	0.69	0.0068
La Larga	15	6	2	0.53	0.0077
Daireaux	16	2	3	0.71	0.0022

Phylogenetic relationships among haplotypes were analyzed by maximum parsimony (MP) and neighbor joining (NJ) methods using PAUP 4.0b10 (Swofford, 2002). MP analysis was performed with heuristic searches with 100 random addition sequence replicates and tree bisection reconnection. Alternatively, phylogenetic relationships were analyzed using Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). We used four independent runs of 5×10^6 iterations of Markov Chain Monte Carlo (MCMC) algorithm. Parameters were sampled with a thinning interval of 100 and the first 2×10^4 iterations were used as burn-in. In all phylogenetic analysis we used sequences of *Ctenomys rionegrensis* (Wlasiuk *et al.*, 2003; Genbank accession numbers AF538366) and *Ctenomys talarum* (Mora *et al.*, 2007; Genbank accession numbers EF531719) as outgroup. The Akaike information criterion and Bayesian information criterion implemented in jModeltest (Posada & Buckley, 2004; Posada, 2008) were used to select the nucleotide substitution model used in NJ and BI analysis. Support of internal nodes was tested using 1000 bootstrap replicates.

The relationship among haplotypes was also analyzed using a haplotype network. Median-joining methods (Bandelt, Forster & Röhl, 1999) as implemented in Network 4.5.1 (<http://www.fluxus-engineering.com>) was used to construct the haplotype network.

The historical demography of *C. porteousi* was inferred using different complementary approaches. First, we tested for sudden demographic expansion using mismatch distribution analyses. We employed the sum of squared deviations (SSD) statistic and the raggedness index (Rg) defined by Harpending (1994) to test the goodness of fit of the observed mismatch distribution to that expected under the sudden expansion model. Estimation and testing were done by bootstrap resampling (10 000 replicates) using ARLEQUIN 3.0 (Excoffier *et al.*, 2005). Second, to test for deviations from neutrality (as would be expected under demographic expansion) we used Tajima's *D* (Tajima, 1989) and Fu's *F*'s (Fu, 1997) tests. Significance of these statistics was tested using 1000 permutations in ARLEQUIN 3.0 (Excoffier *et al.*, 2005). These analyses were carried out separately for each locality and globally, including all sequences as belonging to the same population.

To estimate demographic changes through time we constructed Bayesian skyline plots (BSP) as implemented in BEAST 1.4.8 (Drummond & Rambaut, 2007). This approach

incorporates the uncertainty in the genealogy by using MCMC integration under a coalescent model, where the timing of divergence events provides information about changes in effective population size through time. MCMC tests were run for 2.5×10^7 iterations and sampled every 1000 steps under a relaxed lognormal molecular clock with uniformly distributed priors. The first 10% of iterations were discarded as a burn-in period. To assess the robustness of parameter estimates, four independent chains were run with identical settings. After checking the consistency on the parameter estimation, the chains were combined into a composite chain with 9×10^7 states using LogCombiner 1.4.8 (Drummond & Rambaut, 2007). Log-files were analyzed in TRACER 1.4.8 (Drummond & Rambaut, 2007), and effective sample sizes were used to evaluate MCMC convergence within chains. To convert coalescence times in years from the substitutions per site estimated by BEAST, it is necessary to use an appropriate molecular clock. Because molecular clock estimates are often dubious; we applied three different mutation rates. Initially, we used 1.5 and 6% per million year (Myr), which conservatively bracket the range of mtDNA mutation rates proposed for other small mammals (Martin & Palumbi, 1993). Additionally, we used a mutation rate of $4.42\% \text{ Myr}^{-1}$ estimated for the mitochondrial control region of the genus *Ctenomys* by M. S. Mora *et al.* (unpubl. data). This last estimation is based in sequences for three species of the family Octodontidae (*Spalacopus cyanus*, *Tympanoctomys barrerae* and *Octodon degus*), five species from the family Ctenomyidae (*C. sociabilis*, *C. australis*, *C. leucodon*, *C. pearsoni* and *C. torquatus*), and two direct fossil calibrations (differentiation between the families Ctenomyidae and Octodontidae is at least 9.13 Ma., and 3.5 Ma for the radiation of the genus *Ctenomys*, see Verzi, 1999; Vucetich, Verzi & Hartenberger, 1999; Verzi, Olivares & Morgan, 2010).

Results

Mitochondrial DNA sequence variation

A fragment of 420 bp was obtained for 101 individuals of *C. porteousi*. Along the whole fragment, 17 sites were polymorphic (all transitions) defining 12 haplotypes. Haplotypes sequences were deposited in GenBank under accession numbers JN196432 to JN196443. Overall nucleotide diversity was

0.01 and haplotype diversity was 0.89. Locality values are shown in Table 1.

Population structure and phylogeographical relationships

Genetic differentiation among localities was highly significant ($\Phi_{ST} = 0.37, P < 0.001$). All pairwise comparisons among localities were also significant ($\Phi_{ST} = 0.13-0.74$), indicating a moderate to high degree of population differentiation. Mantel tests did not show a significant correlation between geographic distances and pairwise estimates of population structure ($R = -0.093, P = 0.63$), thus failing to reveal a pattern of isolation by distance.

Results from jModeltest favored the HKY + G + I as the most appropriate evolutionary substitution model. Phylogenetic relationships among haplotypes obtained by different methods used here were highly concordant (only the phylogenetic tree obtained by BI is showed in Fig. 2). The mtDNA haplotypes of *C. porteوسي* conformed a monophyletic clade with a high bootstrap support in the node that separates them from the outgroups. Geographic structure is not clear in the ingroup sequences, showing many polytomies (MP and BI analysis) and low to moderate bootstrap

support (MP and NJ analysis). In general, there is limited geographic structure among localities, recovering all the sites as polyphyletic units.

The haplotype network (Fig. 3) had a complex topology and showed low geographic structure: haplotypes found in the same locality (except for those of Daireaux) were located at very distant parts within the network. There was no commonly distributed haplotype: the most widely distributed haplotype was present in only four localities (H4), and only three haplotypes were shared by more than two sites (H1, H8 and H9). Moreover, half of the haplotypes occurred in only one locality.

Historical demography

Mismatch distribution of pairwise differences among haplotype sequences was bimodal (Fig. 4), contrasting with the unimodal pattern expected in populations that have experienced a recent demographic expansion. However, both SSD ($SSD = 0.022, P = 0.22$) and Harpending's raggedness index ($R_g = 0.04, P = 0.18$) indicated that the observed mismatch distribution did not differ significantly from the distribution expected under population expansion. On the other hand, neutrality tests showed differing results: at a global level,

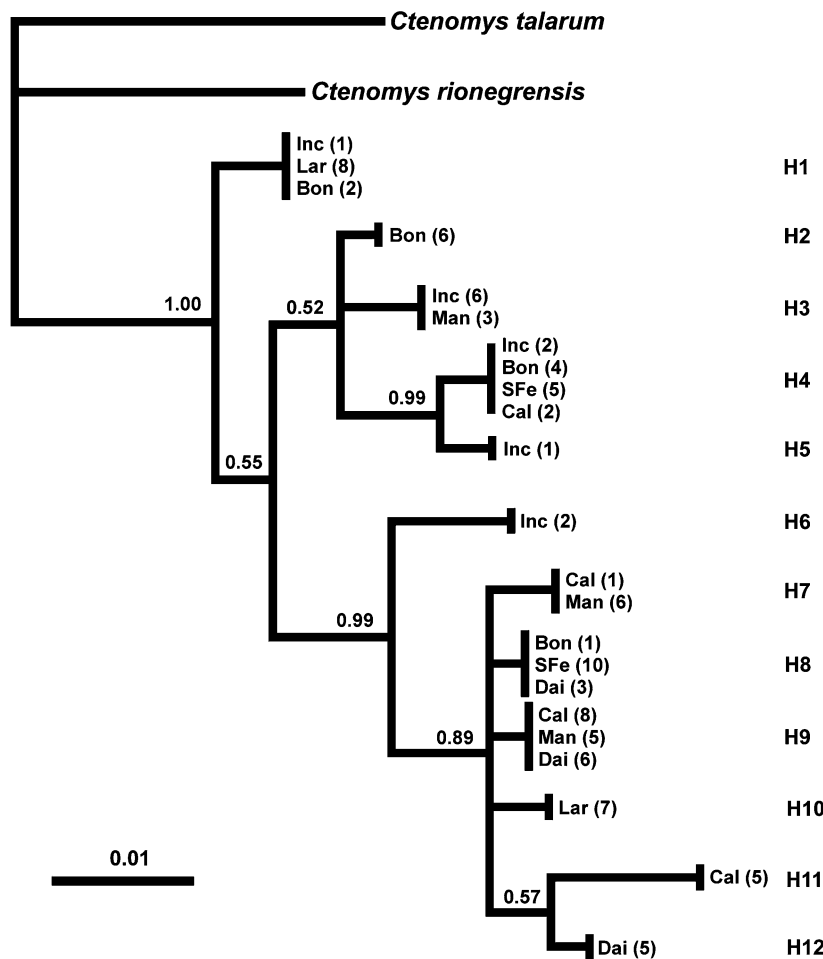


Figure 2 Phylogenetic relationship of *Ctenomys porteوسي* and two outgroups based on mitochondrial control region sequences obtained by Bayesian inference. Posterior probabilities based on 4500 post burn-in trees are shown above the branches. Abbreviations for locations are given in Table 1. Numbers next to locations indicate the sample size of each haplotype at each locality. H1–H12 identifies the haplotype number as in Fig. 3.

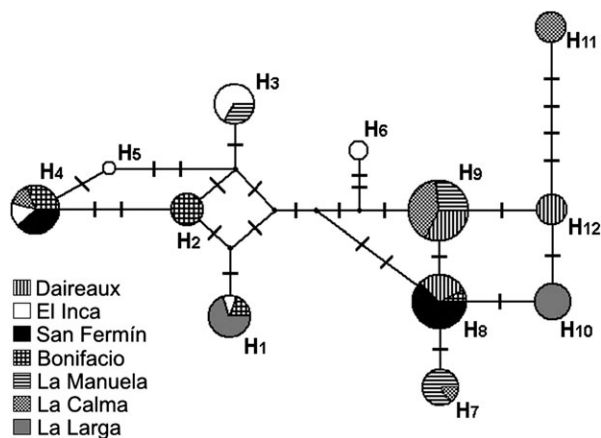


Figure 3 Median-joining haplotype network of *Ctenomys porteousi* mtDNA sequences. Circle sizes are proportional to haplotype frequencies. Haplotype numbers correspond to those of Fig. 2. Shading patterns indicates localities. The crossed marks are nucleotide substitutions inferred in that branch.

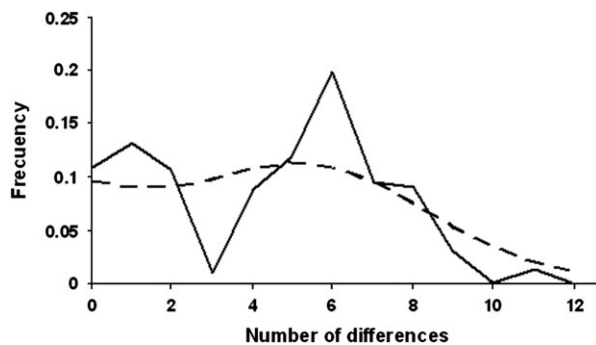


Figure 4 Mismatch pairwise distribution of mitochondrial control region sequences for *Ctenomys porteosui*. Solid line: observed distribution; dashed line: expected distribution under an exponential population expansion model.

Tajima's D test did not show significant deviations of strict neutrality ($D = 1.14$, $P = 0.89$) suggesting demographic stability; but Fu's F_s statistic showed a negative and significant value ($F_s = -25.73$, $P < 0.001$), indicating an excess of low frequency haplotypes, which is expected if *C. porteosui* experienced a recent demographic expansion. The same pattern (significance of Fu but no of Tajima test) was present in each locality when these were analyzed individually (supporting information Table S1).

The demographic scenario inferred from the BSP analysis showed a dual pattern: an initial long time of relatively constant population size followed by a recent period of marked demographic changes (Fig. 5). This period of changes started about 10 000 BP under a mutation rate of $4.42\% \text{ Myr}^{-1}$. Using 1.5 and $6\% \text{ Myr}^{-1}$ mutation rates, the beginning of this period gives 30 000 and 8000 BP, respectively. The first part of these changes implied a reduction in population size of *c.* 75%. A changing rate of decrease in population size is suggested, with steeper declines at the end

of this period. After this decline, an episode of demographic expansion was inferred. Considering a mutation rate of $4.42\% \text{ Myr}^{-1}$ this expansion began about 750 BP, with an interval of 2000–500 BP using 1.5 and $6\% \text{ Myr}^{-1}$ mutation rates, respectively. This tendency continued to nowadays and represents an increase of *c.* 30% in population size.

Discussion

A stepping-stone model is predicted to describe the relationship between gene flow and geographic distance in species with limited dispersal that are distributed in fragmented habitats. As a consequence, it is expected that the relationship between genetic differentiation and geographic distance fits an isolation by distance model (Slatkin, 1993). This pattern is expected to occur in subterranean rodents, which are characterized by present high degree of population structure, mainly as a result of occupy small and unconnected population demes (Wlasiuk *et al.*, 2003; Mora *et al.*, 2010).

Distribution of the genetic variation in *C. porteosui* does not fit to this pattern, possibly reflecting a lack of equilibrium between genetic drift and gene flow among localities. Although half of the haplotypes were restricted to a single locality, some of them have a wide geographic distribution, occurring in very distant localities. Therefore, population differentiation seem not to be produced by a combination of unique or closely related haplotypes, but it is a consequence of distinctive assortments of shared haplotypes.

Genetic equilibrium between the loss of alleles due to drift and their replacement by gene flow is reached when migration remains stable across the geographic range for long enough time (Slatkin, 1993). Demographic processes as well as changes in the geographic range delay the establishment of an isolation by distance pattern. Therefore, population genetic structure would reveal the imprinting of historical population processes, rather than current levels of gene flow (Milá *et al.*, 2000; Turgeon & Bernatchez, 2001; Mora *et al.*, 2007).

The absence of an isolation-by-distance pattern in species with restricted dispersal abilities like *C. porteosui* could be the consequence of a recent expansion of its distributional range (Wlasiuk *et al.*, 2003; Mora *et al.*, 2006). However, since *C. porteosui* is a highly endemic species with a very narrow distributional range, and a very limited availability of suitable habitat (see Mapelli & Kittlein, 2009) this hypothesis seems unlikely at first glance. On the other hand, partial evidence of a population expansion event is provided by Fu's F_s test, suggesting a significant excess of low-frequency haplotypes in *C. porteosui* at a global level. The same pattern was present at each locality, suggesting that the population expansion event included the whole distributional range of this species.

Differences in results of Fu's and Tajima's neutrality tests can be related to their statistical properties. Fu's test is more powerful for detecting demographic change and/or genetic hitchhiking than Tajima's test (Fu, 1997). Another insight of recent population expansion is provided by the results of the

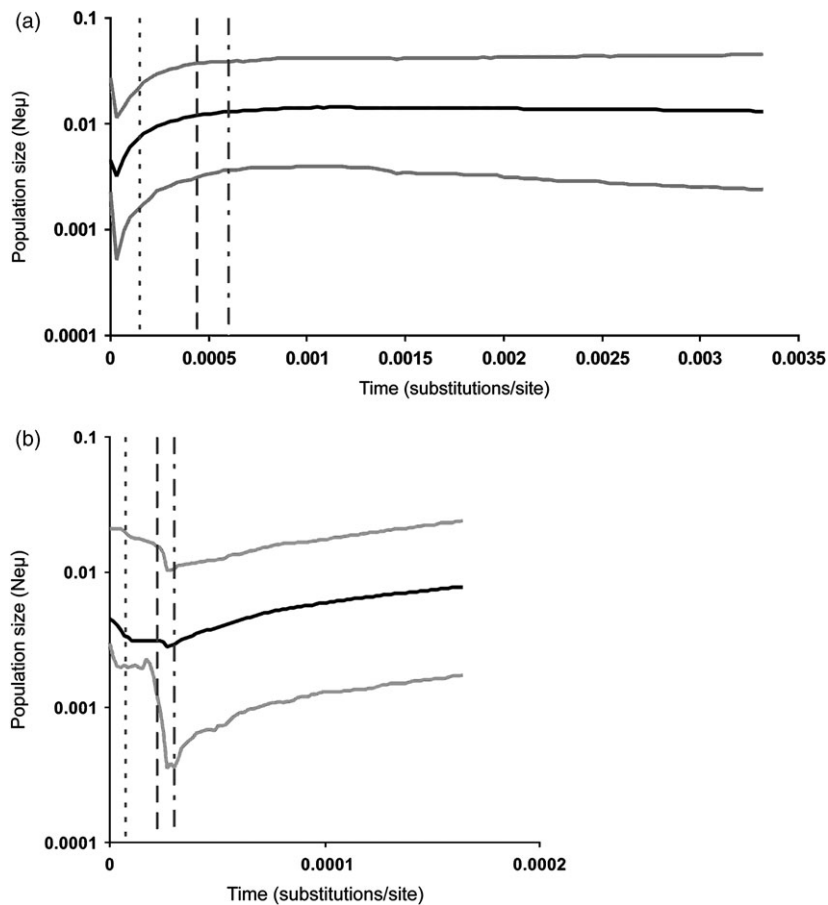


Figure 5 Bayesian skyline plot showing the effective population size fluctuations throughout time in *Ctenomys porteousi*. Black lines represent median estimations and grey lines represent the upper and lower 95% credible intervals. (a) Complete demographic reconstruction. The position of Pleistocene–Holocene boundary (10 000 years) is indicated based on a 1.5% (dotted vertical line), 4.42% (dashed vertical line) and 6% (dashed-and-dotted vertical line) mutation rates. (b) Details of demographic reconstructions for the last years before present. The beginning of Little Ice Age (500 years) is indicated based on a 1.5% (dotted vertical line), 4.42% (dashed vertical line) and 6% (dashed-and-dotted vertical line) mutation rates.

BSP demographic reconstructions, which suggested a recent growth event during the last years before present.

Nevertheless, some results did not suggest a demographic expansion event. Although tests related to mismatch distribution did not reject the population expansion hypothesis, the observed mismatch distribution was clearly not unimodal as expected if the population has recently expanded. In addition, the network of haplotypes is not characterized by a starlike topology and is not dominated by a few haplotypes widely distributed in all localities, as would be expected if a recent expansion had occurred. This apparent contradiction among different analyses would suggest that a single process of demographic expansion does not properly explain the genetic variation in *C. porteousi*.

Some haplotypes had a wide geographic distribution and were present at very distant localities. Given the high Φ_{ST} values recorded, the presence of the same haplotypes in distant localities can not be explained by profuse gene flow among sites and could reflect historical connectivity.

Events associated to strong genetic drift affecting populations after restrictions in gene flow could produce a pattern of haplotype distribution similar to observed in *C. porteousi*. Then, the presence of haplotypes with a wide geographic distribution could be evidence of historical connectivity, and high Φ_{ST} values among sites could be a consequence of

strong effects of genetic drift in populations that evolve under very limited gene flow. Strong reductions in population sizes could produce the elimination of some alleles at low frequencies in some populations (Fernandez-Stolz *et al.*, 2007), leading to a substantial reduction in haplotype diversity in some localities (La Larga and San Fermín, see Table 1). If migration is interrupted by several generations, bottleneck events could result in the elimination of alternative haplotypes at local populations. In this sense, strong genetic drift after reductions in effective population size might explain the occurrence of divergent haplotypes at some localities.

Demographic reconstructions point to a drastic reduction in population size starting around the Pleistocene–Holocene boundary. This episode would have increased genetic drift, with loss of low frequency haplotypes. As we said previously and was also suggested by Glenn, Stephan & Braun (1999) and Golani *et al.* (2007), divergent haplotypes within the same population can occur in species that undergone severe bottleneck processes. As a result of this, however, we would expect that pairs of individuals taken at random from the population differ in average by many nucleotide substitutions; resulting in a mismatch distributions with many modes at large values of pairwise differences (Rogers & Harpending, 1992; Harpending *et al.*, 1998). This is not the

pattern for *C. porteousi* at a global level. Therefore, the distribution of haplotypes among localities was unlikely brought about only by habitat fragmentation and subsequent bottlenecks.

Taking into account all the results in relation to the demographic dynamic in *C. porteousi*, we propose that the distribution of haplotypes among localities was brought about by a complex demographic history, including both population expansions and bottlenecks.

Demographic history of *C. porteousi* populations and environmental effects

The Pampean region, which includes Buenos Aires province, is a large ecotonal biome between the warm and wet Brazilian faunas and the cold and arid western and southern faunas prevailing in Patagonia today (Tonni, Cione & Figini, 1999). Last Cenozoic records in Buenos Aires province suggest climatic fluctuations alternating between arid and cold, and warm and humid conditions. These climatic changes have strongly affected the Pampean fauna, increasing alternatively the proportion of species of Brazilian and Patagonian origin (Tonni *et al.*, 1999).

Middle and Late Pleistocene paleoclimatic indicators in the Pampas suggest cold and dry climatic conditions (Deschamps & Ton, 1992; Iriondo & García, 1993; Quattrocchio *et al.*, 2008). Pampean area was apparently covered by desert vegetation growing on sand dunes and loess fields (Clapperton, 1993), while pollen records suggest strong aeolian activity which may also have caused a decrease of humid grasslands (León & Anderson, 1973; Grill, 1997). These climatic conditions would have sustained a wide distributed plant physiognomy similar to that of habitat patches currently occupied by *C. porteousi*. Since patch occupation in *C. porteousi* is negatively associated to plant cover (Mapelli & Kittlein, 2009), the availability of habitat for this species would have been considerably larger and less fragmented during this period; giving support to the view of larger population sizes and higher stability inferred from BSP analyses for the Middle and Late Pleistocene.

At about 10 000–9000 BP an important rise in temperature occurred in South America (Heusser, Steeter & Stuive, 1981; Clapperton, 1993). Palynological records suggest an increase in temperature and a decrease of arid conditions at Pleistocene–Holocene transition in Buenos Aires province (Borromei, 1995; Grill, 1997; Quattrocchio & Borromei, 1998). A rise of sea level and the flooding of the riverbeds may have favored the spread of inland grasslands, increasing the plant cover (Quattrocchio *et al.*, 2008). Moreover, about 5000–7000 BP an expansion of Brazilian fauna towards south-west suggests a continuous increase in temperature and humidity during the Middle Holocene (Tonni, 1990; Deschamps & Ton, 1992). Several paleoclimatic indicators suggest an increase of warm and humid conditions for the period 1400–600 BP (Cioccale, 1999; Tonni *et al.*, 1999; Prevosti, Bonomo & Tonni, 2004). In summary, several paleoclimatic indicators suggest a significant change in climatic conditions during the period comprising from

10 000 to 600 BP, associated to a marked decrease in aridity. Moreover, paleoclimatic records suggest an accentuation of these conditions at the end of this period.

Subterranean rodents are restricted to friable soils with low vegetation cover, where the cost of burrowing activities is lower and aeration of soils is higher (Vleck, 1979; 1981). Historical demographic reconstruction in *C. porteousi* suggests a reduction of *c.* 75% in population size. This reduction could have probably started around the Pleistocene–Holocene boundary and the rate of decline was higher at the end of this period. According to the paleoenvironmental data and, taking into account the habitat requirements of *Ctenomys* species (Mapelli & Kittlein, 2009), this marked population decrease could have been due to a significant reduction in the habitat availability in response to a rise in temperature and humidity.

A new climate change event is registered starting around 600–500 BP in the Central region of Argentina. This episode has been linked with the Little Ice Age (LIA), which occurred between the XVI and XIX centuries (Cioccale, 1999). During this period, arid and cold conditions were reestablished in the region repeating, with less intensity, climatic conditions prevailing during the Late Pleistocene (Iriondo & García, 1993; Prieto, 1996; Grill, 1997). The palynological records suggest that recurrence of a decrease in vegetation cover during this period (Quattrocchio & Borromei, 1998; Quattrocchio, Grill & Zavala, 1998). This scenario continued until the end of the XIX Century, when the actual climate conditions are established (Cioccale, 1999).

Demographic reconstructions of *C. porteousi* suggest an increase on population size in recent years. Using a wide interval of mutation rates, the beginning of this episode was dated between 2000–500 BP. Taking into account the strong relation of *Ctenomys* species to soils with low vegetation cover (Mapelli & Kittlein, 2009), this expansion could be related to an increase in aridity due to the LIA.

Thus, a relationship seems to exist between the major climatic changes suggested for the Late Pleistocene and Holocene in the Central region of Argentina and the historical demography inferred for *C. porteousi*. Given the match between temporal estimates of the two most relevant demographic changes in this species and the paleoenvironmental changes in the region; we suggest that variations in soil aridity was a key environmental factor responsible for the historical population sizes of *C. porteousi*. Alternative explanations for this complex demographic scenario can not, however, be discarded, particularly considering that the estimated times of demographic changes are based on a single locus.

Conclusions and perspectives

We propose the following scenario for the historical demography of *C. porteousi*: during the Late Pleistocene, populations of this rodent were large and highly connected along different areas of its distributional range, as suggested by the wide geographic distribution of some haplotypes and the

high population size inferred from BSP reconstructions. As a consequence of a decrease in the aridity of the Pampean region that started about 10 000 BP, habitat availability for the species decreased. Suitable habitat started to fragment, populations lost connectivity and decreased in size, favoring the occurrence of bottlenecks and increasing genetic drift. Bottlenecks were intense (75% of the population according to the BSP) and lasted long enough as to eliminate many low frequency haplotypes in isolated demes. A more recent process of population expansion, likely associated to the LIA event, affected the populations; but despite this, present population might be considered relictual when compared to the sizes they reached during the Late Pleistocene.

During the last decades, the area occupied by *C. porteousi* was intensively impacted by human activities. Soil compaction was incremented through forestations and the introduction of an exotic grass *Eragrostis curvula*. This exotic grass quickly reaches very high densities, producing the total exclusion of *C. porteousi* from these areas. On the other hand, the proportion of agricultural land augmented, increasing the rate of habitat alteration. The close associations between habitat suitability and population size inferred from historical demography and recent surveys (Mapelli & Kittlein, 2009) suggest that human impacts on grassland communities are having marked effects on the population dynamics of *C. porteousi*, threatening the viability of this species along much of its distributional range.

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Supporting Information

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

Table S1. Tajima and Fu neutrality tests for each locality.

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