

Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent

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Understanding how animal populations have evolved in response to palaeoenvironmental conditions is essential for predicting the impact of future environmental change on current biodiversity. Analyses of ancient DNA provide a unique opportunity to track population responses to prehistoric environments. We explored the effects of palaeoenvironmental change on the colonial tuco-tuco (*Ctenomys sociabilis*), a highly endemic species of Patagonian rodent that is currently listed as threatened by the IUCN. By combining surveys of modern genetic variation from throughout this species' current geographic range with analyses of DNA samples from fossil material dating back to 10 000 ybp, we demonstrate a striking decline in genetic diversity that is concordant with environmental events in the study region. Our results highlight the importance of non-anthropogenic factors in loss of diversity, including reductions in smaller mammals such as rodents.

Keywords: *Ctenomys sociabilis*; ancient DNA; phylochronology; climatic change; genetic variability; volcanism

1. INTRODUCTION

Tracing genetic lineages through time (phylochronology) bridges the gap between the micro- and macroevolutionary processes that determine faunal diversity (Hadly *et al.* 2004). When coupled with data on biotic and abiotic environments, such analyses bring us closer to a comprehensive understanding of species' responses to environmental change. Although climatic change and habitat loss are identified as contributing to reductions in faunal diversity (McLaughlin *et al.* 2002), few studies have examined the impacts of these processes over evolutionary time-scales. Studies of ancient DNA provide an important means of quantifying the effects of prehistoric events on faunal diversity, including reductions in species abundance and genotypic variability (Willerslev & Cooper 2005). Because studies of ancient DNA allow explicit comparisons of current versus historical patterns of genetic variability, they provide a direct

means of characterizing temporal changes in population- and species-level diversity.

The colonial tuco-tuco (*Ctenomys sociabilis*) is a medium-sized, highly endemic subterranean rodent whose range is currently restricted to an area of Neuquén Province, Argentina, of approximately 1500 km² where it inhabits patches of wet meadow known as 'mallines' (Lacey & Wieczorek 2003). Behaviourally, *C. sociabilis* is the only species of tuco-tuco (*n* greater than 35 species; Woods 1993) known to be social (Lacey 2000). Group living is associated with limited natal dispersal by members of both sexes (Lacey & Wieczorek 2004), which may be reinforced by the patchy distribution of mallín habitats (Lacey & Wieczorek 2003). These behavioural, demographic and ecological attributes are expected to lead to low levels of genetic variability within and high levels of genetic differentiation among local populations. Accordingly, a survey of 15 microsatellite loci revealed very limited genotypic variability within the population of *C. sociabilis* that has been the focus of behavioural and ecological studies of this species (Lacey 2001). Preliminary analyses of ancient DNA samples from a site located approximately 6 km southwest of this population (Estancia Nahuel Huapi) revealed the presence of only a single mitochondrial haplotype over the past 1000 years (Hadly *et al.* 2003), indicating that low levels of genetic variability have persisted in *C. sociabilis* for at least this many generations.

The geographic range of *C. sociabilis* is dominated by a steep west-to-east rainfall gradient created by the rainshadow effect of the Andes (Heusser 2003). Due to this gradient, historical region-wide shifts in climate patterns have caused significant changes in the distribution of the local flora and the location of the mesic forest-arid steppe ecotone where *C. sociabilis* occurs (Markgraf 1983; Pearson & Christie 1985). In addition, this area has a complex history of environmental change associated with volcanism (Heusser 2003), which may have dramatically affected the palaeoenvironment of this species. Collectively, these observations suggest that response to environmental change may be an important component of the evolutionary history of this species, including the low levels of genetic variability and restricted geographic range characteristic of *C. sociabilis* today.

2. MATERIAL AND METHODS

To characterize current haplotypic variability in *C. sociabilis*, five modern populations were sampled from localities spanning the species' current geographic range: (1) Cerro Monte Redondo (*n*=13); (2) Rincon Grande (*n*=9); (3) Altos del Fortin (*n*=9); (4) Paso Coihue (*n*=9); (5) Cerro La Lagunita (*n*=13; figure 1a). Animals were captured and non-destructive tissue samples were collected as described by Lacey (2001). MtDNA was extracted using the Qiagen DNAeasy Kit (Qiagen Inc., Valencia, CA) and cytochrome *b* (1140 bp) was amplified using primers MVZ05 (Smith 1998) and MVZ108 (E. A. Lacey, unpublished data) under the following conditions: 30–60 µg of DNA was added to 1X PCR buffer with 1.5 mM MgCl₂, 0.89 µM dNTP's, 0.22 µM of each primer, and 1.5 U of *Taq* DNA polymerase in total reaction volume of 27 µl (denature 94 °C for 5 min., 36 cycles of 94 °C 1 min., 48 °C 30 s, 72 °C 1 min., 5 min. extension at 72 °C). PCR products were sequenced in both directions on an ABI 377 automated sequencer.

Haplotypic variation in ancient DNA samples was examined using teeth collected from Cueva Trafal (40°43' S, 71°07' W,

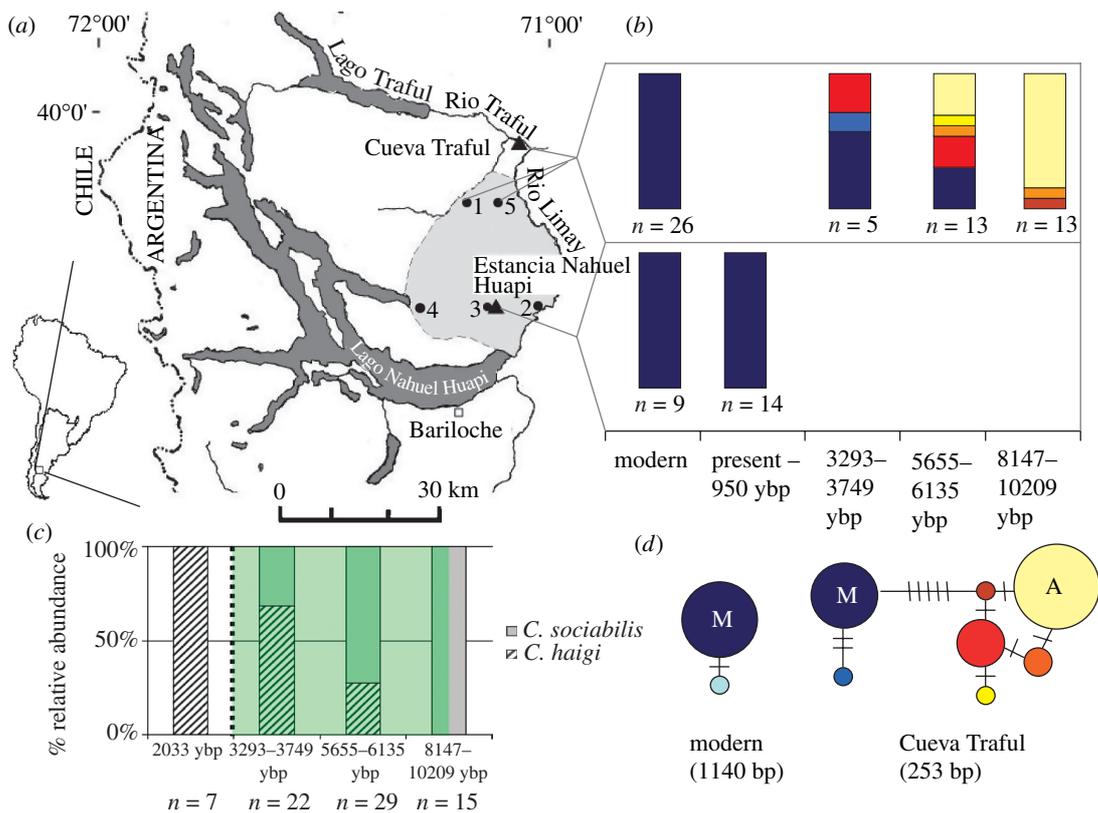


Figure 1. (a) Map of the study area, including prehistoric fossil sites Estancia Nahuel Huapi (ENH) and Cueva Traful (CT). The geographic distribution of *C. sociabilis* is shown in light grey. Numbered dots correspond to modern sampling localities: (1) Cerro Monte Redondo; (2) Rincon Grande; (3) Altos del Fortin; (4) Paso Coihue; (5) Cerro la Lagunita. (b) Frequency of haplotypes (based on 253 bp) detected at ENH and CT, presented in time-intervals corresponding to radiocarbon dates for stratigraphic levels. Also shown are haplotype frequencies for the nearest modern collection localities. (c) Relative abundance of *C. sociabilis* and *C. haigi* at CT over the last 10 000 years by time-interval. Shading denotes an increase in density of *Nothofagus* forest at 8500 ybp; dotted line denotes volcanic eruption with ash deposit in CT. (d) Haplotype networks for cytochrome *b* sequence data from modern and ancient DNA samples. The sizes of the circles indicate the relative abundance of each haplotype; the numbers of tick marks between circles correspond to the number of base pair substitutions that differentiate haplotypes. Colours correspond to those used in (b) to denote haplotype frequencies.

elevation 760 m), a Holocene archaeological site and barn owl roost excavated between 1973 and 1978 (Montero *et al.* 1983). The cave is located in the forest-steppe ecotone approximately 6 km north of the current northern distributional limit for *C. sociabilis*. Due to the deposition of owl pellets, material excavated from the cave provides a continuous temporal sequence of small mammal bones and teeth dating from approximately 2000 to 10 000 ybp (Pearson & Pearson 1981). For genetic analyses, the protocol of Hadly *et al.* (2003) was used to extract DNA from 73 teeth recovered from eight stratigraphic levels (*ca.* 2000–10 000 ybp). For each sample, a total of 253 bp of cytochrome *b* was amplified and sequenced according to strict ancient DNA standards.

3. RESULTS AND DISCUSSION

Only a single cytochrome *b* haplotype was detected in each of the five modern populations of *C. sociabilis* surveyed ($h=0.000$, $\pi=0.000$, $n>9$ animals per population). Four of these populations shared the same haplotype, with the haplotype of the fifth differing by only a single third-position transition (0.09% sequence divergence; figure 1*d*). The predominant haplotype was identical to the comparable portions of the single cytochrome *b* haplotype recovered at Estancia Nahuel Huapi from 14 specimens dating from the present to 950 ybp (figure 1*b*; Hadly *et al.* 2003).

Based on a phylogenetic analysis that included all *Ctenomys* species found in GenBank (39 species, 129 sequences), we identified 43 out of 73 fossil teeth

recovered from Cueva Traful as *C. sociabilis* (see electronic appendix). The remaining 30 teeth were identified as *Ctenomys haigi*, a non-social tuco-tuco found in the same region of Patagonia. In contrast to the modern populations, eight cytochrome *b* haplotypes from *C. sociabilis* were detected among samples from Cueva Traful: the predominant modern haplotype (M) and seven historical variants ($n=33$ sequences; $h=0.71\pm 0.06$, $\pi=0.01283\pm 0.00167$, calculated using DNAsp v. 4.0.6 (Rozas & Rozas 1999), 4.3% overall sequence divergence; figure 1*b*). Thus, prior to *ca.* 3000 ybp, *C. sociabilis* from within an area of Cueva Traful of roughly 19.6 km² (mean hunting radius for barn owls = 2.5 km; Taberlet 1983) were characterized by greater haplotype diversity than occurs today throughout the species' range (Fisher's exact test, $p=0.000$, *s.e.* = 0.000; Raymond & Rousset 1995).

The proportion of dental samples from each stratigraphic level that were identified as *C. sociabilis* indicates that this species declined in relative abundance from approximately 8200 to 3000 ybp (figure 1*c*). This change in relative abundance, in conjunction with the modern absence of *C. sociabilis* from the immediate vicinity of Cueva Traful, suggests a numerical decline and possible range contraction. Although it is difficult to obtain direct evidence

regarding the cause(s) of this decline and associated loss of genetic diversity, further analysis of the Cueva Trafal data suggests that several factors may have contributed to this outcome.

First, consistent changes in vegetation may have altered the habitat available to *C. sociabilis*. Pollen core analyses from this region indicate a sustained increase in the prevalence of *Nothofagus* forest from approximately 8500 to 3000 ybp, which resulted in a local decline in the abundance of the open grassland habitat containing the mallines in which *C. sociabilis* occurs (Markgraf 1983).

Second, a volcanic eruption approximately 3000 ybp (Montero & Silveira 1983) may have exacerbated the decline of *C. sociabilis*. Volcanic eruptions have been shown to reduce abundance and genetic diversity in other vertebrate species, including other *Ctenomys* in the Andean region (Gallardo & Köhler 1994). *C. sociabilis* disappears from Cueva Trafal in the stratigraphic level immediately postdating the tephra layer.

Finally, competition with *C. haigi*, the other local species of ctenomyid, may have contributed to the reduction in *C. sociabilis*. Although the relative abundance of *C. haigi* is low during the early Holocene, it increases steadily throughout the temporal period covered by the Cueva Trafal deposit. Currently, *C. haigi* occupies an extensive area to the north, south, and east of the geographic range of *C. sociabilis*, and is the extant tuco species that occurs in closest proximity to Cueva Trafal.

Although these historical hypotheses require testing, it is clear that *C. sociabilis* experienced a marked reduction in genetic variation during the past 10 000 years. While low levels of genetic variation are often attributed to historical bottlenecks (Amos & Harwood 1998), few studies have tracked directly the genetic changes associated with such events, particularly over a millennial time-scale (but see Shapiro *et al.* 2004). The reduction in genetic variation we have documented is particularly unusual given that (i) *C. sociabilis* is a rodent, rather than a megafaunal mammal, and (ii) no single environmental event (e.g. deglaciation or human over-kill) is clearly associated with this loss of diversity. Absence of genetic diversity has important implications for evolutionary and conservation biology and is particularly important in the context of future climatic modifications, since animal populations may require a repertoire of potential responses to adapt to varying conditions (Lande & Shannon 1996).

How has a species with high prehistoric levels of genetic diversity persisted for the past several thousand years with so little variation? Detailed analyses of additional palaeoecological sites in Neuquén Province should provide a better understanding of the temporal and spatial dynamics of the decrease in relative abundance and genetic diversity detected for *C. sociabilis* at Cueva Trafal. At the same time, comparative analyses of other, non-social tuco-tuco species from this region may yield insights into the role that the distinctive behavioral and demographic attributes of *C. sociabilis* have played in this decline. As our study exemplifies, the ability to track genetic

variation through time provides a powerful tool for identifying and interpreting historical relationships between species and their environments.

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- Amos, W. & Harwood, J. 1998 Factors affecting levels of genetic diversity in natural populations. *Phil. Trans. R. Soc. B* **353**, 177–186. (doi:10.1098/rstb.1998.0200.)
- Gallardo, M. H. & Köhler, N. 1994 Demographic changes and genetic losses in populations of a subterranean rodent (*Ctenomys maulinus brunneus*) affected by a natural catastrophe. *Zeitschrift für Säugetierkunde* **59**, 358–365.
- Hadly, E. A., Van Tuinen, M., Chan, Y. & Heiman, K. 2003 Ancient DNA evidence of prolonged population persistence with negligible genetic diversity in an endemic tuco-tuco (*Ctenomys sociabilis*). *J. Mammal.* **84**, 403–417. (doi:10.1644/1545-1542(2003)084<0403:AD EOPP>2.0.CO;2.)
- Hadly, E. A., Ramakrishnan, U., Chan, Y. L., Van Tuinen, M., O'Keefe, K., Spaeth, P. A. & Conroy, C. J. 2004 Genetic response to climatic change: insights from ancient DNA and phylochronology. *Public Lib. Sci. Biol.* **2**, 1600–1609.
- Heusser, C. J. 2003 Ice age southern Andes: a chronicle of palaeoecological events. In *Developments in quaternary science* (ed. J. Rose), vol. 3, pp. 40–43. New York: Elsevier.
- Lacey, E. A. 2000 Spatial and social systems of subterranean rodents. In *Life underground: the biology of subterranean rodents* (ed. E. A. Lacey, J. L. Patton & G. N. Cameron), pp. 257–296. Chicago, IL: University of Chicago Press.
- Lacey, E. A. 2001 Microsatellite variation in solitary and social tuco-tucos: molecular properties and population dynamics. *Heredity* **86**, 628–637. (doi:10.1046/j.1365-2540.2001.00881.x.)
- Lacey, E. A. & Wiczorek, J. R. 2003 Ecology of sociality in rodents: a ctenomyid perspective. *J. Mammal.* **84**, 1198–1211. (doi:10.1644/BLe-014.)
- Lacey, E. A. & Wiczorek, J. R. 2004 Kinship in colonial tuco-tucos: evidence from group composition and population structure. *Behav. Ecol.* **15**, 988–996. (doi:10.1093/beheco/arh104.)
- Lande, R. & Shannon, S. 1996 The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**, 434–437.
- Markgraf, V. 1983 Late and postglacial vegetational and paleoclimatic changes in subantarctic, temperate, and arid environments in Argentina. *Palynology* **7**, 43–70.
- McLaughlin, J. F., Hellmann, J. J., Boggs, C. L. & Ehrlich, P. R. 2002 Climate change hastens population extinctions. *Proc. Natl Acad. Sci. USA* **99**, 6070–6074. (doi:10.1073/pnas.052131199.)

- Montero, E. A. C. & Silveira, M. J. 1983 Radiocarbon chronology of a tephra layer in Rio Traful Valley, Province of Neuquén, Argentina. *Quaternary of South America and Antarctic Peninsula* **1**, 135–150.
- Montero, E. A. C., Curzio, D. E. & Silveira, M. J. 1983 La estratigrafía de la Cueva Traful I (Provincia del Neuquén). *Praehistoria* **1**, 9–160.
- Pearson, O. P. & Christie, M. I. 1985 Los tuco-tucos (genero *Ctenomys*) de los parques nacionales Lanin y Nahuel Huapi, Argentina. *Hist. Nat.* **5**, 337–343.
- Pearson, O. P. & Pearson, A. K. 1981 La fauna mamíferos pequeños cerca de Cueva Traful, Argentina: pasado y presente. *Praehistoria*, **1**.
- Raymond, M. & Rousset, F. 1995 An exact test for population differentiation. *Evolution* **49**, 1280–1283.
- Rozas, J. & Rozas, R. 1999 DNASP, version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**, 174–175. (doi:10.1093/bioinformatics/15.2.174.)
- Shapiro, B. *et al.* 2004 Rise and fall of the Beringian steppe bison. *Science* **306**, 1561–1565. (doi:10.1126/science.1101074.)
- Smith, M. F. 1998 Phylogenetic relationships and geographic structure in pocket gophers in the genus *Thomomys*. *Mol. Phylogenet. Evol.* **9**, 1–14. (doi:10.1006/mpev.1997.0459.)
- Taberlet, P. 1983 An estimation of the average foraging radius of the barn owl based upon rejection pellets analysis. *Revue D'Ecologie—la Terre et la Vie* **38**, 171–177.
- Willerslev, E. & Cooper, A. 2005 Ancient DNA. *Proc. R. Soc. B* **272**, 3–16. (doi:10.1098/rspb.2004.2813.)
- Woods, C. A. 1993 Suborder Hystricognathi. In *Mammal species of the world: a taxonomic and geographic reference* (ed. D. E. Wilson & D. M. Reeder), pp. 771–806. Washington, DC: Smithsonian Institution Press.

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