Biological Journal of the Linnean Society, 2017, **XX**, 1–17. With 4 figures.

Contrasting patterns of Holocene genetic variation in two parapatric species of *Ctenomys* from Northern Patagonia, Argentina

MAURO N. TAMMONE[1,2*], ULYSES F. J. PARDIÑAS[1] and EILEEN A. LACEY[3]

[1]*Instituto de Diversidad y Evolución Austral (IDEAus-CONICET), 9120 Puerto Madryn, Chubut, Argentina*
[2]*Programa de Estudios Aplicados a la Conservación del Parque Nacional Nahuel Huapi (CENAC-PNNH, CONICET), 8400 Bariloche, Río Negro, Argentina*
[3]*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720, USA*

Received 22 June 2017; revised 26 September 2017; accepted for publication 28 September 2017

Analyses of DNA from fossil specimens can generate critical insights into the demographic histories of natural populations. Previous analyses of fossil specimens from a single cave site in the Limay Valley in Patagonia have revealed striking historical differences in genetic variability between two species of *Ctenomys*, the colonial tuco-tuco (*C. sociabilis*) and the Patagonian tuco-tuco (*C. haigi*). As a first step towards identifying environmental or other factors contributing to this outcome, we assessed whether these differences in variability are generally characteristic of these species. We sequenced a 136-bp fragment of the cytochrome *b* locus for fossil specimens of *Ctenomys* excavated from two additional cave sites in the Limay Valley. Analyses of this expanded data set revealed that while *C. sociabilis* has undergone a pronounced loss of genetic diversity at all three cave sites over the past ~12 000 years, genetic diversity in *C. haigi* has remained relatively constant over the same temporal and geographic scales. The generality of these patterns suggests that although the factors affecting genetic diversity in *C. sociabilis* were widespread in the Limay Valley, impacts on the study species differed, probably due to known behavioural, ecological and demographic differences between these taxa.

ADDITIONAL KEYWORDS: ancient DNA – cytochrome *b* – demographic history – tuco-tucos.

INTRODUCTION

Demographic history is thought to play a critical role in shaping the genetic structures of free-living populations of organisms (Johansson, Primmer & Merila, 2006; Liu *et al.*, 2006; Mora *et al.*, 2007; Mapelli *et al.*, 2012). Historical factors that may impact current patterns of genetic diversity include range expansions or contractions as well as reductions in population size or connectivity (Templeton, Routman & Phillips, 1995; Avise, 2000). Analyses of current genetic structure – particularly those based on samples from multiple localities – can provide valuable insights into the nature and extent of these historical factors, thereby helping to reveal the reasons for modern patterns of variability (Templeton, 1998; Clement, Posada & Crandall, 2000; Kuhner, 2009). Use of current genetic data to identify historical processes or events, however, necessarily relies on statistical inference and thus may be subject to error, particularly when exploring factors occurring over deeper time scales (Ramakrishnan & Hadly, 2009). In contrast, analysis of ancient DNA (aDNA) extracted from fossil samples provides a potentially more direct means of characterizing the historical factors involved in patterns of genetic diversity over time (Gilbert *et al.*, 2005; Campos *et al.*, 2010; de Bruyn *et al.*, 2011).

The tuco-tucos (Rodentia: Ctenomyidae) of the Limay Valley of northwestern Patagonia provide an ideal opportunity to use analyses of aDNA to resolve

*Corresponding author. Current address: CENAC, Fagnano 244, 8400 SC de Bariloche, Argentina. E-mail: mtammone@gmail.com

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

questions regarding patterns of modern genetic variation. The colonial tuco-tuco (*Ctenomys sociabilis*) is endemic to the western Limay Valley and surrounding hills in Neuquén Province (Fig. 1; Pearson & Christie, 1985; Tammone, Lacey & Relva, 2012). A parapatric form, the Patagonian tuco-tuco (*C. haigi*), is widely distributed in the eastern Limay Valley and adjacent areas of Río Negro Province (Fig. 1; Pearson & Christie, 1985). Long-term field studies of these animals have revealed that the two species are characterized by pronounced differences in geographic range, social behaviour, ecology, demography and current genetic structure. In particular, the group-living *C. sociabilis* inhabits a more restricted subset of habitats and displays markedly reduced rates of natal dispersal compared to the solitary, ecologically more generalized *C. haigi* (Lacey, Braude & Wieczorek, 1997, 1998; Lacey & Wieczorek, 2003, 2004). While modern populations of *C. sociabilis* are characterized by extremely limited genetic variability (Lacey, 2001; Chan *et al.*, 2005; Tammone *et al.*, 2016b), genetic variation in *C. haigi* is considerably greater (Lacey, 2001; Lacey, unpublished data). *Ctenomys sociabilis* displays an almost complete lack of variation in mitochondrial (cytochrome *b*) haplotypes throughout its current geographic distribution (Chan *et al.*, 2005), a pattern that seems inconsistent with the more limited movement of individuals between populations and presumably greater potential for local genetic drift in this species compared to *C. haigi*.

The striking differences in modern genetic variation between *C. sociabilis* and *C. haigi* appear to be associated with significant differences in demographic history. Analyses of fossil remains collected from the archaeological site at Cueva Traful I (hereafter, CTI) at the northern end of the Limay Valley have revealed a pronounced loss of genetic diversity in *C. sociabilis* 3000–5000 years before present (Chan *et al.*, 2005); in contrast, no similar reduction in genetic variability was detected for fossil specimens of *C. haigi* collected at this site (Chan & Hadly, 2011). These data suggest that *C. sociabilis* – but not *C. haigi* – was affected by a significant historical event, potential explanations for which include volcanic activity, competitive interactions between the study species and more general changes in environmental conditions (Chan *et al.*, 2005). Because the spatial scales over which these factors would have acted are expected to vary, a critical first step towards evaluating these hypotheses is to assess whether the interspecific differences in genetic variability reported for CTI are generally representative of each study species, having occurred at multiple sites within the historical geographic distributions of these animals.

At least two factors suggest that information obtained from CTI may not be indicative of the overall

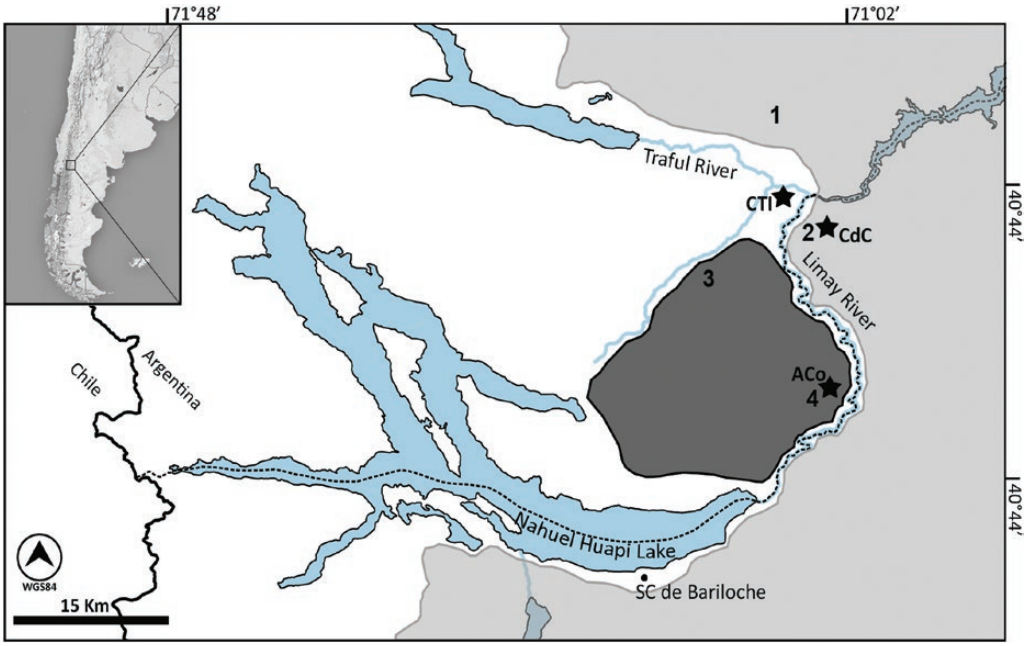

Figure 1. Map of the study area. The current geographic range of *Ctenomys sociabilis* is shown in dark grey while the current geographic range of *C. haigi* is shown in light grey. Numbers indicate the locations of extant populations of tuco-tucos: (1) Río Traful, (2) Valle Encantado, (3) Cerro Monte Redondo and (4) La Lonja. The historical sites sampled were Arroyo Corral (ACo), Cueva del Caballo (CdC) and Cueva Traful I (CTI); stars denote the locations of these cave sites.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

demographic histories of the study species. First, CTI is located on the extreme northeastern edge of the geographic range of *C. sociabilis* (Fig. 1). No extant populations of this species are known to occur in the vicinity of the cave (Pearson & Pearson, 1993; Tammone, 2016), suggesting that *C. sociabilis* may have experienced a contraction in this particular portion of its historical distribution. Second, CTI occurs along the southern shore of the Río Traful, which represents much less of a geographic barrier than the Río Limay and thus the generally parapatric distributions of these species are more likely to come into contact along the Río Traful than at other locations in the Limay Valley, creating the potential for ecological interactions (e.g. interspecific competition) that may not occur elsewhere within the distribution of *C. sociabilis*. As a result, analyses of aDNA from other localities within the distributions of *C. sociabilis* and *C. haigi* are critical to evaluating potential explanations for the historical patterns of genetic change detected at CTI.

To assess the generality of the patterns of genetic change reported for CTI, we characterized genetic variability among fossil specimens of *C. sociabilis* and *C. haigi* obtained from two additional archaeological sites within the Limay Valley region. In contrast to CTI, the sites examined here were located solidly within the geographic range of one or the other of our study species and were associated with extant populations of tuco-tucos. Using radiocarbon-dated remains of tuco-tucos obtained from these sites, we assessed temporal changes in the relative abundance and genetic variability of the study species over the past ~12 000 years (Pleistocene–Holocene boundary; Walker *et al.*, 2008). Genetic variability in the fossil samples was characterized using a portion of the same mitochondrial cytochrome *b* sequences examined by Chan *et al.* (2005). By comparing aDNA variability across species and sites as well as comparing ancient sequences with those obtained from modern populations located near each sampling locality, we characterize temporal changes in genetic variability in *C. sociabilis* and *C. haigi* over larger temporal and spatial scales than have been examined previously. These analyses are critical to ongoing efforts to assess the causal bases for interspecific differences in genetic change over time and provide important new insights into the demographic histories of the study species.

MATERIAL AND METHODS

STUDY SITES

Fossil remains of tuco-tucos (*Ctenomys* spp.) were collected from two archaeological sites located in the hills surrounding the Limay Valley. The Arroyo Corral archaeological locality, consisting of two adjacent deposits (hereafter, collectively referred as ACo), is located on the western side of the valley (Fig. 1), within the current geographic range of *C. sociabilis*. In contrast, the Cueva del Caballo locality (hereafter, CdC) is located in the hills on the eastern side of the valley (Fig. 1), within the current geographic range of *C. haigi*. These sites have been the subject of long-term excavations that have resulted in the collection of hundreds of cranial remains from tuco-tucos, all of which are associated with detailed stratigraphic information (Hajduk *et al.*, 2007; Tammone *et al.*, 2014; Tammone *et al.*, 2016a). Taphonomic analyses of the materials collected at each site suggest that the primary agents of deposition for *Ctenomys* specimens (and other small mammal remains) were Barn Owls (*Tyto furcata*), although evidence of human consumption of tuco-tucos has also been reported for ACo (Tammone *et al.*, 2017). Although both owls and humans are expected to have preyed upon tuco-tucos occurring in the immediate vicinity of this site, activity by the latter is not expected to have biased either the taxonomic identities or the proportions of rodent remains at ACo (Tammone *et al.*, 2017). To facilitate comparisons with previous analyses of historical genetic variation in the study species, published data on fossil *Ctenomys* from CTI (Chan *et al.*, 2005; Chan & Hadly, 2011) were also included in our analyses.

To assess current genetic variation in *C. sociabilis* and *C. haigi*, we used tissue samples collected from extant populations of these species located in close proximity to each archaeological site. Fossil samples from ACo were paired with modern tissue samples (n = 9) collected from the population of *C. sociabilis* at La Lonja (0.8 km from the cave site; Fig. 1). Fossil samples from CdC were paired with modern tissue samples (n = 8) collected from the population of *C. haigi* at Valle Encantado (0.9 km from the cave site). As noted above, no populations of *C. sociabilis* currently occur in the vicinity of CTI; samples (n = 9) from the nearest known population of this species, located at Cerro Monte Redondo (16.2 km from the cave site; Fig. 1), were used as a modern comparison for this cave site. Because CTI occurs at the apparent historical intersection of the ranges of the two study species, modern samples (n = 10) from the nearest known population of *C. haigi* along the Río Traful (5.0 km from the cave site; Fig. 1) were also used as a modern comparison for the CTI site. For all extant populations, animals were live-trapped, after which non-destructive tissue samples were obtained following the procedures in Lacey (2001) and Cutrera, Lacey & Busch (2005); the animals were then released at the point of capture. All field methods were approved by the Animal Care and Use Committee at the University

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

of California, Berkeley, and followed the guidelines of the American Society of Mammalogists for the use of mammals in research (Sikes, Gannon & and the Animal Care and Use Committee of the American Society of Mammalogists, 2016).

Radiocarbon dating and stratigraphy of fossil sites

Eleven of the fossil specimens of *Ctenomys* that yielded aDNA suitable for sequencing were radiocarbon dated using the accelerator mass spectrometry facility housed in the AMS Laboratory at the University of Arizona (USA). The stratigraphic chronology for each site was characterized using these samples as well as existing radiocarbon dates generated as part of zooarchaeological studies at ACo and CdC (Supporting Information, Appendix S1); the latter data were obtained from analyses of charcoal or mammalian bones excavated at each site. Radiocarbon dates were calibrated using the SHCal13 calibration curve (Hogg *et al.*, 2013) and OxCal4.2 calibration software package (Bronk Ramsey, 2009). The stratigraphic profile for each site was compiled using age-depth models, as generated by the R package CLAM; this approach has been shown to produce reliable estimates of the timing and magnitude of stratigraphic changes (Blaauw, 2010; Wright *et al.*, 2017; Supporting Information, Appendix S2). Calibrated dates (empirical and model-estimated) are reported as 95.4% confidence-calibrated ages in years BP (Before Present AD 1950).

For analyses of historical species abundance and paleogenetic diversity (see below), we divided the stratigraphy of each cave site into four temporal intervals (Table 1) corresponding to the estimated dates of the most significant historical environmental changes in this region of Patagonia (Markgraf, 1983; Villarosa *et al.*, 2006; Iglesias *et al.*, 2014). This procedure created a standardized, biologically relevant set of temporal intervals that could be used to compare data across cave sites, including published data from CTI (Ramakrishnan & Hadly, 2009; Chan & Hadly, 2011). Because the time intervals used in this study differ somewhat from those reported by Chan *et al.* (2005) and Chan & Hadly (2011), data on genetic diversity and relative species abundances were analyzed using both our temporal scheme and that employed Chan and colleagues in their analyses of CTI (Supporting Information, Appendix S3).

Sequencing of aDNA

To assess genetic diversity in our fossil samples, DNA was extracted from 89 fossil specimens following stringent anti-contamination procedures for aDNA, as described by Cooper & Poinar (2000), Hadly *et al.* (2003) and Gilbert *et al.* (2005). To minimize the possibility that the same fossil individual was sequenced more than once, DNA was typically extracted only from cheek teeth obtained from left mandibles; for the few stratigraphic layers for which this restriction did not produce reasonable sample sizes, we also extracted DNA from cheek teeth from the right mandible or from the maxilla (Supporting Information, Appendix S4). Extractions were conducted in a facility dedicated to studies of aDNA located on the campus of Stanford University (California, USA) following the protocol of Hadly *et al.* (2003).

Polymerase chain reaction (PCR) amplification of aDNA samples was undertaken in a lab room that was physically separated from the space in which extractions had been conducted and in which no analyses of modern *Ctenomys* DNA had occurred. Each round of extractions included three negative control samples (no tissue) to allow detection of contamination by non-target DNA. A 136-bp fragment of cytochrome *b* was amplified using primers CTENOMYS1 and CTENOMYS2 (Hadly *et al.*, 2003). Although this was less than the total number of

Table 1. Summary of temporal intervals used in this study. Numbers of modern (interval A) and fossil (intervals B, C, D) individuals sequenced for each study species are also given. Modern sites (interval A, corresponding fossil site given in parentheses) for *C. sociabilis* were La Lonja (ACo) and Cerro Monte Redondo (CTI); for *C. haigi*, modern sites were Valle Encantado (CdC) and Río Traful (CTI). No extant population of *C. sociabilis* occurs in the proximity of CdC

Temporal intervals		Environmental factors	*Ctenomys sociabilis*			*Ctenomys haigi*	
			ACo	CdC	CTI	CdC	CTI
A	Present	Anthropogenic impacts*	9	–	9	8	10
B	Late Holocene (0.5–3 Kya)	Volcanism	5	3	–	14	8†
C	Middle Holocene (3–6 Kya)	Vegetation change	5	5	16†	8	20†
D	Early Holocene (6–12 Kya)	Post-glacial	2	6	18†	1	3†

ACo, Arroyo Corral; CdC, Cueva del Caballo; CTI, Cueva Traful I.
*Increase of human land use, including grazing.
†Data from Chan & Hadly (2011).

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

base pairs examined by Chan *et al.*, (2005) and Chan & Hadly (2011), this fragment accounted for ~70% of the variation detected by these authors during analyses of fossil specimens of *Ctenomys* from CTI. The per-sample PCR master mix (10 μL reaction volume) consisted of 5.0 μL of ddH_2O, 1.2 μL (1.56 mg/mL) of bovine serum albumin (BSA), 0.38 μL (0.38 mM each) of dNTPs, 1.15 μL of 10× *Taq*Gold buffer, 1.15 μL (2.8 mM) of $MgCl_2$, 0.21 μL (0.21 μM) of each primer, 0.05 μL (0.25 U) of AmpliTaq Gold 360 and 0.7 μL DNA template. Thermocycling conditions were 95 °C for 10 min followed by 45 cycles at 95 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min. Each PCR reaction included three negative controls (no DNA template) that were used to detect contamination of PCR reagents. Each aDNA extract was subject to at least two independent rounds of amplification. The resulting amplicons were sequenced by Elim BioPharmaceuticals (Hayward, California). All amplicons were sequenced in both directions to minimize the potential for erroneous reads (Hofreiter *et al.*, 2001). The resulting sequences were aligned and edited using Sequencher (version 3.1.1, Gene Code Corporation, Ann Arbor, Michigan).

Sequencing of modern DNA

Modern DNA was extracted from tissue samples collected from extant populations of *C. sociabilis* and *C. haigi* using the DNAeasy tissue extraction kit (Qiagen, Inc.). The same 136-bp fragment of cytochrome *b* used to characterize aDNA samples was amplified from modern extracts using primers CTENOMYS1 and CTENOMYS2. The per-sample master mix (25 μL reaction volume) consisted of 15.0 μL of ddH_2O, 2.5 μL (1 mM each) of dNTP, 3.5 μL of 10× Platinum Taq buffer, 0.6 μL (0.6 mM) of $MgCl_2$, 0.5 μL (0.22 μM) of each primer, 0.3 μL (1.5 U) of Platinum Taq polymerase (Fisher Scientific, Inc.) and 3.0 μL DNA template. PCR thermocycling conditions were the same as those for aDNA samples, with two exceptions: denaturation was conducted at 94 °C and reactions were run for 35 cycles. In addition, the complete 1140-bp cytochrome *b* locus was amplified for each modern sample using the primer pairs and protocol in Tammone *et al.* (2016b). All amplicons from modern DNA samples were purified with 1.5 μL of ExoSAP-IT (USB Corporation, Cleveland, Ohio), diluted 1:4 with H_2O and incubated at 37 °C for 30 min followed by denaturation at 80 °C for 15 min.

For both partial and complete cytochrome *b* modern sequences, amplicons were cycle sequenced using the BigDye Terminator kit (v3.1; Applied Biosystems, Foster City, California). Per-sample cycle sequencing reactions (10 μL reaction volume) consisted of 1.75 μL of 5× BigDye buffer, 0.3 μL of sequencing primer, 0.5 μL of BigDye solution, 6.45 μL of H_2O and 1 μL of purified DNA template. Thermocycling followed the manufacturer's instructions, after which cycle sequencing products were cleaned with Sephadex columns and then run on an ABI 3730 automated sequencer (Applied Biosystems). All analyses of modern DNA samples were completed in the Evolutionary Genetics Laboratory in the Museum of Vertebrate Zoology at the University of California, Berkeley.

Genetic identification of species

While extant populations of tuco-tucos could be reliably be identified to species based on multiple morphological, behavioural and ecological attributes (Lacey & Wieczorek, 2003; Tammone *et al.*, 2016b), reliable identification of fossil remains based on morphological features preserved in these specimens was challenging. Accordingly, identification of fossil *Ctenomys* specimens was completed using the cytochrome *b* sequences obtained from these individuals. Specifically, the sequence obtained from each fossil sample was subjected to a GenBank BLAST search; this procedure served to confirm species identification and to eliminate any potential contaminant sequences from our data set (Hadly *et al.*, 2003; Collins *et al.*, 2013). Finally, to confirm that aDNA sequences assorted based on assigned species identifications, we used PAUP*4.0 (Swofford, 2003) to construct a neighbor-joining tree based on uncorrected p-distance values for the 136-bp portion of cytochrome *b* examined. As references, cytochrome *b* sequences from two fossil specimens from CTI (one per species) were included in this analysis, as were comparable portions of cytochrome *b* sequences from modern specimens of eight species of ctenomyids from the Patagonian-Fuegian region of South America (GenBank accession numbers provided in Fig. 2). Complete cytochrome *b* sequences from modern samples were subjected to the same BLAST and tree construction procedures to confirm species identifications made at the time that members of extant populations of tuco-tucos were captured.

Estimating species abundance

Because morphological traits used to distinguish species of *Ctenomys* from the Nahuel Huapi region (Tammone *et al.*, 2016b) cannot typically be applied to fossil samples, accurate species identification of fossil material could only be accomplished using genetic data. Thus, estimates of the relative abundances of *C. sociabilis* and *C. haigi* at ACo and CdC were limited to the subset of individuals for which cytochrome *b* sequences were available. Accordingly, for each cave site, fossil relative abundance was estimated based on the proportion of all individuals sequenced per temporal period (intervals B–D; Table 1) that was genetically assigned to each study species. To determine the modern relative abundances (interval A; Table 1) of these species,

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

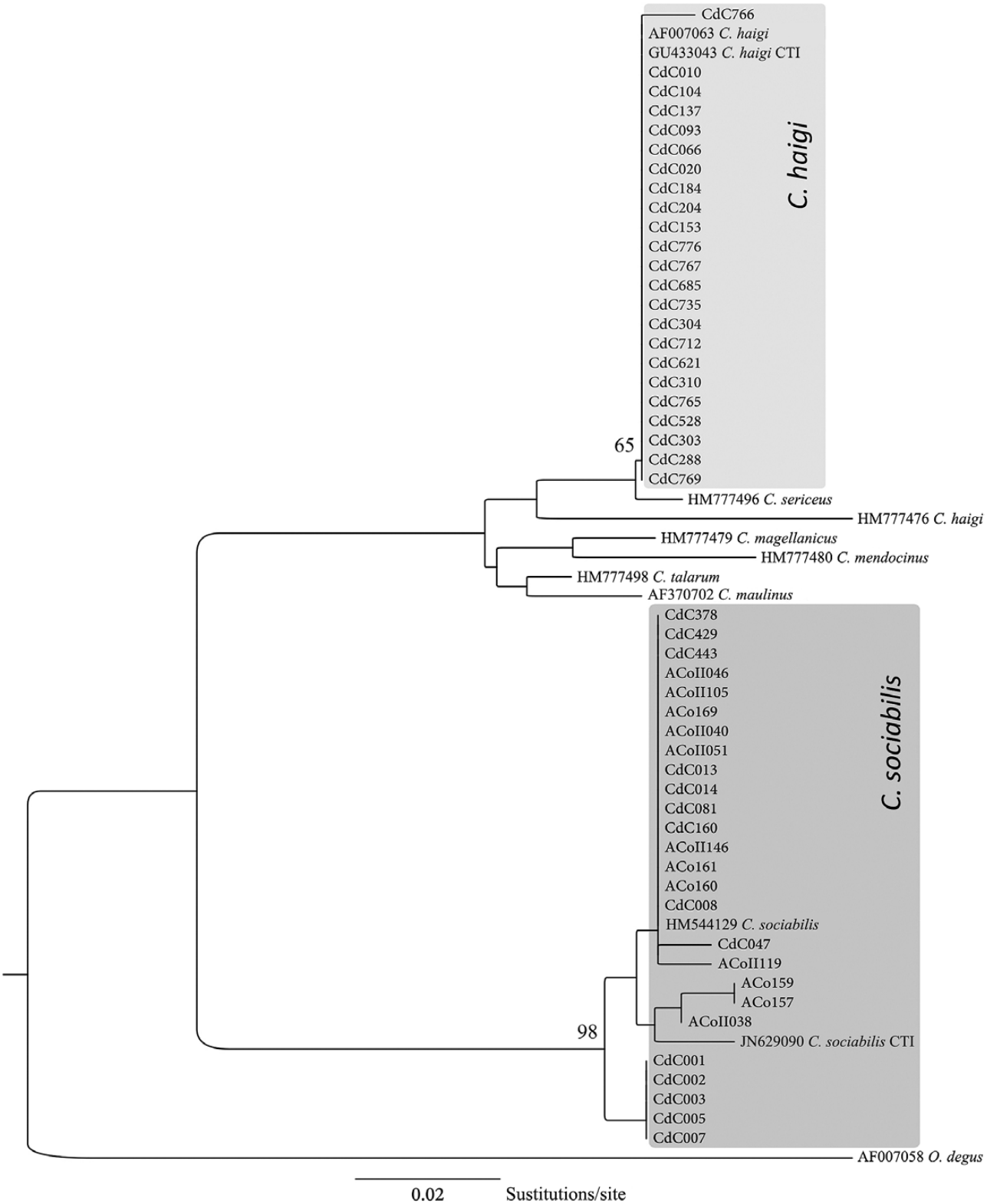

Figure 2. Neighbor-joining tree based on uncorrected p-distances for the 136-bp portion of the cytochrome *b* locus sequenced for fossil specimens from Arroyo Corral (ACo) and Cueva del Caballo (CdC). Also included are comparable sequence fragments from other species of ctenomyids from the Patagonian-Fuegian region of South America. Octodon degus was used

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

we examined the contents of fresh owl pellets collected at ACo, CdC and CTI between 2008 and 2013; species identification were based on the established morphological traits (Tammone *et al.*, 2016b). Taphonomic analyses have revealed that accumulation of fossils at the study sites was due primarily to predation by owls (Pearson & Pearson, 1993; Tammone *et al.*, 2014, 2016a), suggesting that analysis of modern owl pellets provides the most appropriate method of estimating current relative abundances of the study species (see Pardiñas, 1999; Pardiñas & Teta, 2013). Once species identification was complete, for each site the relative abundances of the study species in both modern and fossil samples were estimated using the minimum number of individuals (MNI) procedure (Grayson, 1984; Lyman, 2008); because the same estimation procedure was employed for all temporal periods, any potential biases (e.g. under or over estimation of animal numbers) associated with this analysis should have been comparable for all data sets.

Analyses of genetic diversity

Sequences from fossil tuco-tuco specimens collected at ACo and CdC were aligned with previously published sequences from fossil *C. sociabilis* and *C. haigi* collected at CTI (GenBank accession numbers: *C. sociabilis*: JN629090-94 and DQ402060-66; *C. haigi*: GU433041-46; Chan *et al.*, 2005; Chan & Hadly, 2011). All sequences were aligned using Sequencher v3.1.1 and then trimmed to match the 136-bp portion of cytochrome *b* targeted in this study. Sequences from extant populations of tuco-tucos were aligned using published cytochrome *b* sequences for the study species (GenBank accession numbers: *C. sociabilis*: HM777495 and EU035177; *C. haigi*: HM777476, AF007063 and AF422920) and trimmed to match the 1140 bp comprising the complete gene as well as the 136-bp subset of this locus sequenced for fossils. Fossil sequences from each of the three cave sites were included in all subsequent analyses of genetic variation along with modern sequences from the four extant populations of tuco-tucos sampled (Table 1). Haplotype sequences were deposited in GenBank (haplotype accession numbers: KY568720–KY568728).

Once alignment and editing of fossil sequences were complete, the number of cytochrome *b* haplotypes and the relative frequency of each haplotype were calculated for *C. sociabilis* and *C. haigi* using the R script TempNet v1.4 (Prost & Anderson, 2011). The same script was then used to construct temporally three-dimensional haplotype networks for each species at each cave site. Additionally, for *C. sociabilis*, haplotypes were tracked through time and among sites by constructing a temporal network based on the pooled data set for all three caves sites (Supporting Information, Appendix S5). To quantify genetic diversity through time, we used DnaSP 5.00 (Libardo & Rozas, 2009) to determine the number of variable sites (s), haplotype diversity (h) and nucleotide diversity (π) for each species at each cave site; within sites, separate analyses were run for each standardized time interval (Table 1) and species. Because the sequences within a given interval had been deposited over an extended period of time, standard estimates of π may have been biased. Accordingly, we used the correction for heterochrony (π_h) in Depaulis, Orlando & Hänni (2009, equation 1) to calculate nucleotide diversity. Analyses of variability in modern cytochrome *b* sequences were conducted for both the 136-bp portion and the complete 1140-bp locus using the same parameters, but without the correction for heterochrony. Per cent sequence divergence within and among time periods as well as within and among species was estimated using DnaSP 5.00. Potential departures from neutrality were examined using Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997); both tests were conducted for complete cytochrome *b* sequences from modern populations as well as for the 136-bp portion of this locus sequenced for historical samples from each study species. The significance of these tests was estimated using 1000 bootstrap replicates, as implemented in Arlequin 3.5 (Excoffer & Lischer, 2010). Spatial structuring of genetic variation within time intervals was assessed in DnaSP 5.00 using estimates of pairwise F_{st} between sites.

Due to differences in the number of fossil sequences obtained from CTI vs. ACo and CdC, we employed a resampling procedure to assess the potential effects of sample size on our estimates of historical genetic diversity. This bootstrapping procedure consisted of randomly resampling 12 individuals (n = 100 replicates) from CTI; this number of individuals equalled the smallest historical sample size examined, which was from ACo (Supporting Information, Appendix S6). Mean values of estimates of genetic diversity across replicates were then compared to the full data set for CTI as well as to the full data sets from ACo and CdC.

RESULTS

Radiocarbon analyses indicated that the earliest deposits at ACo dated to the end of the last glacial

as an outgroup. Numbers at nodes including ACo and CdC fossil specimens represent confidence values based on 1000 bootstrap replications of the data set (Felsenstein, 1985). Sequences obtained from GenBank are identified by accession numbers.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

maximum (Late Pleistocene, 23–19 Kya), with subsequent deposits continuing through the Holocene to the present. Fossil remains of small mammals (rodents and marsupials) including *Ctenomys* were present in all levels at this site and were attributed to owl predation (Tammone *et al.*, 2014). At CdC, radiocarbon analyses indicated that deposition of material at this site began by the early Holocene (10–12 kya) and continued to the present. Assemblages of small mammals at this site have also been attributed to owl predation (Tammone *et al.*, 2016a). The overall abundance of *Ctenomys* was similar at ACo and CdC, with deposits from both sites revealing a trend towards decreasing abundance of this genus from the past to the present. More specifically, while the abundance of *Ctenomys* relative to other small mammals ranged from 60 to 25% during the early and middle Holocene, respectively, the relative abundance of *Ctenomys* during the late Holocene was ~15% and fell to ~2.5% in modern owl pellets recovered from ACo and CdC (Tammone, personal observation). A similar trend was reported for CTI (Pearson & Pearson, 1993; Pardiñas & Teta, 2013).

Species identification

Based on our field observations, extant populations of tuco-tucos at La Lonja and Cerro Monte Redondo were identified as *C. sociablis*. In contrast, extant populations at Valle Encantado and Río Traful were identified as *C. haigi*. In all cases, analyses of complete cytochrome *b* sequences from these populations (n = 8–10 individuals per population) confirmed these taxonomic assignments, which were also consistent with the current known geographic distribution of each species.

A total of 49 fossil specimens (ACo: n = 12; CdC: n = 37) were successfully sequenced, resulting in the unambiguous genetic identification of these individuals as either *C. sociabilis* (n = 26) or *C. haigi* (n = 23). Within species, the uncorrected per cent sequence divergence between historical and modern samples (based on 136 bp of cytochrome *b*) was 1.2% for *C. sociabilis* and 0.6% for *C. haigi*. In contrast, between species, uncorrected per cent sequence divergence was 13.4% for modern specimens and 13.7% for fossil specimens; these values are similar to the greatest per cent sequence divergences within *Ctenomys* reported elsewhere (e.g. D'Elía, Lessa & Cook, 1999; Slamovits *et al.*, 2001; Parada *et al.*, 2011), indicating that the two study species are clearly genetically distinct. The neighbor-joining tree constructed from these sequences (Fig. 2) revealed that all specimens from ACo clustered with sequences from modern *C. sociabilis*, while specimens from CdC clustered with modern sequences from both *C. sociabilis* and *C. haigi*, indicating that both study species had been present historically at CdC.

Comparisons of relative species abundances

As indicated above, genetic identification of fossil specimens revealed that *C. sociabilis* was the only species of tuco-tuco present in fossil samples from ACo (Table 1; Supporting Information, Appendix S3). In contrast, analyses of modern owl pellets collected at ACo (*Ctenomys* MNI = 20) revealed that although *C. sociabilis* is currently the predominant species of ctenomyid at this site (70% of MNI), samples of *C. haigi* (the remaining 30% of the sample) were also detected (Supporting Information, Appendix S3). The nearest extant population of *C. haigi* is located *c.* 2 km east of ACo, on the opposite side of the Limay River, suggesting the predation by owls roosting at ACo encompasses both sides of the river.

Patterns of relative abundance at CdC and CTI were similar to one another but differed markedly from that reported for ACo. At both CdC and CTI, genetic identification of fossil specimens indicated that *C. sociabilis* was the most abundant species of tuco-tuco (>80% of specimens) in the oldest layer examined (interval D, 12 000–6000 year BP). The abundance of *C. haigi*, however, increased at both sites during more recent time intervals, rising to >50% in interval C (6000–3000 year BP) and >80% in interval B (3000–500 year BP) (Supporting Information, Appendix S3). Identification of specimens in modern owl pellets revealed that at present, *C. haigi* is the only tuco-tuco evident at either CdC or CTI (MNI = 54 and 7, respectively). For CTI, this finding is consistent with the observation that the extant population of *C. haigi* at Río Traful is closer to this cave site than the nearest known extant population of *C. sociabilis* at Cerro Monte Redondo (Fig. 1). Thus, at all three archaeological sites examined, there has been an increase in the relative abundance of *C. haigi* over the last 12 000 years.

Haplotype variation

When haplotype variation at the 136-bp portion of cytochrome *b* targeted in this study was examined across all temporal periods and sampling localities, levels of genetic variability appeared to be greater for *C. sociabilis* than for *C. haigi*. These analyses revealed a total of 13 haplotypes for *C. sociabilis* (n = 78 sequences) but only four haplotypes for *C. haigi* (n = 72 sequences). For *C. sociabilis*, 12 (92.3%) of the 13 haplotypes detected occurred only at a single locality. The largest number of these private haplotypes was found at CTI (n = 7), followed by ACo (n = 3) and CdC (n = 2); the single remaining haplotype was found at all three historical sites as well as in all extant populations of this species (Fig. 3). For *C. haigi*, three (75%) of the four haplotypes detected occurred only at a single locality.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

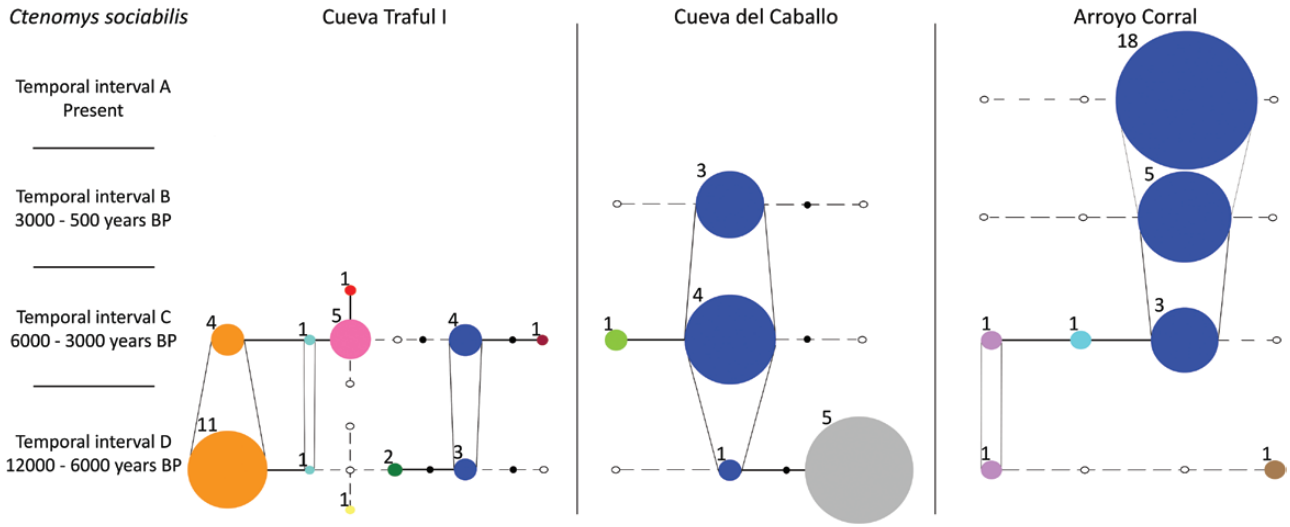

Figure 3. Haplotype networks for the 136-bp fragment of cytochrome *b* examined for fossil specimens and extant populations of *Ctenomys sociabilis*. The different temporal intervals examined are indicated, as is each study site. Each haplotype is depicted with a different colour; the size of each circle denotes the relative frequency of that haplotype, with the number of individuals displaying that haplotype also indicated. Empty circles denote haplotypes not detected during that time interval; base pair differences between haplotypes are indicated by a solid line. Vertical lines link the same haplotype appearing in different time intervals. The absence of haplotypes at Cueva Traful I (CTI) (intervals B and A) and Cueva del Caballo (CdC) (interval A) indicates the absence of samples for *C. sociabilis* for that site by interval combination.

Two of these private haplotypes were found at CdC, with the third found at CTI; the single remaining haplotype occurred at both sites as well as in all modern populations of this species (Fig. 4). Accordingly, overall pairwise estimates of F_{st} for historical populations of *C. sociabilis* ranged from 0.00 to 0.63, while the historical estimate of F_{st} for *C. haigi* was 0.00 (Table 2). Comparisons of historical haplotypes across time periods and cave sites revealed a total of 12 mutations (11 transitions, one transversion) in *C. sociabilis* (n = 78) and ten mutations (seven transitions, three transversions) in *C. haigi* (n = 72), providing no conspicuous evidence of interspecific differences in mutation rate.

Examination of complete cytochrome *b* sequences from modern populations of the study species revealed a very different pattern of haplotype variability. All modern populations of *C. sociabilis* sampled were characterized by a single haplotype, with this same haplotype shared among all populations (Table 3). In contrast, a total of seven different haplotypes were detected in the modern populations of *C. haigi* sampled (Table 3). All of these haplotypes were private, with no haplotypes shared between the two modern populations examined. Accordingly, F_{st} for modern populations of *C. sociabilis* was 0.00, while F_{st} for *C. haigi* was 0.15 (Table 2); this interspecific difference in the spatial structuring of modern haplotypes is the converse of that detected for historical haplotypes.

TEMPORAL CHANGES IN GENETIC DIVERSITY

Analyses of haplotype variation across temporal periods revealed that genetic diversity has been more dynamic in *C. sociabilis* than in *C. haigi*. In the latter species, the same, single haplotype was predominant in all temporal intervals, including modern populations of this species; this same pattern was evident at both CdC and CTI (Fig. 4). In contrast, in *C. sociabilis*, both the number of haplotypes and their relative frequencies varied over time (Fig. 3; Supporting Information, Appendix S5). At each site, there was an almost complete turnover of haplotypes between temporal intervals D and C; at ACo and CdC (the only sites for which more recent samples were available), all but one historical haplotype had disappeared by interval B (Fig. 3; Supporting Information, Appendix S5). Over time, the single haplotype present in modern populations of *C. sociabilis* increased in frequency; this haplotype was present at low frequencies at CTI and CdC during interval D but was not detected at ACo until interval C (Fig. 3). At La Lonja, the extant population of *C. sociabilis* closest to ACo, this haplotype occurred in all modern samples sequenced. Consistent with the distribution of extant populations of this species, no haplotypes for *C. sociabilis* were available in the most recent temporal interval(s) for CTI and CdC. The same single, modern haplotype found at La Lonja was found in all samples from Cerro Monte Redondo, the extant population of *C. sociabilis* nearest to CTI. Thus, while

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

C. haigi has been characterized by a general stability of cytochrome *b* haplotypes over time, *C. sociabilis* appears to have experienced a decrease in haplotype variability, with an increasing tendency for the same single haplotype to dominate more recent – including modern – samples from this species.

Consistent with this interspecific difference in temporal patterns of haplotypic change, other measures of genetic diversity also indicated that variability tended to be greater for *C. sociabilis* during intervals D and C but greater for *C. haigi* during intervals B and A (Table 4). At CdC and CTI (the two sites for which direct comparisons of the study species were possible), measures of the number of variable sites (s), haplotype diversity (h), nucleotide diversity (π) and nucleotide diversity corrected for heterochronous sampling (π_h) were generally greater for *C. sociabilis* during intervals D and C. In contrast, this pattern tended to reverse during intervals B and A (modern haplotypes from Cerro Monte Redondo only; no modern samples for *C. sociabilis* collected near CdC). Indeed, for *C. haigi*, the detection of only single haplotype at CTI resulted in estimates of zero genetic diversity for this site during intervals B and A (Table 4). Comparisons

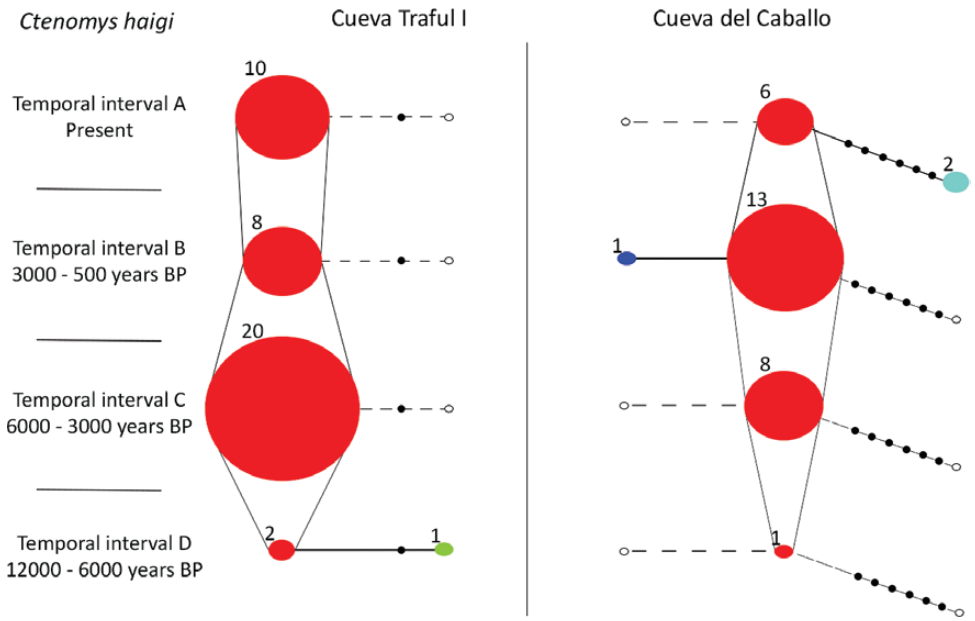

Figure 4. Haplotype networks for the 136-bp fragment of cytochrome *b* examined for fossil specimens and extant populations of *Ctenomys haigi*. The different temporal intervals identified are indicated as is each study site. Each haplotype is depicted with a different colour; the size of each circle denotes the frequency of that haplotype, with the number of individuals displaying that haplotype also indicated. Empty circles denote haplotypes not detected during that time interval; base pair differences between haplotypes are indicated by a solid line. Vertical lines link the same haplotype occurring in different temporal intervals. The absence of haplotypes at Arroyo Corral (ACo) reflects the absence of samples for *C. haigi* for that site.

Table 2. Pairwise F_{st} values among sampling sites within time intervals for complete (1,140-bp) cytochrome b sequences in modern populations (Present) and the 136-bp fragment of the same locus cytochrome b from fossil samples (Kya) of *C. sociabilis* and *C. haigi*

Populations	Species	F_{st}			
		Present	0.5–3 Kya	3–6 Kya	6–12 Kya
CdC–CTI	*Ctenomys sociabilis*	–	–	0.47	0.63
CdC–ACo	*C. sociabilis*	–	0	0.12	0.21
CTI–ACo	*C. sociabilis*	0	–	0.31	0.31
CdC–CTI	*C. haigi*	0.15	0	0	0

ACo, Arroyo Corral; CdC, Cueva del Caballo; CTI, Cueva Traful I.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

Table 3. Genetic diversity and results of neutrality tests for complete (1,140-bp) cytochrome b sequences from modern populations (upper panel) and the 136-bp fragment of cytochrome b from fossil samples (lower panel) of *C. sociabilis* and *C. haigi*. For each site, the number of individuals sequenced (n), number of haplotypes detected, number of variable sites (s), haplotype diversity (h), and nucleotide diversity (π) are shown. Values for Tajima's D, Fu's F_S and associated P-values are also shown

	Population	Species	# sequences	# haplotypes	# private haplotype	s	h	π	Tajima's D		Fu's F_S	
									D	P	F_S	P
Modern	Río Traful	*Ctenomys haigi*	10	4	4	7	0.77	2.20	−0.47	0.34	0.81	0.70
	Valle Encantado	*C. haigi*	8	3	3	79	0.71	33.85	0.60	0.77	12.41	1.00
	La Lonja	*C. sociabilis*	9	1	0	0	0	0	n.a.	n.a.	n.a.	n.a.
	Monte Redondo	*C. sociabilis*	9	1	0	0	0	0	n.a.	n.a.	n.a.	n.a.
Ancient	ACo	*C. sociabilis*	12	4	3	3	0.56	0.01	−0.37	0.36	−0.89	0.16
	CdC	*C. sociabilis*	14	3	2	3	0.58	0.01	0.61	0.76	1.12	0.75
	CTI	*C. sociabilis*	34	8	7	8	0.75	0.01	−0.09	0.51	−1.24	0.25
	CdC	*C. haigi*	23	2	1	1	0.09	0	−1.16	0.17	−0.99	0.06
	CTI	*C. haigi*	31	2	1	2	0.06	0	−1.50	0.02	−0.42	0.15

ACo, Arroyo Corral; CdC, Cueva del Caballo; CTI, Cueva Traful I; n.a., not calculated as absence of genetic variation.

Table 4. Summary of historical levels of genetic diversity for the 136-bp portion of cytochrome b sequenced. For each sampling locality and time period, the number of individuals sequenced (n), number of haplotypes detected, number of variable sites (s), haplotype diversity (h), nucleotide diversity (π), and nucleotide diversity corrected for heterochrony (π_h) are indicated. Modern sites (interval A; corresponding fossil localities given in parentheses) for *C. sociabilis* were La Lonja (ACo) and Cerro Monte Redondo (CTI); for *C. haigi*, modern sites were Valle Encantado (CdC) and Río Traful (CTI). No extant population of *C. sociabilis* occurs in the proximity of CdC

Species	Temporal interval	Site	n	Number of haplotypes	s	h	π	π_h
Ctenomys sociabilis	(A) Present	ACo	9	1	0	0	0	n.a.
		CdC	–	–	–	–	–	–
		CTI	9	1	0	0	0	n.a.
	(B) 0.5–3 Kya	ACo	5	1	0	0	0	0
		CdC	3	1	0	0	0	0
		CTI	0	0	0	0	0	0
	(C) 3–6 Kya	ACo	5	3	2	0.70000	0.00735	0.00156
		CdC	5	2	1	0.40000	0.00294	0.00052
		CTI	16	6	7	0.81667	0.01673	0.00156
	(D) 6–12 Kya	ACo	2	2	3	1.00000	0.02206	0.00327
		CdC	6	2	2	0.33333	0.00490	0.00078
		CTI	18	5	5	0.61438	0.01028	0.00055
C. haigi	(A) Present	CdC	8	2	7	0.42900	0.02206	n.a.
		CTI	10	1	0	0	0	n.a.
	(B) 0.5–3 Kya	CdC	14	2	1	0.14300	0.00105	0.00012
		CTI	8	1	0	0	0	0
	(C) 3–6 Kya	CdC	8	1	0	0	0	0
		CTI	20	1	0	0	0	0
	(D) 6–12 Kya	CdC	1	1	0	0	0	0
		CTI	3	2	2	0.66700	0.00980	0.00887

ACo, Arroyo Corral; CdC, Cueva del Caballo; CTI, Cueva Traful I; n.a., not calculated as not heterochrony between samples.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

of mean estimates of genetic diversity generated by our resampling procedure with those for (1) the full data set for CTI and (2) data from ACo and CdC suggested that the greater variability of *C. sociabilis* at CTI was not due to the larger sample size for this site (Supporting Information, Appendix S6).

Analyses of complete cytochrome *b* sequences from the modern populations sampled revealed striking interspecific differences in genetic diversity, with all measures of diversity equal to zero in *C. sociabilis* populations (Table 3). While the absence of genetic diversity in modern populations of *C. sociabilis* precluded calculations of Tajima's *D* and Fu's F_S for these animals, analyses of ancient samples revealed no evidence of departures from neutrality in this species (Table 3). Similarly, values of *D* and F_S for *C. haigi* did not generally differ from zero (Table 3), although the significantly negative value of Tajima's *D* for ancient samples from CTI ($D = -1.50$, $P = 0.02$; Table 3) is consistent with a population expansion for this species in the area surrounding CTI.

DISCUSSION

Our analyses of fossil and modern specimens of *C. sociablis* and *C. haigi* indicate that these species have been characterized by marked differences in patterns of genetic diversity over the past 12 000 years. Based on the portion of the mitochondrial cytochrome *b* gene targeted in this study, haplotype variation in *C. haigi* has remained relatively constant over this period; in contrast, *C. sociabilis* has experienced a marked reduction in the number of haplotypes detected. Consistent with this pattern, sequence-level measures of genetic diversity have also decreased since the mid-Holocene in *C. sociabilis* but not in *C. haigi*. Comparisons of complete cytochrome *b* sequences from extant populations of tuco-tucos located near each cave site indicated that, currently, *C. sociabilis* is characterized by only a single haplotype; in contrast, multiple haplotypes are present in extant populations of *C. haigi*. These changes in genetic diversity are associated with an increase in abundance of *C. haigi* relative to *C. sociabilis* at each cave site examined. These findings confirm and serve to expand the temporal and geographic scales of the differences in genetic diversity reported by Chan *et al.*, (2005) and Chan & Hadly (2011) for CTI. Thus, our analyses provide critical confirmation that the historical loss of genetic diversity in *C. sociabilis* was not limited to CTI but was instead widespread within the geographic distribution of this species.

These findings may have been influenced by several potential confounding factors. First, because only samples that were genetically identified to species were included in the analyses of genetic diversity, it is possible that interspecific differences in the success of our sequencing protocols resulted in the disproportionate representation of one focal species in our data set. Although this outcome would have affected estimates of relative abundances of the study species, it should not have produced the temporal changes in genetic diversity reported here. Second, estimates of historical levels of genetic diversity may have been affected by the small sample sizes available for some temporal intervals and cave sites. In particular, the number of specimens sequenced tended to be smaller for older time intervals, suggesting that potential underestimation of genetic diversity should have been greatest for those time periods. Contrary to this prediction, for *C. sociabilis*, genetic diversity at all sites was greater during older time intervals, implying that the overall patterns of temporal change in genetic diversity reported here did not result from limited samples sizes for some time periods or sampling localities examined. Finally, although the cytochrome *b* fragment examined here accounted for *c.* 70% of the variation reported previously for fossil *Ctenomys* from CTI (Chan *et al.*, 2005; Chan & Hadly, 2011), it is possible that use of a larger portion of the cytochrome *b* gene would have revealed considerably more historical diversity in the study species, notably *C. haigi*. Despite the greater number of haplotypes detected by Chan & Hadly (2011), their analyses revealed the same tendency for genetic diversity in *C. haigi* to have remained relatively constant over time, suggesting that our more limited data set was sufficient to detect general patterns of temporal change in this species. Nevertheless, we expect that use of additional molecular markers would be informative and future studies of *C. sociabilis* and *C. haigi* will employ more extensive, genome-level analyses of genetic diversity.

LOSS OF GENETIC DIVERSITY IN *C. SOCIABILIS*

Our analyses of fossil samples from ACo and CdC clearly indicate that the loss of genetic diversity reported previously for CTI (Chan *et al.*, 2005) was not limited to this site, but instead occurred at all three localities for which data are available. This finding has important implications for understanding the demographic and evolutionary histories of this species. Notably, because the factor(s) contributing to this decline must have been geographically widespread, it is possible to eliminate some potential explanatory factors, such as highly localized habitat changes that would impact some but not all of the cave sites examined. At the same time, the generality of the genetic decline in *C. sociabilis* raises intriguing questions regarding modern genetic variability in this species. Even if the factors promoting historical losses of diversity were the same for all

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

sites examined, stochastic influences such as localized genetic drift should have resulted in among-population differences in the haplotypes conserved (Slatkin, 1987; Wlasiuk, Garza & Lessa, 2003). Instead, historical loss of genetic diversity in *C. sociabilis* appears to have been associated with conservation of the same single haplotype across all localities sampled (see also Chan *et al.*, 2005). Collectively, these observations suggest that the demographic history of this species is complex and probably reflects the combined effects of multiple environmental factors operating over different spatial and temporal scales.

Interspecific differences in patterns of diversity

Several factors may have contributed to the observed interspecific differences in temporal patterns of genetic change. At the molecular level, variation in mutation rates may generate differential patterns of genetic diversity, including among congeneric species (Johns & Avise, 1998; Sunyaev *et al.*, 2003; Strandberg & Salter, 2004). Based on analyses of cytochrome *b*, *C. sociabilis* tends to be particularly divergent from other ctenomyids (Parada *et al.*, 2011), a pattern that may have arisen if this species has experienced particularly rapid evolution relative to its congeners. Although cytochrome *b* mutation rates for *Ctenomys* have been shown to be higher than those for some other rodent taxa (Lessa & Cook, 1998; Spradling, Hafner & Demastes, 2001; Parada *et al.*, 2011; Tomasco & Lessa, 2011), our comparisons of polymorphic sites in the cytochrome *b* sequences examined here revealed no evidence that mutation rates differ between our study species.

At the population level, the close physical proximity of the study species in the Limay Valley suggests that both have experienced generally similar environmental conditions over the past 12 000 years. As a result, it seems probably that the observed temporal differences in genetic diversity have been influenced by species-specific attributes such as documented differences in the degree of habitat specialization by *C. sociabilis* and *C. haigi*. The Limay Valley and adjacent hills are characterized by arid steppe grassland that is punctuated at irregular intervals by more mesic patches of grasses and shrubs known as mallines (León *et al.*, 1998; Bran, 2000); while populations of *C. haigi* are found in both habitat types, *C. sociabilis* is restricted primarily to mallin areas (Lacey & Wieczorek, 2003; Tammone *et al.*, 2012). Given this difference in habitat use, even general environmental changes in the Limay region may have differentially impacted the study species. During the mid-Holocene, the interface between arid and mesic habitat patches in the Limay region was spatially and temporally dynamic (Heusser & Streeter, 1980; Moreno, 1997) and this variability, combined with higher occurrence of topographic barriers (e.g. rivers) towards the western side of the Limay River, may have contributed to the decline of *C. sociabilis* but not *C. haigi* at the study sites.

In addition to the difference in habitat use, the study species also display several important differences in demography and social behaviour that may have contributed to the observed differences in genetic diversity (see Sands *et al.*, 2015). In particular, *C. sociabilis* is group living, with burrow systems routinely occupied by multiple adult females and their young (Lacey *et al.*, 1997; Lacey & Wieczorek, 2004). Groups arise due to natal philopatry by females and dispersal within the local population is common for both sexes (Lacey & Wieczorek, 2004). In contrast, *C. haigi* is solitary, with no more than one adult per burrow system (Lacey *et al.*, 1998). In this species, all individuals disperse from their natal burrow and settlement within the same local population is rare (Lacey, unpublished data). These differences in dispersal patterns are expected to lead to comparatively reduced gene flow in *C. sociabilis*, which should increase the potential for drift to enhance genetic diversity among local populations of this species compared to *C. haigi* (Slatkin, 1987; Bohonak, 1999). Thus, it seems probably that demographic differences between the study species have contributed to the temporal differences in genetic diversity reported here.

Collectively, these arguments suggest that even under generally homogenous environmental conditions, the study species are likely to have responded differently to changes in habitat conditions. Based on paleogenetic data from CTI, Chan *et al.*, (2005) identified three factors that may have triggered the reduction in genetic diversity in *C. sociabilis*: a catastrophic (volcanic) event, competitive exclusion by *C. haigi* and widespread changes in environmental conditions. We suggest that any of these scenarios would have generated different responses in the two study species. In particular, the greater habitat specialization of *C. sociabilis* would have made this species more sensitive to environmental changes, while its demography would have rendered it more susceptible to disruptions of migration and gene flow. In short, while the specific environmental factors impacting genetic diversity among the tuco-tucos of the Limay region remain to be determined, it is perhaps not surprising that *C. sociabilis* has experienced a greater historical change in genetic variability. More generally, our analyses may have implications for understanding the reported extinctions of other ctenomyids in Patagonia, including sizable populations of *C. magellanicus* in Santa Cruz Province (Hatcher, 1903; Pardiñas, 2013) and *C. emilianus* in Neuquén Province (Thomas &

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

Saint Leger, 1926; Tiranti, 1996; Chebez, Pardiñas & Teta, 2014).

Insights from aDNA

Our analyses of aDNA samples reveal several important aspects of the genetic histories of the study species that would not otherwise have been apparent. For example, our findings provide direct evidence that *C. sociabilis* – but not *C. haigi* – experienced a historical reduction in genetic diversity, resulting in the preservation of a single modern haplotype in the former species (see also Chan *et al.*, 2005; Chan & Hadly, 2011). Further, analyses of fossil sequences revealed that this loss of diversity did not simply result in the preservation of the most common haplotype in each population of *C. sociabilis*; at all sites examined, loss of diversity was associated with a shift to the same, shared haplotype, raising intriguing questions regarding the potential functional significance of this variant. Coalescent-based Bayesian analyses (Chan, Anderson & Hadly, 2006) would probably generate additional insights regarding the possible timing and severity (e.g. reduction in effective population size) of the historical event experienced by *C. sociabilis.* Unfortunately, our current data set is not suitable for such analyses given the low mutation rate for cytochrome *b* and the relatively short temporal window over which genetic diversity was examined, which preclude sufficient accumulation of informative mutations (Drummond *et al.*, 2003). Future studies including more extensive sequences, larger sample sizes and loci characterized by faster mutation rates may help to clarify the nature of the historical event(s) that affected genetic diversity in *C. sociabilis*.

CONCLUSIONS

Our analyses of historical specimens of *C. sociabilis* from ACo and CdC provide critical confirmation that the loss of genetic diversity reported previously for this species (Chan *et al.*, 2005) is a general pattern, occurring at multiple sites within the geographic distribution of this species. Similarly, our comparative analyses of *C. haigi* confirm that this species has undergone relatively little change in genetic diversity over the same temporal period. This generality has important implications for understanding the causal bases for the loss of diversity in *C. sociabilis*. In particular, the consistency of these patterns across sites indicates that (1) environmental changes contributing to this loss of genetic diversity must also have been widespread enough to impact multiple localities and (2) the study species have likely responded differently to changing conditions in the Limay Valley. Modern populations of *C. sociabilis* and *C. haigi* are characterized by pronounced differences in ecology, behaviour and demography that are expected to impact patterns of genetic diversity; assuming that the same differences characterized historical populations of these species, they seem likely to have contributed to the distinct temporal patterns of diversity detected. These findings illustrate the complexity of the factors that shape patterns of genetic variation and underscore the value of using aDNA to explore directly patterns of temporal change in diversity.

ACKNOWLEDGEMENTS

For permission to conduct fieldwork at Arroyo Corral and La Lonja, we thank S. Jones and the Delegación Regional Patagonia of the Administración de Parques Nacionales Argentina. For permission to conduct fieldwork at Cueva del Caballo and Valle Encantado, we thank C. Van Ditmar, as well as the Dirección de Fauna of the Provincia de Río Negro. For access to rodent specimens and other data associated with the excavations at Arroyo Corral and Cueva del Caballo, we thank A. Hajduk, P. Arias and their colleagues. For material collected at Cueva Traful and for the inspiration to study the ctenomyids in the Limay region, we thank the late O. Pearson. E. Hadly generously allowed us to use her laboratory facilities at Stanford University to extract ancient DNA from ctenomyid remains and to learn the procedures associated with genetic analyses of fossil specimens. For assistance with the genetic analyses, we thank J. Hsu and L. Li (Stanford University) as well as L. Smith and B. Lavin (UC Berkeley). Finally, we thank two anonymous reviewers for their helpful comments. Funding for this work was provided by NSF RAPID grant DEB-1201541, the American Society of Mammalogists (Latin American Field Research Award), the Cleveland Zoological Society (Scott Neotropical Fund), the Museum of Vertebrate Zoology (UC Berkeley) and the PICT (Agencia) 2008-0547.

REFERENCES

Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge: Harvard University Press.

Blaauw M. 2010. Methods and code for 'classical' age-modelling of radiocarbon sequences. *Quaternary Geochronology* **5:** 512–518.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. *The Quaternary Review of Biology* **74:** 21–45.

Bran D. 2000. Las regiones ecológicas de la Patagonia y sus principales formaciones vegetales. In: INTA, ed. *Principios de Ecología y Conservación de los Recursos Naturales de la Patagonia*. Buenos Aires, Argentina: INTA, 93–100.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

Bronk Ramsey C. 2009. Bayesian analysis of radiocarbon dates. *Radiocarbon* **51:** 337–360.

Campos PF, Kristensen T, Orlando L, Sher A, Kholodova MV, Götherström A, Hofreiter M, Drucker DG, Kosintsev P, Tikhonov A, Baryshnikov GF, Willerslev E, Gilbert MTP. 2010. Ancient DNA sequences point to a large loss of mitochondrial genetic diversity in the saiga antelope (*Saiga tatarica*) since the Pleistocene. *Molecular Ecology* **19:** 4863–4875.

Chan YL, Anderson CNK, Hadly EA. 2006. Bayesian estimation of the timing and severity of a population bottleneck from ancient DNA. *Public Library of Science, Genetics* **2:** 451–460.

Chan YL, Hadly EA. 2011. Genetic variation over 10000 years in *Ctenomys*: comparative phylochronology provides a temporal perspective on rarity, environmental change and demography. *Molecular Ecology* **20:** 4592–4605.

Chan YL, Lacey EA, Pearson OP, Hadly EA. 2005. Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent. *Biology Letters* **1:** 423–426.

Chebez JC, Pardiñas UFJ, Teta P. 2014. *Mamiferos terrestres de la Patagonia*. Buenos Aires: Vazquez Mazzini Editores.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1659.

Collins CJ, Rawlence NJ, Worthy TH, Scofield RP, Tennyson AJD, Smith I, Knapp M, Waters JM. 2013. Pre-human New Zealand sea lion (*Phocarctos hookeri*) rookeries on mainland New Zealand. *Journal of the Royal Society of New Zealand* **44:** 1–16.

Cooper A, Poinar HD. 2000. Ancient DNA: do it right or not at all. *Science* **289:** 1139.

Cutrera AP, Lacey EA, Busch C. 2005. Genetic structure in a solitary rodent (*Ctenomys talarum*): implications for kinship and dispersal. *Molecular Ecology* **14:** 2511–2523.

D'Elía G, Lessa EP, Cook JA. 1999. Molecular phylogeny of tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae): evaluation of the *mendocinus* species group and the evolution of asymmetric sperm. *Journal of Mammalian Evolution* **6:** 19–38.

de Bruyn M, Hoelzel AR, Carvalho GR, Hofreiter M. 2011. Faunal histories from Holocene ancient DNA. *Trends in Ecology & Evolution* **26:** 405–413.

Depaulis F, Orlando L, Hänni C. 2009. Using classical population genetics tools with heterochroneous data: time matters! *PLoS ONE* **4:** e5541.

Drummond AJ, Pybus OG, Rambaut A, Forsberg R, Rodrigo AG. 2003. Measurably evolving populations. *Trends in Ecology & Evolution* **18:** 481–488.

Excoffer L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10:** 564–567.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147:** 915–925.

Gilbert MTP, Bandelt HJ, Hofreiter M, Barnes I. 2005. Assessing ancient DNA studies. *Trends in Ecology & Evolution* **20:** 541–544.

Grayson DK. 1984. *Quantitative zooarchaeology: topics in the analysis of archaeological faunas*. New York: Academic Press, Inc.

Hadly EA, van Tuinen M, Chan YL, Heiman K. 2003. Ancient DNA evidence of prolonged population persistence with negligible genetic diversity in an endemic tuco-tuco (*Ctenomys sociabilis*). *Journal of Mammalogy* **84:** 403–417.

Hajduk A, Arias Cabal P, Chauvín A, Crivelli E, Albornoz AM, Armendariz Gutiérrez A, Cueto Rapado M, Fernandez M, Fernandez Sánchez M, Fernandez V, Goye S, Lezcano MJ, Tapia Sagarna J, Teira Mayolini LC. 2007. Poblamiento Temprano y arte rupestre en el área del lago Nahuel Huapi y cuenca del río Limay (Pcias. de Río Negro y Neuquén, Argentina) *XVI Congreso Nacional de Arqueología Argentina*. Tras las huellas de las materialidad. Jujuy, Argentina, 8 al 12 de octubre de 2007: Universidad Nacional de Jujuy, 393–399.

Hatcher JB. 1903. Volume I. Narrative of the expedition and geography of Southern Patagonia. *Reports of the Princeton University Expeditions to Patagonia, 1896–1899*. Stuttgart: Schweizerbart Science Publishers, 224.

Heusser CJ, Streeter SS. 1980. Temperature and precipitation record of the past 16,000 years in southern Chile. *Science* **210:** 1345–1347.

Hofreiter M, Serre D, Poinar HD, Kuch M, Paabo S. 2001. Ancient DNA. *Nature Reviews Genetics* **2:** 353–359.

Hogg AG, Hua Q, Blackwell PJ, Niu M, Buck CE, Guilderson TP, Heaton TJ, Palmer JG, Reimer PJ, Reimer RW, Turney CSM, Zimmerman SRH. 2013. SHCal13 Southern Hemisphere calibration, 0–50,000 years cal BP. *Radiocarbon* **55:** 1–15.

Iglesias V, Whitlock C, Markgraf V, Bianchi MIM. 2014. Postglacial history of the Patagonian forest/steppe ecotone (41–43°S). *Quaternary Science Reviews* **94:** 120–135.

Johansson M, Primmer CR, Merila J. 2006. History vs. current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). *Molecular Ecology* **15:** 975–983.

Johns GC, Avise JC. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. *Molecular Biology and Evolution* **15:** 1481–1490.

Kuhner MK. 2009. Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution* **24:** 86–93.

Lacey EA. 2001. Microsatellite variation in solitary and social tuco-tucos: molecular properties and population dynamics. *Heredity* **86:** 628–637.

Lacey EA, Braude SH, Wieczorek JR. 1997. Borrow sharing by colonial tuco-tucos (*Ctenomys sociabilis*). *Journal of Mammalogy* **78:** 556–562.

Lacey EA, Braude SH, Wieczorek JR. 1998. Solitary burrow use by adult Patagonian tuco-tucos (*Ctenomys haigi*). *Journal of Mammalogy* **79:** 986–991.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

Lacey EA, Wieczorek JR. 2003. Ecology of sociality in rodents: a ctenomyid perspective. *Journal of Mammalogy* **84:** 1198–1211.

Lacey EA, Wieczorek JR. 2004. Kinship in colonial tuco-tucos: evidence from group composition and population structure. *Behavioral Ecology* **15:** 988–996.

León RJC, Bran D, Collantes M, Paruelo JM, Soriano A. 1998. Grandes unidades de vegetación de la Patagonia extra andina. *Ecología Austral* **8:** 125–144.

Lessa EP, Cook JA. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Molecular Phylogenetics and Evolution* **9:** 88–99.

Libardo P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Liu H, Prugnolle F, Manica A, Balloux F. 2006. A geographically explicit genetic model of worldwide human-settlement history. *The American Journal of Human Genetics* **79:** 230–237.

Lyman RL. 2008. *Quantitative paleozoology*. Cambridge: Cambridge University Press.

Mapelli FJ, Mora MS, Mirol PM, Kittlein MJ. 2012. Population structure and landscape genetics in the endangered subterranean rodent *Ctenomys porteousi*. *Conservation Genetics* **13:** 165–181.

Markgraf V. 1983. Late and postglacial vegetational and paleoclimatic changes in subantarctic, temperate, and arid environments in Argentina. *Palynology* **7:** 43–70.

Mora MS, Lessa EP, Cutrera AP, Kittlein MJ, Vassallo AI. 2007. Phylogeographical structure in the subterranean tuco-tuco *Ctenomys talarum* (Rodentia: Ctenomyidae): contrasting the demographic consequences of regional and habitat-specific histories. *Molecular Ecology* **16:** 3453–3465.

Moreno P. 1997. Vegetation and climate bear Lago Llanquihue in the Chilean Lake District between 20,200 and 9500 ^{14}C yr BP. *Journal of Quaternary Science* **12:** 485–500.

Parada A, D'Elía G, Bidau CJ, Lessa EP. 2011. Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). *Journal of Mammalogy* **92:** 671–682.

Pardiñas UFJ. 1999. Tafonomía de microvertebrados en yacimientos arqueológicos de Patagonia (Argentina). *Arqueología* **9:** 265–308.

Pardiñas UFJ. 2013. Localidades típicas de micromamíferos en Patagonia: el viaje de J. Hatcher en las nacientes del río Chico, Santa Cruz, Argentina. *Mastozoología Neotropical* **20:** 413–420.

Pardiñas UFJ, Teta P. 2013. Holocene stability and recent dramatic change in micromammalian communities of northwestern Patagonia. *Quaternary International* **305:** 127–140.

Pearson OP, Christie MI. 1985. Los tuco-tucos (género *Ctenomys*) de los Parques Nacionales Lanín y Nahuel Huapi, Argentina. *Historia Natural* **5:** 337–343.

Pearson OP, Pearson AK. 1993. La fauna de mamíferos pequeños de Cueva Traful I, Argentina: pasado y presente. *Præhistoria* **1:** 211–224.

Prost S, Anderson CNK. 2011. TempNet: a method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods in Ecology and Evolution* **2:** 663–667.

Ramakrishnan U, Hadly EA. 2009. Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies. *Molecular Ecology* **18:** 1310–1330.

Sands AF, Matthee S, Mfune JKE, Matthee CA. 2015. The influence of life history and climate driven diversification on the mtDNA phylogeographic structures of two southern African *Mastomys* species (Rodentia: Muridae: Murinae). *Biological Journal of the Linnean Society* **114:** 58–68.

Sikes RS, Gannon WL, and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* **92:** 235–253.

Slamovits CH, Cook JA, Lessa EP, Rossi MS. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae): a phylogenetic approach. *Molecular Biology and Evolution* **18:** 1708–1719.

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. *Science* **236:** 787–792.

Spradling TA, Hafner MS, Demastes JW. 2001. Differences in rate of cytochrome-*b* evolution among species of rodents. *Journal of Mammalogy* **82:** 65–80.

Strandberg AK, Salter LA. 2004. A comparison of methods for estimating the transition:transversion ratio from DNA sequences. *Molecular Phylogenetics and Evolution* **32:** 495–503.

Sunyaev S, Kondrashov FA, Bork P, Ramensky V. 2003. Impact of selection, mutation rate and genetic drift on human genetic variation. *Human Molecular Genetics* **12:** 3325–3330.

Swofford DL. 2003. *PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4*. Sunderland: Sinauer Associates.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123:** 585–595.

Tammone MN. 2016. *Pérdida de diversidad genética: implicaciones para la evolución y la conservación de dos especies de Ctenomys (Rodentia: Ctenomyidae) en Patagonia norte*. Tesis Doctoral, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue.

Tammone MN, Hajduk A, Arias P, Teta P, Lacey EA, Pardiñas UFJ. 2014. Last glacial maximum environments in northwestern Patagonia revealed by fossil small mammals. *Quaternary Research* **82:** 198–208.

Tammone MN, Lacey EA, Hajduk A, Christie M, Pardiñas UFJ. 2016a. The Quaternary record of *Euneomys* (Mammalia, Rodentia, Cricetidae) from northwestern Patagonia: evidence for regional extinction. *Journal of Vertebrate Paleontology* **36:** e1212363.

Tammone MN, Lacey EA, Relva MA. 2012. Habitat use by colonial tuco-tucos (*Ctenomys sociabilis*): specialization, variation, and sociality. *Journal of Mammalogy* **93:** 1409–1419.

Tammone MN, Lavin BR, Pardiñas UFJ, Lacey EA. 2016b. Post-extinction discovery of a population of the highly

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017

endemic colonial tuco-tuco (*Ctenomys sociabilis*). *Journal of Mammalogy* **97:** 1753–1763.

Tammone MN, Lezcano MJ, Lacey EA, Pardiñas UFJ. 2017. Los roedores tuco-tucos (*Ctenomys* sp.) y su relacion con las ocupaciones humanas de cazadores-recolectores en el valle superior del río Limay. *Macroscopia* **7**: 1–11

Templeton AR. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology Resources* **7:** 381–397.

Templeton AR, Routman E, Phillips CA. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinurn*. *Genetics* **140:** 767–782.

Thomas O, Saint Leger J. 1926. The Spedan Lewis South American exploration. V. Mammals obtained by Señor E. Budin in Neuquén. *The Annals and Magazine of Natural History 9th Series* **18:** 635–641.

Tiranti SI. 1996. Small mammals from Chos Malal, Neuquén, Argentina, based upon owl predation and trapping. *Texas Journal of Science* **48:** 303–310.

Tomasco IH, Lessa EP. 2011. The evolution of mitochondrial genomes in subterranean caviomorph rodents: adaptation against a background of purifying selection. *Molecular Phylogenetics and Evolution* **61:** 64–70.

Villarosa G, Outes V, Hajduk A, Montero EAC, Sellés D, Fernandez M, Crivelli E. 2006. Explosive volcanism during the Holocene in the Upper Limay River Basin: the effects of ashfalls on human societies, Northern Patagonia, Argentina. *Quaternary International* **158:** 44–57.

Walker M, Johnsen S, Rasmussen SO, Steffensen J, Popp T, Gibbard P, Hoek P, Lowe J, Andrews J, Björck S, Cwynar L, Hughen K, Kershaw P, Kromer B, Litt T, Lowe D, Nakagawa T, Newnham R, Schwander J. 2008. The Global Stratotype Section and Point (GSSP) for the base of the Holocene Series/Epoch (Quaternary System/Period) in the NGRIP ice core. *Episodes* **31:** 264–267.

Wlasiuk G, Garza JC, Lessa EP. 2003. Genetic and geographic differentiation in the Rio Negro tuco-tuco (*Ctenomys rionegrensis*): inferring the roles of migration and drift from multiple genetic markers. *Evolution* **57:** 913–926.

Wright AJ, Edwards RJ, van de Plassche O, Blaauw M, Parnell AC, van der Borg K, Jong AFM, Roe HM, Selby K, Black S. 2017. Reconstructing the accumulation history of a saltmarsh sediment core: which age-depth model is best? *Quaternary Geochronology* **39:** 35–67.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Radiocarbon dates.
Appendix S2. Age-depth models.
Appendix S3. Chronological schemes.
Appendix S4. *Ctenomys* samples.
Appendix S5. *Ctenomys sociabilis* complete haplotype network.
Appendix S6. Assess sample size.

Downloaded from https://academic.oup.com/biolinnean/article-abstract/doi/10.1093/biolinnean/blx118/4591693
by guest
on 14 November 2017