ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

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

by

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Supplementary Material

Dating of stratigraphic levels

Uncorrected radiocarbon dates for Cueva Traful are shown in Table S1. Capa 1 and Capa 2 were likely bioturbated because radiocarbon dates indicate that they are older than strata below them (Montero et al. 1983).

Ancient DNA protocol and sequence reliability

We extracted DNA from 73 teeth from 9 stratigraphic units using the protocol outlined in Hadly et al (2003). We amplified a 255 bp of cytochrome-b following strict ancient DNA standards, which include (1) no previous amplification of modern Ctenomys DNA in the building, (2) use of primers designed specifically for ctenomyid species, (3) use of negative controls for detection of contamination at all stages of analysis, and (4) spatial and temporal separation of DNA extractions and PCR set-up from PCR amplification (Cooper & Poinar 2000; Hadly et al. 2003). All PCR products were sequenced in both directions to increase the chances of detecting random sequencing errors. To provide independent confirmation of our results, five of the teeth in our sample were broken in half prior to extraction. DNA from three of these teeth was extracted, amplified, and sequenced in the laboratory of Dr. Steven Palumbi (Stanford University); the resulting sequences from the Palumbi lab matched exactly the sequences obtained in our lab.

In addition to these measures, PCR products from three teeth were cloned to confirm the validity of our sequences and to detect nuclear pseudogenes. For two samples, Pfu polymerase was used for amplification. For these samples, 8/8 (from 2036

ypb) and 5/6 (from 5906 ybp) cloning products matched the target sequence exactly; the one non-matching sequence was identified as human using a Blast search on GenBank. A third sample (from 3749 ybp) was amplified using Taq Gold. Eighteen sequences were obtained from this sample after cloning. Twelve of these sequences matched the target exactly. One sequence was highly damaged and although it aligned with the target sequence and blasted as *Ctenomys*, it did not match the target sequence. Four sequences had a single, unique base pair change and one sequence had two unique base pair changes; collectively, these consisted of three first position changes and three second position changes. The random nature of the base pair changes in these sequences suggests Taq error, rather than actual variation in the sequences analyzed.

Finally, the pattern of sequence variation detected provides additional support for the validity of our molecular data. Analyses of transition:transversion ratios and the codon positions of mutations are both concordant with valid mitochondrial sequences. Further, there is no evidence that the number of PCR errors increased as a function of DNA degradation (age). Due to the difficulty in distinguishing between *C. sociabilis* and *C. haigi* based on tooth morphology, we extracted, amplified, and sequenced both species concomittantly. In contrast to the marked discrepancy between historical and modern variability detected in *C. sociabilis*, in *C. haigi* the level of diversity is comparable for modern and ancient samples. Given the similar taphonomy and preservation in the cave site and similar handling of samples from both species, we can think of no reason why degradation and, hence, PCR and sequencing errors should be more pronounced for *C. sociabilis*. Further, haplotypic diversity in *C. sociabilis* from our oldest temporal interval (8147-10209 ybp) is less than that in the more recent intervals (5,655-6,135 and 3,293-

3,749 ybp), suggesting that age (potential for DNA degradation) and haplotypic variability are not directly related.

Species identification

We used a phylogenetic approach to identify ancient *Ctenomys* sequences to species. Specifically, a neighbor-joining tree was constructed using PAUP 4.0b10 (PPC) (Swofford 2003) based on uncorrected p-distance for cytochrome *b* sequences for the 39 species of *Ctenomys* (n = 129 sequences) listed in GenBank (Figure S1). One thousand bootstrap replicates indicated 100% support for a monophyletic clade which includes a modern *C. sociabilis* sequence and the ancient samples from Cueva Traful identified as *C. sociabilis.*

Analysis of divergent clade of ancient *C. sociabilis*

Identifying *Ctenomys* species based solely on genetic variation is difficult and we cannot exclude completely the possibility that the divergent clade detected in Cueva Traful represents a distinct species. However, we believe that this clade is unlikely to represent another species for the following reasons:

1. Univariate and multivariate analyses of incisors indicate that morphology is similar for the modern (n = 8) and ancient (n = 20) *C. sociabilis* clades. Specifically, total length, width, and tip width of incisors did not differ significantly ($p > 0.05$) between clades. Although initially mid-incisor diameter did differ significantly ($p = 0.018$), this result did not persist following Bonferroni

correction of p values ($\alpha < 0.0125$). Further, a principal components analysis indicated extensive overlap between the modern and ancient clade (Figure S2).

2. Intraspecific sequence variation for modern ctenomyid species is comparable to the amount of cytochrome *b* polymorphism observed in the Cueva Traful *C. sociabilis* samples. Although there have been few studies of intraspecific variation in cytochrome *b* for ctenomyids, we compared the amount of variation found in Cueva Traful, to that found within species of ctenomyids listed in GenBank. While the sequences on GenBank are likely biased downward (i.e., toward reduced variability) due to insufficient sampling, the divergence between the ancient and modern clades is in the middle of the range of values obtained for modern ctenomyid species (Table S2).

3. The cytochrome *b* sequence divergence detected within Cueva Traful is at the low end of the range of divergence values found between modern sister species pairs of ctenomyids (Table S3).

Literature Cited

Cooper, A. & Poinar, H. N. 2000 Ancient DNA: Do it right or not at all. *Science* **289**, 1139-1139.

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*). *Journal of Mammalogy* **84**, 403-417.

Montero, E. A. C., Curzio, D. E. & Silveira, M. J. 1983 La estratigrafia de la cueva Traful I (Provincia del Neuquen). *Praehistoria* **1**, 9-160.

Rozas, J. & Rozas, R. 1999 DnaSP,version 3: an integrated program for molecular population genetics and moleculat evolution analysis. *Bioinformatics* **15**, 174-175.

Swofford, D. L. 2003 PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, MA: Sinauer Associates.

Figure S1. A neighbor-joining tree based on p-distance of 232 bp of cytochrome-*b*. The ancient haplotypes identified here as *C. sociabilis* are $\leq$ 4.3% sequence divergent from one another and form a monophyletic clade relative to other modern tuco species as well as ancient material attributed to *C. haigi*.

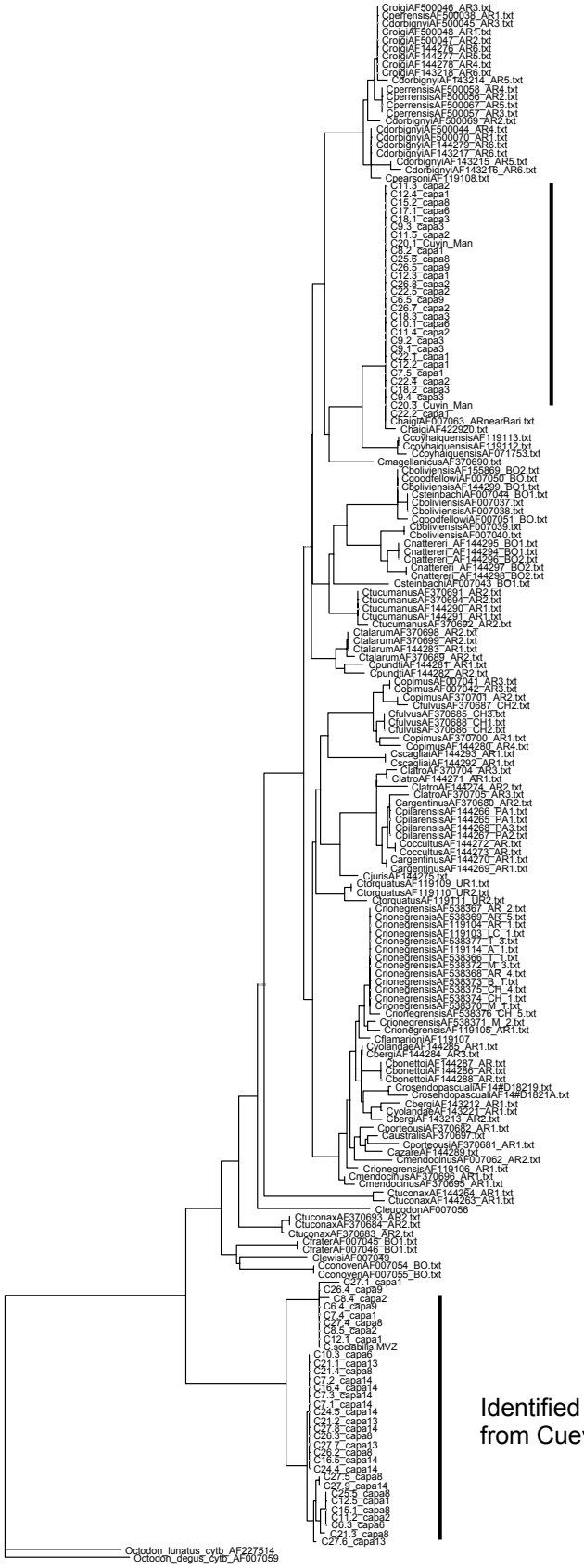
Identified as *C. haigi* from Cueva Traful
Identified as *C. sociabilis* from Cueva Traful
Octodon_lunatus_cytb_AF227514
Octodon_degus_cytb_AF007059
0.005 substitutions/site

Figure S2. Principal components analysis of modern (M) and ancient (A) clades based on incisor morphology.

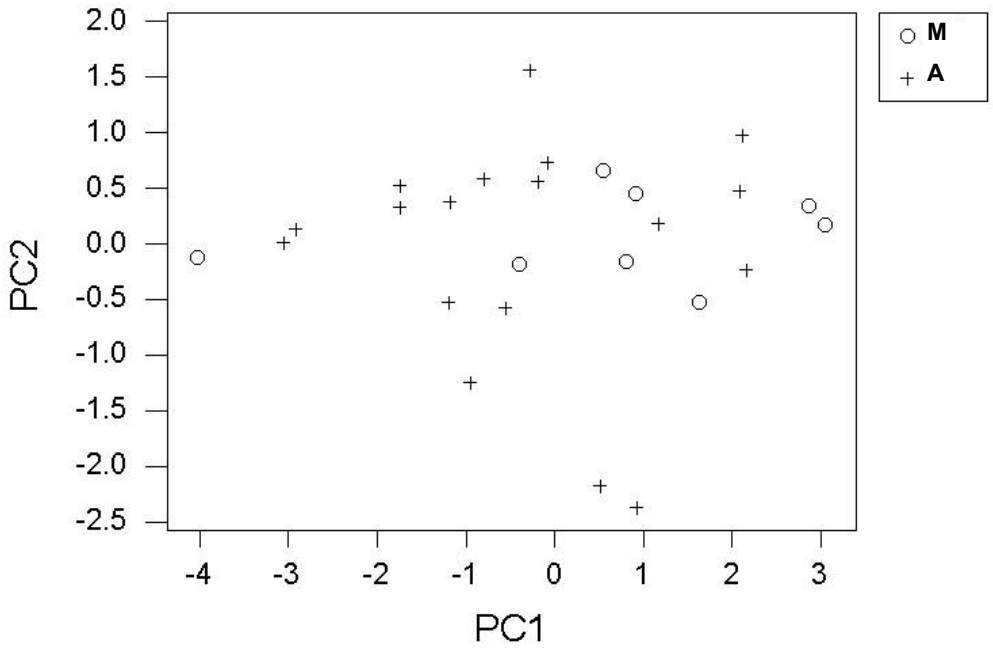

Table S1. Uncorrected radiocarbon dates for Cueva Traful. Letters refer to the location of each sample on excavation grid. * indicates samples from (Montero et al. 1983). Multiple dates from the same interval were averaged for Figure 1.

Interval	^{14}C age ybp		Material	Lab
capa 1 Ir	3,293	±49	jaw bone	NSF-Arizona AMS laboratory
capa 2 Ir	3,749	±94	jaw bone	NSF-Arizona AMS laboratory
capa 3 Hu	2,033	±43	jaw bone	NSF-Arizona AMS laboratory
*capa 3 Iu, Hu	2,230	±40	burned log	Mt. Soledad Radiocarbon Laboratory
*capa 4 Hu	2,720	±40	carbon	Mt. Soledad Radiocarbon Laboratory
*capa 4 Hu	2,720	±40	carbon	Mt. Soledad Radiocarbon Laboratory
capa 5			tephra	volcanic ash
capa 6 Ir	5,906	±84	jaw bone	NSF-Arizona AMS laboratory
capa 8 Hu	5,655	±73	jaw bone	NSF-Arizona AMS laboratory
*capa 9 Hs, Is	6,030	±115	burned log	Teledyne Isotopes
*capa 9 Im	6,240	±60	burned log	Mt. Soledad Radiocarbon Laboratory
*capa 9 Hs, Is	6,030	±115	burned log	Teledyne Isotopes
*capa 9 Im	6,240	±60	burned log	Mt. Soledad Radiocarbon Laboratory
*capa 13 In	7,850	±70	vegetable carbon	Mt. Soledad Radiocarbon Laboratory
*capa 13 In	9,285	±313	vegetable carbon	Laboratorio de Tritio y Radiocarbono LATYR (La Plata)
*capa 13 In	7,308	±285	vegetable carbon	Laboratorio de Tritio y Radiocarbono LATYR (La Plata)
capa 14 Ho	10,209	±96	jaw bone	NSF-Arizona AMS laboratory

Table S2. Amount of intraspecific cytochrome *b* sequence polymorphism (n = 232 bp fragment) within 30 species *Ctenomys* for which sufficient sequence data were available on GenBank (calculated using DNAsp 4.0.6; Rozas & Rozas 1999).

Species	within group average no. differences	standard error	within group average p distance	standard error
C. tuconax	14.4000	2.6010	0.0621	0.0118
C. steinbachi	13.0000	3.4059	0.0560	0.0139
C. boliviensis	6.4000	1.7625	0.0276	0.0072
C. porteousi	5.0000	2.3103	0.0216	0.0089
C. mendocinus	4.6667	1.5817	0.0201	0.0075
C. opimus	4.0000	1.4046	0.0172	0.0063
C. punddti	4.0000	1.7192	0.0172	0.0074
C. latro	3.8333	1.3493	0.0165	0.0062
C. bergi	3.3333	1.3306	0.0144	0.0062
CT C. sociabilis	**2.9720**	**0.9910**	**0.0128**	**0.0040**
C. dorbignyi	2.5556	0.9418	0.0110	0.0044
CT C. haigi	2.0000	0.3094	0.0086	0.0015
C. torquatus	2.0000	1.1256	0.0086	0.0050
C. rosenpaascuali	2.0000	1.4225	0.0086	0.0053
C. nattereri	1.8000	0.9560	0.0078	0.0048
C. fulvus	1.5000	0.8267	0.0065	0.0037
C. goodfellowi	1.0000	0.9660	0.0043	0.0042
C. haigi	1.0000	0.9289	0.0043	0.0038
C. argentinus	0.6667	0.6348	0.0029	0.0028
C. coyhaiquensis	0.6667	0.5971	0.0029	0.0025
C. talarum	0.5000	0.4814	0.0022	0.0022
C. perrensis	0.4000	0.4368	0.0017	0.0017
C. tucomanus	0.4000	0.3704	0.0017	0.0018
C. rionegrensis	0.3366	0.1440	0.0015	0.0007
C. roigi	0.0000	0.0000	0.0000	0.0000
C. conoveri	0.0000	0.0000	0.0000	0.0000
C. frater	0.0000	0.0000	0.0000	0.0000
C. occultus	0.0000	0.0000	0.0000	0.0000
C. scagliai	0.0000	0.0000	0.0000	0.0000
C. bonettoi	0.0000	0.0000	0.0000	0.0000
C. pilarensis	0.0000	0.0000	0.0000	0.0000

Table S3. Amount of interspecific cyt b sequence divergence (n = 232 bp fragment) among sister species pairs of *Ctenomys* (calculated using DNAsp 4.0.6; Rozas & Rozas 1999). Data were obatined from GenBank; sister species were identified following Castillo et al. (2005).

Sister taxa	Between group average no. differences	Standard Error	Between group average p distance	Standard Error
C. frater - C. lewisi	13.0000	3.5800	0.0560	0.0147
C. pearsoni - C. torquatus	9.0000	2.7903	0.0388	0.0115
C. boliviensis - C. steinbachi	8.5000	1.8440	0.0366	0.0073
C. haigi - C. coyhaiquensis	6.8333	2.4294	0.0295	0.0103
CT C. sociabilis	**6.4500**	**2.2397**	**0.0278**	**0.0089**
C. mendocinus - C. rionegrensis	4.7073	1.6016	0.0203	0.0070