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Genetic variation in the South American burrowing rodents of the genus *Ctenomys* (Rodentia: Ctenomyidae)

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Abstract

An electrophomorphic survey of protein variation was done on eleven populations of *Ctenomys* from Argentina. Thirty-four enzyme and protein loci were studied. Estimates of average heterozygosity (H) per population ranged from 0 to 13 percent. Genetic relatedness (Nei's D) is close between most populations (D = .03 to .08) collected from localities as far apart as 1800 kilometers and separated in elevation by as much as 3000 meters. However, two forms (*C. argentinus* and an undescribed species) are more distantly related to one another (D = .35) and to other populations belonging to the *mendocinus* species group. These results are discussed with respect to the evolutionary relationships in this genus and to the hypothesis of reduced molecular variability in fossorial rodents, and they are compared to the well-studied fossorial pocket gophers (genus *Thomomys*) of North America.

Introduction

Except in Australia burrowing rodents occur on all continents where they have independently derived from syntopic, above-ground relatives. Thus, the large number of shared specialized adaptations enabling subterranean existence must result from convergent

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evolution. It is no surprise, therefore, that the morphological and ecological features associated with the fossorial life mode have been of long-standing interest to evolutionary biologists (see, for example, Pearson 1959; Woods 1975; Morlock 1983).

Nevo (1979) presents a comprehensive review of the characteristics of fossorial rodents, arguing that the underground ecological niche also specifies convergently similar patterns of social behavior, reproductive strategies, and population demography. He characterizes the genetic structure of burrowing rodents as one of highly isolated demes of small size. Individuals are viewed as having lower-than-average levels of genic (primarily allozymic) variability due to selection for presumptive uniformity of the subterranean environment (the niche width-genetic variation hypothesis). This general view has been challenged by several authors, based both on observations of quite high levels of genetic variation for several species (e.g., Patton and Yang 1977) and on the overall lack of a correlation between lowered variability and the fossorial life-style (Schnell and Selander 1981).

In South America the most specialized burrowing rodents belong to the genus *Ctenomys* (Family Ctenomyidae, following Woods 1984), colloquially known as tuco-tucos, tunduques, or tacorros in different parts of their range. This genus is a member of the Suborder Hystricomorpha, which has its center of diversity in South America. Clearly, tuco-tucos have independently arrived at the same fossorial life-style as the North American pocket gophers (Family Geomyidae) and the Eurasian mole-rats (Family Spalacidae), each of which is also independently derived within separate subordinal lineages (see Pearson 1959).

As presently considered by systematists, the genus *Ctenomys* is extremely speciose. Thirty-three species are recognized in the most recent summary (Honacki et al. 1982), and new forms are still being described (Travi 1981; Contreras and Berry 1982a, b; Contreras and Contreras 1984). A bewildering array of pelage colorations, body sizes, and a few variable osteological traits were the original bases for describing new species of *Ctenomys*. Additional kinds of characters, such as proteins (Roig and Reig 1969), chromosomes (Reig and Kiblisky 1969; Kiblisky et al. 1977; Gallardo 1979; Freitas and Lessa 1984), and penial sperm morphology (Altuna and Lessa 1985; Feito and Gallardo 1982) are now contributing information about tuco-tuco relationships. The relative recency of origin (Mones and Castiglione 1979; Frailey et al. 1980), wide geographic distribution (Fig. 1), and great taxonomic diversity suggest that very active speciation is underway in the genus.

Molecular characterization of tuco-tuco populations would provide an important perspective on their evolutionary dynamics. For one, these animals provide a further test of Nevo's (1979) niche width-genetic variability hypothesis. Secondly, genetic data can provide a phylogenetic framework in which to judge the present classification of the genus. With these two goals in mind, we have used horizontal starch-gel electrophoresis to examine protein variation in eleven populations of *Ctenomys* from Argentina. Collections came from localities as far apart as 1800 kilometers, with elevations differing by as much as 3000 meters. Results of this initial survey are then compared to data available for other fossorial rodent lineages, and examined with respect to the hypothesis of low genetic variability in homogeneous environments. Finally, we compare the patterns of diversification of tuco-tucos with the well-studied case of the North American fossorial pocket gophers, genus *Thomomys*.

Methods and materials

Tissue preparation and electrophoretic procedures

Tissues and washed red-blood cells were taken from freshly killed animals and immediately frozen. Voucher specimens of skins and/or skulls are deposited in the collection of the Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, California or in the personal collection of J. R.

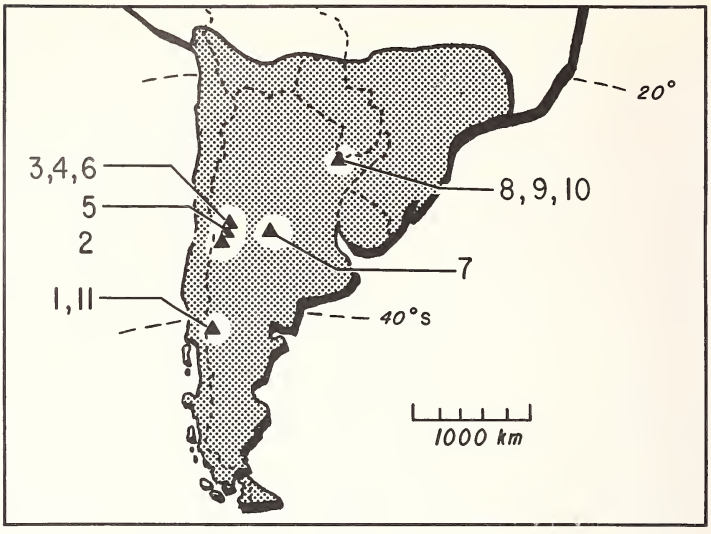

Fig. 1. The approximate distribution of *Ctenomys* in southern South America. Sampled localities are indicated (see Specimens Examined section for specific localities indexed by number)

Contreras. The Diamante and Paramillo samples were collected in 1981, and stored at −20 °C for two years. The remaining samples were collected in January 1984 and stored at −76 °C. Aqueous extracts of kidney (K) and muscle (M) were prepared just prior to electrophoresis in late 1984. Tissues were minced with scissors, mixed with an equal volume of deionized water, and centrifuged at 16,000 RPM for 30 minutes to prepare a cleared, supernatant extract. A hemolysate extract was prepared by centrifuging lysed RBC's or blood drained from the heart (animals from Mercedes).

Standard horizontal starch-gel electrophoresis was performed (Harris and Hopkinson 1976), using Sigma starch throughout the study. Staining recipes are described in Selander et al. (1971) and Harris and Hopkinson (1976). Electrophoretic conditions are presented in Table 1.

Analysis

In cases where more than one allele (electromorph) was found at a locus, the relative mobilities (RM) of the alleles were examined by running them side-by-side on a comparison gel, and the distance the alleles moved from the origin was measured. In all but one case alleles were assigned a relative mobility value by dividing their absolute mobility by that of a standard (RM = 100) allele. The most frequent allele among the samples was chosen as the standard.

Estimates of genetic relationships and variability were computed using the formulae of Nei (1978) and Rogers (1972). The unweighted, pair-group method of analysis (UPGMA) was used to cluster the genetic distance values between populations. All computations were performed using the BIOSYS computer program (Swofford and Selander 1981).

The numbers of animals per sample ranged from one to seventeen. Since sample size can effect the accuracy of the estimates of genetic variability and relatedness (Nei 1978; Gorman and Renzi 1979), an attempt was made to illustrate the effect that small sample sizes can have in altering these estimates. A random sub-sample (three of the 17 animals) from the Mercedes collection was treated separately from the whole sample, and estimates of heterozygosity levels and genetic distances to the other samples were computed.

Specimens examined

C. haigi – [1] Prov. Rio Negro, Depto. Bariloche, 13 km WSW San Carlos de Bariloche, 800 m (MVZ 166421-424). *C. mendocinus* – [2] Prov. Mendoza, Depto. San Carlos, 3 km S Laguna Diamante, 3400 m (MVZ 162935); [3] Prov. Mendoza, Depto. Las Heras, Quebrada de Jaguelitos, Sierras de Uspallata, 3000 m (MVZ 166429-431); [4] Prov. Mendoza, Depto. Las Heras, near Cruz Paramillo, Sierras de Uspallata, 2800 m (MVZ 162936-937); [5] Prov. Mendoza, Depto. Tunuyan, 2 km N Villa Seca, 980 m (MVZ 166417-420); [6] Prov. Mendoza, Depto. Las Heras, 18.5 km N Mendoza, 750 m (MVZ 166426); [7] Prov. San Luis, Depto. Gr. Pedernera, 6 km SE Villa Mercedes, 515 m (MVZ

169022-036, + 2 uncatalogued specimens). *C. bonettoi* – [8] Prov. Chaco, (JRC 0205-0206). *C. dorbignyi* – [9] Prov. Corrientes, (JRC 0198-0202). *C. argentinus* – [10] Prov. Chaco, (JRC 0203-0204). Undescribed species A – [11] Prov. Neuquen, Depto. Los Lagos, Estancia Fortin Chacabuco, 2 km E and 2 km S Cerro Puntudo, 1000 m (MVZ 166425). These eleven localities are plotted in Figure 1.

Table 1

Enzymes and numbers of alleles (electromorphs) resolved in *Ctenomys*

Mitochondrial and supernatant enzymes are denoted by M and S, respectively, while numbered enzymes are identified in order of decreasing relative mobility from the gel origin

Enzyme	Enzyme commission number	Locus abreviation	Number of alleles	Tissue source[1]	Electrophoretic conditions[2]
glycerol-3-phosphate dehydrogenase	1.1.1.8	*Gpd*	4	K	A
sorbitol dehydrogenase	1.1.1.14	*Sordh*	2	K	A
lactate dehydrogenase	1.1.1.27	*Ldh-A*	2	K	F
	1.1.1.27	*Ldh-B*	3	K	F
malate dehydrogenase	1.1.1.37	*Mdh-1*	1	M	A
	1.1.1.37	*Mdh-2*	1	M	A
malic enzyme	1.1.1.40	*Me*	4	K	A
isocitrate dehydrogenase	1.1.1.42	*Icd-1*	2	K	A
	1.1.1.42	*Icd-2*	3	K	A
phosphogluconate dehydrogenase	1.1.1.44	*6-pgd*	3	M	D[3]
glyceraldehyde-phosphate dehydrogenase	1.2.1.12	*Gapdh*	1	K	A[4]
superoxide dismutase	1.15.1.1	*Sod*	1	K	E
purine nucleoside phosphorylase	2.4.2.1	*Np*	3	M	A
glutamate-oxaloacetate transaminase	2.6.1.1	*Got-S*	2	K	A
	2.6.1.1	*Got-M*	1	K	A
creatine kinase	2.7.3.2	*Ck*	1	M	G
phosphoglucomutase	2.7.5.1	*Pgm-2*	1	M	A
esterase	3.1.1.1	*Est-D*	2	K	C
erythrocytic acid phosphatase	3.1.3.2	*Eap*	2	K	F
peptidase	3.4.11	*Pep-B*	5	K	B
	3.4.11	*Pep-C*	4	K	B
	3.4.11	*Pep-S*	2	K	B
prolidase D (proline dipeptidase)	3.4.13.9	*Pep-D*	4	K	B
adenosine deaminase	3.5.4.4	*Ada*	1	M	G
aldolase	4.1.2.13	*Ald*	1	M	D
carbonic anhydrase	4.2.1.1	*Ca-1*	3	H	H
	4.2.1.1	*Ca-2*	1	H	H
aconitase	4.2.1.3	*Acon-2*	3	M	A
glyoxalase I	4.4.1.5	*Glo*	1	M	G
mannose phosphate isomerase	5.3.1.8	*Mpi*	4	M	A
glucophosphate isomerase	5.3.1.9	*Gpi*	2	K	B
albumin protein	–	*Alb*	5	M	C
hemoglobin protein	–	*Hb*	2	K, (H)	C
unidentified protein	–	*Pt-1*	1	M	C

[1] K = kidney, M = muscle, H = hemolysate. – [2] From SELANDER et al. (1971): A – Tris-citrate, pH 8.0, 130 V, 4 hr; B – "Poulik" system, adjusted to pH 9.1, 250 V, 3 hr; C – Lithium hydroxide, pH 8.1, 300 V, 3 hr; D – Tris maleic acid, pH 7.0, 100 V, 4 hr; E – Tris-EDTA-borate, pH 8.2, 200 V, 4 hr. From AYALA et al. (1972): F – Tris-citrate, pH 7.0, 180 V, 3 hr. From HARRIS and HOPKINSON (1976): G – Histidine, pH 7.0, 100 V, 3 hr. From BLAKE (1976): H – Sodium succinate, pH 5.0, 100 V, 3 hr. – [3] NADP was added to the gel after cooking, and before it solidified. – [4] NAD and 2-mercaptoethanol were added to the gel after cooking and before it solidified.

Table 2

Allele frequencies at 22 variable loci in *Ctenomys* rodents

Locus[1] (mobility)	Allele	*haigi* Bariloche	Diamante	Paramillo	Jaguelitos	*mendocinos* Tunuyan	Las Heras	Mercedes	Mercedes[2]	*bonettoi* Chaco	*dorbignyi* Corrientes	*argentinus* Chaco	species A Chacabuco
	N =	4	1	2	3	4	1	17	3	2	5	2	1
Gpd	111										.40		
(19.5)	100	.75		.75	1.00	.88	1.00	.47	.83	1.00	.40	1.00	
	096	.25	1.00			.12		.53	.17		.20		
	078			.25									1.00
Sordh	475											1.00	
(0.8)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
Ldh-A	146							.03					
(−7.9)	100	1.00	1.00	1.00	1.00	1.00	1.00	.97	1.00	1.00	1.00	1.00	1.00
Ldh-B	102									.25			
(58.5)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.75	1.00	1.00	1.00
	097												
Me	104							.06					
(15.7)	100	1.00	1.00	1.00	1.00	1.00	1.00	.74	1.00	1.00	1.00	1.00	
	090							.03					
	null							.17					1.00
Icd-1	100	1.00	.50	1.00	1.00	.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(23.2)	080		.50			.25							
Icd-2	240				.50								
(3.0)	100	1.00	1.00	1.00	.50	1.00	1.00	.97	1.00	1.00	1.00	1.00	
	038							.03					
	027												1.00
6-Pgd	109			.75				.03					
(12.6)	100	1.00	1.00	.25	1.00	1.00	1.00	.94	1.00	1.00	1.00	1.00	
	084							.03					1.00
Np	173						.50						1.00
(10.0)	109											1.00	
	100	1.00	1.00	1.00	1.00	1.00	.50	1.00	1.00	1.00	1.00		
Got-S	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
(18.6)	086											1.00	
Est-D	100	.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(16.5)	069	.50											
Eap	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00
(30.9)	062										1.00		
Pep-B	106					.12		.06					
(36.6)	101							.15	.17				
	100	1.00	1.00	.75	1.00	.88	1.00	.71	.50	1.00	1.00	1.00	1.00

	098	1.00									1.00	1.00	
	097												1.00
Pep-S	108			.25									
(24.2)	100	1.00	1.00	.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pep-D	122		.50	.75							.20		
(32.2)	116										.80	1.00	.50
	111	.12				.25				.25			
	100	.88	.50	.25	1.00	.75	1.00	1.00	1.00	.75			.50
Ca-1	100	.50	1.00	.75		.50							
(−1.5)	180										.60		
	347	.50		.25	1.00	.50	1.00	1.00	1.00	1.00	.40	1.00	1.00
Acon-2	160			.50	.17								
(2.0)	100	1.00	1.00	.50	.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	000												1.00
Mpi	108							.06	.17				
(43.7)	100	.75			1.00	1.00	1.00	.47	.33	.75	.20	1.00	
	099		1.00	1.00				.47	.50	.25	.80		1.00
	094	.25											
Gpi	137					.25							
(13.9)	100	1.00	1.00	1.00	1.00	.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alb[3]	a	.12											
(c = 49.2)	b	.88		.50	.50	1.00		.53	.50			1.00	
	c		.50	.50	.50		1.00	.47	.50				1.00
	d									1.00			
	e		.50								1.00		
Hb	101												
(34.5)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
mean number of alleles/locus		1.2	1.1	1.2	1.1	1.2	1.0	1.4	1.2	1.1	1.1	1.0	1.0
percentage of loci polymorphic		18	9	24	12	21	3	18	12	9	12	0	3
mean heterozygosity (s.e.)		.08 (.03)	.09 –	.13 (.04)	.06 (.03)	.08 (.03)	.03 –	.08 (.03)	.07 (.04)	.04 (.02)	.06 (.03)	0 –	.03 –

[1] The actual mobility (mm) of the 100 allele. – [2] This is a sub-sample, consisting of only 3 of the 17 animals examined. – [3] The albumin alleles are so similar in relative mobility that only a central allele (c) is measured.

Table 2

Allele frequencies at 22 variable loci in *Ctenomys* rodents

Locus[1] (mobility)	Allele	*bonettoi* Bariloche	Diamante	Paramillo	Jagueliños	*mendocinus* Tunuyan	Las Heras	Mercedes	Mercedes[2]	*bonettoi* Chaco	*dorbignyi* Corrientes	*argentinus* Chaco	species A Chacabuco
	N =	4	1	2	3	4	1	17	3	2	5	2	1
Gpd	111										.40		
(19.5)	100	.75		.75	1.00	.88	1.00	.47	.83	1.00	.40	1.00	
	096	.25	1.00			.12		.53	.17		20		
	078			.25									1.00
Sordh	475											1.00	
(0.8)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
Ldh-A	146							.03					
(−7.9)	100	1.00	1.00	1.00	1.00	1.00	1.00	.97	1.00	1.00	1.00	1.00	1.00
Ldh-B	102									.25			
(58.5)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.75	1.00	1.00	1.00
	097												
Me	104							.06					
(15.7)	100	1.00	1.00	1.00	1.00	1.00	1.00	.74	1.00	1.00	1.00	1.00	
	090							.03					
	null							.17					1.00
Icd-1	100	1.00	.50	1.00	1.00	.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(23.2)	080		.50			.25							
Icd-2	240				50								
(3.0)	100	1.00	1.00	1.00	.50	1.00	1.00	.97	1.00	1.00	1.00	1.00	
	038							.03					
	027												1.00
6-Pgd	109			.75				.03					
(12.6)	100	1.00	1.00	.25	1.00	1.00	1.00	.94	1.00	1.00	1.00	1.00	
	084							.03					1.00
Np	173						.50						1.00
(10.0)	109											1.00	
	100	1.00	1.00	1.00	1.00	1.00	.50	1.00	1.00	1.00	1.00		
Got-S	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
(18.6)	086											1.00	
Est-D	100	.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(16.5)	069	.50											
Eap	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00
(30.9)	062										1.00		
Pep-B	106					.12		.06					
(36.6)	101							.15	.17				
	100	1.00	1.00	.75	1.00	.88	1.00	.71	.50	1.00	1.00	1.00	1.00
	092							09	33				
	088			.25									
Pep-C	100		1.00	1.00	.67	1.00	1.00	1.00	1.00	1.00			
(61.5)	099				.33								
	098	1.00									1.00	1.00	
	097												1.00
Pep-S	108			.25									
(24.2)	100	1.00	1.00	75	1.00	1 00	1.00	1.00	1 00	1.00	1.00	1.00	1.00
Pep-D	122		.50	.75							.20		
(32 2)	116										.80	1.00	50
	111	.12				.25				.25			
	100	.88	.50	.25	1.00	.75	1.00	1.00	1.00	.75			.50
Ca-1	100	.50	1.00	.75		50							
(−1.5)	180										.60		
	347	.50		.25	1.00	50	1.00	1.00	1.00	1.00	.40	1.00	1.00
Acon-2	160			.50	.17								
(2.0)	100	1.00	1.00	.50	83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	000												1.00
Mpi	108							.06	.17				
(43.7)	100	.75			1.00	1.00	1.00	47	.33	.75	.20	1.00	
	099		1.00	1.00				47	.50	.25	80		1.00
	094	.25											
Gpi	137					.25							
(13.9)	100	1.00	1.00	1.00	1.00	.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alb[3]	a	.12										1.00	
(c = 49.2)	b	.88		.50	.50	1.00		.53	.50				
	c		.50	.50	.50		1.00	47	.50				1.00
	d									1.00			
	e		50								1.00		
Hb	101												
(34.5)	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
mean number of alleles/locus		1.2	1.1	1.2	1.1	1.2	1.0	1.4	1.2	1.1	1.1	1.0	1.0
percentage of loci polymorphic		18	9	24	12	21	3	18	12	9	12	0	3
mean heterozygosity (s.e.)		.08 (.03)	.09 –	.13 (.04)	.06 (.03)	.08 (.03)	.03 –	.08 (.03)	.07 (.04)	.04 (.02)	.06 (.03)	0 –	.03 –

[1] The actual mobility (mm) of the 100 allele. – [2] This is a sub-sample, consisting of only 3 of the 17 animals examined. – [3] The albumin alleles are so similar in relative mobility that only a central allele (c) is measured.

Results

A total of 34 loci, encoded by 23 enzymes and three non-enzymatic proteins, were studied. All specimens examined were apparently fixed for the same electromorph at 12 of these loci; at the remaining 22 loci one or more animals had variable banding patterns. The patterns of variation observed are consistent with genetically-based differences as reported for humans and other organisms (Harris and Hopkinson 1976). Estimated gene frequencies at these polymorphic loci are presented in Table 2.

Allozyme activity and band patterns

Comments are necessary about the banding patterns we observed at some loci. Populations were highly variable for alleles that showed very small mobility differences at the *Gpd, Mpi,* and *Alb* loci. At the malic enzyme locus (*Me*) a null allele apparently is present in some populations. The Chacabuco specimen was missing a *Me* allozyme, and three animals from Mercedes also lacked such an allozymic band. In none of these samples was there blockage by the counter-staining action of SOD, nor was there any indication of denaturation of the extracts. We conclude that these four animals were homozygous for an inactive (null) gene product. We assume the distribution of the *Me* genotypes in the Mercedes population is in Hardy-Weinberg equilibrium, because this is true at other polymorphic loci in this sample. Since it is technically difficult to identify a heterozygous genotype involving a null allele, our genotype determinations must certainly underestimate the true frequency of the presumptive null allele in this sample. The one esterase that was scored (*Est-D*) is visualized only with the fluorescing substrate 4-methylumbelliferyl acetate. This enzyme does not hydrolyse the alpha-napthyl phosphate substrate. Because of this difference in chemical activity, we assume that this locus is homologous to the *Est-D* locus of humans (Harris and Hopkinson 1976). The acid phosphatase locus that we scored is the one that reacts with 4-methylumbelliferyl phosphate. Although two distantly separated isoenzymes fluoresce on the gels, only one locus is thought to determine these bands in humans (Harris and Hopkinson 1976). But, in frogs two tightly linked loci may produce the two bands commonly seen on gels (Wright et al. 1980). The peptidases of *Ctenomys* have been studied by Lewis (1972), who inferred their homologies to the human isozymes by selective staining with different di- and tri-peptide substrates. We observed the same peptidase patterns seen in this earlier study, as well as tentatively identify another locus (*Pep-S*) that is not very active in the red-cell samples that Lewis studied. On our gels the relative mobilities of the bands of these four loci are (from most to least anodal) *Pep-C, Pep-B, Pep-D,* and *Pep-S*. The allozymes at the albumin (*Alb*) locus are so similar to one another in their relative mobilities that three-digit, numerical identifications of the alleles would not identify most of them. Instead, the alleles are alphabetically identified from *a* to *e* in order of their decreasing mobilities.

Sampling biases

The effect on the sample statistics of the study of only a few individuals is demonstrated in our analysis of a restricted subsample (3 animals) and the full collection (17 specimens) from Mercedes. As expected in a random sampling process, the difference between the large and small collections is the absence of some alleles in the latter sample (Table 2). In the complete collection there were 22 alleles at eight polymorphic loci, while the subsample had only 14 of these electromorphic variants. All of the missing alleles in the subsample were of low frequency ($< .18$) in the full sample. Despite a nearly six-fold increase in the number of animals examined, the mean heterozygosity changed by only one percentage

point (8 to 7 percent) from the complete to the restricted sample (Table 2). The increase from 3 to 17 animals in the sample did not produce very large differences in genetic distance values between Mercedes and the other samples (Table 3). Most of the *D*-value differences between the two Mercedes samples and the other populations examined are about $D = .01$, the maximum observed is only $D = .02$.

Although sample sizes were small for all but one population, polymorphic alleles were shared among localities across the range of our collections. For example, at the *Gpd* locus,

Table 3

Genetic distance estimates between samples of *Ctenomys*, based on 34 protein loci

Values in upper triangular matrix are Nei distances (D), those in the lower one are Rogers' distances

			1	2	3	4	5	6	7a	7b	8	9	10	11
1	*haigi*	Bariloche	—	.12	.09	.05	.04	.08	.06	.06	.07	.12	.14	.37
2	*mendocinus*	Diamante	.34	—	.04	.13	.10	.13	.08	.09	.12	.14	.30	.35
3	*mendocinus*	Paramillo	.31	.24	—	.07	.05	.08	.05	.04	.07	.13	.23	.28
4	*mendocinus*	Jaguelitos	.24	.35	.29	—	.02	.02	.03	.02	.03	.16	.17	.31
5	*mendocinus*	Tunuyan	.21	.30	.26	.18	—	.05	.03	.03	.04	.16	.17	.38
6	*mendocinus*	Las Heras	.29	.34	.30	.16	.23	—	.03	.03	.04	.19	.18	.29
7a	*mendocinus*	Mercedes	.25	.26	.24	.17	.19	.19	—	.00	.04	.14	.20	.28
7b	*mendocinus*	Mercedes[1]	.25	.29	.24	.17	.18	.18	.09	—	.03	.14	.19	.30
8	*bonettoi*	Chaco	.28	.34	.29	.20	.21	.21	.20	.19	—	.15	.19	.34
9	*dorbignyi*	Corrientes	.34	.35	.36	.38	.38	.40	.36	.36	.37	—	.21	.37
10	*argentinus*	Chaco	.36	.51	.46	.40	.39	.41	.42	.41	.42	.43	—	.34
11	species A	Chacabuco	.55	.54	.49	.51	.56	.50	.49	.51	.54	.55	.60	—

[1] Based on sub-sample of only 3 individuals (see text).

the *100* and *096* alleles were both present from Tunuyan north to the Chaco region (*C. bonettoi* and *C. dorbignyi*). A more extreme latitudinal polymorphism exists at the *Pep-D* locus where two alleles (*111* and *100*) are segregating at Bariloche, Tunuyan, and in *C. bonettoi,* across a distance of nearly 1800 kilometers. It seems likely that future studies, using large population samples, will show more examples of such patterns of shared polymorphisms.

Genetic variation within and among populations

The average genetic variability (H) is shown at the bottom of Table 2. Estimated values range from complete homozygosity ($H = 0.0$) in the Chacabuco animal to a maximum value ($H = 0.13$) at the Paramillo locality. The overall, unweighted mean heterozygosity of the 11 populations sampled is nearly 6.2 percent.

The genetic distances among populations and species sampled are given in Table 3, and a summary of relationships is presented as a phenogram based on Nei's D in Figure 2. Four principal groups are distinguishable by genetic distances greater than $D = 0.14$. Three of these major lines are each represented by only one sample, while the fourth lineage contains eight samples that form two distinctive sub-groups separated by a smaller genetic difference. Undescribed Species A, from Chacabuco, is most dissimilar to the remaining 10 population samples. The average distance (Nei's D) of this species to the others is about .34. The second most distantly related sample is *C. argentinus.* An average D-value of about .20 separates this species from the remaining ones. *Ctenomys dorbignyi,* from Corrientes, is next most distinctive, being separated by a distance of about .14 from the remaining eight samples. Within the latter group, two of the high-mountain populations (Diamante and Paramillo) are more similar to one another ($D = 0.04$) than they are to the

last group of six samples, from which they differ by an average distance of .09. The final group, consisting of populations identified as *C. bonettoi*, *C. haigi*, and *C. mendocinus*, are very similar to one another, with the largest average distance among them only .06.

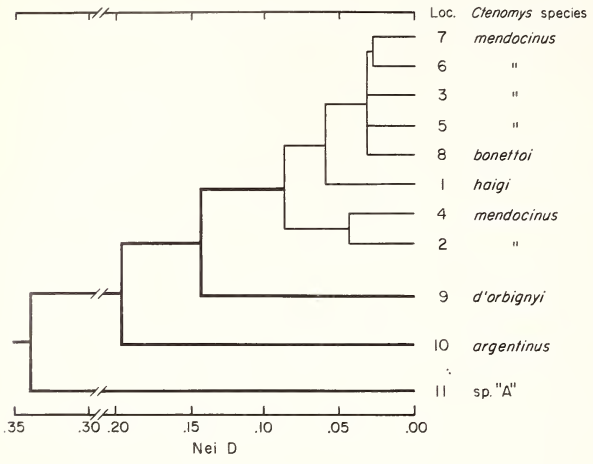

Fig. 2. Phenogram of genetic relationships among 11 populations of tuco-tucos (genus *Ctenomys*) from Argentina, based on Nei's genetic distance (*D*) and clustered by the unweighted pair group method

Discussion

Sample size effects

The effects of sample size on estimates of heterozygosity and genetic distance have been examined theoretically and empirically (Nei 1978; Gorman and Renzi 1979). Both studies concluded that small sample sizes tended to produce a regular overestimation of genetic distance. They also concluded that while the variance in these two statistics increases proportionally with the true genetic heterozygosity, this error is reduced by studying greater numbers of loci. The variance also rapidly declines to a minimum value with increasing sample size. In theory the variance of the genetic distance becomes small and constant when 50 loci are studied using only one specimen per population.

Our use of only 34 loci is somewhat less than ideal for constructing a dendrogram of relationships (Nei et al. 1983). However, the topological reliability of such a tree increases with the genetic difference between pairs of comparisons. We are most confident about the four major branches dividing the eleven populations in the phenogram shown in Figure 2, and less sure about the true relationships among the eight populations of the *mendocinus*-complex.

With regard to the magnitude of error in estimating the true heterozygosity, Gorman and Renzi (1979). determined that a sample of 8 to 12 individuals yields an estimate that is within one percent of the value obtained from much larger samples while only two individuals yields an estimate within 2.5 percent of the one produced with large samples. We only noted a change of one percentage point in the heterozygosity estimate between the subsample of three animals and the full collection of 17 specimens from Mercedes. Our demonstration with these materials, and the work of Nei (1978) and Gorman and Renzi (1979), suggests that the heterozygosity estimated for our five populations consisting of three or more animals are not likely to change by more than one percentage point if more individuals are examined. For the six other populations where only one or two individuals were surveyed, the heterozygosity estimates shown in Table 2 are more likely to change when larger samples are studied.

Phylogenetic relationships

This study provides the first comparative electrophoretic data from which to assess relationships among tuco-tuco populations. The only other molecular analysis of *Ctenomys* relationships is the immunological study of Roig and Reig (1969). Other efforts used to group tuco-tucos have been based on osteological and external morphological (Ellerman 1940; Contreras and Berry 1982b) and spermatozoan characteristics (Feito and Gallardo 1982). Unfortunately, among all of these other studies, there is only inconsequential overlap among the studied populations and our own. Thus, comparisons of alternative data in resolving systematic relationships among tuco-tucos is not possible at present.

The genetic distance estimates in Table 3 range from a Nei's D of .03 to .45. The clustering algorithm produced a tree in which these eleven samples are separable into four comparatively distinct lineages (Fig. 2). Three of these include only a single form, each currently recognized as a distinct species. The fourth contains eight of the populations sampled, including representatives of three nominal species (*mendocinus, haigi,* and *bonettoi*) and populations from all three geographic regions of Argentina from which specimens were available. Despite this taxonomic and geographic diversity, the maximum genetic divergence between members of this group is only $D = .13$ (Table 3), a small amount of differentiation among rodent species (Ayala 1975). More typical D-values for congeneric rodent species are nearly twice this value, at $D = .20$. The general lack of differentiation among members of this fourth group suggests that either the taxonomic treatment of tuco-tuco populations is frequently in error, or that the amount of molecular divergence is unrelated to speciation in this genus.

The current taxonomy of *Ctenomys* is in a state of general chaos. Many species are known only from a few specimens from the type locality, and comprehensive studies of geographic variation in any identified species are virtually non-existent. Most species have parapatric distributions, sequentially replacing one another across the range of the genus (see Fig. 19–23 in Mares and Ojeda 1982). These features make it difficult at present to understand the natural distribution of important biological characteristics that might define biological species in the genus. The variation present in coloration, size, and skull measurements still provides the major diagnostic characters used in the descriptions of new *Ctenomys* species, but this may be insufficient to define the real evolutionary units in the genus.

However, the considerable karyological diversification known for *Ctenomys* populations strongly implies that the genus is comprised of many reproductively isolated species. Reig and Kiblisky (1969) showed that even within the relatively small geographic area of Tucuman Province in Argentina three named forms differed radically in their diploid numbers ($2n = 28, 42,$ and 61). The inferred structural rearrangements that produced these different karyotypes would almost surely render hybrids and their backcrosses sterile or inviable in the event that matings took place between these neighboring forms in the field. Less dramatic, but equivalent types of differences, are present among the populations they studied from the grassland habitats of eastern Argentina and adjacent Uruguay. Similar patterns of interpopulational chromosomal variation have been reported for *Ctenomys* in Chile, Uruguay, and Brazil (Gallardo 1979; Freitas and Lessa 1984; Kiblisky et al. 1977). The karyotypes of our populations are unknown, but it is likely that major chromosomal differences also exist between the four major genetic groups we have identified, and that such karyotypic variation is even present among the populations in the *mendocinus*-complex. This, albeit indirect, contrast between different amounts of protein, karyotypic, and morphological change found in the tuco-tucos re-emphasizes the point that during organic evolution complete independence between different biological systems frequently occurs (reviewed by Schnell and Selander 1981).

Our array of samples is inadequate to estimate the maximum amount of molecular variation in the genus *Ctenomys*. We lacked samples of species from the Chilean lowlands, the Altiplano regions of northern Argentina to Peru, and the Bolivian Chaco which are likely to represent even more distantly related lineages to those we have studied. But the data do suggest two things about the evolutionary history of tuco-tucos. First, the comparatively small amount of genetic divergence among approximately six species sampled from a large geographical area suggests a relatively recent radiation of parts of the group. This is consistent with the paleontological evidence that reports the first appearance of the genus in Pleistocene deposits (Mones and Castiglioni 1979; Frailey et al. 1980).

The second point to emphasize is that the animals of the *mendocinus* lineage are undergoing particularly rapid fragmentation into parapatric species, if the current taxonomy is correct. Because of the small genetic differences present among the tested species of this group, it is likely that further electrophoretic analysis will be useless for establishing unambiguous phyletic relationships among members of this clade (see Sage et al. 1984, for a similar problem in the rapidly evolving cichlid fishes). The successful analysis of the phylogeny of members of the *mendocinus* lineage of tuco-tucos may require the study of molecules more rapidly evolving than structural proteins. Mitochondrial DNA is evolving ten times faster than soluble proteins, and would be a suitable molecule for analysis in such groups (see reviews by Wilson et al. 1985; Avise and Lansman 1983).

Heterozygosity in fossorial rodents

Levels of genetic heterozygosity in tuco-tucos ranges from zero to 13 percent (Table 2), values which nearly span the range reported for other fossorial rodents (Fig. 3a). Despite the sometimes small sample sizes available to us, use of computational corrections recommended by Nei (1978) removes any systematic bias in our estimates towards either over- or under-estimating the actual values for these populations. Moreover, our empirical examination of sample bias in the Mercedes sample changed the estimate by only one percentage point. Thus, we have probably obtained a fairly accurate estimate of variability ranges among *C. mendocinus* populations, at least. These range from 3 to 13 percent in our six samples, providing a weighted mean heterozygosity of .08.

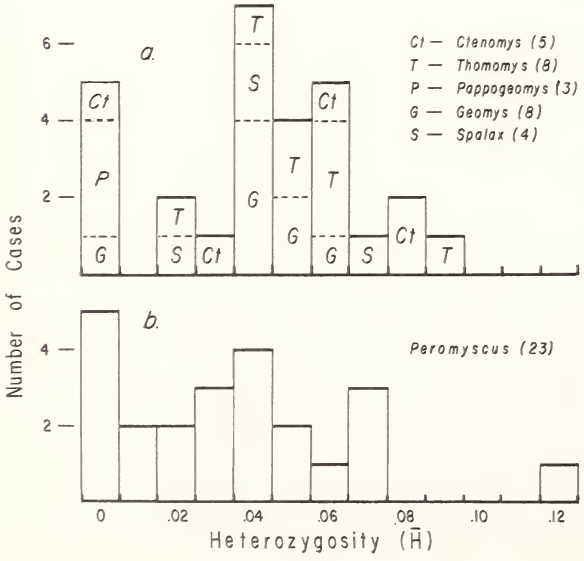

Fig. 3. a: Average heterozygosity ($\bar{H}$) estimates of 28 species or populations of fossorial rodents; b: Average heterozygosity estimates for 23 species of above-ground mice (genus *Peromyscus*). Data for *Ctenomys* are from this study; other data are taken from Nevo et al. (1984)

Little can be said about overall patterns of heterozygosity in *Ctenomys* because so few species and populations have been examined. Among the six species studied, members of the *mendocinus*-lineage do have higher than average levels of variability (*C. haigi,* 8 %; *C. mendocinus,* 8 %, and *C. bonnetoi,* 4 %; see Table 2). The geographic range of *C. mendocinus* shown by Mares and Ojeda (1982: Fig. 21) is the largest of any *Ctenomys* species. Species in the other three lineages that we examined all have relatively much smaller distributions. *Ctenomys argentinus* and *C. dorbignyi* are each known from only two departamentos in Chaco Province (Contreras and Berry 1982b; Contreras and Contreras 1984). Undescribed Species A appears to live only in a single mountain system in one Departamento of Neuquen Province (O. P. Pearson, pers. comm.). It has long been known that environmentally induced fluctuations in the numbers of individuals, or breeding colonies, will more frequently reduce genetic variability through founder events in species consisting of smaller, rather than larger numbers of individuals (Nei et al. 1975). In fact, the degree of geographic fragmentation and the population size of fragments is considered to be the primary proximal factor controlling levels of genic variability in pocket gopher populations (Patton and Yang 1977; Patton 1980).

Nevo (1979) has developed an evolutionary scenario for fossorial rodents in which natural selection directly reduces overall levels of genetic variability below that found in above-ground rodents. The premise is that environmental heterogeneity is the agent which determines genetic variability, with the presumed greater uniformity of the subterranean environment acting to favor homozygosity in structural genes that encode for soluble proteins. Tests of this genic variability-niche width hypothesis have been made in other groups of animals (Ayala 1979; Smith 1981; and Nevo and colleagues, reviewed by Nevo et al. 1984), but the evidence does not regularly support the hypothesis.

An indirect way to examine the hypothesis is to compare levels of genetic variability in groups whose environmental niche-width is putatively greater than that of any fossorial rodent group. For example, the total variation, ecological as well as genetical, in species of the North American deer mice, genus *Peromyscus,* is now very well understood. Yet genetic variation among *Peromyscus* species spans the same range observed in fossorial rodents (Fig. 3b). In the three widely distributed genera of fossorial rodents for which heterozygosity levels are available, the ranges of variation are comparable to those of *Peromyscus* (Fig. 3a, b). This variability exists despite the fact that the amplitude of environmental fluctuation (e.g., air temperature, moisture) experienced by the deer mice species must be much greater than what the fossorial *Thomomys, Geomys,* or *Pappogeomys* experience over the same region of central and western North America. Thus, despite Nevo's (1979) views to the contrary, there is no difference in level of genic variability among fossorial rodents in comparison to above-ground taxa.

It is likely that levels of observed genic variability in both fossorial and non-fossorial rodents is determined by historical factors related to population size and frequency of founder events, rather than by degrees of environmental variability per se (see Patton and Yang 1977, for example). Certainly the great levels of interspecific chromosomal and morphological variation that characterize fossorial rodent lineages adds no support to the proposal that there is greater environmental stability underground than above-ground. We think that agents associated with historical processes such as founder events are more likely to determine levels of genetic variability in these, and other rodents.

Comparative evolutionary patterns of tuco-tucos and other fossorial rodents

Patterns and levels of genetic (both chromosomal and electromorphic) and morphological variation have been extensively studied in the North American geomyid (see Nevo et al. 1974; Patton and Yang 1977; Patton 1980) and Eurasian spalacid (see Wahrman et al. 1969; Nevo and Shaw 1972; Savic and Soldatovic 1984) rodents, both fossorial groups

to which the data presented here for *Ctenomys* can be compared. Electromorphically, two patterns are generally apparent: in *Spalax* and the *talpoides* species group of *Thomomys*, populations show minimal genic differentiation and generally low within population variability, while species in the *bottae* group of *Thomomys* tend to exhibit extensive among population differentiation but high levels of within population heterozygosity. Virtually all fossorial rodents exhibit extensive chromosomal polytypy which, despite a wide variance in the actual cytological mechanisms involved, has been causally or resultantly related to speciation mode (see review by Patton and Sherwood 1983). The ecological and demographic attributes of *Thomomys* populations relating to these patterns of within and among population differentiation have been thoroughly studied (Patton and Yang 1977; Patton 1980; Patton and Feder 1981). Population processes such as gene flow rates, variance in individual reproductive success, and extinction/recolonization rates appear to be the critical factors determining the amounts of within population genic variability and degree of among population diversification.

The limited data we present here for *Ctenomys* clearly conform to the general pattern of genetic variability for *Thomomys* and *Spalax*, thereby reinforcing earlier suggestions that a suite of genetic attributes independent of phylogeny characterizes fossorial rodents. Hence, the extensive data for *Thomomys* and other pocket gophers, as well as those for *Spalax*, strongly suggest that the apparent systematic chaos in *Ctenomys* can be resolved by concordant analyses of genic, chromosomal, and morphological characters through contact zone studies, for example (such as Patton et al. 1979, 1984), and that the phyletic relationships among the resultant taxa can be firmly established (see, for example, Patton and Smith 1981). Moreover, concomitant genetic studies at the local population level with those across geography will establish those processes operating to produce the degrees of differentiation which characterize the genus, and provide further data on generalizations of evolutionary mode in fossorial rodents.

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Zusammenfassung

Genetische Variabilität bei südamerikanischen wühlenden Nagern der Gattung Ctenomys (Rodentia: Ctenomyidae)

An 11 argentinischen Populationen von *Ctenomys* wurde die elektrophoretische erkennbare Proteinvariabilität untersucht. Vierunddreißig Enzym- und Proteinloci wurden verglichen. Schätzungen der durchschnittlichen Heterozygozität (*H*) lagen pro Population bei 0 bis 13 Prozent. Die meisten Populationen wiesen einen hohen genetischen Verwandtschaftsgrad (Nei's *D*) auf (*D* = 0,03 bis 0,08), obwohl sie bis zu 1800 km auseinanderlagen und durch zum Teil 3000 m Höhenunterschied getrennt waren. Zwei Formen jedoch (C. *argentinus* und eine bisher noch nicht beschriebene Art), sind miteinander (*D* = 0,35) und mit anderen Populationen der *mendocinus*-Gruppe entfernter verwandt. Die Ergebnisse werden in bezug auf evolutionäre Verwandtschaften innerhalb der Gattung und in Zusammenhang mit der Hypothese der eingeschränkten genetischen Variabilität wühlender Nagetiere diskutiert. Ferner werden die hier erzielten Ergebnisse mit denen bei wühlenden Taschenratten (Gattung *Thomomys*) Nordamerikas verglichen.

Resumen

Variación genética en los roedores fosoriales y Sudaméricano del genero Ctenomys (Rodentia: Ctenomyidae)

Un estudio electroforético de variación proteina se realizó en once poblaciones de *Ctenomys* de Argentina. Treinte y cuatro loci enzimáticos y proteinos fueron examinados. Las estimaciones de heterocigozidad (*H*) por población fluctuaron entre 0 y 13 por ciento. La relación genética (*D* de Nei) es cercana en la mayoría de las poblaciones (*D* = 0,03) en colectadas hasta en localidades separados

por 1800 Km de distancia o por 3000 m de altura. Sin embargo, dos formas (C. *argentinus* y una especie no descrita) están relacionadas más ligeramente uno de otro ($D = 0{,}35$) y con respecto a otras poblaciones pertenecientes al grupo de especies *mendocinus*. Estos resultados son discutidos en conexión con las relaciones evolutivas en este género y con la hipótesis de variabilidad molecular reducida en roedores fosoriales, y son comparados con los bien estudiados «pocket gophers» fosoriales (genero *Thomomys*) de América del Norte.

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