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Short communication

Protein polymorphism in two species of *Ctenomys* (Rodentia, Ctenomyidae) from Córdoba province, Argentina

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Fossorial rodents of the genus *Ctenomys* are widespread in southern South America, from 17° to 54° S (Cabrera 1961; Reig et al. 1990). The genus comprises 60 recognized species originated by an explosive speciation process, promoted mainly by chromosomal rearrangements (Bidau et al. 1996). At present, systematic relationships among species of *Ctenomys* are poorly known and/or controversial.

Thomas (1902) cited the species *C. bergi* for the NW of Córdoba province, Argentina, being Cruz del Eje the type locality. On the basis of geographic criteria, all the populations from the north of that province were included in that species (Bidau et al. 1996).

Chromosomal studies revealed that individuals from the NE of Córdoba have a diploid number 2 n = 52 (FN = 66) but those proceeding from the NW (Salinas Grandes) presented a karyotype of 2 n = 48 (FN = 90) (Reig et al. 1990). This last form was assigned to *C. bergi* and the former was described as a new species and denominated *C. rosendopascuali* (Contreras 1995).

Several authors have emphasized the importance of the application of biochemical and molecular methods in order to confirm and clarify the taxonomic status of different karyotypic forms of *Ctenomys* (Bidau et al. 1996; Mascheretti et al. 2000). The aim of this study is to analyze the allozymic polymorphism in two populations of *Ctenomys* from the north of Córdoba, Argentina assigned to *C. bergi* and *C. rosendopascuali*, in order to determine their level of differentiation at structural loci.

Fourteen specimens of *C. bergi* from Las Toscas (30°11′ S, 64°54′ W, near an extense salt mine called Salinas Grandes) and 16 individuals of *C. rosendopascualis* obtained in the proximity of the mouth of Xanaes river (Mar Chiquita saline lagoon, 30°55′ S 62°44′ W) were used in this study.

Animals were killed by ether anesthesia, liver and kidneys removed immediately and preserved at –30 °C until used. Homogenates, vertical starch gel electrophoresis and staining procedures were carried out as described by Gardenal et al. (1980) and Gardenal and Blanco (1985). The following enzymes were analyzed (loci scored and E. C. numbers in parenthesis): liver and kidney acid phosphatase (Acp_L-1, Acp_L-2, Acp_K-3, Acp_K-4; 3.1.3.2), aspartate aminotransferase (Aat-1, Aat-2; 2.6.1.1), liver soluble esterases ($Es\text{-}1_L$ to $Es\text{-}6_L$; 3.1.1.1), catalase (Cat; 1.11.1.6), phosphoglucomutase (Pgm-1, Pgm-2; 2.7.5.1), leucine aminopepti-

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dase (Lap-1, Lap-2; 3.4.11.1), malic enzyme (Me; 1.1.1.40), lactate dehydrogenase (Ldh; 1.1.1.27), alcohol dehydrogenase (Adh; 1.1.1.1), glycerophosphate dehydrogenase (Gpdh; 1.1.1.8), malate dehydrogenase (Mdh-1,Mdh-2; 1.1.1.37), isocitrate dehydrogenase (Idh-1, Idh-2; 1.1.1.42), 6-phosphogluconate dehydrogenase (6-Pgdh; 1.1.1.43) and glucose-6-phosphate dehydrogenase (G-6-pdh; 1.1.1.49).

The allele coding for the band migrating fastest to the anode was assigned the number 100; that controlling the fastest cathodic band, –100. The other alleles were numbered according to their relative mobility from the origin. Bands with the same mobility were considered homologous.

Proportion of polymorphic loci (95% and 99% criteria), mean observed and expected heterozygosities, Rogers' genetic distance (1972) and Nei's identity (1975) among populations were calculated using the program Biosys-1 (Swofford and Selander 1989).

Sixteen out of 27 loci analyzed were polymorphic at least in one population. Table 1 shows allele frequencies, proportion of polymorphic loci (P), and observed and expected mean heterozygosity per locus (H_o and H_e) for the two populations analyzed. Locus G-6-pdh was the only one presenting a different allele fixed in each population.

Although crossing tests were not performed, the genetic control of the electrophoretic patterns observed was postulated on the basis of similar polymorphisms described for other rodent species where the Mendelian transmission of variants has been demonstrated (Gardenal and Blanco 1985; Gardenal et al. 1980; García and Gardenal 1989). In all cases, the observed genotypic frequencies did not differ significantly from the expected ones according to the Hardy-Weinberg equilibrium.

Rogers' genetic distance and similarity between the two species was 0.094 and Nei's distance and identity were 0.059 and 0.942, respectively.

Levels of polymorphism revealed in this study for *C. bergi* and *C. rosendopascuali* are particularly high when compared with those reported for other subterranean mammals with low vagility and socially-structured mating system (Nevo et al. 1990). Values of heterozygosity obtained in this study are higher than the mean referred for fossorial rodents (H = 0.0311) and for

Table 1. Allele frequencies, proportion of polymorphic loci (95% and 99% criteria) and observed and expected heterozygocity in *Ctenomys bergi* and *Ctenomys rosendopascuali* from Córdoba province (Argentina).

Locus	Allele	*C. bergi*	*C. rosendopascuali*
Lap-2	100	1.000	0.929
	88	0.000	0.071
Acp_K-1	100	0.067	0.036
	90	0.900	0.857
	81	0.033	0.107
Adh	–100	0.867	0.864
	–50	0.133	0.136
Gpdh	100	1.000	0.923
	60	0.000	0.077
Acp_L-3	100	0.094	0.038
	78	0.906	0.962
Acp_L-4	100	0.031	0.000
	71	0.969	1.000
Aat-1	100	0.000	0.0154
	72	0.969	0.0846
	20	0.031	0.000
Es-1	100	0.844	0.855
	93	0.156	0.115
Es-2	100	0.563	0.731
	94	0.438	0.269
Es-3	100	0.906	0.885
	88	0.094	0.115
Es-4	100	0.000	0.077
	89	0.656	0.615
	85	0.344	0.308
Es-5	100	0.000	0.038
	89	1.000	0.962
Es-6	100	0.813	0.269
	77	0.188	0.731
Pgm-2	100	1.000	0.846
	82	0.000	0.154
Me	100	0.063	0.000
	89	0.938	1.000
G 6pdh	100	1.000	0.000
	87	0.000	1.000
P (95%)		33.33	40.74
P (99%)		40.75	48.15
H_O (%)		10.1	12.8
		(s. e. 3)	(s. e. 3.4)
H_E (%)		9.3	11.7
		(s. e. 2.8)	(s. e. 2.9)

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several species of *Ctenomys* from Bolivia (Cook and Yates 1994), and Chile (Gallardo and Palma 1992), albeit similar to those obtained in 4 species from southern Brazil (H from 0.11 to 0.17) (Moreira et al. 1991). When rapidly evolving loci as esterases are excluded, H_e falls to 0.041 in *C. bergi* and to 0.067 in *C. rosendopascuali*. However, they are still higher than the mean obtained for fossorial rodents, most of them calculated including esterases. Sage et al. (1986) and Ortells and Barrantes (1994) found lower levels of allozymic polymorphism in other species of *Ctenomys* from Argentina. However, estimates were made, in most cases, on the basis of 1 to 4 individuals, which could explain the results obtained by those authors.

Genetic similarity between *C. bergi* and *C. rosendopascuali* is within the range reported for conspecific populations (King 1993). Notwithstanding, in locus G-6-pdh allele '100' is fixed in *C. bergi* and allele '87' has a frequency of 1 in *C. rosendopascuali*, indicating lack of gene exchange between the two forms.

Several cases of interspecific homogeneity in allozymic frequencies have been reported in *Ctenomys*. Gallardo and Palma (1992) found very low levels of genetic differentiation among *Ctenomys* species from Chile, although being very dissimilar in morphological characters and karyotype. Moreira et al. (1991) reported an S value of 0.91 between *C. minutus* and *Ctenomys sp.* from southern Brazil, inhabiting regions separated by 75 km and a wide river.

The genus *Ctenomys* is characterized by a large karyotypic heterogeneity, being one example of "explosive" speciation accompanied by scarce morphological changes (Bidau et al. 1996; Reig et al. 1990). Fixation of chromosomal re-arrangements would be favored by the population structure characteristic of all species in the genus: small, semi-isolated groups with low vagility and continuous extinction, expansion, and re-colonization in a variety of environments (Reig et al. 1990). The low genetic distance between *C. bergi* and *C. rosendopascuali* would be in agreement with the hypothesis of a rapid speciation by chromosomal re-arrangements, with almost no differentiation at structural loci, as those coding for proteins.

On the basis of morphological, morphometric, paleontological, karyological and distributional data, Contreras and Bidau (1999) have proposed a hypothesis on the evolution of the complex genus *Ctenomys*. *C. bergi* would be closely related to the group designated "mendocinus", which comprises several species with very similar karyotypes that have originated from a west-south radiation. *C. rosendopascuali* would integrate a separate lineage, the so called "oriental" group, presenting less stable diploid numbers and particular morphological features such as sperm asymmetry. However, Mascheretti et al. (2000), on the basis of cytochrome b sequences, found a very close relationship between these two species, placing them in the same molecular lineage. Our results would support this last proposal.

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