Z. Säugetierkunde **64** (1999) 30–35

ZEITSCHRIFT FÜR SÄUGETIERKUNDE
INTERNATIONAL JOURNAL OF MAMMALIAN BIOLOGY

Allozyme variation and taxonomic status of *Calomys hummelincki* (Rodentia, Sigmodontinae)

By Beatriz A. Garcia, Angela Martino, Marina B. Chiappero, and Cristina N. Gardenal

Cátedra de Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina and Centro de Inverstigaciones en Ecología y Zonas Aridas, Coro, Estado Falcón, Venezuela

Receipt of Ms. 16. 05. 1994
Acceptance of Ms. 12. 10. 1998

Abstract

The level of genetic polymorphism was analyzed in a population sample of *Calomys hummelincki* from Venezuela. Enzymes and proteins studied by means of gel electrophoresis give information on 30 loci. The proportion of polymorphic loci was 40 %, and mean expected heterozygosity (H_e) was 12.7 %. These values are higher than those reported for most species of rodents in the northern hemisphere, but are comparable to those observed in other Sigmodontinae species from Argentina. Nei's genetic distance (D_N) with the species *C. laucha*, *C. venustus*, and *C. musculinus* ranged from 0.289 to 0.494. D_N values between populations of different Sigmodontinae species are below 0.09. A distance Wagner tree based on modified Rogers' distances shows that *C. hummelincki* is more closely related to *C. venustus* than to *C. laucha*. Our data support the proposal that *C. hummelincki* and *C. laucha* are fully distinct species.

Key words: *Calomys hummelincki*, Sigmodontinae, allozymes, polymorphisms

Introduction

Among the Sigmodontinae rodents (family Muridae) of the tribe Phyllotini, the genus *Calomys* shows a wide distribution in South America. The systematics of this genus, as well as the geographic distribution and ecology of some of its species are still poorly known. The taxonomic status of one of these species, *Calomys hummelincki* (Venezuelan pigmy mouse) has been a matter of controversy. The species was first described by Husson (1960) on the basis of specimens collected on islands of the Caribbean sea and included in the genus *Baiomys*. Later, Hershkovitz (1962) reported on this rodent also from Venezuela, assigning it to the genus *Calomys* as synonym of *C. laucha*. In 1976, Handley recognized the species *Calomys hummelincki* as being different from *C. laucha* and described its geographic distribution in the Orinoco plains and the deserts around the Gulf of Venezuela. The first study on karyologic differences between these species was conducted by Perez Zapata et al. (1987), who reported a karyotype of 2n = 60, FN = 64 for *C. hummelincki*, fairly distinct from that of *C. laucha* (2n = 64, FN = 68).

As a contribution to the knowledge of the systematics and evolution of South American murids of the subfamily Sigmodontinae, we present here an analysis of allozymic polymorphism in *Calomys hummelincki* and an estimation of its genetic distance from the species *C. laucha*, *C. venustus*, and *C. musculinus*.

0044-3468/99/64/01 – 030 $ 12.00/0

Material and methods

Seventeen specimens of *C. hummelincki* collected in Planicie Costera de Adícora (11°55′ N; 69°49′ W) Estado Falcón, Venezuela, were studied. Preparation of tissue homogenates, electrophoretic and staining procedures were carried out as described by Gardenal et al. (1980) and Gardenal and Blanco (1985). The following enzymes were analyzed: soluble esterases (ES-1 to ES-6; E. C. 3.1.1.1), aspartate aminotransferases (AAT-1 and AAT-2; E. C. 2.6.1.1), catalase (CAT; E. C. 1.11.1.6), adenylate kinase (AK; E. C. 2.7.4.3), phosphoglucomutases (PGM-1 and PGM-2; E. C. 2.7.5.1), superoxide dismutases (SOD-1 and SOD-2; E. C. 1.15.1.1), liver acid phosphatase (ACP_L; E. C. 3.1.3.2), kidney acid phosphatase (ACP_K; E. C. 3.1.3.2), malic enzyme (ME; E. C. 1.1.1.40), malate dehydrogenases (MDH-1 and MDH-2, E. C. 1.1.1.37), lactate dehydrogenase (LDH-1 and LDH-2, E. C. 1.1.1.27), NADP-isocitrate dehydrogenases (IDH-1 and IDH-2, E. C. 1.1.1.42), 6-phosphogluconate dehydrogenase (6-PGDH, E. C. 1.1.1.44), glucose-6-phosphate dehydrogenase (G6PDH, E. C. 1.1.1.49), glycerophosphate dehydrogenase (GPDH, E. C. 1.1.1.8), alcohol dehydrogenase (ADH, E. C. 1.1.1.1), and NAD-linked nonspecific dehydrogenase (NDH). In addition, other proteins were studied in serum: transferrin (Tf) haptoglobin (Hpt) and albumin (Alb). Altogether, these proteins give information on genetic variation at 30 loci.

Statistics

Average heterozygosity per individual and proportion of polymorphic loci were estimated from the 30 loci analyzed. Genetic distance (D_N) indices were calculated according to Nei (1972) and Rogers modified by Wright (1978). Calculations were based on the allele frequencies at 20 loci reported previously for comparisons between the species *C. musculinus, C. laucha*, and *C. venustus* (Gardenal et al. 1990). A distance Wagner tree (rooted at midpoit of longest path) was constructed on the basis of modified Rogers' distances (Wright 1978) between the four species. All calculations were performed by using the BIOSYS-1 program (Swofford and Selander 1981).

Results and discussion

Table 1 shows allele frequencies for 12 polymorphic loci in *C. hummelincki*. Expected average heterozygosity (H_e) was 12.7 % and observed average heterozygosity (H_o) was 11.8 %. The proportion of polymorphic loci was 40 %. These values are clearly higher than those reported for most species of rodents in the northern hemisphere (Nevo et al. 1984). Ward et al. (1992) reported an average H value of 6.7 % for 172 species of mammals. Patton et al. (1989) found values ranging from 1.1 to 7.1 % for H and between 7.7 to 21.3 % for P in different species of the tribe Akodontini (subfamily Sigmodontinae, family Muridae) from Peru. The relatively high level of polymorphism observed in *C. hummelincki* is comparable to that of other sigmodontine species of the genera *Calomys* (Gardenal et al. 1980; Gardenal and Blanco, 1985; Gardenal et al. 1990; Garcia et al. 1990) and *Akodon* (Apfelbaum and Blanco 1985), and the species *Graomys griseoflavus* (Theiler and Gardenal 1994) and *Eligmodontia typus* (de Sousa et al. 1996) from Argentina (Tab. 2). Barrantes et al. (1993) found expected H between 3.8 and 11 % in populations of eight species of *Akodon* from Argentina. The last value corresponded to one population of *A. longipilis*. The observed H value was much lower (3.9 %), a result not explained by the authors.

Table 3 presents genetic distance values between *C. hummelincki*, *C. laucha, C. musculinus*, and *C. venustus*. On the basis of modified Rogers' distances (Wright 1978)

Table 1. Allele frequencies in the population sample of *Calomys hummelincki*

Locus	Allele	Frequency	Locus	Allele	Frequency
Es-1	94	0.91	Adh	91	1.00
	91	0.09	Mdh-1	100	1.00
Es-2	95	0.91	Aat-1	79	1.00
	87	0.09	Aat-2	27	1.00
Es-4	70	1.00	Idh-1	69	1.00
Es-5	82	0.43	Idh-2	100	1.00
	72	0.57	Ldh-1	96	1.00
Es-6	75	0.21	Gpdh	81	0.06
	50	0.79		71	0.47
Ndh	100	0.03	Mdh-2	100	1.00
	92	0.85	Acp_k	81	0.82
	85	0.12		70	0.18
Me	67	0.06	Acp_l	100	0.94
	48	0.94		94	0.06
Ldh-2	100	1.00	6Pgdh	a*	0.76
Sod	100	1.00		b	0.24
Cat	a*	0.71	Tf	a*	0.03
	b	0.23		b	0.44
	c	0.06		c	0.53

* Alleles at the loci not analyzed in other species of *Calomys* are designated by letters. The loci Ak, G6pdh, Pgm-1, Pgm-2, Sod-2, Hpt and Alb were monomorphic and not analyzed in other species of *Calomys*. Alleles at the remaining loci are designated by numbers, indicating electrophoretic mobilities of bands relative to those observed in other *Calomys* species by GARDENAL et al. (1990).

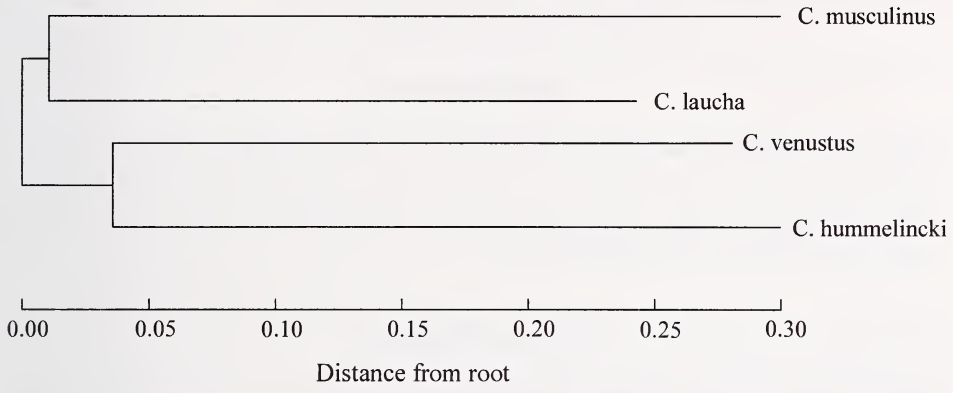
Table 2. Proportion of polymorphic loci (P) and expected heterozygosity (H_e) in different species of South American sigmodontine rodents.

Species	$P_{99\,\%}$	H_e	D_N^*	Reference
C. hummelincki	40	12.7	–	this study
C. laucha	62.5–77.3	11.8–16.3	0.002–0.010	GARDENAL et al. (1990) GARCIA et al. (1990)
C. musculinus	61.0–73.0	14.9–20.0	–	GARDENAL et al. (1980) GARDENAL and BLANCO (1985) GARDENAL et al. (1990)
C. venustus	66.7	14.6	–	GARDENAL et al. (1990)
Akodon dolores	27.8–38.9	13.8–19.2	–	APFELBAUM and BLANCO (1985)
Akodon azarae	16.6–30.0	9.9–11.8	–	APFELBAUM and BLANCO (1985)
Akodon (Peru)	7.7–21.3	1.1–7.1	–	PATTON et al. (1989)
Akodon (Argentina)	6.7–26.7	6.7–11	–	BARRANTES et al. (1993)
Eligmodontia typus	68.0	16.0	0.005–0.015	DE SOUSA and GARDENAL (1996)
Graomys griseoflavus	46.0–66.0	16.0–18.0	0.075–0.093	THEILER and GARDENAL (1994)
Oligoryzomys flavescens	34.6–61.5	5.8–9.7	0.0016–0.0088	CHIAPPERO et al. (1997)

* NEI's (1972) genetic distance between populations

Table 3. Values for modified Rogers' distance (D_R; above the diagonal) and Nei's genetic distance (D_N; below the diagonal) between species of *Calomys*.

	C. hummelincki	*C. laucha*	*C. venustus*	*C. musculinus*
C. hummelincki	**	0.470	0.530	0.591
C. laucha	0.289	**	0.516	0.510
C. venustus	0.378	0.360	**	0.578
C. musculinus	0.494	0.349	0.469	**



Cophenetic correlation = 0.994

Fig. 1. Distance Wagner procedure tree based on modified Rogers' distances between the species *Calomys hummelincki, C. laucha, C. venustus,* and *C. musculinus.*

the distance Wagner tree of figure 1 was constructed. *C. hummelincki* appears more closely related to *C. venustus* than to *C. laucha*. Table 2 includes values of D_N between populations of *C. laucha, E. typus*, and *G. griseoflavus*. These species belong to the same tribe (Phyllotini) as *C. hummelincki*. The highest D_N between populations of the same species was 0.09, while all comparisons between species gave values above 0.29.

The taxonomic status of the Venezuelan pigmy mouse (*C. hummelincki*) has been the subject of controversy (Perez Zapata et al. 1987). Hershkovitz (1962) did not recognize *C. hummelincki* as a separate species and considered it as *C. laucha*.

It was known that *C. laucha* was distributed in a wide area of South America comprising southeastern Brazil, southern Bolivia, Paraguay, Uruguay, and central Argentina. This is very far from the south of the region where *C. hummelincki* is found. When Hershkovitz (1962) proposed this species as a synonym of *C. laucha*, he assumed that its presence in Venezuela could be due to accidental transportation by man.

Data presented here strongly support the proposal that *C. laucha* and *C. hummelincki* are distinct species, providing thus a more rational explanation for the geographic distribution of the species. The genetic distance between *C. laucha* and *C. hummelincki* ($D_N = 0.36$) is within the range accepted for species which have completed their reproductive isolation (Ayala 1982). Five loci (Es-1, Es-4, Adh, Aat-1 and Gpdh) can be utilized as "diagnostic", since the species do not share alleles at these loci. Genetic distances for intraspecific comparisons between populations of *C. laucha, Eligmodontia typus*, and *Graomys griseoflavus*, species closely related to *C. hummelincki*, gave values below 0.09 (Tab. 2).

In an analysis of karyological relationships among species of *Calomys*, Vitullo et al. (1990) described different "groups" of species on the basis of chromosomal characteristics (2n, fundamental number, morphology). According to these criteria, *C. hummelincki* (2n = 60; FN = 64) and *C. laucha* (2n = 64; FN = 68) were included in the same group (Group I), while *C. venustus* (2n = 56; NF = 66) and *C. musculinus* (2n = 38; FN = 56) were included in different groups (II and III, respectively). The study of differentiation in structural genes presented here indicates different relationships between species of *Calomys* than those inferred from cytogenetic analysis. Nevertheless, it has been suggested that in mammals the rate of evolution of morphology, karyotype, and structural genes can be independent, and so the relationships assigned on the basis of these criteria may disagree (Schnell and Selander 1981; Apfelbaum and Reig 1989).

Aknowledgements

The authors thank Dr. Antonio Blanco for advice and critical revision of the manuscript. This work was supported, in part by grants from the Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina, the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and the Antorchas Foundation. C.N.G. and B.A.G. are Career Investigators of CONICET and M.B.C. is a Fellow of CONICOR.

Zusammenfassung

Allozymvariation und taxonomische Stellung von Calomys hummelincki (Rodentia, Sigmodontidae)

Die genetische Variabilität einer Population von *Calomys hummelincki* aus Venezuela wurde mittels Gelelektrophorese von Enzymen und Serumproteinen untersucht. Insgesamt wurden 30 Genloci erfaßt. Die Polymorphierate betrug 40 % und der durchschnittliche erwartete Heterozygotiegrad (H_e) 12,7 %. Diese Werte liegen höher als jene der meisten bisher untersuchten Nagetierarten der Nordhemisphäre. Sie sind jedoch den Angaben über andere Arten der Unterfamilie Sigmodontinae aus Argentinien vergleichbar. Die genetischen Distanzen nach Nei (D_N) zu den Arten *C. laucha, C. venustus* und *C. musculinus* reichten von 0,289 bis 0,494, während jene zwischen Populationen der jeweiligen Arten einen Wert von 0,09 nicht überschritten. Ein auf modifizierten Rogers-Distanzen beruhender Wagner-Baum zeigt, daß *C. hummelincki* mit *C. venustus* näher verwandt ist als mit *C. laucha*. Unsere Daten stützen die Hypothese, daß *C. hummelincki* und *C. laucha* zwei verschiedene Arten sind.

References

Apfelbaum, L. I.; Blanco, A. (1985): Genetic polymorphism in populations of *Akodon rodents*. Canad. J. Genet. Cytol. **27**, 12–17.

Apfelbaum, L. I.; Reig, O. A. (1989): Allozyme genetic distances and evolutionary relationships in species of akodontinae rodents (Cricetidae: Sigmodontinae) Biol. J. Linnean Soc. **38**, 257–280.

Ayala, F. J. (1982): Gradualism versus punctualism in speciation. In: Mechanisms of Speciation: Reproductive Isolation, Morphology, Genetics. Ed. by C. Barigozzi. New York: Alan R. Liss, pp. 51–66.

Barrantes, G. E.; Ortells, M. O.; Reig, O. A. (1993): New studies on allozyme genetic distance and variability in akodontine rodents (Cricetidae) and their systematic implications. Biol. J. Linnean Soc. **48**, 283–298.

Chiappero, M. B.; Calderón, G. E.; Gardenal, C. N. (1997): *Oligoryzomys flavescens* (Rodentia, Muridae): gene flow among populations from central-eastern Argentina. Genetica **101**, 105–113.

De Sousa, G. B.; De Rosa, N.; Gardenal, C. N. (1996): Protein polymorphism in *Eligmodontia typus*. Genetic divergence with other phyllotine cricetids. Genetica **97**, 47–53.

Garcia, B. A.; Gardenal, C. N.; Blanco, A. (1990): Heterozygosity and gene flow in populations of *Calomys laucha* (Rodentia, Cricetidae). Hereditas **112**, 179–186.

Gardenal, C. N.; Blanco, A. (1985): Polimorfismo enzimático en *Calomys musculinus*: nueva estimación. Mendeliana **7**, 3–12.

Gardenal, C. N.; Sabattini, M. S.; Blanco, A. (1980): Enzyme polymorphism in a population of *Calomys musculinus* (Rodentia, Cricetidae). Biochem. Genet. **18**, 563–575.

Gardenal, C. N.; Garcia, B. A.; Sabattini, M. S.; Blanco, A. (1990): Protein polymorphism and genetic distance in South American cricetid rodents of the genus *Calomys*. Genetica **80**, 175–180.

Handley, C. O. (1976): Mammals of the Smithsonian Venezuelan project. Brigham Young Univ. Science Bull. Biol. Ser. **20**, 5: 1–91.

Hershkovitz, P. (1962): Evolution of neotropical cricetine rodents (Muridae), with special reference to the phyllotine group. Fieldiana, Zool. **46**, 1–524.

Husson, A. M. (1960): A new species of the rodent *Baiomys* from Aruba and Curaçao. Stud. Fauna Curaçao **10**, 33–40.

Nei, M. (1972): Genetic distance between populations. Am. Nat. **106**, 238–292.

Nevo, E.; Beiles, A.; Ben-Shlomo, R. (1984): The evolutionary significance of diversity: ecological, demographic and life history correlates. In: Evolutionary Dynamics of Genetic Diversity. Ed. by G. S. Mani. Lecture Notes in Biomathematics **53**, 13–213.

Patton, J. L.; Myers, P.; Smith, M. F. (1989): Electromorphic variation in selected South American akodontine rodents (Muridae: Sigmodontinae), with comments on systematic implications. Z. Säugetierkunde **54**, 347–359.

Perez Zapata, A.; Vitullo, A. D.; Reig, O. A. (1987): Karyotypic and sperm distinction of *Calomys hummelincki* from *Calomys laucha* (Rodentia, Cricetidae). Acta Cientif. Venezolana **38**, 90–93.

Schnell, G. D.; Selander, R. K. (1981): Environmental and morphological correlates of genetic variation in mammals. In: Mammalian Population Genetics. Ed. by H. Smith and J. Joule. Athens: Univ. Georgia Press. p. 60–90.

Swofford, D. L.; Selander, R. K. (1981): BIOSYS-1 a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**, 281–283.

Theiler, G. R.; Gardenal, C. N. (1994): Patterns of evolution in *Graomys griseoflavus* (Rodentia, Cricetidae). I. Protein polymorphism in populations with different chromosome number. Hereditas **120**, 225–229.

Vitullo, A. D.; Espinosa, M. R.; Merani, M. S. (1990): Cytogenetics of Vesper mice, *Calomys* (Rodentia, Cricetidae): Robertsonian variation between *Calomys callidus* and *Calomys venustus*, Z. Säugetierkunde **55**, 99–105.

Ward, R. D.; Skibinski, D. O. F.; Woodward, M. (1992): Protein heterozygosity, protein structure and taxonomic differentiation. In: Evolutionary Biology. Ed. by M. K. Hecht et al. Vol. 26, New York: Plenum Press. pp. 73–158.

Wright, S. (1978): Evolution and the Genetics of Populations. Vol. 4: Variability within and among natural populations. Chicago: Univ. Chicago Press.

Authors' addresses: Cristina N. Gardenal, Beatriz A. García, and Marina B. Chiappero, Cátedra de Química Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla de Correo 35 Sucursal 16, 5016 Córdoba, Argentina and Angela Martino, Centro de Investigaciones en Ecología y Zonas Áridas, Apartado 7506, Coro, Estado Falcón, Venezuela.