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Correlates of systematic differentiation between two closely related allopatric populations of the *Akodon boliviensis* group from NW Argentina (Rodentia: Cricetidae)

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Abstract

Studied external, epigenetic, and continuous morphological characters, karyotypes and allozymic genetic distances of *Akodon alterus* und *Akodon "tucumanensis"*. These forms are closely related deserving for some authors only subspecies status, but they are restricted to quite different biomes in the NW of Argentina. Cytogenetic results demonstrated chromosomal identity between them, as reflected by sharing the same 2n = 40, FN = 40 karyotype with an identical pattern of heterochromatin and a complete G-band arm-to-arm matching. Allozymic analysis revealed a low genetic distance between them, in agreement with previously reported D values for other *Akodon* species. *A. alterus* and *A. "tucumanensis"* can be differentiated by morphological characters and different habitat preferences. Previous reports suggested that adaptative divergence played a minor role in the cladogenesis of *Akodon* and that the main factor in its multifarious speciation may have been the stochastic and sudden fixation of chromosomal mutations eliciting reproductive isolation. *A. "tucumanensis"* and *A. alterus* may be an exception to this rule, or reflect strong habitat differentation in a single species complex. This example could be analyzed within the framework of a lack of correlation between organismic evolution and chromosomal and allozymic evolution.

Introduction

With about 43 living species and a fossil record tracing their origin back to the Pliocene, South American mice of the genus *Akodon* (sensu Reig, 1987, 1989; but see Spotorno 1986) are an interesting case of speedy and bountiful speciation. In view of their morphological resemblance and the frequency of several species packings in the same general biotopes, together with the wide range of chromosomal rearrangements and the high frequency of karyotypic species-specificity, it has been recently suggested that the main factor in *Akodon* multifarious speciation may have been the stochastic and sudden fixation of chromosomal mutations eliciting reproductive isolation. Under this process, ecological equivalence and morphological resemblance are to be expected, with selection acting mainly to tune up minor differentiation, but not being the main original agent of species differentiation (Reig 1989). Additionally, the overall speed of the process predicts small interspecific genetic differentiation, a prediction which has been widely corroborated (Apfelbaum and Reig 1989; Patton et al. 1989).

However, studies aiming to compare morphological, ecological, chromosomal and genetic-distances differences between pairs of closely related forms of *Akodon* supposedly involved in recent speciation, are lacking. These studies may be critical to further understanding the acting forces of speciation in these mice.

We have recently found that the most abundant species of mice in the high alpine steppe

(3000 m) of the mountain valleys at "El Infiernillo" in the Aconquija range of Tucumán Province, was a small pale-coloured akodont, *Akodon alterus*, which has remained unnoticed so far at this location. As previously carried out by Oldfield Thomas' collector Emilio Budin and several other mammalogists, we collected in the subtropical forest and sugar cane plantations of the lowland environments in Tucumán province specimens of a small dark species of *Akodon* currently identified as *Akodon boliviensis tucumanensis*. This species is sympatric and syntopic with *A. puer* (= *A. caenosus*), *A. simulator*, and *A. illuteus*, all of which are clearly separated by their karyotypes, body size, or other minor morphological differences (Barquez et al. 1980; Liascovich et al. 1989). Routine karyotyping demonstrated that the high-grassland pale form is identical in its chromosomal complement to *A. b. tucumanensis*, which suggests a close relationship in spite of their strong ecological differentiation. In contrast with the general model referred to above, this suggests adaptive geographical speciation. We decided then to further study the relationships and degree of morphological, allozymic and chromosomal differentiation among these two forms.

Material and methods

Taxonomy and ecology

Akodon boliviensis, the type species of the genus *Akodon*, was described by Meyen (1833) on specimens from Pichu Pichún, Arequipa Department, Perú. *Akodon tucumanensis* was described by Allen (1901) on specimens from San Miguel de Tucumán, 450 m, Tucumán Province, Argentina. Cabrera (1961) considered the Tucumanian form as a subspecies of *A. boliviensis* proposing the trinomen *A. b. tucumanensis*. Another related species, *A. spegazzini*, was described by Thomas (1897) from lower Río Cachi, Salta Province, Argentina. Recently, Myers et al. (1990) revised the taxonomy of several *Akodon* species from Perú, Bolivia, and the north of Argentina, which they placed together in a "*boliviensis* group", and includes *A. boliviensis, A. spegazzini, A. puer, A. subfuscus* and *A. juninensis*. They suggested that *tucumanensis* is a subspecies of *A. spegazzini*. We study here specimens collected near the type locality of *A. tucumanensis*. As we are not able to decide whether *A. tucumanensis* is a subspecies of *A. boliviensis* or of *A. spegazzini*, we will provisionally refer to our specimens as *A. "tucumanensis"*.

Akodon alterus was described by Thomas (1991a) on specimens from Otro Cerro, at 3000 m, 45 km west of Chumbicha, in La Rioja Province, Argentina. He distinguished them from *A. spegazzini* by their drab brown colour instead of the buffy olive of the latter, and "by the absence of the strong yellowish or buffy suffusion in the fur". He also referred two specimens as *A. alterus* from Chumbicha, at 600 m, Catamarca Province, Argentina, one of which he had previously referred to *A. azarae* (Thomas 1919b). He later (Thomas 1920) described as the same species a series of 8 males and 17 females from La Invernada, at 3800 m and 3 males and 4 females from Potrerillo, at 1500 m, both localities near "Nevado de Famatina", in La Rioja Province. Cabrera (1961) considered *A. alterus* as a synonym of *A. b. tucumanensis*.

We captured 37 specimens (18 males and 19 females) of *A. "tucumanensis"* in August, 1986 and June, 1987 in Quebrada de Los Sosa, Tucumán Province, Argentina. Quebrada de Los Sosa is located approximately 25 km W of the city of San Miguel de Tucumán, on Route 307, at an elevation of 850 m. The area belongs to the Basal Subtropical Forest within the Provincia de Las Yungas (Cabrera 1976). Many epiphytes are present and the forest is largely evergreen. This area is characterized by *Phoebe porphyria* (laurel), *Cedrela lilloi* and *Cedrela angustifolia* (cedro), *Rapanea laetevirens* (palo San Antonio), *Tipuana tipu* (tipa), and *Tabebuia avellanedae* (lapacho) as the most conspicuous trees. Furthermore, there are shrubs, such as *Carica quercifolia* (higuera del monte) and climbing-plants, *Mitostigma latiflorum* and *Cynanchum trilobulatum* (Meyer 1963; Cabrera 1976). Although *A. "tucumanensis"* is the prevailing species in the trapping site (trapping success for this species was 18 % in 1986 and 23 % in 1987) we have also captured specimens of *A. simulator, A. illuteus, Oligoryzomys* sp., *Lutrolina crassicaudata* and *Thylamys* sp.

Additionally, at El Infiernillo, 79 km on Route 307, at an elevation of 3000 m, Tucumán Province, Argentina, we captured 24 specimens (12 males and 12 females) of a small pale akodont mouse identified as *A. alterus*. This locality is separated approximately 220 km from the type locality of *A. alterus* but it belongs to the same biome of high meadow steppe. El Infiernillo belongs to the Provincia Prepuneña (Cabrera 1976) characterized by the grasses *Stipa* (probably *S. saltensis*) and *Festuca* (probably *F. setifolia*), *Trichocereus pasacana* (cardón) covered by the epiphyte *Tillandsia*, and plants of the genus *Azorella, Adesmia*, and *Pycnophyllum*. The trapping success was 60 % in 1986 and 85 % in 1987. *A. alterus* was documented in a rodent guild in which we collected *Oligoryzomys flavescens,*

Phyllotis osilae, *Ctenomys tuconax*, and *Reithrodon auritus*. In the same locality DALBY (1974) reported the finding of the latter, together with *Andinomys edax* and *Calomys musculinus*. His reference to *A. andinus* and *Akodon* sp. may belong to *A. alterus*. We had previously collected *A. simulator* in the same locality but in a more rocky habitat (REIG unpubl.). Comparisons with the type and original specimens of *A. alterus* in the British Museum of Natural History demonstrated that specimens from "El Infiernillo" must be ascribed to this species.

In addition to the differences previously described in the flora of El Infiernillo and Quebrada de Los Sosa, the habitats of both localities are also remarkably different for most abiotic factors. Total rainfall measured at Quebrada de Los Sosa and El Infiernillo are 1432.8 mm/year and 422.8 mm/year, respectively. The rainfall is seasonally distributed with a peak in summer in both localities. However, precipitation exceeds 100 mm from November to March in Quebrada de Los Sosa, while this amount is achieved only in January in El Infiernillo. Moreover, there is a long dry season in El Infiernillo. The annual mean temperatures in Quebrada de Los Sosa and El Infiernillo are 15 °C and 10 °C respectively.

Morphology

Twenty three specimens of *A. alterus* and 27 of *A. "tucumanensis"* were analysed. Skulls were prepared for examination by the papain technique, modified from Luther (SEARLE 1954) further stained with alizarin red "S" (NOBACK and NOBACK 1944). An analysis of epigenetic characters was performed, following GRUNEBERG's (1952) definition and BERRY's (1963) and BERRY and SEARLE (1963) character-state descriptions for *Mus musculus*, and HEDGES' (1969) for *Apodemus sylvaticus* and *Apodemus flavicollis*. We followed Reig's (1977) nomenclature for enameled molar character-states. Bilateral traits were studied separately on either side. Some of the skulls were partly damaged and it was impossible to study all the characters. All determinations were made by the same observer (SB). We first analysed 45 discontinuous characters, but after preliminary analysis we eliminated some traits for obvious redundancy, strong correlation, and ambiguous or difficult recognition. Thus, we studied 35 characters which include the mandibular foramen, frontal wormian, fused frontals, emplacement of the posterior palatine foramen (right and left) and other 30 characters shown in Figures 1, 2 and 3. The MMD statistics modified by SJOVOLD (1973) was applied. Nine morphometric characters were also analysed, as detailed in Figure 1. Discriminant analysis was performed by means of STATGRAF statistical package.

Voucher specimens were deposited in the Museo Municipal de Ciencias Naturales "Lorenzo Scaglia", Mar del Plata, Argentina (MMP).

Cytogenetics

Twelve specimens of *A. alterus* and 26 of *A. tucumanensis* were analysed. Metaphase-chromosome preparations were obtained from bone marrow of animals injected with yeast one day before sacrifice, (LEE and ELDER 1980). Chromosomal spreads were stained with Giemsa or processed for G- and C-bands following SEABRIGHT (1971) and BARROS and PATTON (1985), respectively. Diploid number, fundamental number (FN), and chromosomal morphology for all specimens were determined. Fundamental number is the number of autosomal arms. Chromosomes were classified according to LEVAN et al. (1964). A modification of REIG and KIBLISKY's (1969) size proposal as a percentage of the female haploid set (FHS) was used classifying "large" those chromosomes $> 9\%$ FHS, "medium" those $> 5.5\%$ FHS, but $\leq 9\%$, "small" those $> 2\%$ FHS but $\leq 5.5\%$ FHS, and "minute" those $< 2\%$ FHS.

Allozymic distance

Tissues for electrophoresis and protein staining from 27 specimens of *A. alterus* and 31 of *A. "tucumanensis"* were prepared using the techniques employed by APFELBAUM and REIG (1989). Twenty eight presumptive loci were determined: Acid phosphatase (Acph), Aspartate aminotransferase (Aat-1, Aat-2), Esterase (Es-3, Es-4, Es-5), Fumarase (Fum-1, Fum-2), General Proteins (GP-1, GP-2), Glucose-6 Phosphate dehydrogenase (G-6pdh), Glycerophosphate dehydrogenase (Gpdh), Hemoglobine (Hb), Isocitrate dehydrogenase (Idh-1, Idh-2), Lactate dehydrogenase (Ldh-1, Ldh-2), Leucine aminopeptidase (Lap), Malate dehydrogenase (Mdh-1, Mdh-2), Malic enzyme (Me), Peptidase (Pep-1, Pep-2), Phosphoglucomutase (Pgm-1, Pgm-2, Pgm-3), Superoxide dismutase (Sd), and Xanthine dehydrogenase (Xdh). All differences in electrophoretic mobility were assumed to be of genetic origin and inherited in a Mendelian fashion. Alleles are designated alphabetically by their relative mobility, "a" represents the allele variant that migrates further anodally. Allozymic frequencies for each sample were determined from electrophoretic data and estimated by means of LEVENE's (1949) formula for small samples. Estimates of genic heterozygosity were obtained from the electromorphic genotypes by direct counts and averaged across loci for population estimates of individual variability (H), proportion of polymorphic loci per population (P), and average number of

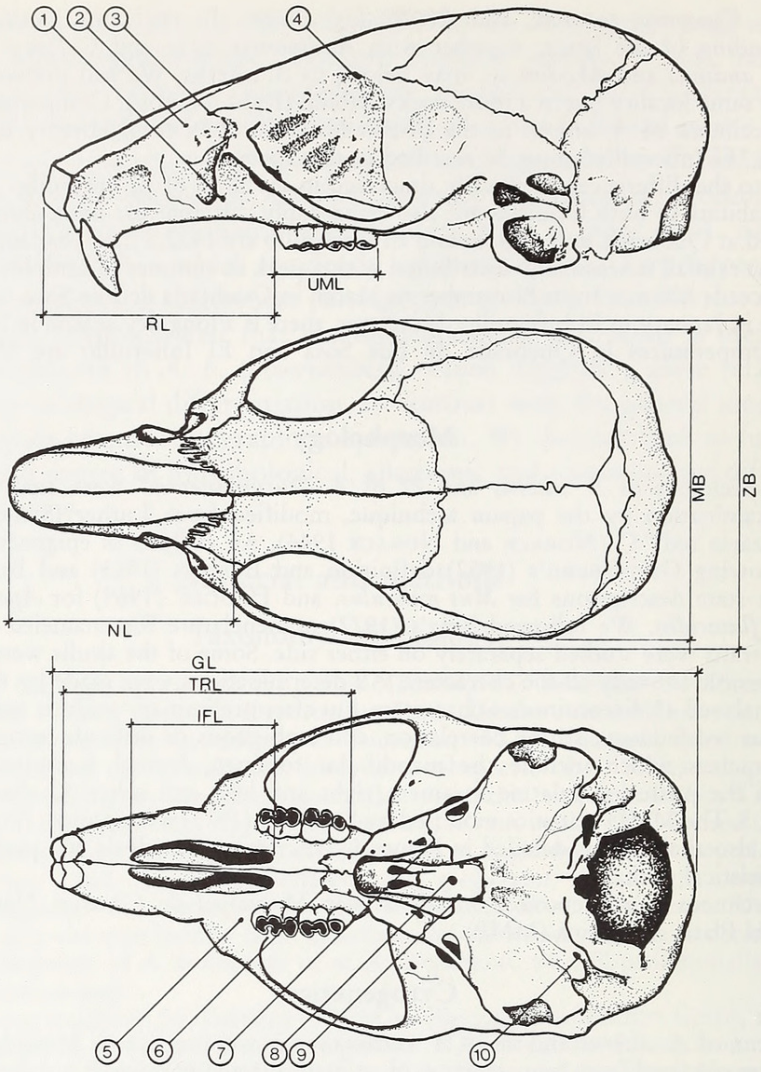

Fig. 1. Lateral (A), dorsal (B), and ventral views of skull of *A. "tucumanensis"*: 1-preorbital foramen I, 2-preorbital foramen II, 3-zygomatic plate foramen, 4-frontal foramen, 5-maxillary foramen, 6-posterior palatine foramen, 7-palatine-alisphenoid suture, 8-foramen sphenoidale medium, 9-ventrolateral sphenoidale foramen, 10-foramen hypoglossi, RL-rostrum length, UML-upper molar length, MB-mastoid breadth, ZB-zygomatic breadth, NL-nasal length, GL-greatest length, RTL-teeth row length, and IFL-incisive foramen length

alleles per locus (A). Genetic distance was estimated to measure the genetic divergence (Nei 1972) using the jackknife approach of Muller and Ayala (1982) as implemented in the program of Sattler and Hilburn (1985).

Results

Morphology

The percentage occurrence of individual non-metrical characters examined in both samples are given in Tables 1 and 2. The value of the mean measure of divergence is $4.405276 \times 10{-}2$ and its standard deviation $1.055756 \times 10{-}2$. The MMD is significant at (approximately) the 0.025 probability level when it is greater than twice its standard deviation (Sjovold 1973; Nevez 1984).

The following characters show strong differences in frequency between the two forms or are exclusive in one of them (Tables 1, 2), thus affording the main variables to

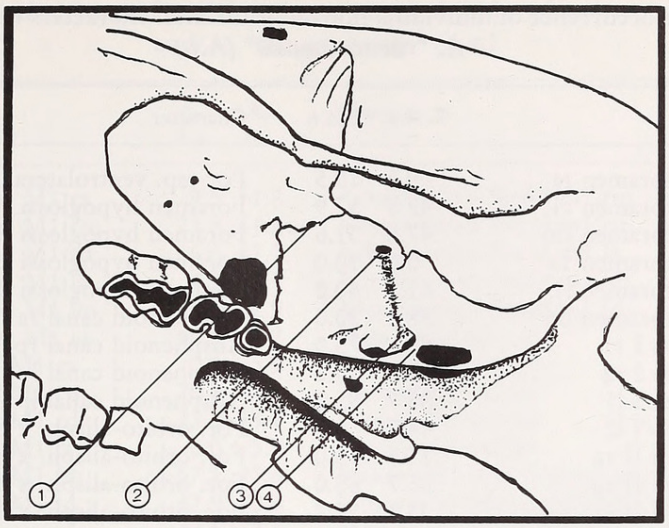

Fig. 2. Detail of the ventro-lateral view of skull of *A. "tucumanensis"*: 1-orbito-alisphenoid foramen, 2-upper buccino masticatory foramen, 34-buccino masticatory foramen, and 4-alisphenoid strut

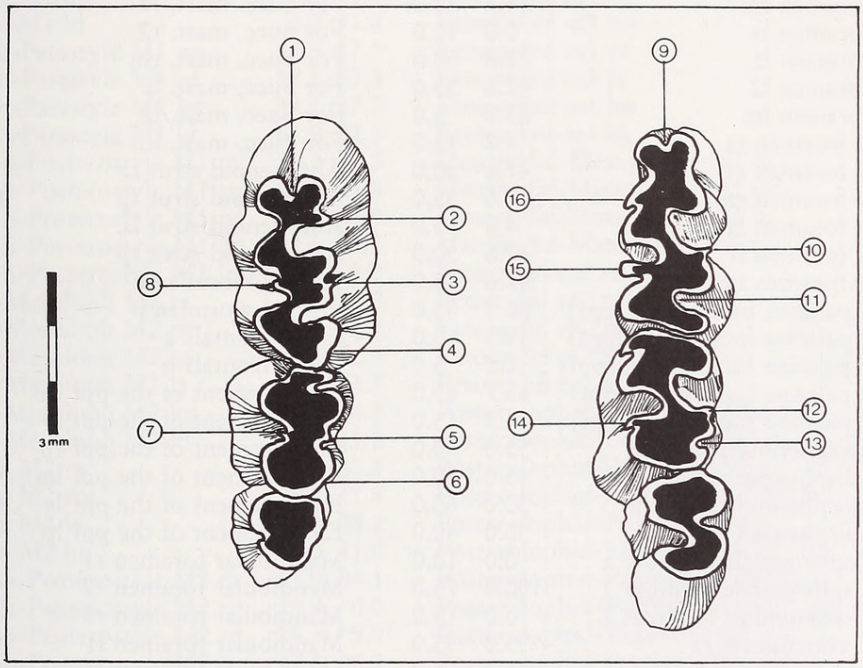

Fig. 3. Left upper molar row (A) and left lower molar row (B) of a female of *A. alterus* from El Infiernillo, Tucumán Province, Argentina: 1-anteromedian flexus of M1; 2-anteroloph-parastyle of M1; 3-mesostyle-mesoloph of M1; 4-posteroloph-posterostyle of M1; 5-mesostyle-mesoloph of M2; 6-posteroloph-posterostyle of M2; 7-enterostyle of M2; 8-enterostyle of M1; 9-anteromedian flexid of m1; 10-mesostylid-mesolophid of m1; 11-posterolophid-posterostylid of m1; 12-mesostylid-mesolophid of m2; 13-posterolophid-posterostylid of m2; 14-ectostylid of m2; 15-ectostylid of m1; and 16-protostylid of m1

distinguish the two forms: posterior palatine foramen (triple), frontal foramen (absent or present), foramen sphenoidale medium (absent and double), mandibular foramen (triple), mesostyle-mesoloph M1 (absent or present), enterostyle M1 (moderate or well-developed), anteromedian flexid (absent or present), posteroloph-posterostyle M1 (moderate or well-developed), posterolophid-posterostylid of m1 (absent and moderatly developed), posteroloph-posterostyle of M2 (moderate or well-developed).

Table 1. **Percentage occurrence of individual non-metrical skull characters of *A. alterus (A.a.)* and *A. "tucumanensis" (A.t.)***

Character	% *A.a.*	% *A.t.*	Character	% *A.a.*	% *A.t.*
Zygomatic plate foramen ra	9.5	10.5	For esp. ventrolateral lp	0.0	10.0
Zygomatic plate foramen r1	42.8	57.9	Foramen hypoglossi r1	65.0	79.0
Zygomatic plate foramen rm	47.6	31.6	Foramen hypoglossi r2	35.0	21.1
Zygomatic plate foramen 1a	0.0	10.0	Foramen hypoglossi l1	55.0	73.7
Zygomatic plate foramen l1	61.9	60.0	Foramen hypoglossi l2	45.0	26.3
Zygomatic plate foramen lm	38.1	30.0	Alisphenoid canal ra	100.0	90.0
Preorbital foramen I r1	85.7	75.0	Alisphenoid canal rp	0.0	10.0
Preorbital foramen I r2	14.3	25.0	Alisphenoid canal la	90.5	80.0
Preorbital foramen I l1	85.7	95.0	Alisphenoid canal lp	9.5	20.0
Preorbital foramen I l2	14.3	5.0	For. orbito-alisph. x' ra	71.4	75.0
Preorbital foramen II ra	14.3	35.0	For. orbito-alisph. x' rp	28.6	25.0
Preorbital foramen II rp	85.7	65.0	For. orbito-alisph. x' la	76.2	80.0
Preorbital foramen II la	33.3	52.7	For. orbito-alisph. x' lp	23.8	20.0
Preorbital foramen II lp	66.7	47.3	For. buccino-mast. sup. ra	23.8	30.0
Frontal foramen ra	0.0	5.0	For. buccino-mast. sup. rp	76.2	70.0
Frontal foramen r1	33.3	45.0	For. buccino-mast. sup. la	28.6	25.0
Frontal foramen r2	52.4	30.0	For. buccino-mast. sup. lp	71.4	75.0
Frontal foramen rm	14.3	20.0	For bucc. mast. ra	0.0	10.0
Frontal foramen la	0.0	10.0	For bucc. mast. r2	80.6	65.0
Frontal foramen l1	28.6	50.0	For bucc. mast. rm	19.4	25.0
Frontal foramen l2	47.6	35.0	For bucc. mast. la	9.5	20.0
Frontal foramen lm	23.8	5.0	For bucc. mast. l2	61.9	70.0
Maxillary foramen ra	4.8	15.0	For bucc. mast. lm	28.6	10.0
Maxillary foramen r1	47.6	50.0	Alisphenoid strut ra	0.0	10.0
Maxillary foramen rm	47.6	35.0	Alisphenoid strut rp	100.0	90.0
Maxillary foramen la	4.8	5.0	Alisphenoid strut la	9.5	10.0
Maxillary foramen l1	47.6	50.0	Alisphenoid strut rp	90.5	90.0
Maxillary foramen lm	47.6	45.0	Frontal wormian a	71.4	90.0
Posterior palatine foramen r1 (ppf)	80.9	85.0	Frontal wormian p	28.6	10.0
Posterior palatine foramen r2 (ppf)	19.1	10.0	Fused frontals a	19.1	15.0
Posterior palatine foramen rm (ppf)	0.0	5.0	Fused frontals p	80.9	85.0
Posterior palatine foramen l1 (ppf)	85.7	85.0	Emplacement of the ppf rm	23.8	15.8
Posterior palatine foramen l2 (ppf)	14.3	15.0	Emplacement of the ppf rs	19.0	26.3
Palatine-alisphenoid suture rn	55.0	80.0	Emplacement of the ppf rp	57.1	57.9
Palatine-alisphenoid suture rt	45.0	20.0	Emplacement of the ppf lm	42.9	10.5
Palatine-alisphenoid suture ln	50.0	60.0	Emplacement of the ppf ls	14.3	31.6
Palatine-alisphenoid suture lt	50.0	40.0	Emplacement of the ppf lp	42.8	57.9
Foramen sphenoidale medium a	0.0	10.0	Mandibular foramen r1	85.7	90.0
Foramen sphenoidale medium 1	100.0	75.0	Mandibular foramen r2	4.8	10.0
Foramen sphenoidale medium 2	0.0	15.0	Mandibular foramen r3	9.6	0.0
For esp. ventrolateral ra	95.0	95.0	Mandibular foramen l1	80.9	90.0
For esp. ventrolateral rp	5.0	5.0	Mandibular foramen l2	14.3	10.0
For esp. ventrolateral la	100.0	90.0	Mandibular foramen l3	4.8	0.0

r = right, l = left, a = absent, p = present, 1 = simple, 2 = double, m = multiple, n = anterior, t = transverse.

Regarding the morphometric analysis, highly significant differences were found between both groups (DF = 9, X2 = 25.29, at level = 0.003). Analysis of the discriminant function indicates that the most important variables in the differentiation were in order of precedence lower molar length (LML), rostrum length (RL), and length of incisive foramen (IFL) (see Table 3).

Cytogenetics

A. "tucumanensis" and *A. alterus* have an identical 2n = 40, FN = 40 karyotype (Fig. 4). Pairs 1 to 18 are constituted by telocentric chromosomes, while pair 19 is made of

Table 2. **Percentage occurrence of individual non-metrical molar characters of *A. alterus* (A.a.) and *A. "tucumanensis"* (A.t.)**

Character	% A.a.	% A.t.	Character	% A.a.	% A.t.
Anteromedian flexus M1 ra	9.5	11.8	Antermedian flexid m1 rm	4.8	23.5
Anteromedian flexus M1 rm	23.8	41.2	Antermedian flexid m1 rd	95.2	76.5
Anteromedian flexus M1 rd	66.6	47.0	Antermedian flexid m1 la	0.0	5.9
Anteromedian flexus M1 la	4.9	5.9	Antermedian flexid m1 lm	4.8	23.5
Anteromedian flexus M1 lm	28.7	52.9	Antermedian flexid m1 ld	95.2	70.6
Anteromedian flexus M1 ld	66.5	41.2	Protostylid m1 ra	4.8	11.8
Mesostyle-Mesolph M1 ra	0.0	5.9	Protostylid m1 rm	14.3	23.5
Mesostyle-Mesolph M1 rm	76.2	29.4	Protostylid m1 rd	80.9	64.7
Mesostyle-Mesolph M1 rd	23.8	64.7	Protostylid m1 lm	14.3	29.4
Mesostyle-Mesolph M1 la	0.0	5.9	Protostylid m1 ld	85.7	70.6
Mesostyle-Mesolph M1 lm	57.1	23.6	Mesostylid-Mesolophid m1 ra	23.8	29.4
Mesostyle-Mesolph M1 ld	42.9	70.6	Mesostylid-Mesolophid m1 rm	71.4	52.9
Enterostyle M1 ra	47.6	2.4	Mesostylid-Mesolophid m1 rd	4.8	17.6
Enterostyle M1 rm	52.4	5.9	Mesostylid-Mesolophid m1 la	14.3	47.1
Enterostyle M1 rd	0.0	11.7	Mesostylid-Mesolophid m1 lm	85.7	47.1
Enterostyle M1 la	52.4	76.4	Mesostylid-Mesolophid m1 ld	0.0	5.9
Enterostyle M1 lm	47.6	11.9	Ectostylyd m1 ra	4.8	5.9
Enterostyle M1 ld	0.0	11.8	Ectostylyd m1 rm	71.4	58.8
Anteroloph-Parastyle M1 rm	42.9	17.7	Ectostylyd m1 rd	23.8	35.3
Anteroloph-Parastyle M1 rd	57.1	82.3	Ectostylyd m1 la	4.8	11.8
Anteroloph-Parastyle M1 lm	38.1	17.7	Ectostylyd m1 lm	61.9	58.8
Anteroloph-Parastyle M1 id	61.9	82.3	Ectostylyd m1 ld	33.3	29.4
Posteroloph-Posterostyle M1 ra	38.1	82.3	Mesostylid-Mesolophid m2 ra	66.7	70.6
Posteroloph-Posterostyle M1 rm	61.9	11.8	Mesostylid-Mesolophid m2 rm	33.3	29.4
Posteroloph-Posterostyle M1 rd	0.0	5.9	Mesostylid-Mesolophid m2 la	57.1	64.7
Posteroloph-Posterostyle M1 la	28.6	94.1	Mesostylid-Mesolophid m2 lm	42.9	35.3
Posteroloph-Posterostyle M1 lm	71.4	5.9	Ectostylid m2 ra	47.6	76.5
Mesostyle-Mesoloph M2 ra	9.5	5.0	Ectostylid m2 rm	52.4	23.5
Mesostyle-Mesoloph M2 rm	85.7	58.8	Ectostylid m2 la	47.6	76.5
Mesostyle-Mesoloph M2 rd	4.8	11.9	Ectostylid m2 lm	52.4	17.7
Mesostyle-Mesoloph M2 la	9.5	23.5	Ectostylid m2 ld	0.0	5.9
Mesostyle-Mesoloph M2 lm	85.7	64.7	Posterolophid-Posterostylid m1 ra	19.1	0.0
Mesostyle-Mesoloph M2 ld	4.8	11.8	Posterolophid-Posterostylid m1 rm	0.0	5.9
Enterostyle M2 ra	95.2	88.2	Posterolophid-Posterostylid m1 rd	80.9	94.1
Enterostyle M2 rm	4.8	11.8	Posterolophid-Posterostylid m1 la	19.1	0.0
Enterostyle M2 la	100.0	88.2	Posterolophid-Posterostylid m1 lm	0.0	5.9
Enterostyle M2 lm	0.0	11.8	Posterolophid-Posterostylid m1 ld	80.9	94.1
Posteroloph-Posterostyle M2 ra	61.9	94.1	Posterolophid-Posterostylid m2 ra	4.8	23.5
Posteroloph-Posterostyle M2 rm	23.8	0.0	Posterolophid-Posterostylid m2 rm	52.4	41.2
Posteroloph-Posterostyle M2 rd	14.3	5.9	Posterolophid-Posterostylid m2 rd	42.8	35.3
Posteroloph-Posterostyle M2 la	47.6	88.2	Posterolophid-Posterostylid m2 la	4.8	17.7
Posteroloph-Posterostyle M2 lm	38.1	5.9	Posterolophid-Posterostylid m2 lm	42.8	47.1
Posteroloph-Posterostyle M2 ld	14.3	5.9	Posterolophid-Posterostylid m2 ld	52.4	35.3

e = right, l = left, a = absent, m = moderate, d = development.

metacentric minute chromosomes. Pair 1 is large, easy to identify by size from the remaining autosomal pairs. Pairs 2 to 6 are medium-sized and pairs 7 to 18 are small autosomes gradually decreasing in size. The X is subtelocentric and the Y is submetacentric, representing 8.40 % and 3.21 % of the complement, respectively. A polymorphism of the sexual pair has been found in females of both species. Two types of females were found: females with XX sex chromosomes, and females with XXd sex chromosomes being the Xd an X-chromosome with a gross deletion in its long arm. In the present report over a total of 19 *A. "tucumanensis"* females analyzed, 9 were XX and 10 were XXd and over a total of 12 *A. alterus* females, 8 were XX and 4 were XXd. The Xd represents a 3.23 % of

Table 3. **Measurements (mm) of *A. alterus* (A.a.) and *A. "tucumanensis" (A.t.)* holotypes, and samples of El Infiernillo and Quebrada de Los Sosa**

	A.a.			*A.t.*		
	X	SD	Type ♂ BMNH 19.2.7.44	X	SD	Type ♀ BMNH 0.7.9.13
Greatest length	23.9	0.8	–	23.6	0.9	–
Rostrum length	8.2	0.0	–	8.6	0.0	–
Nasal length	8.2	0.0	–	8.5	0.1	–
Zygomatic breadth	12.5	0.0	–	12.6	0.4	–
Mastoid breadth	11.4	0.0	–	11.3	0.0	11.7
Teeth row length	10.5	0.0	–	10.7	0.0	–
Incisive foramen length	6.0	0.0	–	6.1	0.0	–
Upper molar length	4.5	0.0	4.0	4.4	0.0	4.2
Lower molar length	4.6	0.0	4.4	4.4	0.0	4.2

X = mean; SD = standard deviation.

the complement in *A. "tucumanensis"*, and a 3.93 % in *A. alterus*. It is of interest to notice that in spite of the X polymorphism the sex ratio was nearly 1:1 in the samples of both species.

C-bands (Fig. 4) showed that constitutive heterochromatin is located in the whole short arm of the X, the Xd-chromosome and the Y, in both species. Figure 5 shows a total G-band correspondence of chromosome arms between *A. "tucumanensis"* and *A. alterus*.

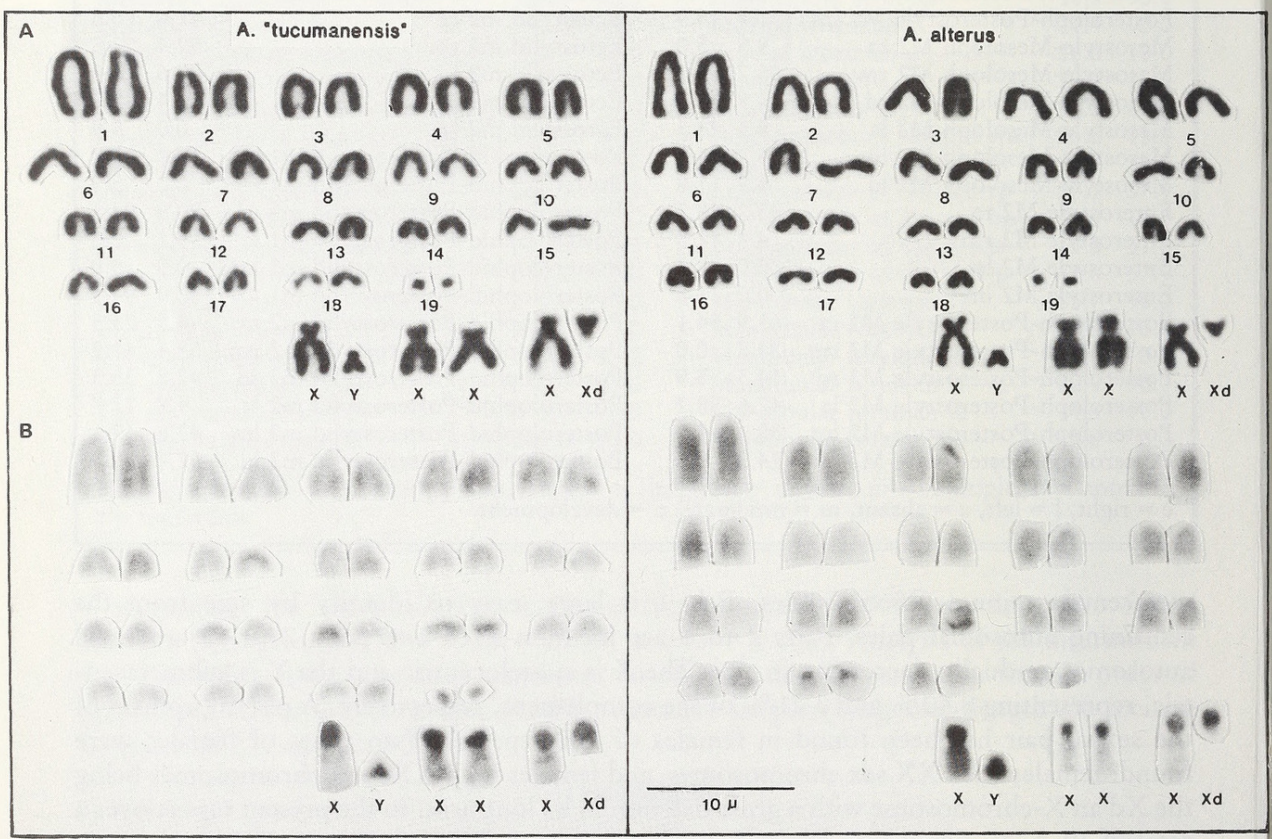

Fig. 4. A. "tucumanensis" (left) and *A. alterus* (right) Giemsa-stained karyotypes (A), and C-bands (B)

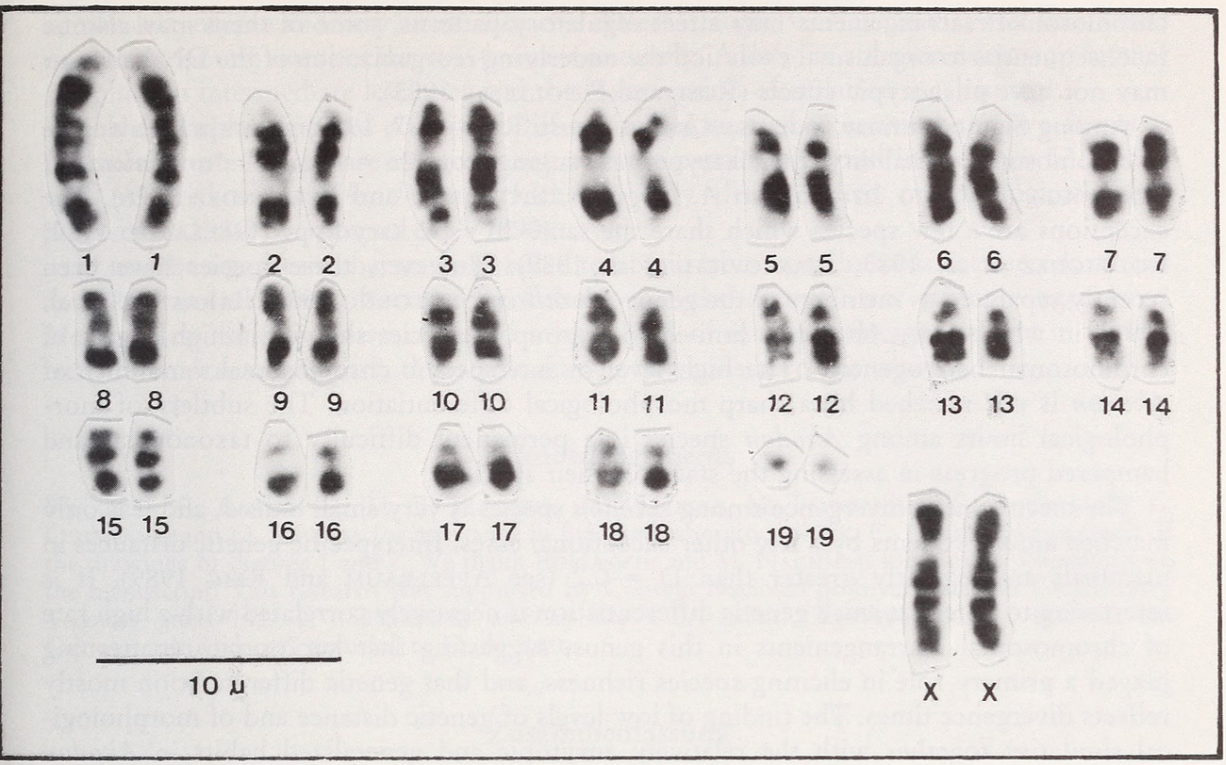

Fig. 5. G-banding pattern comparison between *A. "tucumanensis"* and *A. alterus*. Each pair is composed of one autosome of *A. "tucumanensis"* (left) and one of *A. alterus* (right)

Allozymic distance

Of the 28 loci examined electrophoretically, 20 (71.4 %) were monomorphic and fixed for the same allele in the two samples. The remaining 8 loci (28.6 %) were polymorphic in one of the two analysed species. The value of (A) was 1.082 in *A. alterus* and 1.111 in *A. "tucumanensis"*, (H) was 7.6 % in *A. alterus* and 10 % in *A. "tucumanensis"*, and (P) was 21.42 % in *A. alterus* and 25 % in *A. "tucumanensis"*. Estimates of genetic distance (D) values between the two forms showed an average value of D = 0.0280.

Discussion

There is ample evidence that organismic evolution is not always accompanied by chromosomal and allozymic changes. Against the conclusion of Wilson et al. (1975) several taxa show high chromosomal differences among synmorphic species, while others show a high degree of organismal differentiation within karyotypic invariance (Reig 1984). In rodents in which chromosomal variability is the rule, a correlation between the rate of chromosomal change and speciation rate has been suggested (Wilson et al. 1975; Bush et al. 1977; Aguilera 1980; Imai 1983). Based on the correlation between high species-diversity and high karyotypic heterogeneity patterns, Reig (1989) suggested that chromosomal repatterning could be a prime causative factor of speciogenesis in the akodontine and in other groups of rodents.

Modern theoretical developments suggest that phenotypic evolution is the result of changes in developmental programs strongly mediated by the evolution of regulatory genes with direct influence on epigenesis (Arthur 1984). Changes in structural genes, whatever their adaptative or neutral character, may be inconsequential to evolution. Although

chromosomal rearrangements may affect regulatory patterns, some of them may also be inconsequential to organismal evolution the underlying reorganization of the DNA may or may not have phenotypic effects (Rose and Doolittle 1983).

Among Sigmodontinae rodents, *Akodon* (sensu Reig 1987, 1989) shows a high degree of chromosomal variability, their karyotypes ranging from 2n = 14 (in *A. "arviculoides"*, Yonenaga (1972) to 2n = 52 (in *A. longipilis*, Spotorno and Fernandez 1976). The exceptions are a few species which share the same 2n = 52 karyotype (Gallardo 1982; Rodriguez et al. 1983; Liascovich et al. 1989). However, these species have been recently separated as members of the genus *Abrothrix* (Spotorno 1986; Barrantes et al. 1991), in which case, *Akodon* is limited to a group of species showing a high degree of chromosomal heterogeneity. The high level of interspecific chromosomal variability of *Akodon* is not matched by a sharp morphological differentiation. The subtlety of morphological limits among *Akodon* species is a permanent difficulty to taxonomists and hampered progress in assessing the status of their species.

The mean genetic divergence among *Akodon* species is very small indeed, and it is only matched among rodents by a few other exceptional cases. Interspecific genetic distances in mammals are regularly greater than D = 0.2 (see Apfelbaum and Reig 1989). It is interesting to note that small genetic differentiation is negatively correlated with a high rate of chromosomal rearrangements in this genus, suggesting that karyotypic repatterning played a primary role in eliciting species richness, and that genetic differentiation mostly reflects divergence times. The finding of low levels of genetic distance and of morphological similarity together with the relatively eurytopic and generalized habits in *Akodon* species allow us to speculate that adaptative divergence played a minor role in its speciose cladogenesis. Our results of allozymic analysis revealed a low genetic distance of D = 0.028 between *A. "tucumanensis"* and *A. alterus*. This figure agrees with previously reported values for other *Akodon* species. For example, genetic distance between pairwise comparison with *A. longipilis/A. xanthorhinus* (D = 0.022) *A. iniscatus/A. neocenus* was D = 0.021 and *A. "tucumanensis"/A. molinae* was D = 0.030. However, slightly high D values were found among *A. "tucumanensis"/A. puer* (D = 0.122), *A. "tucumanensis", A. albiventer/A. kempi* (D = 0.190), and *A. azarae/A. cursor* (D = 0.154) (Apfelbaum and Reig 1989; Patton et al. 1989; Barrantes et al. 1991).

Cytogenetic results have shown chromosomal conservatism between *A. "tucumanensis"* and *A. alterus* as reflected by a complete G-band correspondence. Moreover, both species share the same chromosomal polymorphism in the X-chromosome. This kind of chromosomal variability was also found in other species of the genus which belong to the group of species of around 40 or more reduced diploid numbers (*A. azarae*, Bianchi and Contreras 1967; Lizarralde et al. 1982; *A. puer*, Kajon et al. 1984; Vitullo et al. 1986; *A. neocenus* (cited as *A. varius*), Bianchi et al. 1971; *A. cursor*, Yonenaga-Yassuda 1979). The meaning of the X polymorphism in *Akodon azarae* has been discussed by Bianchi et al. (1989) and Solari et al. (1989).

However, these two forms can be differentiated by their external (coloration and body size), epigenetic, and continuous morphological characters. Additionally, both forms inhabit quite different ecological habitats in allopatric distribution; *A. alterus* inhabits the high mountain steppes, whereas *A. "tucumanensis"* inhabits the humid lower mountain forests.

The morphological differentiation between our samples of *A. alterus* and *A. "tucumanensis"* is indeed comparable to that found in different local populations of small mammals inhabiting different habitats. This would suggest that Cabrera was not wrong in placing *alterus* as a junior synonym of *boliviensis* , or, that both are mere subspecies of the same species. The small genetic distance, and the lack of chromosomal difference between the two forms would reinforce this view. However, as there is a strong ecological and a less marked morphological differentiation between them, and as several *Akodon* species share

similar karyotypes and very small allozyme differentiation, we believe that any conclusion on the taxonomic status of the two forms is untimely. A definite solution must wait further sampling in intermediate localities and the investigation of isolation mechanisms.

If the morphologic and ecological differences are enough to consider the two forms as full species, our results are in agreement with the pattern observed in the 2n = 52 species now referred to as *Abrothrix* , which are only distinguished morphologically (see SPOTORNO 1986; SPOTORNO et al. 1990).

It may be significant that we have failed to obtain laboratory hybrids between *A. alterus* and *A. "tucumanensis"* because the behavior of the couples was strongly aggressive. Nevertheless, since we also observed a high level of intraspecific aggressiness in mating trials of *A. "tucumanensis"*, this result may not be considered conclusive.

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Zusammenfassung

Korrelate von systematischer Differenzierung bei zwei nahe verwandten allopatrischen Populationen von Akodon aus der Boliviensis-Gruppe des Nordwestens von Argentinien (Rodentia, Cricetidae)

A. alterus und *A. „tucumanensis"* wurden bezüglich ihrer äußerlichen, epigenetischen und kontinuierlichen morphologischen Merkmale, der Karyotypen und der allozymischen, genetischen Abstände untersucht. Diese beiden Arten scheinen eng miteinander verwandt zu sein, leben aber in zwei verschiedenen Biotopen im Nordwesten von Argentinien. Die cytogenetischen Ergebnisse zeigen eine große Übereinstimmung zwischen beiden Arten: eine gleiche diploide Zahl der Chromosomen (2n = 40), eine gleiche fundamentale Zahl (NF = 40), eine gleiche Heterochromatin-Verteilung und eine vollständige Homologie der G-Bandenmuster. Die allozymische Analyse ergab ferner eine geringe genetische Distanz zwischen beiden Arten. Dieses stimmt mit Werten bei Gegenüberstellung von anderen überein. *A. alterus* und *A. „tucumanensis"* können aber durch ihre morphologischen Merkmale und die verschiedenen Habitate voneinander unterschieden werden. Frühere Untersuchungen weisen darauf hin, daß die adaptive Divergenz eine geringe Rolle in der Kladogenese von *Akodon* gespielt hat, und daß sich die große artliche Vielfalt auf eine stochastische und schnelle Fixierung von chromosomischen Mutationen zurückführen läßt, welche zur reproduktiven Isolation geführt haben. Wir fanden heraus, daß *A. „tucumanensis"* und *A. alterus* eine Ausnahme von dieser Regel darstellen. Dieses Beispiel kann im Kontext mit der Abwesenheit von Korrelationen zwischen organismischer Evolution und chromosomischer, allozymischer Evolution analysiert werden.

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