Karyotypic Relationship between *Akodon azarae* and *A. boliviensis* (Rodentia, Sigmodontinae)

J. A. Lisanti^{1,*}, E. Pinna-Senn¹, M. I. Ortiz¹,
G. Dalmasso¹ and S. Parisi de Fabro²

¹Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales,
Universidad Nacional de Río Cuarto. (5800) Río Cuarto, Argentina

²Instituto de Biología Celular, Facultad de Ciencias Médicas,
Universidad Nacional de Córdoba, Argentina

Accepted March 3, 2000

Summary The genus *Akodon*, comprising more than 60% of the species of the Akodontini, widely distributed in South America, presents many interesting cytogenetic attributes, such as an ample karyotypic variation ($2n=10-52$), intraspecific and intrapopulational polymorphisms of the autosomes and of the gonosomes, and, as in both species here studied, XY fertile females. We report a cytogenetic comparative study of *A. azarae* ($2n=38$) and of *A. boliviensis* ($2n=40$) specimens. In these species, only the sex chromosomes, the first autosomal pair and the small bibrachial autosome characteristic of the genus, can be identified in conventional preparations. Chromosome relative lengths of each species were determined from G-banded karyotypes, and a schematic representation of the G-banding patterns is presented. The comparison of these patterns shows that 16 autosomal pairs are “shared” chromosomes, corresponding to 88.3% of the autosomal complement of *A. azarae* and to 87.5% of that of *A. boliviensis*. Furthermore, *A. azarae* pair 2 (8.23% of its autosomal complement) has a banding pattern homologue to pair 15 and most of pair 12 of *A. boliviensis*, which indicates that this chromosome of *A. azarae* would basically result from a tandem translocation. Apparently, no correspondence exists between the remaining pair 16 of *A. azarae* and pair 11 of *A. boliviensis*.

Key words Akodontini, *Akodon boliviensis*, *Akodon azarae*, Chromosomes, G-bands.

The genus *Akodon* (Reig 1986, 1987) is the most polytypic of the akodontine tribe of the South American Sigmodontinae, comprising more than 60% of the species of the tribe (Apfelbaum and Reig 1989, Reig 1987). Its species are extensively distributed in South America, and can be found in different habitats through most part of the subcontinent. This genus is cytogenetically very interesting, not only because of its systematic complexity, but also due to the considerable karyotypic variation existent ($2n=10-52$), the intraspecific variation concerning the autosomes and the sex chromosomes, and the presence of species with fertile XY females (Bárquez *et al.* 1980, Bianchi and Merani 1984, Bianchi *et al.* 1971, 1979a, b, 1993, Fagundes *et al.* 1997, 1998, Gallardo 1982, Geise *et al.* 1998, Ortiz *et al.* 1998, Pinna Senn *et al.* 1993, Reig 1987, Silva and Yonenaga-Yassuda 1998, Vitullo *et al.* 1986, Wittouck *et al.* 1995).

This paper studies the karyotypic relationship between *Akodon azarae* and *Akodon boliviensis* by means of the comparison of their G-banding patterns.

Materials and methods

Ten specimens of *Akodon azarae* (6 males, 3 XX and 1 XY females) from Chucul, and the same number of specimens (6 males, 2 XX and 2 XY females) of *Akodon boliviensis* trapped near

* Corresponding author.

Table 1. Relative lengths ($\bar{x} \pm s.d.$) of *A. azarae* and *A. boliviensis* chromosomes, expressed as percentage of the genome and of the autosomal set. For X and Y chromosomes, the total length (upper row) and the short arm length (lower row) are given. Short arm lengths of *A. azarae* pair 18 and *A. boliviensis* pair 19 were not measured

Pair	<i>A. azarae</i>		Pair	<i>A. boliviensis</i>	
	% genome	% autosomal set		% genome	% autosomal set
1	10.74 $\pm$ 0.56	11.69 $\pm$ 0.63	1	10.30 $\pm$ 0.37	11.09 $\pm$ 0.43
2	7.56 $\pm$ 0.14	8.23 $\pm$ 0.11	2	6.97 $\pm$ 0.40	7.50 $\pm$ 0.42
3	7.36 $\pm$ 0.29	8.02 $\pm$ 0.33	3	6.88 $\pm$ 0.13	7.41 $\pm$ 0.15
4	6.65 $\pm$ 0.25	7.24 $\pm$ 0.29	4	6.78 $\pm$ 0.42	7.30 $\pm$ 0.44
5	6.63 $\pm$ 0.19	7.22 $\pm$ 0.19	5	6.07 $\pm$ 0.18	6.54 $\pm$ 0.20
6	6.33 $\pm$ 0.31	6.89 $\pm$ 0.35	6	5.62 $\pm$ 0.25	6.06 $\pm$ 0.26
7	5.83 $\pm$ 0.29	6.35 $\pm$ 0.29	7	5.13 $\pm$ 0.35	5.52 $\pm$ 0.38
8	5.19 $\pm$ 0.19	5.65 $\pm$ 0.18	8	5.06 $\pm$ 0.34	5.45 $\pm$ 0.36
9	4.91 $\pm$ 0.07	5.35 $\pm$ 0.06	9	4.61 $\pm$ 0.18	4.96 $\pm$ 0.18
10	4.33 $\pm$ 0.11	4.71 $\pm$ 0.13	10	4.47 $\pm$ 0.29	4.82 $\pm$ 0.31
11	4.11 $\pm$ 0.22	4.48 $\pm$ 0.25	11	4.08 $\pm$ 0.35	4.40 $\pm$ 0.38
12	4.10 $\pm$ 0.20	4.47 $\pm$ 0.20	12	3.93 $\pm$ 0.10	4.23 $\pm$ 0.10
13	3.74 $\pm$ 0.25	4.07 $\pm$ 0.27	13	3.88 $\pm$ 0.14	4.18 $\pm$ 0.14
14	3.46 $\pm$ 0.19	3.77 $\pm$ 0.21	14	3.73 $\pm$ 0.15	4.02 $\pm$ 0.15
15	3.23 $\pm$ 0.15	3.51 $\pm$ 0.16	15	3.56 $\pm$ 0.33	3.84 $\pm$ 0.36
16	3.18 $\pm$ 0.24	3.46 $\pm$ 0.26	16	3.54 $\pm$ 0.17	3.81 $\pm$ 0.17
17	2.85 $\pm$ 0.06	3.11 $\pm$ 0.07	17	3.33 $\pm$ 0.17	3.56 $\pm$ 0.20
18	1.65 $\pm$ 0.29	1.80 $\pm$ 0.32	18	3.02 $\pm$ 0.35	3.25 $\pm$ 0.37
			19	1.91 $\pm$ 0.22	2.06 $\pm$ 0.35
X	8.13 $\pm$ 0.50		X	7.16 $\pm$ 0.27	
	1.31 $\pm$ 0.43			1.68 $\pm$ 0.23	
Y	1.54 $\pm$ 0.13		Y	2.80 $\pm$ 0.36	
	0.65 $\pm$ 0.07			1.06 $\pm$ 0.20	

Parador "El Cóndor" (Pampa de Achala), both sites in the province of Córdoba, Argentina, were studied. Chromosome preparations were obtained from bone marrow by conventional procedures, following the *in vivo* injection of colchicine. Metaphase cells were spread onto clean slides and air dried, and stored at -20°C until use. G-banding was obtained by trypsin digestion (Seabright 1971). The staining of the preparations was done with Giemsa.

The relative lengths of the different pairs was determined on 5 G-banded karyotypes of *A. azarae* (from 2 males) and the same number of banded karyotypes of *A. boliviensis* (from 3 males), and were expressed as percentage of the female haploid genome and of the autosomal set (Table 1).

Results and discussion

In accordance with previous descriptions (Bianchi *et al.* 1971, Ortiz *et al.* 1998), the specimens of *A. azarae* showed a karyotype composed of 17 telocentric autosome pairs, a small bibrachial one which is characteristic of the genus, a subterminal X and a bibrachial Y that is the smallest chromosome of the karyotype. Only the first pair, the small bibrachial and the sex chromosomes are recognizable in unbanded cells. Similarly, the karyotype of *A. boliviensis* coincides with that previously found in this species (Bianchi *et al.* 1971, Pinna Senn *et al.* 1993): 18 telocentric and a small metacentric autosomal pairs, a submetacentric X and a bibrachial Y, intermediate in size between pairs 18 and 19. Again, only the first pair, the small bibrachial and the gonosomes can be identified in unbanded metaphases. With respect to C-bands, it has been shown that in *A. azarae*, centromeric bands can be found in the autosomes, excepting the small biarmed one; the Y chromo-

some is heterochromatic, and the X presents several bands that originate a polymorphic pattern (Ortiz *et al.* 1998). In *A. boliviensis*, on the other hand, the only heterochromatic regions observed are the short arm of the X chromosome and the long arm of the Y (Pinna Senn *et al.* 1993).

The relative lengths of *A. azarae* and *A. boliviensis* chromosomes determined on G-banded karyotypes are shown in Table 1. Those of the chromosomes that are identifiable in conventionally stained cells can be compared with previously published data (Bianchi *et al.* 1971). With respect to *A. azarae*, our values for pairs 1 and 18 are 10.74 and 1.65, respectively, against 10.9 and 1.7 obtained by Bianchi *et al.* (1971); with respect to the gonosomes our relative lengths are 8.13 for the X and 1.54 for the Y chromosome, comparable to 8.2 and 1.5 of those authors.

As regards to *A. boliviensis*, our relative lengths corresponding to pairs 1 and 19 are 10.30 and 1.91, while the results of Bianchi *et al.* (1971) are 11.1 and 1.7 in *A. b. tucumanensis* and 10.6 and 1.8 in *A. b. spegazzinii*. The X and Y lengths here determined are 7.16 and 2.80, in comparison to 8.9 and 2.7 (*A. b. tucumanensis*) and 8.8 and 3.6 (*A. b. spegazzinii*) obtained by Bianchi *et al.*

Fig. 1. G-banded chromosomes of *A. azarae*. Two complete male karyotypes, with different degrees of chromosome condensation, are shown. Below: the corresponding metaphase plates. The bar represents 10 μ m.

(1971). However, it must be taken into account that a considerable structural variation of the X chromosome is observed in different populations of *A. boliviensis* (Bárquez *et al.* 1980, Bianchi *et al.* 1971), most probably due to different amounts of constitutive heterochromatin in its short arm (Pinna Senn *et al.* 1993).

G-banded karyotypes of *A. azarae* and *A. boliviensis* are shown in Figs. 1 and 2 and a schematic representation of the banding patterns in Fig. 3. In addition, a comparison of the G-banded chromosomes of the 2 species is presented in Fig. 4. As can be observed, 16 pairs of each species have similar banding patterns and relative lengths, and can be considered as conserved chromosomes. The comparison of the relative lengths of the matched pairs was done dividing, in each match, the largest length by the smallest one. Except for the 18/19 match (the first number indicates the *A. azarae* chromosome pair), this ratio presented values between 1.01 and 1.09. For the 18/19 match, corresponding to the small metacentrics, this value was 1.16, a value that could reflect a real difference or, more probably, considering the lower relative lengths obtained for the *A. boliviensis* meta-centric by Bianchi *et al.* (1971), could result from measurement errors due to the small length of

Fig. 2. G-banded chromosomes of *A. boliviensis*. Two complete male karyotypes, with different degrees of chromosome condensation, are shown. Below: the corresponding metaphase plates. The bar represents 10 μ m.

Fig. 3. Schematic representation of the G-banding patterns of *A. azarae* (upper) and *A. boliviensis* (lower). The idiograms correspond to the relative lengths (percentage of the genome) of Table 1.

these chromosomes and the lack of bands on most of their arms.

These data (Fig. 4, Table 1) indicates that about 88% of the autosomal set of *A. azarae* and *A. boliviensis* (88.31% and 87.53%, respectively) corresponds to "shared chromosomes" (Bianchi and Merani 1984).

Pairs 2 and 16 from *A. azarae*, and pairs 11, 12 and 15 from *A. boliviensis* remain apart. Nevertheless, there exists considerable banding homology (Fig. 4) between *A. boliviensis* chromosome 15 and most of the proximal region of *A. azarae* pair 2, and also between most of pair 12 of *A. boliviensis* and the distal portion of that chromosome of *A. azarae*, which would suggest that *A. azarae* chromosome 2 could result from a tandem translocation, plus additional rearrangements such as translocation of *A. boliviensis* chromosome 12 distal band to other place in the genome and addition of heterochromatic material in the centric region. On the other hand, there is no correspondence between chromosome 16 of *A. azarae* and chromosome 11 of *A. boliviensis*, neither in size nor in banding pattern. A better clarification of the karyotypic relationship between these two species would depend on the employment of more powerful techniques such as fluorescent *in situ* hybridization (FISH) and chromosome painting.

Cytogenetically, *A. azarae* belongs to a group of species with intermediate chromosome numbers that would have diversified during the Southeastern radiation from the proto-Puna center of origin, is now distributed in the Argentinian prairies and humid pampa, in Mesopotamia and in

Fig. 4. Comparison of G-banded chromosomes of *A. azarae* and *A. boliviensis* done with the more elongated karyotypes of Figs. 1 and 2. In each matched pair, the left chromosome belongs to *A. azarae* (A) and the right one to *A. boliviensis* (B). In the group of three chromosomes of the lower row, the two chromosomes of *A. boliviensis* and the one of *A. azarae* are indicated. The remaining chromosomes in this last row correspond to those with no apparent homolog.

Uruguay (Reig 1987), and includes species such as *A. boliviensis*, *A. dolores*, *A. molinae*, *A. simulator* and *A. albiventer*.

The karyotypic relationship between *A. azarae* and *A. boliviensis* is higher than those found by other authors (Bianchi *et al.* 1976, Bianchi and Merani 1984) between the first species and others of the genus by them examined, including *A. molinae*, *A. dolores* and *A. varius*. It seems to exist, as indicated by these authors, a notable karyotypic conservation among this group of species.

On the other hand, an analysis of allozymic genetic distances between *A. azarae*, *A. molinae*, *A. boliviensis* and *A. neocenus* (= *A. varius*), among other species, shows a tight relationship between *A. boliviensis* and *A. molinae* and a greater distance between these and *A. azarae* (Apfelbaum and Reig 1989). Moreover, in a study based on the mitochondrial gene cytochrome b, Smith and Patton (1993) place *A. azarae* and *A. toba* together within a not very well sustained clade which includes *A. boliviensis* and other species of the so-called "*boliviensis* group". *A. toba* presents a karyotype which, at least in non banded preparations, appears similar to that of *A. molinae*, showing also the polymorphism of pair 1 characteristic of the latter species (Myers 1989). It is evidently necessary the contribution of new comparative cytogenetical and molecular data in order to discuss adequately these results.

Acknowledgements

The authors wish to thank Dr. J. J. Polop for providing the animals used in this study and for his critical reading of the manuscript, Dr. I. E. DiTada for his support, and Mr. Marcos Torres for his technical work in the field. This work was partly financed with a grant from the Secretaría de Ciencia y Técnica of the U.N.R.C.

References

- Apfelbaum, L. I. and Reig, O. A. 1989. Allozyme genetic distances and evolutionary relationships in species of akodontine rodents (Cricetidae: Sigmodontinae). *Biol. J. Linn. Soc.* **38**: 257–280.
- Bárquez, R. M., Williams, D. F., Mares, M. A. and Genoways, H. H. 1980. Karyology and morphometrics of three species of *Akodon* (Mammalia: Muridae) from Northwestern Argentina. *Ann. Carnegie Mus.* **49**: 379–403.
- Bianchi, N. O., Vidap-Rioja, L. and Bianchi, M. S. 1976. Cytogenetics of South American Akodont rodents (Cricetidae). II. Interspecific homologies of G-banding patterns. *Cytologia* **41**: 139–144.
- and Merani, S. 1984. Cytogenetics of South American Akodont rodents (Cricetidae). X. Karyological distances at generic and intergeneric levels. *J. Mammal.* **65**: 206–219.
- , Reig, O. A., Molina, O. J. and Dulout, F. N. 1971. Cytogenetics of South American Akodont rodents (Cricetidae). I. A progress report of Argentinian and Venezuelan forms. *Evolution* **25**: 724–736.
- , Merani, S. and Lizarralde, M. 1979a. Cytogenetics of South American Akodont rodents (Cricetidae). VI. Polymorphism in *Akodon dolores* (Thomas). *Genetica* **50**: 99–104.
- , —, Larramendy, M., Lizarralde, M. and Oliveira, D. 1979b. Cytogenetics of South American Akodont rodents (Cricetidae). V. Segregation of chromosome 1 polymorphism in *Akodon molinae*. *Experientia* **35**: 1438–1439.
- , Bianchi, M. S., Bailliet, G. and De la Chapelle, A. 1993. Characterization and sequencing of the sex determining region Y gene (*Sry*) in *Akodon* (Cricetidae) species with sex reversed females. *Chromosoma* **102**: 389–395.
- Fagundes, V., Scalzi-Martin, J. M., Sims, K., Hozier, J. and Yonenaga-Yassuda, Y. 1997. Zoo-FISH of a microdissection DNA library and G-banding patterns reveals the homeology between the Brazilian rodents *Akodon cursor* and *A. montensis*. *Cytogenet. Cell Genet.* **78**: 224–228.
- , Christoff, A. U. and Yonenaga-Yassuda, Y. 1998. Extraordinary chromosomal polymorphism with 28 different karyotypes in the neotropical species *Akodon cursor* (Muridae, Sigmodontinae), one of the smallest diploid number in rodents ($2n=16, 15$ and 14). *Heredity* **129**: 263–274.
- Gallardo, M. H. 1982. Chromosomal homology in southern *Akodon*. *Experientia* **38**: 1485–1487.
- Geise, L., Canavez, F. C. and Seuánez, H. N. 1998. Comparative karyology in *Akodon* (Rodentia, Sigmodontinae) from Southeastern Brazil. *J. Heredity* **89**: 158–163.
- Myers, P. 1989. A Preliminary Revision of the *varius* Group of *Akodon* (*A. dayi*, *dolores*, *molinae*, *neocenus*, *simulator*, *toba*, and *varius*). In: Redford, K. H. and Eisenberg, J. F. (eds.). *Advances in Neotropical Mammalogy. The Sand-*

- hill Crane Press, Inc., Gainesville, Florida. pp. 5–54.
- Ortiz, M. I., Dalmasso, G., Dezi, R., Pinna-Senn, E. and Lisanti, J. A. 1998. A C-band polymorphism of the X chromosome in *Akodon azarae* (Rodentia, Cricetidae). *Cytologia* **63**: 365–369.
- Pinna Senn, E., de Barale, G. D., Polop, J., Ortiz, M. I., Provencal, M. C. and Lisanti, J. A. 1993. Estudio cariotípico y morfológico en una población de *Akodon* sp. (Rodentia, Cricetidae) de Pampa de Achala. *Mendeliana* **10**: 59–70.
- Reig, O. A. 1986. Diversity Patterns and Differentiation of High Andean Rodents. In: Vuilleumier, F. and Monasterio, M. (eds.). *High Altitude Tropical Biogeography*. Oxford Univ. Press.
- 1987. An assessment of the systematics and evolution of the Akodontini, with description of new fossil species of *Akodon* (Cricetidae: Sigmodontinae). *Fieldiana Zool. (New Series)* **39**: 347–399.
- Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet* **2**: 971–972.
- Silva, M. J. de J. and Yonenaga-Yassuda, Y. 1998. Karyotype and chromosomal polymorphism of an undescribed *Akodon* from central Brazil, a species with the lowest known diploid chromosome number in rodents. *Cytogenet. Cell Genet.* **81**: 46–50.
- Smith, M. and Patton, J. L. 1993. The diversification of South American murid rodents: evidence for mitochondrial DNA sequence data for the akodontine tribe. *Biol. J. Linn. Soc.* **50**: 149–177.
- Vitullo, A., Merani, M., Reig, O., Kajon, A., Scaglia, O., Espinosa, M. and Pérez-Zapata, A. 1986. Cytogenetics of South American Akodont rodents (Cricetidae): new karyotypes and chromosomal banding patterns of Argentinian and Uruguayan forms. *J. Mammal.* **67**: 69–80.
- Wittouck, P., Pinna Senn, E., Soñez, C. A., Provencal, M. C., Polop, J. J. and Lisanti, J. A. 1995. Chromosomal and synaptonemal complex analysis of Robertsonian polymorphisms in *Akodon dolores* and *Akodon molinae* (Rodentia, Cricetidae) and their hybrids. *Cytologia* **60**: 93–102.
-