Z. Säugetierkunde 59 (1994) 14–20

ISSN 0044-3468

Cytogenetic analysis of autosomal polymorphism in *Graomys griseoflavus* (Rodentia, Cricetidae)

By A. Zambelli, Lidia Vidal-Rioja, and R. Wainberg

Instituto Multidisciplinario de Biología Celular, La Plata, Argentina and Cátedra de Biología General, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina

Receipt of Ms. 1. 12. 1992
Acceptance of Ms. 8. 6. 1993

Abstract

South American phyllotine *Graomys griseoflavus* specimens were collected in eight localities of central Argentina and cytogenetically analysed. These populations comprised the following karyomorphs: 2n = 42, 41, 38, 37, 36, 35 and 34. These chromosome polymorphisms resulted from Robertsonian fusions (RFs). A pericentric inversion (PI) in two different autosomal pairs are described. The numerical karyotype variability is explained by successive RFs, starting from a karyotype with 2n = 42.

Introduction

Graomys griseoflavus (Waterhouse, 1837) is a South American phyllotine rodent showing a high degree of chromosomal polymorphisms. Previously, Wainberg and Fronza (1974a, b) reported preliminary data in one population of Argentina that then was misdetermined as *Phyllotis griseoflavus griseoflavus*. Later, according to a revision of the taxa (Pearson and Patton 1976) these specimens were recognized as *Graomys griseoflavus* (Reig pers. comm.). Wainberg and Fronza (1974a, b) described diploid numbers of 2n = 38, 37 and 36 for specimens collected in Chasicó (Buenos Aires province, Argentina) and suggested RFs as a possible mechanism for the karyotypic variability found.

Some reports sustain the idea that rodents are in active speciation processes (Greenbaum et al. 1978; Hsu and Arrighi 1968; Reig 1984). Supporting evidence are chromosomal polymorphisms observed in many species. In *Mus domesticus* for instance, there were defined more than 110 different Robertsonian fusions (RFs). The presence of several Robertsonian populations is regarded as an example of stasipatric speciation (Redi and Capanna 1988). Cricetid rodents that show high chromosomal variability have been considered models for studies on chromosomal rearrangements, speciation and evolution (Bianchi et al. 1971, 1979; Hood et al. 1984; Nachman and Myers 1989; Nachman 1992). In the genus *Eligmodontia* Zambelli et al. (1992) described the occurrence of RFs in a polymorphic system 2n = 34-33-32, where the 2n = 33 (heterokaryomorph) males showed a trivalent in diakinesis-metaphase I. So far, in all the models described the contributing acrocentric chromosomes have a random chance to fuse.

In the present study we describe the so far unknown 2n = 42, 41, 35 and 34 karyomorphs of *G. griseoflavus* and present G-banding analysis that confirms the occurrence of RFs and pericentric inversions (PIs). We suggest a hypothesis explaining the karyotypic variability by successive RFs that occurred non-randomly.

Material and methods

We processed seventy-two animals collected at the following eight localities of central Argentina (Fig. 1): Chasicó (Buenos Aires province); La Carrera (Catamarca province); Divisadero Largo (Mendoza province); Salicas and General Belgrano (La Rioja province) and Deán Funes, Laguna Larga and Santiago Temple (Córdoba province). Table 1 shows the number of specimens caught in each locality, and the diploid numbers (2n) found.

Bone marrow metaphase spreads were obtained by a modification of the technique of Rothfelds and Siminovich (1958). To enhance bone marrow cell proliferation all specimens were previously treated with a yeast suspension (Lee and Elder 1980). Meiotic preparations from testes were done as indicated by Evans et al. (1964). Chromosomal G-banding was obtained according to Seabright (1971). Homozygous and heterozygous terms were abbreviated Hm and Ht, respectively.

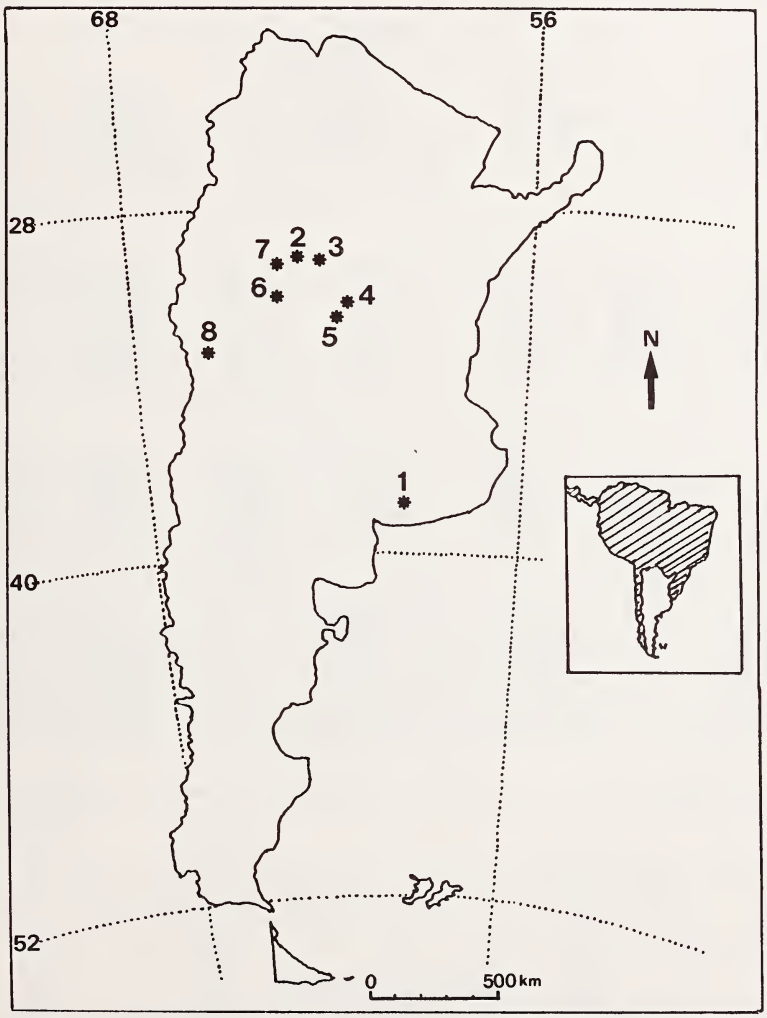

Fig. 1. Map showing the localities where specimens of *G. griseoflavus* were collected. 1: Chasicó; 2: La Carrera; 3: Deán Funes; 4: Santiago Temple; 5: Laguna Larga; 6: General Belgrano; 7: Salicas; 8: Divisadero Largo

Table 1. **Numbers of specimens of the different karyomorphs of *G. griseoflavus* collected in each locality**

Locality	42	41	38	37	36	35	34
1. Chasicó	–	–	12	9	4	–	–
2. La Carrera	1	–	1	4	1	–	–
3. Deán Funes	4	1	–	–	1	–	–
4. Santiago Temple	7	–	–	–	–	–	–
5. Laguna Larga	4	–	–	–	–	–	–
6. General Belgrano	6	–	–	–	–	–	–
7. Salicas	–	–	1	1	2	–	–
8. Divisadero Largo	–	–	–	–	4	2	1

Results

Robertsonian fusions

The 2n = 42 karyomorph comprises twenty pairs of autosomes (Fig. 2a). Chromosomes 1–18 are acrocentric gradually decreasing in size (large to small). The pair 19 is a medium sized submetacentric and pair 20 is a small submetacentric. In some animals chromosome 4 can be submetacentric (see below pericentric inversions). The X is a large submetacentric and the Y is a small acrocentric chromosome.

The 2n = 38 karyomorph shows fourteen pairs of acrocentric and two additional pairs of large submetacentric autosomes. These features constitute the main difference to the 2n = 42 karyomorph (Fig. 2b).

The G-banding pattern analysis of 2n = 42 and 2n = 38 karyomorphs allowed us to conclude that the two large submetacentric pairs observed in 2n = 38 animals resulted from RFs between the acrocentric chromosomes 15/17 and 16/18 pairs of 2n = 42 specimens (RF15–17 and RF16–18, respectively) (Fig. 2, Tab. 2).

It was previously proposed that the very large submetacentric chromosome of the 2n = 37 karyomorph was produced by a RF occurring in the 2n = 38 karyomorph. This assumption was based on morphological chromosomal comparisons between these karyomorphs and on the presence of one trivalent in diakinesis-metaphase I of 2n = 37 males. We identified by G-band comparison that the RF involves autosomes 1 and 6 (RF1–6) (Figs. 2, 3a, Tab. 2). RF1–6 is also present as heterozygous in the 2n = 41 karyomorph and as homozygous in the 2n = 36 karyomorph (Tab. 2, Fig. 3b). Diakinesis-metaphases I of 2n = 36 males showed 17 bivalents plus a symmetrical very large sized RF1–6 bivalent (Fig. 3e).

In the 2n = 35 karyomorph there was a decrease of two acrocentric and the appearence of one very large submetacentric chromosome produced which according to G-banding arose by centric fusion of autosome 2 and 5 (RF2–5) (Tab. 2). Diakinesis-metaphases I of

Table 2. **Different Robertsonian fusions found in each karyomorph of *G. griseoflavus***

2n	RF1–6	RF2–5	RF15–17	RF16–18
42	–	–	–	–
41	Ht	–	–	–
38	–	–	Hm	Hm
37	Ht	–	Hm	Hm
36	Hm	–	Hm	Hm
35	Hm	Ht	Hm	Hm
34	Hm	Hm	Hm	Hm

(–) = absence of RF.

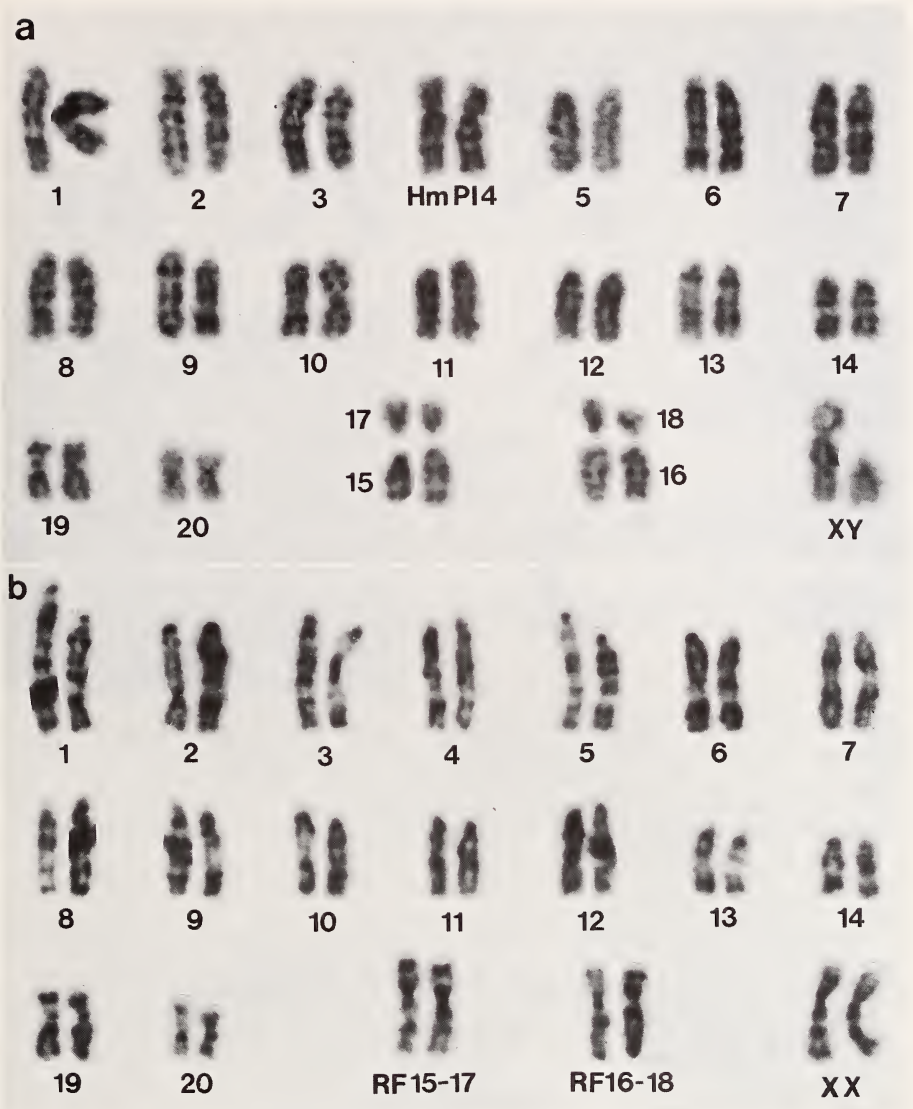

Fig. 2. G-banded karyotypes with 2n = 42 (a) and 2n = 38 (b) of *G. griseoflavus*. HmPI = homozygous pericentric inversion

2n = 35 specimens showed 15 bivalents, one very large bivalent (homozygous RF1–6) and one trivalent, which is formed by chromosomes RF2–5, 2 and 5 (Fig. 3f).

In the 2n = 34 karyomorph the RF2–5 was homozygous (Fig. 3c, Tab. 2).

Pericentric inversions

In our survey, we found two autosomal acrocentric pairs that underwent pericentric inversion (PI), giving rise to submetacentric elements. G-band analysis showed that this rearrangement involved chromosomes 4 and 13 (PI4 and PI13, respectively) (Figs. 2a, 3d).

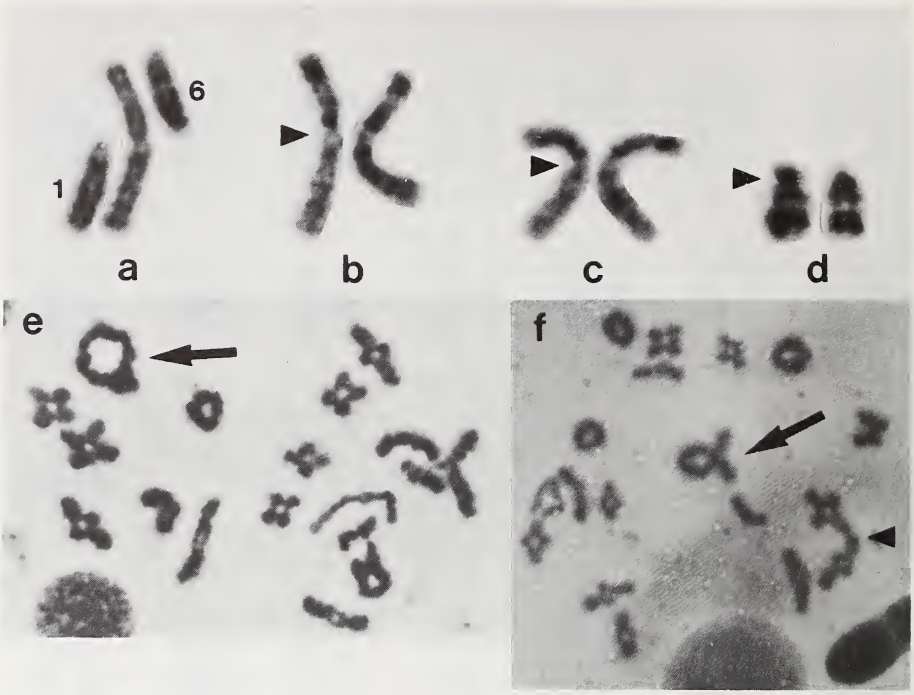

Fig. 3. a: G-banded RF1–6 and its acrocentric homologues (1 and 6 autosomes) from a 2n = 37 karyomorph; b: G-banded RF1–6 chromosome pair from a 2n = 36 karyomorph; c: G-banded RF2–5 chromosome pair from a 2n = 34 karyomorph; d: Heterozygous pericentric inversion of pair 13 from a 2n = 37 karyomorph; e–f: Diakinesis-metaphaseI figures from 2n = 36 (e) and 2n = 35 (f) karyomorphs. The arrows point to the very large RF-bivalent; the arrow head points to the trivalent

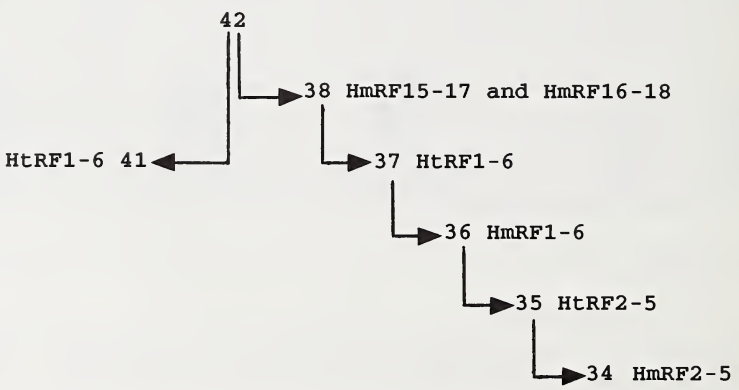

Fig. 4. Sequence of Robertsonian fusions explaining the karyotypic divergence of *G. griseoflavus*

The PI4 was found in 2n = 42, 38, 37 and 36 karyomorphs. The PI13 was found in 2n = 38, 37 and 36 karyomorphs. Within a population both PIs may show a homozygous or heterozygous state or even be absent.

Discussion

The phyllotine rodent *Graomys griseoflavus* exhibits a high degree of chromosomal polymorphisms produced by two pericentric inversions and four Robertsonian fusions. Cytogenetic findings suggest that the latter rearrangements occurred non-randomly and can be summarized as follows: a. the RF15–17 and RF16–18 (present in 2n = 38–34 complex) were always found in a homozygous state; b. all 2n = 37–36 individuals were heterozygous and homozygous respectively for RF1–6; c. all 2n = 35–34 animals examined were homozygous for RF1–6 and heterozygous and homozygous respectively, for RF2–5. These findings suggest a sequencial occurrence of Robertsonian fusions. Thus, HmRF15–17 and HmRF16–18 (uncertain who was first) were followed by HtRF1–6, HmRF1–6, HtRF2–5 and HmRF2–5. On these grounds, we propose a sequence of Robertsonian events that lead to the karyotypic divergence observed, with the 2n = 42 as the common ancestral karyomorph (Fig. 4). This assumption agrees with the view of Gardner and Patton (1976) that karyotypic evolution in Neotropical cricetids decreases the chromosomal number via Robertsonian fusions.

To explain the karyotype divergence from 2n = 42 to 2n = 38 we assumed the existence of the 2n = 41, 40 and 39 karyomorphs. At least, the 2n = 41 and 39 individuals should be heterozygous for RF15–17 or RF16–18. Thus far, in fifty-one wild animals studied only individuals homozygous for these Robertsonian fusions were found. Thus, we assumme that the heterozygous animals may be not viable or that its frequency is low. To test these hypotheses we performed experimental crosses to asses the segregation of Robertsonian chromosomes. In matings between 2n = 42/41, 2n = 38/37, 2n = 38/36 and 2n = 37/37 individuals the F1 and F2 progenies showed normal meiosis and RF15–17, RF16–18 or RF1–6 segregating in a Mendelian fashion. On the other hand, when matings between 2n = 42/38 and 2n = 42/37 individuals were tried under the same laboratory breeding conditions, no offsprings were obtained. Probably, the unsuccesful breedings were due to the inviability of heterozygous RF15–17 and RF16–18 embryos.

There is evidence that Robertsonian fusions may be involved in speciation processes only when they cause reproductive failure, e. g. in the case of meiosis nondisjunction (King 1987). In *Graomys griseoflavus* the inviability of heterozygous RF15–17 and/or RF16–18 products may produce reproductive isolation of 2n = 42–41 and 2n = 38–37–36 (and probably also 2n = 35–34) karyomorphs, while heterozygous RF1–6 and RF2–5 (this latter always present together with HmRF1–6) would not induce reproductive isolation.

According to the remarkable chromosomal polymorphism described in *Graomys griseoflavus* we suggest that this species is evolving actively and therefore represents an interesting model for speciation and chromosomal evolutionary studies.

Acknowledgements

We thank to Dr. Noemí Gardenal for providing some specimens included in this work and to Anibal Ramos for the technical assistance. Our appreciation go to Dr. N. O. Bianchi and Dr. K. Fredga for critical reading and suggestions on this manuscript. This paper was supported by CONICET grant PIDs 3085300/85 to R. W. and 3066400/88 to L.V.R.

Zusammenfassung

Cytogenetische Analysen von Autosomen-Polymorphismen bei Graomys griseoflavus (Rodentia, Cricetidae)

Graomys griseoflavus aus Südamerika wurden an acht verschiedenen Orten gefangen und cytogenetisch untersucht. In diesen Populationen wurden Karyotypen mit folgenden Chromosomenzahlen gefunden: 2n = 42, 41, 38, 37, 36, 35 und 34. Diese numerischen Karyotypvariationen werden auf Robertsonsche Fusionen (RFs) zurückgeführt. Es wurden vier verschiedene RFs beschrieben, die acht akrocentrische Autosomen als Fusionspartner betreffen. Weiterhin wurden pericentrische Inversionen

an zwei verschiedenen Autosomen festgestellt. Die zahlenmäßigen Karyotypvariationen werden durch aufeinanderfolgende RFs erklärt, wobei als Ausgangskaryotyp der mit 2n = 42 postuliert wird.

References

Bianchi, N. O.; Merani, S.; Lizarralde, M. (1979): Cytogenetics of the South-American akodon rodents (Cricetidae). VI. Polymorphism in *Akodon dolores* (Thomas). Genetica **50**, 99–104.

Bianchi, N. O.; Reig, O. A.; Dulout, F. N. (1971): Cytogenetics of the South American akodon rodents (Cricetidae). I. A progress report of Argentinian and Venezuelan forms. Evolution **25**, 125–135.

Evans, E. P.; Breckton, G.; Ford, C. E. (1964): An air-drying method for meiotic preparations from mammalian testes. Cytogenetics **3**, 289–294.

Gardner, A. L.; Patton, J. L. (1976): Karyotypic variation in oryzomine rodents (Cricetidae) with comments on chromosomal evolution in the neotropical cricetine complex. Occas. Papers Mus. Zool., Lousiana State Univ. **49**, 1–48.

Greenbaum, I. F.; Baker, R. J.; Ramsey, P. R. (1978): Chromosomal evolution and its implication concerning the mode of speciation in three species of deer mice of the genus *Peromyscus*. Evolution **32**, 646–654.

Hood, C. S.; Robbins, L. W.; Baker, R. J.; Shellhammer, H. S. (1984): Chromosomal studies and evolutionary relationships of an endangered species, *Reithrodontomys raviventris*. J. Mammalogy **65**, 665–667.

Hsu, T. C.; Arrighi, F. E. (1968): Chromosomes of *Peromyscus* (Rodentia, Cricetidae). I. Evolutionary trends in 20 species. Cytogenetics **7**, 417–446.

King, M. (1987): Chromosomal rearrangements, speciation and the theoretical approach. Heredity **59**, 1–6.

Lee, M.; Elder, F. (1980): Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenet. Cell Genet. **26**, 36–40.

Nachman, N. W. (1992): Geographic patterns of chromosomal variation in South American marsh rats, *Holochilus brasiliensis* and *H. vulpinus*. Cytogenet. Cell Genet. **61**, 10–16.

Nachman, M. W.; Myers, P. (1989): Exceptional chromosomal mutations in a rodent population are not strongly underdominant Proc. Natl. Acad. Sci. USA **86**, 6666–6670.

Pearson, O. P.; Patton, J. L. (1976): Relationships among South American phyllotine rodents based on chromosome analysis. J. Mammalogy **57**, 339–350.

Redi, C. A.; Capanna, E. (1988): Robertsonian heterozygotes in the house mouse and fate of their germ cells. in: The Cytogenetics of Mammalian Autosomal Rearrangements. Ed. by A. Daniel. New York: Liss. Pp. 315–359.

Reig, O. A. (1984): Geographic distribution and evolutionary history of South American muroids (Cricetidae, Sigmodontinae). Rev. Brasil. Genet. **7**, 333–365.

Rothfels, K. H.; Siminovich, L. (1958): An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technol. **33**, 73–77.

Seabright, M. (1971). A rapid banding technique for human chromosomes. Lancet **2**, 971–972.

Wainberg, R. L.; Fronza, T. G. (1974a): Autosomic polymorphism in *Phyllotis griseoflavus griseoflavus* Waterhouse, 1837 (Rodentia, Cricetidae). Boll. Zool. **41**, 19–24.

– – (1974b): Autosomic polymorphism in the South-American (Argentinean) rodent *Phyllotis griseoflavus griseoflavus* Waterhouse, 1837 (Cricetidae) with karyotypes 2n = 38, 37 and 36. II. The complement 2n = 36. First Intern. Theriol. Congr. Moscow. Vol. 2, 293–294.

Zambelli, A.; Dysenchauz, F.; Ramos, A.; de Rosa, N.; Wainberg, R.; Reig, O. A. (1992): Cytogenetics and karyosystematics of phyllotine rodents (Cricetidae, Sigmodontinae). III. New data on the distribution and variability of karyomorphs of the genus *Eligmodontia*. Z. Säugetierkunde **57**, 155–162.

Authors' addresses: Andrés Zambelli and Lidia Vidal-Rioja, IMBICE, CC403 (1900) La Plata, Argentina; Ricardo Wainberg, Cátedra de Biología General, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas 47 y 115, Universidad Nacional de La Plata, (1900) La Plata, Argentina