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Research Articles

Cytogenetics of vesper mice, *Calomys* (Sigmodontinae): a new karyotype from the Puna region and its implication for chromosomal phylogeny

M. B. Espinosa[a], A. Lasserre[a], M. Piantanida[b] and A. D. Vitullo[a,*]

[a]*Centro de Estudios Farmacológicos y Botánicos, CEFYBO-CONICET, Buenos Aires (Argentina)*
[b]*División Mastozoología, Museo Argentino de Ciencias Naturales ''Bernardino Rivadavia'', Buenos Aires (Argentina)*
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Abstract. The karyotype of specimens identified as *Calomys lepidus*, trapped at 3600 m above sea level in the Puna region, northwestern Argentina, was studied. All specimens analysed showed a $2n = 44$ ($NF_a = 68$) asymmetrical karyotype with 13 pairs of metacentric/submetacentric autosomes and 7 pairs of telocentric chromosomes. The X was a medium-sized submetacentric and the Y a small submetacentric chromosome. This karyotype was quite different from that previously described for *C. lepidus* from Peru ($2n = 36$, $NF_a = 68$). However, both karyotypes may be easily interrelated by means of four centric fusions, and the chromosome complement of Punian *C. lepidus* fitted into a previously proposed chromosomal phylogeny of the genus. In addition, the spermatozoa of specimens corresponded to a morphological pattern previously described for other species of *Calomys*.
Key words. Chromosome evolution; sperm morphology; *Calomys lepidus*; Rodentia; Sigmodontinae.

The South American vesper mouse, *Calomys*, occurs in a variety of habitats including montane grasslands, brushy areas, and forest fringes in Argentina, Bolivia, Brazil, Paraguay, Peru, Uruguay and Venezuela [1]. Nine species are currently recognized for the genus although a generic revision is pending [2].

In the last fifteen years, increasing knowledge of cytogenetics of the genus led to an improved recognition of the number of species and their relationships. An early revision of the genus reduced the number of species to only four forms, namely *C. lepidus*, *C. sorellus*, *C. laucha* and *C. callosus* [3]. More recently, cytogenetic studies showed that most of the specimens classified as *C. callosus* or *C. laucha* belonged in distinct, well-differentiated species, and the original classification proposed by Thomas [4] was mostly revalidated [5–7].

A pathway of chromosomal evolution was proposed by Vitullo et al. [7] in which the different species of *Calomys* may be progressively derived from a $2n = 70$ all-telocentric ancestral karyotype through a basic series of 17 centric fusions. At the top of this fusion pathway, the most transformed, all-biarmed karyotype is displayed by *C. lepidus* from Peru, which shows a $2n = 36$ chromosomal complement [8].

We describe here a new karyotype of specimens identified as *C. lepidus*, caught in the Puna region, northwestern Argentina, and we analyse it in the light of a previous chromosomal phylogeny.

Materials and methods

Chromosomal analysis was performed in eight vesper mouse specimens (four males and four females) trapped at Laguna de Pozuelos, province of Jujuy, northwestern Argentina, at 3600 m above sea level. Animals were identified as *Calomys lepidus* by their external morphological characteristics. Voucher specimens were preserved in the collection of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina.

To improve the mitotic index, animals were injected twice, 24 h apart, with a yeast suspension [9] and chromosomes were obtained from bone marrow. They were injected intraperitoneally with colchicine (1 μg/g body weight) and killed 1.5 to 2.0 h later by cervical dislocation. Hypotonic treatment of bone marrow suspensions was performed in 0.075 M KCl for 25 min at 37 °C. Cells were then fixed in freshly prepared cold 3:1 methanol:acetic acid. Chromosomal spreads were air-dried and Giemsa stained or processed for G- and C-banding [10, 11]. The classification of chromosomes followed the nomenclature of Levan et al. [12]. Fundamental numbers are referred to as NF_a, autosomal arms only.

Sperm samples were prepared as previously described [13] and stained with a modified Giemsa solution [14]. Sperm cells were examined under brightfield microscopy and measured with an ocular micrometer at a magnification of 400×.

* Corresponding author. Present address: Cleveland Clinic Research Institute, Department of Cancer Biology NN10, 9500 Euclid Avenue, Cleveland (Ohio 44195, USA), Fax +1 216 445 6269 or +1 216 795 1190, e-mail: vitulla@cesmtp.ccf.org.

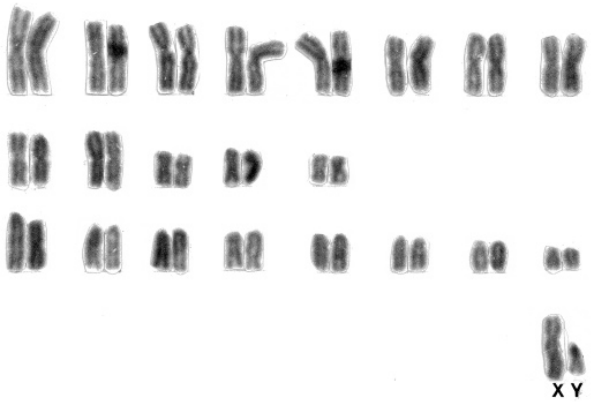

Figure 1. Giemsa stained karyotype of *Calomys lepidus* (2n = 44, $NF_a = 68$) from the Puna region, NW Argentina.

Results

The eight specimens collected in the Puna region showed all the external characteristics of *C. lepidus* as originally described by Thomas [15]. They were compared by Dr. O. Pearson with specimens of *C. lepidus* from Peru and Bolivia preserved in the Museum of Vertebrate Zoology, University of California, Berkeley, USA. This comparison showed that skins and skulls of specimens from Argentina were identical to Peruvian and Bolivian individuals (O. P. Pearson, personal communication). Animals trapped in NW Argentina were therefore undoubtedly *C. lepidus*.

All animals showed a similar 2n = 44 ($NF_a = 68$) karyotype (fig. 1). This karyotype was clearly asymmetric with autosomes arranged into two groups, metacentric-submetacentric (pairs 1–13) and telocentric (pairs 14–21) chromosomes. Sex chromosomes were XX/XY with a medium-sized submetacentric X, and a small subtelocentric Y chromosome as identified by G- and C-banding (figs. 2 and 3).

Constitutive heterochromatin was localized on the pericentromeric region of all pairs of homologues (fig. 3). While telocentric autosomes showed conspicuous C bands, most of the metacentric-submetacentric chromosomes displayed scarce constitutive heterochromatin.

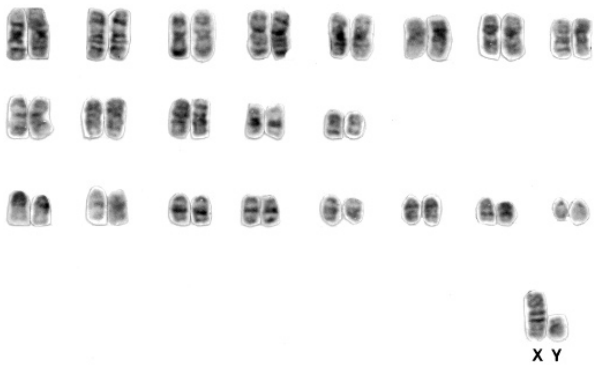

Figure 2. G-banded karyotype of *C. lepidus* (2n = 44, $NF_a = 68$).

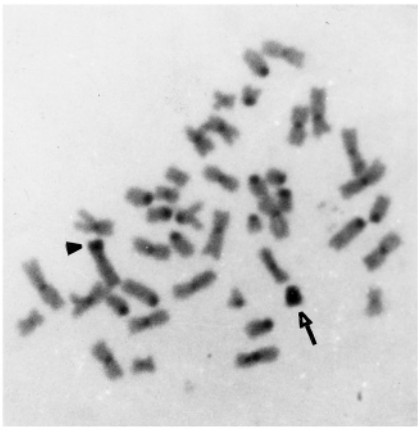

Figure 3. Representative C-banded metaphase of *C. lepidus*. A terminal band on the X chromosome (arrowhead) and the fully C-positive Y chromosome (open arrow) are indicated.

The X chromosome showed a distinctive terminal C band which extended over the distal half of the short arm, and the Y chromosome was fully C positive (fig. 3).

Spermatozoa from mature males showed hooked heads with a roughly polygonal nucleus; the head base was flat and the tail inserted eccentrically and ipsilateral to the hook (fig. 4a). Sperm cells showed an average total length of 77.62 ± 1.85 μm (n = 60) with a main longitudinal head axis of 7.01 ± 0.68 μm (n = 60) and a 70.63 ± 1.56 μm (n = 60) flagellum.

Discussion

The 2n = 44, $NF_a = 68$ karyotype found in specimens of *C. lepidus* from northwestern Argentina has so far not been described for any other species of the genus. An all-biarmed 2n = 36 karyotype was previously reported for *C. lepidus* from two different localities in Peru [8]. Although G-banded karyotypes from Peruvian specimens are not available for an arm-to-arm comparison with our specimens, both karyotypes have the same number of autosomal arms ($NF_a = 68$) and four centric fusions/fissions may account for the difference in diploid number.

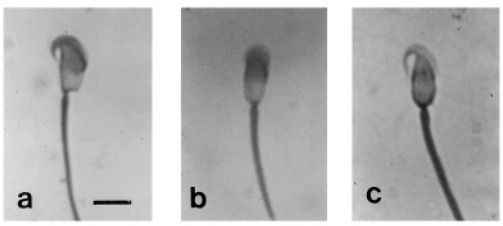

Figure 4. Sperm morphological patterns in *Calomys* species: (*a*) sperm type found in *C. lepidus*; the same pattern has been described for *C. musculinus* [20] and *C. hummelincki* [17], (*b*) hookless sperm type found in *C. laucha* [20], and (*c*) sperm type displayed by *C. callidus* [20] and *C. venustus*. Bar = 5 μm.

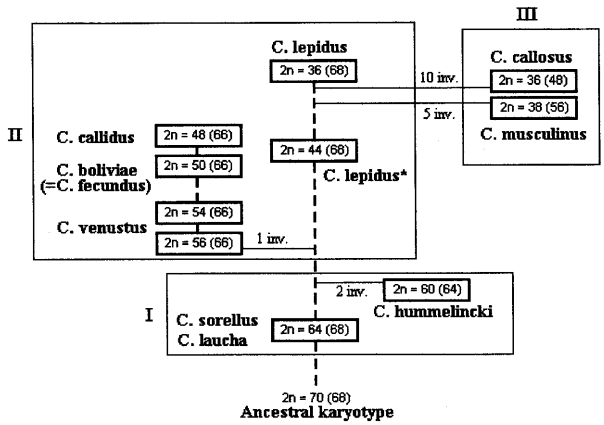

Figure 5. Chromosome evolution in the genus *Calomys*. Each vertical dash indicates a Robertsonian fusion (thus, 17 fusions between the hypothetical ancestral karyotype and Peruvian *C. lepidus*). Horizontal lines show superimposed pericentric inversions. Squares enclose the species belonging in chromosomal groups I, II and III (see text). Numbers in parenthesis are NF_a. The asterisk on *C. lepidus* indicates the karyotype described in this study.

A karyotype of 68 telocentric autosomes ($2n = 70$, $NF_a = 68$) was interpreted by Pearson and Patton [8] as the ancestral condition for all the phyllotini rodents. Departing from this all-telocentric ancestral karyotype, we have shown [7] that *Calomys* species may have developed in progressive steps through a basic series of 17 cumulative centric fusions, involving a minimum of other chromosomal changes (fig. 5). The karyotype of Punian *C. lepidus* fitted well within the main path of chromosome transformation (fig. 5), departing from the ancestral condition by the accumulation of 13 centric fusions.

In this chromosomal phylogeny, *Calomys* species have been classed into three groups according to their karyological characteristics [7]. Group I includes karyotypes with the highest chromosome numbers, mostly telocentric and closely related to the ancestral condition. Group II species show asymmetric karyotypes with intermediate diploid numbers, and group III involves highly transformed karyotypes quite distant from the ancestral condition (fig. 5). We formerly included Peruvian *C. lepidus* in group III since it showed the lowest diploid number for the genus, placed at the opposite extreme from the ancestral condition (fig. 5). In the light of the results reported here, it seems more reasonable to include *C. lepidus*, both Peruvian and Argentinian forms, in group II (fig. 5) since its karyotype shows more affinity to *C. venustus–C. boliviae–C. callidus* group than to group III species. First, the Punian *C. lepidus* karyotype is clearly asymmetric. Pearson and Patton [8] in their chromosomal analysis of the genus suggested that the *C. lepidus* karyotype was more closely related to *C. boliviae* (=*C. fecundus*) than to either *C. musculinus* or *C. callosus*. In addition, C bands in Punian *C. lepidus* appear to be of a similar type to those found in group II. Group I species *C. laucha* and *C. hummelincki* have scarce pericentromeric heterochromatin in all autosomes [16, 17]. In group II *C. callidus* [6] and *C. venustus* (Vitullo, unpublished results) have conspicuous C bands in telocentric chromosomes and very scarce or absent C bands in biarmed autosomes. This is essentially the pattern we found for Punian *C. lepidus* (see fig. 3). Conversely, group III *C. musculinus* display important blocks of pericentromeric heterochromatin which extend to proximal segments of chromosome arms [18].

Sperm morphology in *Calomys* correlates well with chromosomal evolution depicted in figure 5 [19]. Ancestral, hookless spermatozoa are found in *C. laucha* (fig. 4b), a species closely related to the ancestral karyotype. Modified sperm types appear soon in chromosomal evolution, as seen in *C. hummelincki* (fig. 4a), and are maintained in further chromosomally transformed species as *C. musculinus* (figs. 4a and 5). The sperm type found in Punian *C. lepidus* is essentially the same as that found in *C. hummelincki* and *C. musculinus*. Although the *C. lepidus* spermatozoon is different from that shown by other group II species (i.e. *C. callidus* and *C. venustus* (fig. 4c)), it is worth noting that this pattern is shared by species belonging in the three chromosomal groups.

Finally, it must be considered that Peruvian and Punian *C. lepidus* karyotypes may represent chromosomal races of the same species or fully separate species. Nevertheless, we consider it more appropriate to retain the name *C. lepidus* for Punian specimens until further studies on reproductive isolation between these populations can be performed.

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