Chromosome divergence of *Octodon lunatus* and *Abrocoma bennetti* and the origins of Octodontoidea (Rodentia: Historcognathi)

**Divergencia cromosómica de Octodon lunatus y Abrocoma bennetti y los orígenes de los Octodontoidea (Rodentia: Historcognathi)**

**ABSTRACT**

Octodontoidea have 2n from 10 to 102, and NF from 16 to 202, the largest ranges known for a mammal family group. Although 4 out of the 7 genera have very similar karyotypes to the one found in *Octodon degus* 2n=58, NF=116, other two genera are extremely divergent ones. We describe and compare here the undescribed chromosome data from seven specimens of *Octodon lunatus* 2n=78, NF=128, and of thirteen specimens of *Abrocoma bennetti* 2n=64, NF=114, from the related monogenic *Abrocomidae*. Karyotype and chromosome analysis based on shape, size, and G-, C-, and AgAs bands detected 20 and 7 telocentric pairs respectively. Most of these characters were previously unknown in non-*Ctenomys* octodontoids. Some large metacentric chromosomes differed among species, and the differences in G bands were more abundant than what would be expected from their 2n and NF. C bands were very heterogenous within karyotypes. The general cytogenetics features of *Abrocoma* were nearer to those of 4 octodontid genera than to those of *Chinchilla*, and consistent with the classical position of *Abrocomidae* within Octodontoidea. Given that *Abrocoma* is a predominantly northern genus, as it is *Octodon lunatus* among chilean Octodontidae, the northern origin of the whole group is suggested.

**Keywords:** evolution, karyo-idiogram, karyograph, Chinchilloidea

**INTRODUCTION**

One of the major events in the evolution of South American mammals was the radiation of Southern Andean Octodontoidea, the second most diverse clade among the twelve endemic families of New World histricognath rodents (Patterson & Pascual 1972). Although the diversification of such monophyletic group (Nedhal et al 1994) seems to be linked to the rise of the Andes, details are largely unknown, since most of the species from its morphologically well distinct families have been poorly studied by molecular or other modern methods.

At a first glance, we might expect that such large divergences might be associated with large chromosomal divergences.
Nevertheless, as four divergent living genera of the most diverse Octodontidae were known to have species with very similar karyotypes to that of *Octodon degus* 2n=58 and NF=116, it was suggested “that diversification of the main adaptively different lineages of octodontines took place without major chromosome re-patterning” (Reig 1989).

With the recent descriptions of the highest chromosome and arm numbers for a mammal karyotype, 2n=102, NF=202 in the octodontid *Tympanoctomys barrerae* (Contreras et al. 1990), of the C-banding karyotypes in some Octodontoids (Gallardo, 1992), and of the *Ctenomys steinbachi* karyotype of 2n=10, NF=16 (Anderson et al. 1987), the situation have been reversed. The superfamily now exhibits the largest range of 2n and NF values known for a mammalian family group.

We report and analyze here the chromosomes of two rare species from Central Chile, *Octodon lunatus* and *Abrocoma bennetti*. The latter belongs to Abrocomidae, traditionally considered a closely related family to Octodontidae and Ctenomyidae. All are usually included within the superfamily Octodontoidea (Patterson & Pascual 1972). Nevertheless, it has been recently suggested that Abrocomidae might belong to the more recent superfamily Chinchilloidea (Glanz & Anderson 1990).

The chromosome analyses of both species, further cytogenetic data from the related octodontids *Octodon degus*, *Spalacopus cyanus* and *Tympanoctomys barrerae*, and morphological and biogeographic information, will demonstrate that the karyotypes of *Octodon lunatus* and *Abrocoma bennetti* represent intermediate conditions between two divergent extremes: *Tympanoctomys* and the rest of Octodontoidea (Contreras et al. 1990). This have been called a bidirectional trend in karyotype evolution (Gallardo 1992). It will be also shown that those data are consistent with the traditional position of Abrocomidae within Octodontoidea, suggesting a northern origin and a southern diversification of Octodontidae along the Andes.

**MATERIALS AND METHODS**

*Specimens.* All the studied animals were collected in the field. Skulls and skins were prepared as voucher specimens and are deposited in the collection of the Laboratorio de Citogenética, Facultad de Medicina, Universidad de Chile (LCM) and of the Museo Nacional de Historia Natural.

Taxa, original localities, altitude above sea level (in meters), and number of examined specimens with LCM numbers (in parenthesis) are as follows. **ABROCOMIDAE, Abrocoma bennetti:** 2 km SE Las Tacas, IV Region, 50 m (1: 287); 3 km NE Aucó, IV Region, ca. 1050 m (2: 443, 444); Las Breas, IV Region, ca 2000 m (2: 368, 442); La Dehesa, E Santiago, RM, ca. 850 m (8: 001, 005, 007, 303, 418, 421, 422, 423). **OCTODONTIDAE, Octodon degus:** Los Molles, IV Region, 50 m (2: 184, 1619); La Dehesa, RM, ca. 850 m (12: 267, 268, 310-316, 320-323). *O. lunatus:* 5 km NE Aucó, IV Región (2: 1032, 1286); 2 km NE Peñuelas, V Región (5: 1617-1619, 1676, 1677). *Spalacopus cyanus:* 10 km W Catapilco, V Región (6: 269, 270, 272-275); Farellones, RM, ca. 2800 m (5: 336, 630, 767, 837, 838); Lagunillas, RM, ca. 2600 m (1: 340). *Tympanoctomys barrerae:* Salinas 40 km N of Desaguadero, Mendoza, Argentina, ca 500 m (1: 1076).

*Chromosome analysis.* Chromosomes were obtained from bone marrow cells using the conventional in vivo colchicine, hypotonic method, preceded by yeast injection to improve the mitotic index (Lee & Elder 1980). Some metaphase cells were stained with 2% Giemsa, or treated with C (Crossen 1972, Sumner 1972) and G banding techniques (Chiarelli, 1972). The nucleolar organizing regions were detected by silver staining procedures (Quack & Noel 1977).

Giemsa stained chromosomes were first measured on photographic enlargements and their relative lengths calculated as percentages the female haploid set (FHS; see Reig & Kiblisky 1969, Massarini et al. 1991). They were classified as large, medium or small sized, when their relative lengths were > 9%, 9 - 5.5% or < 5.5% of
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This standard procedure assumes a constancy in the DNA amount per cell. When substantial interspecific variations in C bands were detected later, we also compared absolute chromosome lengths and displayed them within a karyidiogram, a bivariate plot allowing detailed chromosome comparisons (Spotorno et al. 1985).

Some G-banded karyotypes were compared from selected metaphases of male and female specimens from each taxa. Chromosome pairs were classified according to their G band pattern similarities as totally corresponding (homologous), partially corresponding (homeologous) or unique among the different taxa (Walker et al. 1992).

RESULTS

The karyotype of Octodon lunatus was strikingly asymmetric in the size and shape of its elements (Fig. 1). Among its 78 chromosomes, 20 pairs were small and telocentric in shape, with no visible short arms, and the other 20 were small subtelocentric, submetacentric or metacentric ones. Since the largest chromosome (5.1% of the female haploid set) was a single submetacentric element in males and were 2 in females, they probably are the X; the presumed Y was a very small one (1.44% of the FHS). The submetacentric pair 22 (3.6% of the FHS) exhibited a distinct interstitial secondary constriction at its long arm (Fig. 1).

The 64 small chromosomes of Abrocoma bennetti also exhibited a certain asymmetry in shape. Only 7 pairs were totally telocentric ones, the remaining 25 pairs having all other possible shapes (Fig. 1). The largest chromosome (5.9% of the FHS) was also the presumptive X, and the probable Y had a 2.6% FHS, with a metacentric shape. The single secondary constriction was consistently observed in the autosome pair 12 (3.5% FHS). These features are basically similar to those described by Gallardo 1992, with some differences in the size of the second telocentric (number 26 in his Fig. 3).

There were many similarities, as well as striking differences, between these karyotypes and those described for Octodon degus, Spalacopus cyanus (Fernández-Donoso 1968, Reig et al. 1972) and Tympanoctomys barrerae already reported (Contreras et al. 1990). Such similarities in size and shape are shown in the comparative karyidiogram of Fig. 2.

On the other hand, the 7 and 20 wholly telocentric chromosomes here found in Octodon lunatus and Abrocoma bennetti respectively, were completely absent as such in the karyotypes of all the other species. Moreover, we expected that many metacentric and submetacentric chromosomes would overlapped in the karyidiogram, since they were abundant in karyotypes having very similar 2n and NF. Nevertheless, most chromosomes were distributed all over this area of the karyidiogram (Fig. 2). For example, chromosome 1 from O. degus was much larger than the largest autosome of O. lunatus (number 21 in Fig. 2), and both were very different than the nearest in A. bennetti (number 8).

G bands allowed reasonable identifications of some chromosomes and chromosome arms between karyotypes (Fig. 3). Clear examples are the almost complete similarities in kind and sequence of G-bands shown by: a) the X chromosomes of the four species (being Spalacopus the most divergent); b) the chromosomes bearing the secondary constriction in all the four karyotypes (chromosome numbers as follows: lunatus 23, bennetti 12, degus 4 and cyanus 5); and c) A. bennetti 1 and O. lunatus 3 and also with the long arm of O. degus 3 (Figs. 3 and 5). In concordance with the divergent morphologies of the abundant metacentric chromosomes, we were not able to establish reasonable correspondences among most of these elements when compared in detail.

C-bands were relatively constant among different cells and individuals within a species. Since nominal subspecies have been described for Spalacopus cyanus from the mountains and the coast of Chile, we examined individuals from two such different populations. No differences in C-
Fig. 1. Chromosomes of *Octodon lunatus* male, specimen LCM 1032 (upper); and *Abrocoma bennetti* female, LCM 287 (lower).

Cromosomas de *Octodon lunatus* macho, especímen LCM 1032 (arriba); y *Abrocoma bennetti* hembra, LCM 287 (abajo).
bands (Fig. 4b) nor in G-bands (not illustrated) were detected among them.

C bands also revealed an heterogenous distribution of constitutive heterochromatin within some karyotypes. Small but clear C-bands were present at the centromeric region of many chromosomes from Octodon degus and Spalacopus cyanus (Fig. 4), but they were absent in ten chromosomal pairs of the former and six ones of the latter. Most of the latter were subtelocentric ones, and their centromeric region exhibited stained G-bands (Fig. 3). Conversely, most metacentric chromosomes with centromeric C bands showed light G-bands in the centromeric regions.

The C-bands of Octodon lunatus and Abrocoma bennetti were also pericentromeric and of small sizes (male 1032 and female 287, respectively, not illustrated here). The exception was a single chromosome in Octodon lunatus, probably the Y, with a large pericentromeric C-band in the proximal region of the long arm.

Some homeologies between species chromosomes were evident through G- and C-band comparisons. For instance, the short arm of autosome 1 of Spalacopus cyanus corresponded with the long arm of the autosome 1 of Octodon degus (Fig. 5). This band sequence was also identical to those shown by the telocentric autosome 1 of Octodon lunatus (Fig. 4). We were not able to identify this chromosomal portion in the Abrocoma genome.

A large and unique C band was evident in the subcentromeric region of Spalacopus 1 long arm (Fig. 4b; see also Gallardo 1992). The staining of this band was lighter than that of the small centromeric one, suggesting differences in condensation or base composition. This feature was not evident in the previous description mentioned, a difference probably derived from the different technique used. This particular portion of heterochromatin exhibited distinct G banding (Fig. 4b and 5).

AgAs-stained bands were consistently present in a single chromosome pair having similar size and shape among all karyotypes (Fig. 2 and 6). They are at the same place where secondary constrictions appeared under other staining procedures. This AgAs-positive band was usually larger in the homologue with a greater length, probably representing a functional condition.

DISCUSSION

After the cytogenetic description of almost all species from all genera of Octodontoida s.s., a general picture on the chromosome evolution of the three most diverse families seems to emerge. This can be seen graphically by means of two synthetic diagrams: the taxic curve (Fig. 7) and the karyograph (Fig. 8).

The taxic curve shows the diversity in number of species of the different clades of Octodontoida s.s.; the diploid numbers have been added in this particular case. An asymmetrical hollow curve distribution is observed, which is one of the most remarkable characteristic on the distribution of intrataxonomical diversity of many living organisms (see full discussion in Reig 1989). Although a very similar curve was obtained by such author for Octodontidae genera (including Ctenomys), his interpretation of chromosomal variation in this group was limited to four non-Ctenomys species karyotypes by that time. Now we have information for eleven species included in such a graph.

After his discussion of the 2n=58 karyotype as the probable primitive one, and as an exception to his main thesis, Reig (1989) concluded: "These data also suggest that diversification of the main adaptively different lineages of octodontines took place without major chromosomal repatterning, and show that chromosomal invariance in them is related to absence or poverty in species differentiation" (p. 264). Further species additions, and particularly the large increase in diploid number ranges and variations described for Octodon (present report) and Aconaemys species (Gallardo & Reise 1992), as well as the gross G-band divergences among 2n=58 karyotypes here detected, strongly suggest that much more genome diversity is included among the Octodontoida lineages. Our data thus reinforces the main thesis.
of Reig (1989) that morphological and ecological diversification took place in association with major chromosomal changes.

The karyograph of Fig. 8 displays most of the known diploid and fundamental numbers of Octodontoidea species. Most exhibit predominantly metacentric karyotypes (towards the upper diagonal in Fig. 7). The telocentric chromosomes of *Octodon lunatus* and *Abrocoma bennetti* represent a striking departure of such gene...

**Fig. 2.** Comparative karyo-idiogram of chromosome lengths from *Spalacopus cyanus* (some chromosomes), *Octodon degus*, *O. lunatus* and *Abrocoma bennetti*. Chromosome nomenclature according to Levan et al. 1964; i= centromeric index. Some chromosomes of interest are marked with numbers, sex chromosomes with X or Y, and NOR bearing chromosomes with arrow-heads.

Cario-idiograma comparativo de las longitudes cromosómicas de *Spalacopus cyanus* (algunos cromosomas), *Octodon degus*, *O. lunatus* y *Abrocoma bennetti*. La nomenclatura cromosómica sigue a Levan et al. 1974; i= índice centromérico. Algunos cromosomas de interés están marcados con números, cromosomas sexuales con X o Y, y cromosomas portadores de NOR con puntas de flechas.

Telocentric chromosomes like those present in *A. bennetti* and *O. lunatus* karyotypes, probably represent primitive conditions within Octodontoidea. First, they are actually present in Abrocomidae, a family usually considered as the most related outgroup of present Octodontoidea and Ctenomyidae (Patterson & Pascual 1972). Second, they are also present in all the three families, since telocentric chromosomes have been also described in a few species of the Ctenomyidae phyletic line, for instance *Ctenomys torquatus* (Reig & Kiblisky 1969) and *C. sociabilis* (Gallardo 1991). Third, although metacentric chromosomes are the most frequent condition within octodontids (Contreras et al. 1990), a closer analysis shows that some exhibit divergent morphologies (Figs. 2 and 5), demonstrating they are not homologous elements, i.e. they were not inherited as such from a recent common ancestor. In short, metacentric chromosomes seem to have evolved independently in two or perhaps the three phyletic lines, probably through parallel centric fusions of the presumptive ancestral telocentric elements. This point of view has been also suggested for ctenomyids (OrteLLs 1990), octodontids (Contreras et al. 1994), and for two genera of muroid rodents living in the same regions, *Eligmodontia* and *Auliscomys* (Spotorno et al. 1994).

Therefore, it appears that chromosome rearrangements have been much more frequent and complex than what was initially suggested by the apparent similarities in the 2n and NF exhibited by the three families. This agrees with paleontological data that indicate a relatively long time of divergence for the group (Patterson & Pascual 1972).

The Octodontoidea genomes have been accumulating different amounts and kinds of heterochromatin, here detected through C bands, in many phyletic lines. The small centromeric C-bands observed in the primitive *Abrocoma* as well as in *Ctenomys sociabilis* (Gallardo 1991 and 1992) contrast with the well marked and large ones detected in most of the chromosomal...
Fig. 3. G-banded chromosomes of: a. *O. lunatus* male, LCM 1032; b. *Abrocoma bennetti* female, LCM 287; c. *Octodon degus* male, LCM 253; and d. *Spalacopus cyanus* male, LCM 340. Chromosomes numbers are from the original karyotype descriptions.

Fig. 4. C banded metaphases of: a. *Octodon degus*, male, LCM 267; b. *Spalacopus cyanus*, two males; in every pair, left chromosome is from LCM 751, and right one, from LCM 340.

short arms of *Tympanoctomys barrerae* (Contreras et al. 1990), and also in the pericentromeric region of some chromosomes of many species, such as *Octodon degus*, *Spalacopus cyanus* (Fig. 4 and Gallardo 1991), *Ctenomys opimus*, *Octodontomys gliroides* (Gallardo 1991) and other *Ctenomys* species (Massarini et al. 1991). Sometimes, such heterochromatin involved many whole short arms, as in four species of *Ctenomys* (Massarini et al. 1991).

It is most probable that the main mechanism of such heterochromatin increases was the amplification of the same short sequences of repetitive DNA, given that a DNA probe from a species of *Ctenomys* hybridized with differing amounts of the DNA extracted from other *Ctenomys* and *Octodontomys* species (Rossi et al. 1990). In situ hybridization of the same DNA probe with different chromosomes of other Octodontoida would be a definitive proof of this hypothesis.

In any case, amplifications of DNA sequences to give whole heterochromatic arms seem to have occurred independently in *Tympanoctomys barrerae* and in a few *Ctenomys* genomes, thus increasing the total number of chromosome arms in these octodontid and ctenomyid phyletic lines. Since these new arms would increase the total number of chromosome arms, this would explain some of the extreme high NF values, as shown in Fig. 7.

Although many chromosomes seem to have been affected in such a process of heterochromatin amplification, a few regions remained unaffected in different genomes. The lack of heterochromatin actually shown by only some submetacentric chromosomes here observed in *Octodon*, *Spalacopus* (Fig. 4) and in a few *Ctenomys* species (Gallardo 1992; Massarini et al. 1991), in contrast with the usual amplification to all the chromosomes of a karyotype (an example in Walker et al. 1979), suggests an incomplete process (for instance, Walker et al. 1991), or more probably, the existence of some constraints in the diffusion or fixation of heterochromatin accumulation. Interchromosomal associations, like those described for *O. degus* and *C. opimus* (Fernández-Donoso & Berríos 1993), might be one of the mechanisms favouring differential diffusion or isolation of heterochromatic portions within particular genomes.

The cytogenetic features of *Abrocoma* are of some interest since the phylogenetic position of the monogeneric Abrocomidae have been recently changed from the superfamily Octodontoida to the Chinchilloidea (Glanz & Anderson 1990). Although a detailed chromosome comparison among all these taxa should wait the description of chinchillids G-bands, the general cytogenetic features of *Abrocoma bennetti* are more near to those of 4 Octodontidae genera than to those of *Chinchilla* (Fig. 7). This argues in favour of the classical position of Abrocomidae within Octodontoida.

A few hints about the probable northern origin of the main phyletic lines of Octodontoida arise from these data. First, *Abrocomidae* is a rather northern family, having three of the four living species
Fig. 6. AgAs NOR metaphases from: a. *Octodon lunatus* male, LCM 1286; b. *Tympanoctomys barrerae* male, LCM 1076; c. *O. degus* male, LCM 429; d. *Spalacopus cyanus* male, LCM 340. NOR bearing chromosomes marked with arrow-heads.

distributed in the Altiplano subregion (Glanz & Anderson 1990). Second, the whole group of *Ctenomys* species with the primitive condition of symmetric sperm heads, which is also shared by the rest of Octodontoidea, have also northern distributions; the remaining *Ctenomys* with the derived condition of asymmetric sperm heads, have southern distributions (Gallardo 1991, Roldan et al. 1992). Third, among the Octodontidae s.s., *O. lunatus*, having a primitive karyotype, lives in central Chile, occupying a more northern territory than those of *O. bridgesi* and *O. pacificus* (Hutterer 1994). Fourth, among all Octodontoidea, only the southern Octodontidae s.s. species share the derived condition of 2-2 penial spikes (Contreras et al. 1994), with the relatively northern *Octomys* and *Tympanoctomys* retaining the primitive 1-1 condition. Therefore, the geographic distribution of many independent characters suggest a northern origin for an initial radiation in the central Andes.

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**Fig. 7.** Taxic curve having an asymmetric distribution of living species diversity in extant genera of Octodontoidea (data from Reig 1989, Ortells et al. 1990, Contreras et al. 1990 and further additions here reported).

Curva tóxica con una distribución asimétrica de la diversidad de las especies vivas en los actuales géneros de Octodontoidea (datos de Reig 1989, Ortells et al. 1990, Contreras et al. 1990, y adiciones aquí reportadas).
through which the three main Octodontoidea phyletic lines arose, and a later expansion to the xeric environments of the southern Andes (Spotorno 1979, Contreras et al. 1987). The recent description of the earliest South American hystricomorph rodent in a Tinguiririca fauna from Central Chile (Wyss et al. 1993) suggests that such drier habitats are much older than what was previously thought.

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Fig. 8. Karyograph of known karyotypes of Octodontoidea. Limits for possible values indicated by the two diagonals; karyotypes with only metacentric chromosomes fall on the upper diagonal. Arrows show possible directions of chromosome change. Black Ctenomys signs mark species with asymmetrical sperms (from Roldan et al. 1992). The value for Chinchilla lanigera included for comparison. (modified from Contreras et al. 1990, with further data from Anderson et al. 1987, and Gallardo & Reise 1992).


