The largest known chromosome number for a mammal, in a South American desert rodent

L. C. Contreras[a], J. C. Torres-Mura[b] and A. E. Spotorno[c]

[a]Departamento de Biología, Universidad de La Serena, Casilla 599, La Serena (Chile), [b]Sección Zoología, Museo Nacional de Historia Natural, Casilla 787, Santiago (Chile), and [c]Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago 7 (Chile)
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Summary. Tympanoctomys barrerae, a desert specialist member of the family Octodontidae, until now thought to be conservative, and ancestral to South American hystricognath rodents, presents the highest diploid chromosome number (2n = 102) known in a mammal. Unexpectedly, its karyotype was found to be composed mainly of metacentric to sub-metacentric chromosomes. Mechanisms by which such a karyotype may have been derived are discussed.
Key words. Rodent; hystricognath; octodontid; chromosome; karyology; heterochromatin.

Among the 14 families of South American hystricognath rodents, the family Octodontidae, with nine living species, is one of the five most diverse[1]. Octodontids are particularly important for the understanding of the evolution of hystricognath rodents because the oldest and most primitive caviomorphs have been assigned to this family[2]. Unfortunately, it is one of the least studied families; the evolutionary relationships among species are uncertain, distributional ranges are poorly defined, systematic classification is dubious, and ecological studies are scarce[3,4]. *Tympanoctomys barrerae* is certainly the least known octodontid species. It was discovered in 1939 and although it has remarkable adaptive specializations, typically found in desert rodents from other continents, it has remained practically unconsidered by biologists. Only three papers report on its biology[5-7], and despite extensive and intensive trapping efforts in the Monte Desert, no additional specimens have been collected since its discovery. We recently captured some specimens 40 km north of Desaguadero, Mendoza Province, Argentina (33° 10′ S, 67° 15′ W, 500 m above the sea level). In this work we describe the analysis of the karyotype of this rare species as part of a project on the ecology and evolution of Octodontidae. Our work has resulted in the finding of the largest known diploid chromosome and arm number for mammals.

Methods

Five specimens were processed for chromosome analysis. Chromosome metaphases were obtained from bone-marrow using the standard colchicine-hypotonic pretreatment-airdrying technique[8]. Slides were Giemsa stained and C-bands were induced using the method of Summer[9]. From these plates we chose those with the best morphology and the fewest chromosomal overlaps. Because of the large number of chromosomes, such plates were rather rare. Each chromosome was cut out and the length of the straightest chromatid was measured with a dial caliper. Data from a male and a female were then plotted in a karyo-idiogram (fig. 2), which allows the separation of chromosomes by size and centromeric position[10].

Results

After examining 16 metaphase plates we concluded that the diploid number is 2n = 102 (fig. 1). The karyotype of *T. barrerae* (figs 1 and 2) is composed of 36 metacentric chromosome pairs, 11 submetacentrics, two subtelocentrics, and a telocentric one. Such predominance of metacentric and submetacentric chromosomes is also exhibited by other octodontids[11-15]. In addition to the telocentric chromosome (number 50 in fig. 1) and a small submetacentric one only present in male cells, two marker chromosomes are clearly distinct in *T. barrerae*; the largest (4.52 μm) metacentric, which is most probably the X chromosome, and a medium-sized (3 μm) submetacentric element with a secondary constriction in the long arm (number 40 in figs 1 and 2). A similar marker chromosome, probably NOR-bearing, is also shared by other octodontoid families (Ctenomyidae, Echimyidae, and Capromyidae), and is different from the one shared by chinchillids, hydrochoerids, and cuniculids[16].

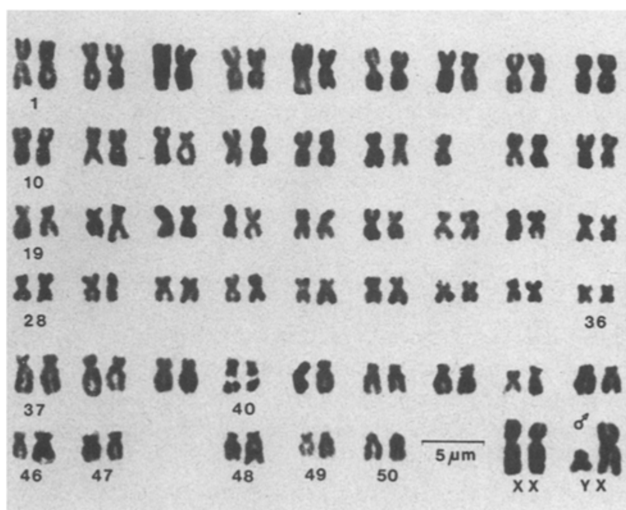

Figure 1. Karyotype of a female *Tympanoctomys barrerae* (558 LCC). One chromosome of pair 16 is missing. The sex chromosomes of a male (560 LCC) are shown at the lower right corner (YX).

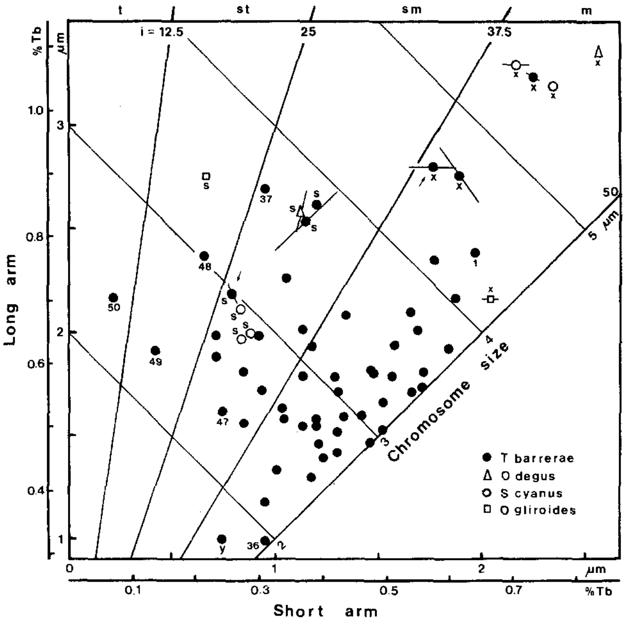

Figure 2. Haploid karyo-idiogram of female *Tympanoctomys barrerae* karyotype from figure 1. Each dot represents the average length of both arms of the straightest chromatid of each chromosome pair. The X and secondary constriction S-bearing chromosomes of that karyotype are indicated by arrows. The X and S-bearing chromosomes of two other plates of *T. barrerae* are also shown. The absolute sizes of the X and S-bearing chromosomes of other metaphasic plates of *T. barrerae* and of other octodontid species are also represented. The percentage scales (%Tb) correspond to the individual of figure 1. The lines through the X and S-bearing chromosomes represent the range of a pair of each metaphasic plate. The Y-chromosomes of a male (560 LCC) *T. barrerae* is also represented. (i = centromeric index; m, metacentric; sm, submetacentric; st, subtelocentric; and t, telocentric chromosomes according to Levan et al.[23].

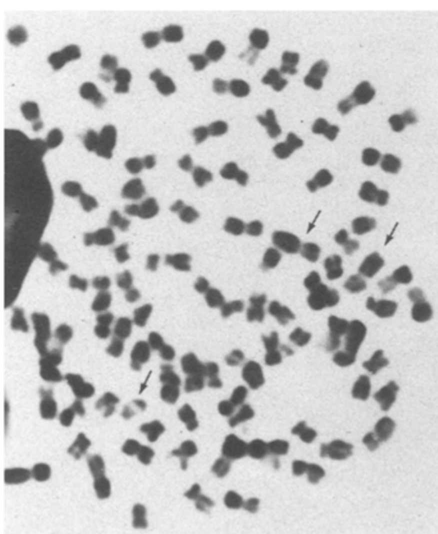

Figure 3. C-banded metaphase of *T. barrerae*. Note the presence of large segments with heterochromatin. Arrows indicate X and secondary constriction-bearing chromosomes.

Discussion

Most mammals have between 30 and 60 chromosomes (diploid); the highest value on record being 92 for the South American cricetine rodent *Anatomys leander*[17]. The 102 chromosomes of *T. barrerae* considerably exceed this value; the number is also a record for reptiles and birds[18]. The arm number is also exceptional. Most mammals present arm numbers (NF) between 55 and 100. The highest value so far recorded is that for the North American heteromyid rodent *Dipodomys ordii* with NF = 134[17]. Since *T. barrerae* has a single telocentric chromosome pair, its NF = 202, an absolute record by any standard, even considering that South American hystricognath families are characterized by high diploid and NF numbers[17].

The highly specialized biology of this species, with long hind legs, bipedal locomotion, large tympanic bullae, a large kidney with a high medullary index, a restricted chaenopod diet and a salty habitat[19], immediately sug-

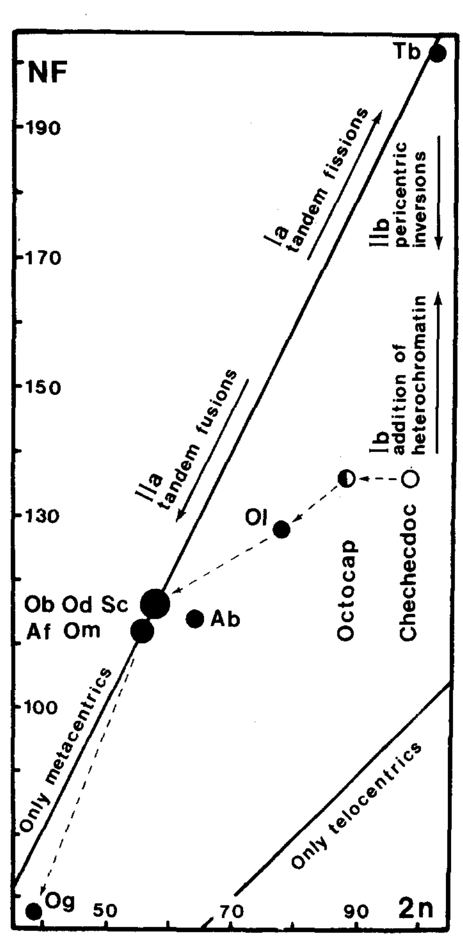

Figure 4. Karyograph of known octodontid and abrocomid species. Each dot represent a karyotype; the position depends on diploid numbers and arm number. Karyotypes lacking telocentric chromosomes fall along the left diagonal. Continuous arrows indicate processes of change. Dashed arrows represent karyotype derivations. Closed circles represent living species (Af = *Aconaemys fuscus*, 2n = 56, NF = 112; Ob = *Octodon bridgesi*, 2n = 58, NF = 116; Od = *O. degus*, 2n = 58, NF = 116; Og = *Octodontomys gliroides*, 2n = 38, NF = 68; Sc = *Spalacopus cyanus*, 2n = 58, NF = 116[11–15]; Ab = *Abrocoma bennetti*, 2n = 64, NF = 114; Ol = *O. lunatus*, 2n = 78, NF = 128[24]; Om = *Octomys mimax*, 2n = 56, NF = 112[25]; Tb = *Tympanoctomys barrerae*, 2n = 120, NF = 202. Open circles correspond to hypothetical ancestors (Chechecdoc and Octacap) proposed by George and Weir[16]. The symbol for Octacap is half shaded because there is one living hystricognath with that karyotype, *Geocapromys brownii*.

gests that its karyotype also may be highly derived. In such a case, three main cytogenetic mechanisms could be envisaged: tetraploidy, tandem fissions (processes Ia in fig. 4), and the addition of heterochromatic arms (Ib, fig. 4). The results of our chromosome analysis argue against tetraploidy; if this were the mechanism, the chromosomes in figure 2 would appear in clustered pairs, which is not the case. On the other hand, tandem fissions would require at least 22 new centromeres, which would be unlikely.

The third mechanism is much more plausible; it implies an increase in the amount of total DNA [20]. An analysis of marker chromosomes within Octodontidae provides some support for this. Although the absolute sizes of the secondary constriction-bearing and the X chromosomes are roughly the same among four octodontid species (fig. 2), their relative sizes (expressed as percentage of the female haploid set, %Tb) are very much diminished in *T. barrerae*. For instance, the X chromosome appears with 1.46% (0.55% short arm + 0.91% long arm, fig. 2), a clear difference from the widespread constancy of 5% for mammalian X chromosomes [21]. Therefore, as their absolute sizes and shapes have remained similar (fig. 2), it is reasonable to conclude that *T. barrerae* must have increased its total genome size in relation to the other related species.

An ancestral hystricognath karyotype of $2n = 98$ has already been postulated [16] (fig. 4). This figure is strikingly near to the $2n = 102$ reported here, and if the postulate is correct, large amounts of heterochromatin should be expected in the *T. barrerae* genome. This is true in the few chromosome plates which we stained with the C-banding technique (fig. 3). Most of this heterochromatin involves whole arms, suggesting that the ancestral chromosomes were telocentric, a fact fully consistent with our recent description of other related karyotypes which have several telocentric pairs (see fig. 4).

The extreme alternative hypothesis assumes that such a high chromosome number is a characteristic of the ancestral state. In this case, direct derivation of the other octodontid karyotypes could be explained through numerous tandem fusions with heterochromatin losses (IIa in fig. 4), a mechanism already documented for the origin of the lowest known 2n in mammals (*Muntjacus muntjack* $2n = 6$ and 7, $NF = 12$) [22]. The lack of high 2n karyotypes among other mammalian species, as far as is known at present, makes this possibility appear less likely.

Our discovery means that the family Octodontidae is the mammalian family with the greatest chromosomal diversity, with respect to both diploid and arm numbers, and probably DNA content. This agrees with the known fossil record, suggesting that this family is a very old lineage that has experienced an extensive evolutionary radiation since the Oligocene.

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1 Honacki, J. H., Kinman, K. E., and Koeple, J. W., Mammal Species of the World. Allen Press Inc. and The Assoc. Syst. Coll., Lawrence, Kansas 1982.
2 Patterson, B., and Wood, A. E., Bull. Mus. comp. Zool. *120* (1982) 279.
3 Mares, M. A., and Ojeda, R. A., in: Mammalian Biology, in: South America, vol. 6, p. 393. Eds M. A. Mares and H. H. Genoways. Pymatuning Lab. Ecol., Linesville, Pennsylvania 1982.
4 Contreras, L. C., Torres-Mura, J. C., and Yañez, J. L., in: Studies in Neotropical Mammalogy: Essays in honor of Phillip Herskovitz, p. 401. Eds B. D. Patterson and R. M. Timm. Fieldiana, Zool. new ser. *39* (1987).
5 De La Barrera, J. M., Revta Inst. Bact. *9* (1940) 565.
6 Lawrence, B., Proc. New Engl. Zool. Club *18* (1941) 43.
7 Yepes, J., Revta Argent. Zoogeogr. *2* (1942) 69.
8 Hsu, T. C., and Patton, J. L., in: Comparative Mammalian Cytogenetics. Ed. K. Benirschke. Springer Verlag, New York 1969.
9 Summer, A. T., Exp. Cell Res. *75* (1972) 304.
10 Spotorno, A. E., in: El Nucleo los Cromosomas y la Evolución, p. 133. Ed. R. Fernandez-Donoso. Unesco, Santiago 1985.
11 Fernandez, R., Archos Biol. Med. exp. (Santiago) *5* (1968) 33.
12 George, W., and Weir, B., Chromosoma *37* (1972) 53.
13 Reig, O. A., Spotorno, A., and Fernandez, R., Biol. J. Linn. Soc. *4* (1972) 29.
14 Venegas, W., Boln Soc. Biol. Concepción *47* (1974) 207.
15 Venegas, W., Boln Soc. Biol. Concepción *49* (1975) 7.
16 George, W., and Weir, B. J., in: The Biology of Hystricomorph Rodents, p. 79. Eds I. W. Rowlands and B. J. Weir. Symp. Zool. Soc., London 1974.
17 Imai, H. T., and Crozier, R. H., Am. Nat. *116* (1980) 537.
18 Chiarelli, A. B., and Capanna, E., Cytotaxonomy and Vertebrate Evolution. Academic Press, London 1973.
19 Torres-Mura, J. C., Lemus, M. L., and Contreras, L. C., J. Mammal. *70* (1989) 646.
20 Mazrimas, J. A., and Hatch, F. T., Nature *240* (1972) 102.
21 Ohno, S., Becak, W., and Becak, M. L., Chromosoma *15* (1964) 14.
22 Shi, L. M., Ye, Y., and Duan, X., Cytogenet. Cell Genet. *26* (1980) 22.
23 Levan, A., Fredga, K., and Sandberg, A. A., Hereditas *52* (1964) 201.
24 Spotorno, A. E., Walker, L., Contreras, L. C., Pincheira, J., and Fernandez, D. R., Archos Biol. Med. exp. *21* (1988) R527.
25 Contreras, L. C., Silva, S., Torres-Mura, J. C., and Spotorno, A. E., Archos Biol. Med. exp. *21* (1988) R527.

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