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Chromosome polymorphism in *Ctenomys minutus* (Rodentia-Octodontidae)

Thales Renato O. de Freitas

ABSTRACT

A sample of 101 specimens of *Ctenomys minutus* was collected along its geographic range. Eight karyotypes (2n = 42, 45, 46a, 46b, 47, 48, 49 and 50) were found. The chromosome polymorphisms were due to Robertsonian rearrangements and tandem fusions. The distribution of polymorphisms indicated three population blocks: northern (2n = 49 and 50), central (2n = 46a, 47, and 48) and southern (2n = 42, 45, and 46b). These findings suggest that this species is undergoing a speciation process due to geographic isolation.

INTRODUCTION

The caviomorph fossorial rodents of the genus *Ctenomys* form a large group of 56 living species (Reig *et al.*, 1990). Chromosome number among species ranges from 2n = 10 to 70. Chromosome polymorphisms have been described for some species. Kiblisky *et al.* (1977) reported 2n = 56, 64 and 70 for *C. pearsoni* and 2n = 44 for *C. torquatus* in Uruguay, whereas Freitas and Lessa (1984) described 2n = 44 and 46 for *C. torquatus* in the South of Brazil. In Bolivia, *C. boliviensis* was described as having 2n = 36, 42, and 44 by Anderson *et al.* (1987). In northwestern Argentina, *C. perrensis* varies from 2n = 54 to 58 while *C. rionegrensis* in Uruguay shows 2n = 52 and 56 (Ortells *et al.*, 1990). In the southeastern portion of Argentina, three species from the Mendocinus group, *C. mendocinus*, *C. porteousi* and *C. chasiquensis*, have 2n = 47/48 (Massarini *et al.*, 1991).

Departamento de Genética, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brasil. E-mail: trof@if1.if.ufrgs.br.

MATERIAL AND METHODS

A sample of 101 specimens of *C. minutus* Nehring, 1887, 46 males and 55 females, was collected in the northeastern coastal plains of the States of Rio Grande do Sul and Santa Catarina, Brazil (Figure 1 and Table I). Their skulls and skins were deposited in the collection of the Departamento de Genética, Universidade Federal do Rio Grande do Sul.

The chromosome slides were obtained according to the technique of Lee and Elder (1980). G- and C-bands, and the characterization of the nucleolus organizer regions (NOR) were obtained following Seabright's (1971), Sumner's (1972), and Howell and Black's (1980) techniques, respectively.

RESULTS

Seven diploid numbers and eight karyotypes (Figure 2) were found (2n = 42, 45, 46a, 46b, 47, 48, 49 and 50). The sex pair was the same, with a submetacentric X and an acrocentric Y-chromosome, in all karyotypes. The form with 2n = 50 (Figure 2A) consisted of 14 biarmed and 10 acrocentric pairs. Specimens with 2n = 49 (Figure 2B) had a heteromorphic pair, 14

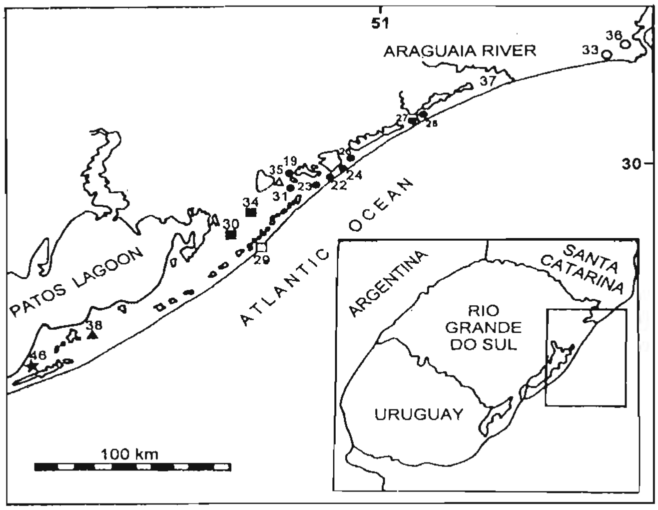

Figure 1 - Collecting localities in the coastal plain of Rio Grande do Sul and Santa Catarina States. (46) Tavares; (38) Mostardas; (30) Palmares do Sul; (34) Passinhos; (29) Cidreira Lake; (31) Osório; (35) Barros Lake; (19) Emboaba Lake; (23) Traíras Lake; (22) Caiera Lake; (24) Praia do Barco; (26) Capão Novo; (27) Torres; (28) Passo das Torres; (37) Morro dos Conventos; (33) and (36) Jaguaruna. Open circles indicate 2n = 49 and 50; closed circles, 2n = 46a; closed squares, 2n = 48; open triangle, 2n = 47; open square, 2n = 48 and 47; closed triangle, 2n = 42 and closed star, 2n = 46b and 45.

Table I - Number of specimens of *Ctenomys minutus* studied and collection localities.

Population block	Locality	Number of collection site	Animals karyotyped
North	Jaguaruna	36	4
		33	8
Central	Morro dos Conventos	37	6
	Passo das Torres	28	3
	Torres	27	2
	Capão Novo	26	3
	Praia do Barco	24	5
	Caiera Lake	22	2
	Traíras Lake	23	5
	Osório	31	12
	Emboaba Lake	19	5
	Barros Lake	35	8
	Fortaleza Lake	29	12
	Passinhos	34	4
	Palmares do Sul	30	8
South	Mostardas	38	8
	Tavares	46	6

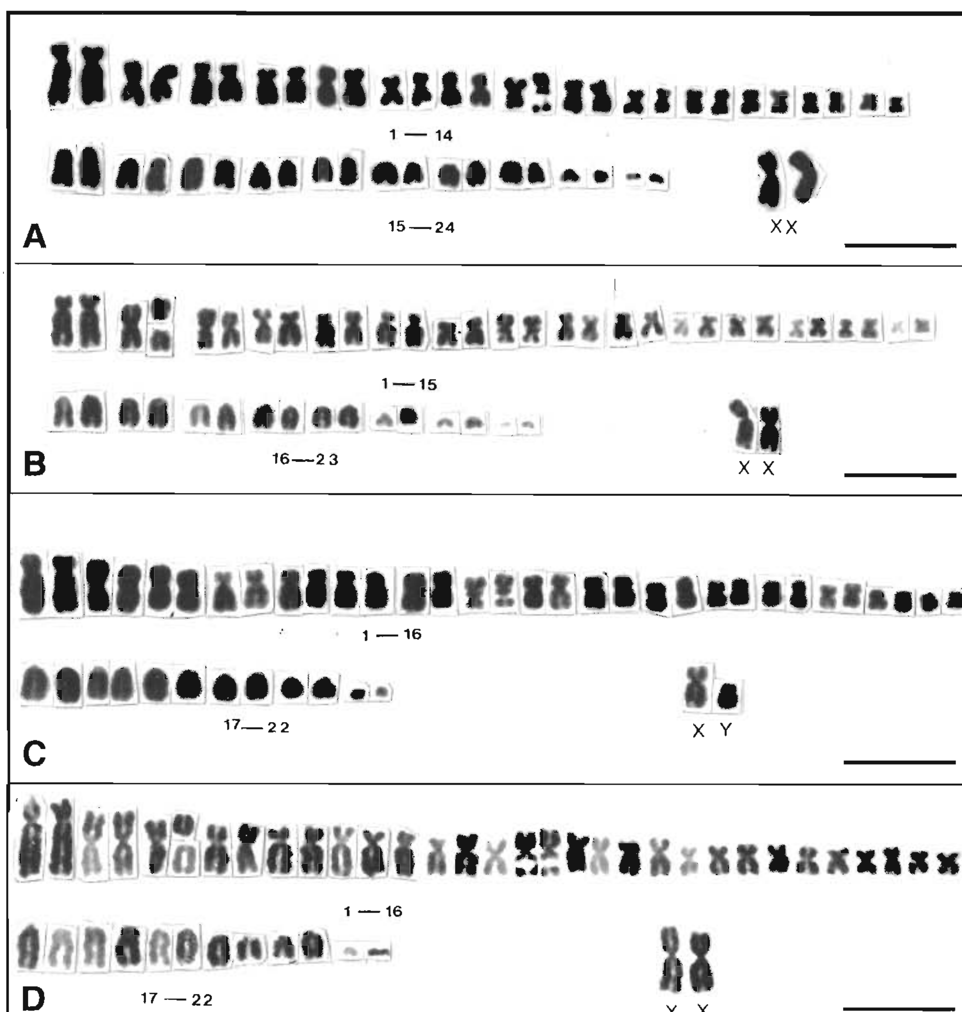

Figure 2 - Eight different standard stained karyotypes found in *Ctenomys minutus*. A, 2n = 50 and B, 2n = 49 from Jaguaruna; C, 2n = 46a; D, 2n = 47, and E, 2n = 48 from Torres to Palmares; F, 2n = 42 from Mostardas; G, 2n = 46b and 2n = 45 from Tavares. The bar is 10 μ.

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biarmed, and eight acrocentric pairs. The 2n = 46a karyotype (Figure 2C) was composed of 16 meta-submetacentric and six acrocentric pairs. Karyotype 2n = 47 (Figure 2D) presented a heteromorphic pair while that with 2n = 48 (Figure 2E) was formed by 15 biarmed and eight acrocentric pairs. Animals with 2n = 42 (Figure 2F) showed 20 biarmed and three acrocentric pairs. Karyotype 2n = 46b (Figure 2G) had 16 biarmed and six acrocentric pairs, while the 2n = 45 karyotype (Figure 2H) differed from the 2n = 46b by the presence of a heteromorphic pair. In the forms 2n = 50, 49, 46a, 47, and 48, the smallest chromosome pair was an acrocentric dot. The nucleolus organizer region was located in the secondary constriction of the long arm of pair 8 in animals with 2n = 50, 49, 46a, 48, 42, and 46b and in pair 9 in those with 2n = 47 and 45 (Figure 3).

The eight karyotypes were distributed along a 330-km transect of the coastal plain of Rio Grande do Sul and Santa Catarina (Figure 1). In Jaguaruna beach (localities 33 and 36), 2n = 49 and 2n = 50 were found. Karyotype 2n = 46a extended from the southern banks of the Ararangua River (locality 37) to Emboaba Lake (locality 19), a distance of about 135 km. A hybrid population with 2n = 46a, 47, and 48 was found along the eastern banks of Barros Lake (locality 35). Diploid numbers of 2n = 47 and 48 were found along the banks of the Fortaleza Lake (locality 29). In Passinhos (locality 34) and Palmares do Sul (locality 30) only karyotype 2n = 48 was observed. Cytotype 2n = 42 was found in Mostardas (locality 38), while 2n = 45 and 46b were observed in the south, in Tavares (locality 46).

G-banding patterns were analyzed to study chromosome rearrangements. The 2n = 50 karyotype was found in the northern part of *C. minutus'* distribution (Figure 4A) and this form was used as a standard for comparing the other karyotypes found in this species (Figure 4B). The metacentric pair 2 and the acrocentric pairs 16, 17, 19, 20, 22, 23, and 24 from 2n = 50 were found in other karyotypes as chromosomes or chromosome arms. The variant from 2n = 50 was 2n = 49 which had the 20/17 fusion in heteromorphic form. The diploid number 2n = 46a presented two fusions,

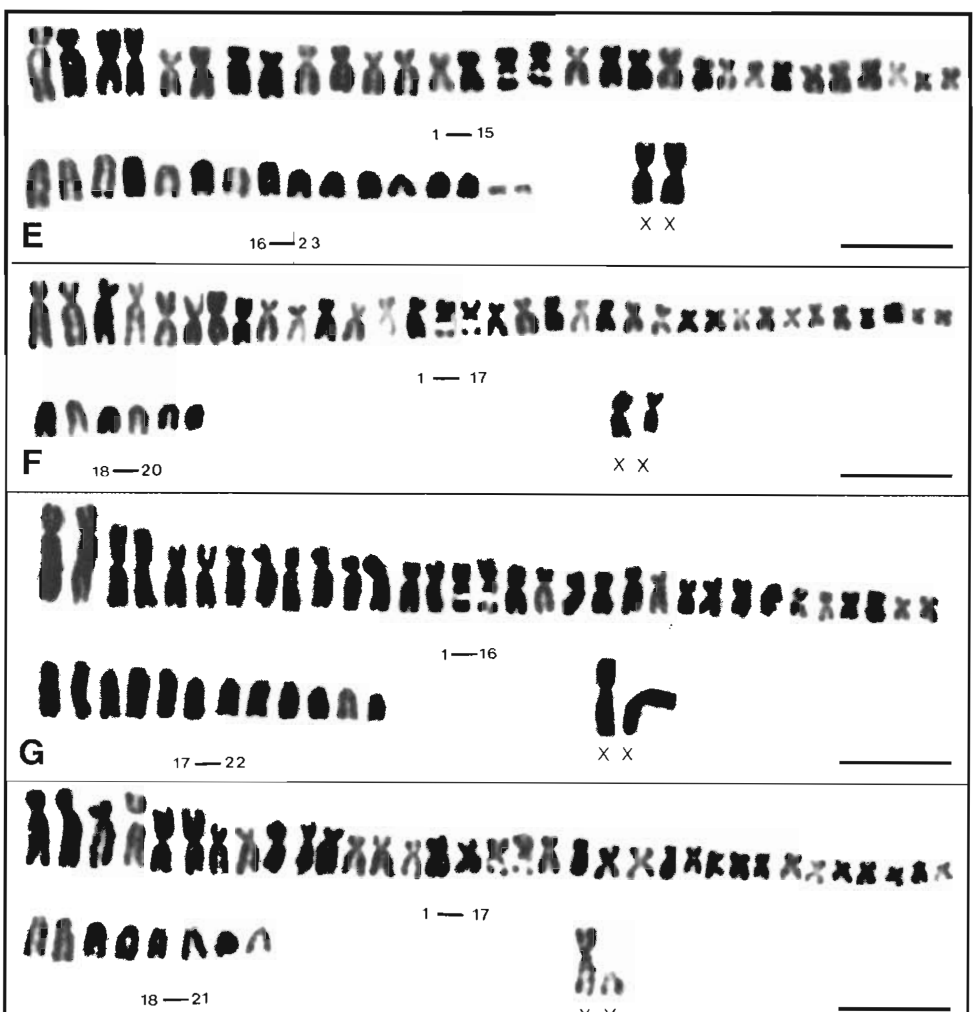

Figure 2 - Continued.

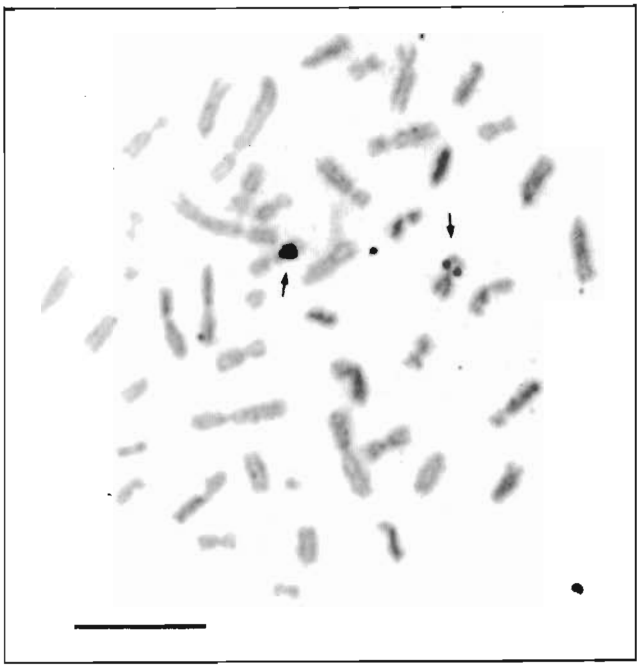

Figure 3 - NOR pattern found in 2n = 46a. The bar is 10 μ. Arrows indicate location of NOR stain.

20/17 and 23/19. The karyotype 2n = 48 presented the same fusions, plus a fission in chromosome 2 (2p and 2q) that was responsible for the heterozygote 2n = 47. The diploid number 2n = 42 had the fusions 20/17 and 23/19 and the metacentric pair 2. However, among pairs 16, 24, and 22, two different rearrangements occurred forming a new chromosome: a tandem fusion between chromosomes 24 and 16, followed by a centric fusion with chromosome 22. Finally, the 2n = 46b form was the most divergent, having 1) 2q as an acrocentric chromosome, 2) 2p as a metacentric chromosome due to a pericentric inversion ($2p^{inv}$), and 3) chromosome 22 and a new one formed due to the tandem fusion 24/16 as two isolated chromosomes.

The constitutive heterochromatin of *C. minutus* showed a highly variable pattern. Animals with 2n = 46a and 48 had a positive autosomal C-band, only in the pair with the secondary constriction (Figures 5A and B). The X did not have positive C-bands (Figure 5A), while the Y had two blocks of positive C-bands in its long arm (Figure 5B). Specimens from Jaguaruna beach (localities 33 and 36) showed constitutive heterochromatin in the centromeric region of 54% of the autosomes and in the X, with the long arm of the Y-chromosome being entirely heterochromatic (Figure 5C).

DISCUSSION

The results of the present report confirm the large karyotypic variability of the genus *Ctenomys* (Reig and Kiblisky, 1969; Ortells *et al.*, 1990). Based on our findings we conclude that eight different karyotypes of *Ctenomys minutus* have originated by Robertsonian rearrangements and tandem fusions. Similar findings

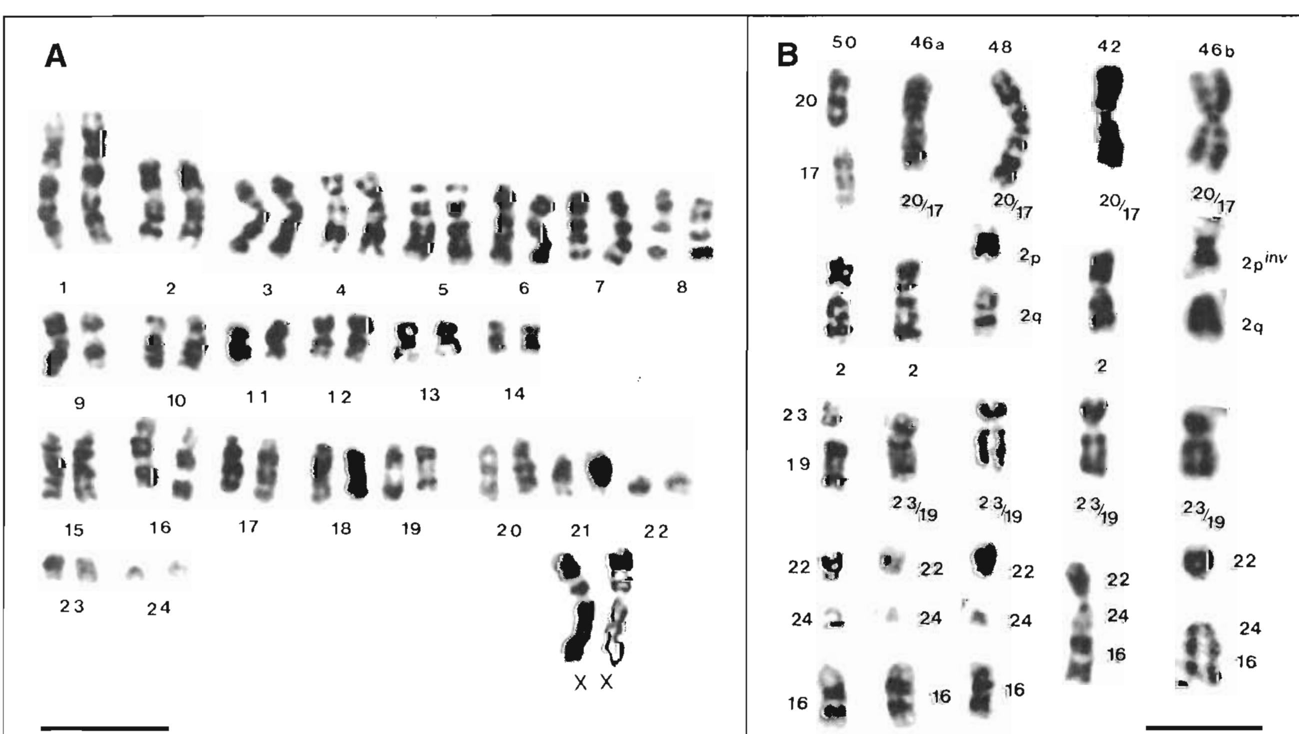

Figure 4 - A, G-band pattern found in a female with 2n = 50. B, Rearrangements determining karyotype variation. The bar is 10 μ.

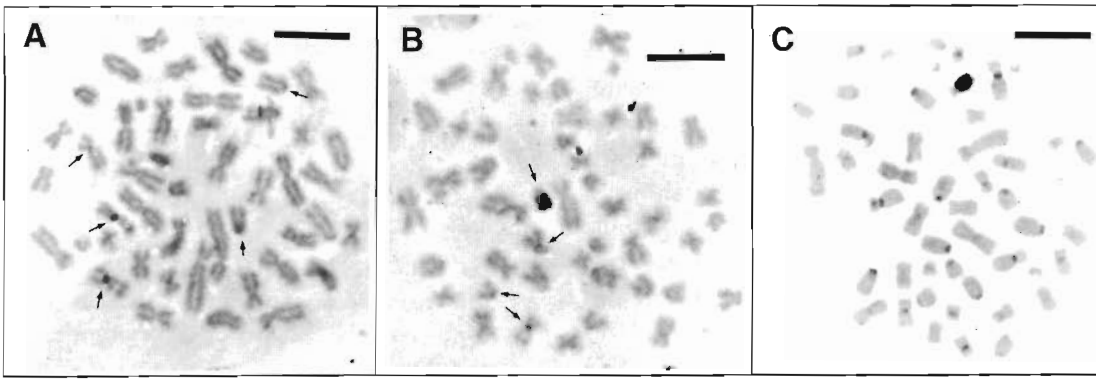

Figure 5 - Heterochromatin variation in *Ctenomys minutus*. A, Female with 2n = 48. B, Male with 2n = 48: the Y-chromosome presents a large heterochromatic block. C, Karyotype with 2n = 50 with heterochromatic blocks in almost all the chromosomes. The bar is 10 μ. Arrows indicate C-bands.

have been reported for other subterranean rodents, *Spalax ehrenbergi* and *Thomomys* (Thaeler Jr., 1974; Patton, 1980; Wahrman *et al.*, 1985; Nevo, 1986). There is a hybrid zone, as found in other subterranean species, e.g., *Spalax ehrenbergi* and *Thomomys talpoides* (Thaeler, 1974; Patton *et al.*, 1979; Nevo, 1986).

The relationship between the geographical distribution of the specimens and the chromosome rearrangements showed the existence of three population blocks. The northern block was characterized by 2n = 49 and 50. The 20/17 and 23/19 fusions in 2n = 46a and the 2p-2q fission in 2n = 47 and 48 were characteristic of the central block. The southern block was mainly characterized by the presence of the new submetacentric chromosome (22, 24 and 16) and an inversion of $2p^{inv}$. In the central and southern blocks, chromosomes with arms 20/17 and 23/19 were biarmed chromosomes, while in the northern block they changed to acrocentric. Chromosomes 2 and their arms also changed from the central to the southern block. Fissions were recorded from the central to the northern block, whereas fusions were found from the central to the southern block. Each of the three population blocks of *C. minutus* had a predominant karyotype, suggesting mainly intra-block gene flow, with geographic barriers hindering gene flow between blocks. The Ararangua River separates the northern from the central block, and marshes and wet lands isolate the central from the southern block.

Chromosomal variation in fossorial rodents has been reported as having two origins: population structure (Patton, 1985) or ecological adaptation (Nevo 1986; Wahrman *et al.*, 1969, 1985). The results reported here show that *C. minutus* is a very complex species. Chromosome variation is an important evolutionary process in this species. Geographic barriers established populational blocks, which in turn gave rise to three chromosome races. The 2n = 46a and 2n = 50 group is formed by polymorphic populations. The 2n = 42 group is more complex than the others because of rearrangements due to tandem and centric fusions. The rearrangements found in each block may represent meiotic barriers similar to those found by Capanna *et al.* (1976), Capanna (1984), and Corti and Ciabatti (1990) in the genus *Mus*. Since geographic barriers are also known in *C. minutus*, it would be interesting to perform a barrier efficiency meiotic test among these cytotypes.

These findings show the absence of a relationship between similarity at the gene level and chromosome variation in *C. minutus*. Moreira *et al.* (1991) made a biochemical analysis of the same animals studied herein. No correlation was detected between the enzymatic variability (based on 12 loci for all populations) and the geographic distances among demes. The *S* values ranged from 0.96 to 0.66. The highest similarity index (0.96) was found between populations 35 and 29, that are far away (35 km) from each other and that presented 2n = 46a, 47 and 48, and 2n = 48 and 47, respectively. Populations 31 (2n = 46a) and 35 had a low degree of similarity (0.84) though they are close geographically (about 10 km). Other examples are populations 36 (2n = 50) and 37 (2n = 46a), chromosomally different, separated by the Ararangua River, but showing high genetic similarity.

The genus *Ctenomys* has different C-banding patterns, and thus different amounts of constitutive heterochromatin. Reig *et al.* (1990) classified the species of *Ctenomys*, using C-banding, into three groups: 1) negative C-bands in some pairs; 2) positive C-bands in the centromeric region, and 3) strongly positive C-bands in the short arms and centromeric regions of the chromosomes. The species studied herein can be classified into the first two groups. Karyotypes 2n = 49 and 50 have C-bands in centromeric regions, while the other forms have positive C-bands in the chromosome with the secondary constriction and in the Y only. Constitutive heterochromatin varies in relation to the distribution of karyotypes: 2n = 50, in the north, presents a greater amount of constitutive heterochromatin than other populations in the south. This variation was also found in *C. flamarioni* (Freitas, 1990, 1994) in which the amount of constitutive heterochromatin shows a gradient from north to south along the geographic distribution. Redi *et al.* (1990) and Garagna *et al.* (1993) suggest that in *Mus m. domesticus* there is a relation between heterochromatin amount variation and frequency of chromosome rearrangements. This fact is also observed in *C. minutus*. Two events occur at the same time: chromosome rearrangements and variation in quantity of constitutive heterochromatin. Molecular studies would explain the relation between chromosome rearrangements and constitutive heterochromatin variation.

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RESUMO

Uma amostra de 101 exemplares de *Ctenomys minutus* foi coletada ao longo de sua distribuição geográfica e apresentou oito cariótipos (2n = 42, 45, 46a, 46b, 47,48, 49 e 50). Os polimorfismos cromossômicos deveram-se a rearranjos Robertsonianos e fusões *in tandem*. A distribuição dos polimorfismos evidenciou três blocos populacionais: ao norte (2n = 49 e 50), no centro (2n = 46a, 47 e 48) e ao sul (2n = 42, 45 e 46b). Estes dados sugerem que esta espécie está passando por um processo de especiação devido ao isolamento geográfico.

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