



## Karyological geographic variation of *Oligoryzomys nigripes* Olfers, 1818 (Rodentia, Cricetidae) from Brazil

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### Abstract

The karyotypes of 85 specimens of *Oligoryzomys nigripes* (Rodentia, Sigmodontinae) collected in the Cerrado and Atlantic Forest of seven states of Brazil were analyzed. Eighty four specimens presented a karyotype with  $2n = 62$  and one individual had  $2n = 61$  due to a monosomy of the X chromosome. High levels of intra- and inter-population karyotypic variability, due to sex chromosomes heteromorphisms and pericentric inversions in four autosomes (pairs 2, 3, 4 and 8), led to a variation of the autosomal arm numbers (fundamental number, FN) from 78 to 82. Synaptonemal complex analyses revealed normal meiosis in males heterozygous for pericentric inversions. We found 39 different cytotypes, 27 of which are herein described for the first time. A literature survey revealed 46 described karyotypes for *O. nigripes*. We tested the hypothesis that chromosomal variants frequencies are dependent on geographical distribution and we propose a model for the karyotypical evolution of *Oligoryzomys nigripes* with  $2n = 62/\text{FN} = 78-82$ .

**Key words:** pericentric inversion, *Oryzomyini*, *Oligoryzomys nigripes*, Rodentia, clinal variation.

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### Introduction

The genus *Oligoryzomys* Bangs, 1900 comprises 18 species distributed throughout South America and parts of Central America (Musser and Carleton, 2005; Bonvicino and Weksler, 1998; Weksler and Bonvicino, 2005). According to the last estimates, there are eleven species spread throughout most of the Brazilian biomes: rain forest (Amazonian and Atlantic Forests), open and dry areas (Cerrado, Caatinga and Restinga) and flooding-prone areas (Pampas and Pantanal) (Lima *et al.*, 2003; Weksler and Bonvicino, 2005).

Specimens with very similar external morphology and karyotypes ( $2n = 62$ ) collected in Brazil were classified as three different species: *O. nigripes* Olfers, 1818; *O. delticola* Thomas, 1917; and *O. eliurus* Wagner, 1845. Geographic distribution was the main feature used in the identification of specimens (Myers and Carleton 1981; Bonvicino and Weksler, 1998).

*Oligoryzomys nigripes* was first described as *Mus nigripes* (Olfers 1818). Over a century later, Myers and Carleton (1981) proposed a neotype for *O. nigripes* from a sample collected in Paraguay, near the type locality. The specimens showed  $2n = 62$  and  $\text{FN} = 80-81$ .

Specimens collected in the Paraná river delta (Buenos Aires, Argentina) were considered distinct from the sympatric species *O. flavescens* and were classified as *Oligoryzomys delticola* by Thomas (1917), who disregarded the original description of *O. nigripes* (Olfers, 1818). Brum-Zorrilla *et al.* (1988) reported the karyotypes of *O. delticola* from Uruguay with  $2n = 62/\text{FN} = 80-81$ , very similar to those described for *O. nigripes* by Myers and Carleton (1981). Sbalqueiro (PhD Thesis. Universidade Federal do Rio Grande do Sul. Porto Alegre, Brazil, 1989) described karyotypes with  $2n = 62/\text{FN} = 79-82$  for *O. delticola* from Southern Brazil. Espinosa and Reig (1991) studied specimens of *O. delticola* collected in the type locality (Buenos Aires) and found karyotypes with  $2n = 62/\text{FN} = 81-82$ . More recently, Bonvicino and Weksler (1998), based on karyotypes and GTG-banding similarities, considered *O. delticola* as a junior synonym of *O. nigripes*.

Wagner (1845) described *Oligoryzomys eliurus* from the type locality Itararé, São Paulo, Brazil, but did not per-

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form any comparative analyses with other *Oligoryzomys* species. Morphological and cranial studies later indicated that *O. eliurus* was a junior synonym of *O. nigripes* (Cabrera, 1961; Myers and Carleton, 1981; Bonvicino and Weksler 1998). Andrades-Miranda *et al.* (2001) reported karyotypes with  $2n = 62/FN = 64$  for *O. eliurus* and  $2n = 62/FN = 79-82$  for *O. nigripes*. Specimens of both species were found in sympatry in the Cerrado of the state of Goiás, Brazil. Bonvicino and Weksler (1998) identified a karyotype with  $2n = 62/FN = 64$  in specimens of *O. fornesi* Massoia (1973), collected in Goiás.

Considering the morphological and karyological data reported in the literature, we support the status of *O. delticola* and *O. eliurus* as junior synonyms of *O. nigripes* and consider the karyotypes with  $2n = 62/FN = 78-82$  to be related to *O. nigripes*.

Most *O. nigripes* populations analyzed showed pericentric inversion polymorphisms in autosome pairs 2, 3, 4 and 8 (Brum-Zorrilla *et al.*, 1988; Almeida and Yonenaga-Yassuda, 1991; Bonvicino *et al.*, 2001). The pericentric inversions did not seem to result in any selective disadvantages for heterozygotes (Bonvicino *et al.*, 2001).

In this work, we present data on the karyotypes of 85 specimens from wild populations of *O. nigripes* from Brazil, which showed high levels of sex chromosome and autosome variation. The compilation of our data with litera-

ture reports revealed the existence of 46 karyotype variants (cytotypes) with  $2n = 61-62$  and  $FN = 78-82$ , in specimens from Brazil and Uruguay.

## Material and Methods

### Specimens

Our sample consisted of 85 specimens of *Oligoryzomys nigripes* trapped in seven states of Brazil in areas of Caatinga (Ceará), Cerrado (Minas Gerais and Mato Grosso do Sul), and Atlantic forest (Bahia, Espírito Santo, São Paulo and Rio Grande do Sul) (Table 1, Figure 1, Appendix 1). Skins and skulls were deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP) and Museu de Biologia Professor Mello Leitão (MBML).

### Cytogenetic studies

Metaphase preparations were obtained from bone marrow and testis, after *in vivo* injection of a 0.1% colchicine solution (1mL/100g of body weight). Cells were suspended in a 0.075M KCl solution for 25-30 min at 37 °C, fixed in 3:1 methanol: acetic acid, spread onto clean slides and air-dried. GTG- and CBG-banding were performed according to Seabright (1971) and Sumner (1972), respectively. At least 20 metaphases per individual were analyzed to define the diploid and fundamental numbers

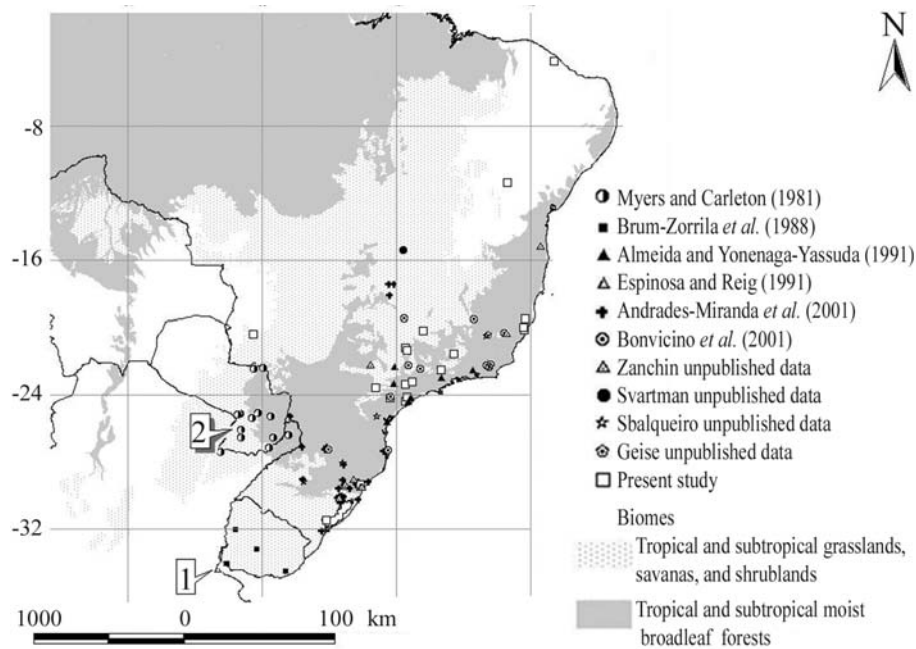
**Table 1** - Cytotypes of *Oligoryzomys nigripes* with  $2n = 62$  and  $FN = 78-82$ .

Cytotype	2n	FN	Autosome pairs <sup>1</sup>				Sexual pair <sup>2</sup>	n	Specimens <sup>3</sup>	Locality <sup>4</sup>	Reference <sup>5</sup>
			2	3	4	8					
1	62	78	A	A	M	M	XaXb	1	CIT986	23	Present study
2	62	78	A	A	M	M	XaYa	3	CIT990, CIT992, CIT1018	23	Present study
3	62	78	A	A	M	M	XaYc	1	CIT1263	24	Present study
4	62	78	M	A	A	M	XcXa	2	-	1	Zanchin 1988 <sup>5a</sup>
5	62	79	M	A	H	M	XaXb	1	LGA116	2	Present study
6	62	79	M	A	M	H	XaYa	1	LGA391	3	Present study
7	62	79	M	A	M	H	XaXa	1	LGA413	4	Present study
8	62	80	M	H	H	M	XcXc	1	LGA115	2	Present study
9	62	80	M	H	H	M	XaXb	1	LGA404	4	Present study
10	62	80	M	A	M	M	XaXa	1	CIT1283	19	Present study
								1	BIO837	5	Present study <sup>5b</sup>
								1	-	6	Zanchin 1988 <sup>5a</sup>
11	62	80	M	A	M	M	XaXb	1	LGA916	7	Present study
								1	CIT355	15	Present study
								1	CIT117	8	Present study <sup>5b</sup>
								1	-	6	Zanchin 1988 <sup>5a</sup>
12	62	80	M	A	M	M	XaXc	1	-	9	Zanchin 1988 <sup>5a</sup>
13	62	80	M	A	M	M	XbXb	1	LGA619	10	Present study
14	62	80	M	A	M	M	XcXc	2	-	6	Zanchin 1988 <sup>5a</sup>
15	62	80	M	A	M	M	XaYa	2	LGA615, LGA620	10	Present study
								1	CIT1051	19	Present study
								10	-	6	Zanchin 1988 <sup>5a</sup>
16	62	80	M	A	M	M	XaYb	1	LGA101	2	Present study
								1	LGA408	4	Present study

Table 1 (cont.)

Cytotype	2n	FN	Autosome pairs <sup>1</sup>				Sexual pair <sup>2</sup>	n	Specimens <sup>3</sup>	Locality <sup>4</sup>	Reference <sup>5</sup>
			2	3	4	8					
17	62	80	M	A	M	M	XaYc	1	CIT133	14	Present study <sup>5b</sup>
								2	CIT122, CIT137	8	Present study <sup>5b</sup>
								1	LGA45	2	Present study
								1	-	11	Svartman 1989 <sup>5b</sup>
18	62	80	M	A	M	M	XbYc	1	LGA388	3	Present study
								1	-	9	Zanchin 1988 <sup>5a</sup>
								1	CIT1038	19	Present study
19	62	80	M	A	M	M	XaYd	1	CIT192	14	Present study
20	62	80	M	H	M	M	XcYa	1	LGA65	2	Present study
								2	-	6	Zanchin 1988 <sup>5a</sup>
21	61	81	M	H	M	M	Xa0	1	LGA125	2	Present study
22	62	81	M	H	M	M	XaXa	2	LGA39, LGA64	2	Present study
								2	-	9	Zanchin 1988 <sup>5a</sup>
								2	CIT120, CIT125	8	Present study <sup>5b</sup>
								2	-	6	Zanchin 1988 <sup>5a</sup>
								4	-	6	Zanchin 1988 <sup>5a</sup>
23	62	81	M	H	M	M	XaXb	2	LGA182, LGA820	2	Present study
								1	LGA928	12	Present study
								3	BIO793, BIO794, CIT121	8	Present study <sup>5b</sup>
								1	CIT963	17	Present study
								1	CIT329	18	Present study
								1	CIT350	21	Present study
								1	-	8	Zanchin 1988 <sup>5a</sup>
								1	-	6	Zanchin 1988 <sup>5a</sup>
24	62	81	M	H	M	M	XaXc	5	-	6	Zanchin 1988 <sup>5a</sup>
25	62	81	M	H	M	M	XbXc	2	LGA114, LGA183	2	Present study
26	62	81	M	H	M	M	XbXb	1	LGA882	13	Present study
27	62	81	M	H	M	M	XaYa	1	LGA58	2	Present study
								1	CIT351	21	Present study
								1	-	9	Zanchin 1988 <sup>5a</sup>
								6	-	6	Zanchin 1988 <sup>5a</sup>
28	62	81	M	H	M	M	XaYb	1	LGA403	4	Present study
								1	CIT1042	19	Present study
29	62	81	M	H	M	M	XaYc	4	LGA55, LGA56, LGA59, LGA63	2	Present study
								2	BIO633, CIT123	8	Present study <sup>5b</sup>
								1	CIT939	16	Present study
								1	BIO831	5	Present study <sup>5b</sup>
30	62	81	M	H	M	M	XaYd	1	LGA406	4	Present study
								1	BIO797	8	Present study <sup>5b</sup>
								1	CIT339	14	Present study
								1	LGA122	2	Present study
31	62	81	M	H	M	M	XbYc	1	LGA126	2	Present study
32	62	81	M	H	M	M	XcYa	1	LGA110	2	Present study
33	62	81	M	H	M	M	XcYb	1	LGA110	2	Present study
								3	-	6	Zanchin 1988 <sup>5a</sup>
34	62	81	M	H	M	M	XcYd	1	LGA927	7	Present study
35	62	82	M	M	M	M	Xa0	1	-	9	Zanchin 1988 <sup>5a</sup>
36	62	82	M	M	M	M	XaXa	1	LGA124	2	Present study
								1	CIT119	8	Present study <sup>5b</sup>
								1	CIT1283	20	Present study
								1	CIT343	21	Present study
								1	LGA108	2	Present study
37	62	82	M	M	M	M	XaXb	2	LGA917, LGA918	7	Present study
								1	CIT57	8	Present study <sup>5b</sup>





**Figure 1** - Collection localities of *Oligoryzomys nigripes* ( $2n = 62/\text{FN} = 78-82$ ). 1 and 2 represent the type localities for *Oligoryzomys delticola* and *Oligoryzomys nigripes*, respectively.

Minas Gerais MG), Bahia (BA), Ceará (CE) and Mato Grosso do Sul (MS) in Brazil; and Uruguay (URU).

The correlation between the frequencies of the chromosome variants and the geographic areas was tested with the chi-square test ( $\chi^2$ ) with a p-value of 0.05, assuming that the chromosome heteromorphisms are randomly distributed in the populations.

## Results

### Karyotype variability

Thirty-nine cytotypes were detected in our sample, due to variations of chromosomes 2, 3, 4, 8, and of the sex chromosomes. Twenty-seven of these cytotypes had not yet been described. A literature review showed the presence of 46 cytotypes among 143 *O. nigripes* specimens (Table 1).

Eighty four out of 85 specimens of *Oligoryzomys nigripes* presented  $2n = 62$ . One specimen from Espírito Santo (LGA 125) had  $2n = 61$  due to a monosomy of the X chromosome (Table 1). The number of autosome arms (FN) varied from 78 to 82 as a result of pericentric inversions in pairs 2, 3, 4 and 8. Heteromorphisms of the X and Y chromosomes were also detected.

In the karyotypes analyzed, pair 1 was a large submetacentric; pair 2 was a large submetacentric (2SM) or a large acrocentric (2A); pairs 3 and 4 were present as homomorphic metacentrics (3M/4M), homomorphic acrocentrics (3A/4A) or were heteromorphic metacentric/acrocentric (3H/4H); pair 8 was metacentric (8M) or heteromorphic metacentric/acrocentric (8H); pairs 5, 6, 7, 9, 10 and 11 were medium to small meta/submetacentrics; and pairs

12 to 30 were acrocentrics with gradual variation in size (Figure 2 and 3a). G-banding patterns analyses allowed us to confirm that pericentric inversions were responsible for the polymorphisms of pairs 2, 3, 4 and 8. The acrocentric form of pair 2 is herein described for the first time.

Three different morphologies were observed for the X chromosome and four Y chromosome variants were identified. The sex chromosomes were classified as Xa, Xb, Ya, Yb and Yc according to Almeida and Yonenaga-Yassuda (1991). The Xc and Yd forms are herein described for the first time. Xa is a large submetacentric, Xb is a large subtelocentric and Xc is a large metacentric, all similar in size to pair 1, although Xa and Xc are slightly larger than Xb. Ya is a medium metacentric, similar in size to the long arm of Xa; Yb is a small submetacentric, equivalent in size to half of the long arm of Xa; Yc is a medium submetacentric, similar in size to the long arm of Xa; and Yd is a small subtelocentric, equivalent in size to half of the long arm of Xa (Figure 3b). The size variation of the X and Y chromosomes were due to differences in constitutive heterochromatin content.

After FISH with a telomeric probe, signals were restricted to the telomeres of all chromosomes. No interstitial telomeric sites (ITS) neither signals associated with heterochromatic regions were observed (Figure 3c).

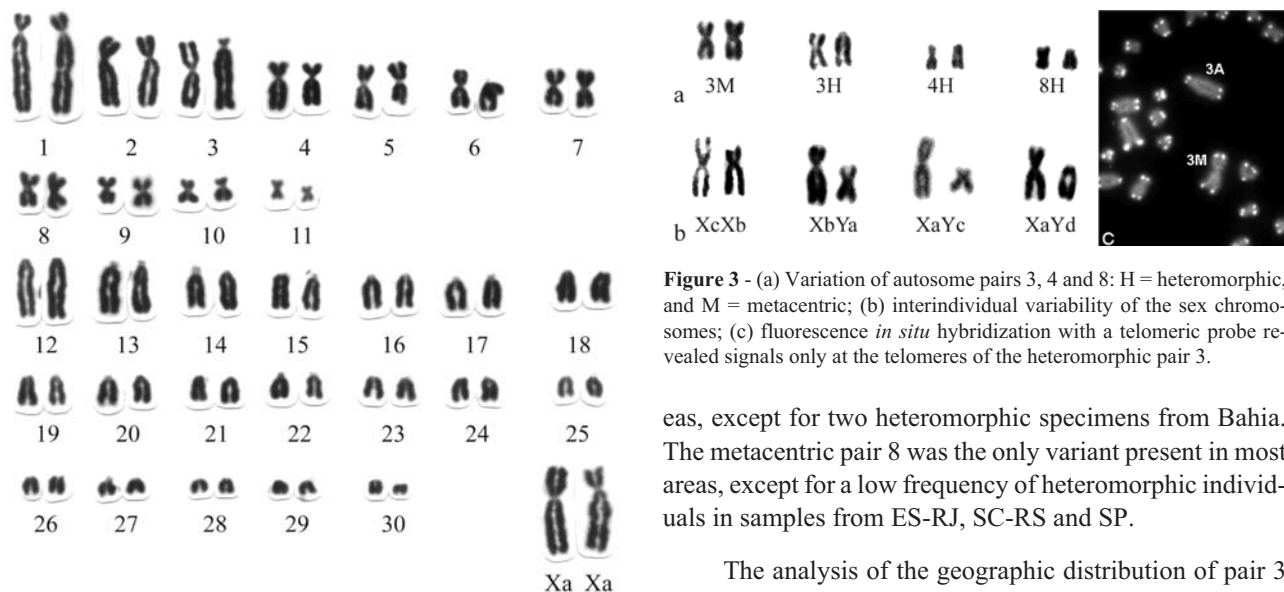
Analyses of meiotic cells revealed that the heterochromatic short arm of the X chromosome pairs with the euchromatic long arm of the Y chromosome (Figure 4a-b). Synapsis starts interstitially and proceeds towards the tips of the chromosomes. At their maximum level of pairing,

**Table 2** - Constitution of pair 3 in *Oligoryzomys nigripes* with  $2n = 62/FN = 78-82$ .

Referred as	2n	FN	Pair 3 form			n	Reference <sup>1</sup>
			3A	3H	3M		
<i>O. nigripes</i>	62	78-82	20	23	14	57	Zanchin 1988 <sup>1a</sup>
<i>O. nigripes</i>	62	80-82	-	1	-	1	Svartman 1989 <sup>1b</sup>
<i>O. delticola</i>	62	79-82	18	10	7	35	Sbalqueiro 1989 <sup>1c</sup>
<i>O. nigripes</i>	62	80-82	38	65	36	139	Almeida and Yonenaga-Yassuda 1991
<i>O. nigripes</i>	62	82	-	-	6	6	Geise 1995 <sup>1d</sup>
<i>O. nigripes</i>	62	80-82	8	50	39	97	Bonvicino <i>et al.</i> 2001
<i>O. delticola</i>	62	80-81	4	3	-	7	Brum-Zorrilla <i>et al.</i> 1988
<i>O. delticola</i>	62	81-82	-	1	4	5	Espinosa and Reig 1991
<i>O. nigripes</i>	62	80-81	NI	NI	NI	35	Myers and Carleton 1981
<i>O. nigripes</i>	62	78-82	26	39	20	85	Present study
Total						467	

$2n$  = diploid number; FN = fundamental number, *i.e.* number of autosomal arms; 3A = acrocentric pair 3; 3H = heteromorphic pair 3; 3M = metacentric pair 3; n = number of individuals. NI = not identified.

<sup>1a</sup>Zanchin NIT (1988) Estudos cromossômicos em orizomíinos e equimídeos da Mata Atlântica. PhD thesis. Universidade Federal do Rio Grande do Sul. Porto Alegre. Rio Grande do Sul. Brazil. <sup>1b</sup>Svartman M (1989) Levantamento cariotípico de roedores da região do Distrito Federal. Master Thesis. São Paulo, São Paulo, Brasil. <sup>1c</sup>Sbalqueiro IJ (1989) Análises cromossômicas e filogenéticas em algumas espécies de roedores da região Sul do Brasil. PhD Thesis. Porto Alegre, Rio Grande do Sul, Brazil. <sup>1d</sup>Geise L (1995) Os roedores Sigmodontinae (Rodentia, Muridae) do Estado do Rio de Janeiro: sistemática, citogenética, distribuição e variação geográfica. PhD Thesis. Universidade Federal do Rio de Janeiro. Rio de Janeiro, Rio de Janeiro, Brazil.



**Figure 2** - Conventional stained karyotype of *Oligoryzomys nigripes* ( $2n = 62/FN = 80$ ). Pair 3 is heteromorphic and the X chromosomes are large submetacentrics (XaXa).

half of the Y and about 20% of the X were paired (Figure 4c-d).

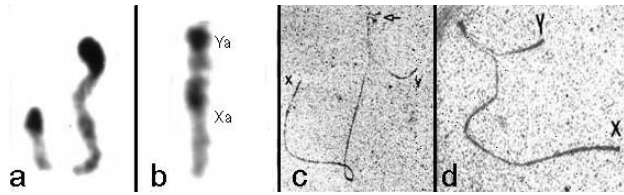
### Geographic patterns of chromosome variation

The submetacentric form of pair 2 occurred in all populations, except from Ceará, where the acrocentric form was exclusive. Pair 4 was metacentric in all geographic ar-

**Figure 3** - (a) Variation of autosome pairs 3, 4 and 8: H = heteromorphic, and M = metacentric; (b) interindividual variability of the sex chromosomes; (c) fluorescence *in situ* hybridization with a telomeric probe revealed signals only at the telomeres of the heteromorphic pair 3.

reas, except for two heteromorphic specimens from Bahia. The metacentric pair 8 was the only variant present in most areas, except for a low frequency of heteromorphic individuals in samples from ES-RJ, SC-RS and SP.

The analysis of the geographic distribution of pair 3 (3A, 3H and 3M) and Y chromosome variants (Ya, Yb, Yc and Yd) showed a clinal distribution pattern. The cline starts in Central Brazil and progresses to the south (SC-RS and SP) towards the northeast (BA-CE). The acrocentric form of chromosome 3 (3A) was the only one observed in the central region (MS), its frequency decreased in the URU and SC-RS regions (40-57%) and SP, mg, ES-RJ (0-50%) and it was exclusive in the northernmost populations (100%). Accordingly, 3M was very frequent in the areas of SP, MG, ES-RJ (38-76%) and less frequent in the southern and central regions (SP, SC-RS, URU and MS) (0-20%) (Figure 5a). The chi-square test pointed to a non-



**Figure 4** - (a-b) C-banded sex chromosomes of *O. nigripes* in diplotene: (a) dissociated sex chromosomes; (b) end-to-end pairing of the long arm of Ya and the short arm of Xa; (c-d) Electron micrograph of silver-stained spreads of early pachytene nuclei: (c) pairing of Xa and Yb with interstitial synaptic initiation; (d) total pairing of the short arm of Xa and the long arm of Yb; unpaired axes show heteropiconosis (arrow). Xa = large submetacentric, Ya = medium submetacentric, Yb = small metacentric.

random geographic distribution of chromosome 3 variants (p-value = 0.00).

Ya was the most frequently found Y chromosome variant. It predominated in the central (MS), southern (RS-SC) and northern (BA, CE) areas, and its frequency decreased in the southern areas (SP, MG, ES-RJ). Yb was predominant in populations from SP and Yc was predominant in ES-RJ populations. Yd was the least frequent Y chromosome variant and it was only found in SP and SC-RS samples (Figure 5b). Clinal variation was observed for Ya and Yc. The chi-square test indicated that the Y chromosome variants are not randomly distributed (p-value = 0.00).

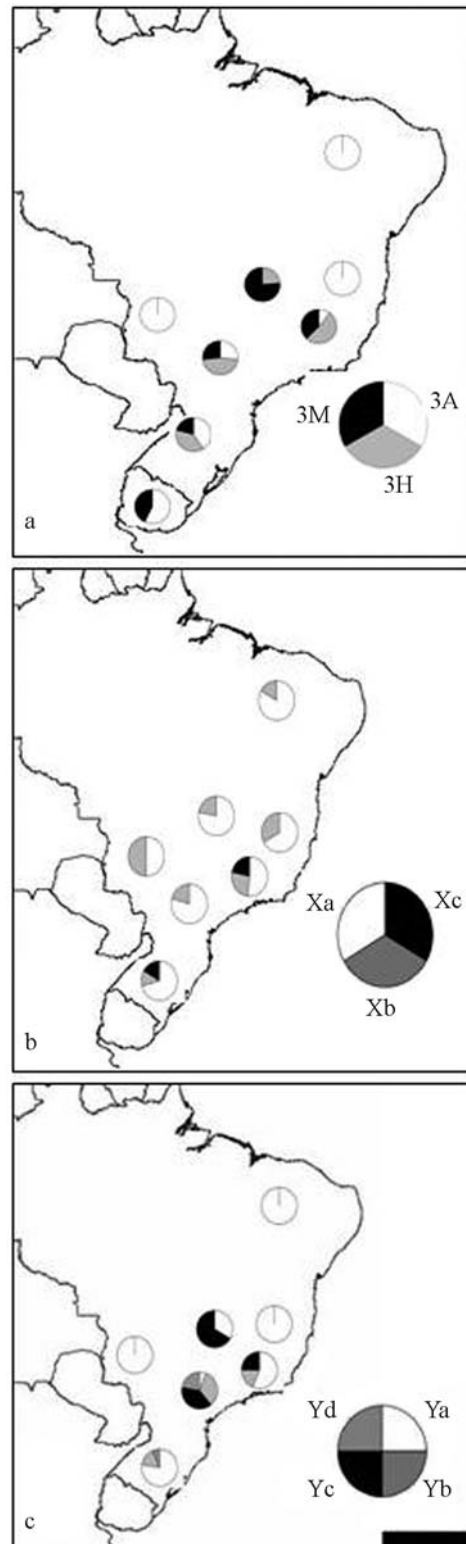
A significant difference in the geographic distribution of X chromosome morphological variants was observed: Xa was the most frequent form in all populations (47.5%; n = 143), followed by Xb (22.5%; n = 143), and Xc was only observed in ES-RJ and RS-SC populations (7.7%; n = 143). The distribution of the X chromosome variants did not follow a clear clinal pattern (Figure 5c). Nevertheless, the chi-square test revealed significant differences in the frequencies of X chromosome variants among populations (p-value = 0.005).

## Discussion

### Chromosomal polymorphisms

*Oligoryzomys nigripes* is one of the most polymorphic species of Neotropical rodents. A total of 432 karyotypes would be expected considering all combinations of the polymorphisms involving pairs 2 (2A or 2M), 3 (3A, 3H or 3M), 4 (4A, 4H or 4M), 8 (8H or 8M), X (Xa, Xb or Xc) and Y (Ya, Yb, Yc or Yd). So far, only 46 of these cytotypes have been observed, representing only 10.6% of the theoretically predicted forms.

The heteromorphism of pair 3 is widespread in the species. The synaptonemal complexes analyses indicated that these heteromorphisms do not pose a problem for the synapsis and segregation of the homologues during meiosis in heterozygotes. Several genetic mechanisms seem responsible for overcoming meiotic disturbance in heteromorphic rodents, such as the occurrence of heterosynapsis



**Figure 5** - Geographical distributions of the frequencies of the polymorphic forms of (a) pair 3, (b) X chromosomes and (c) Y chromosomes.

(Fagundes *et al.*, 1998) and a low frequency of chiasmata between inverted segments (Wang *et al.*, 2003a). Moreover, wild rodent populations showed high numbers of heterozygous individuals without any evidence of fertility re-

duction (Greenbaum and Reed, 1984; Hale, 1986). Mechanisms as the ones mentioned above are likely to be operating in *Oligoryzomys nigripes* populations, thus reducing the deleterious effects associated with heterozygous pericentric inversions.

Almeida and Yonenaga-Yassuda (1991) described the frequencies of heterozygotes and homozygotes for pair 3 in populations from São Paulo, which were shown to be in Hardy-Weinberg equilibrium.

Sex chromosomes polymorphisms are common in Oryzomyini and are usually due to constitutive heterochromatin additions/deletions (Almeida and Yonenaga-Yassuda, 1991). Three variants of Y chromosomes were found in *O. nigripes* populations from the states of São Paulo and Rio de Janeiro. This variation was attributed to either constitutive heterochromatin duplications or pericentric inversions (Almeida and Yonenaga-Yassuda, 1991).

Five out of 11 other *Oligoryzomys* species showed sex chromosomes polymorphisms due to constitutive heterochromatin variation: *O. stramineus* (V.M. Furtado unpublished data), *O. magellanicus* (Gallardo and Palma, 1990), *O. longicaudatus* (Gallardo and González, 1977), *O. flavescens* (Espinosa and Reig, 1991) and *O. fulvescens* (Haiduk *et al.*, 1979).

Multani *et al.* (2001) classified the constitutive heterochromatin of mammals into three types: telomeric, nontelomeric and a combination of both. Different types of repetitive sequences have been observed in rodents. Heterogeneity of sex chromosomes heterochromatin was either attributed to differences in DNA-protein associations (Murer-Orlando and Richer, 1983) or to repetitive elements and sequence composition (Modi, 1993). Our data revealed that the heterochromatin of the sex chromosomes of *Oligoryzomys nigripes* is not related to repetitive telomeric sequences, and thus we believe that they are associated to DNA-protein association.

### Frequencies

Only 10.6% of the expected cytotypes to occur in *O. nigripes* have already been found (46 out of 432). The chi-square test showed significant differences in the distribution frequency of the variants of pair 3, the X and Y chromosomes, indicating that some variants are exclusive to specific geographical areas. Thus, the total number of possible theoretical combinations ( $n = 432$ ) is unlikely to occur.

In the rodent *Akodon cursor*, Fagundes *et al.* (1998) proposed that 81 karyotypic combinations were expected to exist, although only 34.6% of them had been observed.

The occurrence of a rearrangement in a particular geographical area could be related to a positive adaptive value. Baker *et al.* (1983) proposed that natural selection may favor the fixation of a chromosomal rearrangement that promotes a genetic benefit to the carrier.

In an attempt to correlate karyotypic with environmental features, Confalonieri and Colombo (1989) studied 103 individuals from six *Trimerotropis pallidipennis* (Orthoptera) populations from Argentina, distributed along an altitudinal gradient. These authors observed a high correlation between frequencies of chromosomal inversions and altitude. Later, Confalonieri (1994) investigated 281 individuals from 17 Argentinian populations and detected a positive correlation between the frequency of heterozygotes and the altitude and longitude in which they lived. According to Confalonieri (1994), *T. pallidipennis* fits into a balanced model in which the selection coefficient varies according to the altitudinal, latitudinal and longitudinal gradients. The major selective forces could be climatic and/or ecological variables associated to these gradients, such as temperature, humidity, oxygen rate, atmosphere pressure, precipitation and length of daylight.

Most studies that described the occurrence of chromosomal rearrangements in wild rodent populations made no correlation to environmental components. The major difficulty is determining patterns of rearrangements occurrence because of either small sampling or insufficient number of geographical areas studied (Bianchi *et al.*, 1969; Liascovich *et al.*, 1990; Almeida and Yonenaga-Yassuda, 1991; Sbalqueiro *et al.*, 1991; Castro *et al.*, 1991; Andrades-Miranda *et al.*, 2001; Wang *et al.*, 2003b).

Although our data showed a high correlation between specific chromosome variants and the geographic area where they were found, we were unable to associate specific cytotypes to any ecological or environmental features in *Oligoryzomys nigripes*.

### A model of karyotypic evolution for *Oligoryzomys nigripes*

We propose a hypothetical evolutionary pattern and radiation scenario for *Oligoryzomys* based on the hypothesis that the original differentiation area of the oryzomyines was the Andean region of Ecuador, Colombia and Venezuela (Savage, 1974; Reig, 1978, 1984, 1986). The ancestral oryzomyine (proto-oryzomyini) had reached the northwestern coast of South America and quickly dispersed into the highlands that emerged during the Inferior Miocene. The subsequent Andean elevation of the Cordillera had increased the heterogeneity of the Andean environments, leading to the differentiation of the ancestral lineage of Oryzomyini which adapted to the different habitats of the mountainous jungle. Other oryzomyines had simultaneously diversified into habitats of lower elevations, invading the lowlands of the northwestern Andes and later reaching southern lower areas. It is likely that *Oligoryzomys* originated with adaptations to live in low prairies and with herbivory trends (Reig, 1984).

Indeed, we clearly observed that the geographical distribution of *O. nigripes* coincides with tropical and subtropical humid forests (Figure 1), suggesting that this sort of



environment could play the role of a dispersal corridor for *Oligoryzomys*, as previously suggested by Reig (1984).

Considering that the lowlands of the western Brazilian and the Paraguayan prairies could be the region where *Oligoryzomys* originated, the populations of these areas could carry the most primitive characters of the genus. Based on this idea, we suggest that the karyotype with  $2n = 62/FN = 80$  is the *O. nigripes* primitive karyotype. This karyotype is composed of a submetacentric pair 2, an acrocentric pair 3 and metacentric pairs 4 and 8. These are the chromosome variants present in all specimens from Mato Grosso do Sul and in most individuals from Paraguay. Subsequently, specimens showing the acrocentric form of pair 2, the heteromorphic and metacentric forms of pair 3, the heteromorphic and acrocentric forms of pair 4, and the heteromorphic form of pair 8, would have originated during the radiation of the ancestral group towards the lowlands of southern Brazil, Uruguay and Argentina, reaching the Atlantic coast of southern and northeastern Brazil.

Populations in lowland islands of Argentina, Uruguay and Santa Catarina/Rio Grande do Sul showed acrocentric or heteromorphic pair 3 and metacentric pairs 4 and 8. Some lineages with the most derived karyotypical constitution (metacentric pair 3, heteromorphic and acrocentric pair 4 and heteromorphic pair 8) would have spread towards Minas Gerais, São Paulo, Rio de Janeiro/Espírito Santo and Bahia (to the eastern and northern portions of the geographical distribution). Lineages from Ceará (northeast) have an acrocentric pair 2, a derived condition.

The clinal distribution of the chromosomal polymorphisms corroborates this hypothesis. The acrocentric pair 3 and metacentric pairs 4 and 8 ( $FN = 80$ ) prevailed in the western populations. Southern populations presented individuals with pericentric inversion of pair 3, but the acrocentric form predominated. The frequency of the acrocentric pair 3 form decreased in eastern and northern populations (São Paulo, Rio de Janeiro/Espírito Santo, Minas Gerais and Bahia), while the frequency of the presumed derived metacentric form increased.

The clinal variation observed regarding the Y chromosome reinforces the differences among the populations from west/south and east/north: the frequency of  $Y_a$  decreases and the frequency of  $Y_c$  increases towards the east and north of the geographic distribution.

This work presents new data that allowed us to hypothesize an evolutionary model for *Oligoryzomys nigripes* based on chromosome variants. We also correlated chromosome variants to specific geographical areas which can be further investigated in order to evaluate the adaptive value of chromosome rearrangements as proposed by Barker (1983).

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## Appendix

For some of the records, Global Position System (GPS) information provided by the authors were used, and in some cases the geographic position of the municipality administrative center collected from the Brazilian Institute of Geography and Statistics (IBGE) was used.

**Present Study and Silva MJJ [Estudos cromossômicos e de complexos sinaptonêmicos em roedores brasileiros da Tribo Oryzomyini (Cricetidae, Rodentia). MSc Dissertation, Universidade de São Paulo, São Paulo, Brazil, 1994]** - Brazil. Ceará: Pacoti (04°13' S, 38°55' W). Bahia: Morro do chapéu (11°36' S, 41°38' W). Espírito Santo: Santa Tereza (19°55' S, 40°36' W), Duas Bocas (20°16' S, 40°28' W), Santa Maria de Jetibá (20°02' S, 40°44' W). Minas Gerais: Caxambu (21°58' S, 44°55' W), Serra da Canastra (20°18' S, 46°35' W). São Paulo: Águas de Santa Bárbara (23°58' S, 49°25' W), Iguape (24°43' S, 47°33' W), Iporanga (24°07' S, 47°38' W), Intervalos (24°12' S, 48°30' W), Pilar do Sul (23°48' S, 47°42' W), Araçariçuama (23°26' S, 47°03' W), Santo Antônio (22°49' S, 45°39' W), Ribeirão Preto (21°19' S, 47°48' W), Luís Antônio (21°33' S, 47°42' W), Juquiá (24°19' S, 47°38' W). Mato Grosso do Sul: Serra da Bodoquena (20°42' S, 56°51' W). Rio Grande do Sul: Maquine (29°40' S, 50°10' W), São Francisco de Paula (31°46' S, 52°19' W).

**Almeida and Yonenaga-Yassuda (1991)** - Brazil. São Paulo: Iguape (24°43' S, 47°32' W), Taubaté (23°01' S, 45°32' W), Pedro de Toledo (24°16' S, 47°13' W), Itapetininga (23°36' S, 48°02' W), Santa Maria da Serra (22°33' S, 48°10' W). Rio de Janeiro: Itaguaí (22°52' S, 43°46' W). Paraná: Guaratuba (25°52' S, 48°34' W).

**Andrades-Miranda et al. (2001)** - Brazil. Goiás: Ipameri (17°43' S, 48°09' W), Caldas Novas (17°44' S, 48°37' W), Corumbáiba (18°08' S, 48°33' W). Paraná:

Parque Nacional do Iguaçu (25°30' S, 54°30' W). Rio Grande do Sul: Derrubadas (27°15' S, 53°52' W), Aratiba (27°23' S, 52°19' W), Muitos Capões (28°19' S, 51°10' 60W), Ivaí (29°01' S, 53°47' W), Tupanciretã (29°04' S, 53°51' W), Caxias do Sul (29°10' S, 51°10' W), Tainhas (29°16' S, 50°17' W), São Francisco de Paula (31°46' S, 52°19' W), Charqueadas (29°58' S, 51°37' W), Sapiranga (29°37' S, 51°00' W), Riozinho (29°38' S, 50°27' W), Faxinal (30°18' S, 51°40' W), Torres (29°21' S, 49°43' W), Rio Jacuí (30°05' S, 51°36' W), Belém Novo (30°11' S, 51°10' W), Osório (29°53' S, 50°16' W), Tramandaí (29°58' S, 50°07' W), Quintão (30°20' S, 50°16' W), Tapes (30°40' S, 51°22' W), Mostardas (31°06' S, 50°57' W), Rio Grande (32°01' S, 52°04' W). Santa Catarina: Florianópolis (27°34' S, 48°34' W), Concórdia (27°13' S, 52°01' W).

**Bonvicino *et al.* (2001)** - Brazil. Minas Gerais: Peirópolis (19°43' S, 47°45' W), Juramento (16°49' S, 43°34' W), Alto Caparaó (20°31' S, 41°53' W). Rio de Janeiro: Teresópolis (22°25' S, 42°58' W), Nova Friburgo (22°16' S, 42°31' W), Sumidouro (22°03' S, 42°40' W). São Paulo: Rio Claro (22°23' S, 47°32' W), Intervales (24°12' S, 48°30' W), Pedreira (22°43' S, 46°55' W). Santa Catarina: Florianópolis (27°34' S, 48°34' W), Ita (27°16' S, 52°19' W).

**Brum-Zorrilla *et al.* (1987)** - Uruguay. Maldonado (34°58' S, 54°57' W), Paysandu (32°04' S, 57°55' W), Colônia (34°09' S, 58°10' W), Durazno (33°24' S, 56°30' W).

**Myers and Carleton (1982)** - Argentina. Las Palmas (27°04' S, 58°42' W), Caraguatay (26°37' S, 54°46' W). Paraguay. Ibycuí (26°01' S, 57°02' W), Carayaó (25°10' S, 56°23' W), Cerro Corá (22°37' S, 55°58' W), Pedro Juan Caballero (22°34' S, 56°46' W), Sommerfeld Colony

(25°25' S, 55°43' W), Central (25°16' S, 57°34' W), Asunción (25°16' S, 57°40' W), Itapua (27°19' S, 55°53' W), San Rafael (27°07' S, 56°22' W), Rio Pirapó (26°51' S, 55°31' W), Sapucay (25°40' S, 56°55' W), San Ygnacio (26°52' S, 57°02' W).

**Svartman M (Levantamento cariotípico de roedores da região do Distrito Federal. MSc Dissertation, Universidade de São Paulo, São Paulo, Brazil, 1989)** - Brazil: Brasília (15°46' S, 47°55' W).

**Zanchin NIT (Estudos cromossômicos em orizomídeos e equimídeos da Mata Atlântica. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, 1988)** - Brazil. Bahia: Rio de Uma (15°18' S, 39°04' W). Espírito Santo: Monte Verde (20°38' S, 41°47' W). Rio Grande do Sul: Morro Alto (29°46' S, 51°11' W), Emboaba (29°58' S, 50°12' W), Faxinal (30°18' S, 51°40' W), Alto Ferrabraz (29°35' S, 50°56' W), Picada Verão (29°35' S, 51°02' W), Pontal Morro Alto (29°48' S, 50°09' W). São Paulo: Casa Grande (22°25' S, 49°55' W).

**Sbalqueiro ij (análises cromossômicas e filogenéticas em algumas espécies de roedores da região Sul do Brasil. PhD Thesis, Porto Alegre, Brazil 1989)** - Brazil. Paraná: Piraquara (25°26' S, 49°03' W). Rio Grande do Sul: Taim (32°02' S, 52°05' W). Esmeralda (28°03' S, 51°11' W), Porto Alegre (30°01' S, 51°13' W), Tupanciretã (29°04' S, 53°50' W).

**Geise L [Os roedores Sigmodontinae (Rodentia, Muridae) do Estado do Rio de Janeiro: sistemática, citogenética, distribuição e variação geográfica. PhD Thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 1995]** - Brazil. Minas Gerais: Viçosa (20°45' S, 42°53' W). Rio de Janeiro: Nova Friburgo (22°26' S, 42°32' W).

**Espinosa and Reig (1991)** - Argentina. Buenos Aires: Isla Ella (34°40' S, 58°30' W).

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