CARYOLOGIA Vol. 60, no. 3: 233-244, 2007

Meiotic effects of Robertsonian translocations in tuco-tucos of the *Ctenomys perrensi* superspecies (Rodentia: Ctenomyidae)

LANZONE *[1,2] CECILIA, MABEL D. GIMÉNEZ [1,3], JUAN L. SANTOS [4] and CLAUDIO J. BIDAU [5]

[1] Laboratorio de Genética Evolutiva, Universidad Nacional de Misiones, (3300) Posadas, Misiones, Argentina.
[2] Grupo de Investigaciones de la Biodiversidad, Instituto Argentino de Investigaciones en Zonas Áridas, CRICYT, CONICET, Parque Gral. San Martín, Av. Ruiz Leal s/n, CC 507, (5500) Mendoza, Argentina. Tel. (0261)-44280080.
[3] Department of Biology, University of York, PO Box 373, York YO10 5YW, UK.
[4] Departamento de Genética, Facultad de Biología, Universidad Complutense de Madrid, (28040) Madrid, Spain.
[5] Laboratório de Biologia e Parasitologia de mamíferos Silvestres Reservatórios, Departamento de Medicína Tropical, Instituto Oswaldo Cruz, (21046-900) Rio de Janeiro, Brazil.

Abstract — Meiotic behaviour was studied in males of the *Ctenomys perrensi* superspecies from Argentina that show variations in their chromosome number mostly due to Robertsonian translocations (Rb). A significant positive correlation between cell chiasma frequencies and total chromosome numbers was found. The reduction in chiasma frequency observed in individuals with Rb rearrangements occurred mainly at expenses of proximal and interstitial chiasmata, although significant differences between Rb trivalents and bivalents both in chiasma distribution and univalent frequency were also observed. Nevertheless, not all changes in chiasma distributions could be ascribed to Rb rearrangements. Chromosome synapsis was analysed in Rb hetero- and homozygotes. Trivalents showed a high frequency of synapsis at pericentromeric regions, suggesting no mechanical incompatibilities or delay in the synaptonemal complex formation in such regions. The relationship between chiasma distributions and synaptic patterns is discussed and a hypothesis about the possible role of telocentric or subtelocentric regions in these processes is proposed. It is also concluded that one or two Rb translocations in heterozygosis have weak direct effects on the fertility of the male carriers; therefore *C. perrensi* superspecies may be prone to maintain Rb chromosomal rearrangements.

Key words: chiasma frequency, chiasma localisation, chromosomal rearrangements, *Ctenomys*, synaptonemal complex.

INTRODUCTION

Robertsonian (Rb) translocations are common as chromosome polymorphisms or fixed differences between populations (BAKER *et al.* 1985; BIDAU 1990; HATFIELD *et al.* 1992; NACHMAN 1992a; SEARLE 1993; SEARLE *et al.* 1993; ROGATCHEVA *et al.* 1997; 1998; BIDAU and MARTÍ 2002; PIÁLEK *et al.* 2005). In Rb heterozygous meiosis, the bi-armed chromosome and its acrocentric homologues must pair, form chiasmata, orientate and segregate disjunctionally which is that expected in balanced polymorphisms, but not when Rb rearrangements act as isolating mechanisms (WHITE 1978; HEWITT 1979; JOHN 1983; KING 1993; RIESEBERG 2001). Secondary contact between populations differing for Rb translocations produces hybrid zones where hybrids may show decreased fertility relative to the parental forms. Synaptic failure, association of unsynapsed autosomal segments with asynaptic regions of sex chromosomes, and other factors affecting early presynaptic stages have been related to decreases of fertility in Rb heterozygotes (KING 1993). Furthermore, Rb rearrangements usually modify chiasma frequency and distribution patterns, which may disrupt groups of coadapted genes (BIDAU *et al.* 2001).

Fossorial *Ctenomys* rodents (tuco-tucos) are endemic to South America, and represent the most chromosomally variable mammal genus. *Ctenomys* includes 67 named species (BIDAU 2006, and personal observations), and chromosome numbers range from 2n=10 in *C. steinbachi* to 2n=70 in *C. dorbignyi* and *C. pearsoni* (KIBLISKY *et al.* 1977; ANDERSON *et al.* 1987; ORTELLS

* Corresponding author: e-mail: celanzone@lab.cricyt.edu.ar

et al. 1990; ARGÜELLES *et al.* 2001). Karyotypes of *Ctenomys* species are diverse, owing to a wide range of rearrangements (ORTELLS *et al.* 1990; ORTELLS 1995; BRAGGIO *et al.* 2000; GIMÉNEZ *et al.* 1997; 1999; 2002; ARGÜELLES *et al.* 2001; BIDAU 2006). However, meiotic effects of chromosomal rearrangements are poorly understood in this genus (LANZONE *et al.* 2002).

A group of closely related *Ctenomys* populations, inhabiting Corrientes province (Argentina), the *C. perrensi* superspecies (ORTELLS 1995; GIMÉNEZ *et al.* 2002; LANZONE *et al.* 2002), shows a heterogeneous chromosomal constitution. Diploid numbers range from 40 to 70 mainly due to a system of Rb translocations (ORTELLS *et al.* 1990; GIMÉNEZ *et al.* 2002). Since this superspecies is of recent evolutionary origin (GIMÉNEZ *et al.* 2002), it is feasible that hybridisation between chromosomally distinct populations could produce hybrids with reduced fertility due to meiotic disturbances. If the chromosomal rearrangements are of recent origin, compensatory mechanisms for the restoration of fertility, and the maintenance of polymorphysm, might have not yet arisen. However, comparison of molecular and chromosomal data has shown that relatively neutral variants of Rb chromosomes are formed and maintained more frequently in some taxa than in others (SEARLE 1993; NACHMAN and SEARLE 1995; COLANGELO *et al.* 2005). Previous results in *Ctenomys*, indicate that the sex chromosome pair displays a pattern of axis differentiation during pachytene that leads to full synapsis of both X and Y chromosomes (LANZONE *et al.* 2002). This behaviour might prevent possible interactions with unsynapsed regions of Rb trivalents that could lead to infertility (KING 1993; TURNER *et al.* 2005).

In this paper, we analyse chiasma frequencies, chiasma distributions and synaptic patterns of Rb hetero- and homozygotes males of the *C. perrensi* superspecies to assess the effects of chromosomal repatterning in this group of tuco-tucos.

MATERIALS AND METHODS

Trapping locality and the number of specimens of *Ctenomys perrensi* superspecies *("C. perrensi")* analysed in this study are indicated in Table 1 and Fig. 1. In addition, specimens of different species, namely: *C. roigi* and *C. dorbignyi* that belong to the same species complex (GIMÉNEZ *et al.* 2002), *C. opimus*, a species with low chromosome number from a different lineage, and an Octodontid (the sister familiy of the Ctenomyidae) with 2n= 102, *Tympanoctomys barrerae*, that were not heterozygous for Rb translocations were used as controls (Table 1). Karyotypying followed a bone marrow short-term culture protocol (GIMÉNEZ *et al.* 1999). Meiotic preparations were made according to EVANS *et al.* (1964). C-banding was used to detect centromere location and chiasma positioning. The procedure used was that of EIBERG (1974): slides were aged at room temperature 7 - 10 days, incubated in Earle's solution, pH= 8.5-9.0 at 85°C for 30 min, washed with distilled water, air-dried, and stained with 4% buffered Giemsa. Chiasmata were recorded at diakinesis as: proximal (P), interstitial (I) and distal (D) relative to the centromere (BIDAU *et al.* 2001). Means, standard deviations and coefficients of variation ([sd/mean]*100) were calculated. Normality of data was estimated through the Kolmogorov-Smirnov test (ZAR 1984; 1999). For the purposes of regression, mean chiasma frequencies were *log*-transformed and the *arcsin* transformation was applied to coefficients of variation (STEELE and TORRIE 1980). Analysis of Variance (ANOVA), non-parametric correlation and linear and non-linear regressions were employed for data analyses. The XY bivalent, which normally shows a distal chiasma (LANZONE *et al.* 2002), was excluded from both the chiasma analyses and the calculation of the number of chromosome arms (Fundamental Number, FN), which then became in the Autosomal FN (AFN). Both parameters were *log*-transformed for statistical purposes.

Observations of synaptonemal complexes (SC) were carried out according to the method described by LANZONE *et al.* (2002). Briefly, meiotic cells were released in TC 199 medium and centrifuged 3 times for 15 min at 1500 rpm. The spreading medium (1% Triton X-100, pH=7.5) was added on a plastic-coated slide with two drops of the cell suspension and left for 12 min. The cells were fixed with 4% paraformaldehyde. Slides were stained with aqueous $AgNO_3$ (50%), selected cells transferred to EM grids and examined under a Jeol 1010 electron microscope.

RESULTS

Meiotic characteristics of tuco-tucos and chiasma responses to variations in diploid number - Populations along the Saladas- Mburucuyá transect were polymorphic for Rb rearrangements: all karyomorphs but one were heterozygous for one or two Rb translocations (Fig. 2; Table 1). The three males from Chavarría formed 29 bivalents in

Table 1 — Localities, geographic coordinates, diploid chromosome numbers (2n) and number of Rb trivalents (III), autosomal fundamental number (AFN), and mean frequencies of total (T), proximal (P), interstitial (I) and distal (D) chiasmata per cell (±SD) in males of the *Ctenomys perrensi* superspecies and in males from the selected control species of *Ctenomys* and *Tympanoctomys*. The asterisks indicates those individuals selected for SC study.

Taxon	Locality	Coord.	2n (III)	AFN	Mean cell chiasma frequencies ± SD			
					T	P	I	D
"C. perrensi"	Curuzú Laurel	27°56' S 57°30' W	42* (0)	72	28.90±1.73	1.80±1.40	6.10±1.29	21.00±3.65
	San Miguel	28°01' S 57°36' W	44 (0)	72	29.80±1.55	2.10±0.99	6.50±1.65	21.20±2.86
	Estancia Rosarito (Km. 48)	28°06' S 58°17' W	51 (1)	76	32.00±0.71	2.20±0.84	6.00±2.35	23.80±2.59
			52* (2)	76	32.00±1.66	5.11±1.05	7.44±1.67	19.44±2.46
	Pago Alegre (Km. 28)	28°05' S 58°22' W	56* (2)	80	34.44±2.01	4.33±1.00	6.00±2.12	24.11±2.32
	Pago de los Deseos (Km. 10)	28°15'S 58°31' W	56* (2)	80	35.14±1.68	3.71±1.60	7.86±1.86	23.57±3.15
	Pje. Sto. Domingo (Km. 7,5)	28°15' S 58°33' W	55 (1)	78	34.89±1.54	2.22±0.83	5.44±1.42	27.22±2.49
			54 (2)	78	34.40±1.14	4.40±1.14	6.80±1.64	23.20±1.48
	Chavarria	28°56' S 58°36' W	58 (0)	80	38.56±1.33	5.44±0.73	6.22±1.09	26.89±1.90
			58 (0)	80	37.89±1.05	5.89±1.05	6.11±1.83	25.67±2.87
			58 (0)	80	38.17±1.17	5.67±1.21	6.83±1.33	25.67±2.07
	Saladas	28°16' S 58°39' W	54 (0)	82	35.50±2.56	2.50±0.93	6.75±1.28	26.25±3.77
			55 (1)	82	35.25±1.91	1.50±0.76	5.88±1.64	27.88±3.04
	Santa Rosa	28°11' S 58°07' W	66 (0)	80	37.33±2.70	8.83±1.17	10.17±1.17	18.33±2.50
			65* (1)	80	38.78±1.20	8.44±1.13	9.89±1.05	20.44±1.67
C. roigi	Costa Mansión	28°02' S 58°49' W	48 (0)	76	31.00±0.00	2.50±2.12	4.00±0.00	24.50±2.12
C. dorbignyi	Mbarigüí	27°33' S 57°31' W	70 (0)	80	40.13±2.75	6.20±1.92	6.40±2.19	27.40±3.97
C. opimus	Piedra del Molino	25°11' S 65°51' W	26 (0)	48	21.00±1.58	3.20±1.30	4.00±1.22	13.80±1.79
T. barrerae	El Nihuil	35°02' S 68°40' W	102 (0)	198	55.50±2.56	8.50±3.66	7.88±2.80	40.38±4.53

meiosis. One of the analysed males from Santa Rosa showed 33 bivalents (2n= 66), while the other one had 2n= 65 and showed a Rb trivalent. On the other hand, specimens from Curuzú Laurel and San Miguel had either 22 or 21 bivalents (Table 1; Fig. 2). Karyotypic and chiasmatic data of individuals from control species are shown in Table 1; any of them was heterozygous for Rb translocations.

An ANOVA was conducted to determine whether total chiasma frequency varied among "*C. perrensi"* karyotypes. Homogeneity of variances was determined by the Bartlett's test (SOKAL and ROHLF 1998) (χ^2=16.82; df=14; 0.20 <P< 0.30). Differences among karyotypes were statistically highly significant (F=27.97; df=1,14, P<0.01).

Since the diploid chromosome number (2n) and the AFN departed from normality despite *log*-transformation, non-parametric Spearman correlations were calculated between 2n, AFN, the frequencies of T, P, I and D chiasmata, and their respective coefficients of variation (CV) (Table 2). It can be observed that in "*C. perrensi*" both total (T) and proximal (P) chiasmata increase significantly with 2n. T chiasmata also increase with AFN; however, a partial correlation analysis using 2n as a control variable, eliminated the statistical significance of the T/AFN correlation (r= 0.496; df= 12; *P*= 0.071). It is noteworthy that the coefficient of variation of P chiasmata was negatively correlated with 2n (Table 2; Fig. 3b) and also with the frequency of P chiasmata (Fig. 3c). Similar tendencies and correlations were obtained when the control species were included (Table 2), with the exception of a significant negative correlation between interstitial (I) and 2n.

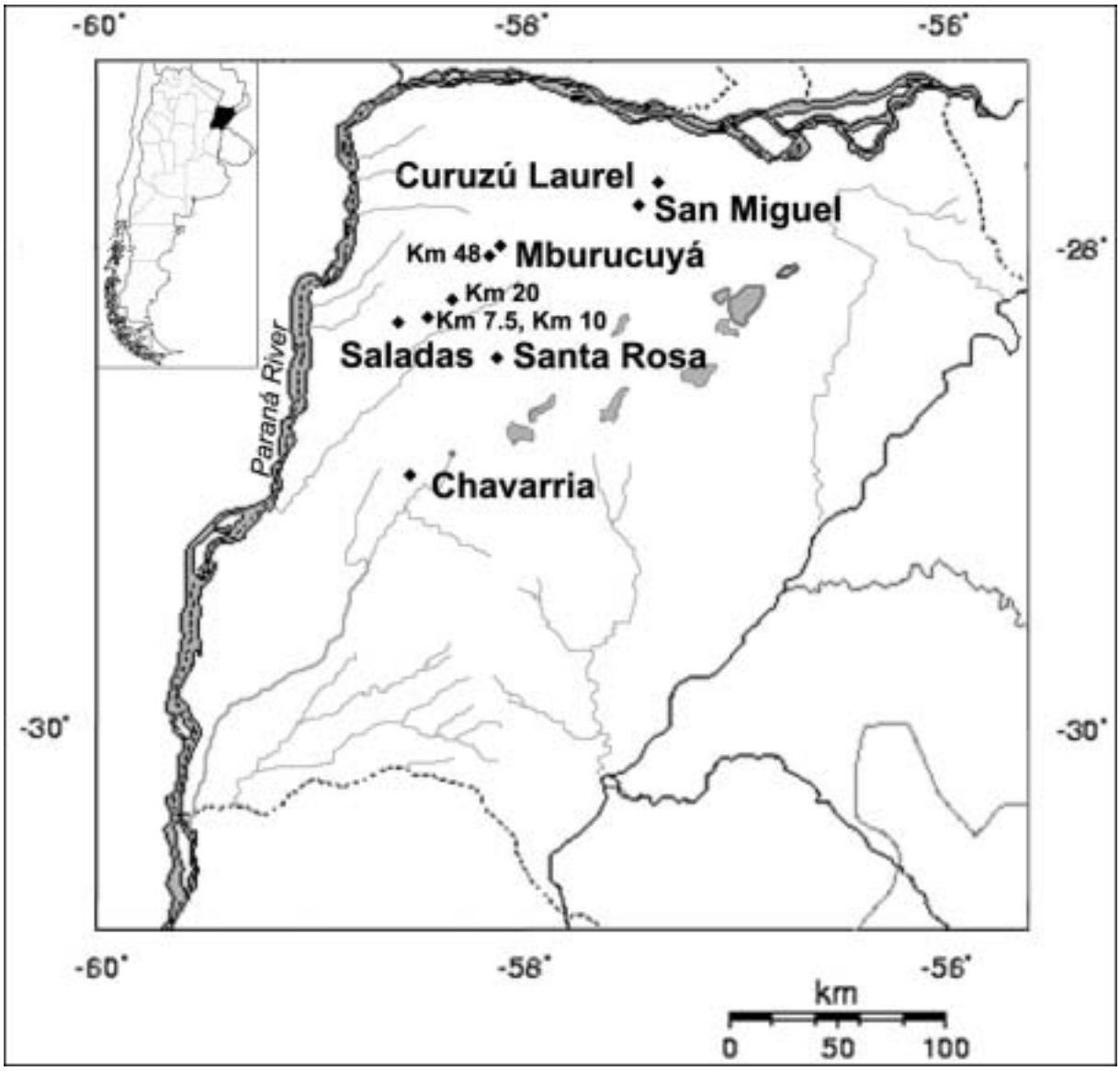

Fig. 1 — Sampling localities of the *Ctenomys perrensi* superspecies.

Also, the correlation between T and AFN lost its statistical significance when 2n was the control variable (r= 0.31; df= 16; *P*= 0.356) although that between D and AFN was maintained (r- 0510; df= 16; *P*= 0.031).

Despite many of the relationships were not linear, we have calculated linear regressions between T, P, I and D chiasmata to best visualise the variations of chiasma frequencies in relation to 2n. Nevertheless, we have included the best non-linear models obtained to understand best the dynamics of the chiasmata/chromosome morpholgy relationship (Table 3). In "*C. perrensi*" there were positive correlations between T, P and I chiasmata frequencies and the chromosome number (Table 3; Fig. 3a). However, the frequency of D chiasmata was not related to the variation in chromosome number (Fig. 3a; Table 3) although a complex non-linear relationship between D and 2n may exist (Table 3). When the control species were included in the analyses the same tendencies were found (Table 3; Fig. 3b).

All Rb trivalents exhibited similar chiasma frequencies and distributions, despite having originated from independent Rb translocations (contingency χ^2= 12.41; d.f.= 20; 0.900<P<0.975). However, all trivalents had higher frequencies of P and I chiasmata than the rest of the chromosome complement (contingency χ^2=121.41; gl=2; P<0.001) (Table 4).

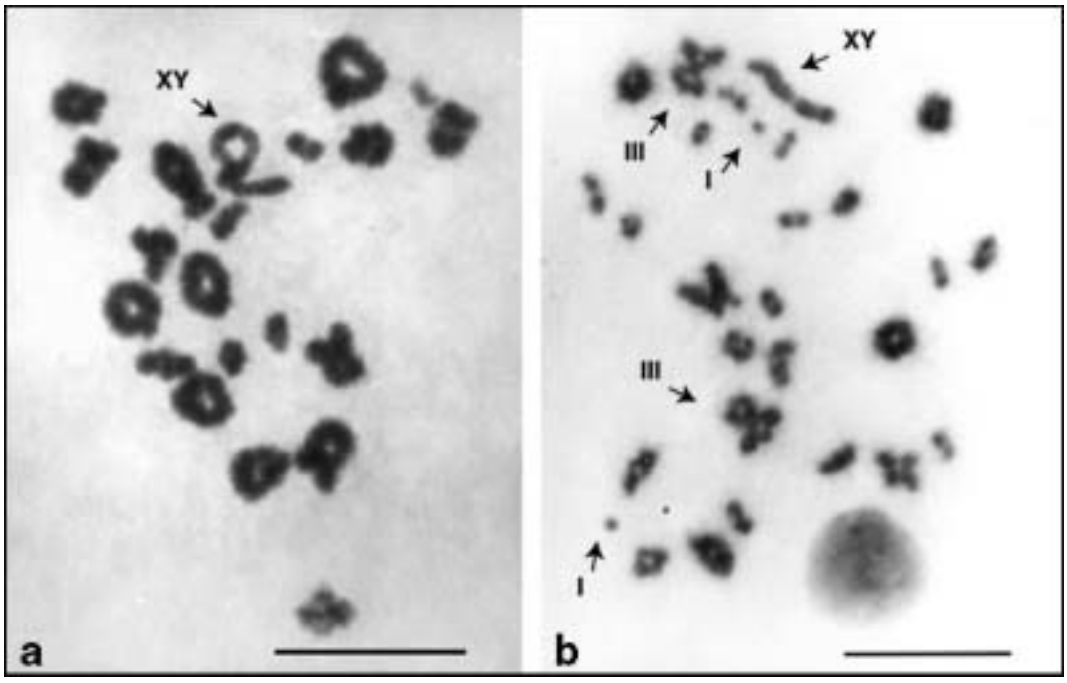

Fig. 2 — Diakinesis **c**ells from individuals of "*Ctenomys perrensi*" with different chromosome constitutions. (a) 2n = 42, structural homozygote. (b) 2n = 56, double Robertsonian heterozygote. Note the presence of two autosomal univalents (arrows). III = Robertsonian trivalent, I = univalent, XY = sex pair. Bar = 10 μm.

Table 2 — Spearman correlation coefficients between *log*-transformed total (T), proximal (P), interstitial (I) and distal (D) mean chiasma frequencies, their respective *arcsin*-transformed coeffcients of variation (CV), and diploid chromosome numbers (2n) and AFNs (*log*-transformed) of all karyotypes in the *C. perrensi* superspecies (upper rows) and when the control species were added to the analyses (lower rows). Probability (in italics) is indicated for all significant correlations. Two marginally significant correlations are shown in bold type. ns = non-significant.

Taxon		Spearman's *rho*							
		arcsin Chiasma Frequencies				Coefficients of Variation			
		T	P	I	D	CVT	CVP	CVI	CVD
"*C. perrensi*"	2n	0.892 *0.000*	0.801 *0.000*	ns	ns	ns	-0.766 *0.001*	ns	ns
"*C. perrensi*"	AFN	0.758 *0.001*	ns	ns	**0.492** ***0.063***	ns	ns	ns	ns
All species	2n	0.947 *0.000*	0.808 *0.000*	0.566 *0.012*	**0.438** ***0.061***	ns	-0.570 *0.011*	ns	ns
All species	AFN	0.841 *0.000*	ns	ns	0.639 *0.003*	ns	ns	ns	ns

Incidence of univalents - Autosomal univalents, originated from chiasma failure in bivalents and trivalents, were recorded at diakinesis (Table 4; Fig. 2b). Individuals heterozygous for Rb translocations tended to show a higher frequency of univalents per cell (18.03%) than homozygotes (6.90%). Desynapsis of the XY pair was observed at relatively low frequencies (0-20%), although the Santa Rosa males had the highest frequency of XY dissociation, 30% (Table 5).

Analysis of Synaptonemal Complexes (SCs) - Six complete pachytene nuclei from the Curuzú Laurel male (2n=42) (Table 1), were selected and measured on photographic enlargements. The total average autosomal haploid SC length was 258.06 ± 10.38 μm. In addition, 53 trivalents from thirty nuclei belonging to four individuals were studied (Table 1).

At pachytene, trivalents presented different patterns of synapsis although neither complex

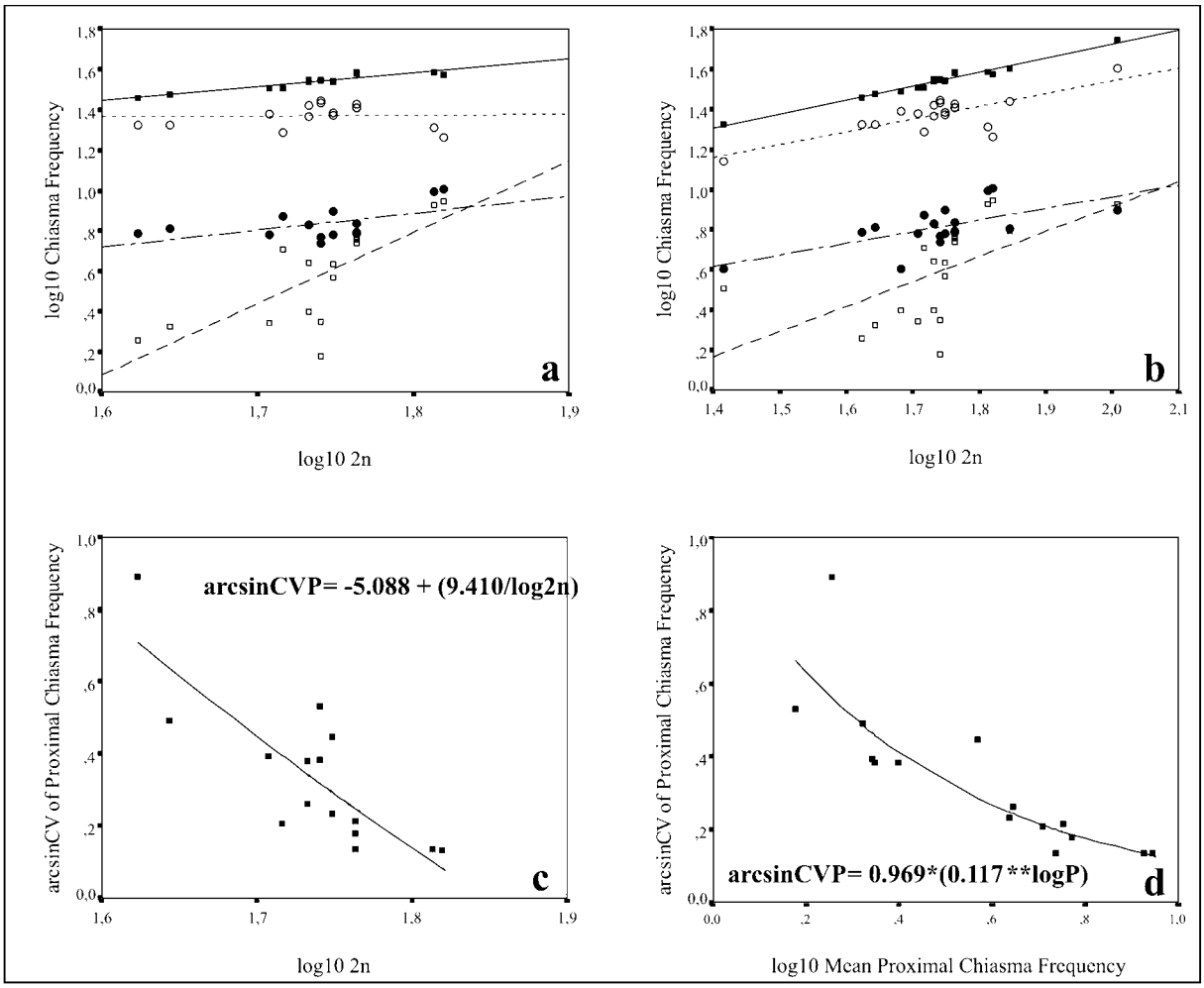

Fig. 3 — Linear regressions between log-transformed total (■), proximal (□), interstitial (●) and distal (○) chiasma frequencies and log-2n of, **a.** Males of "*C. perrensi*" with different karyotypes (2n= 42-66) and **b**. Males of all species analysed (2n= 42-102) (see Table 3 for regression equations). **c**. Non-linear regression (inverse model) between the coefficient of variation (*arcsin*CV) of proximal chiasma frequency and 2n in males of "*C. perrensi*" with different karyotypes. **d**. Non-linear regression (compound model) between the coefficient of variation (*arcsin*CV) of proximal chiasma frequency and log-transformed proximal chiasma frequencies of males of "*C. perrensi*" with different karyotypes.

Table 3 — Linear and non-linear regression equations between *log*-transformed total (T), proximal (P), interstitial (I) and distal (D) chiasma frequencies and the log 2n in the *C. perrensi* superspecies (upper row of each pair of equations) and when the control species were added to the analyses (lower row of each pair). F= F statistic; *P*= statistical significance.

Relationship	Linear regression	F	*P*	Non-linear regression	F	*P*
T vs. 2n	T= 0.324 + 0.701*2n	73.26	0.000	T= e**[1.216+(-1.361/2n)]	79.05	0.000
	T= 0.320 + 0.703*2n	507.23	0.000	T= 0.689 + (1.588**2n)	551.92	0.000
P vs. 2n	P= - 5.581 + 3.541*2n	16.76	0.001	P= 6.499+(-10.293/2n)	15.71	0.002
	P= - 1.575 + 1.245*2n	8.72	0.009	P= 5.842 – 7.481*2n + 2.555*2n**2	5.80	0.013
I vs. 2n	I= - 0.636 + 0.846 *2n	5.80	0.032	I= 11.884 – 11.721*2n**2 + 4.633*2n**3	10.22	0.003
	I= - 0.198 + 0.581 *2n	11.72	0.003	I= e**[1.048 + (-2.189/2n)]	13.76	0.002
D vs. 2n	D= 1.289 + 0.047*2n	0.020	0.879	D= -19.286 – 18.004*2n - 2.021*2n**3	5.10	0.025
	D= 0.272 + 0.635 *2n	24.24	0.000	D= e**[1.085 + (-1.330/2n)]	25.10	0.000

Table 4 — Relative frequencies of proximal (P), interstitial (I) and distal (D) chiasmata in bivalents (II) and trivalents (III) of "*Ctenomys perrensi*" heterozygous for Rb translocations.

Individual	II			III		
	P	I	D	P	I	D
2n=51	0.05	0.18	0.77	0.25	0.33	0.42
2n=52	0.14	0.22	0.64	0.32	0.29	0.39
2n=54	0.09	0.21	0.70	0.39	0.09	0.52
2n=55 (Km 7.5)	0.05	0.15	0.80	0.29	019	0.52
2n=55 (Saladas)	0.03	0.17	0.80	0.19	0.19	0.62
2n=56 (Km 10)	0.07	0.22	0.71	0.29	0.27	0.44
2n=56 (Pago Alegre)	0.10	0.15	0.75	0.36	0.33	0.31

Table 5 — Frequency of univalents at diakinesis in different karyomorphs of the *Ctenomys perrensi* superspecies. Univalents produced by bivalents (II), Rb trivalents (III) or the sex pair (XY) are discriminated.

Number of heterozygous Rb translocations	2n	Frequency of autosomal univalents			XY univalents	Number of cells
		II	III	Total		
1	55	0.2	0.2	0.4	0.1	9
1	51	0	0	0	0	5
1	55	0	0.1	0.1	0	8
2	56	0	0.1	0.1	0	7
0	54	0.1	0	0.1	0.1	8
2	52	0	0.2	0.2	0.1	9
2	54	0.2	0	0.2	0.2	5
2	56	0.2	0	0.2	0.1	9
0	58	0.1	0	0.1	0.1	9
0	58	0	0	0	0.1	9
0	58	0.2	0	0.2	0.2	6
0	44	0	0	0	0	10
0	42	0	0	0	0.1	10
0	66	0.2	0	0.2	0.3	6
1	65	0	0	0	0.3	9

multivalent SCs nor associations with the XY pair were observed (Fig. 4). Thus, 24% of trivalents (13/53) showed complete synapsis (Fig. 4a). In 49% of configurations side arms involving both acrocentric chromosomes (25/53) or fold-back loops in one of the acrocentrics (1/53) were observed, suggesting non-homologous synapsis at pericentromeric regions (Fig.4b). In 27% of trivalents (14/53) some asynapsis at the centromeric ends of acrocentric chromosomes was recorded (Figs.4c, d and e). C*is* configurations of the acrocentric centromeres with respect to the metacentric centromere were more frequent than those in *trans*, (Figs. 4c, d and e).

DISCUSSION

Examples of polymorphic Rb fusions are found in mammals as *Mus musculus domesticus, Holochilus brasiliensis, Sorex* spp. and *Suncus murinus* (Nachman 1992a; b; Zima *et al.* 1994; Rogatcheva *et al.* 1997; 1998; Bidau *et al.* 2001), and insects (Bidau 1990; Bidau and Martí 2002). Apart from an instantaneous decrease of interchromosomal recombination by reducing independently assorting elements, fusions reduce intrachromosomal recombination and modify chiasma patterns correlated with the number of Rb configurations present (Bidau 1990; Nachman 1992b; Bidau *et al.* 2001; Bidau and Martí 2002; Castiglia and Capanna 2002; Dumas and Britton-Davidian 2002).

In tuco-tucos of the *C. perrensi* superspecies a comparable repatterning of chiasmata occurs. Karyomorphs with different Rb translocations show a reduction of chiasma frequency and changes in chiasma distribution, suggesting that Rb translocations may be involved in the control of recombination thus, determining the potential amount of variability released by different karyomorphs in different populations (Bidau *et al.* 2001) (however, see below). The mechanics involved in chiasma repatterning are poorly known (Martí and Bidau 2001). In *Ctenomys*, the reduc-

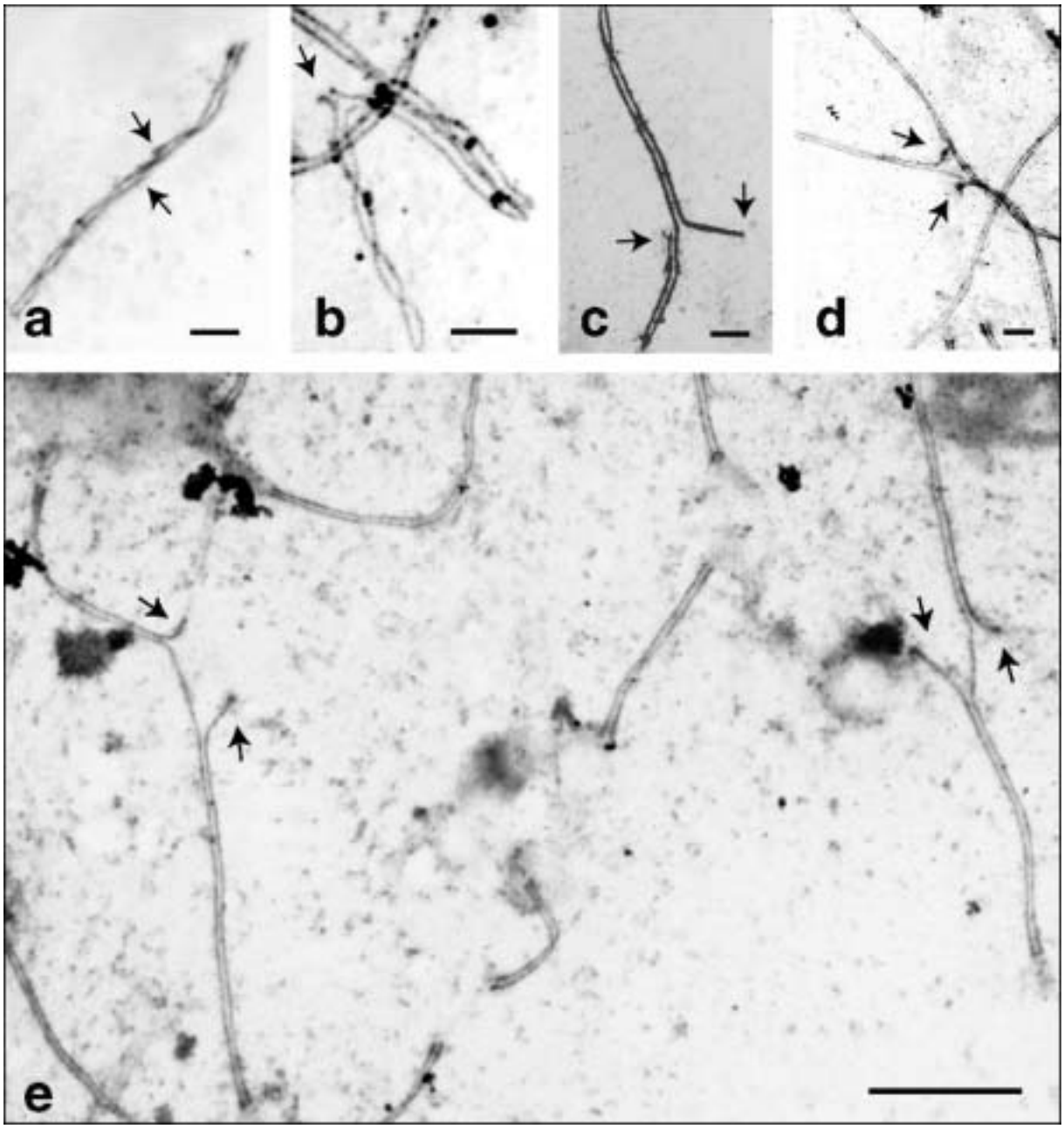

Fig. 4 — Selected pericentromeric regions of the acrocentric chromosomes (arrows) in pachytene Robertsonian trivalents of *"C. perrensi"* illustrating different synaptic features. a. Full linear synapsis between the three chromosomes. b. Side arm SC formed by heterosynapsis between the pericentromeric regions of both acrocentric chromosomes. c. Asynapsis leading to one free acrocentric end. Acrocentric centromeres in *trans* configuration. d. Small asynapsed region comprising both centromeric ends of acrocentric chromosomes with terminal thickenings. e. Asynapsis leading to two acrocentric ends in trivalents of a double Rb heterozygote. Note the different configurations of the centromeres, in *trans* (right) and in *cis* (left). Bar = 1 μm.

tion of total chiasma frequency is produced mainly by the elimination of non-distal chiasmata as has been reported in other species. Nevertheless, several points are worth of discussion.

i) Although there is a general decrease of total chiasma frequency when the chromosome number decrease, it is clear from the comparison within the "*C. perrensi*" group that not all chromosomal variation is due to Rb translocations since AFN varies between 72 and 82. Furthermore, this is more obvious when the control species are incorporated to the analyses. In this case,

AFN (48 to 198 autosomal chromosome arms) varies much more than 2n (26 to 102 chromosomes). In both instances, the relationship between AFN and 2n is a complex non-linear (cubic) one indicating that a number of other chromosomal rearrangements, that could also affect chiasmata (albeit in different ways), have certainly played an important role in chromosomal evolution of the group. This fact could also explain the non-linearity of some of the responses of chiasma frequencies to change in diploid number, while in cases where Rb translocations are the only source of 2n variation (without AFN variation) responses are always basically linear (Bidau 1990; 1993; Bidau and Martí 1995; Bidau *et al.* 2001; Castiglia and Capanna 2002; Dumas and Britton-Davidian 2002).

ii) It has recently been suggested that in mammals, reciprocal recombination (and thus frequency of chiasmata) is proportional to the number of chromosome arms in the karyotype (Pardo Manuel de Villena and Sapienza 2001). Within our sample, we found such a correspondence between chiasmata and AFN but, its significance dissapeared when 2n was included as a control variable, which was not tested by these authors in their sample of distantly related species. This result suggests that the number of chromosome arms may predict chiasma frequency when Rb translocations are the main mechanism of chromosome change in morphology, but not when other rearrangements are involved. This is not to say that the number of chromosome arms does not affect chiasma frequency, but the total number of chromosomes is a more realiable predictor of the amount of recombination within a species.

iii) Unlike other studies, we did not find statistically significant relationships between chiasma frequencies and their variability. Usually, when only Rb variation is involved, higher chiasma frequencies are positively associated with higher variability of T, P or I chiasmata (Bidau 1990; Bidau and Martí 2002). In the case reported here, the only significant relationships were those between CVP and 2n, and CVP and P chiasma frequency but both were, surprisingly, negative. Proximal chiasmata are the first to be suppresssed when Rb fusions occur because of the problems they impose on segregation of trivalents (Bidau 1990; Bidau and Martí 1995), thus a reduction of P chiasmata is accompanied by a decrease in their variability. The situation here observed is difficult to explain but again it may be due to the fact that multiple (and not only Rb) rearrangements have operated in this group. Since the number of metacentrics increases from the highest to the lowest 2n values in our samples, and metacentrics usually have less P chiasmata than expected, this could imply an opposite trend to the decrease in the number of P chiasmata with 2n, determining an increase in their variability. In other species (i. e. *D. pratensis,* Martí and Bidau 2001) chiasma formation is directly related to pairing and synaptic patterns. Thus, chiasmata would have a P-D distribution in acrocentric bivalents and a D-D distribution in metacentric ones if synapsis starts at the ends of chromosomes and proceeds towards the centromeres (Bidau 1990; 1993; Bidau and Martí 1995; Martí and Bidau 1995; 2001). In male meiosis of tuco-tucos, synapsis is normally initiated at chromosome ends, thus chiasma repatterning may obey the same principles as in *D. pratensis* (Bidau 1990; 1993; 1996; Martí and Bidau 2001): chiasmata tend to be more probably formed in chromosomal regions that pair first and remain synapsed longer. The former would explain why the frequency of I chiasmata decreases less than half than that of P chiasmata when chromosome number decreases as a consequence of Rb translocations and, why P chiasmata would be the less likely to be produced in Rb configurations.

This model of chiasma repatterning explains the chiasmatic behaviour of Rb metacentric bivalents and trivalents if the underlying mechanism of chiasma formation is identical for both types of Rb configurations (Bidau 1993; Bidau and Martí 1995). However, in the case of *Ctenomys*, Rb trivalents showed higher than expected frequencies of P and I chiasmata, while Rb bivalents behaved according to the model. Comparable results were found in Rb trivalents of *Mus musculus domesticus* (Bidau *et al.* 2001; Castiglia and Capanna 2002) and perhaps, in *Holochilus brasiliensis* (Nachman 1992b). Thus, what is the plausible mechanism behind the differences in chiasma formation between Rb heterozygotes and homozygotes in *Ctenomys*? A testable hypothesis is that telomeric or subtelomeric regions might be involved in recombination initiation. If Rb translocations of *Ctenomys* lead to the loss of telomeric regions of acrocentrics then, Rb metacentric bivalents might be devoid of them in pericentromeric regions of chromosomes while Rb trivalents would have two extra telomeric regions as represented by both acrocentric chromosomes. The latter may explain the excess of P and I chiasmata in trivalents. Moreover, if both telomeric and subtelomeric regions of the acrocentrics in the trivalent promote

chiasma formation independently, a cumulative effect on chiasma formation is expected in absence of chiasma interference across the centromere. In *Ctenomys* almost 50% of analysed trivalents presented non-homologous synapsis between the pericentromeric regions of both acrocentric chromosomes, and over 24% between these regions and the metacentric chromosome. This high frequency of synapsis in the pericentromeric regions suggests no mechanical incompatibilities or SC delay formation in the centromeric region of trivalents, as was described in mouse trivalents with crossover suppression (DAVISSON and AKESON 1993). Thus, SC formation may be a major factor related to chiasma distribution in tucotucos. The similarities in chiasma patterns observed in non related species with Rb translocations suggest that the effect of chiasma repatterning may be related to a general mechanism concerning the formation of metacentric chromosomes (BIDAU *et al.* 2001; DUMAS and BRITTON-DAVIDIAN 2002). At pachytene, the sex bivalent and most of Rb trivalents showed full synapsis that would prevent the deleterious effects produced by the maintenance of unsynapsed regions at the end of pachytene (LANZONE *et al.* 2002; TURNER *et al.* 2005). These findings suggest the existence of mechanisms that reduce the harmful effects of heterozygous chromosome configurations. Also, the higher frequency of univalents at diakinesis in Rb heterozygous individuals suggests the possibility of generating unbalanced gametes. However, the lack of correlation between the presence of univalents and the amount of trivalents in a given individual may indicate some influence of the genetic background and of the particular characteristics of the chromosomes involved, as was reported for other mammals (SEARLE 1993; NACHMAN and SEARLE 1995; ROGATCHEVA *et al.* 1998). The present study supports the idea that one or two Rb translocations in heterozygosis have weak direct effects on the fertility of the male carriers.

Some authors have proposed that relatively neutral variants of Rb chromosomes are formed and maintained more frequently in some taxa than in others (SEARLE 1993; NACHMAN and SEARLE 1995; COLANGELO *et al.* 2005). The maintenance of the chromosome polymorphism displayed by the *C. perrensi* superspecies may be promoted by the synaptic behaviour of sex chromosomes and Rb trivalents (LANZONE *et al.* 2002, this study). Also, the presence of proximal and interstitial chiasmata in trivalents may help maintain high levels of recombination in heterozygotes, perhaps balancing the effect of the reduction of interchromosomal recombination (BIDAU *et al.* 2001). Our data suggest that the *C. perrensi* superspecies may be "resistant" to the effects of Rb chromosomal rearrangements. However, no individual with more than two heterozygous Rb configurations has yet been found. We conclude that extreme Rb variation within the *C. perrensi* superspecies is probably not related to evolutionary divergence within this group, supporting the lack of substantial molecular differentiation between chromosomally distinct populations, and even Linnean species (MASCHERETTI *et al.* 2000; GIMÉNEZ *et al.* 2002). It is plausible that present chromosomal variation has been inherited from highly chromosomally polymorphic ancestors (GIMÉNEZ *et al.* 2002).

Acknowledgments — We are especially indebted to Pavel Borodin for his critical reading of a former draft of this manuscript. We thank Nelly Horak for her assistance with the English version and Esteban Braggio for his assistance during field work and his constant support and encouragement throughout this study. CL and CJB are affiliated to the CONICET (Argentina) and CJB is very grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil for a Visiting Researcher's grant that allowed the completion of this paper.

REFERENCES

ANDERSON S., YATES T.L. and COOK J.A, 1987 — *Notes on Bolivian mammals. 4. The genus Ctenomys in the eastern lowlands*. Am. Mus. Novit., 2891: 1-20.

ARGÜELLES C.F., SUÁREZ P., GIMÉNEZ M.D. and BIDAU C.J., 2001 — *Intraspecific chromosome variation between different populations of Ctenomys dorbignyi (Rodentia, Ctenomyidae) from Argentina*. Acta Theriologica, 46: 363-373.

BAKER R.J., BICKHAM J.W. and ARNOLD M.L., 1985 — *Chromosomal evolution in Rhogeessa (Chiroptera: Vespertilionidae): Possible speciation by centric fissions*. Evolution, 39: 233-243.

BIDAU C.J. and MARTÍ D.A., 1995 — *Male and female meiosis in Robertsonian heterozygotes of Dichroplus pratensis (Acrididae)*. In: P.E. Brandham and M.D. Bennett (Eds), "Kew Chromosome Conference IV", pp. 381-396. Royal Botanic Gardens Press, Kew.

BIDAU C.J. and MARTÍ D.A., 2002 — *Geographic distribution of Robertsonian fusions in Dichroplus pratensis (Melanoplinae, Acrididae): the centralmarginal hypothesis reanalysed*. Cytogenet. Genome Res., 96: 66-74.

BIDAU C.J., 1990 — *The complex Robertsonian system of Dichroplus pratensis (Melanoplinae, Acrididae). II*

Effects of the fusion polymorphisms on chiasma frequency and distribution. Heredity, 64: 145-159.

BIDAU C.J., 1993 — *Causes of chiasma repatterning due to centric fusions*. Braz. J. Genet. 16: 283-296.

BIDAU C.J., 1996 — *Chiasma repatterning in hybrids between chromosomal races of the grasshopper Dichroplus pratensis (Melanoplinae, Acrididae)*. Cytobios, 85: 91-110.

BIDAU C.J., 2006 — *Familia Ctenomyidae* Lesson, 1828. In: R.J. Barquez, M.M. Díaz, and R.A. Ojeda (Eds.), "Mamíferos de Argentina: Sistemática y Distribución". SAREM, Tucumán, Argentina (in press)

BIDAU C.J., GIMÉNEZ M.D., PALMER C.L. and SEARLE J.B., 2001 — *The effects of Robertsonian fusion on chiasma frequency and distribution in the house mouse (Mus musculus domesticus) from a hybrid zone in northern Scotland*. Heredity, 87: 305-313.

BRAGGIO E., GIMÉNEZ M.D., CONTRERAS J.R., JUSTO E.R. and BIDAU C.J., 2000 — *Karyotypic variation in populations of Ctenomys (Rodentia, Ctenomyidae) from La Pampa province (Argentina).* Caryologia, 52: 131-140.

CASTIGLIA R. and CAPANNA E., 2002 — *Chiasma repatterning across a chromosomal hybrid zone between chromosomal races of Mus musculus domesticus*. Genetica, 114: 36-40.

COLANGELO P., CORTI M., VERHEYEN E., ANNESSI F., OGUGE N., MAKUNDI R.H. and VERHEYEN W., 2005 — *Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus Tatera (Rodentia: Gerbillinae) in Africa*. Mol. Phylogenet. Evol., 35: 556-568.

DAVISSON M.T. and AKESON E.C., 1993 — *Recombination suppression by heterozygous Robertsonian chromosomes in the mouse*. Genetics, 133: 649-667.

DUMAS D. and BRITTON-DAVIDIAN J., 2002 — *Chromosomal rearrangements and evolution of recombination: comparison of chiasma distribution patterns in standard and Robertsonian populations of the house mouse*. Genetics, 162: 1355-1366.

EIBERG H., 1974 — *New selective giemsa technique for human chromosomes, Cd staining*. Nature, 248: 55.

EVANS E.P., BRECKON G., and FORD C.E., 1964 — *An air-drying method for meiotic preparations from mammalian testes*. Cytogenetics, 3: 289-294.

GIMÉNEZ M.D., CONTRERAS J.R. and BIDAU C.J., 1997 — *Chromosomal variation in Ctenomys pilarensis, a recently described species from eastern Paraguay (Rodentia, Ctenomyidae)*. Mammalia, 61: 385-398.

GIMÉNEZ M.D., BIDAU C.J., ARGÜELLES C.F. and CONTRERAS J.R., 1999 — *Chromosomal characterization and relationship between two new species of Ctenomys (Rodentia, Ctenomyidae) from northern Córdoba province, Argentina*. Z Säugetierkunde, 64: 91-106.

GIMÉNEZ M.D., MIROL P.M., BIDAU C.J. and SEARLE J.B., 2002 — *Molecular analysis of populations of Ctenomys (Caviomorpha, Rodentia) with high karyotypic variability*. Cytogenet. Genome Res., 96: 130-136.

HATFIELD T., BARTON N. and SEARLE J.B., 1992 — *A model of a hybrid zone between two chromosomal races of the common shrew (Sorex araneus).* Evolution, 46: 1129-1145.

HEWITT G.M., 1979 — *Orthoptera. Grasshoppers and Crickets*. In: B. John (Ed), "Animal Cytogenetics, Vol. 3 Insecta 1", p. 170. Gebrüder Borntraeger, Berlin-Stuttgart.

JOHN B., 1983 — *The role of chromosome change in the evolution of orthopteroid insects*. In: A.K. Sharma, and A. Sharma (Eds), "Chromosomes in the Evolution of Eukaryotic Groups", pp. 110. CRC Press, Boca Raton.

KIBLISKY P., BRUM-ZORRILLA N., PÉREZ G. and SAEZ F.A., 1977 — *Variabilidad cromosómica entre diversas poblaciones del roedor cavador del género Ctenomys (Rodentia, Octodontoidae)*. Mendeliana, 2: 85-93.

KING M., 1993 — *Species Evolution: the role of chromosome change*. University Press, Cambridge.

LANZONE C., BIDAU C.J., GIMÉNEZ M.D. and SANTOS J.L., 2002 — *Synaptic behaviour and morphological modifications of the X and Y chromosomes during pachytene in three species of Ctenomys (Rodentia, Caviomorpha, Ctenomyidae)*. Genome, 45: 1110-1115.

MARTÍ D.A. and BIDAU C.J., 2001 — *Synapsis in Robertsonian heterozygotes and homozygotes of Dichroplus pratensis (Melanoplinae, Acrididae) and its relationship with chiasma patterns*. Hereditas, 134: 245-254.

MARTÍ D.A. and BIDAU J.C., 1995 — *Male and female meiosis in natural population of Dichroplus pratensis (Acrididae) polymorphic for Robertsonian translocation: A study of chiasma frequency and distribution*. Hereditas, 123: 227-235.

MASCHERETTI S., MIROL P., GIMENEZ M.D., BIDAU C.J., CONTRERAS J.R. and SEARLE J.B., 2000 — *Phylogenetics of the speciose and chromosomally variable genus Ctenomys (Ctenomyidae, Octodontoidea), based on mitochondrial cytochrome b sequences* Biol. J. Linn. Soc., 70: 361-376.

NACHMAN M.W. and SEARLE J.B., 1995 — *Why is the house mouse so variable?*. Trends Ecol. Evol., 10: 397-402.

NACHMAN M.W., 1992a — *Geographic patterns of chromosomal variation in South American marsh rats, Holochilus brasiliensis and H. vulpinus*. Cytogenet. Cell. Genet., 61: 10-16.

NACHMAN M.W., 1992b — *Meiotic studies of Robertsonian polymorphisms in the South American marsh rat, Holochilus brasiliensis.* Cytogenet. Cell. Genet., 61: 17-24.

ORTELLS M.O., 1995 — *Phylogenetic analysis of G-banded karyotypes among the South American subterranean rodents of the genus Ctenomys (Caviomorpha: Octodontidae), with special reference to chromosomal evolution and speciation.* Biol. J. Linn. Soc., 54: 43-70.

ORTELLS M.O., CONTRERAS J.R. and REIG O.A., 1990 — *New Ctenomys karyotypes (Rodentia, Octodonti-*

dae) from north-eastern Argentina and from Paraguay confirm the extreme chromosomal multiformity of the genus. Genetica, 82: 189-201.

PARDO MANUEL DE VILLENA F. and SAPIENZA C., 2001 — *Recombination is proportional to the number of chromosome arms in mammals*. Mamm. Genome, 12: 318-322.

PIÁLEK J., HAUFFE H.C. and SEARLE J.B., 2005 — *Chromosomal variation in the house mouse: review*. Biol. J. Linn. Soc., 84: 535-563.

RIESEBERG L.H., 2001 — *Chromosomal rearrangements and speciation*. Trends. Ecol. Evol., 16: 351-358.

ROGATCHEVA M.B., BORODIN P.M., ODA S.I. and SEARLE J.B., 1997 — *Robertsonian chromosomal variation in the house musk shrew (Suncus murinus, Insectivora: Soricidae) and colonization history of the species*. Genome, 40: 18-24.

ROGATCHEVA M.B., ODA S.I., AXENOVICH T.I., AULCHENKO Y.S., SEARLE J.B. and BORODIN P.M., 1998 — *Chromosomal segregation and fertility in Robertsonian chromosomal heterozygotes of the house musk shrew (Suncus murinus, Insectivora, Soricidae).* Heredity, 81: 335-341.

SEARLE J.B., 1993 — *Chromosomal hybrid zones in eutherian mammals*. In: R. Harrison (Ed), "Hybrid Zones and Evolutionary Process", pp. 309-353. Oxford University Press, New York.

SEARLE J.B., NAVARRO Y.N. and GANEM G., 1993 — *Further studies of a staggered hybrid zone in Mus musculus domesticus (the house mouse)*. Heredity, 71: 523-531.

SOKAL R.R. and ROHLF F.J., 1998 — *Biometry. The principles and practice of statistics in biological research*. In: W.H. Freeman (Ed) 3°ed,, pp. 887. San Francisco.

STEEL R.G.D. and TORRIE J.H., 1980 — *Principles and Procedures of Statistics*. 2nd Edit. In. McGeaw-Hill (Ed), p. 633. New York.

TURNER J.M.A., MAHADEVAIAH S.K., FERNÁNDEZ-CAPETILLO O., NUSSENZWEIG XU X. DENG CH-X. and BURGOYNE P.S., 2005 — *Silencing of unsynapsed meiotic chromosomes in the mouse*. Nat. Genet., 37: 41-47.

WHITE M.J.D., 1978 — *Modes of Speciation*. In: W.H. Freeman (Ed), pp. 455. San Francisco.

ZAR J.H., 1984 — *Biostatistical Analysis*. 2th Edit. Prentice Hall, p. 713 Englewoods Cliffs, NJ.

ZAR J.H., 1999 — *Biostatistical Analysis*. 4th Edit. Prentice Hall, p. 663 + Appendixes. Upper Saddle River, NJ.

ZIMA J., SEARLE J.B. and MACHOLAN M., 1994 — T*he cytogenetics of the Sorex araneus group and related topics*. F. Zool., 43: 1-116.

Received August 28th 2006; accepted January 1th 2007