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Chromosome synapsis and recombination in simple and complex chromosomal heterozygotes of tuco-tuco (*Ctenomys talarum*: Rodentia: Ctenomyidae)

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Abstract The chromosomal speciation hypothesis suggests that irregularities in synapsis, recombination, and segregation in heterozygotes for chromosome rearrangements may restrict gene flow between karyotypically distinct populations and promote speciation. *Ctenomys talarum* is a South American subterranean rodent inhabiting the coastal regions of Argentina, whose populations polymorphic for Robertsonian and tandem translocations seem to have a very restricted gene flow. To test if chromosomal differences are involved in isolation among its populations, we examined chromosome pairing, recombination, and meiotic silencing of unsynapsed chromatin in male meiosis of simple and complex translocation heterozygotes using immunolocalization of the MLH1 marking mature recombination nodules and phosphorylated histone γH2A.X marking unrepaired double-strand breaks. We observed small asynaptic areas labeled by γH2A.X in pericentromeric regions of the chromosomes involved in the trivalents and quadrivalents. We also observed a decrease of recombination frequency and a distalization of the crossover distribution in the heterozygotes and metacentric homozygotes compared to acrocentric homozygotes. We suggest that the asynapsis of the pericentromeric regions are unlikely to induce germ cell death and decrease fertility of the heterozygotes; however, suppressed recombination in pericentromeric areas of the multivalents may reduce gene flow between chromosomally different populations of the Talas tuco-tuco.

Keywords Meiosis · synaptonemal complex · Robertsonian translocation · whole-arm reciprocal translocation · MLH1 · SYCP3 · gammaH2A.X · gene flow · chromosome speciation

Abbreviations

ACA	Anti-centromere antibodies
AMCA	7-Amino-4-methylcoumarin-3-acetic acid
Cy3	Orange fluorescing cyanine
DAPI	4′-6-diamidino-2-phenylindole
γH2A.X	Phosphorylated histone H2A.X
FITC	Fluorescein isothiocyanate
MLH1	MutL Homolog 1
Rb	Robertsonian translocation
SC	Synaptonemal complex
S.D.	Standard deviation
SYCP3	Synaptonemal complex protein 3
t	Reciprocal translocation

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Introduction

The chromosomal speciation hypothesis suggests that fixation of different chromosomal rearrangements across local populations of the same species may promote speciation. The traditional model of this hypothesis puts emphasis on sterility or reduced fertility of hybrids, which may result from incorrect or incomplete pairing and altered chromosomal recombination following germ cell death or incorrect chromosome segregation and the generation of unbalanced gametes (King 1995; White 1978). A more recent model of the chromosomal speciation hypothesis emphasizes a reduction of gene flow across hybrid zones due to recombination suppression around rearrangement breakpoints in chromosomal heterozygotes (Faria and Navarro 2010; Jackson 2011; Livingstone and Rieseberg 2004; Rieseberg 2001).

Several studies of hybrids between chromosomal races or karyotypically different subspecies of mammals have detected various meiotic irregularities which may reduce fertility (Axenovich et al. 1998; Banaszek et al. 2000; Borodin et al. 2006; Castiglia and Capanna 2000; Ishak et al. 1992; Rogatcheva et al. 1998; Searle 1986). Substantial changes in the recombination pattern in heterozygotes for Robertsonian translocations compared to homozygotes has also been observed in the two species of mammals studied so far: the house mouse (*Mus musculus*) and the common shrew (*Sorex araneus*) (Bidau et al. 2001; Borodin et al. 2008; Dumas and Britton-Davidian 2002; Merico et al. 2013). Usually, complex translocation heterozygotes (i.e., the heterozygotes for two or more metacentric chromosomes with monobrachial homology) demonstrate more severe meiotic disturbances than simple Robertsonian heterozygotes (Baker and Bickham 1986; Banaszek et al. 2000; Mercer et al. 1992; Merico et al. 2013).

Tuco-tucos, South American subterranean rodents of the genus *Ctenomys* (Rodentia: Ctenomyidae), are often considered an example of chromosomal speciation (Kiblisky and Reig 1966; Reig and Kiblisky 1969). The genus includes about 60 species, which emerged within the last 0.6 million years (Castillo et al. 2005; Lessa and Cook 1998). This high rate of diversification, one of the highest among mammals, has been ascribed to a combination of several peculiarities of tuco-tucos, including small effective population size, patchy distribution, spatial isolation, limited dispersal capacity, high dependence on specific soil types, and extensive chromosomal variation (Busch et al. 2000). Populations and species of tuco-tucos show extensive karyotypic heterogeneity due to Robertsonian and tandem translocations, having diploid numbers ranging from $2n=10$, FN=20 to $2n=70$, FN=80 (Lopes et al. 2013; Massarini et al. 2002; Ortells 1995).

Although the idea of such chromosomal variation being involved in speciation in the genus seems very appealing, there is actually no evidence that chromosome rearrangements play a role in genetic structuring of populations and species of tuco-tuco. Chromosomal polymorphisms and polytypisms were studied in various species of tuco-tuco (Freitas 1997; Garcia et al. 2000; Gava and Freitas 2003; Lanzone et al. 2002; Massarini et al. 2002; Ortells 1995), but the role of chromosomal differences in promoting isolation has never been demonstrated. We are aware of only one study on the effects of chromosomal heterozygosity on chromosome pairing and recombination in the genus. Lanzone et al. (2007) demonstrated orderly chromosome synapsis and non-altered chiasma count in simple Robertsonian heterozygotes derived from natural populations of the *Ctenomys perrensi* species group. They concluded that Robertsonian variation was unrelated to the divergence within this group. This conclusion is supported by the low molecular differentiation and the evidence of gene flow between chromosomally distinct populations of the *C. perrensi* species complex, which show diploid number variation from 42 to 70 (Fernández et al. 2012; Giménez et al. 2002; Mirol et al. 2010).

In this study, we examined chromosome synapsis and recombination at the male meiotic prophase in simple and complex heterozygotes for Robertsonian and whole-arm reciprocal translocations isolated from natural populations of the Talas tuco-tuco *Ctenomys talarum* with the aim to explore their role in the genetic structuring of populations. This species occurs along the coast of Buenos Aires Province in Argentina. It shows high karyotypic variability for a series of Robertsonian and tandem translocations (Massarini et al. 1991, 2002; Ortells 1995; Reig and Kiblisky 1969). Unlike the species and populations of the *C. perrensi* group mentioned above, there seems to be restricted gene flow among some of the populations of *C. talarum* as evidenced by molecular markers (Mora et al. 2013).

We analyzed chromosome synapsis by visualizing synaptonemal complexes (SC) with antibodies to SYCP3, the main protein of the lateral elements of the

SC. We estimated the number and distribution of crossover points along the arms of the chromosomes involved in translocations using antibodies to MLH1, the mammalian homolog of the MutL mismatch repair protein (Anderson et al. 1999; Baker et al. 1996). Immunolocalization of γH2A.X, a phosphorylated form of histone H2A.X, a marker for DNA double-strain breaks (DSBs) (Rogakou et al. 1998) at SC spreads allowed us to reveal unrepaired DSBs in asynapsed chromosome regions, which may activate pachytene checkpoint (Turner et al. 2005).

Material and methods

Thirteen adult males of *C. talarum* were trapped during the breeding season in November 2012 in three localities along the Atlantic coast of Buenos Aires Province, Argentina: near to Cerro de la Gloria (35° 58.975′ S; 57° 27.012′ W), Costa del Este (36° 37.076′ S; 56° 42.119′ W), and Villa Gesell (37°11.497′ S; 56°57.383′ W).

Trapping, handling, and euthanasia of animals were performed according to the protocols approved by the Animal Care and Use Committees at the Institute of Cytology and Genetics of the Russian Academy of Sciences and the Bernardino Rivadavia Argentine Museum of Natural Sciences. All institutional and national guidelines for the care and use of laboratory animals were followed.

Testicular cell spreads were prepared using drying-down technique (Peters et al. 1997). Immunostaining was performed as described by Anderson et al. (1999). Primary antibodies used in this study were as follows: rabbit polyclonal to SYCP3 (1:500; ab150292, Abcam), mouse monoclonal to MLH1 (1:50; ab14206, Abcam), rabbit polyclonal to γH2A.X (phospho S139) (1:200; ab2893, Abcam), and human anti-centromere (ACA) (1:100; Cat#15-235-0001, Antibodies Inc.). Secondary antibodies used were Cy3-conjugated goat anti-rabbit (1:500; Cat#111-165-144, Jackson ImmunoResearch), FITC-conjugated goat anti-mouse (1:50; Cat#115-095-003, Jackson ImmunoResearch), FITC-conjugated goat anti-rabbit (1:200; Cat#111-095-003, Jackson ImmunoResearch), FITC-conjugated donkey anti-human (1:100; Cat#709-095-149, Jackson ImmunoResearch), and AMCA-conjugated donkey anti-human (1:100; Cat#709-155-149, Jackson ImmunoResearch). All antibodies were diluted in PBT (3 % bovine serum albumin, 0.1 % Tween 20 in phosphate buffered saline). A solution of 10 % PBT was used to perform a blocking reaction. Primary antibody incubations were performed overnight in a humid chamber at 37 °C; secondary antibody incubations were performed for 1 h at 37 °C. Finally, slides were mounted in Vectashield with or without DAPI (Vector Laboratories).

The preparations were visualized with an Axioplan 2 imaging microscope (Carl Zeiss) equipped with a CCD camera (CV M300, JAI), CHROMA filter sets, and ISIS4 image-processing package (MetaSystems GmbH). Brightness and contrast of all images were enhanced using Corel PaintShop Photo Pro X3 (Corel Corp).

The centromeres were identified by ACA foci. MLH1 signals were only scored if they were localized on SCs. The length of the SC of each chromosome arm was measured in micrometers and the positions of centromeres and MLH1 foci in relation to the centromere were recorded using MicroMeasure 3.3 (Reeves 2001).

We identified individual SCs by their relative lengths, centromeric index, and DAPI pattern. To generate recombination maps, we calculated the absolute position of each MLH1 focus multiplying the relative position of each focus by the average absolute length for the appropriate chromosome arm. These data were pooled for each arm and graphed to represent a recombination map. We measured absolute distances between two MLH1 foci across the centromere in homozygotes and heterozygotes for Robertsonian fusions and reciprocal translocations. The relative distances were calculated as fractions of metacentric chromosome length.

Statistica 6.0 software package (StatSoft) was used for descriptive statistics, Fisher's exact test and ANOVA.

Results

Karyotypes

All four males (## 10–13) trapped near Villa Gesell had the same karyotype $2n=48$. The same diploid chromosome number was scored in the specimens trapped 5 km south from our trapping site by Massarini et al. (2002). We took this karyotype as standard. Karyotypes of the specimens trapped in other sites differed from the standard one for one or more chromosomes. We identified variable chromosomes by their relative sizes and

centromeric indexes (Table 1). We also took into account their synaptic configurations and DAPI patterns of surrounding chromatin. Figure 1 shows ideograms of variable SCs in different karyotypes of *C. talarum*. Chromosome nomenclature follows Massarini et al. (2002).

All five males (## 1–5) trapped near Cerro de la Gloria, at 140 km to the north from Villa Gesell, had $2n=46$. They were homozygous for a fusion of two small acrocentric chromosomes (probably B2 and B3) (Fig. 1, ## 1–5).

The third trapping site (Costa del Este) was located at 64 km to the northeast from the Villa Gesell trapping site and at 95 km to the southeast from Cerro de la Gloria. Each of the four males trapped in Costa del Este, in an area of a few dozen square meters, had its own karyotype (Fig. 1, ## 6–9).

Male # 6 ($2n=45$) had a trivalent (Fig. 2, # 6) and a small metacentric bivalent, which was not present in Villa Gesell and Cerro de la Gloria karyotypes. We suggest that the male was heterozygous for the fusion of B2 and B3, which discriminated Villa Gesell and Cerro de la Gloria karyotypes, and homozygous for a fusion of the small submetacentric (A11) and the smallest acrocentric (B6) chromosomes (Fig. 1, # 6).

Male # 9 ($2n=44$) was homozygous for two fusions. One involved the largest submetacentric (A1) and a small acrocentric chromosome, apparently the same B3. Another fusion involved two acrocentrics: the small (B2) and the smallest (B6) ones (Fig. 1, # 9).

Table 1 Relative lengths and centromeric indexes (±S.D.) of the SCs of variable chromosomes

Chromosome	Relative length[a]		Centromeric index	
A1	9.33	±0.50	0.05	±0.04
A2	7.20	±0.41	0.10	±0.04
A11	4.16	±0.10	0.13	±0.03
A15	3.51	±0.28	0.42	±0.04
B2	3.69	±0.43	0.11	±0.09
B3	2.38	±0.29	0.13	±0.08
B6	1.07	±0.16	0.07	±0.10
Rb B3A1	11.33	±0.52	0.18	±0.02
tA15pA2q	8.26	±0.51	0.21	±0.03
Rb B3B2	6.09	±0.66	0.34	±0.05
Rb B6A11	5.13	±0.28	0.12	±0.03
tA2pA15q	2.59	±0.30	0.31	±0.06

[a] Percent of total autosomal SC length

Male # 7 ($2n=44$) had a quadrivalent involving RbA1B3 (presented in male # 9 karyotype) and RbB2B3 (presented in Cerro de la Gloria karyotype) and their submetacentric and acrocentric partners A1 and B2 (Fig. 2, # 7). It was also homozygous for RbA11B6 (Fig. 1, # 7), the same fusion that was detected in the male # 6.

Male # 8 ($2n=44$) had a quadrivalent which apparently involved the products of whole-arm reciprocal translocation between the second largest submetacentric (A2) and a small metacentric (A15) chromosomes (Fig. 2, # 8). It was also homozygous for RbA11B6 found in the males # 6 and # 7 (Fig. 1, # 8).

Synapsis

Average total length of autosomal SC of *C. talarum* was 239.1±30.9 μm. This is rather close to the estimate obtained by electron microscopic analysis of the SCs in *C. perrensi* (258.1±10.4 μm: Lanzone et al. 2007). The pattern of chromosome pairing in the specimens examined was similar to that described for *C. perrensi* and two other species of tuco-tuco (Lanzone et al. 2002, 2007). At early pachytene, Yq was completely paired with the distal part of Xq, while Yp, Xp, and a proximal part of Xq remained unpaired. We observed a signal of γH2A.X over the asynapsed parts of the sex chromosomes, but it was absent over the synapsed parts (Fig. 3a). At midpachytene, Yp was paired with Xq and the signal of γH2A.X was detected all over the sex bivalent (Fig. 3b). At late pachytene, the sex bivalent showed multiple splits of the axes and intensive labelling of its chromatin by γH2A.X antibodies (Fig. 3c).

In the heterozygotes, we found a wide variety of heteromorphic synaptic configurations. We observed completely paired trivalents in 33 out of 54 (61±7 %) pachytene cells of the simple Robertsonian heterozygote # 6 (Fig. 2a, # 6–1). In this configuration, the short arms of the acrocentrics displayed non-homologous synapsis with the metacentric partner in the pericentromeric region. In 15±5 % cells, we found side arms formed by the short arms of the acrocentric partners non-homologously paired to each other (Fig. 2, # 6–2). The remaining 24±6 % cells displayed an asynapsis of the most part of the short arm (an average 78 % of its length) of either one or both twin acrocentrics (Fig. 2, # 6–3). We detected only one case where asynapsis extended at

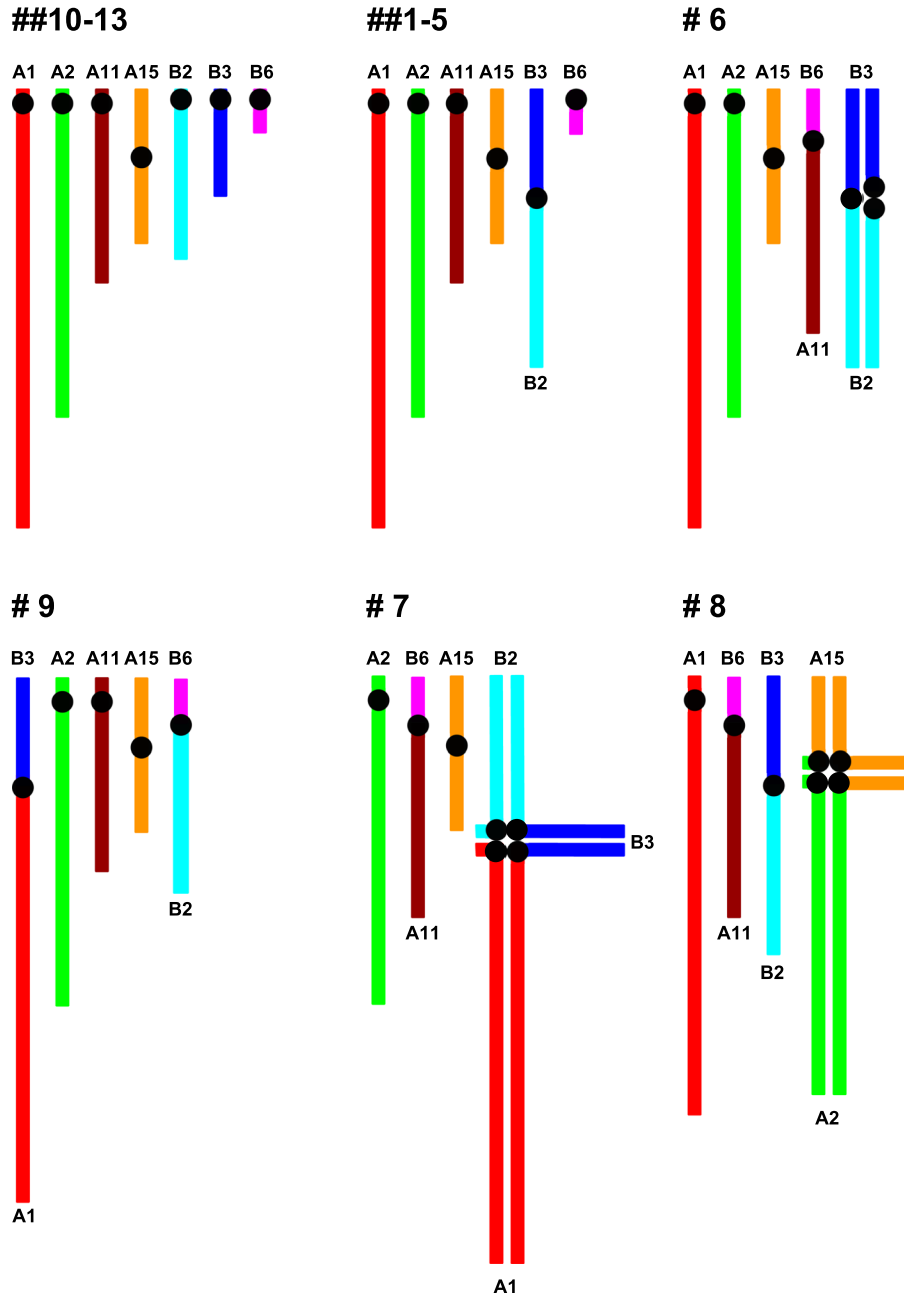
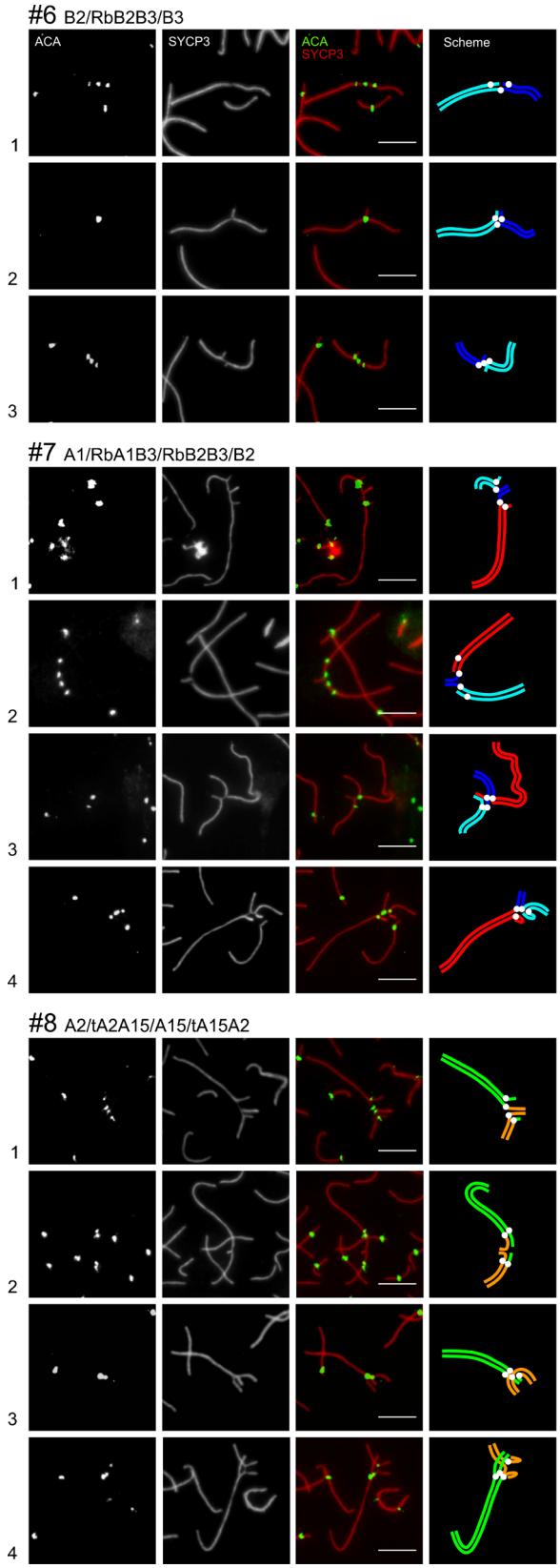


Fig. 1 Ideograms of variable SCs of *C. talarum* based on their relative lengths, centromeric indexes, and synaptic configurations. Males ## *10–13* (Villa Gesell), males ## *1–5* (Cerro de la Gloria), males ## *6–9* (Costa del Este)

the long arm of the acrocentric partner affecting 20 % of its length.

The frequency and extent of asynapsis were substantially higher in complex heterozygotes. We analyzed 103 pachytene cells in the male # 7. Nearly half (53± 5 %) showed asynapsis of one or both short arms of the submetacentric (A1) and acrocentric (B2) partners in the quadrivalent with the RbA1B3 and RbB2B3 (Fig. 2, # 7–1). In 40±5 % of cells, A1p and B2p were paired non-homologously with the pericentromeric regions of the partner's A1q and B2q (Fig. 2, #7–2). In one cell, we observed non-homologous pairing between the A1p and B2p (Fig. 2, # 7–3). In six cells, A1p and B2p formed foldbacks of self-synapsis (Fig. 2, # 7–4).



◀ **Fig. 2** Synaptic configurations in surface spread pachytene spermatocytes of *C. talarum*. Male # *6*, trivalent B2/RbB2B3/B3. *1* Complete synapsis. *2* Side arm involving B2p and B3p. *3* Asynapsis of B2p and B3p. Male # *7*, quadrivalent A1/RbA1B3/RbB2B3/B2. *1* Asynapsis of A1p and B2p. *2* Heterosynapsis of A1p and B2p with pericentromeric regions of chromosome B3. *3* Non-homologous synapsis between A1p and B2p. *4* Self-synapsis (foldbacks) of A1p and B2p. Male # *8*, quadrivalent A2/tA2A15/A15/tA15A2. *1* Asynapsis of A2p arms. *2* Heterosynapsis of A2p arms with the pericentromeric regions of A15p arms. *3* Complete synapsis. *4* Self-synapsis (foldbacks) of A2p arms. Chromosome color keys in the schemes are the same as in Fig. 1. *White* indicates centromeres. *Scale bar*=5 μm

Most of 92 pachytene cells examined (84±4 %) in male # 8 showed asynapsis of the A2p arms in the quadrivalent involving the products of whole-arm reciprocal translocation between the chromosomes A2 and A15 (Fig. 2, #8–1). In eight cells, A2p arms were paired non-homologously with the pericentromeric regions of the A15p arms (Fig. 2, # 8–2). Only three cells contained the completely paired quadrivalent (Fig. 2, # 8–3). In four cells, we observed self-synapsis of A2p arms (Fig. 2, # 8–4).

Thus, in most cases, we observed asynapsis of the short (and putatively heterochromatic) arms of the chromosomes. A two-way Fisher's exact test showed a decrease in the frequency of asynapsed configurations from early to late pachytene in the pooled sample of the cells of all three heterozygotes (P=0.04). In early and midpachytene cells, the asynaptic regions were labeled with γH2A.X antibodies (Fig. 3a, b). In the late pachytene cells (with XY bivalents densely compacted and heavily labeled by γH2A.X antibodies), we did not detect the γH2A.X signal at the heteromorphic synaptic configurations (Fig. 3c).

Recombination

MLH1 foci are considered as reliable markers of mature recombination nodules (Anderson et al. 1999). In tuco-tuco, they occurred at the autosomal SCs at the middle and late pachytene (Fig. 4). The mean (±S.D.) number of MLH1 foci at autosomes was 27.3±3.8 foci per cell. The XY pairing region usually contained a single MLH1 focus. The MLH1 foci were evenly distributed at the interval between 0.10 and 0.70 of the Yp axis length from the Yp telomere. Thus, the pseudoautosomal region covers about a half of the Yp axis length.

To estimate in centimorgans (cM), the recombination length of the male genome of *C. talarum*, we multiplied

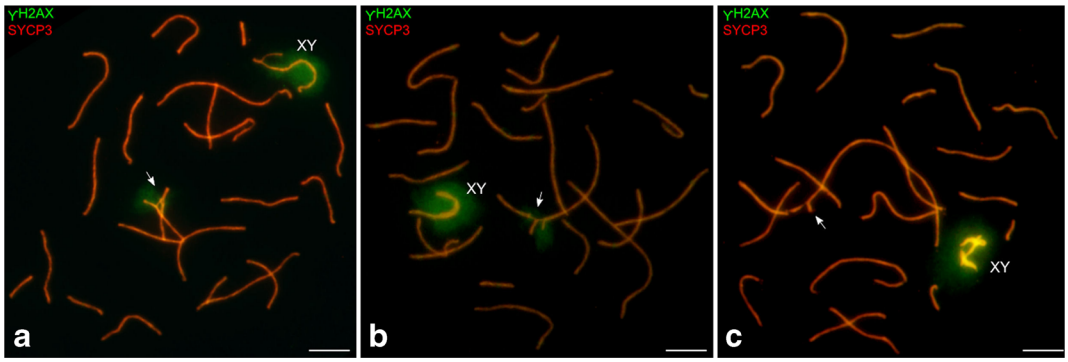


Fig. 3 Immunolocalization of phosphorylated histone γH2A.X at the SC of *C. talarum*. **a** early pachytene (male # 8), **b** midpachytene (male # 8), **c** late pachytene (male # 7). *Arrows* indicate quadrivalents, *XY* sex bivalents. *Scale bar*=5 μm

the average number of MLH1 foci per cell by 50 map units (one recombination event=50 cM), which gave 1,414.5 cM. The genome size of *C. talarum* is unknown. Haploid DNA content in the closely related species *C. porteousi* is 3.20 pg (Rossi et al. 1995), i.e., its genome size is approximately 3,100 Mb. Supposing that the genome sizes of these two species are roughly similar, we estimated a recombination rate in tuco-tuco as 0.46 cM/Mb. This is close to the estimate obtained by MLH1 mapping for male mice (0.44 cM/Mb: Anderson et al. 1999).

To estimate the effects of structural heterozygosity on recombination frequency and distribution in the chromosomes involved in translocations, we compared the number and distribution of MLH1 foci along the same arms in homozygous (bivalents) and heterozygous (multivalents) synaptic configurations (Table 2, Fig. 5). Two-way ANOVA detected a significant effect of karyotype on the number of MLH1 foci per chromosome arm for all arms examined but A15q. Most arms showed higher number of MLH1 foci in non-fused homozygotes than in heterozygotes and fused homozygotes (Table 2). Figure 5 shows MLH1 focus distribution along the arms of the variable chromosomes in various synaptic configurations. In all configurations, we observed a decreased frequency of MLH1 near the centromeres. However, the suppressive centromeric effect was more pronounced in the heterozygotes and fused homozygotes than in non-fused homozygotes (Fig. 5).

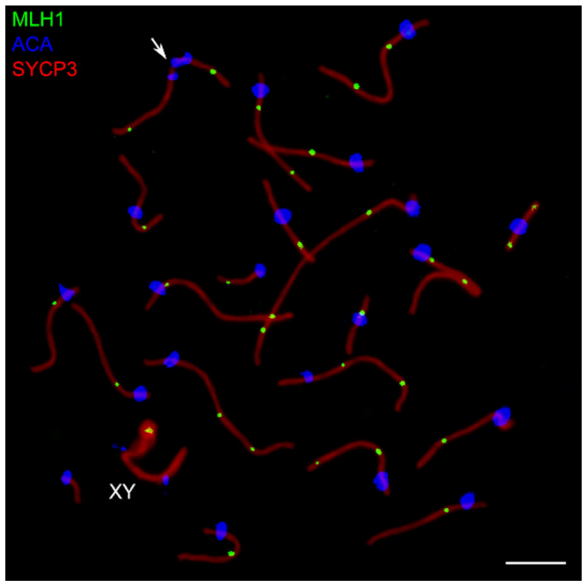


Fig. 4 Immunolocalization of MLH1 at the SC of *C. talarum* (male # 6). *Arrow* indicates trivalent B2/RbB2B3/B3; *XY* sex bivalent. *Scale bar*=5 μm

Average distances between two neighboring MLH1 across the centromere were similar in heterozygotes and homozygotes for the fused variant chromosomes: 11.9±5.3 μm and 11.7±5.2 μm for A1B3 (t_{37}=0.16; p=0.88); 9.0±2.1 μm and 9.8±2.5 μm for B2B3 (t_{54}=1.45; p=0.15).

Discussion

Chromosomal variation

Despite the small sample size, our study revealed a rather complex structure of chromosomal variation of *C. talarum* inhabiting the Atlantic coast of Buenos Aires Province. In 13 specimens examined, we detected a polymorphism for five translocations: RbB2B3, RbA11B6, RbA1B3, RbB2B6, and tA2A15. Using C- and G-band staining of metaphase chromosomes of 72 specimens, Massarini et al. (2002) also found chromosomal polymorphism in this area. They suggested that

Table 2 The number (±S.D.) of MLH1 foci at the SCs of fused and not fused chromosomes in different synaptic configurations

Combination	Arm	Karyotype						Effect of karyotype		
		Homozygous not fused		Heterozygous		Homozygous fused		*F*	df	*P*
Rb B3A1	A1	1.93	±0.53	1.86	±0.44[a]	1.66	±0.55	50.9	2,185	<0.001
	B3	0.86	±0.35	0.90	±0.31[a]	0.37	±0.49	19.3	2,134	<0.001
Rb B3B2	B2	0.98	±0.30	0.69	±0.47[b]	1.00	±0.45	17.7	2,152	<0.001
	B3	0.86	±0.35	0.53	±0.51[b]	0.52	±0.50	10.8	2,180	<0.001
	B2	0.98	±0.30	0.79	±0.41[a]	1.00	±0.45	8.3	2,132	<0.001
tA2A15	A2q	1.58	±0.57	1.72	±0.52[c]			18.6	1,178	<0.001
	A15q			0.72	±0.44[c]	0.69	±0.46	1.5	1,178	0.226
	A15p			0.91	±0.25[c]	0.27	±0.44	70.5	1,178	<0.001

[a] As part of the quadrivalent A1/RbA1B3/RbB2B3/B2

[b] As part of the trivalent B2/RbB2B3/B3

[c] As part of the quadrivalent A2/tA2A15/A15/tA15A2

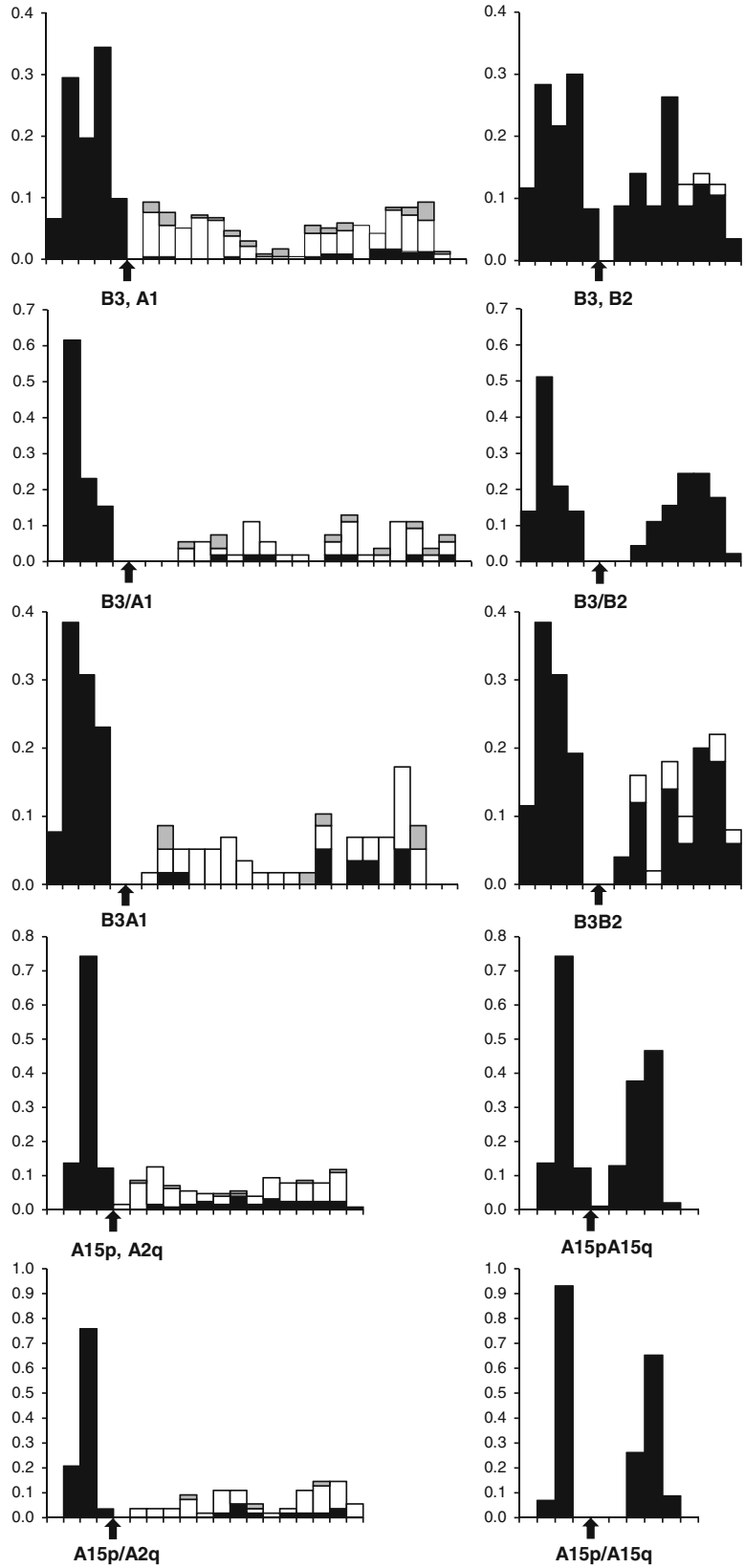
the polymorphism was due to a Robertsonian fusion and a tandem fusion of two different chromosome pairs: RbB4B5 and tA2A15. This discrepancy might be due to the differences in sampling time (10 years) and place (dozen kilometers) or/and the resolution of the method of karyotyping. Analysis of surface spread SC in structural heterozygotes provides much higher resolution in detecting complex rearrangements than G- and C-band staining (Borodin et al. 1992) or Zoo-FISH of mitotic metaphase chromosomes (Bakloushinskaya et al. 2012). Occurrence of multivalents in the pachytene cells of the specimens from Costa del Este unambiguously indicates the presence of complex rearrangements with monobrachial homology.

The rearrangements could have occurred due to independent fusions of the same chromosomes in different combinations or by whole-arm reciprocal translocations. It is known that most short arms of the autosomes of *C. talarum* are heterochromatic and contain repeated DNA sequences (Rossi et al. 1995). Rearrangements involving these arms are not expected to produce strong phenotypic effects (Novello et al. 2010; Reig et al. 1992). They may be maintained in populations of tuco-tuco and get fixed because of the characteristic features of this species: small population size and limited dispersal (Busch et al. 2000).

The populations of Villa Gesell and Cerro de la Gloria differ from each other for one chromosome fusion RbB2B3. In Costa del Este, we found two heterozygous carriers of this fusion. Apparently, this site is close to the center of a hybrid zone between Villa Gesell and Cerro de la Gloria chromosome races. However, in Costa del Este, we also found the carriers of four other metacentric chromosomes, which were not present in either Villa Gesell or Cerro de la Gloria. This indicates the presence of another not yet described chromosome race (or races) nearby, which contributes to the hybrid zone. Massarini et al. (2002) also noted that the samples from the North and South ends of their transect were karyotypically similar to each other and differ from the samples taken from the center (near Costa del Este).

Apparently, the gene and chromosome flow along the Atlantic coast is restricted. Mora et al. (2013) made pairwise comparison of genetic distances (Φst) amongst mitochondrial haplotypes between several localities sampled geographically close to the ones studied here. They found that the populations located near Costa del Este showed larger genetic distances from the populations located near Villa Gesell and Cerro de la Gloria than the latter two populations from each other. This result is consistent with the chromosomal distances found here.

Fig. 5 Distribution of MLH1 foci along the arms of the chromosomes involved in rearrangements. The *X*-axis shows the position of MLH1 foci in relation to the centromere (*arrow*), at the SC of a particular arm (indicated below the axis) in homozygotes for a non-fused state (separated by coma), heterozygotes (separated by slash), and homozygotes for a fused state (fused). The marks on the *X*-axis are separated by 1 μm of the SC. The *Y*-axis indicates the frequency of MLH1 foci in each 1 μm interval. Stacked columns show the frequency for the bivalents containing MLH1 foci at each interval; *black columns* indicate arms with a single MLH1 focus; the *white columns* indicate those with two MLH1 foci, grey—with three MLH1


B3, A1
B3, B2
B3/A1
B3/B2
B3A1
B3B2
A15p, A2q
A15pA15q
A15p/A2q
A15p/A15q

Asynapsis and heterosynapsis around translocation breakpoints

Extensive asynapsis at pachytene may trigger meiotic silencing of unsynapsed chromatin and result in pachytene arrest due to transcriptional inactivation of essential meiotic genes (Baarends et al. 2005; Turner et al. 2005). This in turn may lead to a reduction of fertility in the carriers. However, studies on the mouse (Burgoyne et al. 2009; Manterola et al. 2009) and human (Sciurano et al. 2007) showed that surveillance mechanisms of pachytene checkpoints can ignore a limited degree of asynapsis.

Most cells of structural heterozygotes in *C. talarum* showed asynapsis around the breakpoints of rearrangements. Even at the midpachytene, more than half of spermatocytes with quadrivalents contained asynapsed regions, although the frequency of cells with asynapsis tended to decrease through pachytene. Apparently, the delay in synaptic progression near the centromeres in multivalents was determined by topological constraints. It has been suggested that at zygotene, the centromeres of the acrocentric partners are anchored to the nuclear membrane via telomeres of the short arms, while the centromere of the metacentric partner is not (Garagna et al. 2001; Hultén 2011; Manterola et al. 2009).

In our study, the relative length of unpaired SCs was rather small. Asynapsis was restricted by the short arms of the acrocentrics and pericentromeric regions of the metacentrics. These regions are presumably heterochromatic and gene-free. Phosphorylated γH2A.X almost disappeared from the asynapsed regions at late pachytene. For these reasons, we suggest that pachytene checkpoints are insensitive to these pairing disturbances. This is in agreement with a study of mice carrying multiple Robertsonian translocations that form multiple trivalents with asynapsis near centromeric regions (Manterola et al. 2009).

Thus, pairing defects which we observed in the structural heterozygotes were unlikely to induce germ cell death and in this way to reduce gene flow between the parental chromosome races. However, an asynapsis around centromeres in the multivalents may affect recombination frequency and distribution, which in turn may affect chromosome segregation.

Recombination suppression

The modern version of the chromosome speciation hypothesis ascribes a reduction of gene flow between karyotypically different populations to a recombination suppression in structural heterozygotes around the breakpoints of the rearrangements (Brown and O'neill 2010; Faria and Navarro 2010; Merico et al. 2013; Rieseberg 2001).

We observed a tendency for a reduction of the number of crossovers per arm from acrocentric homozygotes to heterozygotes to metacentric homozygotes. This is in line with findings in mice on reduction of chiasma count in homozygotes and heterozygotes for the Robertsonian translocations in comparison with acrocentric homozygotes (Bidau et al. 2001; Davisson and Akeson 1993; Dumas and Britton-Davidian 2002). The same tendency has been confirmed by various methods of recombination scoring on various species of animals (Marti and Bidau 2001; Ostberg et al. 2013; Searle 1986).

Another important effect of structural heterozygosity on recombination detected in this study was a distalization of the crossover distribution in heterozygotes and to a lesser extent in the metacentric homozygotes compared to acrocentric homozygotes. The causes of the shift in distributions were probably different in heterozygotes and metacentric homozygotes. In heterozygotes, it appears to be determined by misalignment followed by asynapsis and non-homologous synapsis that we detected around the centromeres in most multivalents. In the homozygotes, for the fused variant chromosomes, the distalization of the crossovers was seemingly determined by transcentromere crossover interference: the occurrence of a crossover in one arm of the metacentric bivalent which reduces the probability of a crossover occurring nearby in another arm (Borodin et al. 2008; Colombo and Jones 1997).

A similar shift of distribution of MLH1 foci and chiasma from centromeres in trivalents comparing to homologous acrocentric bivalents was observed in shrews (Borodin et al. 2008) and mice (Bidau et al. 2001; Dumas and Britton-Davidian 2002; Gimenez et al. 2013; Merico et al. 2013). A stronger suppression of recombination in the pericentromeric area in structural heterozygotes was an apparent consequence of a delayed alignment of the centromeres of acrocentric and metacentric partners discussed above.

Chromosomal rearrangements and gene flow

The Talas tuco-tuco occurs along the Atlantic coast of Buenos Aires Province distributed in three main patches: Northern coastal (including the three populations analyzed here), Southern coastal, and Inland. Mora et al (2013) presented a comprehensive study of genetic structure of the three groups of populations, which demonstrated that although the overall pattern of differentiation among populations followed an isolation by distance model, within the Northern and Southern groups, there was a lack of equilibrium between gene flow and local genetic drift. Moreover, estimates of gene flow among populations were near zero. Within this context, the two effects of translocation heterozygosity detected in this study, namely reduction of crossover number and distalization of their distribution, may play a significant role in restriction of the gene flow across the hybrid zone in two different ways.

Firstly, a strong suppression of recombination around centromeres may conserve linkage disequilibrium between the alleles located in the pericentromeric area and promote further divergence of genetic content of parental chromosomes. Studies of gene flow between mouse chromosomal races demonstrated its stronger restriction near the centromeres of Rb chromosomes than in other regions (Franchini et al. 2010; Gimenez et al. 2013). This reduction in gene flow is probably very difficult to detect with the set of molecular markers used in population studies of *Ctenomys* at present.

Secondly, reduced numbers of crossovers in particular arms of multivalents may increase the incidence of chromosome non-disjunction at metaphase I, which is already high in complex heterozygotes with monobrachial homology (Baker and Bickham 1986). This in turn may lead to reduced fertility of the chromosomal heterozygotes and restricted gene flow between karyotypically different populations. However, this effect can only be observed in the hybrid zones where complex chromosomal heterozygotes occur.

In conclusion, although the transient asynapsis is unlikely to induce germ cell death and decrease fertility of the heterozygotes, the suppressed recombination in pericentromeric areas and non-disjunction of the chromosomes involved in multivalents may impede gene flow between chromosomally different populations of the Talas tuco-tuco and thus may contribute to the observed pattern of population differentiation, potentially the first evidence for chromosomal speciation in this group.

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