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Robertsonian chromosome polymorphism of *Akodon molinae* (Rodentia: Sigmodontinae): analysis of trivalents in meiotic prophase

Polimorfismo cromosómico Robertsoniano de *Akodon molinae* (Rodentia: Sigmodontinae): análisis de trivalentes en la profase meiótica

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ABSTRACT

Akodon molinae (with 2n = 42-43-44 and an FN = 44) shows a remarkable polymorphism of chromosome 1 in natural and laboratory populations. Specimens 2n = 42, named single homozygotes (SH), have a chromosome pair 1 formed by two large metacentric chromosomes. Specimens 2n = 3, heterozygotes (Ht), have one chromosome 1 and two medium-sized subtelocentric chromosomes, 1a and 1b, which are homologous with the long and short arms of chromosome 1 respectively. Specimens 2n = 44 are double homozygotes (DH), with just two pairs of medium-sized subtelocentric chromosomes, 1a and 1b. Analysis of meiotic metaphases I and II showed that anomalous segregation occurs more frequently in spermatocytes carrying the 1a and 1b chromosomes. This would disturb gametogenesis and other reproductive and developmental processes, producing a marked decrease in viability of DH individuals. There is, as yet, no satisfactory explanation for these phenomena. To investigate structural elements which might explain such segregational anomalies, we have studied bivalent and trivalent synapsis in pachytene spermatocytes from SH, Ht and DH specimens. Of a total of 80 spermatocyte nuclei microspreads, the following results were obtained: of 16 microspreads from two SH individuals, 20 autosomic bivalents plus the XY bivalent were observed; of 48 microspreads from three Ht individuals, 19 autosomic bivalents, 1 trivalent and an XY bivalent were seen; and of the 16 microspreads from two DH individuals, 21 autosomic bivalents plus the XY bivalent were found. Trivalents analysed showed complete pairing between the short arms of 1a and 1b, and having an apparently normal synaptonemal complex (SC) with lengths of 1 and 2.8 μm. The trivalent SC showed three telomeric ends, corresponding to arms: q1 and q1a; p1 and q1b; and p1a and p1b, with attachment plates to the nuclear envelope of normal organisation. None of the trivalents showed asynapsis or desynapsis between p1a and p1b, nor an association with the XY bivalent. In 70 % of spermatocytes studied, the XY bivalent showed complete pairing between X and Y, with SC formation along the whole length of the Y chromosome. The remaining 30 % showed partial pairing, with an SC length which varied from the common end. Based on these findings and those of previous studies, we discuss: 1.- that the obliged configuration of the trivalent, with SC formation between the short arms of 1a and 1b, helps to assure a quasi normal segregation between 1, 1a and 1b in anaphase I of Ht meiosis; and 2.- that co-existence in trivalents of chromosomes 1, 1a and 1b in Ht individuals, breaks down the structural and functional integrity of the short arms of 1a and 1b, producing an accumulative damage which would also explain the decreased viability of individuals bearing these chromosomes.

Key words: *Akodon molinae*, Sigmodontinae, Robertsonian chromosome polymorphism, trivalents, synaptonemal complex.

RESUMEN

Akodon molinae con 2n = 42-43-44 y FN = 44 presenta un notable polimorfismo en el cromosoma 1 en poblaciones naturales y de laboratorio, los individuos 2n = 42 tienen un par 1 formado por dos cromosomas metacéntricos grandes y son denominados homocigotos simples (SH); los individuos 2n = 43, heterocigotos (Ht), presentan un cromosoma 1 y dos cromosomas subtelocéntricos de tamaño medio 1a and 1b, que son homólogos con los brazos largo y corto del 1, respectivamente; y los individuos 2n = 44 que son los doble homocigotos (DH) y presentan dos pares de cromosomas subtelocéntricos 1a y 1b. Análisis de la metafases I y II meióticas han demostrado que se producen segregaciones anómalas con una alta frecuencia en los espermatocitos portadores de los cromosomas 1a and 1b. Ello alteraría a otros procesos, como la gametogénesis, la reproducción y el desarrollo, disminuyendo la viabilidad individual y poblacional de los DH. No ha habido una explicación satisfactoria para estos fenómenos. Para investigar elementos estructurales que pudiesen explicar tales alteraciones segregacionales, se estudió la sinapsis de bivalentes y trivalentes en

espermatocitos en paquiteno de ejemplares SH, Ht y DH. De un total de 80 microesparcidos de núcleos de espermatocitos: en 16 núcleos de ejemplares SH se observaron 20 bivalentes autosómicos más el bivalente XY; en 48 núcleos de Ht se observaron 19 bivalentes autosómicos, 1 trivalente y un bivalente XY; y en 16 núcleos de DH se observaron 21 bivalentes autosómicos más el bivalente XY. Los trivalentes analizados mostraron apareamiento completo entre los brazos cortos de 1a y 1b conformándose un complejo sinaptonémico (CS) aparentemente normal con una longitud entre 1 µm y 2,8 µm. El CS de los trivalentes presentó tres extremos teloméricos correspondientes a los brazos: q1 y q1a, p1 y q1b y p1a y p1b, con placas de inserción a la envoltura nuclear de organización normal. En ninguno de los trivalentes se observó asinapsis o desinapsis entre p1a y p1b, ni asociaciones con el bivalente XY. En el 70 % de los espermatocitos estudiados el bivalente XY mostró apareamiento completo entre X e Y, con formación de CS en toda la longitud del cromosoma Y. El 30 % restante presentó apareamiento parcial con un CS de longitud variable a partir del extremo común. Sobre la base de los resultados de este trabajo y los antecedentes existentes, se discute: 1.- que la configuración obligada del trivalente con formación de CS entre los brazos cortos de 1a y 1b, contribuye a asegurar la segregación cuasi normal entre 1, 1a y 1b en la I anafase de la meiosis de los Ht; y 2.- que la coexistencia en los trivalentes de los cromosomas 1, 1a y 1b en los Ht, erosiona la integridad estructural y funcional de los brazos cortos de 1a y 1b, daño que sería acumulativo y que explicaría la viabilidad disminuída de los individuos portadores de estos cromosomas.

Palabras clave: *Akodon molinae*, Sigmodontinae, polimorfismo cromosómico Robertsoniano, trivalentes, complejo sinaptonémico.

INTRODUCTION

Akodon molinae forms part of the Akodontini tribe of the Sigmodontinae subfamily, one of the groups with the largest generic and specific diversity in the Muridae family (Hershkovitz 1966, Reig 1980, 1984, Wilson & Reeder 1993). Cytogenetic studies of species from the genus *Akodon*, have revealed that these species have very varied diploid numbers and chromosome polymorphisms (Bianchi et al. 1971, 1973, 1979a, 1979b, Yonenaga 1972, Kasahara & Yonenaga-Yassuda 1984). Their chromosomes also show great morphological diversity and varied sizes, as a result of different types of rearrangements, which include Robertsonian fusions, pericentric inversions, translocations in tandem, loss of centromeres, telomeres, and loss or gain of heterochromatin. Some rearrangements are also associated with the persistence of intersticial telomeric sequences, as seen in *Akodon cursor* (Fagundes et al. 1997, 1998) and the new *Akodon* sp species 2n = 10, recently described by Silva & Yonenaga-Yassuda (1998).

Within the genus *Akodon*, the species *A. molinae* shows a remarkable polymorphism of chromosome 1. In natural populations individuals can have 42, 43 or 44 chromosomes. Specimens with 42 chromosomes have the pair 1 formed by two large metacentric chromosomes that are easily identifiable. Specimens with 43 chromosomes have one chromosome 1 and two medium-sized subtelocentric chromosomes, 1a and 1b, which pair with the long and short arms of chromosome 1, respectively (Bianchi et al. 1979b). Specimens with 44 chromosomes have two pairs of medium-sized subtelocentric chromosomes 1a and 1b (Bianchi et al. 1979b).

Different *Akodon* lineages, 2n = 42, 2n = 43 y 2n = 44, have been able to reproduce and survive in captivity (Merani & Lizarralde 1980). Studies measuring reproductive viability, using indices based on litter size, litter number or on the number of successful crosses (Bianchi et al. 1979b), have shown that significant differences exist between distinct lineages. Bianchi et al. (1979b) demonstrated that the fertility index of crosses between individuals double homozygotes for 1a and 1b, is approximately 100 times less than that shown by crosses between simple homozygotes for the metacentric chromosome 1, and approximately 40 times less than that from crosses between heterozygotes.

Furthermore, studies perfomed in meiotic metaphases I and II in each of the individual lineages, show that anomalous segregation occurs more frequently in *A. molinae* spermatocytes carrying the 1a and 1b chromosomes, that is Ht and DH (Merani et al. 1980). Analysis of the DNA content of male sperm from the different *A. molinae* lineages, confirm these observations, in the sense that chromosomes 1a and 1b from DH individuals, show the highest aneuploidy indices (Redi et al. 1982).

In meiosis, segregation anomalies of chromosomes forming part of a trivalent are not surprising. However, no satisfactory explanation has been found for the anomalous segregation which would exist in double homozygotic 1a1a and 1b1b individuals. These two pairs of chromosomes give rise to bivalents that are clearly differentiated in metaphase I of meiosis (Merani et al. 1980), and that should segregate normally.

In the present work, we have studied the structure and organization of the synaptonemal complex (SC) and the pairing of autosomal and sex chromosomes in pachytene spermatocytes of *A. molinae*. We examined the ultrastructure of trivalents which should be formed in 2n = 43 specimens, as well as that of bivalents from 2n = 42 and 2n = 44 homozygotes, in the hope that we might find structural elements to explain the differences observed in the segregation of these chromosomes. We evaluate and discuss the role of meiotic mechanisms involved in the progression of the polymorphism of chromosome 1, mainly intrinsic nuclear factors that affect Robertsonian trivalents during pachytene, which might influence the behavior and segregation of chromosomes 1a and 1b.

MATERIAL AND METHODS

Animals

We studied a total of 10 adult males of *A. molinae*, aged 3 months. The animals (three 2n = 42, four 2n = 43 and three 2n = 44), were transported in separate cages to our laboratory in Santiago from the laboratory colony of the Instituto Multidisciplinario de Biología Celular, La Plata, Argentina. The only difference between the three karyotypes occurs through a Robertsonian rearrangement combined with two pericentric inversions (Bianchi et al. 1973). The animals with 2n = 42 (simple homozygotes or SH) have two large metacentric chromosomes 1; animals with 2n = 43 (heterozygotes or Ht) have one chromosome 1 and two subtelocentric chromosomes 1a and 1b, homologues to the long and short arms of chromosome 1, respectively; animals with 2n = 44 (double homozygotes or DH) have a pair of 1a and a pair of 1b chromosomes. The DH and SH samples came from lineages in which the progenitor karyotype was determined in vivo by the "diffusion microchamber technique" (Bianchi et al. 1975). The Ht samples came from F1 of crosses done between DH x SH progenitors.

Chromosomes and bands

A specimen of each chromosome formula was used to study somatic chromosomes. Specimens were injected with colchicine 1 μg ml^{-1} and killed one hour later. Chromosome spreads were prepared from bone marrow, according to routine procedures. GBG banding was obtained using Seabright (1971) trypsin method and chromosomal C-heterochromatin was revealed by the CBG banding method (Arrighi & Hsu 1971).

Spermatocyte nuclear spreads

Two DH, three Ht and two SH specimens were used for analysis of spermatocytes in meiotic prophase. Electronic microspreads were obtained according to the method described by Solari (1998). A cellular suspension was obtained from germinal cells which were rich in pachytene spermatocytes. This suspension was dropped onto a 0.5 % NaCl solution. Cells which floated in this hypotonic solution were collected by adhesion to a plastic film, obtained by dipping a slide in a 0.55 % Falcon solution in chloroform. Cells were fixed in 4 % paraformaldehyde, 0.03 % SDS in sodium borate buffer at pH 8, for 10 minutes. Preparations were washed in 0.4 % Photoflo (Kodak), at pH 8 and left to dry at room temperature. Then, they were contrasted in 1 % ethanolic PTA, washed in ethanol and left to dry. The film of each slide came away when floated in water and under these conditions, parallel bar copper grids were placed on the film. The film and grids were collected with parafilm and left to dry. The grids were recut, taking care not to disturb the attached film with microspreads. The quality of material collected from grids was controlled by phase contrast microscopy. Later, selected grids were observed and photographed in a Siemens Elminskop electronic microscope, using 35 or 60 mm b/w Kodalite film.

RESULTS

Except for the polymorphic group of chromosomes 1, 1a and 1b, the chromosome complement of *A. molinae* was composed of 19 pairs of telocentric chromosomes, including the XY sex chromosome pair and a metacentric pair, the smallest of the karyotype. All chromosomes were positive for CBG pericentric heterochromatin, except for chromosome 1 and the Y chromosome. Chromosome 1 accounted for approximately 18 % of the length of the haploid set; chromosomes 1a and 1b, for 10 and 9 %, respectively as was previously described (Bianchi et al. 1973, Merani et al. 1980). Chromosomes 1a and 1b had the same GBG banding pattern as the p and q arms of chromosome 1 (Fig. 1). Chromosomes 1a and 1b, which are subtelocentrics, had abundant pericentric heterochromatin, which was not present in the metacentric chromosome 1 (Fig. 1).

The meiotic karyotypes obtained from microspreads of pachytene spermatocyte nuclei in meiotic prophase, allowed us to study, at good resolution, the synapsis established in autosomic bivalents and in sex chromosomes.

Fig. 1. Mitotic chromosomes 1, 1a and 1b from an heterozygous male of *A. molinae*, 2n = 43. (a) CBG banding: chromosome 1 has no centromeric heterochromatin, chromosomes 1a and 1b show conspicuous masses of C-heterochromatin in the centromere region; (b) GTG banding: the equivalent G-banding patterns between chromosome 1, 1a and 1b are shown.

Cromosomas mitóticos 1, 1a and 1b de un macho heterocigoto de *A. molinae* 2n = 43. (a) bandeo CBG: cromosoma 1 no tiene heterocromatina centromérica, los cromosomas 1a and 1b muestran gran cantidad de heterocromatina C en la región centromérica; (b) bandeo GTG: se muestra el patrón equivalente de bandeo G entre los cromosomas 1 y 1a y 1b.

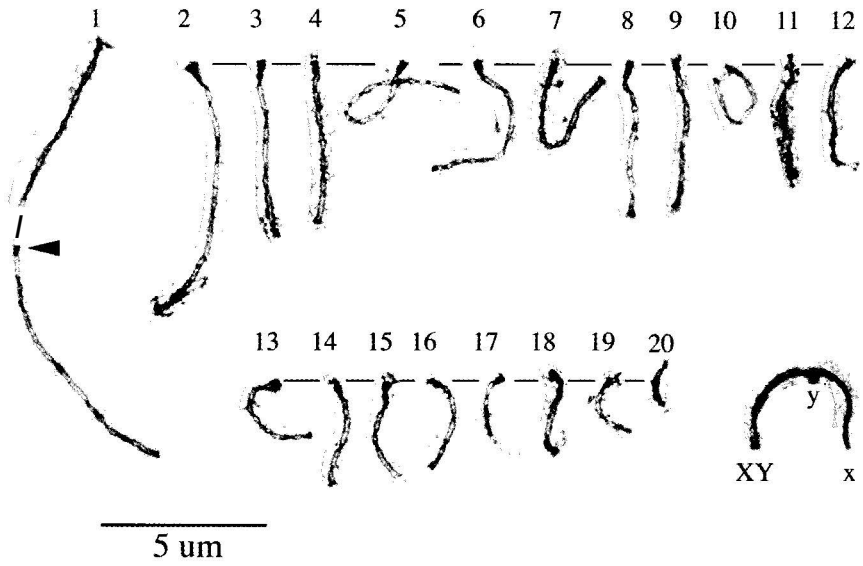
In 16 nuclei from two SH individuals 2n = 42, complete synapsis was found in the 20 autosomic bivalents and in the sex bivalent (Fig. 2). Of the autosomic bivalents, 18 were telocentric and only two, the largest number 1 and the smallest number 20, were metacentric. Centromeric regions of the telocentric bivalents were seen to be more electronically dense, probably due to the persistence of condensed pericentromeric heterochromatin. However, in the centromeric region of the metacentric chromosome 1, this denseness was not seen. We frequently saw multiple turns in the trajectories of the autosomic synaptic complexes and recombination nodes towards the middle-distal and telomeric extremes of bivalents. The Y chromosome was completely paired with the X chromosome. This synapsis extended from the telomeric region at the common attachment end at the nuclear envelope, to just below half the length of the thick axis of the X chromosome (Fig. 2).

In 48 spermatocytes from three heterozygote individuals (Ht 2n = 43) we found: a trivalent, 19 autosomic bivalents and the XY bivalent. The trivalent showed complete synapsis between the metacentric chromosome 1 and the subtelocentric chromosomes 1a and 1b (Fig. 3).

For most of the spermatocytes studied, the Y chromosome appeared to be in total synapsis with the X chromosome. According to the configuration shown by the XY bivalent, some of the microspreads seemed to reveal that in the nucleus, both paired sex chromosomes had formed an arc, whose union with the nuclear envelope would be given by the XY common end and by the non-pairing extreme of the X chromosome (Fig. 3).

In 16 spermatocyte nuclei from two double homozygotes, DH individuals (2n = 44), 21 autosomic bivalents and the XY bivalent were seen. Autosomic bivalents of greater relative length and with a small short arm were identified as chromosome pairs 1a and 1b, in synapsis. For most of the cells studied, the Y chromosome showed total synapsis with the X chromosome, whose axis appeared thickened towards its non-pairing end (Fig. 4).

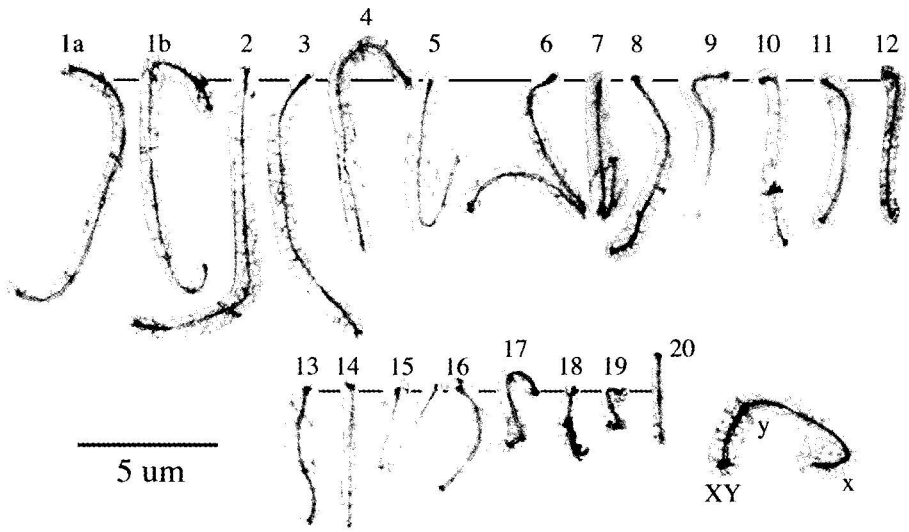
Detailed analysis of trivalents revealed that in 100% of cases, total synapsis was produced between the three involved chromosomes. This synapsis included the SC formed between the long arms of the subtelocentrics 1a and 1b and the respective homologous arms of the metacentric chromosome, and the SC formed between the heterologous short arms of chromosomes 1a and 1b which also showed variation in length from 1 to 2.8 μm (Fig. 5). Trivalents had three functional telomeric ends, with normally constituted attach-



Akodon molinae 2n=42 (SH)

Fig. 2. Meiotic karyotype of an *A. molinae* male SH 2n = 42 with 20 autosomic bivalents and the XY bivalent in complete synapsis. 18 autosomal bivalents are telocentric and the1 and 20 are metacentric. Arrowhead indicates the centromere of the metacentric 1. The X and Y chromosomes are in complete synapsis. XY: common end of the XY bivalent; y: single telomere of the Y; x: single telomere of the X.

Cariotipo meiótico de un macho SH 2n = 42 de *A. molinae* con 20 bivalentes autosómicos y el bivalente XY en sinapsis completa. 18 bivalentes autosómicos son telocéntricos y el 1 y 20 son metacéntricos. Punta de flecha señala el centrómero del metacéntrico 1. Los cromosomas X e Y estan en sinapsis completa. XY: extremo conjunto del bivalente XY; y: telómero simple del Y; x: telómero simple del X.



Akodon molinae 2n=44 (DH)

Fig. 3. Meiotic karyotype of an *A. molinae* male Ht, 2n = 43 with one trivalent, 19 autosomic bivalents and the XY bivalent. Arrowhead indicates the SC formed between the short arms of 1a and 1b. The X and Y chromosomes are in complete synapsis. XY: common end of the XY bivalent; y: single telomere of the Y; x: single telomere of the X.

Cariotipo meiótico de un macho Ht 2n = 43 de *A. molinae* con un trivalente, 19 bivalentes autosómicos y el bivalente XY. La flecha señala el CS entre los brazos cortos de 1a y 1b Los cromosomas X e Y estan en sinapsis completa. XY: extremo conjunto del bivalente XY; y: telómero simple del Y; x: telómero simple del X.

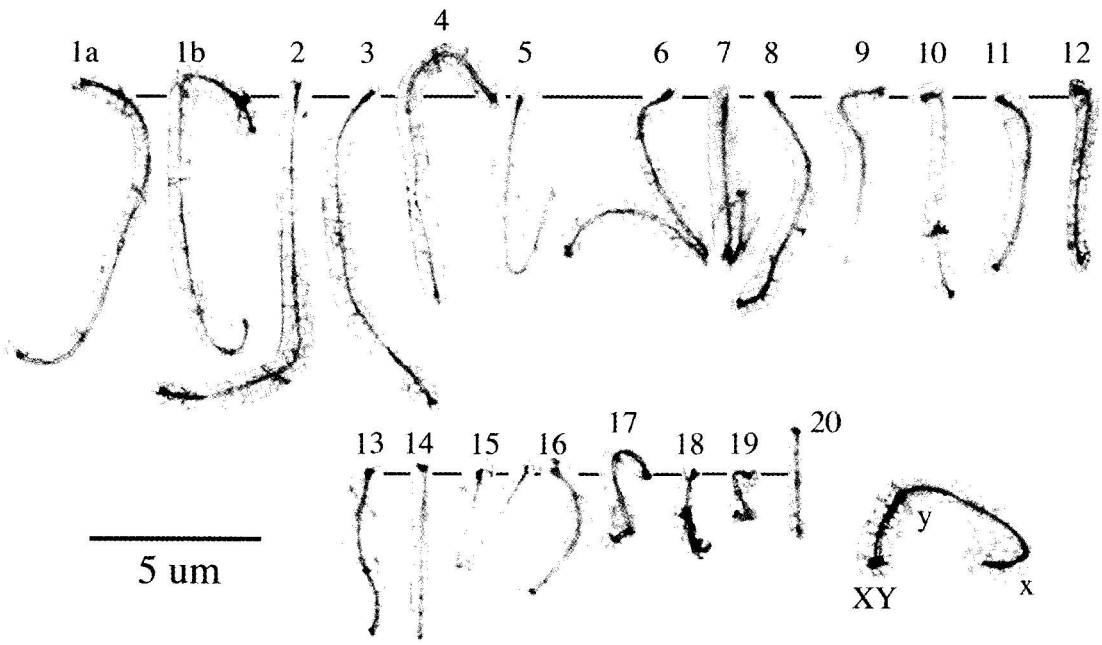


Fig. 4. Meiotic karyotype of a male *A. molinae* DH, 2n = 44 with 21 autosomic bivalents and the XY bivalent. Bivalents 1a and 1b show small short arms The X and Y chromosomes are in complete synapsis. XY: common end of the XY bivalent; y: single telomere of the Y; x: single telomere of the X.

Cariotipo meiótico de un macho DH 2n = 44 de *A molinae* con 21 bivalentes autosómicos y el bivalente XY. Los bivalentes 1a y 1b presentan un pequeño brazo corto Los cromosomas X e Y estan en sinapsis completa. XY: extremo conjunto del bivalente XY; y: telómero simple del Y; x: telómero simple del X.

ment plates, which would attach the SC with the nuclear envelope in three places, as shown in Fig. 5g. Remains of the nuclear envelope, which were still sticked to attachment plates, could also be seen in the third telomeric extreme of various trivalents (Figs. 5a, 5b y 5d). Many bivalents and trivalents also presented recombination nodules between homologous regions in synapsis. In trivalents, these nodules were generally located at distal positions with respect to the centromeres of the involved chromosomes.

DISCUSSION

A chromosome polymorphism can be operationally defined as the occurrence of alternative structural varieties in one or more pairs of homologous chromosomes, which as homo- and heterozygotes would have been established in a natural population or reproductive community. The frequency of homo- and heterozygote individuals would fluctuate around a relatively constant average from one generation to another. A polymorphism can disappear from the population if individuals bearing one of the chromosomal varieties are better adapted to the prevailing environmental conditions. In this case, the survival and reproduction of the better adapted variant would be strongly encouraged by natural selection at the expense of alternative forms.

Polymorphism of chromosome 1 in Akodon molinae

Polymorphism of the chromosome 1 in *A. molinae* (2n = 42-43-44, with an FN 44), has been extensively studied (Bianchi et al. 1973, Bianchi et al. 1979b) in specimens collected directly from natural populations as well as in experimental crosses between specimens of the three lineages, maintained and reproduced under laboratory conditions for various generations (Merani et al. 1980, Redi et al. 1982).

The homology of chromosomes 1a and 1b with the arms of chromosome I was confirmed in the present work by their equivalent pattern of G-

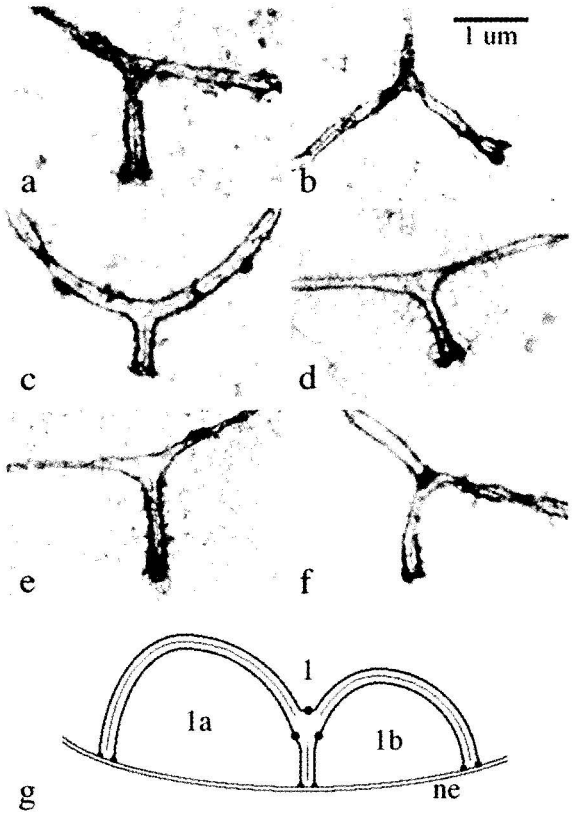


Fig. 5. a-f: detail of 6 trivalents from *A. molinae* Ht 2n = 43 Synapsis between short arms of chromosomes 1a and 1b show normal organization, except in the join between the three chromosomes -in Y form- where medial element of the SC is sketchy or disappears (c, d y e). Remains of the nuclear envelope can be seen in the attachment plates of telomeric ends. The SC segment of 1a and 1b short arms is variable in length. g: a trivalent 1, 1a and 1b, with its attachment to the nuclear envelope via three telomeres. ne: nuclear envelope.

a-f: detalle de 6 trivalentes de *A. molinae* Ht 2n = 43. La sinapsis entre los brazos cortos de los cromosomas 1a y 1b está normalmente organizada, salvo en el encuentro entre los tres cromosomas -en forma de Y- donde el elemento medial del SC se desdibuja o desaparece (c, d y e). Se aprecia restos de envoltura nuclear en las placas de adhesión en los extremos teloméricos. El segmento de SC de los brazos cortos de 1a y 1b es de longitud variable. g: trivalente 1, 1a y 1b, con inserciones a la envoltura nuclear via tres telómeros. ne: envoltura nuclear.

banding (Bianchi et al. 1973), by the appearance of trivalents in male first meiotic metaphase (Merani et al. 1980), as well as by the apparently normal SC organised along the whole extension of trivalents during pachytene of meiotic prophase as is shown in the present work.

The polymorphism of chromosome 1 is not exclusive to *A. molinae*. The same rearrangement, with similar chromosome structure and segregational behavior, is also present in *A. dolores*. This species has chromosomal formulae 2n = 34-35-36-38-39-40, with a NF 44 (Bianchi & Merani 1980).

Apfelbaum & Blanco (1984), in their studies of genetic similarity between species of the genus *Akodon* - similarity estimated according to electrophoretic patterns corresponding to 23 loci - observed that *A. molinae* and *A. dolores* were situated within the range of conspecific populations. Merani et al. (1983) obtained fertile hybrids between *A. molinae* and *A. dolores*, and Vidal-Rioja et al. (1982), in a study performed in various *Akodon* species, demonstrated that highly repeated DNA sequences in *A. molinae* and *A. dolores* showed a marked degree of similarity. From these evidences, Apfelbaum & Blanco (1984) suggested that *A. dolores* and *A. molinae* represented geographic chromosomal races of the same species.

In *A. dolores*, the chromosomes and polymorphisms that give rise to the large diploid number variation in this species, appear as Robertsonian fusions, in which the metacentric chromosomes derived from centric fusions conserve the pericentromeric heterochromatin present in their telocentric homologues. These chromosomes do not have short arms and the polymorphism types and frequencies show a clear correlation with the geographic distribution of the natural populations (Bianchi & Merani 1980).

The polymorphism of chromosome 1, shared by *A. dolores* and *A. molinae*, is independent of the geographical distribution of the natural populations in both species (Bianchi & Merani 1980). Chromosome 1 is a metacentric with no pericentromeric heterochromatin, and chromosomes 1a and 1b are subtelocentric with conspicuous short arms and have abundant pericentromeric heterochromatin (Bianchi et al. 1973).

By simple parsimony, the presence of chromosome 1 polymorphism in *A. molinae* and *A. dolores* strongly suggests that this would have been present in an ancestor common to both species. Metacentric chromosome 2 and polymorphisms of chromosomes 3, 4 and 5 of *A. dolores* (Bianchi & Merani 1980), would have appeared in natural populations bearing a karyotype similar to that of *A. molinae*, after both species had differentiated.

On the other hand, Bianchi and coworkers have observed that carriers of chomosomes 1a and 1b, would have a depressed viability and that maintenance of both chromosomes in natural

populations and under laboratory conditions, would, in some way, be assured by Ht individuals. These proposals have been supported by much evidence which we summarize below: (i) a very low representation of DH individuals in natural populations (Bianchi et al. 1979); (ii) low fertility of DH individuals in crosses made in the laboratory (only 0.5 % of that shown by SH animals, Bianchi & Merani 1980); (iii) a high frequency of segregational anomalies in DH individuals in meiotic divisions I and II (Merani et al. 1980, Redi et al. 1982); (iv) a significantly lower frequency than that expected for DH individuals from controlled Ht x Ht crosses in the laboratory. Furthermore, Ht individuals show decreased fertility in laboratory populations (30 % less than SH individuals) and have abnormal segregation levels in the second meiotic division which are similar to those observed in DH individuals (Merani et al. 1980, Redi et al. 1982).

Thus, Ht and DH individuals bearing chomosomes 1a and 1b, are affected at various levels of biological organisation, such as in meiosis, gametogenesis, reproduction and pre- and postnatal development. These results indicate that, even under conditions in which important environmental selective factors have been controlled, chromosomes 1a and 1b would be being strongly selected agaist.

Analysis of chromosome pairing and of the synaptonemal complexes

The study of chromosome pairing and of the SC in spermatocytes from the three *A. molinae* lineages gives us an analysis perspective which, although more complex, allows us to explain some of the mechanisms, which would assure quasi-normal segregation of trivalents in meiosis I of Ht individuals.

A detailed study of bivalents in spermatocytes from DH and SH individuals reveals normal chromosome pairings, and the number of bivalents formed are as expected. In DH individuals, bivalentes 1a and 1b were normally organized, and we were unable to detect any atypical element which might have indicated an altered meiotic prophase. The anomalies which have been described relative to chromosomes 1a and 1b in DH and Ht individuals, manifest mainly in the segregation phases of these chromosomes, as well as in gamete differentiation or in post-zygotic development.

No structural alterations of synapsis were observed in the pairing between chromosomes 1, 1a and 1b. The presence of only one trivalent per nucleus, could mean that the additional delay, which arises from the complete closure of the SC formed between the heterologous sectors, is not significant in the nuclear context for loosening the transition between zygotene and pachytene. The ending of chromosome pairing is one of the fundamental check-points for normal continuity of meiotic prophase (Odorisio et al. 1998), as has been observed in *Mus domesticus* heterozygotes for two or more Robertsonian rearrangements and which form trivalents (Redi et al. 1988, Redi et al. 1990). At the same time, the relatively early saturation of pairing between the heterologous segments of the trivalent, would prevent their participation in other ectopic associations in the peripheral region of the nucleus, for example, with the non-pairing segment of the X chromosome of the XY bivalent. No trivalent was observed associated with the XY bivalent. Ectopic relations with the sex chromosomes have been frequently observed in other mammalian species and in man, which have demonstrated that they can be highly deleterious for the normal evolution of pachytene (Forejt et al. 1981, Rosenmann et al. 1985).

In middle pachytene however, the XY bivalent showed total pairing of the Y chromosome with the X chromosome. Attachment of the common end of the XY bivalent SC to the nuclear envelope and the respective insertions of their independent telomeric ends, would maintain the sexual chromatin extensively adhered to the nuclear envelope. This organisation of the XY bivalent, would not favour ectopic interactions with the short arms of a trivalent in eventual asynapsis. In advanced pachytene, the X and Y chromosomes are paired by a small segment similar to the PAR (pseudo autosomal region) of other mammal species. Pairing between X and Y in this species, would seem to be produced between the distal segments of both chromosomes, which should result in an end to end association in metaphase I of meiosis.

Analysis of trivalents also reveals great variability in the length of the SC segment corresponding to heterologous pairing of chromosomes 1a and 1b. Such pairing would mainly involve the telomeres, centromeres and heterochromatin of the short arms of these chromosomes. Variation in length can be explained by different degrees of longitudinal adjustment of the SC, as has been observed in *M. domesticus* (Poorman et al. 1981, Moses et al. 1982, Moses 1984), and which has been suggested for other *Akodon* species and other mammals (Yonenaga-Yassuda 1979, Hale 1986, Greenbaum et al. 1990, Silva & Yonenaga-Yassuda 1998). We can not ignore that part of

these length variations could be due to artefactual stretching produced by the microspread technique. However, the SC of this heterologous segment appeared normally constituted, with lateral and medial elements and well developed attachment plates to the nuclear envelope. In all trivalents studied, the SC had a normal constitution, except for the small triangular segment located in the center of the Y shaped convergence of the three chromosomes. In this area, the medial element of the SC appeared deformed or was sketchy, or showed no organization at all.

Since the trivalents are attached to the nuclear envelope by three functional telomeres, this makes for an organisation which assures an opposed position of the short arms of chromosomes 1a and 1b with respect to chromosome 1 (Fig. 5). Terminal associations between proximal telomeres of chromosomes 1a and 1b, together with the extensive pairing and chiasma formation between the involved chromosomes, would contribute to orientate the centromeres of chromosome 1 with respect to chromosomes 1a and 1b, towards opposite poles in metaphase I of meiosis (Fig. 6).

Thus, the regular organization of a trivalent that derives in a quasi-normal segregation of the involved chromosomes in anaphase I, would indeed help to maintain the polymorphism in natural and laboratory *A. molinae* populations.

Segregation anomalies and viability of chromosomes 1a and 1b

In spermatocytes from Ht individuals, the presence of trivalents creates new facts and circumstances, which are not present in homozygote forms:

(i) Chromosomes 1, 1a and 1b are paired, forcing them to be located in the same nuclear space area of the meiocyte; (ii) due to the action of proximal telomeres of the short arms of 1a and 1b, a third point of attachment to the nuclear envelope appear; (iii) the axes of the short arms of 1a and 1b, which are heterologous, come together and form a SC; (iv) at the meeting point of chromosomes that form the trivalent SC, the centromeres of the three involved chromosomes become very close to each other (Fig. 5 and 6, Fernández-Donoso 1982b, Fernández-Donoso & Berríos 1985).

The organization and behavior of the small SC segment formed by the short arms of chromosomes 1a and 1b and the absence of recombination nodules in this same segment, lead us to assume that synaptic adjustment and possibly, a suppression of the crossing over event has occurred, as it has been observed in *Peromyscus* (Greenbaum & Reed 1984, Hale 1986, Grenbaum et al. 1990), *M. domesticus* (Davisson & Akeson 1993), and which has been proposed for other *Akodon* species (Silva & Yonenaga-Yassuda 1998).

However, the intimate juxtaposition, which is produced in this heterologous pairing, might not be innocuous for chromosomes 1a and 1b. The

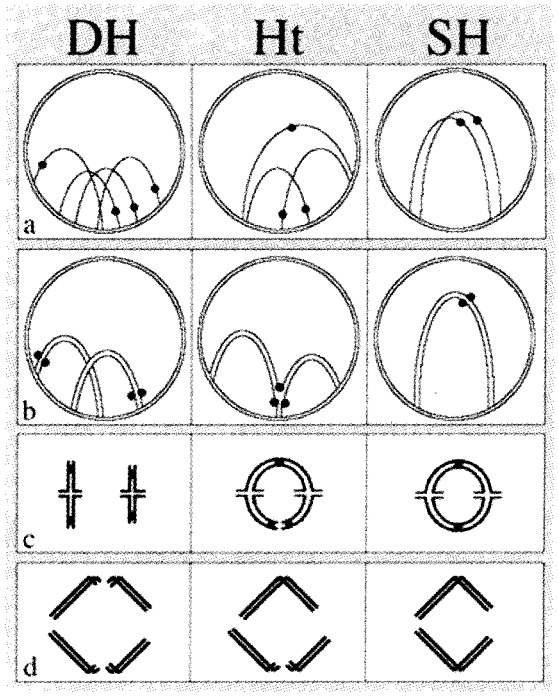


Fig. 6. Stages of meiosis I in DH, Ht and SH *A. molinae*. Row a, chromosomal axes in leptozygotene: homologous chromosomes begin to pair from their telomeric ends attached to the nuclear envelope. Row b, in pachytene pairing is completed. In Ht, chromosomes 1, 1a and 1b form a trivalent attached to the nuclear envelope via three telomeric ends. Row c, diacinesis - metaphase I with only one chiasma per chromosomal arm. Row d, segregation occurring in anafase I. Segregation of polymorphic chromosomes to opposite poles would be quasi normal in Ht individuals. (Modified from Fernández-Donoso 1982b).

Etapas de la I meiosis en *A. molinae* DH, Ht y SH. Fila a, ejes cromosómicos en lepto - cigoteno: los homólogos se aparean por sus extremos teloméricos unidos a la envoltura nuclear. Fila b, en paquiteno el apareamiento es completo. En los Ht, los cromosomas 1, 1a y 1b forman un trivalente unido a la envoltura nuclear por tres telómeros. Fila c, la diacinesis - I metafase meiótica con sólo un quiasma por brazo cromosómico. Fila d, segregación en la anafase I. En individuos Ht la segregación a polos opuestos de los cromosomas polimórficos sería cuasi normal. (Modificado de Fernández-Donoso 1982b).

segregational anomalies of chromosomes 1a and 1b in meiosis II of Ht and in meiosis I and II of DH individuals, as well as their decreased viability, might be other of the consequences of trivalent formation in Ht individuals.

With this in mind, we visualize at least two possible effects of the obliged configuration of trivalents: (1) induction of a Robertsonian translocation between telocentric or sub-telocentric chromosome partners while they are forming the trivalent with an homologous metacentric chromosome during pachytene of meiotic prophase, a phenomenon which would vary in frequency between cells and individuals; or (2) erosion of the integrity and structure of the proximal ends of telocentic chromosomes, or of the short arms of sub-telocentric chromosomes, while they are forming the trivalent during pachytene, via interchromosomal genic conversion and/or transposition and duplication of DNA sequences. These phenomena are thought to damage the centromeres and proximal telomeres, being very frequent in all heterocygote individuals and of variable intensity in different cells.

With respect to the first effect, Redi et al. (1990) have carried out a clear and extensive analysis of the rapid establishment and accumulation of Robertsonian chromosomes in natural populations of *Mus domesticus*, and have proposed a "genomic scenario", which models the conditions of nuclear architecture and the molecular mechanisms of interchange between chromosomes explaining these conditions. The close chromosomic association, which is produced between centromeres and telomeres of trivalent telocentric partners, as well as the changes in DNA metabolism with persistence of the endogenously generated nicking activity in pachytene of male structural heterozygotes (Hotta et al. 1977, 1979, Stubbs & Stern 1986), would make it possible for the Rb metacentric chromosome to induce fusion of its telocentric partners in heterozygotes (Fernández-Donoso 1982a, Dover et al. 1984, Imai 1988, Haaf et al. 1989, Redi et al. 1990). Possibly, similar mechanisms were present in the Robertsonian fusion attained by cromosome number 2, and would be the working mechanisms in the polymorphic chromosomes number 3, 4 and 5, in *A. dolores*.

The second effect would take place, when short arms of significant length are present in a trivalent with subtelocentric partners. However, it is not necessarily an alternative to the first one; both could happen simultaneously. To a greater or lesser extent, both effects can be the consequence of the action of intrinsic nuclear factors, among which the concurrence of the particular structure and function of the SC (Sybenga 1999), the association between meiotic chromosomes (Berríos & Fernández-Donoso 1990), typical and/or atypical recombination forms (Choo et al. 1988, Storlazzi et al. 1995, among others) and the molecular dynamics of the genome (molecular drive; Dover 1982, 1988, John & Miklos 1988), would play a fundamental role. In recent studies of meiotic recombination in mammals, Ashley has found hyper-recombination sites - situated in the telomere or very near to them - which function as recombinational "hot spots" in the absence of recombination nodules (Ashley et al. 1993, Ashley 1994). Also, analysis of alpha satellite DNA (pTRA-2) distribution in the short arms of human nucleolar chromosomes suggests DNA interchange between non-homologous chromosomes via ectopic recombinations (Choo et al. 1988, Moens 1994).

From this standpoint, we believe that in *A. molinae* and *A. dolores*, the structural micro arrangements induced in the short arms of chromosomes 1a and 1b while they were forming part of a trivalent, may damage them, widely affecting the segregation and viability of these chromosomes in Ht and DH individuals. In other words, in the trivalent formed in Ht individuals of both species, genic transformation and molecular alterations of the centromeres and telomeres, as well as the interchange of heterochromatic segments by means of ectopic recombinations, would be producing a progressive erosion in the coadapted structure of the short arms of the original forms 1a and 1b. On the other hand, the successful viability of SH individuals in both species demonstrates the outcome of chromosome 1 and that these genomes can function without the short arms of 1a and 1b.

Thus, the erosion of 1a and 1b short arms might account for the segregational anomalies in meiosis II of Ht and in meiosis I and II of DH and for the hyper or hypo haploidies in sperms and early embryonic mortality in such individuals, all of which would lead to a decreased litter size and the poor representation of chromosomes 1a and 1b, in both natural and laboratory populations.

CONCLUSIONS

1. The formation of the trivalent in heterozygotes, would yield a quasi-normal segregation between chromosomes 1, and 1a and 1b, so that heterozygotes would be able to withstand the spread of chomosome 1, as well as of chromosomes 1a and 1b, with eroded short arms.

2. Spreading from Ht individuals, chromosomes 1a and 1b with damaged short arms would have accumulated among DH individuals. The Ht would possess intrinsic nuclear advantages with respect to DH individuals due to the presence of chromosome 1. The metacentric form would be favoured in the reproductive community, because of the maintainance of its integrity and its normal segregation.

3. The polymorphism of chromosome 1 in *A. molinae* and *A. dolores* would represent an advanced stage in the progressive disappearance of chromosomes 1a and 1b, which would be being displaced by chromosome 1.

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