Hexavalents in spermatocytes of Robertsonian heterozygotes between *Mus m. domesticus* 2n=26 from the Vulcano and Lipari Islands (Aeolian Archipelago, Italy)

Soledad Berríos,[1] Raúl Fernández-Donoso,[1] Jesús Page,[2] Eliana Ayarza,[3] Ernesto Capanna,[4] Emanuela Solano,[4] Riccardo Castiglia[4]

[1]Programa Genética, Instituto Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[2]Departamento de Biología Celular, Facultad de Ciencias, Universidad Autónoma de Madrid, Spain
[3]Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile, Independencia, Chile
[4]Department of Biology and Biotechnology "Charles Darwin", La Sapienza University of Rome, Italy

Abstract

The size and shape of the chromosomes, as well as the chromosomal domains that compose them, are determinants in the distribution and interaction between the bivalents within the nucleus of spermatocytes in prophase I of meiosis. Thus the nuclear architecture characteristic of the karyotype of a species can be modified by chromosomal changes such as Robertsonian (RB) chromosomes. In this study we analysed the meiotic prophase nuclear organization of the heterozygous spermatocytes from *Mus musculus domesticus* 2n=26, and the synaptic configuration of the hexavalent formed by the dependent Rb chromosomes Rbs 6.16, 16.10, 10.15, 15.17 and the telocentric chromosomes 6 and 17. Spreads of 88 pachytene spermatocytes from two males were studied and in all of them five metacentric bivalents, four telocentric bivalents, one hexavalent and the XY bivalent were observed. About 48% of the hexavalents formed a chain or a ring of synapsed chromosomes, the latter closed by synapsis between the short arms of telocentric chromosomes 6 and 17. About 52% of hexavalents formed an open chain of 10 synapsed chromosomal arms belonging to 6 chromosomes. In about half of the unsynapsed hexavalents one of the telocentric chromosome short arms appears associated with the X chromosome single axis, which was otherwise normally paired with the Y chromosome. The cluster of pericentromeric heterochromatin mostly determines the hexavalent's nuclear configuration, dragging the centromeric regions and all the chromosomes towards the nuclear envelope similar to an association of five telocentric bivalents. These reiterated encounters between these chromosomes restrict the interactions with other chromosomal domains and might favour eventual rearrangements within the metacentric, telocentric or hexavalent chromosome subsets. The unsynapsed short arms of telocentric chromosomes frequently bound to the single axis of the X chromosome could further complicate the already complex segregation of hexavalent chromosomes.

Correspondence: Soledad Berríos, Programa Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile, 1027 Independencia Ave., Santiago 8380453, Chile.
Tel. +56.2.29786462.
E-mail: sberrios@med.uchile.cl

Keywords: Hexavalents; Robertsonian chromosomes; mouse spermatocytes; nuclear architecture.

Contributions: EA, JP, RFD, experimental design and performance; SB, EC, ES, RC, data analysis; SB, manuscript drafting.

Conflict of interest: the authors report no conflict of interest.

Funding: This work was supported by VID Universidad de Chile (Chile) and CGL2014-53106-P from the Ministerio de Economía y Competitividad (Spain).

Received for publication: 24 January 2018.
Accepted for publication: 12 February 2018.

European Journal of Histochemistry 2018; 62:2894
doi:10.4081/ejh.2018.2894

Introduction

Robertsonian (Rb) translocations are frequently present in natural populations of the house mouse *Mus musculus domesticus*.[1] Rb translocations involve double-strand DNA breaks at the centromere in two telocentric (acrocentric) chromosomes, followed by repair (fusion) ligating the respective long arms, creating a metacentric Rb chromosome.[2,3] Thus the emergence of Rb metacentric chromosomes leads to the reduction of the diploid number of the standard karyotype of 40 telocentric chromosomes of the domestic mouse. This natural process has produced more than 40 different chromosomal races, ranging from 2n=40 to 2n=22.[4] Another mechanism of metacentric formation involves whole arm reciprocal translocation (WART).[5] A WART is an exchange of chromosome arms between two metacentrics or between a metacentric and a telocentric chromosome. This process generates a new metacentric with a different arm combination.[4]

In the emergence of new Rb chromosomes or in the encounter of two different homozygous populations, Rb heterozygotes are produced. According to the homology between Rb chromosomes present in heterozygotes, two behaviours can be observed in the chromosomal synapse of prophase I of meiosis; in single or multiple heterozygotes, Rb metacentric chromosomes and the respective homologous telocentric chromosomes form trivalents, while in heterozygous complexes, homologous chromosome arms of different Rb metacentric chromosomes produce rings or chains of several synapsed chromosomes.[6-8]

The cytogenetic analysis of *M. m. domesticus* from all the seven islands that form the Aeolian Archipelago shows the four Rb races with a large number of shared metacentric chromosomes: 2n = 36/37 on Panarea, 2n = 34 on Alicudi, 2n = 26 on Lipari and Stromboli, and a different 2n = 26 race on Vulcano. The standard karyotype was found on Salina and Filicudi.[9,10] Chromosome differences between mice from Vulcano and Lipari can produce, in laboratory breeding conditions, hybrid mice that show a multivalent chain of six chromosomes composed of four metacentric and two telocentric chromosomes in their meiotic diakineses.[9,10]

We have shown that the Rb chromosomes change the nuclear architecture of house mouse spermatocytes in meiotic prophase. This not only means that the chromosome domains of the derived Rb chromosome have a different location in the nuclear space of the spermatocyte, but also that the probability of interaction between chromosome domains of non-homologous chromosomes changes.[11] The meiotic nuclear organization of the heterozygotes of crosses between Lipari and Vulcano mice are of enormous interest. The heterozygotes share the same diploid chromosome number as homozygous individuals, four pairs of telocentric chromosomes and seven pairs of Rb chromosomes; however, four Rb metacentric chromosomes present monobrachial homology, which in the meiotic synapse is

known to generate a chain of synapsed chromosomes.[9,12]

In this study we analyse the meiotic prophase nuclear organization of the heterozygous spermatocytes of *Mus m. domesticus* 2n=26 and the synaptic configuration of the hexavalent formed by the dependent Rb chromosomes Rbs 6.16, 16.10, 10.15, 15.17 and the telocentric chromosomes 6 and 17. We describe the meiotic organization of the hexavalents and discuss the possible segregation consequences of synapsed chromosomes in closed or non-closed chain configuration.

Materials and Methods

Hybrids

We analysed spermatocytes from two three month-old males of *Mus m. domesticus* 2n = 26. Heterozygous mice were generated by mating strain 2n = 26 mice from Lipari and Vulcano Islands (Figure 1a). For collection of the original samples see Solano *et al*.[9]

The Rb chromosomes present in hybrids were the following: Rb (1.2, 3.9, 4.13, 5.14, 8.12, 6.16, 16.10, 10.15, 15.17) (Figure 1a). Mice were maintained at 22°C with a light/dark cycle of 12/12 hours and fed *ad libitum*. Procedures involving the use of the mice were reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universidad de Chile, and by the Ethics Committee of the Universidad Autónoma de Madrid.

Spermatocyte nuclear spreads

Spermatocyte spreads were obtained following the procedure described by Peters *et al*.[13] Briefly, a testicular cell suspension in 100 mM sucrose was spread onto a slide dipped in 1% paraformaldehyde in distilled water containing 0.15% Triton X-100 then left to dry for two hours in a moist chamber. The slides were subsequently washed with 0.08% Photoflo (Kodak, Rochester, NY, USA), air-dried, and rehydrated in PBS.

Immunochemical identification of chromosome pairings

The slides were incubated for 1 h at 37°C in a moist chamber with the primary antibodies rabbit anti-SYCP3 1:100 (ab15093; Abcam, Cambridge, UK) and mouse anti-CENPA 1:200 (ab13939; Abcam). Then the slides were incubated for 1 h at room temperature with the secondary antibodies: FITC-conjugated goat anti-rabbit and Texas Red-conjugated goat anti-mouse IgG (1:200) (Jackson ImmunoResearch, West Grove, PA, USA). Slides were counterstained with 1 µg/mL DAPI (4,6-diamidino-2-phenylindole). Finally, slides were rinsed in PBS and mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA).

Observations were made using a Nikon (Tokyo, Japan) Optiphot or Olympus BX61

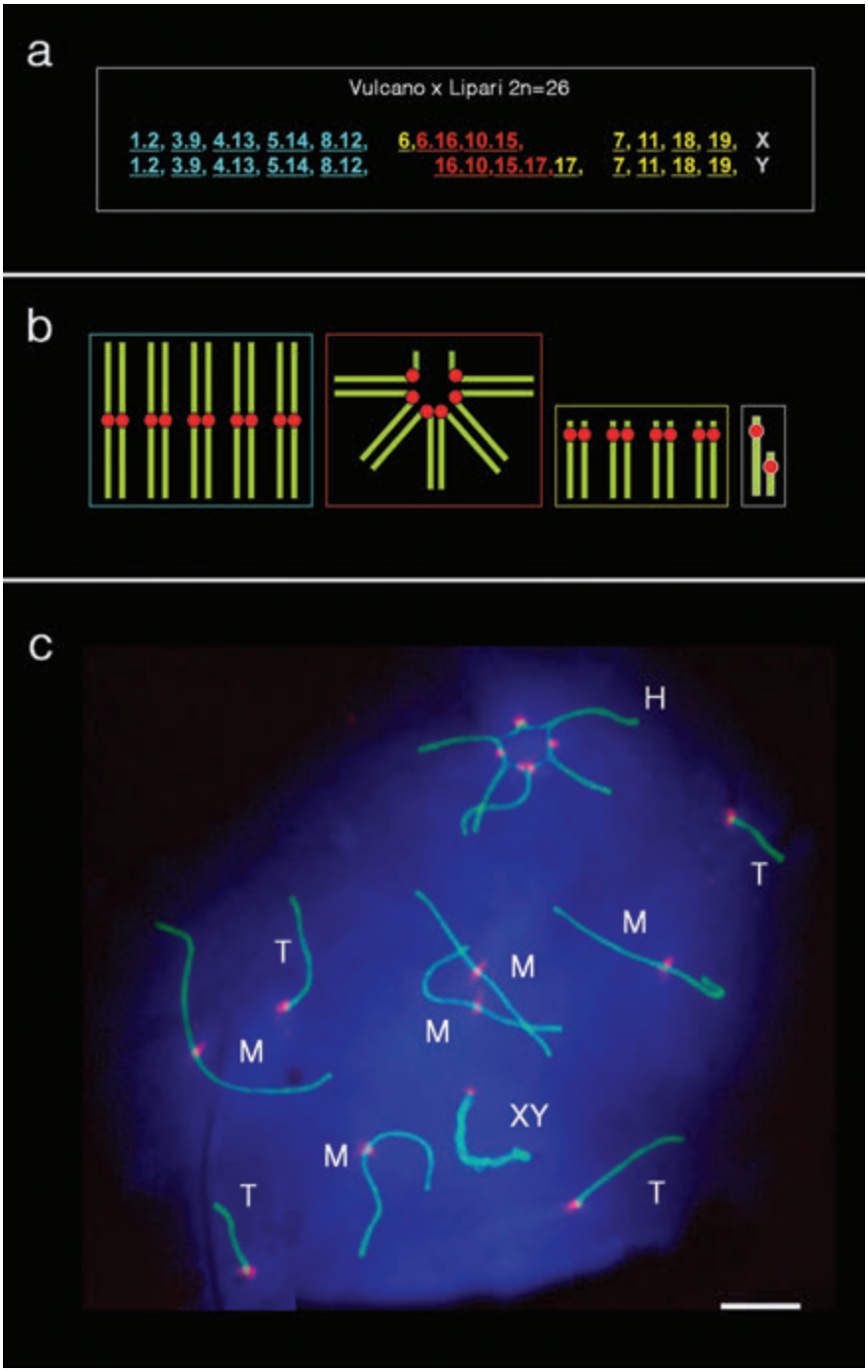

Figure 1. Mitotic and meiotic chromosomes of the hybrid male 2n=26 resulting from the cross between Vulcano and Lipari mice. a) Mitotic karyotype: five pairs of metacentric Rb chromosomes, four pairs of telocentric chromosomes, the sex chromosomes X and Y, and the remaining 6 chromosomes, 6, 6.16, 16.10, 10.15, 15.17 and 17, that present monobrachial homology. b) Meiotic chromosomes: five metacentric bivalents, one hexavalent, four telocentric bivalents and the sex bivalent. c) Nuclear spread of a pachytene spermatocyte: five metacentric bivalents (M), four telocentric bivalents (T), one hexavalent (H) and a complete synapsed XY bivalent (XY); the synaptonemal complexes were labelled with FITC anti-SYCP3 antibodies (green), the centromeres with Texas Red anti CENP-A antibodies (red) and the nuclear DNA was stained with DAPI (blue); scale bar: 2 µm.

microscope equipped with epifluorescence optics, and the images were photographed with a DS camera control unit DS-L1 Nikon or captured with an Olympus DP70 digital camera. All images were processed using Adobe Photoshop CS5.1 software or the public domain software ImageJ (National Institutes of Health, United States; http://rsb.info.nih.gov/ij).

Results

Germ cells in different stages of differentiation were observed in all the nuclear micro spreads examined. A relative proportion of 2:3 spermatids was estimated with respect to the number of spermatocytes I. Five metacentric bivalents, four telocentric bivalents, a hexavalent and the XY bivalent were observed in all spermatocytes in pachytene treated with immunocytochemistry to identify the synaptonemal complex and the position of the centromeres (Figure 1 b,c). No spermatocytes were seen with unsynapsed chromosomes or with absence of hexavalents.

In 43 of the 88 spermatocytes examined (49%) the hexavalent was found forming a closed chain (ring) of synapsed chromosomes (Figure 1c and 2a). In these cases, the short arms of telocentric chromosomes 6 and 17 are joined by synapses with the presence of the SCP1 protein (*not shown*).

In open-chain hexavalents the short arms of chromosomes 6 and 17 were not bound to each other (Figure 2 b,c). In 22 of 44 of the open hexavalents examined (50%), one of these asynaptic axes was bound to the single axis of the X chromosome, which was otherwise normally paired with the Y chromosome (Figure 2c).

When a ring of 6 synapsed chromosomes configures a closed hexavalent, at the meiotic prophase nucleus all the synaptonemal complexes describe arcs in which the proximal telomeres and centromeres converge and are immersed in a central block of pericentromeric heterochromatin from the 6 chromosomes. The block of heterochromatin is broadly attached to the nuclear envelope similar to an association of five telocentric bivalents of *Mus*. The distal telomeres are attached circularly to the nuclear envelope at the surrounding perimeter of this heterochromatin cluster (Figure 3a). Alternatively, a linear chain of six synapsed chromosomes configures the open hexavalent, whose free extremes correspond to the short arms of chromosomes 6 and 17. Each synapsed chromosome describes an arc whose proximal telomeres and centromeres form part of an elongated block of pericentromeric heterochromatin and the distal telomeres are attached to the neighbouring surface of the nuclear envelope. Half of them appeared bound to the non-paired axis of the X chromosome (Figure 3b).

Discussion

Mouse heterozygotes derived from wild populations have shown that the presence of one or three trivalents at meiosis may have little effect on fertility,[14-16] whereas many trivalents or longer chains or rings may reduce fertility to the point of sterility.[12,16] Chromosome differences between mice from Vulcano and Lipari produce hybrid mice that carry a chain of six chromosomes composed of four metacentrics and two telocentric chromosomes. Preliminary analysis of fertility by Solano and Castiglia showed that fertility in hybrids is reduced compared to homozygotes.[9]

In our observations, the Lipari-Vulcano heterozygote males have a complete germ cell line, although with a lower proportion of spermatids than expected considering the number of spermatocytes I. Apparently, the spermatocytes develop normally throughout the meiotic prophase, at least in terms of synapsis, both the pairs of homologous chromosomes that form bivalents and those involved in the hexavalent. In fact, the nuclear configuration of hexavalents closely resembles that of the associated telocentric bivalents through their pericentromeric heterochromatin in pachytene spermatocytes of 2n = 40 individuals.[17] The associations of bivalents observed in those pachytene spermatocytes would originate very early within prophase I. Indeed, a block of pericentromeric heterochromatin would be formed associating all the chromosomes by their proximal ends during the formation of the bouquet in leptotene.[18,19] Then, with the progress of prophase and the increase in the nuclear volume, the initial large cluster of heterochromatin that associated all the bivalents is disaggregated into smaller fragments. As a result of this phenomenon, groups of different numbers of associated bivalents would remain scattered on the surface of the nuclear envelope.[17] Following this line of thought, the initial cluster of heterochromatin of the early

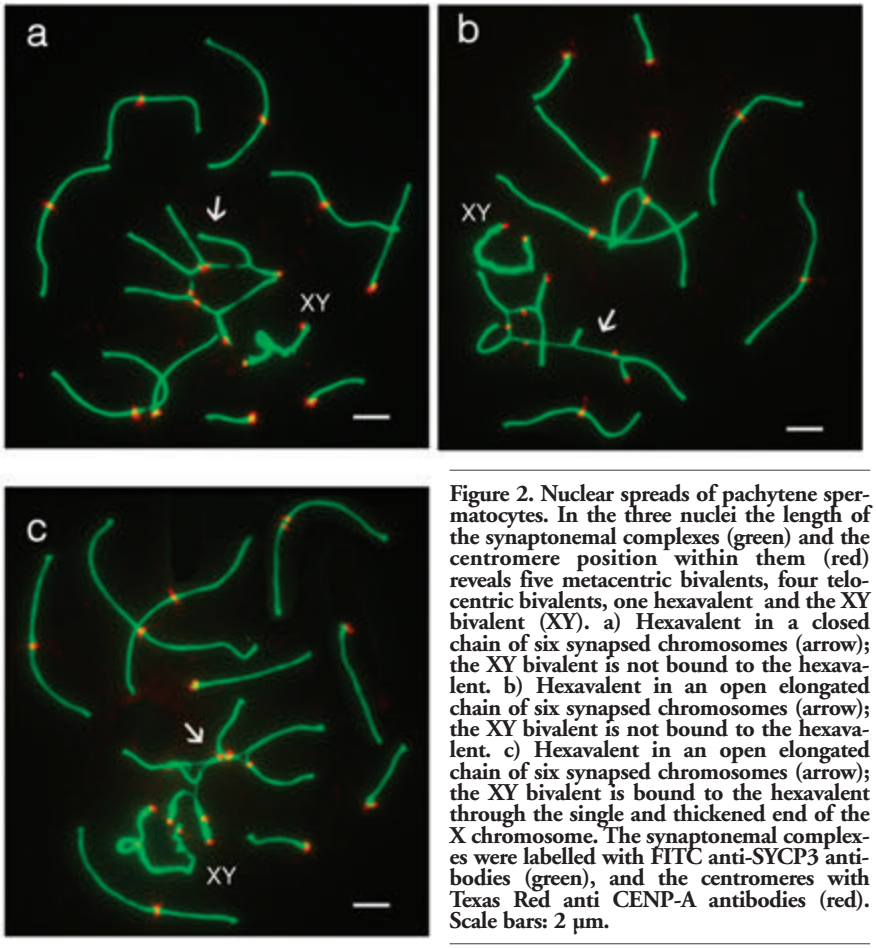

Figure 2. Nuclear spreads of pachytene spermatocytes. In the three nuclei the length of the synaptonemal complexes (green) and the centromere position within them (red) reveals five metacentric bivalents, four telocentric bivalents, one hexavalent and the XY bivalent (XY). a) Hexavalent in a closed chain of six synapsed chromosomes (arrow); the XY bivalent is not bound to the hexavalent. b) Hexavalent in an open elongated chain of six synapsed chromosomes (arrow); the XY bivalent is not bound to the hexavalent. c) Hexavalent in an open elongated chain of six synapsed chromosomes (arrow); the XY bivalent is bound to the hexavalent through the single and thickened end of the X chromosome. The synaptonemal complexes were labelled with FITC anti-SYCP3 antibodies (green), and the centromeres with Texas Red anti CENP-A antibodies (red). Scale bars: 2 µm.

prophase may contribute to the hexavalent formation, and its preservation in pachytene is not a disturbing element because in the meiotic nuclear organization of *Mus* it is common that several bivalents remain associated through their pericentromeric heterochromatin. In fact, we did not observe pachytene spermatocytes with extended lack of synapse or with appreciable nuclear alterations. We also did not observe spermatocytes in which the Meiotic Silence Unsynapsed Chromosomes (MSUC), which is accomplished by a complex series of epigenetic modifications in the chromatin, had been extensively triggered.[20,21]

Thus, the observed reduction in the expected number of spermatids is possibly due to difficulties in the segregation of the chromosomes involved in the hexavalent that should be manifested in the meiotic divisions.[22] Also, the asynaptic axes of the short arms of chromosomes 6 and 17, and particularly their ectopic association with the X chromosome, would further complicate the already predictably difficult segregation of the chromosomes committed in the hexavalent. In order to produce balanced gametes, the hexavalent can only segregate as the chromosomes present in the gametes of individuals from Lipari or Vulcano Islands. Any other combination or a new Rb fusion between chromosomes 6 and 17 would necessarily lead to unbalanced gametes and consequently to trisomic or monosomic descendents. This evidence indicates that a mechanism of reproductive isolation has been generated between mice from the Lipari and Vulcano islands.

The hexavalent not only implies the synapses of six chromosomes but also the restriction of the possible interactions between the remaining chromosomes. We showed that in the house mouse with an all-acrocentric karyotype the chromosome interactions are very broad and with practically the same probability between the 19 autosomal bivalents.[23] However, chromosome interactions would be strongly restricted in the meiosis of the Lipari-Vulcano heterozygotes. Six chromosomes would be obligatory together in the hexavalent and only the 5 metacentric bivalents among themselves and the 4 telocentric bivalents among themselves would be available for associations. This nuclear organization would favour the occurrence of chromosome changes within each of these three chromosomal subsets: WARTS among the five metacentric bivalents or among the hexavalent chromosomes and Rb fusions between the four telocentric bivalents.[11,24]

The hexavalent is a complex structural organization strongly settled over the nuclear envelope through telomeres and pericentromeric heterochromatin coming from six synapsed chromosomes, and this to a large extent determines the nuclear organization and possible interactions between the other chromosomes. The unsynaptic axes of the heterologous regions bound to the sex chromosomes may be additional perturbing factors to the progression of meiosis mostly by altering chromosome segregation. The effects exerted by the chromosome changes present in Robertsonian heterozygotes may be better understood knowing the configuration that these chromosomal rearrangements may adopt in the first meiotic prophase nuclei; both chromosome malsegregation and the associated germ cell losses become more intelligible thanks to the cytochemical descriptive analysis of chromosome pairing.

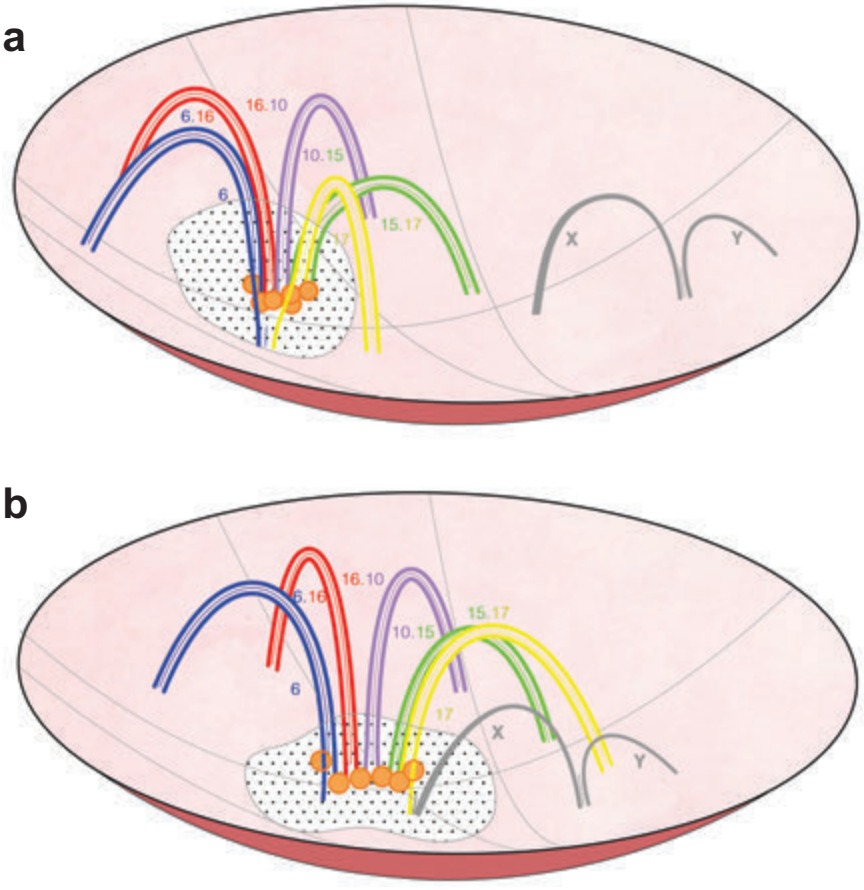

Figure 3. Synaptic configuration of the hexavalent in the meiotic prophase nucleus. a) Closed chain of six synapsed chromosomes: 6 (blue), 6.16 (blue-red), 16.10 (red-purple), 10.15 (purple-green), 15.17 (green-yellow) and 17 (yellow); the telocentric chromosomes 6 and 17 are bound to each other through their short arms; the sex bivalent is not bound to the hexavalent; all synapsed chromosomes describe arcs whose proximal telomeres and centromeres are immersed in a central block of pericentromeric heterochromatin coming from the six chromosomes (dotted area), which in turn is broadly attached to the nuclear envelope; the distal telomeres are attached to the nuclear envelope circularly at the surrounding perimeter of this heterochromatin cluster. b) Open chain of six synapsed chromosomes: 6 (blue), 6.16 (blue-red), 16.10 (red-purple), 10.15 (purple and green, 15.17 (green-yellow) and 17 (yellow); the short arms of the telocentric chromosomes 6 and 17 are not bound to each other and one of them is bound to the single axis of the X chromosome; each synapsed chromosome describes an arc whose proximal telomeres and centromeres are forming part of an elongated block of pericentromeric heterochromatin and the distal telomeres are attached in the neighboring surface of the nuclear envelope.

References

1. Capanna E, Gropp A, Winking H, Noack G, Civitelli MV. Robertsonian metacentrics in the mouse. Chromosoma 1976;58:341-53.
2. Redi CA, Garagna S, Hilscher W, Winking H. The effects of some Robertsonian chromosome combinations on the seminiferous epithelium of the mouse. J Embryol Exp Morphol 1985;85:1-19.
3. Garagna S, Marziliano N, Zuccotti M, Searle JB, Capanna E, Redi CA. Pericentromeric organization at the fusion point of mouse Robertsonian translocation chromosomes. Proc Natl Acad Sci USA 2001;98:171-5.
4. Piálek J, Hauffe HC, Searle JB. Chromosomal variation in the house mouse. Biol J Linn Soc 2005;84:535-63.
5. Capanna E, Redi CA. Whole-arm reciprocal translocation (WART) between Robertsonian chromosomes: finding of a Robertsonian heterozygous mouse with karyotype derived through WARTs. Chromosome Res 1995;3:135-7.
6. Wallace BM, Searle JB, Everett CA. The effect of multiple simple Robertsonian heterozygosity on chromosome pairing and fertility of wild-stock house mice (Mus musculus domesticus). Cytogenet Genome Res 2002;96:276-86.
7. Matveevsky S, Bakloushinskaya I, Tambovtseva V, Romanenko S, Kolomiets O. Analysis of meiotic chromosome structure and behavior in Robertsonian heterozygotes of Ellobius tancrei (Rodentia, Cricetidae): a case of monobrachial homology. Comp Cytogenet 2015;9:691-706.
8. Berríos S, Fernández-Donoso R, Ayarza E. Synaptic configuration of quadrivalents and their association with the XY bivalent in spermatocytes of Robertsonian heterozygotes of Mus domesticus. Biol Res 2017;50:38.
9. Solano E, Castiglia R, Capanna E. Chromosomal evolution of the house mouse, Mus musculus domesticus, in the Aeolian Archipelago (Sicily, Italy). Biol J Linn Soc 2009;96:194-202.
10. Capanna E, Castiglia R, Solano E. Men and mice: mouse population genetics in the Aeolian archipelago. S. Casellato, P. Burighel and A. Minelli, Editors. Life and time: The evolution of life and its history. Cleup; Padova: 2009.
11. Berríos S, Manieu C, López-Fenner J, Ayarza E, Page J, González M, et al. Robertsonian chromosomes and the nuclear architecture of mouse meiotic prophase spermatocytes. Biol Res 2014;47:16-29.
12. Hauffe HC, Searle JB: Chromosomal heterozygosity and fertility in house mouse (Mus musculus domesticus) in Northern Italy. Genetics 1998;150: 1143-54.
13. Peters AH, Plug AW, Van Vugt MJ, De Boer P: A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. Chromosome Res 1997; 5:66–68.
14. Britton-Davidian J, Sonjaya H, Catalan J, Cattaneo-Berrebi G. Robertsonian heterozygosisty in wild mice: fertility an transmission rates in Rb(16.17) translocation heterozygotes. Genetica 1990;80:171-4.
15. Wallace BM, Searle JB; Everett CA. Male meiosis and gametogenesis in wild house mice (Mus musculus domesticus) from a chromosomal hybrid zone; a comparison between 'simple' Robertsonian heterozygotes and homozygotes. Cytogenet. Cell Genet 1992;61:211-20.
16. Castiglia R, Capanna E. Contact zone between chromosomal races of Mus musculus domesticus. 2. Fertility and segregation in laboratory-reared and wild mice multiple heterozygous for multiple Robertsonian rearrangements. Heredity 2000;85:147-57
17. Berríos S, Manterola M, Prieto Z, Lopez-Fenner J, Page J, Fernández-Donoso R. Model of chromosome associations in Mus domesticus spermatocytes. Biol Res 2010;43:275-85.
18. Scherthan H. A bouquet makes ends meet. Nat Rev Mol Cell Biol 2001; 2:621-7.
19. Berríos S. Nuclear architecture of mouse spermatocytes: Chromosome topology, heterochromatin, and nucleolus. Cytogenet Genome Res 2017; 151:61-71.
20. Burgoyne PS, Mahadevaiah SK, Turner JM. The consequences of asynapsis for mammalian meiosis. Nat Rev Genet 2009;10:207-16.
21. Naumova AK, Fayer S, Leung J, Boateng KA, Camerini-Otero RD, Taketo T. Dynamics of response to asynapsis and meiotic silencing in spermatocytes from Robertsonian translocation carriers. PLoS One 2013;8:e75970.
22. Redi CA, Garagna S, Zuccotti M. Robertsonian chromosome formation and fixation: the genomic scenario. Biol J Linn Soc 1990;41:235-55.
23. López-Fenner J, Berríos S, Manieu C, Page J, Fernández-Donoso R. Bivalent associations in Mus domesticus 2n=40 spermatocytes. Are they random? Bull Math Biol 2014;76:1941-52.
24. Solano E, Castiglia R, Corti M. A new chromosomal race of the house mouse, Mus musculus domesticus, in the Vulcano Island-Aeolian Archipelago, Italy. Hereditas 2007;144:75-7.