Analysis of meiotic chromosome structure and behavior in Robertsonian heterozygotes of *Ellobius tancrei* (Rodentia, Cricetidae): a case of monobrachial homology

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Abstract

Synaptonemal complex (SC) chains were revealed in semisterile intraspecific F1 hybrids of *Ellobius tancrei* Blasius, 1884 (2n = 49, NF=56 and 2n=50, NF=56), heterozygous for Robertsonian (Rb) translocations. Chains were formed by Rb submetaeucentrics with monobrachial homology. Chromosome synapsis in spermatocytes of these hybrids was disturbed, apparently because of the problematic release of the chromosomes from the SC chains. These hybrids suffer from low fertility, and our data support the opinion that this is because a formation of Rb metacentrics with monobrachial homology within different races of the same species might be an initial event for the divergence of chromosomal forms.

Keywords

Meiosis, synaptonemal complex, SC multivalent, hybrid, fertility
Introduction

Many authors have described the significant impact of chromosomal rearrangements as reproductive barriers in the speciation process (White 1945, Matthey 1951, 1953, Capanna 1982, Baker and Bickham 1986, Sites and Moritz 1987, King 1987, 1993, Vorontsov 1989, Nunes et al. 2011, etc.). W. Robertson first described centric fusions, which join two acrocentrics into one metacentric chromosome, in grasshoppers (Robertson 1916). Named Robertsonian (Rb) translocations, they are widespread among animals, including mammals, and might result in chromosome polymorphism and/or species diversification (Moses et al. 1979, Lyapunova et al. 1980, Searle et al. 1991, Stanyon et al. 2002, Volobouev et al. 2007, and others).

Heterozygosity for the Rb translocations may seriously affect the segregation of chromosomes during meiosis. In heterozygotes, the first division of meiosis will take place without complications only if both acrocentrics, homologous to Rb metacentric arms, move into one pole, whereas the metacentric moves to the opposite pole of the nucleus. Otherwise, if the Rb metacentric and one of the homologous acrocentrics go to the same pole of the nucleus, an inadequate segregation of chromosomal arms will take place and aneuploid gametes will develop (Cappanna et al. 1976, Demin et al. 1983, Redi et al. 1985, Britton-Davidian et al. 1990, Bogdanov and Kolomiets 2007).

A study of the structure and behavior of the synaptonemal complex (SC) is crucial for understanding chromosome synapsis, particularly in heterozygous animals. SC is a skeleton of meiotic bivalents, which form between homologous chromosomes in prophase I of meiosis. The SC analysis allows us to trace all details of the formation of its axial elements, from the beginning of pairing and synapsis at the zygotene, especially synapsis and its correction in heterozygotes for chromosome rearrangements at the pachytene and desynapsis dynamics at diplotene.

The eastern mole vole *Ellobius tancrei* (Cricetidae, Rodentia) is an interesting example of the wide variability of chromosome numbers (from 2n = 30 to 2n = 54 with a stable NF = 56) (Vorontsov and Radzhabli 1967, Lyapunova et al. 1980, 2010, Bakloushinskaya et al. 2013). Unlike *Mus musculus domesticus* Schwarz & Schwarz, 1943 (Cappanna et al. 1976, Pialek et al. 2005) and *Sorex araneus* Linnaeus, 1758 (Searle et al. 1991, Wojcik et al. 2003), in which the variability due to Rb translocations is observed over the entire species range, for the eastern mole vole, such an area is limited to a ~150 km territory in the Pamir-Alay mountains.

Applying comparative chromosome painting (Zoo-FISH) helped to prove the occurrence of non-homologous Rb translocations in different populations of *E. tancrei* (Bakloushinskaya et al. 2010). To evaluate the role of such chromosomal aberrations in the formation of reproductive barriers between intraspecific forms, we investigated the meiotic prophase I of hybrids that cross chromosomal forms with monobrachial homology (MBH) of Rb chromosomes (2n=48 and 2n=50; 2n=50 and 2n=50).
Materials and methods

Animals

The specimens studied by us were obtained from laboratory colonies. Hybrid animals were bred in the laboratory from crosses between animals, homozygous for the diagnostic fusions. Animals were kept under standard conditions with free access to food at the facility of the Koltzov Institute of Developmental Biology RAS. Animals were treated according established international protocols, such as the Guidelines for Humane Endpoints for Animals Used in Biomedical Research, and Regulations for Laboratory Practice in Russian Federation, and under the supervision of the Ethics Committee for Animal Research of the Koltzov Institute of Developmental Biology.

As a control we study SCs of two adult males with 2n=54, obtained from two points in Tajikistan: near Miskinobod 38° 39.78’ N; 69° 33.29’ E, 1780 m above sea level, and Panchkotan valley, the left bank of the Sorbo River 38° 45.27’ N; 69° 17.6’ E, 1265 m above sea level. Parental forms for F1 hybrids originated from the right bank of the Surkhob River, close to the airport Garm, 39° 0.28’ N; 70° 17.77’ E, 1310 m above sea level (2n=48), and from the opposite bank of the Surkhob River near the Voydara settlement 38° 58.9’ N; 70° 14.71’ E, 1440 m above sea level (2n = 50). Eight F1 hybrids (2n = 49), five newborn females and three adult males (not younger than 1 year), were investigated. For the second intraspecific crossing we use animals from Voidara (2n=50) and from the Varzob Valley, near the Khodzha-Obi-Garm settlement 38° 53.53’ N; 68° 46.52’ E, 2020 m above sea level (2n = 50). Five adult males of F1 hybrids (2n = 50) were investigated.

Laboratory analysis

Chromosomes from bone marrow (Ford and Hamerton 1956) were prepared from all animals; tissues of four specimens of all parental forms were used for tissue culturing. Fibroblast cell lines were prepared as previously described (Sitnikova et al. 2007). Full sets of paints derived from flow-sorted chromosomes of the field vole Microtus agrestis Linnaeus, 1761 (Sitnikova et al. 2007) were used. FISH was performed according to previously published protocols (Yang et al. 1999). G-banding was carried out for all metaphase chromosomes prior to FISH using trypsin treatments (Seabright 1971).

The suspensions of oocytes and spermatocytes were prepared by the method described by Kolomiets et al. (2010), and spreads were prepared and fixed using the technique of Navarro et al. (1981) with some modifications. Slides coated with poly-L-lysine were used for immunostaining and slides coated with plastic Falcon for electron microscopic study.
The slides were stained with 50% AgNO$_3$ in a humid chamber at 56 °C for 3 hours after which they were washed four times in distilled water and air dried. The stained slides were observed by light microscope and suitably spread cells were selected. Plastic circles were cut out with a diamond tap and transferred onto grids. The slides were then examined under a JEM 100B electron microscope.

The slides were washed in PBS. Whole mount SCs were blocked with HB (holding buffer: PBS, 0.3% BSA, 0.005% Triton X-100). The slides were incubated overnight at 4 ºC with rabbit polyclonal antibodies against the human lateral element protein SCP3 (Abcam, 15093Ab, UK Cambridge, UK) diluted to a concentration of 1:200 in ADB (Antibody Dilution Buffer: PBS, 3% BSA, 0.05% Triton X-100), human anti-centromere antibodies ACA, 1:200 (Antibody Incorporated, California, USA), and mouse monoclonal antibodies to human mismatch repair protein MLH1, 1:50 (Abcam, Cambridge, UK). The slides were washed in PBS and incubated with goat anti-rabbit Alexa Fluore 488 conjugated antibodies (1:800, Abcam, Cambridge, UK) and goat anti-human Alexa Fluore 546 conjugated antibodies (1:800) at 37 ºC for 60 min. The slides were washed with PBS, rinsed briefly with distilled water, dried and mounted in Vectashield with DAPI (Vector Laboratories).

Meiotic cellular suspensions from testes for post-pachytene stages analysis were prepared using the air-drying method (Evans et al. 1964).

The slides were analyzed with an Axioimager D1 microscope CHROMA filter sets (Carl Zeiss, Jena, Germany) equipped with a Axiocam HRm CCD camera (Carl Zeiss), and image-processing AxioVision Release 4.6.3. software (Carl Zeiss, Germany). Images were processed using Adobe Photoshop CS3 Extended. Measurements of autosomal bivalents and their ranking in each cell were made in order to determine relative lengths (MicroMeasure 3.3, Colorado, USA) using the STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA).

Results

Heterozygous karyotypes of *E. tancrei*

Karyotypes of parental forms from opposite banks of the Surkhob River were published earlier (Bakloushinskaya et al. 2010) and were described according to the nomenclature of the *E. tancrei* chromosomes (2n = 54) (Bakloushinskaya et al. 2012). The form with 2n = 48 had six Rb metacentrics [2Rb(2.11), 2Rb(5.9), and 2Rb(3.18)] while the form with 2n = 50, “Voidara,” had four Rb metacentrics [2Rb(2.18 and 2Rb(5.9)]. There were five Rb metacentrics, three of them with MBH in the F1 hybrids [Rb(2.11), Rb(2.18) and Rb(3.18)] (Fig. 1a).

We identified the Rb translocations in the form with 2n = 50, “Khodza Obi-Garm”, which had four Rb metacentrics [2Rb(4.12) and, 2Rb(9.13)]. F1 hybrids of crossings “Khodza Obi-Garm” with 2n = 50, “Voidara” had four different Rb metacentrics, two of them with MBH [Rb(5.9) and Rb(9.13)] (Fig. 3a).
Figure 1. Chromosome synopsis in pachytene spermatocytes of F1 hybrid *E. tancrei* (2n = 49, NF = 56). a The scheme reflects a prognosis for chromosome synopsis in prophase I of meiosis [M1=Rb(3.18), M2=Rb(2.18), and M3=Rb(2.11)] b Closed SC pentavalent. Immunostaining with antibodies to SC protein 3 (green) and to the centromere ACA (red) c The scheme of chromosome synopsis in the structure of SC pentavalent (see Fig. 1b). Black dots mark centromere positions d Electron micrograph of spread spermatocyte from F1 hybrid. Closed SC pentavalent is formed from three metacentrics with monobrachial homology (M1, M2, and M3) and two acrocentrics (A1, A2). The arrow shows the fragment of SC between the short arms of homologous acrocentrics. Gaps are marked with asterisks. A sex bivalent (XX) does not associate with the multivalent. Nb – nucleolus-like body e The scheme of chromosome synopsis in the structure of SC pentavalent (see Fig. 1d) f A complex case of the association of sex bivalent (XX) g The scheme of the association of sex bivalent (XX) with an autosome (see Fig. 1f) h An association of sex bivalent (XX) i The scheme of the association of sex bivalent (XX) with an autosome (see Fig. 1h). Scale bars: 1 μm (b, d, i); 2 μm (e, f, h).
Intraspecific hybridization

As a control, we used *E. tancrei* with 2n=54, which is typical for the species. Their fertility was 2.37±0.22 (71 litters, 168 pups). In crossing type I (48 × 50), 24 litters and 62 hybrids F1 were obtained. Litter size was 2.58 ± 1.02. In inbred crosses 11 litters with 16 hybrids F2 were obtained. A litter size was estimated as 1.27±0.47, and the mean litter size was lower comparing the control and parents (p<0.01).

In crossing type II (50 × 50), 35 litters and 106 hybrids F1 were obtained. Litter size was 2.94 ± 0.63. In inbred crosses, 44 litters with 71 hybrids F2 were obtained. A litter size was estimated as 1.61±0.84, with the mean litter size was lower when comparing the control and parents (p<0.01).

Spermatocytes and oocytes of hybrids with 2n=49

We analyzed a total of 106 spermatocytes of the hybrids (Table 1), including 88 at the pachytene stage. We identified a closed SC pentavalent, formed by three metacentrics and two acrocentrics, at the pachytene stage (Fig. 1a, b, d). A short SC fragment was formed between the pericentromeric regions of the nonhomologous acrocentrics, which are determined by the presence in these chromosome regions of large C-heterochromatic blocks (Fig. 1c, e).

The number of spermatocytes, for which the structure of the sex bivalent and SC multivalent were defined, was less than the total number of cells. This may occur due to weak or partial staining of the axial elements of SC and XX bivalents, which was previously observed in *E. talpinus* (Kolomiets et al. 2010). It may also be caused by strong tensions and numerous gaps in SC structure of multivalent (Fig. 1e). A closed XX bivalent was identified in a significant part of pachytene nuclei (0.84±0.03SE (standard error)). An electrondense nucleolus-like body (Nb) was formed on one of the axial elements of the sex bivalent (Fig. 1). High rates of sex bivalent associations with SC autosomes were observed in the hybrids; the occurrence was significantly higher (p<0.05) than that found in the 54-chromosome form (0.48±0.06 and 0.17±0.03, respectively). This association could have arisen due to heterosynapsis of the axial elements of the sex bivalent with axial elements of the SC bivalents (Fig. 1f, g). As associations, we classified the cases of single and multiple overlapping SC bivalents on the sex bivalent, which occurred in slightly more than half of all associations (Fig. 1h, i).

We studied 59 oocytes in total. According to our observations, in cell suspensions of ovaries, the number of oocytes was lower in hybrids (three to six nuclei per slide) than in ordinary of *Ellobius talpinus* Pallas, 1770 females (40–60 nuclei per slide) (Kolomiets et al. 2010).

There are 21 autosomal SC bivalents in pachytene oocytes. One SC bivalent was formed by metacentric chromosomes, another SC bivalent appeared due to single pair of non-Rb submetacentrics, and the other 19 SC bivalents were formed by acrocentrics. The rest of chromosomes presented as SC pentavalent and sex (XX) bivalent (Fig. 2a–c).
The XX bivalent that was formed between the two largest acrocentrics was reliably identified via immunostaining of centromeric proteins. Sex chromosomes form the SC, which is indistinguishable from autosomal SCs.

The SC pentavalent was formed by three submetacentrics with MBH, as well as two acrocentrics (Fig. 2a–c), as in spermatocytes (Fig. 1c, e). The SC pentavalent was usually closed due to the non-homologous synapsis of the short arms of the acrocentrics (Table 1). Apparently, nuclear architecture changes in hybrid oocytes, resulting in extension of chromosomes and formation of gaps in the SC axial elements of multivalents (Table 1), similar to what happens in spermatocytes. The formation of SC multivalents in the male and female meiocytes was identical with the only difference in the ability to associate with sex bivalents.

**Table 1.** Synaptic characteristics of multivalents and sex bivalents in the spermatocytes and oocytes of homozygotes and heterozygotes of *E. tancrei*.

| Name                        | Number of cells | Sex (XX) bivalent associations (±SE) | SC multivalent (±SE) (Penta-/tetravalent) |
|-----------------------------|----------------|-------------------------------------|------------------------------------------|
|                             |                |                                     | With gaps | Closed | Open |
| *E. tancrei* 2n=54, male    | 102            | 0.17±0.03 1                         | –         | –      | –    |
| *E. tancrei* hybrid 2n=49, male | 106          | 0.48±0.06 1,2                       | 0.10±0.07 3 | 0.69±0.09 | 0.31±0.02 |
| *E. tancrei* hybrid 2n=49, female | 59          | Sex bivalent behaves as autosomes.  | 0.29±0.03 3,4 | 0.62±0.04 | 0.38±0.03 5 |
| *E. tancrei* hybrid 2n=50, male | 94           | 0.08±0.04 2                         | 0.11±0.04 4 | –      | 0.96±0.06 5 |

SC – synaptonemal complex, 1, 2, 3, 4, 5 Significant difference (p<0.05)

**Figure 2.** Spreads of oocytes from intraspecific F1 hybrid *E. tancrei* (2n= 49, NF = 56). Immunostaining with antibodies to SCP 3 (green) and to the centromere (ACA, red). The yellow arrow marks the SC pentavalent. **a** Early-mid pachytene oocyte: 21 SC bivalents, SC pentavalent, XX bivalent. **b** SC pentavalent (yellow dotted line) from Fig. 2a. **c** Scheme of SC pentavalent (see Figs 2a, b). The chromosomes forming the SC pentavalent shown in Fig. 1a. Scale bar: 5 μm.
Figure 3. Chromosome synapsis in pachytene spermatocytes and diakinesis/metaphase I cell of F1 hybrid *E. tancrei* (2n = 50, NF = 56). a The scheme reflects a prognosis for chromosome synapsis in prophase I of meiosis [Trivalent №1 Rb(2/2.18/18), trivalent №2 Rb(4/4.12/12), and tetravalent Rb(5/5.9/9.13/13)]. M – metacentric, A – acrocentric b Electron micrograph of part of the spread spermatocyte. Nb – DAPI-positive nucleolus-like body c Spermatocyte is stained with DAPI (blue). Immunostaining with antibodies to SCP 3 (green) and to MLH1 (yellow) d Autosome’s axial element (A) stiking to Nb of sex bivalent (autosome – sex chromosome association) e Open SC trivalent f Diakinesis/Metaphase I cell showing 18 pairing elements, XX bivalent, two univalents (Un), two trivalents (Tr) and tetravalent. Scale bar: 5 μm.
Spermatocytes of hybrids with 2n=50

We analyzed 94 spermatocytes of the F1 hybrid (Table 1). At the pachytene nuclei we distinguished 19 SC and XX bivalents, one SC tetravalent and two SC trivalents. The sex XX bivalent was closed in most of the nuclei (0.87±0.04). Typical SC was formed at the telomeric zones, and usually did not participate in associations with other chromosomes (0.08±0.04). This characteristic was lower compared to the typical E. tancrei with 2n=54 (Table 1). One of the few spermatocytes with the autosome – XX association is shown in Fig. 3d. One or two DAPI-positive and electrondense Nbs are formed on one of the axial elements of the sex bivalents (Fig. 3).

The SC tetravalent was usually open (0.96±0.06). It was formed by two Rb metacentrics with MBH and two acrocentrics (Fig. 3). Recombination nodules were detected in synaptic sites of the tetravalent. As a rule, the SC tetravalent did not associate with the sex bivalent, but sometimes we observed end-to-end association of the XX axial elements and the acrocentric bivalents (Fig. 3c).

Each SC trivalent was formed by one Rb metacentric and two acrocentrics (Fig. 3c). Arms of SC trivalents had one recombination nodule. In the most of the nuclei, SC trivalents were closed (0.71±0.03 and 0.63±0.03 for two trivalents, p>0.05). However, the open trivalents (Fig. 3e) sometimes associated with the tetravalents (Fig. 3b).

Small acrocentrics did not undergo synapsis in some pachytene nuclei, and univalents were discovered (Fig. 3c). These data correlated with diakinesis samples: for 92 cells studied, 45 univalents were identified. The diakinesis/metaphase I cell demonstrated 18 pairing elements, XX bivalent, two univalents, two trivalents and tetravalent (Fig. 3f). Mature sperm were frequently seen in meiotic preparations of both types of the heterozygous mole vole (Fig. 4).

Discussion

Previous studies support the suggestion that the single and multiple Rb fusions may be involved in the process of speciation because accumulation of Rb translocations (including MBH) in different populations of the species can lead to reproductive isolation between them (Gropp and Winking 1981; Baker and Bickham 1986; Hauffe and Searle 1998). The complex compounds, which formed in meiocytes of heterozygous animals, can disturb the synapsis and dissociation of chromosomes during meiosis, leading to the death of spermatocytes, or the formation of unbalanced gametes, and eventually to the hybrid’s sterility.

The impact of simple and complex SC configurations on the progression of spermatogenesis and fertility varied among different groups of animals. Simple and complex SC configurations are well studied in different races of shrews (Narain and Fredga 1998, Pavlova et al. 2008, Matveevsky et al. 2012, etc.). Trivalents in Cricetulus hybrids spermatocytes (Matveevsky et al. 2014) and pentavalent in heterozygous Allocricetulus spermatocytes led to only partial germ cell death (Gureeva et al. 2015).
In *Mus musculus domesticus*, the impact of Rb translocation in meiotic events and, in general, the formation of gametes, may be insignificant or significant, depending on the number of Rb fusions in the heterozygotes and, therefore, the characteristics and organization of SC chains in the meiotic nucleus. Heterozygous mice with four and seven trivalents had abnormal pairing such as SC trivalent associations with each other and with XY bivalent that might be the cause of infertility (Wallace et al., 2002). Hybrids between monobrachially homologous Rb races of the house mouse from the island of Madeira had two MBH Rb metacentrics, and thereafter in meiosis chromosomes, they formed one chain-of-four configuration (tetravalent). In such semi-sterile hybrids aneuploidy and germ cell death were higher than in homozygous mice (Nunes et al. 2011). It is interesting, that two types of hybrids with MBH Rb metacentrics and SC tetravalents, had a different fertility. Mice heterozygous by Rb(6/6.15/15.4/4) were fertile, but mice with Rb(4/4.6/6.15/15) were sterile (Mahadevaiah et al. 1990). The last type of hybrids demonstrated a high index of asynapsis in SC tetravalents, and a low number of spermatocytes. A study of the SC in *M. domesticus* hybrids obtained by crossing forms from Campobasso and Cittaducale is of particular interest (Johannisson and Winking 1994). In these hybrids, a closed ring multivalent formed which included 16 Rb metacentrics. In hybrids, obtained by crossing forms from Sicily and Alpie Orobie, an open SC multivalent formed which included 15 Rb metacentrics. Johannisson and Winking (1994) noticed that the formation of closed SC multivalents
Analysis of meiotic chromosome structure and behavior in Robertsonian heterozygotes...

(SC rings) due to spermatogenic normality. At the same time, the open SC multivalents (SC chains) were often associated with sex bivalent. The mice had a complete spermatogenic breakdown at the spermatocyte I level.

Fertility of lemurs was also variable depending on the number of Rb fusions. Hybrids with three to six SC trivalents were usually fertile. Heterozygous lemurs with eight trivalents in spermatocytes were viable, but had reduced fertility. Hybrids with complex SC configurations, including ones formed by Rb metacentrics that shared monobrachial homology, were sterile (Ratomponirina et al., 1988). In spermatocytes of such hybrids defects of synapsis and the association of sex chromosomes with multivalent were revealed. In spermatocytes of lemurs heterozygous for two MBH, Rb metacentrics found an open SC tetravalent with the terminal association with the XY (Djlelati et al. 1997; Rumpler 2004). The authors suggested that this may lead to a partial loss of meiocytes. The case is similar to associations in E. tancrei F1 hybrids with 2n=50. At the same time, Dutrillaux and Rampler (1977) found a small number of spermatocytes with pentavalents in fertile hybrids of lemurs.

Studied heterozygous E. tancrei had approximately half the level of fertility in comparison with parental individuals and animals with 2n=54 (see also Lyapunova et al. 1990). Despite the similar parameters of hybrid fertility in spermatocytes, chromosome synapsis with different scenarios was observed. Hybrids with 2n = 49 had an increased level of XX association with other chromosomes, while heterozygotes with 2n = 50 had surprisingly low indices, even in comparison with the animals with 2n = 54. Such associations, a failure of displacement of sex bivalent to the nuclei periphery, and SC configurations are signs of hybrid spermatocytes’ pachytene arrest.

Previously, we have studied other variants of intraspecific hybrids. In the nuclei of spermatocytes of F1 (2n = 44), hybrids from crossing 2n = 54 and 2n = 34 were expected to have six SC bivalents, the sex bivalent, and 10 SC trivalents, each of which was formed as a result of the synopsis of one Rb metacentric and two acrocentrics. However, the expected pattern could be observed only in a single nucleus at the late pachytene. It was found that chromosome synapsis in forming trivalents in most cases was slower than was bivalent synapsis (Kolomiets et al. 1985, 1986, Bogdanov et al. 1986). The process of forming the axial elements slows down in the central part of the Rb metacentrics from SC trivalents, which were highly extended between the points of attachment to the nuclear envelope. There were gaps in the pericentromeric area of the metacentric’s axis until the middle pachytene.

Formation of complex SC configurations (SC chains and rings) in heterozygotes for multiple Rb translocation is accompanied by extended preservation zones of asynapsis in centromeric regions of acrocentrics and metacentrics (Homolka et al. 2007, Mahadevaiah et al. 2008). However, it is known that asynapted chromatin sites undergo transcriptional inactivation, which in fact leads to a block (arrest) of meiosis. According to our observations (unpublished data), the DAPI-positive Nbs of mole vole XX bivalents are chromatin inactivation sites. The apparently unsynapsed site of the SC bivalent anchored to the Nb of XX (Fig. 3d), is a transcriptionally silent region. If the genetically important part of the genome undergoes inactivation, it can lead to germ cell death.
Signs of transcriptional inactivation of asynapted chromatin involving the proteins gamma-H2AX, ATR, and SUMO1 were detected in mice heterozygous for eight Rb translocations. Such inactivation, however, did not lead to disturbances in the formation of the sex body in most of the spermatocytes nuclei (Manterola et al. 2009). This points out the low activity of the pachytene arrest. However, apoptosis of spermatocytes was observed during the metaphase due to defects of the spindle apparatus. The low efficiency of the pachytene checkpoints in relation to the Rb translocations apparently determined the circulation of the Rb metacentrics in natural populations and their roles in the evolution of karyotypes. Perhaps there is low genetic significance of the silenced asynapted centromeric chromatin regions of the SC multivalents, and the SC chains reduce the activity of the checkpoint in the pachytene.

Baker and Bickham (1986) proposed a model of chromosomal speciation by monobrachial centric fusion. Fixation of metacentric chromosomes with homology formed as a result of independent fusion of acrocentric chromosomes can entail reproductive isolation of the population and further speciation due to the accumulation of genetic differences (Capanna 1982, Baker and Bickham 1986). Our data support the possibility of speciation by monobrachial centric fusions in *E. tancrei* as suggested by previous studies for other animals (Capanna et al. 1976, Baverstock et al. 1983, Baker et al. 1985, see review by King 1993). This evolutionary mechanism is likely widely distributed in nature and, along with other chromosomal rearrangements, led to species differentiation.

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**References**

Baker RJ, Bickham JW (1986) Speciation by monobrachial centric fusions. Proceedings of the National Academy of Sciences of the USA 83: 8245–8248. doi: 10.2307/2408359

Baker RJ, Bickham JW, Arnold ML (1985) Chromosomal evolution in *Rhogeessa* (Chiroptera: Vespertilionidae): Possible speciation by centric fusions. Evolution 39: 233–243. doi: 10.2307/2408359

Bakloushinskaya IYu, Romanenko SA, Graphodatsky AS, Matveevsky SN, Lyapunova EA, Kolomiets OL (2010) The role of chromosome rearrangements in the evolution of mole voles
of the genus *Ellobius* (Rodentia, Mammalia). Russian Journal of Genetics 46: 1143–1145. doi: 10.1134/S1022795410090346

Baklushinskaya IYu, Matveevsky SN, Romanenko SA, Serdukova NA, Kolomiets OL, Spangenberg VE, Lyapunova EA, Graphodatsky AS (2012) A comparative analysis of the mole vole sibling species *Ellobius tancrei* and *E. talpinus* (Cricetidae, Rodentia) through chromosome painting and examination of synaptonemal complex structures in hybrids. Cytogenetic and Genome Research 136: 199–207. doi: 10.1159/000336459

Baklushinskaya I, Romanenko S, Serdukova N, Graphodatsky A, Lyapunova E (2013) A new form of the mole vole *Ellobius tancrei* Blasius, 1884 (Mammalia, Rodentia) with the lowest chromosome number. Comparative Cytogenetics 7: 163–169. doi: 10.3897/compcytogen.v7i2.5350

Baverstock PR, Gelder M, Jahnke A (1983) Chromosome evolution in Australian *Rattus* - G-banding and hybrid meiosis. Genetica 60: 93–103. doi: 10.1007/BF00127495

Britton-Davidian J, Sonjaya H, Catalan J, Cattaneo-Berrebi G (1990) Robertsonian heterozygosity in wild mice: fertility and transmission rates in Rb(16:17) translocation heterozygotes. Genetica 80: 171–174. doi: 10.1007/BF00137322

Bogdanov YuF, Kolomiets OL, Lyapunova EA, Yanina IYu, Mazurova TF (1986) Synaptonemal complexes and chromosome chains in the rodent *Ellobius talpinus* heterozygous for ten Robertsonian translocations. Chromosoma 94: 94–102. doi: 10.1007/BF00286986

Bogdanov YuF, Kolomiets OL (2007) Synaptonemal complex as an indicator of the dynamics of meiosis and chromosome variation. Moscow, 360 pp. [In Russian]

Capanna E (1982) Robertsonian numerical variation in animal speciation: *Mus musculus* an emblematic model. In: Barigozzi C (Ed.) Mechanisms of speciation. Alan R Liss, New York, 155–177.

Capanna E, Gropp A, Winking H, Noack G, Civirelli MV (1976) Robertsonian metacentrics in the mouse. Chromosoma 58: 341–353. doi: 10.1007/BF00292842

Demin YuS, Safronova LD, Panevina ND (1983) Cytogenetics of robertsonian translocation in mammals. Proceedings of the USSR Academy of Sciences 272(1): 204–207. [In Russian]

Djlelati R, Brun B, Rumpler Y (1997) Meiotic study of hybrids in the genus *Eulemur* and taxonomic considerations. American Journal of Primatology 42(3): 235–245. doi: 10.1002/(SICI)1098-2345(1997)42:3<235::AID-AJP6>3.0.CO;2-Y

Dutrillaux B, Rumpler Y (1977) Chromosomal evolution in Malagasy lemurs. Cytogenetic and Cell Genetics 18(4):197–211. doi: 10.1159/000130763

Evans EP, Breckon G, Ford CE (1964) An air-drying method for meiotic preparations from mammalian testes. Cytogenetics 3: 289–294. doi: 10.1159/000129818

Ford CE, Hamerton JL (1956) A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technology 31: 247–251. doi: 10.3109/1052095609113814

Gropp A, Winking H (1981) Robertsonian translocations: Cytology, meiosis, segregation patterns and biological consequences of heterozygosity. Symposia of the Zoological Society of London 47: 141–181.

Gureeva AV, Feoktistova NYu, Matveevsky SN, Kolomiets OL, Surov AV (2015) Speciation of Eversman and Mongolian hamsters (*Allocricetulus*, Cricetinae): experimental hybridization. Zoologicheskyy Zhurnal 94: 614–620. doi: 10.7868/S0044513415050062 [In Russian]

Hauffe HC, Scarle JB (1998) Chromosomal heterozygosity and fertility in house mice (*Mus musculus domesticus*) from Northern Italy. Genetics 150: 1143–1154.
Homolka D, Ivanek R, Capkova J, Jansa P, Forejt J (2007) Chromosomal rearrangement interferes with meiotic X chromosome inactivation. Genome Research 17(10): 1431–1437. doi: 10.1101/gr.6520107

Johannisson R, Winking H (1994) Synaptonemal complexes of chains and rings in mice heterozygous for multiple Robertsonian translocations. Chromosome Research 2: 137–145. doi: 10.1007/BF01553492

King M (1987) Chromosomal rearrangements, speciation and the theoretical approach. Heredity 59: 1–6. doi: 10.1038/hdy.1987.90

King M (1993) Species Evolution: The role of chromosome change. Cambridge, 336 pp.

Kolomiets OL, Lyapunova EA, Mazurova TF, Yanina IY, Bogdanov YF (1985) Varying ways of formation of trivalent synaptonemal complexes in heterozygote hybrids by Robertson's translocation. In: Sozinov AA (Ed.) Molecular mechanisms of genetic processes. Molecular genetics, evolution and molecular-genetic bases of selection. Moscow, 72–84. [In Russian]

Kolomiets OL, Lyapunova EA, Mazurova TF, Yanina IY, Bogdanov YF (1986) Participation of heterochromatin in formation of synaptonemal complex chains in animals heterozygous for multiple Robertsonian translocation. Russian Journal of Genetics 22: 273–283. [In Russian]

Kolomiets OL, Matveevsky SN, Bakloushinskaya IYu (2010) Sexual dimorphism in prophase I of meiosis in mole vole (Ellobius talpinus Pallas) with isomorphic (XX) chromosomes in males and females. Comparative Cytogenetics 4: 55–66. doi: 10.3897/compcytogen.v4i1.25

Lyapunova EA, Bakloushinskaya IYu, Kolomiets OL, Mazurova TF (1990) Analysis of fertility of hybrids of multi chromosomal forms in mole voles of the superspecies Ellobius tancrei differing in a single pair of Robertsonian metacentrics. Proceedings of the USSR Academy of Sciences 310: 721–723. [In Russian]

Lyapunova EA, Vorontsov NN, Korobitsina KV, Ivanitskaya EYu, Borisov YuM, Yakimenko LV, Dovgal VY (1980) A Robertsonian fan in Ellobius talpinus. Genetica 52/53: 239–247. doi: 10.1007/BF00121833

Mahadevaiah SK, Bourc’His D, de Rooij DG, Bestor TH, Turner JM, Burgoyne PS (2008) Extensive meiotic asynapsis in mice antagonises meiotic silencing of unsynapsed chromatin and consequently disrupts meiotic sex chromosome inactivation. Journal of Cell Biology 182:263–276. doi: 10.1083/jcb.200710195

Mahadevaiah SK, Setterfield LA, Mittwoch U (1990) Pachytene pairing and sperm counts in mice with single Robertsonian translocations and monobrachial compounds. Cytogenetic and Cell Genetics 53: 26–31. doi: 10.1159/000132889

Manterola M, Page J, Vasco C, Berrios S, Parra M, Viera A, Rufas J, Zuccotti M, Garagna S, Fernández-Donoso R (2009) A high incidence of meiotic silencing of unsynapsed chromatin is not associated with substantial pachytene loss in heterozygous male mice carrying multiple simple Robertsonian translocations. PLoS Genetics 8: 1–14. doi: 10.1371/journal.pgen.1000625
Matthey R (1951) The chromosomes of the vertebrates. Advances in Genetics 4: 159–180. doi: 10.1016/S0065-2660(08)60234-5
Matthey R (1953) La formule chromosomique et le problème de la détermination sexuelle chez *Ellobius lutescens* Thomas (Rodentia—Muridae—Microtinae). Archiv der Julius Klaus-Stiftung für Vererbungsforschung 28: 65–73.
Matveevsky SN, Pavlova SV, Acaeva MM, Kolomiets OL (2012) Synaptonemal complex analysis of interracial hybrids between the Moscow and Nereoosa chromosomal races of the common shrew *Sorex araneus* showing regular formation of a complex meiotic configuration (ring-of-four). Comparative Cytogenetics 6: 301–314. doi: 10.3897/CompCytogen.v6i3.3701
Matveevsky SN, Malygin VM, Lebedev VS, Poplavskaya NS, Surov AV, Kolomiets OL (2014) Sporadic disorders in the meiotic prophase I in *Cricetulus barabensis* hybrids (Cricetidae, Rodentia) do not lead to reproductive isolation between karyomorphs. Caryologia 67: 150–155. doi: 10.1080/00087114.2014.931636
Moses MJ, Karatsis PA, Hamilton AE (1979) Synaptonemal complex analysis of heteromorphic trivalents in lemur hybrids. Chromosoma 70: 141–160. doi: 10.1007/BF00288403
Narain Y, Fredga K (1998) Spermatogenesis in common shrews, *Sorex araneus*, from a hybrid one with extensive Robertsonian polymorphism. Cytogenetic and Cell Genetics 80: 158–164. doi: 10.1159/000014973
Navarro J, Vidal F, Quitart M (1981) A method for the sequential study of SC by light and electron microscopy. Human Genetics 59: 419–423. doi: 10.1007/BF00295483
Nunes AC, Catalan J, Lopez J, da Graça Ramalhinho M, da Luz Mathias M, Britton-Davidian J (2011) Fertility assessment in hybrids between monobrachially homologous Rb races of the house mouse from the island of Madeira: implications for modes of chromosomal evolution. Heredity 106: 348–356. doi: 10.1038/hdy.2010.74
Pavlova SV, Kolomiets OL, Bulatova N, Searle JB (2008) Demonstration of a WART in a hybrid zone of the common shrew (*Sorex araneus* Linnaeus, 1758). Comparative Cytogenetics 2: 115–120.
Pialek J, Hauffe HC, Searle JB (2005) Chromosomal variation in the house mouse. Biological Journal of the Linnean Society 84: 535–563. doi: 10.1111/j.1095-8312.2005.00454.x
Ratomonpinirina C, Brun B, Rumpler Y (1988) Synaptonemal complexes in Robertsonian translocation heterozygous in lemurs. In: Brandham PE (Ed.) Kew chromosome Conference 3. HMSO, London, 65–73.
Redi CA, Garagna S, Hilscher B, Winking H (1985) The effects of some Robertsonian chromosome combinations on the seminiferous epithelium of the mouse. Journal of Embryology and Experimental Morphology 85: 1–19.
Robertson WRB (1916) Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae: V-shaped chromosomes and their significance in Acrididae, Locustidae, and Gryllidae: chromosomes and variation. Journal of Morphology 27: 179–181. doi: 10.1002/jmor.1050270202
Rumpler Y (2004) Complementary approaches of cytogenetics and molecular biology to the taxonomy and study of speciation processes in Lemurs. Evolutionary Anthropology 13: 67–78. doi: 10.1002/evan.20010
Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2: 971–972. doi: 10.1016/S0140-6736(71)90287-X

Searle JB, Fedyk S, Fredga K, Hausser J, Volobouev VT (1991) Nomenclature for the chromosomes of the common shrew (Sorex araneus). Memorandes de la Societe Vaudoise des Sciences Naturelles 19: 13–22.

Sites JW, Moritz C (1987) Chromosomal evolution and speciation revisited. Systematic Zoology 36(2): 153–174. doi: 10.2307/2413266

Sitnikova NA, Romanenko SA, O’Brien PCM, Perelman PL, Fu B, Rubtsova NV, Serdukov NA, Golenishchev FN, Trifonov VA, Ferguson-Smith MA, Yang F, Graphodatsky AS (2007) Chromosomal evolution of Arvicolinae (Cricetidae, Rodentia). I. The genome homology of tundra vole, field vole, mouse and golden hamster revealed by comparative chromosome painting. Chromosome Research 15: 447–456. doi: 10.1007/s10577-007-1137-y

Stanyon R, Koehler U, Consigliere S (2002) Chromosome painting reveals that galagos have highly derived karyotypes. Journal of Physical Anthropology 117: 319–326. doi: 10.1002/ajpa.10047

Volobouev VT, Aniskin VM, Sicard B, Dobigny G, Granjon L (2007) Systematics and phylogeny of West African gerbils of the genus Gerbilliscus (Muridae: Gerbillinae) inferred from comparative G- and C-banding chromosomal analyses. Cytogenetic and Genome Research 116: 269–281. doi: 10.1159/000100411

Vorontsov NN (1989) The problem of species and speciation. International Studies in the Philosophy of Science 3: 173–189. doi: 10.1080/02698598908573334

Vorontsov NN, Radzhabli SI (1967) Chromosomal sets and cytogenetic differentiation of two forms within the superspecies Ellobius talpinus Pall. Tsitologiia 9: 848–852. [In Russian]

Wallace BMN, Searle JB, Everett CA (2002) The effect of multiple simple Robertsonian heterozygosity on chromosome pairing and fertility of wild-stock house mice (Mus musculus domesticus). Cytogenetic and Genome Research 96: 276–286. doi: 10.1159/000063054

White MJD (1945) Animal cytology and evolution. Cambridge, 375 pp.

Wójcik JM, Borodin PM, Fedyk S, Fredga K, Hausser J, Mishta A, Orlov VN, Searle JB, Volobuev VT, Zima J (2003) The list of the chromosome races of the common shrew Sorex araneus (updated 2002). Mammalia 62: 169–179. doi: 10.1515/mamm.2003.67.2.169

Yang F, O’Brien PCM, Milne BS, Graphodatsky AS, Solanky N, Trifonov V, Rens W, Sargan D, Ferguson-Smith MA (1999) A complete comparative chromosome map for the dog, red fox, and human and its integration with canine genetic maps. Genomics 62: 189–202. doi: 10.1006/geno.1999.5989