1 *Article*

2 **Chromosome pairing and recombination in male hybrids between two chromosome races of the**

3 **common shrew (*Sorex araneus* L., Soricidae, Eulipotyphla)**

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14 **Abstract:** The hybrid zone between chromosome races of the common shrew (*Sorex araneus*)

15 provides an exceptional model to study the potential role of chromosome rearrangements in the

16 initial steps of speciation. The Novosibirsk and Tomsk races differ by a series of Robertsonian

17 fusions with monobrachial homology. They form a narrow hybrid zone and generate hybrids with

18 both simple (chain of three chromosomes) and complex (chain of eight or nine) synaptic

19 configurations. Using immunolocalisation of the meiotic proteins, we examined chromosome

20 pairing and recombination in males from the hybrid zone. Homozygotes and simple heterozygotes

21 for Robertsonian fusions showed a low frequency of synaptic aberrations (< 10%). The carriers of

22 complex synaptic configurations showed multiple pairing abnormalities, which might lead to

23 reduced fertility. Recombination frequency in the proximal regions of most chromosomes of all

24 karyotypes was much lower than in the other regions. The strong suppression of recombination in

25 the pericentromeric regions and co-segregation of race specific chromosomes involved in the long

26 chains would be expected to lead to linkage disequilibrium between genes located there. Genic

27 differentiation, together with the high frequency of pairing aberrations in male carriers of the long

28 chains, might contribute to maintenance of the narrow hybrid zone.

29 **Keywords:** common shrew, meiotic chromosomes, *Sorex araneus*, synaptonemal complex, hybrids,

30 MLH1

31

32 **1. Introduction**

33   The role of chromosome rearrangements in speciation is a subject of discussion. The traditional

34 underdominance model suggests that interspecies hybrids heterozygous for chromosome

35 rearrangements should display various irregularities in meiotic chromosome pairing, recombination

36 and segregation and therefore become completely or partially sterile due to meiotic arrest or

37 formation of unbalanced gametes [1]. This model faces serious theoretical difficulties, because

38 rearrangements causing sterility have a very low chance to spread in populations [2]. A more

39 convincing modification of this model presumes that accumulation of several different selectively

40 neutral chromosome rearrangements in different geographically isolated populations may lead to

41 chromosomal incompatibility in the hybrids and their sterility [3–5]. Gene flow between

42 chromosomally different populations, races or species may also be restricted due to recombination

43 suppression near the breakpoints of the chromosome rearrangements [6].

44   The common shrew, *Sorex araneus*, is a model species to examine various scenarios of

45 chromosomal speciation. This species shows a high level of chromosome variation. It has been

46 suggested that ancestral karyotype of the common shrew consisted mostly of uniarmed (acrocentric)
47 chromosomes [7]. The consecutive fixation of various centric or Robertsonian fusions (Rbs) and
48 whole-arm reciprocal translocations (WARTs) gave rise a wide variety of combinations of biarmed
49 (metacentric) and acrocentric chromosomes in extant chromosome races. About 70 chromosome races
50 have been described so far [8]. These races are distributed in a parapatric fashion and generate many
51 hybrid zones [9].
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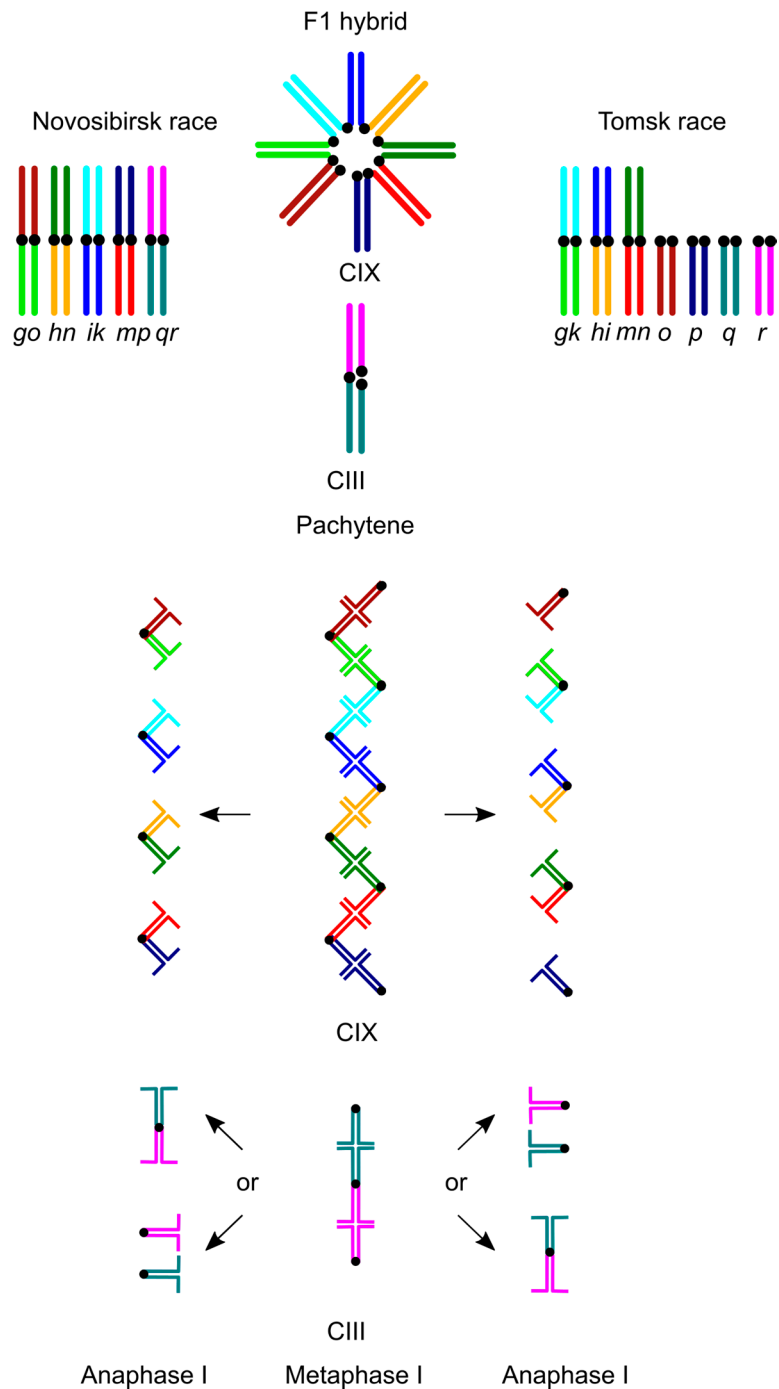
55 **Figure 1.** Expected meiotic configurations of the shrews of Novosibirsk and Tomsk races
56 and their hybrids at pachytene (top row) and later in the first meiotic division (bottom row).
57 Homologous arms are labelled and colour coded. Only balanced anaphase I complements
58 are shown.

59   The hybrid zone between Novosibirsk and Tomsk races is of a special interest. The races differ
60 for a series of chromosome rearrangements (Figure 1). They form a narrow hybrid zone and generate
61 hybrids with both simple and complex synaptic configurations [10,11]. The simple configurations are
62 trivalents, or chains of three (CIII), involving metacentric and twin acrocentric chromosomes. The
63 complex chain configurations occur due to synapsis of eight (CVIII) or nine (CIX) metacentric and
64 acrocentric chromosomes with monobrachial homology (Figure 1).
65   Studies on gene flow across this zone have given contradictory results. On the one hand, analysis
66 of population structure using several microsatellite markers revealed rather weak differentiation
67 between the races; genetic differences between the Novosibirsk and Tomsk races were less than the
68 inter-population differences within each race [12]. On the other hand, significant difference in
69 morphology between the races has been demonstrated. The Novosibirsk shrews and the hybrids are
70 significantly smaller than the Tomsk shrews [13]. Phenotypic differentiation in geometric shape of
71 skulls and mandibles across this zone was found to be greater than expected under the assumption
72 of unrestricted gene flow during the estimated time since contact [14]. Interestingly, the
73 morphological clines coincide with CIX chromosome clines of about nine km width, while the cline
74 of the CIII chromosomes is much wider (about 53 km). The difference in the cline widths is parallel
75 with the expected level of meiotic abnormalities in CIX and CIII chromosome carriers [11,14].
76   In order to determine the role of chromosomal heterozygosity in the restriction of gene flow
77 between the races, we examined chromosome pairing and recombination in shrews of various
78 karyotypes trapped in the hybrid zone between the Tomsk and Novosibirsk chromosome races. To
79 visualise synaptonemal complexes (SC) and the sites of crossing over we used fluorescently-labelled
80 antibodies to SYCP3, a protein of the lateral elements of SC, and MLH1, a mismatch repair protein of
81 mature recombination nodules. This approach has been successfully applied for mapping the sites of
82 recombination in several species of mammals [15–17], including the common shrew [18]

2. Materials and Methods

84   *2.1. Animals.* Sixteen juvenile male shrews were used in this study. The animals were collected
85 with Sherman live traps in the hybrid zone between the Novosibirsk and Tomsk chromosome races
86 (54°47' N; 83°25′ E) located 30 km southeast from Novosibirsk city (West Siberia, Russia) [10].
87 Experiments described in this manuscript were carried out in accordance with the approved national
88 guidelines for the care and use of laboratory animals. All experiments were approved by the Ethics
89 Committee on Animal Care and Use of the Institute of Cytology and Genetics of Siberian Department
90 of the Russian Academy of Sciences, Russia (approval No. 35 of October 26, 2016).
91   *2.2. Karyotyping.* Bone marrow chromosome spreads were prepared according to Searle [19] and
92 G-band stained [20]. We followed the chromosome nomenclature suggested by Searle et al. [21].
93 Chromosome arms and acrocentric chromosomes were presented by italicised letters. Metacentric
94 chromosomes were described by two letters (the first for the long arm, the second for the short arm).
95 Heterozygotes for a metacentric and twin acrocentric chromosomes, for example *qr* and *q*, *r*, are
96 described as *q/r*. Heterozygotes forming larger meiotic chains are described by a list of single
97 chromosomes involved in chain in a way to indicate the pairing with a '/' between the chromosomes.
98 All animals used in this study were homozygous for the chromosomes *af*, *bc*, *jl*, *tu*, and had the sex
99 trivalent X/Y_1Y_2. Karyotypic categories of the shrews were defined by the complement of diagnostic
100 metacentric chromosomes: *go*, *ik*, *hn*, *mp* for the Novosibirsk race and *gk*, *hi*, *mn* for the Tomsk race
101 (Figure 1). The karyotypes of all individuals examined are listed in Table 1.
102   *2.3. Meiotic chromosome preparation, staining and identification.* Spermatocyte spreads were
103 prepared from testis tissue using the drying-down technique [22]. A subset of cell spreads was
104 processed for imaging by electron microscopy. Cells were stained with silver nitrate [23] and slides
105 were covered with plastic film. After light microscopic examination, cells were transferred to
106 specimen grids, examined and photographed with an electron microscope JEM-100 (JEOL, Japan) at
107 80 kV. The remainder of cell spreads was processed for imaging by fluorescent microscopy. The
108 immunostaining protocol was similar to that of Anderson et al. [16]. The slides were incubated for 2
109 h at 37°C with a rabbit polyclonal antibody against rat lateral element protein SYCP3 (Abcam) diluted

110   to a concentration of 1:1000, and a mouse monoclonal antibody to mouse mismatch repair protein
111   MLH1 (Abcam) at 1:50 dilution in 3% bovine serum albumin (BSA) in phosphate buffered saline
112   (PBS). Slides were washed in 1xPBS and incubated for 40 min at 37°C with donkey anti-rabbit Cy3-
113   conjugated antibodies (Jackson) at 1:200 dilution and goat anti-mouse FITC-conjugated antibodies
114   (Jackson) at 1:400 dilution. Slides were washed with PBS, dried, and mounted in Vectashield with
115   DAPI (Vector Laboratories) to stain DNA and reduce fluorescence fading.
116   The preparations were visualised with an Axioplan 2 Imaging microscope (Carl Zeiss, Germany)
117   equipped with a CCD camera (CV M300, JAI Corporation, Japan), CHROMA filter sets and ISIS4
118   image-processing package (MetaSystems GmbH, Germany). Brightness and contrast of all images
119   were enhanced using Corel PaintShop Photo Pro X3. Only cells containing complete sets of
120   chromosomes were analysed. Each chromosome arm was identified by its relative size and specific
121   DAPI pattern according to Belonogova et al. [24]. The centromere position for each SC was identified
122   by DAPI-banding. MLH1 signals were only scored if they were localised on an SC. The length of the
123   SC of each chromosome arm was measured in micrometres using MicroMeasure 3.3 [25] and the
124   positions of MLH1 foci in relation to the centromere were recorded. To generate recombination maps,
125   we calculated the absolute position of each MLH1 focus multiplying the relative position of each
126   focus by the average absolute length for the appropriate chromosome arm. These data were pooled
127   for each arm and graphed to represent a recombination map. Statistica 6.0 software package (StatSoft)
128   was used for descriptive statistics.

129   **3. Results**

130   *3.1. Karyotype variability in the hybrid zone*
131   Table 1 shows karyotypes and synaptic characteristics of the male shrews subjected to SC
132   analysis. Among them, we detected ten carriers of diagnostic chromosomes of the Novosibirsk race,
133   three carriers of diagnostic chromosomes of Tomsk race and three complex heterozygotes for these
134   chromosomes.

135

136   **Table 1. The frequency of synaptic aberrations**

ID	Synaptic type	Diagnostic chromosome set	Karyotype	Number of cells scored	Frequency of cells with synaptic aberrations
1	Homozygote	Novosibirsk	*go, hn, ik, mp, qr*	29	0.07
2	Homozygote	Novosibirsk	*go, hn, ik, mp, qr*	67	0.03
3	Homozygote	Novosibirsk	*go, hn, ik, mp, qr*	31	0.06
4	Homozygote	Tomsk	*gk, hi, mn, o, p, qr*	76	0.00
5	Homozygote	Tomsk	*gk, hi, mn, o, p, q, r*	13	0.23
6	CIII	Tomsk	*gk, hi, mn, o, p, q/r*	74	0.07
7	CIII	Novosibirsk	*go, hn, ik, mp, q/r*	44	0.00
8	CIII	Novosibirsk	*g/o, hn, ik, mp, qr*	21	0.10
9	CIII	Novosibirsk	*go, hn, ik, m/p, qr*	78	0.03
10	2 CIII	Novosibirsk	*go, hn, ik, m/p, q/r*	21	0.10
11	2 CIII	Novosibirsk	*go, hn, ik, m/p, q/r*	19	0.00
12	2 CIII	Novosibirsk	*g/o, hn, ik, mp, q/r*	94	0.10
13	2 CIII	Novosibirsk	*go, hn, ik, m/p, q/r*	12	0.33
14	CVIII	Hybrid	*o/og/gk/ki/ih/hn/nm/m, p, qr*	69	0.48
15	CVIII+CIII	Hybrid	*o/og/gk/ki/ih/hn/nm/m, p, q/r*	39	0.44
16	CIX+CIII	Hybrid	*o/og/gk/ki/ih/hn/nm/mp/p, q/r*	126	0.79

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139   Among the carriers of the Novosibirsk karyotype, five specimens were simple heterozygotes for
140   *q/r*, four for *m/p*, two for *g/o*. One of the carriers of the Tomsk race diagnostic chromosomes was
141   heterozygous for *q/r*, one was homozygous for metacentric chromosome *qr* typical for the
142   Novosibirsk race, and one was homozygous for acrocentric chromosomes *q* and *r* typical for the
143   Tomsk race. One of the complex heterozygotes was also homozygous for the Novosibirsk variant of
144   chromosome *qr*, while other two were heterozygous for *q/r*. Among the complex heterozygotes, one
145   had CIX and two had CVIII. The chromosome chain in CVIII specimens was truncated because they
146   were homozygous for acrocentric chromosome *p*.
147   The karyotype variability in the hybrid zone makes impossible reliable discrimination between
148   'purebred' representatives of parental races, F_1 hybrids between them and recombinant progeny of
149   the F_1 hybrids and their backcrosses to parental races. For this reason, we grouped the specimens
150   according to complexity of synaptic configurations occurring in meiosis rather than to their origin
151   inferred from the sets of diagnostic chromosomes.
152   *3.2. Synaptic aberrations*
153   In the majority of pachytene cells of the homozygotes and the simple heterozygotes for one or
154   two CIII, we observed orderly paired autosomes. The sex trivalent X/Y_1Y_2 followed its usual pairing
155   pattern [18,26,27]: the autosomal part of Xq (arm *d*) and the whole Y_2 were completely synapsed,
156   while the Y_1 (arm *s*) was partly paired with the end of Xp (arm *e*) in a short pseudoautosomal region
157   (Figure 2A). Pachytene cells of simple heterozygotes for one or two CIII usually contained completely
158   synapsed trivalents with or without formation of side arms between pericentromeric regions of the
159   twin acrocentrics. Pairing abnormalities, such as partial asynapsis of the chromosome ends in the
160   bivalents and pericentromeric regions of the twin acrocentrics in the trivalents or complete asynapsis
161   of one of the twin acrocentrics (Figure 2B) were rare in most specimens of these karyotypes. Two
162   specimens #5 and #13 demonstrated a rather high frequency of pairing abnormalities, but only a few
163   cells were suitable for SC analysis in these specimens (Table 1). The differences in frequency of pairing
164   abnormalities between the homozygotes and simple heterozygotes for one or two CIII were not
165   significant (Fisher's exact test p > 0.05).
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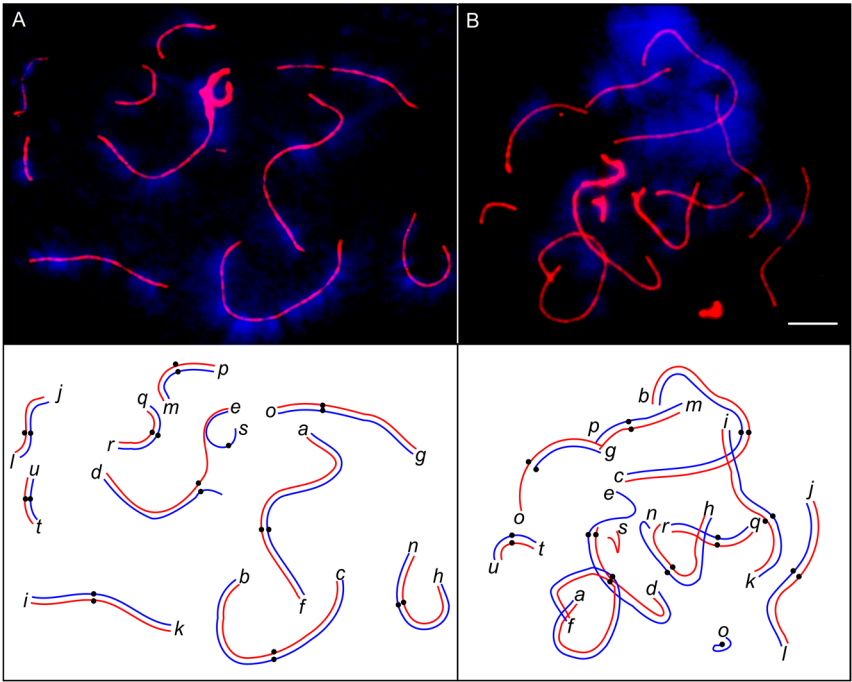
169   **Figure. 2.** Fluorescent micrographs and interpretative diagrams of pachytene spermatocytes of
170   a normal homozygote (A) and CIII heterozygote (B) after immunolocalisation of SYCP3 (red) and
171   DAPI counterstaining (blue). Centromeres are marked with black circles on the diagrams. Letters
172   indicate distal ends of the chromosome arms. Bar 5 μm.

173   About half of the pachytene cells of the males with a CVIII configuration and most pachytene
174 cells of the male with a CIX configuration showed synaptic abnormalities (Table 1, Figure 3). The
175 difference in the frequency of synaptic aberrations between CVIII and CIX karyotypes, as well as the
176 differences between complex heterozygotes on the one hand and homozygotes and simple
177 heterozygotes on the other hand were significant (Fisher's exact test p < 0.01).
178   A considerable part of the pericentromeric SCs of the multivalents usually appeared unpaired
179 and had a reduced intensity of SYCP3 and AgNOR staining. We often observed large unstained gaps
180 in these regions even when all arms of the multivalents were synapsed (Figure 3A, 4). Matveevsky et
181 al. [28] also observed such gaps in tetravalents of hybrid male shrews. Complete asynapsis of one or
182 more chromosome arms resulted in dissociation of the expected CVIII and CIX configurations into
183 two or more novel configurations. For example we observed g*k*/go/o, *hn*/*mn* and *hi*/*ki* with the shared
184 arms paired homologously and other arms involved in non-homologous synapsis (Figure 3B).
185   CVIII and CIX multivalents were often associated with the configurations formed by invariable
186 chromosomes. Most often we observed an association between the CIX and the sex trivalent (Figure
187 3C). The remaining nuclei contained multivalents or their elements associated or paired with *af* or *bc*
188 bivalents or with both of them (Figure 3B). Chromosomes *jl*, *qr* and *tu* were seldom found attached
189 to the chain.
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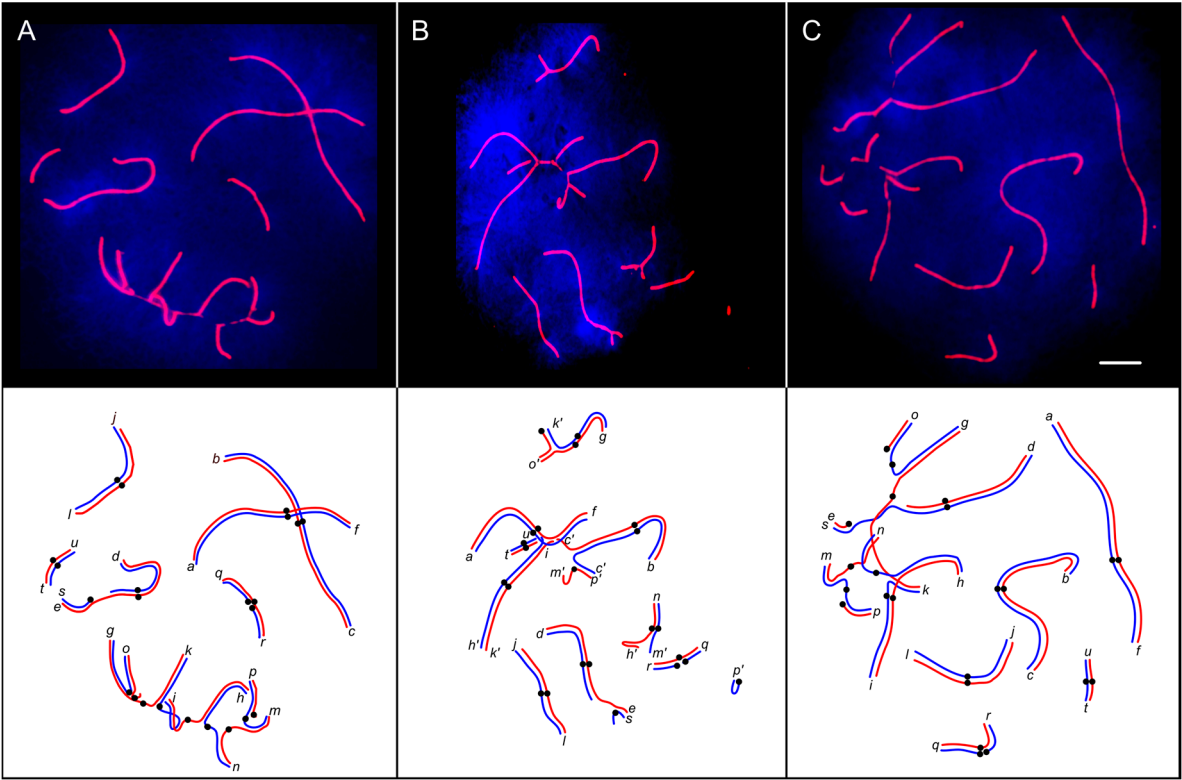
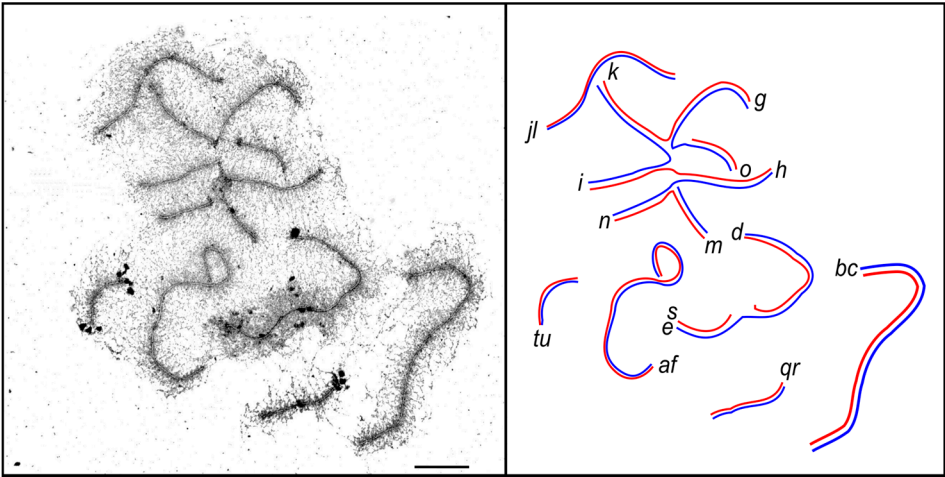

192   **Figure 3.** Fluorescent micrographs and interpretative diagrams of pachytene spermatocytes of a
193 CVIII heterozygote after immunolocalisation of SYCP3 (red) and DAPI counterstaining (blue).
194 Centromeres are marked with black circles in the diagrams. Letters indicate distal ends of the
195 chromosome arms. A prime following the letter indicates that it is paired non-homologously or
196 completely unpaired. Bar 5 μm.
197   (A) homologous synapsis of all arms in a CVIII multivalent with partial asynapsis around
198 centromeres; (B) dissociation of a CVIII multivalent into novel shorter configurations g*k*/go/o,
199 *bc*/*bc*/*mp*, *hn*/*mn*, *hi*/*ki* and univalent *p*; C: association between the sex trivalent and the CVIII
200 configuration.

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203   **Figure 4.** Electron microphotograph and interpretative diagram of a pachytene spermatocyte of
204   a CVIII heterozygote after AgNOR staining. Letters indicate chromosomes and chromosome arms.
205   Bar 5 μm.
206
207   The arms that contribute to the CIX configuration varied in the frequency of asynapsis (Figure
208   5). We did not observe a pairing failure in the arm *g*, while the arm *n* was unpaired significantly more
209   often than any other arm (Fisher's exact test with Bonferroni correction on multiple testing $p<0.002$).
210   There was no correlation between the frequency of asynapsis and the arm size ($p>0.05$).
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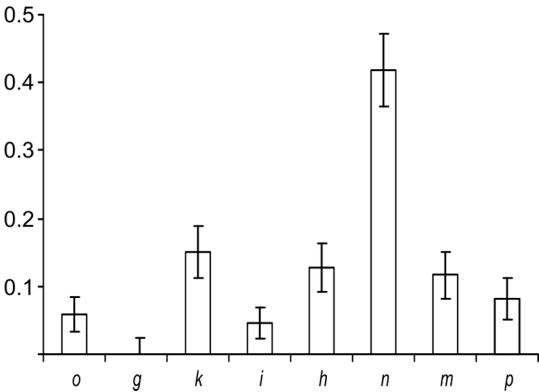
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213   **Figure 5.** Frequency of asynapsis in the chromosome arms involved in the CIX configuration. Bars
214   show S.D. Letters indicate chromosome arms.
215
216   *3.3. MLH1 foci number and distribution*
217   We used MLH1 foci as markers of recombination points along the SCs (Figure 6). Table 2 shows
218   the average number of MLH1 foci per cell for all shrews examined. We also included in the Table 2
219   our estimates of the expected number of obligate chiasmata for each karyotype. We expected that at
220   least one chiasma per chromosome is necessary for a proper segregation of the chromosomes
221   involved into bivalents and one per arm for an orderly segregation of trivalents and longer
222   multivalents. The male with a CIX configuration, which showed the highest frequency of synaptic
223   abnormalities, had the lowest MLH1 foci number among the shrews examined. It was lower than the
224   expected number of obligate chiasmata. In the other shrews, the observed number of MLH1 foci was
225   higher than expected number of obligate chiasmata.
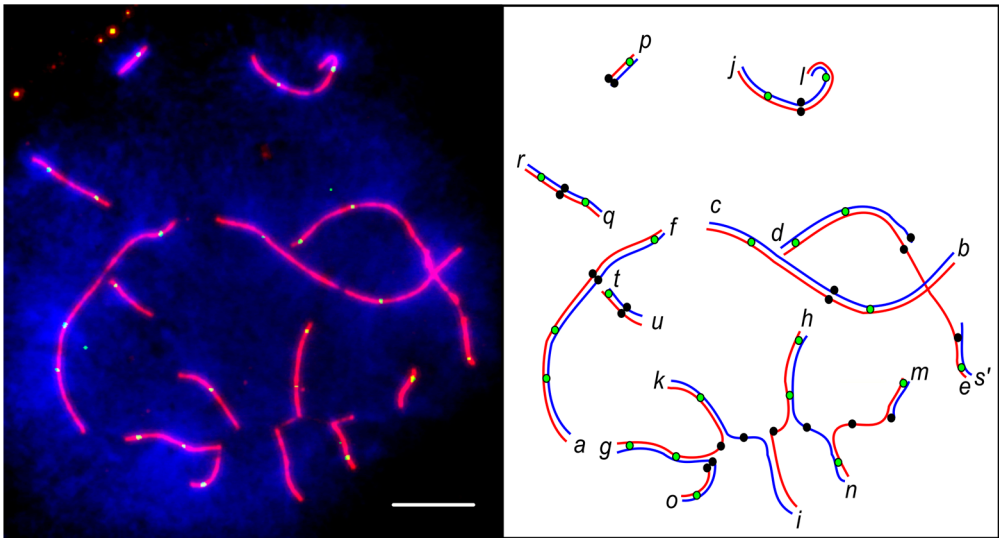
Figure 6. Fluorescent microphotograph and interpretative diagram of pachytene spermatocyte of a CVIII heterozygote after immunolocalisation of SYCP3 (red) and MLH1 (green) and DAPI counterstaining (blue). Centromeres are marked with black circles on the diagram. MLH1 foci marked with green circles. Letters indicate distal ends of the chromosome arms. Bar 5 μm.

Supplementary Table S1 shows the mean number of MLH1 foci per chromosome arm in different karyotypes. Despite a high frequency of synaptic aberration detected in CVIII carriers we did not find a significant reduction of MLH1 foci number either for the arms of invariable chromosomes or for the arms of the chain forming chromosomes (p>0.05 in each pairwise comparison).

The distribution of MLH1 foci along the SCs of the Siberian shrews matched the pattern described for the shrews sampled from Oxford-Wirral hybrid zone [18]. For all chromosome arms in all karyotypes examined we observed pronounced peaks at the distal regions and a deficiency of the foci in pericentromeric regions (Figures 7-8). Large and some medium sized chromosome arms showed a multi- or bi-modal MLH1 foci distribution. However, even in these arms proximal peaks were lower than the distal ones. Medium and small sized arms, including those involved in the CVIII and the CIII showed a gradual decrease of MLH1 foci number from distal to medial chromosome regions

Table 2. Average number of MLH1 foci per cell (scored in the cells without synaptic abnormalities) and expected number of obligate chiasmata in the shrews of different karyotypes

ID	Synaptic type	Karyotype	Number of cells scored	Average number of MLH1 foci per cell ± S.D.	Expected number of obligate chiasmata
1	homozygote	*go, hn, ik, mp, qr*	6	18.6 ± 3.1	11
2	homozygote	*go, hn, ik, mp, qr*	13	19.2 ± 3.4	11
4	homozygote	*gk, hi, mn, o, p, qr*	6	19.5 ± 2.4	12
5	homozygote	*gk, hi, mn, o, p, q, r*	7	19.8 ± 2.1	13
6	CIII	*gk, hi, mn, o, p, q/r*	15	22.1 ± 3.7	13
8	CIII	*g/o, hn, ik, mp, qr*	14	20.6 ± 3.4	13
12	2 CIII	*g/o, hn, ik, mp, q/r*	27	19.1 ± 1.9	13
14	CVIII	*o/og/gk/ki/ih/hn/nm/m, p, qr*	19	21.1 ± 2.5	15
15	CVIII+CIII	*o/og/gk/ki/ih/hn/nm/m, p, q/r*	17	22.1 ± 2.8	16
16	CIX+CIII	*o/og/gk/ki/ih/hn/nm/mp/p, q/r*	5	14.1 ± 2.5	16

249   All chain forming arms except arms *g* and *h* contained very few MLH1 foci in their proximal
250 regions. We observed an especially wide and deep pericentromeric valley of MLH1 frequency for
251 arm *n* (Figure 8), characterised by the highest frequency of asynapsis in the CVIII (Figure 5).
252   Each arm displayed its individual pattern of MLH1 foci distribution. It was not substantially
253 altered by the arm combination in the metacentric homozygotes. For example, the arm *g* showed a
254 similar pattern in the *go* and *gk* combinations. The patterns of individual arms also did not differ
255 significantly between homozygotes for metacentric chromosome including a particular arm and
256 complex heterozygotes for this arm (Figure 8).
257   Having found extensive asynapsis in the pericentromeric regions in the chain configuration
258 (Figures 3, 6) we expected to observe a substantial distalisation of the MLH1 focus distribution in the
259 complex heterozygotes. It was not the case. Although in all arms compared the distance between
260 centromeres and MLH1 foci was slightly higher (by 0.4 µm on average) in complex heterozygotes
261 (CVIII and CIX) than in metacentric homozygotes, the difference were not significant (p>0.05)
262 (Supplementary Table S2). Apparently, recombination in the proximal regions is so strongly
263 suppressed in normal homozygous karyotype that partial asynapsis in these regions does not make
264 it any stronger.

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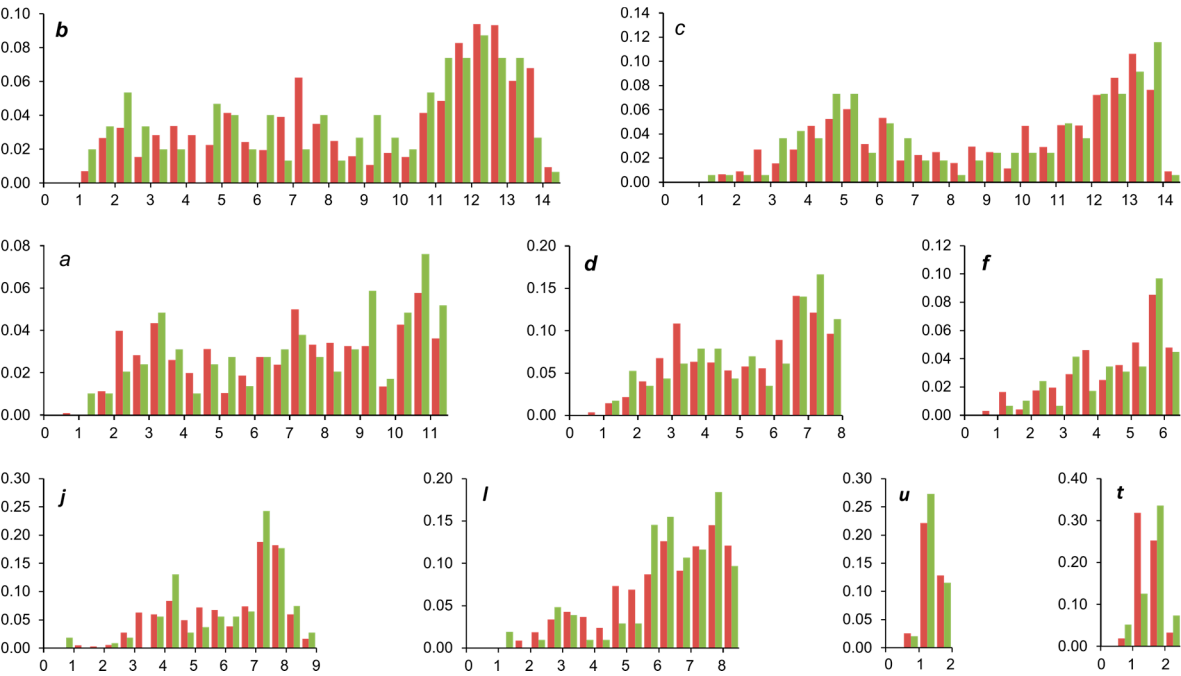
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268   **Figure 7.** Distribution of MLH1 foci along the arms of invariable chromosomes.
269   The X-axis shows the position of MLH1 foci in relation to the centromere (marked by 0) for the
270 SC of the particular arm (indicated at the top of each graph) in homozygotes for all metacentric
271 chromosomes (red) and complex heterozygotes with a CVIII configuration (green). The marks on the
272 X-axis are separated by 1 µm of the SC. The Y-axis indicates the frequency of MLH1 foci in each 0.5
273 µm interval.

Figure 8. Distribution of MLH1 foci along the arms of variable chromosomes.
The X-axis shows the position of MLH1 foci in relation to the centromere (marked by 0) at the SC of particular arm (indicated at the top of each graph) in homozygotes for the Novosibirsk race (red) and Tomsk race (blue) derived metacentric chromosomes, complex heterozygotes with a CVIII configuration (green) and simple heterozygotes (violet). The marks on the X-axis are separated by 1 μm of the SC. The Y-axis indicates the frequency of MLH1 foci in each 0.5 μm interval.

4. Discussion

282

283   Here we demonstrated a very high rate of synaptic aberration in male complex heterozygotes
284   for chromosome rearrangements with monobrachial homology derived from the hybrid zone
285   between the Novosibirsk and Tomsk chromosome races of the common shrew (Table 1) and strong
286   recombination suppression in pericentromeric regions of most chromosomes. How may these factors
287   affect gene flow across the hybrid zone?
288   Partial or complete asynapsis of the chromosomes involved in the chain was the abnormality
289   most often seen. Asynapsis is a well known cause of impaired fertility due to meiotic arrest and germ
290   cell loss [29]. Completion of synapsis at pachytene is monitored by a checkpoint mechanism, which
291   triggers apoptosis in the cells with incomplete synapsis [30–32]. However, at least half of the germ
292   cells in CVIII carriers showed normal chromosome synapsis and recombination. These cells can
293   complete meiosis and form viable gametes. Earlier Pavlova et al. [33,34] and Matveevsky et al. [28]
294   detected normal pairing of the expected configurations at diakinesis-metaphase I and active sperm
295   of normal morphology in male carriers of ring of four (RIV) and CXI from the Moscow-Neroosa and
296   Moscow-Seliger hybrid zones, respectively. Studies in other hybrid zones of the common shrew
297   suggest decreases in fertility in heterozygotes, particularly complex heterozygotes, but not to a
298   massive degree [35–38]. This indicates that despite a high frequency of pachytene cells with aberrant
299   chromosome pairing the male carriers of complex meiotic configuration generally can produce viable
300   gametes, although likely in reduced numbers.
301   Analysis of the distribution of molecular markers in the hybrid zones of the common shrew
302   showed no evidence of reduced gene flow resulting from male hybrid sterility or reduced fertility.
303   There was no difference in a degree of genetic differentiation within and between the Novosibirsk
304   and Tomsk races; the markers located in common and race-specific chromosomes showed the same
305   level of differentiation between the races [12]. No linkage disequilibrium was detected between the
306   race specific Y chromosome haplotypes and the race specific autosome complement in the
307   Novosibirsk-Tomsk hybrid zone [39]. These indirect data indicate that pairing abnormalities in
308   hybrid CVIII and CIX carriers detected in this study do not lead to complete sterility of males.
309   Female meiosis in the hybrids has not been studied yet due to technical difficulties. However,
310   studies at other mammalian species indicate that female meiosis is less vulnerable to chromosomal
311   heterozygosity and genetic incompatibility than male meiosis. It is also known that meiotic
312   checkpoints in female germ cells are less sensitive to incomplete synapsis than in male germ cells (see
313   [40] for review). For this reason, we suppose that the fertility in female hybrid shrews should be
314   affected less than in males.
315   The overall recombination rate was rather similar in all shrews examined, except the male CIX
316   carrier showing a very high frequency of pairing aberration (Table 2). In the homozygous, CIII and
317   CVIII karyotypes it was higher than the expected number of obligatory chiasmata sufficient for
318   correct chromosome segregation at MI (Table 2). Thus, correct segregation of chromosomes is
319   possible in the male hybrids, though direct studies are needed to estimate the frequency of meiotic
320   non-disjunction in this hybrid zone. Certainly the narrowness of the Novosibirsk-Tomsk hybrid zone
321   indicates that there is a degree of infertility associated with presence of the CVIII or CIX configuration
322   in hybrids [11], either attributable to germ cell death (reduced reproductive lifespan of females,
323   greater frequency of males with insufficient numbers of sperm) or non-disjunction (greater embryo
324   death) [35].
325   Figure 1 shows the balanced segregation of the CIX, when all chromosomes derived from Tomsk
326   race move to one pole and all Novosibirsk derived chromosomes to another pole. Of course,
327   recombination in the terminal and medial regions of the chromosomes lead to the production of
328   recombined chromosomes. However, a strong suppression of recombination around the centromeres
329   spanning from a quarter to a half of the arm length in the chain forming metacentric chromosomes
330   enforces linkage disequilibrium between all parental alleles located in the pericentromeric regions. If
331   in geographic isolation chromosome races become fixed around the centromeres with alternative
332   epistatically-interacting coadaptive genes or supergenes, then such supergenes could still be

333 maintained after the races come into contact. Such pericentromeric supergenes has been found in
334 several species of plants (see [41] and references therein). In the shrew hybrids, these supergenes
335 could be dispersed over multiple pericentromeric regions of the chromosomes involved in long
336 meiotic chain, and therefore would segregate as a united race specific 'super-supergene'. It has been
337 shown that the gene flow between mouse chromosome races is more strongly restricted near the
338 centromeres of Robertsonian chromosomes than in other regions [42–44]. In the case of Novosibirsk-
339 Tomsk hybrid zone we would also expect strongly restricted gene flow in the proximal regions. This
340 reduction in gene flow is probably very difficult to detect with the set of chromosomes specific
341 microsatellite makers available at present in the common shrew [12]. More makers with known
342 subchromosomal localisation have to be developed.

343   Linkage disequilibrium due to crossover suppression in pericentromeric regions in the complex
344 chromosome heterozygotes occurring in the hybrid zone might keep together race-specific alleles
345 controlling morphological traits and thus contribute to the phenotypic differentiation of the parental
346 races [13,14]. Linkage disequilibrium for genes controlling local adaptations may also affect the width
347 of clines for the chromosomes containing such genes. Polyakov at al. [45] demonstrated altitudinal
348 partitioning of the Novosibirsk and Tomsk races. This partitioning marks a border between two
349 rather different biotopes: forest steppe at low altitude and taiga at the high. It has been shown that
350 the structure of the cline for the CIX chromosomes closely follows that predicted by a model including
351 altitude, while the CIII cline does not fit it so well [11].

352   In conclusion, the meiotic studies of hybrids from the Novosibirsk-Tomsk hybrid zone of the
353 common shrew show indications that both fertility reduction and recombination suppression may
354 contribute to reduced gene flow, in line with previous findings for the best studied house mouse
355 chromosome hybrid zone [43].

356 **Supplementary Materials:** The following is available online at www.mdpi.com/link:
357   Table S1. Number of MLH1 foci per chromosome arm
358   Table S2. Mean (± S.D.) distance of MLH1 foci from centromere in the chromosome arms
359 involved in different synaptic configurations.

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369 N.B., A.T., J.S. and P.B. wrote the manuscript.

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