

Reduced meiotic fitness in hybrids with heterozygosity for heterochromatin in the speciating *Mus terricolor* complex

TIKARAM SHARMA[†], AMIT BARDHAN and MIN BAHADUR*

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

*Department of Zoology, North Bengal University, Darjeeling 734 430, India

[†]Corresponding author (Fax, 91-542-2368457; Email, tsharma123@yahoo.co.in)

Mus terricolor I, II and III are the three chromosomal species which differ in stable autosomal short-arm heterochromatin variations established in homozygous condition. Analysis of meiosis in the laboratory-generated F₁ male hybrids from crosses (both ways) between *M. terricolor* I and II and between *M. terricolor* I and III shows high frequencies of pairing abnormalities at pachytene. The backcross (N3 generation) male hybrids between *M. terricolor* I and II have meiotic abnormalities as in the F₁ male hybrids, though to a lesser extent. They show difference in pairing abnormalities in the different karyotypic forms; the backcross hybrids heterozygous for the heterochromatic short arms have more anomalies compared to the homokaryotypic hybrids. This suggests a negative influence of the heterochromatin heterozygosity in meiotic pairing. The results indicate a role for heterochromatin variations in the development of a reproductive barrier in the speciating *M. terricolor* complex.

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1. Introduction

Mus terricolor (formerly known as *Mus dunni*) and *Mus booduga* are the two co-existing sibling species of pygmy field mice of India. In contrast to the highly conservative *Mus musculus*-like karyotype in *M. booduga* ($2n = 40$) with all acrocentric chromosomes in the complement, in *M. terricolor* species-specific centric heterochromatin has evolved, and its repatterning has resulted in acrocentric and submetacentric autosomes in the complements (Sharma and Sharma 1998). There are three apparently non-overlapping chromosomal species *M. terricolor* I, II and III (called as *terricolor* I, II and III, respectively); their karyotypes ($2n = 40$) differ in fixed autosomal short-arm heterochromatin variations established in homozygous condition (Sharma 1996; Sharma and Sharma 1998; Sharma *et al* 2002). While *terricolor* I is characterized by all acrocentric autosomes with minute but distinct heterochromatic short arms, *terricolor* II and *terricolor* III possess large C-positive heterochromatic

short-arms on the autosome pairs 1 and 3, and 1, 3 and 6, respectively. All the three chromosomal species however possess identical sex chromosomes, a large submetacentric X chromosome with a heterochromatic short arm and a heterochromatic segment at the distal end of the long-arm, and a large acrocentric Y chromosome which is totally heterochromatic (see figure 1, Bardhan and Sharma 2000a; Sharma *et al* 2002).

In this actively speciating group of mice, Robertsonian fusions (involving different autosomes) and pericentric inversions (in autosomes 2 and 5) have been encountered in high frequency in wild populations. However, they exist only in polymorphic states, and interestingly, their heterozygosity has no effect on fertility (Bardhan and Sharma 2000a). In both the types of heterozygotes some alterations in the process of homologous synapsis occur that could negate the possible negative influence of heterozygosity of the polymorphic chromosomal rearrangements on meiotic progression and fertility (Bardhan and Sharma 2000a). In the chromosomal speciation in ani-

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mals, normally centric fusions and pericentric inversions are associated (White 1978; King 1981; Capanna 1982; Shaw 1981; Baker and Bickham 1986; reviewed by King 1993). But, in the evolutionary divergence of the *terricolor* complex, these chromosomal rearrangements do not seem to be of any consequence.

The experimental hybrids, among the chromosomal species of *terricolor*, show post-zygotic reproductive barrier manifested in the form of spermatogenic impairment (see §3). In the present paper meiotic analysis has been carried out to understand the cytogenetic mechanism of the spermatogenic impairment, and whether the stable autosomal heterochromatin variations established in homozygous condition have any implication in the process. The results are discussed in the light of relevance of the heterochromatin variations in developing reproductive barrier among the *terricolor* chromosomal species.

2. Materials and methods

2.1 Mice

M. terricolor I was collected from Varanasi in northern India; *terricolor* II from Mysore in interior of southern India; and *terricolor* III from Chennai in coastal region of southern India. The mice were collected by digging burrows in cultivated wheat and paddy fields.

2.2 Breeding and hybridization

Males and females of the same chromosomal species were pair-mated for routine laboratory breeding in mouse cages in animal room with a provision for automatic 12 h light-dark cycle. Inbreeding was avoided by separating about three weeks old males and females in different cages and by monitoring the mice regularly.

About one month old first generation pups born in the laboratory were used for hybridizations. For backcrossing, about one month old female hybrids were pair-mated with one to two months old males of one of the parental types. To obtain backcross hybrids with homozygous acrocentric autosomes 1 and 3, female hybrids from *terricolor* I \times II crosses were mated with *terricolor* I males. Mating of female hybrids from *terricolor* I \times II crosses with *terricolor* II males produced hybrids with homozygous submetacentric autosomes 1 and 3 with heterochromatic short arms. Both these crosses also produced hybrids which were heterozygous for the heterochromatic short arms in the autosomes 1 and/or 3.

2.3 Cytology and histology

When the mice were 2–3 months old and sexually mature they were sacrificed for study. Mitotic chromosomes

were prepared from bone marrow of colchicine-injected mice following conventional method. C-banding was done according to the BSG-technique of Sumner (1972) and G-banding following Seabright (1971). Karyotypes were arranged according to the Committee on Standardized Genetic Nomenclature for Mice (1972). Air-dried male meiotic chromosomes were prepared following Evans *et al* (1964). Synaptonemal complexes (SCs) were prepared from spermatocytes, using the surface-spreading technique of Fletcher (1979). SCs from female mice were prepared using foetuses collected from advanced-pregnant mice; spreading was done according to the method of Dietrich and Mulder (1983) with minor modifications. SCs were stained either cytochemically with silver nitrate, or immunocytoologically using rabbit 'B' serum raised against hamster SCs (Moens *et al* 1987). The method of Dobson *et al* (1994) was followed for immune staining.

Sections of testis and ovary (5–6 μ m) were obtained following routine histological techniques. For each batch of histological preparations, one control tissue (testis or ovary) from fertile laboratory mouse was processed parallelly to rule out preparational artefacts. Sections were stained with haematoxylin and eosin. Twenty-five cross sections were observed from each animal. Cytological and histological analyses were made on the same hybrids, and results composed.

2.4 Identification of spermatocytes

Spermatocytes were classified into pachytene and diplotene mainly on the basis of morphology of the XY bivalent (table 1 and figure 4 in Bardhan and Sharma 2000b). Pachytene was sub-staged into early- and mid-/late-pachytenes on the extent of XY synapsis (Bardhan and Sharma 2000b). Gametogenic and meiotic analyses were done on F₁ hybrids obtained from all six possible combinations of crosses. In all the crosses the female parent has been mentioned first, unless otherwise stated.

3. Results

All the hybrids had $2n = 40$ chromosomes in their complements, which meant absence of centric fusions in them. The hybrids however had pericentric inversions in the autosome pairs 2 and/or 5 in homozygous or heterozygous condition. It has already been shown (Bardhan and Sharma 2000a) that such inversion heterozygosity has no apparent effect on meiotic progression and fertility. Hybrids between *terricolor* I and II had heterozygosity for heterochromatic short arms on two autosomal pairs (1 and 3). Hybrids between *terricolor* I and III had heterozygosity for heterochromatic short arms on three

Table 1. Spermatogenic features in F₁ hybrids among the three chromosomal species *M. terricolor* I, II and III.

Spermatogenic features ^a	Type of cross ^b /hybrid Nos ^c	Remark
Group I		
Nuclear condensation in spermatocytes, coarse chromatin clumped and pycnotic suggesting progressive degeneration; exfoliation; lack of elongated spermatids and sperm in many tubules (sperm were however present in a few tubules in these hybrids).	I × II (5); II × I (5) I × III (6); III × I (5) II × III (1); III × II (2)	Incomplete spermatogenic impairment
Group II		
Tubules atrophied, filled with epithelial vacuoles; spermatocytes had nuclear condensation and pycnosis; presence of only a few spermatogenic cells; total lack of elongated spermatids and sperm.	I × II (1); II × I (2) I × III (2); III × I (3) II × III (0); III × II (0)	Complete spermatogenic impairment
Group III		
Seminiferous tubules with normal association of spermatogenic cells and without any degenerative feature.	I × II (1); II × I (1) I × III (0); III × I (1) II × III (7); III × II (7)	Normal spermatogenesis

^aSpermatogenic features are qualitatively classified into groups I, II and III, based on the extent of abnormalities.

^b*M. terricolor* I, II and III are designated as I, II and III, respectively. In each cross, the female parent is mentioned first.

^cNumbers of hybrids in each group are given within parentheses, in each type of cross.

autosomal pairs (1, 3 and 6). In hybrids between *terricolor* II and III only one pair of autosomes (6) was heterozygous for heterochromatic short-arm.

3.1 Gametogenesis was affected only in the male hybrids

Histological analysis of testis of F₁ hybrids showed that spermatogenesis was affected to different degrees, resulting in incomplete or complete spermatogenic impairment in *terricolor* I × II (both ways) and *terricolor* I × III (both ways) hybrids (table 1 and figure 1). The hybrids from crosses between *terricolor* II × III (both ways) however had only some degree of spermatogenic impairment. The spermatogenesis was largely normal in them (table 1 and figure 1). Two males each of *terricolor* I, II and III were analysed as controls; all had normal spermatogenesis.

3.2 Meiotic synapsis was aberrant in *terricolor* I × II and *terricolor* I × III hybrid males

Five F₁ male hybrids from two *terricolor* I × II crosses, and six from three reverse crosses; and, six F₁ male hybrids from three *terricolor* I × III crosses, and five from three reverse crosses, were analysed for meiotic synapsis (data summarized in table 2). One hundred complete pachytene spreads were observed from each hybrid. All the pachytenes had 19 autosomal SCs and the XY bivalent, but the latter was dissociated in some nuclei. The

spread nuclei had different extent of general synaptic abnormalities of the autosomal SCs (figure 2). Normal pachytenes had fully paired linear autosomal SCs (figure 2A). Abnormal pachytenes, on the other hand, had terminal and/or interstitial unpaired axial segments in the autosomal SCs (figure 2B-E). Lack of synapsis was extensive in many mid-/late-pachytenes, that sometimes resulted in complete separation of the axial elements except for one or a few terminal and/or interstitial connections (figure 2B,D). Such pairing abnormalities would possibly lead to atresia of the spermatocytes. Intriguingly however, XY synapsis was unaffected even in pachytenes that had extensive synaptic abnormalities of the autosomes (figure 2C,D). The frequency of abnormal pachytenes varied widely among the hybrids (15–81% in *terricolor* I × II hybrids; 29–74% in *terricolor* I × III hybrids). The average frequency of synaptic abnormalities is summarized in table 2.

The unpaired axial segments at pachytene differed in appearance from the unpaired desynapsed axes at diplotene. Desynapsis at diplotene proceeded in a coordinated manner, generally starting terminally and then interstitially in a few bivalents. The desynapsed axes had a weaker staining (Bardhan and Sharma 2000b). During extensive desynapsis at late-diplotene, the unpaired axes had a discontinuous staining pattern while the telomeric ends were intensely stained. Lack of complete synapsis did not have a consistent pattern in the abnormal pachytenes of hybrids. The unpaired axes were terminal and/or interstitial at several points along the bivalents, and were occasion-

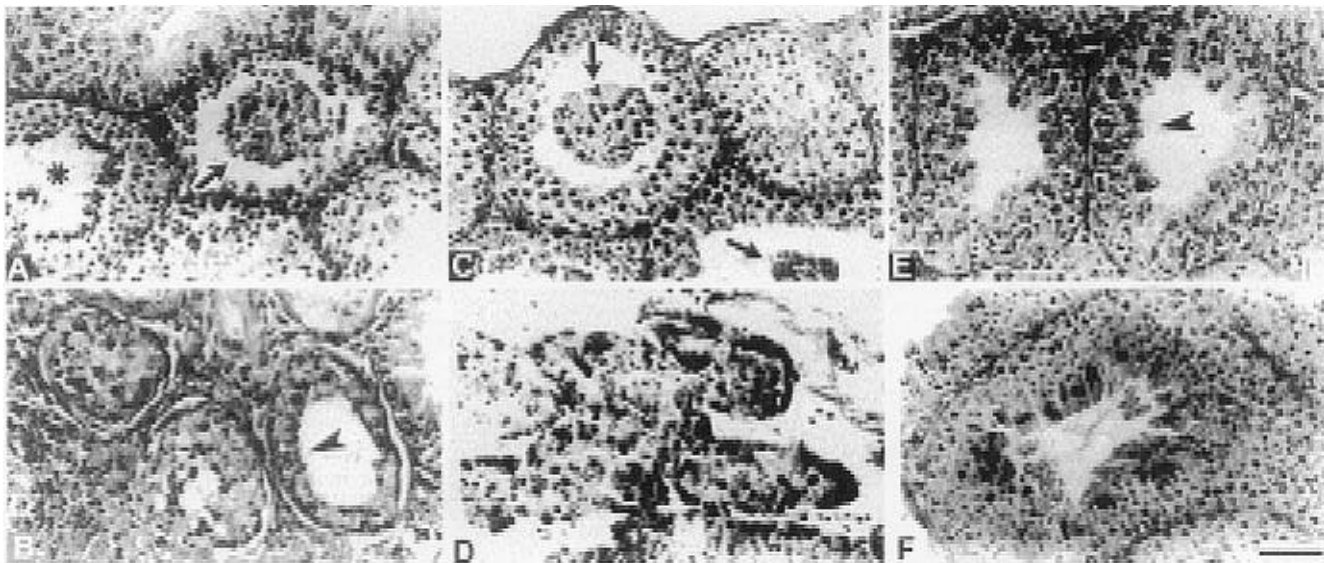


Figure 1. Testis sections in I × II hybrids (A, B), in I × III hybrids (C, D). (A, C) Mild spermatogenic abnormalities; note exfoliation of spermatocytes and spermatids into tubular lumen (arrow), and loose association of germinal cells. One normal tubule lined with testicular sperm (asterisk) is seen in (A). (B, D) Severely atrophied seminiferous tubules. (E) Mild spermatogenic abnormalities in II and III hybrid; elongated spermatids are absent along the lumens (arrowhead). (F) Normal spermatogenesis in II × III hybrid; size of the seminiferous tubule is much larger and the germinal cells are well organized. Note the difference in size, cellular associations and the tubular lumen between (F) and (A-E). Bar = 1 µm.

Table 2. Summarized average frequency of synaptic abnormalities and X-Y dissociation in the male hybrids among *M. terricolor* I, II and III.

Cross/hybrid nos ^a	Frequency-distribution (%) of pachytene on the basis of SC phenotype ^b		X-Y dissociation (%)	
	Autosomal synapsis at pachytene		Pachytene	Dia/MI ^c
	Complete synapsis	Partial synapsis		
I × II (5)	48.8	51.2	17.8	16.3 (257)
II × I (6)	48.8	51.2	18.0	17.9 (217)
I × III (6)	52.5	47.5	26.6	23.3 (159)
III × I (5)	47.0	53.0	20.8	19.5 (256)
II × III (4)	86.5	13.5	17.0	18.2 (148)
III × II (4)	98.5	1.5	13.5	13.7 (146)

^aNumbers of hybrids in each type of cross are given within parentheses.
^bOne hundred pachytene spreads were analysed from each hybrid.
^cThe Nos of diakinesis/metaphase-I observed in each type of cross are given within parentheses.

ally twisted and intact for their entire length (figure 2B-D). In the hybrids the diplotene nuclei, identified according to Bardhan and Sharma (2000b), were all normal (figure 2F). It appeared that the spermatocytes having pairing abnormalities do not progress beyond pachytene, they degenerate.

Analysis of meiotic synapsis in the wild *terricolor* showed normal synaptic progression. The autosomal SCs were completely synapsed at the onset of pachytene. A total of 351 pachytene nuclei observed from nine mice

had fully paired linear autosomal SCs. The heterochromatic short arms however showed delayed synapsis at mid- to late-pachytene.

3.3 Meiotic synapsis was largely normal in *terricolor* II × III hybrid males

Four F₁ male hybrids from two crosses between *terricolor* II and III, and four from two reverse crosses were analysed (data summarized in table 2). One hundred

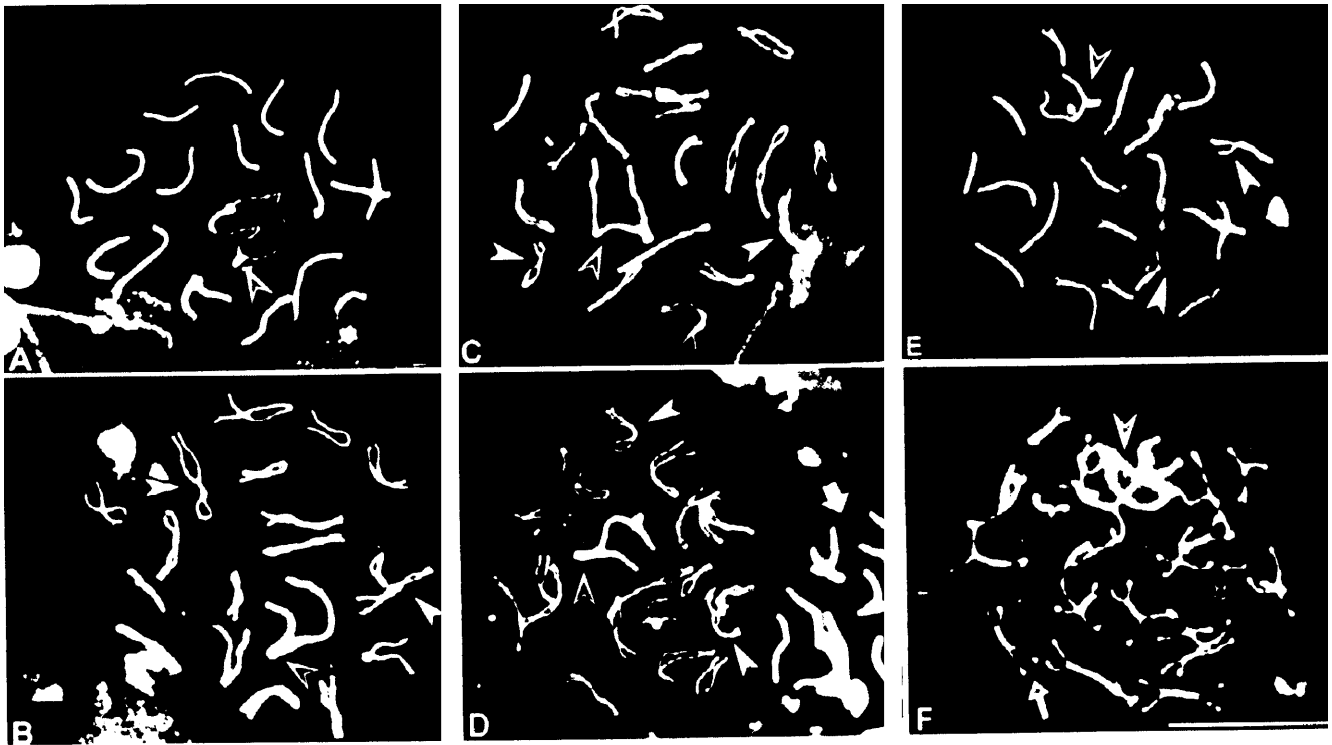


Figure 2. SCs in male hybrids. A normal mid/late-pachytene spermatocyte in a hybrid between I and III; all the autosomal SCs are fully synapsed while the XY (open arrow) has a short paired segment (A). Extensive synaptic abnormalities at mid-/late-pachytene in hybrids between I and III (B) and between I and II (C, D); the SCs show lack of complete synapsis with unpaired axial elements that were often twisted (arrowheads). Abnormal synapsis in (B) and (D) resulted in complete axial-separation of most of the SCs. Part of a normal pachytene with fully synapsed (solid arrow) autosomal SCs (D). A mid-/late-pachytene in a hybrid between II and III (E); some SCs have terminal unpaired axial elements (arrowhead). A normal mid-/late-diplotene spermatocyte in a hybrid between II and III (F); the desynapsed axes are stained less intensely (open arrowhead) while the telomeric ends are darkly stained. The XY is associated end-to-end in diplotene (F), but has a short paired segment in pachytene (A-E). The pattern of axial separation is also different between the pachytene and diplotene; in pachytene, the unpaired segments have no consistent pattern of separation and are often twisted (B, C, D); in diplotene, the desynapsed unpaired segments are mostly terminal without any twisting (F). The XY bivalent is shown by open arrow in all the figures. Bar = 10 μ m.

complete pachytene spreads were observed from each hybrid. Synaptic progression was mostly normal and only a low frequency of the pachytene spreads had pairing abnormalities; some of the autosomal SCs had unpaired axial elements while others were completely synapsed (figure 2E).

3.4 XY synapsis at pachytene was unaffected

The X and Y chromosomes showed different degrees of dissociation in all the F_1 hybrids analysed (table 2). Similar frequency of X-Y dissociation was also observed in the wild individuals (data not shown), suggesting that the observed dissociation was not due to hybridity.

3.5 X-Y dissociation frequency at diakinesis/metaphase-I was unaltered

The XY bivalent was dissociated in 10–30% of diakinesis/metaphase-I nuclei in the hybrids. Some hybrids had

dissociation frequency as low as 4%, while others had as high as 37%. SC analysis from the same hybrids showed similar dissociation frequency at pachytene suggesting that the X-Y dissociation resulted from their asynapsis at pachytene. The average frequencies of X-Y dissociation are summarized in table 2. The X-Y dissociation frequency in the F_1 hybrids did not differ from the parental types (data not shown), which precluded effect of hybridity on the dissociation.

3.6 Absence of interaction between the asynapsed autosomal segments and the XY bivalent

In the F_1 hybrids, 2–10% of the pachytene spreads showed physical proximity of unpaired/partially-paired autosomes to the XY bivalent. Similar physical proximity was also observed in the parental types, which suggested that this was not a specific interaction between the XY bivalent and the unpaired autosomal segment(s) in the hybrids.

3.7 Synaptic abnormalities in female hybrids

Four hybrid female fetuses from two *terricolor* II \times I crosses, and four hybrid female fetuses from two *terricolor* III \times I crosses, and three from one reverse cross were utilized for analysis of pachytene synapsis during female meiosis. Female hybrids from the *terricolor* II \times III cross were not analysed because the hybrid males from such crosses had low level of synaptic abnormalities. Two female fetuses each of *terricolor* I, II and III were analysed as controls.

All the F₁ female hybrids had synaptic abnormalities (figure 3) similar to those seen in the F₁ male hybrids, but in much lower frequency (data summarized in table 3). Only 10–13% of the pachytenes had synaptic abnormalities, not observed in the parental type female fetuses. In the parental types, synapsis was normal in all the pachytenes with linear, completely paired SCs, except for the heterochromatic short arms that were unpaired in some nuclei. Normal pachytene in the hybrids had similar appearance. Abnormal synapsis, on the other hand,

included lack of complete pairing of the SCs with interstitial and/or terminal asynapsed axial segments.

The largely normal meiosis in the female hybrids was consistent with their apparently normal ovarian histology, and normal litter sizes of female hybrids in backcrosses (data not shown).

Table 3. Summarized average frequency of synaptic abnormalities in F₁ female hybrids between *M. terricolor* chromosomal species.

Cross/hybrid Nos ^a	Pachytenes observed	Pachytenes with incomplete synapsis	Frequency of aberrant synapsis (%)
II \times I (4)	225	23	10.2
I \times III (3)	152	20	13.1
III \times I (4)	223	28	12.5

^aThe numbers of hybrids in each type of cross are given within parentheses.

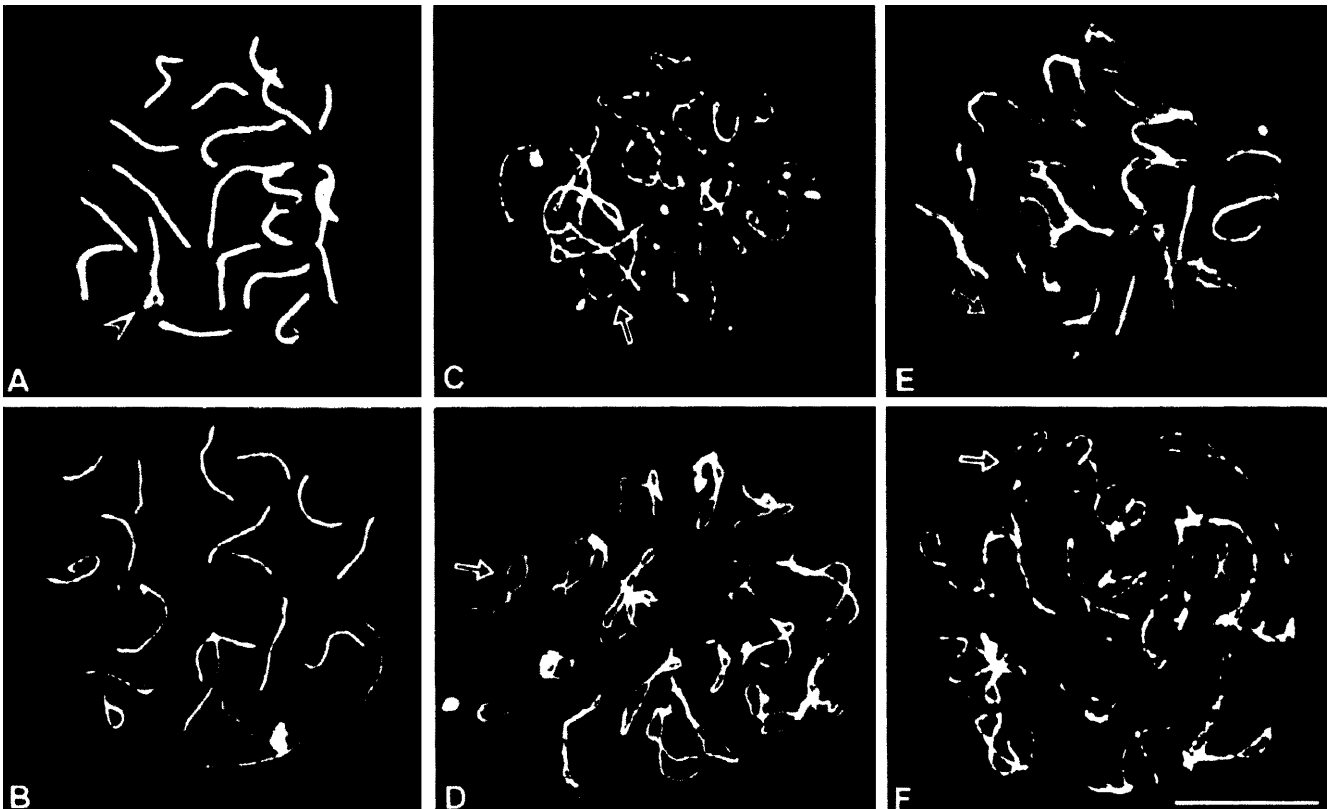


Figure 3. Examples of surface-spread SCs in female. (A, B) Normal pachytene with completely paired SCs, except for occasional asynapsis of the heterochromatic short arm (as in A, shown by arrowhead). (C–F) Abnormal pachytenes in hybrids between *terricolor* I and II (C, D), and between *terricolor* I and III (E, F); the SCs are not completely synapsed and have unpaired axial elements (arrows). Abnormal synapsis sometimes resulted in complete separation of the axial elements but for few interstitial and/or terminal connections (D, F). Bar = 10 μ m.

3.8 Backcross hybrids of different karyotypes differed in the extent of synaptic abnormalities

To address the question whether the observed synaptic abnormalities in the hybrids were either due to diverged genic factor(s), or due to heterozygosity for the fixed variations of the autosomal short arm heterochromatin; homozygosity and heterozygosity for the heterochromatic arms were obtained in the otherwise largely similar genetic background by repeated backcrossings. Female *terricolor* II \times I hybrids were backcrossed for three generations to *terricolor* I and *terricolor* II males, and the third generation (N3) backcross male hybrids were analysed for synaptic progression. Every care was taken to avoid mixing of mice, even by chance, while backcrossing. The hybrids analysed were karyotypically of three types: (i) both the autosome pairs 1 and 3 were homozygous for acrocentrics ($1^A/1^A$, $3^A/3^A$); (ii) both the pairs were heterozygous for submetacentrics with heterochromatic short arms ($1^A/1^S$, $3^A/3^S$); and, (iii) both were homozygous for submetacentrics with heterochromatic short arms ($1^S/1^S$, $3^S/3^S$).

The rationale was: (i) if genetic divergence was the sole cause of the synaptic abnormalities in the hybrids, a large difference in the extent of synaptic abnormalities between

the homo- and hetero-karyotypic forms would not be expected; conversely, (ii) if heterozygosity for the heterochromatic short arms was the sole cause, there would be no abnormality in the homokaryotypic forms, while the heterokaryotypic forms would exhibit similar aberration-frequency as observed in the F_1 hybrids. The backcross data are presented in figure 4.

Pachytene analysis of the N3 male hybrids showed synaptic abnormalities as seen in the F_1 hybrids, though the frequency of abnormal pachytene was comparatively lower. Interestingly, the extent of abnormalities differed between the homokaryotypic and heterokaryotypic mice. Hybrids with either of the homozygous karyotypes had comparable level of abnormalities: 10–16% in the hybrids with autosome pairs 1 and 3 homozygous for acrocentrics, and 14–18% in the hybrids with autosome pairs 1 and 3 homozygous for submetacentrics. In contrast, the hybrids with autosome pairs 1 and 3 heterozygous for the heterochromatic short arms had higher level (30–41%) of pachytenes with synaptic abnormalities (figure 4).

4. Discussion

The experimental data suggest that in the *terricolor* complex postzygotic reproductive barrier has developed

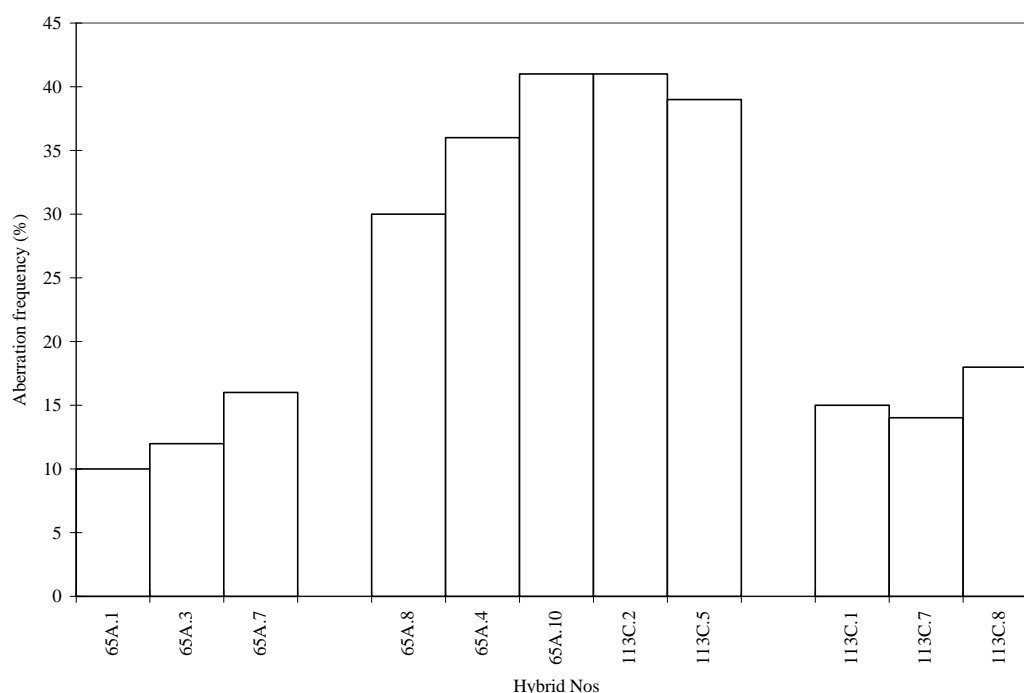


Figure 4. Chart showing frequencies of synaptic abnormalities in N3 generation backcross hybrids. Hybrid Nos 65A.1, 65A.3 and 65A.7 had $1^A/1^A$, $3^A/3^A$ genotype; hybrids 65A.8, 65A.4, 65A.10, 113C.2 and 113C.5 had $1^A/1^S$, $3^A/3^S$ genotype; while hybrids 113C.1, 113C.7 and 113C.8 were $1^S/1^S$, $3^S/3^S$ genotype.

which manifests in the form of reduction in fertility in the F₁ male hybrids. Meiotic analysis carried out in the same hybrids that were used for the histological analysis revealed that the hybrids that had severe gametogenic abnormalities also had high frequency of meiotic abnormalities. Conversely, the hybrids with low frequency of synaptic abnormalities had less severe or hardly detectable effect on gametogenesis. These meiotic phenotypes differed from those reported in hybrids of a majority of the mammalian species, including *M. musculus*. In majority of the mammalian hybrids with male-specific sterility, the X chromosome physically interacts with unpaired autosomal segment(s) at pachytene (for example, Forejt *et al* 1981; Setterfield *et al* 1988; Matsuda *et al* 1992; Hale *et al* 1993). This interaction supposedly results in improper gene expression during spermatogenesis and subsequently leads to spermatogenic malfunction (Lifschytz and Lindsley 1972; Forejt *et al* 1981; Jaafar *et al* 1993; reviewed by Forejt 1996). An alternative cytogenetic mechanism of reduction in fertility in male hybrids has been found in some mice. Asynapsis or precocious desynapsis of the X and Y chromosomes due to divergence of the pseudoautosomal region (Matsuda *et al* 1991; Hale *et al* 1993) leads to a high level of dissociation of X-Y at diakinesis/metaphase-I and to spermatogenic malfunction or reduction in testis weight (Imai *et al* 1981; Matsuda *et al* 1982, 1991, 1992). The lack of both these cytological phenotypes in the *terrlicolor* hybrids implies a different mechanism for the observed spermatogenic impairment.

High frequency of general synaptic failure could adversely influence meiotic progression by causing cell-death (Miklos 1974; Burgoyne and Baker 1984), and this could be responsible for the observed spermatogenic malfunction. That pairing failure could reduce fertility is supported by observations in mouse and human males carrying chromosomal aberrations (Burgoyne *et al* 1985; Chandley *et al* 1986; Batanian and Hulten 1987). The presence of pairing abnormality in the *terrlicolor* female hybrids indicated that the same mechanism of gametogenic impairment could operate also in the females. However, the manifestation of the impairment observed in the males could be due to: (i) greater extent of synaptic abnormalities in the male hybrids; and (ii) difference between the male and female gametogenesis (Hale *et al* 1993).

The general synaptic failure apparently indicates that the reason for the malfunction might be genic (Forejt and Ivanyi 1975). The reduction in the frequency of synaptic abnormality in the N3 hybrids as compared to the F1 hybrids is in agreement with the involvement of diverged genic factor(s). However, if genetic divergence was the only cause of the synaptic abnormality, the markedly lesser frequency of abnormality in the *terrlicolor* II × III hybrids (as compared to the rest of the hybrids) would be rather unexpected, because *terrlicolor* II and III have the

highest genetic distance in the complex ($D = 0.155$ between *terrlicolor* II and III, 0.082 between *terrlicolor* I and II, and 0.114 between *terrlicolor* I and III; Singh and Sharma 1997). In such a scenario, the backcross results (figure 4) could best be explained as follows.

Some diverged genic factor(s) could lead to abnormal synapsis, regardless whether there was heterochromatin heterozygosity or homozygosity. The N3 hybrids homozygotes for the presence/absence of the heterochromatic short arms displayed synaptic abnormalities. Additionally, heterozygosity for the fixed variations of the autosomal short arm heterochromatin could adversely affect synaptic progression in the hybrid genic background because the N3 hybrids with such heterozygosity had elevated levels of abnormalities as compared to the hybrids with homozygous karyotypes. Moreover, the effect of the heterochromatin heterozygosity could even be dose-dependent. The number of the autosomal pairs heterozygous for the heterochromatic short arms (two and three in hybrids between *terrlicolor* I and II, and between *terrlicolor* I and III, respectively; one in hybrids between *terrlicolor* II and III) could influence the extent of the abnormalities.

That heterochromatin heterozygosity affects progression of meiotic synapsis in a hybrid genic background in the *M. terrlicolor* complex is of significance in the speciation process. It has been shown by molecular-genetic dissection that heterochromatin can play an important role in meiotic synapsis and segregation (McKee *et al* 1992; Hawley *et al* 1992; Dernburg *et al* 1996; Karpen *et al* 1996). Heterochromatin may also house transposable elements (see Dimitri and Junakovic 1999) and meiotic drive factors (see Lyttle 1993) which could play a significant role in species differentiation by reducing meiotic fitness of the hybrids and thereby generating fertility barrier (Shaw 1994; Dimitri and Junakovic 1999). In a recent review (Redi *et al* 2001) attention has been drawn to heterochromatin's role in speciation but hard evidence is still lacking. Notwithstanding that the mechanistic explanation remains to be worked out, the heterochromatin's role in cladogenesis (splitting of population) in the speciating *M. terrlicolor* complex is essentially clear.

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Corresponding editor: DURGADAS P KASBEKAR