Modulation of Prdm9-controlled meiotic chromosome asynapsis overrides hybrid sterility in mice

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Abstract

The infertility of hybrids between closely related species is one of the reproductive isolation mechanisms leading to speciation. Prdm9, the only known vertebrate hybrid sterility gene causes failure of meiotic chromosome synapsis and infertility in male hybrids between mouse strains derived from two mouse subspecies. Within species Prdm9 determines the sites of programmed DNA double-strand breaks and meiotic recombination hotspots. To investigate the relation between Prdm9-controlled meiotic arrest and asynapsis, we inserted random stretches of consubspecific homology on several autosomal pairs in sterile hybrids and analyzed their ability to form synaptonemal complexes and rescue male fertility. Twenty-seven or more Mb of consubspecific homology fully restored synapsis in a given autosomal pair and we predicted that two symmetric DSBs or more per chromosome are necessary for successful meiosis. We hypothesize that impaired recombination between evolutionary diverged homologous chromosomes could function as one of the mechanisms of hybrid sterility occurring in various sexually reproducing species.
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Introduction

Hybrid sterility (HS) is a postzygotic reproductive isolation mechanism that safeguards speciation by restricting gene flow between related taxa. HS is a universal phenomenon observed in many eukaryotic inter-species hybrids, including yeast, plants, insects, birds, and mammals (COYNE and ORR 2004; MAHESHWARI and BARBASH 2010). In the early days of genetics, HS had been difficult to accommodate in Darwin's theory of evolution by natural selection until the Bateson - Dobzhansky - Muller incompatibility (BDMI) hypothesis (MULLER and PONTECORVO 1942; DOBZHANSKY 1951; ORR 1996) explicated HS and, more generally, any hybrid incompatibility, as a consequence of the independent divergence of mutually interacting genes and resulting aberrant interaction of the new alleles, previously not tested by natural selection. HS has several common features across various sexually reproducing eukaryotic species. Haldane's rule posits that if one sex of the F1 offspring of two different animal races is absent, rare, or sterile, it is the heterogametic sex (XY or ZW) (HALDANE 1922). Another common feature refers to the disproportionately large role of Chr X compared to autosomes in reproductive isolation (PRESGRAVES 2008). More recently, interaction between selfish genomic elements causing meiotic drive and their suppressors has been implicated in some instances of reproductive isolation (ORR 2005; ZHANG et al. 2015).

The molecular mechanisms underlying HS remain an unresolved question. Historically, genic and chromosomal mechanisms of HS were hypothesized, but the latter were soon dismissed as unlikely on the grounds that large chromosomal rearrangements do not segregate with HS genetic factors (DOBZHANSKY 1951). Other possible forms of non-genic chromosomal HS were not considered because of the
limited knowledge of the carrier of genetic information at the time. Thus, for the last 80 years or so, the focus on the genic control of HS prevailed (DOBZHANSKY 1951; ORR 1996; FORSDYKE 2017). In studies mapping HS genes, the Drosophila group of species has been the model of choice, yet only five Drosophila HS genes, namely OdsH, JYAlpha, Ovd, agt, and Taf1, have been identified so far, none of which has a known interacting gene predicted by the BDMI hypothesis (TING et al. 1998; MASLY et al. 2006; PHADNIS and ORR 2009). The low success rate of the positional cloning of HS genes was explained by the oligogenic or polygenic nature of HS phenotypes and by the inherent difficulty in genetically dissecting the phenotype that prevents its own transfer to progeny.

Over forty years ago, we introduced the house mouse (Mus musculus) as a mammalian model for the genetic analysis of HS. The first HS locus Hst1 was genetically mapped in crosses of laboratory inbred strains (predominantly of Mus musculus domesticus (Mmd) origin) with wild Mus musculus musculus (Mmm) mice (FOREJT and IVANYI 1974). Later, we developed PWD/Ph and PWK/Ph inbred strains purely from the wild Mmm mice of Central Bohemia (GREGOROVA and FOREJT 2000) and used them in the positional cloning of Hst1 by high-resolution genetic crosses and physical mapping (GREGOROVA et al. 1996; TRACHTULEC et al. 1997). Finally, we identified the Hst1 locus with the PR domain containing 9 gene (Prdm9) (MIHOLA et al. 2009), coding for histone H3 lysine 4/lysine 36 methyltransferase (POWERS et al. 2016) the first and still the only HS gene known in vertebrates. Most of the tested laboratory inbred strains share either the Prdm9^{Dom2} or Prdm9^{Dom3} allele. (PARVANOV et al. 2010; BRUNSCHWIG et al. 2012). The former allele was found in inbred strains producing sterile male hybrids when crossed with PWD females, while the Prdm9^{Dom3}
was observed in the strains that yielded quasi-fertile males in the same type of inter-
subspecific crosses (FOREJT et al. 2012).

The male sterility of (PWD x C57BL/6)F1 (henceforth PB6F1) hybrids depends on the
interaction of the heterozygous allelic combination $Prdm9^{Msc}/Prdm9^{Dom2}$ with the
PWD allelic form of the X-linked Hybrid sterility X chromosome 2, $Hstx2^{Msc}$ locus
(DZUR-GEJDOSOVA et al. 2012; BHATTACHARYYA et al. 2014). For the sake of clarity
and to stress the origin of the alleles we will use $Prdm9^{PWD}$, $Prdm9^{B6}$ and $Hstx2^{PWD}$ in
the rest of this paper. Any other tested allelic combination of these two major HS
genes yields fully fertile or subfertile male hybrids (DZUR-GEJDOSOVA et al. 2012;
FLACHS et al. 2012). The proper allelic combination of $Prdm9$ and $Hstx2$ genes is
necessary but not sufficient to completely govern HS because less than 10% instead
of expected 25% of (PWD x B6) x B6 male backcross progeny replicated the infertility
of male PB6F1 hybrids (DZUR-GEJDOSOVA et al. 2012). Initially, we explained this
'missing heritability' by assuming additional genic interaction of three or more
additional HS genes with a small effect that escaped the genetic screen (DZUR-
GEJDOSOVA et al. 2012). However, an alternative, non-genic explanation emerged
from the analysis of meiotic phenotypes of sterile hybrids. The failure of multiple
autosomal pairs to synapse properly, the persistence of phosphorylated histone
γH2AX indicating unrepaired DNA double-strand breaks (DSBs) on unsynapsed
chromosomes, and the disturbed transcriptional inactivation of sex chromosomes at
the first meiotic prophase were the most prominent features observed in
approximately 90% of primary spermatocytes of infertile PB6F1 inter-subspecific
hybrids (BHATTACHARYYA et al. 2013; BHATTACHARYYA et al. 2014). We suggested that
the failure of chromosomes to synapse could be related to their fast-evolving
nongenic DNA divergence. Because failure of proper synapsis in pachytene
spermatocytes is known to interfere with the normal progression of the first meiotic division (Mahadevaiah et al. 2008; Burgoyne et al. 2009), we assumed that the asynapsis could be the ultimate cause of the sterility of male hybrids. Recently, the role of PRDM9 zinc finger domain binding sites within noncoding genomic DNA has been demonstrated in PB6F1 male HS. Replacement of the mouse sequence encoding the PRDM9 zinc-finger array with the orthologous human sequence reversed sterility in (PWD x B6-Prdm9Hu)F1 hybrid males (Davies et al. 2016). In PB6F1 hybrids, roughly 70% of Prdm9-directed DSBs hotspots identified by the DMC1 ChIP-seq method were enriched on the 'nonself' homologous chromosome, since the DSBs determined by the B6 allele of Prdm9 were found predominantly or exclusively on PWD chromosomes, and vice versa. Such hotspots were designated as asymmetric DSB hotspots. Chromosome-specific quantification of asymmetry correlated well with the asynapsis rate across five arbitrarily chosen chromosomes of PB6F1 hybrids (Davies et al. 2016; Smagulova et al. 2016). Another, non-exclusive interpretation of DMC1 ChIP-seq data pointed to significant enrichment of PRDM9-independent hotspots in the PB6F1 hybrid testis, which occurs in promoters and other regulatory motifs and which is characteristic of spermatogenic arrest in Prdm9 knockout males (Smagulova et al. 2016). Recently, one third of PRDM9-dependent DSBs was reported within sequences that have at least some repetitive character and indicating that inappropriately high DSB levels in retroposons and other repetitive elements may contribute to the infertility seen in some mouse hybrids (Yamada et al. 2017).

In this work, we studied the relationship between meiotic chromosome asynapsis, intersubspecific heterozygosity and male HS in a series of PB6F1 hybrids carrying recombinant chromosomes with Mmm/Mmm consubspecific (belonging to the same
subspecies) PWD/PWD homozygous intervals on *Mmm/Mmd* intersubspecific (belonging to different subspecies) PWD/B6 heterozygous background. We report the restoration of synapsis of intersubspecific chromosome pairs in the presence of 27 Mb or more of consubspecific sequence, and the reversal of HS by targeted suppression of asynapsis in the four most asynapsis-sensitive chromosomes. Thus the asymmetry of PRDM9 hotspots is linked to failure of synapsis of homologous chromosomes and to meiotic arrest of sterile hybrid males.

**Results**

**Small chromosomes are more susceptible to asynapsis in sterile F1 hybrids**

First, we ascertained the frequency of meiotic asynapsis separately for each chromosome pair of PB6F1 hybrid males by combining fluorescence in-situ hybridization (FISH) to decorate chromatin from individual chromosomes with immunostaining of the synaptonemal complex protein 3 (SYCP3) a major component of axial/lateral elements to visualize synaptonemal complexes, and a HORMA domain-containing protein-2, HORMAD2 (WOJTAŠ et al. 2012), to identify the axial elements of unsynapsed chromosomes (*figure supplement 1A*). Altogether 4168 pachynemas from 40 PB6F1 hybrid males were analyzed. All autosomes of hybrid males displayed a certain degree of asynapsis, classified as complete, partial, or intermingled, with frequencies ranging from 2.6% (Chr 1) to 42.2% (Chr 19) (*Figure 1A - table supplement 1*). A strong bias was evident towards higher asynapsis rates in the five smallest autosomes (*P < 5.3x10^-14*, comparison of GLMM models, *Figure 1A*). Recently, SPO11 oligos released during the processing of DSBs were
sequenced, mapped and quantified at chromosome-wide scale in male mice of the B6 laboratory inbred strain (LANGE et al. 2016). This information, together with the estimated frequency of asymmetric DSB hotspots in PBF1 hybrids (DAVIES et al. 2016; SMAGULOVA et al. 2016) enabled us to calculate the possible correlation between the number of symmetric DSBs per chromosome and synapsis between intersubspecific homologs. The calculation is based on and limited by the following premises: (i) the overall densities of DSBs on individual chromosomes of B6 and PB6F1 hybrid males are similar, (ii) approximately 250 DSBs occur per leptotene/zygotene cell (KAUPPI et al. 2013), and (iii) the 0.28 proportion of symmetric DSB hotspots in (PWD x B6)F1 hybrid males (DAVIES et al. 2016) is constant in all autosomes. Under these conditions, a strong negative correlation (Spearman’s ρ= -0.76, P = 0.0002) of asynapsis rate with symmetric DSB hotspots (LANGE et al. 2016) can be seen (Figure 1B - table supplement 2). This correlation is stronger than the correlation of the asynapsis rate with the chromosomal physical length (Spearman’s ρ=-0.68, P = 0.0013). Importantly, the asynapsis rate cannot be better explained by the chromosomal length (P = 0.709, comparison of GLMM models), when it is controlled for the symmetric DSB hotspots (SPO11 oligos), while it is better explained by the symmetric DSB hotspots (SPO11 oligos) when controlled for chromosomal length (P = 0.046, comparison of GLMM models). Thus our findings indicate that asynapsis of a pair of homologous chromosomes depends on the presence of a certain minimum number of symmetric DSB hotspots.

Asynapsed chromosomes are devoid of active euchromatin

The Cot-1 euchromatin RNA (ecRNA) is localized throughout the active interphase chromosome territory in cis and does not diffuse into the rest of the nucleus like other types of RNAs. EcRNA is mainly composed of repeat-rich noncoding RNA in active,
open euchromatin (HALL et al. 2014). Accordingly, we used Cot-1 DNA as a probe for ecRNA FISH to compare the ecRNA distribution in asynapsed and synapsed chromosomes. Pachytene spermatocytes of PB6F1 hybrids were examined for subnuclear localization of active chromatin and asynapsed chromosomes using confocal fluorescence microscopy after Cot-1 RNA FISH and HORMAD2 immunolabeling. Fluorescence signal quantification revealed that subnuclear regions of asynapsed chromosomes composed of sex chromosomes and/or autosomal univalents were lacking active euchromatin in contrast to other regions of the pachytene nuclei (Figure supplement 1B - video supplement 1). We propose that the absence of active euchromatin is a consequence of the meiotic synapsis failure of intersubspecific chromosomes, known as meiotic silencing of unsynapsed chromatin (MSUC (BURGOYNE et al. 2009)), which can act as an epigenic component contributing to the meiotic phenotypes of sterile hybrids. Our findings are in agreement with the transcriptional under-expression of genes on several autosomes and disruption of meiotic sex chromosome inactivation (MSCI) in leptotene/zygotene spermatocytes from semi-sterile Mmm x Mmd male hybrids with Prdm9^{PWK/LEWES} and Hstx2^{PWK} or Hstx2^{LEWES} allelic combinations (LARSON et al. 2016).

The minimal length of consubspecific sequence necessary to rescue meiotic chromosome synapsis

We have shown previously that meiotic asynapsis affects intersubspecific (PWD/B6) but not consubspecific (PWD/PWD) pairs of homologous chromosomes in sterile male hybrids from crosses of PWD females and B6.PWD-Chr # consomic males (GREGOROVA et al. 2008; BHATTACHARYYA et al. 2013). Here, we searched for the minimum length of the PWD/PWD consubspecific sequence that still could secure synapsis of a chromosome and potentially restore fertility in the hybrids. Instead of
substituting the whole PWD chromosome for its B6 homolog, we generated recombinant PWD/B6 and B6/PWD (centromere/telomere) chromosomes. To do that, we crossed the male hybrids between two B6.PWD-Chr# consomic strains and a PWD female to match the intact maternal PWD homologs and estimated the minimum size and location of consubspecific PWD/PWD stretches needed for synapsis rescue as shown in Figure 2A. In three such generated 'two-chromosome crosses' (hereafter 2-chr cross) we investigated the effect of the PWD/PWD consubspecific intervals on the asynapsis rate in six different chromosomes - two in a given experiment, namely Chr 5 & Chr 12 (Figure 2B - table supplement 3 and 4), Chr 7 & Chr 15 (Figure 2B - table supplement 5 and 6) and Chr 17 & Chr 18 (Figure 2B - table supplement 7 and 8). Altogether over 12,000 pachynemas from 122 chromosomes were examined. All male progeny of the 2-chr crosses were fully sterile, with low testis weight and the absence of sperm in the epididymis. The analysis of data from six recombinant chromosomes revealed the following common features:

- Introduction by recombination of 27 Mb or more of a consubspecific (PWD/PWD) interval into a pair of intersubspecific (PWD/B6) homologs effectively suppressed the asynapsis rate below the baseline of 5 % in all six studied autosomes (Figure 2B). The efficiency of synapsis rescue was gradual with an apparent change point (Figure 2B). To describe the pattern in the data a segmented regression model was used (see Materials and Methods). The model based on the data pooled from all 2-chr crosses was selected as the best model with an estimated change point estimate at 27.14 Mb (19.36; 34.91) (95% CI) (see table supplement 9). The slope of the decrease of asynapsis in the region of consubspecific intervals shorter than 27.14 Mb
was different for the respective chromosomes, reflecting the asynapsis rate in respective chromosomes in F1 cross (P < 3 × 10^{-11} F-test).

- In spite of the known role of subtelomeric (bouquet) association in chromosome pairing (ISHIGURO et al. 2014; SCHERTHAN et al. 2014), the location of the consubspecific sequence at the telomeric end was not essential for synapsis. The PWD/PWD intervals of sufficient size located at the centromeric (proximal), interstitial, or telomeric (distal) positions rescued synapsis as well (Figure 2B - table supplements 3-8).

Reversal of hybrid sterility by targeted suppression of asynapsis of four of the most asynapsis-sensitive autosomes

The above experiments have shown that a randomly located consubspecific PWD/PWD interval with a 27 or more Mb on otherwise intersubspecific PWD/B6 background is sufficient to restore the pachytene synapsis of a given autosomal pair.

To check the causal relationship between meiotic chromosome asynapsis and HS, we attempted to reverse HS by reducing the asynapsis in the four most asynapsis-prone chromosomes. Provided that hybrid male sterility is directly dependent on chromosome synapsis we predicted, by multiplying the probabilities of the synapsis of individual chromosomes obtained in F1 hybrids, that complete elimination of asynapsis of four of the shortest autosomes (excluding Chr 17 to avoid Prdm9^{PWD/PWD} interference) could increase the proportion of primary spermatocytes with the full set of synapsed autosomes up to 26.7% and potentially abolish apoptosis of these cells to yield around 5 million sperm cells in the epididymis of the hybrid males.

To evaluate the above prediction experimentally, random intervals of consomic Chrs 15^{PWD}, 16^{PWD}, 18^{PWD}, and 19^{PWD} were transferred on the genetic background of B6
mice in a three-generation cross as shown in Figure 3A. Eleven G3 males selected for maximal extent of PWD sequence on these chromosomes were crossed to PWD females (Figure 3A -table supplement 10). The resulting G4 hybrid male progeny (hereafter 4-chr cross) displayed various degrees of PWD homozygosity in the studied consomic autosomes on otherwise intersubspecific PWD/B6 genetic background. As predicted, a significant fraction of hybrid males indeed showed partial rescue of spermatogenesis. While in the PB6F1 cross, 100% of hybrid males displayed no sperm in the epididymis, in the 4-chr cross, only 51.7% of 87 G4 males were aspermic, 19.5% had a 0.01–0.74 x 10^6 sperm count, and 28.7% had 1.0–13.7 x 10^6 sperm cells (Figure 3B - figure supplement 2, table supplement 11).

Next, we asked whether the reversal of meiotic arrest correlates with the recovery of meiotic synapsis of recombined chromosomes and with the size of PWD/PWD consubspecific stretches in the four manipulated chromosomes. Eighteen G4 males were deliberately selected according to their fertility parameters, 13 with HS partial rescue, displaying sperm cells in the epididymis (0.1 x 10^6–6.9 x 10^6), and five aspermic controls. The meiotic analysis of over 6500 pachynemas from the genotyped males indeed confirmed the prediction based on the results of 2-chr crosses. The nonrecombinant PWD/PWD consubspecific bivalents were always fully synapsed, while all nonrecombinant PWD/B6 intersubspecific pairs revealed the highest frequencies of asynapsis. All recombinant chromosomes with consubspecific intervals of sufficient length (Figure 3B -table supplement 12; see table supplement 9 for change point estimates) effectively restored synapsis. Moreover, the presence of sperm cells corresponded with the rescue of synapsis of consomic chromosomes. As a rule of thumb, the hybrids had sperm when asynapsis was suppressed in at least three of four segregating chromosomes and when the probability of all four consomic
chromosomes being synapsed was > 0.7. Chrs 16, 18, and 19 contributed the strongest effect. *(Figure 3C - table supplement 12).*

Evidence for a trans-effect on the rate of asynapsis

Provided the probability of failure of the synapsis of each chromosome was completely independent of the rest of the hybrid genome, the asynapsis rate of the nonrecombinant intersubspecific chromosome pairs would be the same in F1 hybrids, 2-chr crosses, and 4-chr cross. Moreover, the frequency of pachynemas with all chromosomes synapsed could be predicted from the observed frequencies of the synopsis of individual chromosomes. Such predicted values would be close to the values directly read from the meiotic spreads and would lie along the diagonal in *Figure 4*. As shown below, both types of analysis clearly revealed that the asynapsis rate of a particular chromosome depends on the synopsis status of other chromosomes. In PB6F1 hybrids, the observed 13.1 % (11.4 %, 14.9 %) (95 % CI) of fully synapsed pachynemas was double *(P = 0.023, Mann-Whitney test)* the rate expected by the multiplication of the observed synopsis rates 6.6 % (5.3 %, 8.1 %) of individual chromosomes *(Figure 4)* indicating a trans effect of synapsed autosomes on the probability of the asynapsis of other intersubspecific chromosome pairs. The tendency is more pronounced in data from 2-chr crosses and 4-chr cross experiments. The most straightforward comparison is between the nonrecombinant PWD/B6 intersubspecific chromosomes from F1 hybrids and 2-chr cross or 4-chr crosses, where the asynapsis rate is dramatically reduced to 58–91 % of the respective F1 hybrids' rates. Moreover, the difference between the observed and expected ratios of asynaptic pachynemas increased with increasing probability of sperm cells in the epididymis *(Figure 4 - table supplement 13)*. In 4-chr cross, the trans effect was analyzed further by comparing the asynapsis rate of a given
nonrecombinant PWD/B6 pair with the other three analyzed chromosomes. *Figure supplement 3* shows a negative correlation (from $r = -0.45$ for Chr 16 to $r = -0.88$ for Chr 15, linear regression slope is $\beta_{3\_chrs\_syn} = -0.19$, $P = 0.0003$) between the asynapsis rate of the nonrecombinant chromosome and the probability of synapsis of the other three studied chromosomes.

It has to be emphasized that in the animals with identical *Prdm9* and *Hstx2* and mostly intersubspecific chromosomes investigated in this work, the *cis*-effect of the PWD/PWD length in a given chromosome dominates over the *trans*-effect. Namely, the *trans*-effect can be more clearly observed when conditioned on the *cis*-effect of the PWD/PWD length in a given chromosome ($P = 0.002$, F-test), while it is harder to detect ($P = 0.042$, F-test) when the PWD/PWD length in a given chromosome is not taken into account. On the other hand, the asynapsis rate critically depends on the *cis*-effect of the PWD/PWD length in a given chromosome ($P < 2 \times 10^{-16}$, F-test) when the *trans*-effect is not taken into account.

To conclude, the described *trans* effect enhances the probability of the successful pairing of intersubspecific chromosomes in males with identical *Prdm9* and *Hstx2*. The mechanism is unknown, but it is tempting to speculate that a rate-limiting step of an anti-recombination mismatch repair machinery (*SPIES* and *FISHEL* 2015; *CHAKRABORTY* and *ALANI* 2016) could be involved as elaborated in Discussion.

**Discussion**

While the genic control of HS and meiotic synapsis in PBF1 hybrids can be demonstrated by complete restitution of fertility and meiotic pairing in males with *Prdm9*<sup>PWD/PWD</sup> or *Prdm9*<sup>PWD/B6Hu</sup> genotypes and partial recovery in *Prdm9*<sup>PWD/C3H</sup>
males (DZUR-GEJDOsoVA et al. 2012; BHATTACHARYYA et al. 2013; DAVIES et al. 2016),
a chromosome-autonomous nature of asynapsis became apparent in experiments
where PB6F1 hybrids carried a single pair of PWD/PWD consubspecific homologs.
The males remained sterile, but synapsis of the particular consubspecific pair was
completely restored (BHATTACHARYYA et al. 2013). Such regulation of meiotic
asynapsis in PBF1 hybrids can be explained by a combined effect of the
chromosome-autonomous interaction of homologs operating in cis and Prdm9/Hstx2
incompatibility operating in trans. Here we separated the non-genic chromosome
autonomous from genic control mechanisms by keeping the sterility-determining
allelic combination of the Prdm9PWD/Prdm9B6 gene and Hstx2PWD locus constant in all
crosses, while successively introgressing stretches of the PWD/PWD consubspecific
sequence into eight PWD/B6 intersubspecific autosomal pairs.

The meiotic asynapsis rate correlates with the presumed paucity of symmetric DSBs
in individual chromosomes in sterile hybrids

Davies and coworkers (DAVIES et al. 2016) found that the DNA-binding zinc finger
domain of PRDM9 molecule is responsible for sterility in PB6F1 hybrids. In the sterile
hybrids, most PRDM9PWD-specific hotspots reside on B6 chromosomes and, vice
versa, most of the PRDM9B6 binding sites are activated on PWD chromosomes. This
asymmetry could be explained in part by erosion of the PRDM9 binding sites due to
preferential transmission to progeny of the mutated hotspots motifs (BOULTON et al.
1997; MYERS et al. 2010). In a parallel study, (SMAGULOVA et al. 2016) authors
identified a novel class of strong hotspots absent in PWD and B6 parents apparently
related to asymmetric hotspots described in (DAVIES et al. 2016). Moreover, Prdm9-
independent 'default' hotspots were particularly enriched in Chr X and we noticed that
percentage of these 'default' hotspots in autosomes correlates with the present data
on asynapsis rate in F1 hybrids (Spearman’s $\rho = 0.69$, $P = 0.0012$). We assume that these Prdm9-independent hotspots could represent the late-forming DSBs on unsynapsed chromosomes and, as such, they may be a consequence rather than the cause of meiotic asynapsis (see KAUPPI et al. 2013).

We found that meiotic asynapsis affects each autosomal pair in PB6F1 intersubspecific hybrids at distinctively unequal rates, with shorter chromosomes affected more often than the longer ones. A similar pattern of higher sensitivity of smaller autosomes to the synapsis failure was observed in mice with lowered dosages of SPO11 (KAUPPI et al. 2013) and consequent twofold DSB reduction. The fact that the asynapsis rate of sterile F1 hybrids correlates better with SPO11-oligo derived DSB density (inferred from B6 mouse strain data (LANGE et al. 2016) than with the chromosome length brings the first experimental evidence for the idea (DAVIES et al. 2016) linking the asynapsis in sterile PB6F1 hybrids to insufficient number of symmetric DSB hotspots.

Small stretches of consubspecific sequence restore the synapsis of intersubspecific chromosomes

Provided that a shortage of symmetric DSB hotspots (DAVIES et al. 2016) is the ultimate cause of failure of meiotic synapsis of intersubspecific homologs, then by exchanging the asymmetric hotspots for the symmetric ones the full synapsis could be restored. To experimentally test this prediction, we constructed pairs of PWD/B6 intersubspecific homologs with stretches of PWD/PWD consubspecific intervals, which by definition cannot carry asymmetric hotspots. We found that chromosomes with 27 Mb or longer stretches of consubspecific PWD/PWD sequence always rescued full synapsis in male hybrids between PWD females and consomic B6.PWD-
Chr # males. The position of the consubspecific interval along the chromosome was not critical for synapsis rescue, in accordance with the finding that synaptonemal complexes nucleate at multiple recombination sites in each chromosome (Zickler and Kleckner 2015; Finsterbusch et al. 2016). We assume that the presence of symmetric DSB hotspots within the PWD/PWD homozygous stretches exceeded the threshold of a minimum number of symmetric DSBs thus rescuing normal meiotic synapsis.

Allowing for the assumptions enumerated in in the first section of Results the number of DSBs necessary for proper synapsis of a given chromosome can be estimated based on the expected distribution of symmetric DSB hotspots on all autosomes and their asynapsis ratios in sterile F1 hybrids (Table supplement 2). We aimed to model how the induction and repair of DSBs influence proper meiotic synapsis, and tried to estimate the minimum number of symmetric DSBs per chromosome sufficient for full meiotic synapsis. Our model predicts that in approximately 25% of cases, a chromosome is asynapsed because there is no symmetric DSB (median of $P(\text{asynapsis}) / P(0\text{ symmetric DSBs}) = 4.5$). Assuming a critical threshold of the required DSBs, the remaining 75% of asynapsis could occur on chromosomes with one symmetric DSB. It is consistent with our data that all asynapsis occurs when there is 0 or 1 symmetric DSBs (median of $P(\text{asynapsis}) / P(0 \text{ or symmetric DSBs}) = 1.0$) and that two symmetric DSBs per chromosome could be sufficient for full development of the synaptonemal complex, as shown in Figure 5 for PB6F1 hybrids. The same conclusion also holds true for 2-chr cross and 4-chr cross experiments (Figure 5 - figure supplement 4 and 5). The deviations from the diagonal in the Figure 5 depicting 4-chr cross can be explained by the trans-effect described in the Results section.
It has been tacitly assumed, that the early meiotic arrest in sterile PBF1 males is due to the high incidence of asynapsed chromosomes in zygotene/pachytene spermatocytes of PBF1 hybrid males (Bhattacharyya et al. 2013). Here we provided the first experimental evidence on linkage between Prdm9-controlled asynapsis and meiotic arrest in PBF1 hybrid males by targeted suppression of asynapsis rate in selected autosomes. The suppression of asynapsis in any of three combinations of two PWD/B6 recombinant chromosomes (2-chr cross experiment) did not improve the spermatogenic arrest. However, when hybrid genomes with four short recombinant chromosomes (that are most sensitive to asynapsis) were constructed, almost 50% of males had sperm in the epididymis and normal testis size. By improving synopsis in three of the four manipulated chromosomes (representing ~11% of the genome) with randomly located PWD/PWD homozygous intervals, more than 40% of pachynemas regained full chromosome synapsis and yielded sperm. These results show that the high incidence of primary spermatocytes with asynaptic chromosomes is indeed the factor determining the extent of meiotic arrest in intersubspecific PBF1 hybrids.

The specific feature of PBF1 hybrid sterility consists in the inter-homolog interactions controlled by the Prdm9 and Hstx2 genes. The interhomolog incompatibility caused by single nucleotide variation in PRDM9 binding sites, which are situated away from coding genes (Baudat et al. 2013) is proposed to make the asymmetric sites difficult to repair (Magulova et al. 2016). We hypothesize that building the meiotic barrier between (sub)species begins with genome-wide accumulation of single nucleotide variants within PRDM9 binding sites and leading to gene conversion-guided meiotic
In the subspecific hybrids the asymmetric binding sites can be more difficult to repair using the homolog from the other subspecies as a template because of the strict requirements for sequence identity resulting in the rejection of mismatched heteroduplex intermediates as demonstrated in yeast (Sugawara et al. 2004). Originally, such an inter-species barrier was proposed by Radman and colleagues (Rayssiguier et al. 1989; Stambuk and Radman 1998) to prevent homeologous recombination between E. coli and Salmonella typhiurium. Among eukaryotes, the role of the mismatch repair system in reproductive isolation was reported in Saccharomyces species (Hunter et al. 1996; Greig et al. 2003; Liti et al. 2006). An exciting possibility arises that the heteroduplex rejection activity of mismatch repair machinery could operate as a mechanism gradually restricting gene flow between related (sub)species, a conditio sine qua non of speciation.

Materials and Methods

Mice, Ethics Statement and Genotyping. The mice were maintained at the Institute of Molecular Genetics in Prague and Vestec, Czech Republic. The project was approved by the Animal Care and use Committee of the Institute of Molecular Genetics AS CR, protocol No 141/2012. The principles of laboratory animal care, Czech Act No. 246/1992 Sb., compatible with EU Council Directive 86/609/EEC and Appendix of the Council of Europe Convention ETS, were observed. SSLP markers used for genotyping consomic chromosomes in 2-chr crosses and 4-chr cross are listed in table supplement 14. Detailed descriptions of inbred mouse strains, fertility testing and genotyping are given in the Supplementary text.

Antibodies for the immunostaining of spread spermatocytes. Detailed descriptions of the preparation of meiotic spreads and the visualization of SYCP3,
γH2AFX, and HORMAD2 proteins on spreads of meiotic cells are given in the
Supplementary Materials and Methods.

DNA and RNA FISH. To identify individual chromosomes, XMP Xcyting Mouse
Chromosome N Whole Painting Probes (Metasystem) were used for DNA FISH.
Identification of nascent RNA in pachytene spermatocytes was performed as
previously described (MAHADEVAIAH et al. 2009), with modifications. A detailed
description is given in the Supplementary Materials and Methods.

Microscopy and image capture. Images were observed using a Nikon Eclipse 400
epifluorescence microscope and captured with a DS-QiMc mono-chrome camera
(Nikon). Images were analyzed using the NIS elements program. A detailed
description is given in the Supplementary Materials and Methods.

Statistics

All calculations were performed in the statistical environment R 3.2.2. For modeling
the dependence between the asynapsis rate and the number of symmetric DSBs, we
determined the probabilistic distribution of the number of symmetric DSBs. The
distribution was determined by simulation and with parameters based on previous
studies: (i) The number of DSBs per cell (BHATTACHARYYA et al. 2013) was modeled
as an observation from the normal distribution N(250, sd=20). (ii) We assumed a
number of DSBs proportional to Spo11 oligos (LANGE et al. 2016) in each autosome.
(iii) The positions of DSBs in the particular autosome were simulated from the
uniform distribution, U(0, Autosome length). (iv) For the intersubspecific part of the
autosome, the number of symmetric DSBs was simulated from the binominal
distribution Bi(n=N_DSBs_in_het_part, p=0.28 (DAVIES et al. 2016; SMAGULOVA et al.
2016) For the consubspecific part of the autosome, all DSBs were taken as
symmetric. The total number of symmetric DSBs in the autosome was taken as the sum of symmetric DSBs in the respective parts.

Steps (i) - (iv) were performed in N=100000 simulations to obtain a probabilistic distribution. Further details on statistical evaluation are given in the Supplementary Materials and Methods.

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Figure 1. The asynapsis rate of individual autosomes in sterile male (PWD x B6)F1 hybrids. (A) Mean asynapsis rate ± S.E (based on GLMM model). Intermingled asynapsis refers to overlaps of two or more asynapsed chromosomes within the DNA FISH cloud of chromatin. Smaller chromosomes had higher asynapsis rate (GLMM model, P < 1.1x10^{-13}). Concurrently, the chromosomes with higher asynapsis rate were also more involved in complete rather than partial asynapsis (GLMM model, P < 6.3x10^{-5}). Proportion of complete and partial asynapsis was controlled by the asynapsis rate rather the chromosomal length (test for effect of the length when controlled for the asynapsis rate, 0.491). (B) Negative correlation (Spearman’s ρ = -0.76, P = 0.0002) between asynapsis rate and mean expected number of symmetric DSBs (29) based on the chromosome-wide distribution of SPO11 oligos in fertile B6 males (30).

The following table supplement is available for figure 1.

Table supplement 1. Asynapsis rate of individual chromosomes of (PWD x B6)F1 males.
Figure 2. The effect of consubspecific PWD/PWD stretches of genomic sequence on pachytene synapsis, 2-chr cross. (A) The F1 hybrid males of two consomic strains (generation 1 - G1, Chr 5 and Chr 12 shown here) were crossed to PWD females to produce generation 2 - G2 sterile F1 hybrids with random recombinant consomic chromosomes 5 and 12. Using whole chromosome probes, the asynapsis rate of the consomic chromosomes was scored by DNA FISH. (B) Combination of two chromosomes (5+12, 7+15 and 17+18) were challenged in each experiment. The localization of PWD homozygous sequence with respect to centromere, interstitial part of the chromosome, telomere, or on both ends is distinguished by color (see also Tables Supplement 3 – Supplement 8). The average length between the minimum and maximum of the consubspecific sequence is plotted. The mean asynapsis rate of a given chromosome is regularly higher in PB6F1 hybrids (dashed line) than in 2-chr cross. For explanation see Fig. 4 and the chapter on the trans-effect dependent variation in asynapsis rate. Loess curve with 95% CI.

The following table supplements are available for figure 2.

Table supplement 3. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 5.

Table supplement 4. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 12.

Table supplement 5. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 7.

Table supplement 6. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 15.

Table supplement 7. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 17.

Table supplement 8. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 18.
Figure 3. The effect of consubspecific PWD/PWD stretches of genomic sequence on pachytene synapsis and meiotic progression, 4-chr cross. (A) Scheme of a four-generation cross resulting in F1 hybrids with four recombinant consomic chromosomes. (B) The asynapsis rate related to the size and chromosomal position of the consubspecific PWD/PWD sequence in four consomic chromosomes (15, 16, 18 and 19, see also Table 1). The localization of PWD homozygous sequence with respect to centromere, interstitial part of the chromosome, telomere, or on both ends is distinguished by color (see also Tables 1). (C) Number of sperm in epididymis is a function of probability of synapsis of all four consomic chromosomes. The complete meiotic arrest is reversed in males having 70% or higher chance of all four chromosomes synapsed. See also Table Supplement 11. Loess curve with 95% CI.

The following table and figure supplements are available for figure 3.

Table supplement 9. Change point estimates of the minimal length [Mb] of PWD/PWD homozygosity showing detectable affect on synapsis rate.

Table supplement 10. Eleven G3 male parents selected for the 4-chr cross experiment.

Table supplement 11. The fertility parameters of hybrids of the 4-chr cross experiment.

Table supplement 12. Four-chr cross. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chrs 15, 16, 18 and 19 and on fertility parameters.

Figure supplement 2. The fertility parameters of hybrids of the 4-chr cross experiment.
Figure 4. The trans-acting effect of consubspecific PWD/PWD stretches increases the probability of full synapsis of PWD/B6 heterosubspecific homologs in males 2-chr cross and 4-chr cross experiments. The expected rate of synapsed pachynemas was calculated for each mouse in 2-chr cross and 4-chr cross experiments by multiplication of observed synapsis rates (i.e. assuming independence) of FISH analyzed chromosomes (e.g. chrs 15, 16, 18 and 19 in 4-chr cross) and observed PB6F1 synapsis rates of the remaining autosomes. Asynapsis rate was calculated as a complement to synapsis rate. The difference between expected and observed overall asynapsis is most pronounced in 4-chr cross males with the lowest expected overall asynapsis rate. Recovery of spermatogenesis signaled by the presence of sperm in the epididymis occurs when more than 40% of pachynemas are fully synapsed. SC is sperm count.

The following table supplement is available for figure 4.

Table supplement 13. Four-chr cross experiment. The relation between observed and calculated rate of pachytene asynapsis and fertility parameters.
Figure 5. Two or more symmetric DSBs can be sufficient for synapsis. The probability of less than 1 symmetric DSBs per chromosome is ~4 times lower than the asynapsis rate observed in PB6F1 hybrid males, (i.e. estimate of probability of asynapsis) implying ~75% of all asynapsis occurs when there is 1 or more repairable DSBs. The probability of less than 2 symmetric DSBs is a good estimate of the probability of asynapsis, while the probability of less than 3 symmetric DSBs overestimates the probability of asynapsis. This shows that in the simplest explanation two or more symmetric DSBs could be sufficient for synapsis. The probabilistic distribution of symmetric DSBs is calculated based on the model described in the Discussion.

The following figure supplements are available for figure 5.

Figure supplement 4. Two and more symmetric DSBs can be sufficient for synapsis in 2-chr cross males. See Fig. 5 for details.

Figure supplement 5. Two or more symmetric DSBs can be sufficient for synapsis in 4-chr cross hybrid males. See Fig. 5 for details.
Supplementary Information for

Modulation of Prdm9-controlled meiotic chromosome asynapsis overrides hybrid sterility in mice

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This Supplementary Information contains the supplementary materials and methods, figure supplements and table supplements


Supplementary Materials and Methods

Mice, ethics statement. The project was approved by the Animal Care and use Committee of the Institute of Molecular Genetics AS CR, protocol No 141/2012. The principles of laboratory animal care, Czech Act No. 246/1992 Sb., compatible with EU Council Directive 86/609/EEC and Appendix of the Council of Europe Convention ETS, were observed. SSLP markers used for genotyping consomic chromosomes in 2-chr crosses and 4-chr cross are listed in Table supplement 14.

The PWD/Ph inbred strain originated from a single pair of wild mice of the Mus musculus subspecies trapped in 1972 in Central Bohemia, Czech Republic (Gregorova and Forejt, 2000). The C57BL/6J (B6) inbred strain was purchased from The Jackson Laboratory. The panel of 27 chromosome substitution strains C57BL/6J-Chr #PWD (abbreviated B6.PWD-Chr #) was prepared in our laboratory and is maintained by the Institute of Molecular Genetics AS CR, Division BIOCEV, Vestec, Czech Republic, and by The Jackson laboratory, Bar Harbor, Maine, U.S.A. All mice were maintained in a 12-h light/12-h dark cycle in a specific pathogen-free barrier facility. The mice had ad libitum access to a standard rodent diet (ST-1, 3.4% fat; VELAZ) and water. All males were killed at age 60–70 d.

Immunostaining and image capture

For immunocytochemistry, the spread nuclei were prepared as described (Anderson et al. 1999) with modifications. Briefly, a single-cell suspension of spermatogenic cells in 0.1M sucrose with Protease inhibitors (Roche) was dropped on 1% paraformaldehyde-treated slides and allowed to settle for 3 hours in a humidified box at 4°C. After brief H2O and PBS washing and blocking with 5% goat sera in PBS (vol/vol), the cells were immunolabeled using a standard protocol with the following antibodies: anti-HORMAD2 (1:700, rabbit polyclonal
antibody, gift from Attila Toth) and SYCP3 (1:50, mouse monoclonal antibody, Santa Cruz, #74569). Secondary antibodies were used at 1:500 dilutions and incubated at room temperature for 60 min; goat anti-Rabbit IgG-AlexaFluor568 (MolecularProbes, A-11036) and goat anti-Mouse IgG-AlexaFluor647 (MolecularProbes, A-21235). The images were acquired and examined in a Nikon Eclipse 400 microscope with a motorized stage control using a Plan Fluor objective, 60x (MRH00601; Nikon) and captured using a DS-QiMc monochrome CCD camera (Nikon) and the NIS-Elements program (Nikon). The images were processed using the Adobe Photoshop CS software (Adobe Systems).

**Combined immunofluorescence staining with DNA FISH or RNA FISH**

XMP XCyting Mouse Chromosome N Whole Painting Probes (Metasystems) were used for DNA FISH analysis of asynapsis of all autosomes, one at a time, as described (Turner et al. 2005), with slight modifications. Testes from 8-week-old mice were dissected and spread meiocyte nuclei were prepared as described previously (Mahadevaiah et al. 2009) with a modification, which relies on reversed sequence of RNA FISH and immunofluorescence staining (Turner et al. 2005). Briefly, after cell fixation and permeabilization, the immunofluorescent labeling was performed for 90 min at 20°C with primary anti HORMAD2 and anti SYCP3 antibodies. Secondary antibodies were selected as above and incubated at room temperature for 60 min. After washing and postfixation steps, the immunostained nuclei were processed with RNA fluorescence in situ hybridization. The Cot-1 DNA biotin-labeled probe was incubated overnight at 37°C, and then the hybridized biotinylated Cot-1 probe was labelled with FITC-avidin conjugate and the fluorescent signal was amplified as described previously (Chaumeil et al, 2008). The images of the immunofluorescence stained
and cot-1 RNA FISH-labeled spread spermatocytes were examined and photographed using confocal microscope DMI6000CEL – Leica TCS SP8.

Statistics

The effects of the number of Spo11 oligos and the chromosomal length on the asynapsis rate were investigated using a GLMM model. In all the used GLMM models in this work, the asynapsis was modeled as a binary response to fixed effects under investigation and a random intercept for each animal. In F1 hybrids 95 % confidence intervals of observed rate of asynapsed pachynemas and expected rated of asynapsed pachynemas were calculated by bootstrap. The estimates of mean asynapsis rate in respective chromosomes, their standard errors and 95 % confidence intervals were based on GLMM model. In 2-chr crosses and 4-chr cross 95 % confidence intervals of asynapsis rate were constructed based on the likelihood ratio to capture also the uncertainty in the cases when zero asynapsis rate per mouse and chromosome was observed.

Based on the nature of the dependence between the asynapsis rate and the length of consubspecific PWD/PWD region on chromosomes of 2-chr crosses and 4-chr cross, we fitted the data by segmented two-part continuous regression models (Vito et al. 2003)

We fitted the models for all the chromosomes separately (Table supplement 9) being aware of the limitations caused by the lack of animals having specific lengths of consubspecific PWD/PWD region in the respective chromosomes. As the best model describing the dependence of asynapsis rate on the lengths of PWD/PWD intervals we selected piecewise linear models fitting 1) pooled data from 2-chr crosses and 2) pooled data from both 2-chr crosses and 4-chr cross. Those models are not severely affected by the lack of observations with specific lengths of PWD/PWD segment nor by outliers.
All calculations were performed in R 3.2.2, the change point models and GLMM models were fitted using the packages segmented and lme4, respectively (Vito et al. 2008, Douglas et al. 2015).

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Figure supplement 1. Asynapsis of heterosubspecific homologs in PB6F1 pachynemas. (A) Partial (arrowheads) and complete (arrows) asynapsis of Chr 19, 11 and 13. HORMAD2-labeled chromosomes with synapsis defects often form tangles via nonhomologous pairing. (B) Asynapsed chromosomes are embedded in transcriptionally silenced chromatin visualized by the lack of ecRNA detected by Cot1 RNA FISH. See also video 1.
Figure supplement 2. Fertility parameters of G4 males from the 4-chr cross. Rescue of HS meiotic arrest is detectable in males with the weight of paired testes >70 mg and >0.1 x 10^6 of sperm in the epididymis. The males selected for DNA FISH/HORMAD2 analysis of asynapsis are highlighted in turquoise. All males share the sterility-determining allelic combination of Prdm9^PWD/B6 and Hstx2^PWD. Loess curve with 95% CI.
Figure supplement 3. Asynapsis rate of individual nonrecombinant consomic PWD/B6 chromosomes modified in trans by the probability of synapsis of the remaining 3 consomic chromosomes in 4-chr cross in individual males compared to PB6F1 hybrids. Asynapsis rate of a given chromosome is in negative correlation (r = -0.88, -0.45, -0.80, -0.67 for Chrs 15, 16, 18, 19 respectively) with the probability that all other 3 analyzed chromosomes are synapsed. The slope of linear regression is $\beta_{\text{3_chrs_syn}} = -0.19$, overall $P = 0.0003$. 
Figure supplement 4. Two and more symmetric DSBs can be sufficient for synopsis in 2-chr cross males. See Fig. 5 for details.
**Figure supplement 5.** Two or more symmetric DSBs can be sufficient for synapsis in 4-chr cross hybrid males. See Fig. 5 for details.
Table supplement 1. Asynapsis rate of individual chromosomes of (PWD x B6)F1 hybrid males

| Chromosome | Asynapsis Complete | Asynapsis Partial | Asynapsis Intermingled | Complete Asynapsis | Partial Asynapsis | Intermingled Asynapsis | Number of Males per Chr | Analyzed cells Complete | Analyzed cells Partial | Analyzed cells Intermingled |
|------------|--------------------|-------------------|-----------------------|-------------------|-------------------|-----------------------|------------------------|------------------------|------------------------|------------------------|
| chr1       | 2.6                | 1.2; 5.8          | 97.4                  | 0.4               | 2.2               | 0.0                   | 2                      | 125; 102               | 1; 0                   | 5; 0                   | 0; 0                   |
| chr2       | 2.7                | 1.3; 5.6          | 97.3                  | 1.6               | 1.2               | 0.0                   | 2                      | 103; 152               | 1; 3                   | 0; 3                   | 0; 0                   |
| chr3       | 11.6               | 7.8; 16.8         | 88.4                  | 6.5               | 2.5               | 2.5                   | 2                      | 81; 118                | 4; 9                   | 2; 3                   | 4; 1                   |
| chr4       | 6.5                | 3.9; 10.7         | 93.5                  | 0.5               | 4.6               | 1.4                   | 2                      | 110; 105               | 1; 0                   | 7; 3                   | 2; 1                   |
| chr5       | 10.6               | 7.6; 14.6         | 89.4                  | 8.3               | 1.3               | 1.0                   | 3                      | 85; 89; 129            | 6; 9; 10               | 0; 3; 1                | 0; 2; 1                |
| chr6       | 5.1                | 2.7; 9.6          | 94.9                  | 1.1               | 4.0               | 0.0                   | 2                      | 70; 105                | 1; 1                   | 2; 5                   | 0; 0                   |
| chr7       | 12.8               | 8.8; 18.2         | 87.2                  | 8.7               | 4.1               | 0.0                   | 2                      | 94; 102                | 10; 7                  | 2; 6                   | 0; 0                   |
| chr8       | 10.7               | 7.3; 15.4         | 89.3                  | 3.6               | 7.1               | 0.0                   | 2                      | 93; 132                | 3; 5                   | 10; 6                  | 0; 0                   |
| chr9       | 5.0                | 2.8; 8.8          | 95.0                  | 0.5               | 4.5               | 0.0                   | 2                      | 107; 113               | 0; 1                   | 3; 7                   | 0; 0                   |
| chr10      | 12.6               | 8.8; 17.6         | 87.4                  | 4.9               | 5.8               | 1.8                   | 2                      | 114; 109               | 8; 3                   | 3; 10                  | 2; 2                   |
| chr11      | 5.9                | 3.4; 10.2         | 94.1                  | 1.0               | 1.5               | 3.5                   | 2                      | 126; 76                | 2; 0                   | 2; 1                   | 5; 2                   |
| chr12      | 12.6               | 8.5; 18.2         | 87.4                  | 10.4              | 2.2               | 0.0                   | 2                      | 92; 91                 | 10; 9                  | 2; 2                   | 0; 0                   |
| chr13      | 4.4                | 2.6; 7.4          | 95.6                  | 3.5               | 1.0               | 0.0                   | 3                      | 112; 106; 97           | 3; 4; 4                | 2; 0; 1                | 0; 0; 0                |
| chr14      | 7.8                | 5.2; 11.7         | 92.2                  | 1.9               | 4.8               | 1.1                   | 2                      | 129; 139               | 4; 1                   | 3; 10                  | 0; 3                   |
| chr15      | 20.1               | 15.3; 26.0        | 79.9                  | 15.0              | 4.7               | 0.5                   | 2                      | 102; 112               | 18; 14                 | 3; 7                   | 0; 1                   |
| chr16      | 22.8               | 17.5; 29.1        | 77.2                  | 15.3              | 6.4               | 1.0                   | 2                      | 111; 91                | 16; 15                 | 7; 6                   | 2; 0                   |
| chr17      | 14.6               | 10.4; 20.1        | 85.4                  | 12.1              | 2.4               | 0.0                   | 2                      | 111; 95                | 12; 13                 | 5; 0                   | 0; 0                   |
| chr18      | 30.5               | 24.1; 37.7        | 69.5                  | 29.9              | 0.6               | 0.0                   | 2                      | 92; 82                 | 29; 23                 | 1; 0                   | 0; 0                   |
| chr19      | 42.2               | 34.9; 49.8        | 57.8                  | 39.8              | 2.4               | 0.0                   | 2                      | 80; 86                 | 36; 30                 | 1; 3                   | 0; 0                   |

Synapsis and classes of asynapsis are based on the GLMM model to take into account the unequal numbers of cells analyzed per males.
Table supplement 3. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 5 and on fertility parameters.

| Male ID  | Distance from the centromere of Chr 5 (total length 151.8 Mb) | PWD/PWD length | Asynapsis of Chr 5 | Cells analyzed | Testes weight | Sperm count |
|----------|-------------------------------------------------------------|----------------|---------------------|----------------|--------------|-------------|
| 4,9      | 5,2                                                          | 9,8            | 24,5                | 28,1           | 45,0         | 61,4        |
| 69064    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 69133    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 67874    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 65083    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 67730    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 67878    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 67882    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 67876    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69062    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69733    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69137    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69846    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69328    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69186    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69184    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69281    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69331    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69440    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69441    | PB                                                          | PB             | PB                  | PB             | PB           | PB          |
| 69136    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 69847    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 65082    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 67883    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |
| 67977    | PP                                                          | PP             | PP                  | PP             | PP           | PP          |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/66 and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals. Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.
Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.

Table supplement 4. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 12 and on fertility parameters.

| Male ID | Distance from centromere of Chr 12 (total length 120.1 Mb) | PWD/PWD length | Asynapsis of Chr 12 | Cells analyzed | Testes weight mg | Sperm count 10^4 |
|---------|----------------------------------------------------------|-----------------|---------------------|----------------|-----------------|-----------------|
|         | 5.4 | 7.6 | 15.7 | 19.8 | 30.8 | 35.0 | 54.6 | 91.0 | 103.4 | 113.8 |
| 69064   | PB  | PB  | PB  | PB  | PB  | PB  | PP  | PP  | PP  | PP  | PP  | PB  | 91.0 | 103.4 | 0.0 | 0.0 | 2.0 | 100 | 54.0 | 0.0 |
| 69777   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PP  | PP  | PP  | PP  | PB  | 48.8 | 78.8 | 0.0 | 0.0 | 2.0 | 100 | 67.0 | 0.0 |
| 67876   | PP  | PP  | PP  | PP  | PP  | PP  | PP  | PB  | PB  | PB  | PB  | 35.0 | 54.6 | 1.0 | 0.1 | 4.3 | 100 | 69.0 | 0.0 |
| 69144   | PP  | PP  | PP  | PP  | PP  | PP  | PB  | PB  | PB  | PB  | PP  | PP  | 36.5 | 59.9 | 1.0 | 0.1 | 4.3 | 100 | 66.0 | 0.0 |
| 69760   | PP  | PP  | PP  | PP  | PP  | PB  | PB  | PB  | PB  | PB  | 19.8 | 30.8 | 1.0 | 0.1 | 4.3 | 100 | 65.0 | 0.0 |
| 69133   | PP  | PP  | PP  | PP  | PP  | PB  | PB  | PB  | PB  | PB  | 19.8 | 30.8 | 3.0 | 0.8 | 7.6 | 100 | 64.0 | 0.0 |
| 67973   | PP  | PP  | PP  | PP  | PP  | PB  | PB  | PB  | PB  | PB  | 12.2 | 25.4 | 6.0 | 2.4 | 11.8 | 100 | 67.0 | 0.0 |
| 69281   | PP  | PP  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 7.6 | 15.7 | 4.0 | 1.3 | 9.0 | 100 | 71.0 | 0.0 |
| 67974   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 10.0 | 5.1 | 16.9 | 100 | 69.0 | 0.0 |
| 69136   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 9.0 | 4.4 | 15.6 | 100 | 61.0 | 0.0 |
| 69138   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 9.0 | 4.4 | 15.6 | 100 | 55.0 | 0.0 |
| 69184   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 8.0 | 3.7 | 14.4 | 100 | 71.0 | 0.0 |
| 69282   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 6.0 | 2.4 | 11.8 | 100 | 67.0 | 0.0 |
| 69328   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 12.0 | 6.6 | 19.3 | 100 | 66.0 | 0.0 |
| 69331   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 5.0 | 1.8 | 10.4 | 100 | 63.0 | 0.0 |
| 69411   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 8.0 | 3.7 | 14.4 | 100 | 68.0 | 0.0 |
| 69663   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 4.0 | 1.3 | 9.0 | 100 | 70.0 | 0.0 |
| 69664   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 8.0 | 3.7 | 14.4 | 100 | 71.0 | 0.0 |
| 69847   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 0.0 | 0.0 | 5.0 | 1.8 | 10.4 | 100 | 63.0 | 0.0 |
| 69186   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 6.3 | 16.7 | 10.0 | 5.1 | 16.9 | 100 | 82.0 | 0.0 |
| 69763   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 6.3 | 16.7 | 3.0 | 0.8 | 7.6 | 100 | 68.0 | 0.0 |
| 69427   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 6.3 | 16.7 | 0.0 | 0.0 | 2.0 | 100 | 68.0 | 0.0 |
| 60882   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | 6.3 | 16.7 | 0.0 | 0.0 | 2.0 | 100 | 71.0 | 0.0 |
| 69062   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PP  | PP  | PP  | 29.1 | 65.5 | 2.0 | 0.3 | 6.0 | 100 | 59.0 | 0.0 |
| 69083   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PP  | PP  | PP  | 29.1 | 65.5 | 1.0 | 0.1 | 4.3 | 100 | 57.0 | 0.0 |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/B6 and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals.

Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.
Table supplement 5. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 7 and on fertility parameters

| Male ID | Distance from the centromere of Chr 7 (total length 145.4 Mb) | PWD/PWD length | Asynapsis of Chr 5 | Cells analyzed | Testes weight | Sperm count |
|---------|---------------------------------------------------------------|----------------|---------------------|---------------|--------------|-------------|
|         |                                                               | Mb, range | % | 95% CI | N | mg | 10⁶ |
| 69468   | PP | PP | PP | PB | PB | PP | 26.4; 44.7 | 4.0 | 1.3; 9.0 | 100 | 56.0 | 0.0 |
| 69745   | PP | PP | PP | PB | PB | PB | 24.6; 25.7 | 11.0 | 5.9; 18.1 | 100 | 64.0 | 0.0 |
| 69744   | PP | PP | PB | PB | PB | PB | 18.0; 24.6 | 8.0 | 3.7; 14.4 | 100 | 65.0 | 0.0 |
| 69934   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 10.0 | 5.1; 16.9 | 100 | 68.0 | 0.0 |
| 69935   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 9.0 | 4.4; 15.6 | 100 | 64.0 | 0.0 |
| 69878   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 8.0 | 3.7; 14.4 | 100 | 65.0 | 0.0 |
| 69627   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 8.0 | 3.7; 14.4 | 100 | 64.0 | 0.0 |
| 69467   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 7.0 | 3.1; 13.1 | 100 | 58.0 | 0.0 |
| 69626   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 3.0 | 0.8; 7.6 | 100 | 63.0 | 0.0 |
| 69780   | PB | PB | PB | PB | PB | PB | 0.0; 0.0 | 1.0 | 0.1; 4.3 | 100 | 60.0 | 0.0 |
| 69572   | PB | PB | PB | PB | PB | PB | 0.7; 14.4 | 8.0 | 3.7; 14.4 | 100 | 57.0 | 0.0 |
| 37007   | PB | PB | PB | PB | PB | PB | 0.7; 14.4 | 4.0 | 1.2; 9.1 | 98 | 69.0 | 0.0 |
| 69625   | PB | PB | PB | PB | PB | PB | 0.7; 14.4 | 4.0 | 1.2; 9.1 | 98 | 69.0 | 0.0 |
| 69877   | PB | PB | PB | PB | PB | PB | 14.4; 25.7 | 1.0 | 0.1; 4.3 | 100 | 56.0 | 0.0 |
| 69781   | PB | PB | PB | PB | PB | PB | 100.7; 119.0 | 3.0 | 0.8; 7.6 | 100 | 62.0 | 0.0 |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/B6 and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals.

Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.
Table supplement 6. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 15 and on fertility parameters.

| Male ID | Distance from centromere of Chr 15 (total length 104.0 Mb) | PWD/PWD length | Asynapsis of Chr 15 | Cells analyzed | Testes weight | Sperm count |
|---------|-----------------------------------------------------------|----------------|---------------------|---------------|--------------|-------------|
|         | 9.3            | 40.0           | 63.0               | 70.2          | 97.0         | 97.8        | 100.6       | 101.9       | 102.8       | 103.4       | Mb, range   | %          | 95% CI      | N  | mg | 10⁶ |
| 69571   | PP             | PB             | PB                 | PB            | PB           | PB          | 40.0       | 63.0       | 2.0     | 0.3       | 6.0         | 100         | 69.0        | 0.0 |
| 69623   | PP             | PB             | PB                 | PB            | PB           | PB          | 9.3        | 40.0       | 2.0     | 0.3       | 6.0         | 100         | 65.0        | 0.0 |
| 69783   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 3.0     | 0.8       | 7.6         | 100         | 56.0        | 0.0 |
| 69784   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 11.0    | 5.9       | 18.1        | 100         | 60.0        | 0.0 |
| 69566   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 13.0    | 7.4       | 20.5        | 100         | 72.0        | 0.0 |
| 69469   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 56.0        | 0.0 |
| 69565   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 68.0        | 0.0 |
| 69625   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 65.0        | 0.0 |
| 69624   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 19.0    | 12.1      | 27.4        | 100         | 59.0        | 0.0 |
| 69746   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 65.0        | 0.0 |
| 69743   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 19.0    | 12.1      | 27.4        | 100         | 62.0        | 0.0 |
| 69781   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 65.0        | 0.0 |
| 69878   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 65.0        | 0.0 |
| 69879   | PB             | PB             | PB                 | PB            | PB           | PB          | 0.0        | 0.0        | 16.0    | 9.7       | 24.0        | 100         | 58.0        | 0.0 |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/B6 and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals.

Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.
Table supplement 7. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 17 and on fertility parameters.

| Male ID | Distance from centromere of Chr 17 (total length 95.0 Mb) | PWD/PWD length | Asynapsis of Chr 17 | Cells analyzed | Testes weight | Sperm count |
|---------|----------------------------------------------------------|----------------|---------------------|----------------|--------------|-------------|
|         | 4.8 | 15 | 15.6 | 30.9 | 34.7 | 45.4 | 52.7 | 65.3 | 73.8 | 93.2 | Mb, range | % | 95% CI | N | mg | 10^6 |
| 64979   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 18.8 | 12.0; 27.1 | 101 | 72.0 | 0.0 |
| 66707   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 14.9 | 8.9; 22.7 | 101 | 63.0 | 0.0 |
| 64855   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 14.9 | 8.9; 22.7 | 101 | 73.0 | 0.0 |
| 66954   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 14.6 | 8.7; 22.3 | 103 | 71.0 | 0.0 |
| 64574   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 11.7 | 6.5; 18.8 | 103 | 68.0 | 0.0 |
| 64977   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 11.2 | 6.3; 17.8 | 116 | 63.0 | 0.0 |
| 64718   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 12.5 | 6.9; 20.1 | 96  | 68.0 | 0.0 |
| 64849   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 0.0; 0.0 | 7.0  | 3.1; 13.0 | 102 | 76.0 | 0.0 |
| 66515   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 1.8; 21.2 | 16.0 | 9.5; 24.4 | 92  | 75.0 | 0.0 |
| 66514   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 1.8; 21.2 | 14.0 | 8.1; 21.7 | 100 | 71.0 | 0.0 |
| 64851   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 1.8; 21.2 | 10.5 | 5.6; 17.3 | 105 | 69.0 | 0.0 |
| 64719   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 1.8; 21.2 | 5.8  | 2.4; 11.3 | 106 | 62.0 | 0.0 |
| 66819   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 1.8; 21.2 | 3.4  | 0.9; 8.2  | 100 | 72.0 | 0.0 |
| 66813   | PB  | PB  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 21.2; 29.7 | 1.0  | 0.1; 4.2  | 104 | 68.0 | 0.0 |
| 66876   | PB  | PB  | PB   | PB   | PB   | PB   | PP   | PP   | PP   | PP   | PP  | 29.7; 42.3 | 0.0  | 0.0; 2.0  | 97  | 58.0 | 0.0 |
| 64575   | PB  | PB  | PB   | PB   | PB   | PP   | PP   | PP   | PP   | PP   | PP  | 42.3; 49.6 | 0.0  | 0.0; 1.9  | 103 | 73.0 | 0.0 |
| 64922   | PB  | PB  | PB   | PB   | PB   | PP   | PP   | PP   | PP   | PP   | PP  | 42.3; 49.6 | 2.8  | 0.7; 7.1  | 108 | 69.0 | 0.0 |
| 64975   | PP  | PP  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 4.8; 15.0 | 7.9  | 3.7; 14.2 | 101 | 80.0 | 0.0 |
| 66532   | PP  | PP  | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB   | PB  | 4.8; 15.0 | 6.0  | 2.4; 11.8 | 100 | 72.0 | 0.0 |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/Bb and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals.
Table supplement 8. Two-chr cross. The effect of the size and location of PWD/PWD conspecific intervals on asynapsis of Chr 18 and on fertility para

| Male ID | Distance from centromere of Chr 18 (90.7 Mb) | PWD/PWD length | Asynapsis of Chr 18 | Cells analyzed | Testes weight | Sperm count |
|---------|---------------------------------------------|-----------------|---------------------|---------------|--------------|-------------|
|         | 3,8 | 25,0 | 57,4 | 76,9 | 89,6 | Mb, range | % | 95% CI | N | mg | 10^6 |
| 64574   | PB  | PB  | PB  | PB  | PB  | 0.0; 0.0 | 30.8 | 22.5; 40.1 | 104 | 68,0 | 0,0 |
| 64851   | PB  | PB  | PB  | PB  | PB  | 0.0; 0.0 | 21.4 | 14.2; 30.0 | 103 | 69,0 | 0,0 |
| 64719   | PB  | PB  | PB  | PB  | PB  | 0.0; 0.0 | 19.3 | 12.7; 27.4 | 109 | 62,0 | 0,0 |
| 64976   | PB  | PB  | PB  | PP  | PP  | 1.1; 13.8 | 28.0 | 19.8; 37.3 | 100 | 71,0 | 0,0 |
| 66876   | PB  | PB  | PP  | PP  | PP  | 13.8; 33.3 | 9.4  | 3.9; 18.1 | 64  | 58,0 | 0,0 |
| 66877   | PB  | PB  | PP  | PP  | PP  | 13.8; 33.3 | 1.0  | 0.1; 4.3  | 101 | 64,0 | 0,0 |
| 66532   | PB  | PB  | PP  | PP  | PP  | 13.8; 33.3 | 0.0  | 0.0; 2.5  | 78  | 72,0 | 0,0 |
| 66954   | PB  | PB  | PP  | PP  | PP  | 33.3; 65.7 | 0.0  | 0.0; 1.9  | 102 | 71,0 | 0,0 |
| 66813   | PB  | PB  | PP  | PP  | PP  | 33.3; 65.7 | 0.0  | 0.0; 1.7  | 113 | 68,0 | 0,0 |
| 64922   | PP  | PP  | PP  | PP  | PP  | 90.7; 90.7 | 1.8  | 0.3; 5.5  | 109 | 69,0 | 0,0 |
| 64718   | PP  | PP  | PP  | PP  | PP  | 90.7; 90.7 | 0.0  | 0.0; 2.0  | 100 | 68,0 | 0,0 |
| 64975   | PP  | PP  | PP  | PP  | PP  | 90.7; 90.7 | 0.0  | 0.0; 2.0  | 100 | 80,0 | 0,0 |
| 64855   | PP  | PP  | PP  | PP  | PP  | 76.9; 89.6 | 0.0  | 0.0; 1.9  | 103 | 73,0 | 0,0 |
| 64849   | PP  | PP  | PP  | PP  | PP  | 76.9; 89.6 | 0.0  | 0.0; 2.0  | 100 | 76,0 | 0,0 |
| 64575   | PP  | PP  | PP  | PP  | PP  | 76.9; 89.6 | 2.0  | 0.3; 6.0  | 101 | 73,0 | 0,0 |
| 64968   | PP  | PP  | PP  | PP  | PP  | 76.9; 89.6 | 3.0  | 0.8; 7.6  | 101 | 73,0 | 0,0 |
| 64979   | PP  | PP  | PP  | PP  | PB  | 57.4; 76.9 | 1.0  | 0.1; 4.3  | 100 | 72,0 | 0,0 |
| 66872   | PP  | PP  | PP  | PP  | PB  | 57.4; 76.9 | 2.0  | 0.3; 6.0  | 103 | 73,0 | 0,0 |
| 66514   | PP  | PP  | PP  | PP  | PB  | 57.4; 76.9 | 3.0  | 0.8; 7.6  | 100 | 71,0 | 0,0 |
| 64977   | PP  | PP  | PB  | PB  | PB  | 25.0; 57.4 | 4.8  | 1.7; 10.0 | 104 | 63,0 | 0,0 |
| 66707   | PP  | PB  | PB  | PB  | PB  | 3.8; 25.0 | 4.9  | 1.8; 10.2 | 103 | NA  | 0,0 |
| 66815   | PP  | PB  | PB  | PB  | PB  | 3.8; 25.0 | 11.0 | 6.0; 17.8 | 109 | 75,0 | 0,0 |

The minimal and maximal length of PWD/PWD interval was determined by positions of the DNA markers used for genotyping. White and orange intervals are heterozygous PWD/B6 and homozygous PWD/PWD, respectively. Recombination breaks are located in gray intervals. Sperm count refers to the number of sperm cells in both epididymis, TW is the wet weight of paired testes in milligrams.
Table supplement 9. Change point estimates of the minimal length [Mb] of PWD/PWD homozygosity showing detectable effect on asynapsis rate

| Chr#   | Experiment                  | ^1Change point estimate | ^2Change point lower bound | ^3Change point upper bound |
|--------|-----------------------------|-------------------------|----------------------------|---------------------------|
| Chr5   | 2-chr cross                 | 19.50                   | 14.57                      | 24.44                     |
| Chr7   | 2-chr cross                 | ^4NA                    | NA                         | NA                        |
| Chr12  | 2-chr cross                 | 20.87                   | 6.80                       | 34.95                     |
| Chr15  | 2-chr cross                 | 8.61                    | -2.87                      | 20.09                     |
| Chr17  | 2-chr cross                 | 29.31                   | 10.29                      | 48.33                     |
| Chr18  | 2-chr cross                 | 25.32                   | 19.68                      | 30.96                     |
| Chr15  | 4-chr cross                 | 2.00                    | 0.71                       | 3.29                      |
| Chr16  | 4-chr cross                 | 12.80                   | 8.43                       | 17.16                     |
| Chr18  | 4-chr cross                 | 33.42                   | 10.80                      | 56.04                     |
| Chr19  | 4-chr cross                 | 19.16                   | 12.26                      | 26.06                     |
| Chr15  | 2-chr cross & 4-chr cross   | 7.32                    | 1.54                       | 13.10                     |
| Chr18  | 2-chr cross & 4-chr cross   | 27.13                   | 21.32                      | 32.94                     |
| Chr5,7,12,15,17,18 | 2-chr cross | 27.14                   | 19.36                      | 34.91                     |
| Chr5,7,12,15-19 | 2-chr cross & 4-chr cross | 28.40                   | 23.18                      | 33.63                     |

^1 Change point estimate of the length [Mb] of PWD/PWD homozygosity in the piecewise linear regression model. For the lengths of PWD/PWD homozygosity greater than the change point, the asynapsis rate is modelled as constant.

^2 Lower bound of 95% CI.

^3 Upper bound of 95% CI.

^4 Change point cannot be determined.

^5 The estimate is influenced by two outliers with mouse ID 38759 and 38737.
Table supplement 10. Eleven G3 male parents selected for the 4-Chr cross experiment

| Male ID | Chr 15 (104 Mb) | Chr 16 (98.2 Mb) | Chr 18 (90.7 Mb) | Chr 19 (61.4 Mb) |
|---------|-----------------|-----------------|-----------------|-----------------|
|         | 9.3 | 39.9 | 63.3 | 70.0 | 103.3 | 6.2 | 20.2 | 29.3 | 42.7 | 67.6 | 97.0 | 3.8 | 34.0 | 45.1 | 57.6 | 89.7 | 3.3 | 21.9 | 33.0 | 42.4 | 59.7 |
| 37487   | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  |
| 37504   | B   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37507   | B   | B   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | B   | B   | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  | PB  |
| 37715   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37852   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 377871  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37746   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37634   | B   | B   | B   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37616   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37709   | B   | B   | B   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |
| 37862   | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | PB  | B   | B   | PB  | PB  | PB  |

PB - PWD/B6 heterozygosity
B - B6/B6 homozygosity
Table supplement 11. The fertility parameters of hybrids of the 4-chr cross experiment

| Male ID | TW (mg) | Sperm x 10^11 |
|---------|---------|----------------|
| 38754   | 51      | 0.00           |
| 38428   | 57      | 0.00           |
| 38735   | 58      | 0.00           |
| 38088   | 59      | 0.00           |
| 38089   | 60      | 0.00           |
| 38753   | 60      | 0.00           |
| 38726   | 61      | 0.00           |
| 38751   | 61      | 0.00           |
| 38755   | 61      | 0.00           |
| 38455   | 62      | 0.00           |
| 38090   | 63      | 0.00           |
| 38085   | 64      | 0.00           |
| 38087   | 64      | 0.00           |
| 38448   | 64      | 0.00           |
| 38738   | 65      | 0.00           |
| 38739   | 65      | 0.00           |
| 38721   | 65      | 0.00           |
| 38728   | 65      | 0.00           |
| 38822   | 65      | 0.00           |
| 38430   | 66      | 0.00           |
| 38432   | 66      | 0.00           |
| 38710   | 66      | 0.00           |
| 38084   | 67      | 0.00           |
| 38434   | 67      | 0.00           |
| 38814   | 67      | 0.00           |
| 38752   | 68      | 0.00           |
| 38783   | 68      | 0.00           |
| 38817   | 68      | 0.00           |
| 38758   | 69      | 0.00           |
| 38821   | 69      | 0.00           |
| 38740   | 70      | 0.00           |
| 38766   | 70      | 0.00           |
| 38813   | 70      | 0.00           |
| 38426   | 71      | 0.00           |
| 38468   | 71      | 0.00           |
| 38765   | 72      | 0.00           |
| 38767   | 73      | 0.00           |
| 38819   | 73      | 0.00           |
| 38792   | 74      | 0.00           |
| 38818   | 74      | 0.00           |
| 38820   | 74      | 0.00           |
| 38815   | 75      | 0.00           |
| 75890   | 68      | 0.00           |
| 79414   | 65      | 0.00           |
| 38712   | 76      | 0.00           |
| 38733   | 77      | 0.01           |
| 38732   | 73      | 0.01           |
| 38561   | 78      | 0.01           |
| 38713   | 77      | 0.03           |
| 38727   | 72      | 0.03           |
| 38757   | 72      | 0.04           |
| 38427   | 64      | 0.06           |
| 38429   | 64      | 0.09           |
| 38425   | 75      | 0.10           |
| 38556   | 63      | 0.17           |
| 38744   | 66      | 0.21           |
| 38086   | 73      | 0.23           |
| 38725   | 69      | 0.29           |
| 38852   | 75      | 0.34           |
| 38842   | 89      | 0.43           |
| 38759   | 81      | 0.60           |
| 38858   | 73      | 0.74           |
| 38765   | 81      | 1.02           |
| 38736   | 81      | 1.10           |
| 38764   | 84      | 1.10           |
| 38741   | 81      | 1.20           |
| 38805   | 83      | 1.51           |
| 38565   | 70      | 1.61           |
| 38794   | 90      | 1.80           |
| 38737   | 94      | 1.90           |
| 75889   | 90      | 2.10           |
| 38692   | 89      | 2.20           |
| 79415   | 83      | 2.20           |
| 38724   | 71      | 2.80           |
| 79417   | 90      | 2.80           |
| 38742   | 103     | 3.90           |
| 38433   | 78      | 4.20           |
| 38816   | 106     | 4.20           |
| 38756   | 85      | 4.70           |
| 38650   | 82      | 5.40           |
| 38652   | 90      | 5.60           |
| 38831   | 81      | 6.40           |
| 79416   | 95      | 6.40           |
| 38555   | 98      | 6.87           |
| 38793   | 117     | 10.20          |
| 75891   | 131     | 12.40          |
| 79413   | 108     | 13.70          |
Table supplement 12. Four-chr cross. The effect of the size and location of PWD/PWD consubspecific intervals on asynapsis of Chr 15, 16, 18, and 19 and on fertility parameters.

| Male ID | Distance from the centromere of Chr 15 | Asynapsis of Chr 15 | Distance from the centromere of Chr 16 | Asynapsis of Chr 16 | Dist. from the centromere of Chr 18 | Asynapsis of Chr 18 | Dist. from the centromere of Chr 19 | Asynapsis of Chr 19 | SpERM count | Cells analyzed | PWD/PWD length in Chr 15, 16, 18, 19 |
|---------|----------------------------------------|---------------------|----------------------------------------|---------------------|----------------------------------|---------------------|----------------------------------|---------------------|-------------|----------------|----------------------------------|
|         | (total length 124.0 Mbp)               | (total length 98.2 Mbp) | (total length 90.7 Mbp)               | (total length 81.4 Mbp) |                                  | (total length 61.4 Mbp) |                                  |                                  |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |
| PB      | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0                   | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 | 0.0; 2.0; 4.0; 10.0              | 0.0; 2.0; 4.0; 10.0 |             |                         |                                  |

The reference and maximal length of PWD/PWD interval was determined by positions of the GbM markers used for gene mapping. White and orange entries are heterozygous PWD/PWD and homozygous PWD/PWD, respectively. Reconstruction breaks are located in gray intervals.

1)SpERM count refers to the number of sperm cells in both epididymis.  
2)The average between minimum and maximum PWD/PWD consubspecific sequence is given.
Table supplement 13. Four-chr cross experiment. The relation between observed and calculated rate of pachytene asynapsis and fertility parameters

| Male ID | Testes (mg) | Sperm No x 10^6 | Frequency of Chr synapsed | Probability of all 4 chr synapsed | Probability of all 19 synapsed | Probability of asynaptic pachy | Obs ratio of asynaptic pachy | Obs ratio of all 19 synapsed |
|---------|-------------|-----------------|---------------------------|-----------------------------------|--------------------------------|------------------------------|-----------------------------|-----------------------------|
| 38435   | 62          | 0               | 0.750                     | 0.810                             | 0.960                          | 0.610                        | 0.670                        | 0.294                       | 0.079                       | 0.921                       | 0.790                       | 0.210                       |
| 38087   | 64          | 0               | 0.860                     | 0.820                             | 0.900                          | 0.427                        | 0.114                        | 0.862                       | 0.860                       | 0.660                       | 0.340                       |                             |
| 75414   | 65          | 0               | 0.979                     | 0.719                             | 0.740                          | 0.518                        | 0.138                        | 0.862                       | 0.580                       | 0.420                       |                             |
| 38432   | 66          | 0               | 1.000                     | 0.793                             | 0.759                          | 0.567                        | 0.151                        | 0.849                       | 0.360                       | 0.640                       |                             |
| 75890   | 68          | 0               | 0.983                     | 0.959                             | 0.793                          | 0.567                        | 0.151                        | 0.849                       | 0.630                       | 0.370                       |                             |
| 38425   | 73          | 0.1             | 1.000                     | 0.990                             | 0.770                          | 0.762                        | 0.193                        | 0.807                       | 0.580                       | 0.420                       |                             |
| 38086   | 73          | 0.23            | 0.980                     | 0.960                             | 0.990                          | 1.000                        | 0.931                        | 0.249                        | 0.751                       | 0.360                       | 0.640                       |                             |
| 38759   | 81          | 0.6             | 0.970                     | 1.000                             | 0.850                          | 1.000                        | 0.825                        | 0.220                        | 0.780                       | 0.490                       | 0.510                       |                             |
| 38736   | 81          | 1.1             | 0.960                     | 0.820                             | 0.920                          | 1.000                        | 0.724                        | 0.193                        | 0.807                       | 0.480                       | 0.520                       |                             |
| 38741   | 81          | 1.2             | 0.950                     | 0.990                             | 1.000                          | 0.980                        | 0.922                        | 0.246                        | 0.754                       | 0.480                       | 0.520                       |                             |
| 38737   | 94          | 1.9             | 0.990                     | 0.820                             | 1.000                          | 1.000                        | 0.812                        | 0.217                        | 0.783                       | 0.400                       | 0.600                       |                             |
| 38692   | 89          | 2.2             | 0.920                     | 1.000                             | 1.000                          | 1.000                        | 0.920                        | 0.246                        | 0.754                       | 0.280                       | 0.720                       |                             |
| 38433   | 78          | 4.2             | 1.000                     | 1.000                             | 1.000                          | 1.000                        | 1.000                        | 0.267                        | 0.733                       | 0.280                       | 0.720                       |                             |
| 38756   | 85          | 4.7             | 0.870                     | 1.000                             | 1.000                          | 1.000                        | 0.870                        | 0.232                        | 0.768                       | 0.390                       | 0.610                       |                             |
| 38650   | 82          | 5.4             | 0.980                     | 1.000                             | 1.000                          | 1.000                        | 0.980                        | 0.262                        | 0.738                       | 0.360                       | 0.640                       |                             |
| 38652   | 90          | 5.6             | 0.940                     | 1.000                             | 1.000                          | 1.000                        | 0.940                        | 0.251                        | 0.749                       | 0.330                       | 0.670                       |                             |
| 38431   | 81          | 6.4             | 0.970                     | 1.000                             | 1.000                          | 1.000                        | 0.970                        | 0.259                        | 0.741                       | 0.460                       | 0.540                       |                             |
| 38555   | 98          | 6.9             | 0.980                     | 0.960                             | 1.000                          | 1.000                        | 0.941                        | 0.251                        | 0.749                       | 0.255                       | 0.745                       |                             |
| Chr No. | SSLP marker | Mbp |
|---------|-------------|-----|
| 5       | D5Mit49     | 5.20|
| 5       | D5Mit178    | 9.83|
| 5       | D5Mit49     | 24.97|
| 5       | D5Mit386    | 28.10|
| 5       | D5Mit106    | 44.99|
| 5       | D5Mit302    | 61.43|
| 5       | D5Mit303    | 64.10|
| 5       | D5Mit277    | 107.06|
| 5       | D5Mit136    | 118.73|
| 5       | D5Mit138    | 125.10|
| 5       | D5Mit140    | 126.95|
| 5       | D5Mit65     | 127.72|
| 5       | D5Mit63     | 132.13|
| 5       | D5Mit326    | 137.43|
| 5       | D5Mit102    | 151.35|
| 7       | D7Mit21     | 3.27|
| 7       | D7Mit191    | 18.89|
| 7       | D7Mit180    | 24.63|
| 7       | D7Mit265    | 25.68|
| 7       | D7Mit246    | 30.30|
| 7       | D7Mit31     | 87.49|
| 7       | D7Mit66     | 119.89|
| 7       | D7Mit71     | 131.03|
| 7       | D7Mit259    | 144.76|
| 12      | D12Mit37    | 5.37|
| 12      | D12Mit215   | 7.61|
| 12      | D12Mit49    | 15.71|
| 12      | D12Mit11    | 22.39|
| 12      | D12Mit136   | 30.17|
| 12      | D12Mit147   | 35.72|
| 12      | D12Mit285   | 54.65|
| 12      | D12Mit118   | 91.51|
| 12      | D12Mit9     | 103.40|
| 12      | D12Mit20    | 113.36|
| 15      | D15Mit175   | 9.37|
| 15      | D15Mit130   | 20.81|
| 15      | D15Mit49    | 26.04|
| 15      | D15Mit138   | 40.03|
| 15      | D15Mit113   | 50.40|
| 15      | D15Mit170   | 63.52|
| 15      | D15Mit234   | 65.07|
| 15      | D15Mit667   | 70.20|
| 15      | D15Mit125   | 83.89|
| 15      | D15Mit161   | 97.01|
| 15      | D15Mit643   | 97.99|
| 15      | D15Mit987   | 100.80|
| 15      | D15Mit680   | 102.03|
| 15      | D15Mit616   | 102.99|
| 15      | D15Mit679   | 103.47|
| 16      | D16Mit55    | 6.24|
| 16      | D16Mit165   | 14.00|
| 16      | D16Mit29    | 20.20|
| 16      | D16Mit57    | 29.30|
| 16      | D16Mit12    | 39.12|
| 16      | D16Mit681   | 42.81|
| 16      | D16Mit76    | 67.72|
| 16      | D16Mit71    | 96.91|
| 17      | D17Mit19    | 4.81|
| 17      | D17Mit34    | 15.00|
| 17      | D17Mit34    | 15.60|
| 17      | D17Mit81    | 30.90|
| 17      | D17Mit63    | 34.74|
| 17      | D17Mit50    | 45.36|
| 17      | D17Mit139   | 52.66|
| 17      | D17Mit152   | 65.34|
| 17      | D17Mit93    | 73.80|
| 17      | D17Mit122   | 83.06|
| 17      | D17Mit155   | 84.50|
| 17      | D17Mit123   | 93.20|
| 18      | D18Mit66    | 3.84|
| 18      | D18Mit22    | 24.95|
| 18      | D18Mit64    | 33.43|
| 18      | D18Mit70    | 34.85|
| 18      | D18Mit149   | 45.00|
| 18      | D18Mit63    | 52.43|
| 18      | D18Mit55    | 53.16|
| 18      | D18Mit64    | 53.43|
| 18      | D18Mit124   | 57.46|
| 18      | D18Mit184   | 66.82|
| 18      | D18Mit209   | 66.97|
| 18      | D18Mit185   | 69.03|
| 18      | D18Mit7    | 76.85|
| 18      | D18Mit25    | 89.57|
| 19      | D19Mit31    | 5.78|
| 19      | D19Mit61    | 15.53|
| 19      | D19Mit128   | 17.25|
| 19      | D19Mit66    | 21.44|
| 19      | D19Mit64    | 32.94|
| 19      | D19Mit11    | 42.40|
| 19      | D19Mit71    | 59.60|