Meiotic Recognition of Evolutionarily Diverged Homologs: Chromosomal Hybrid Sterility Revisited

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Associate editor: Dr. Harmit Malik

Abstract

Hybrid sterility (HS) is an early postzygotic reproductive isolation mechanism observed in all sexually reproducing species. Infertility of hybrids prevents gene flow between incipient species and leads to speciation. While *Drosophila* studies have focused almost exclusively on the genic control of HS, two other model species, *Mus musculus* and budding yeast, provided the first experimental evidence of hybrid sterility governed by the nongenic effects of DNA sequence divergence. Here, we propose that the nongenic effect of increasing DNA divergence between closely related species may impair mutual recognition of homologous chromosomes and disrupt their synapsis. Unsynapsed or mispaired homologs can induce early meiotic arrest, or their random segregation can cause aneuploidy of spermatids and sperm cells. Impaired recognition of homologs may thus act as a universal chromosomal checkpoint contributing to the complexity of genetic control of HS. Chromosomal HS controlled by the *Prdm9* gene in mice and HS driven by the mismatch repair machinery in yeast are currently the most advanced examples of chromosomal homology search-based HS. More focus on the cellular and molecular phenotypes of meiosis will be needed to further validate the role of homolog recognition in hybrid sterility and speciation.

Key words: **meiotic pairing, reproductive isolation, speciation, chromosomal sterility, *Prdm9*, antirecombination.**

"Nothing in Biology Makes Sense Except in the Light of Evolution" (Dobzhansky 1973)

Understanding the mechanisms of speciation remains a primary goal of evolutionary genetics. History begins with Darwin's book *"On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life"* (Darwin 1859), a Big Bang for evolutionary biology, much like Mendel's article *"Versuche über Pflanzenhybriden"* for genetics. Interestingly, at first, the two theories were considered incompatible (see Coyne and Orr 2004 for details), and it took over 30 years for the theory of natural selection to be reconciled with the principles of genetic variation in the Modern Synthesis (Dobzhansky 1951; Stebbins 1958; Mayr 1963).

In the classic allopatric model, the speciation begins with the geographic split of a population, followed by the accumulation of random mutations during the independent evolution of separated parts. After secondary contact, the diverged populations either coalesce and mix newly acquired mutations in the reunited population or become incipient subspecies if they have evolved partial or complete reproductive isolation (fig. 1). Once a reproductive barrier is created, free gene flow between taxa cannot be restored, and the process of their genetic alienation becomes irreversible (Coyne and Orr 2004). The importance of reproductive isolation is reflected in the biological concept of species, which defines species as "a group of individuals fully fertile inter se, but barred from interbreeding with other similar groups" (Dobzhansky 1935; Mayr 1963). Reproductive isolation between taxa can be prezygotic, preventing the formation of a fertilized egg, or postzygotic, causing hybrid inviability or sterility. Interest in infertility of interspecific hybrids is documented since Aristotle's discussion of the sterility of mules, the hybrids between a mare and donkey. Pioneering genetic studies of hybrid sterility (HS) were carried out in the 1930s by Dobzhansky, Haldane, Muller, and others, mainly on interspecific hybrids of *Drosophila*. These and later studies revealed two rules common to all sexually reproducing species, namely the predominant involvement of the X chromosome in control of hybrid sterility, known as the "large X-effect" or "Coyne's rule" (Coyne 2018; Presgraves and Meiklejohn 2021), and the preferential involvement of the heterogametic sex in sterility or inviability of hybrids (XY males and ZW females), referred to as Haldane's rule (Haldane 1922).

From a geneticist's point of view, some basic questions remain: What is the genetic architecture of hybrid sterility? Is it based on similar gene pathways in different species, or is it heterogeneous, based on random incompatibilities of functionally interacting genes, as suggested by the Dobzhansky–Muller model? Furthermore, what is the role of selfish genetic elements and their suppressors in

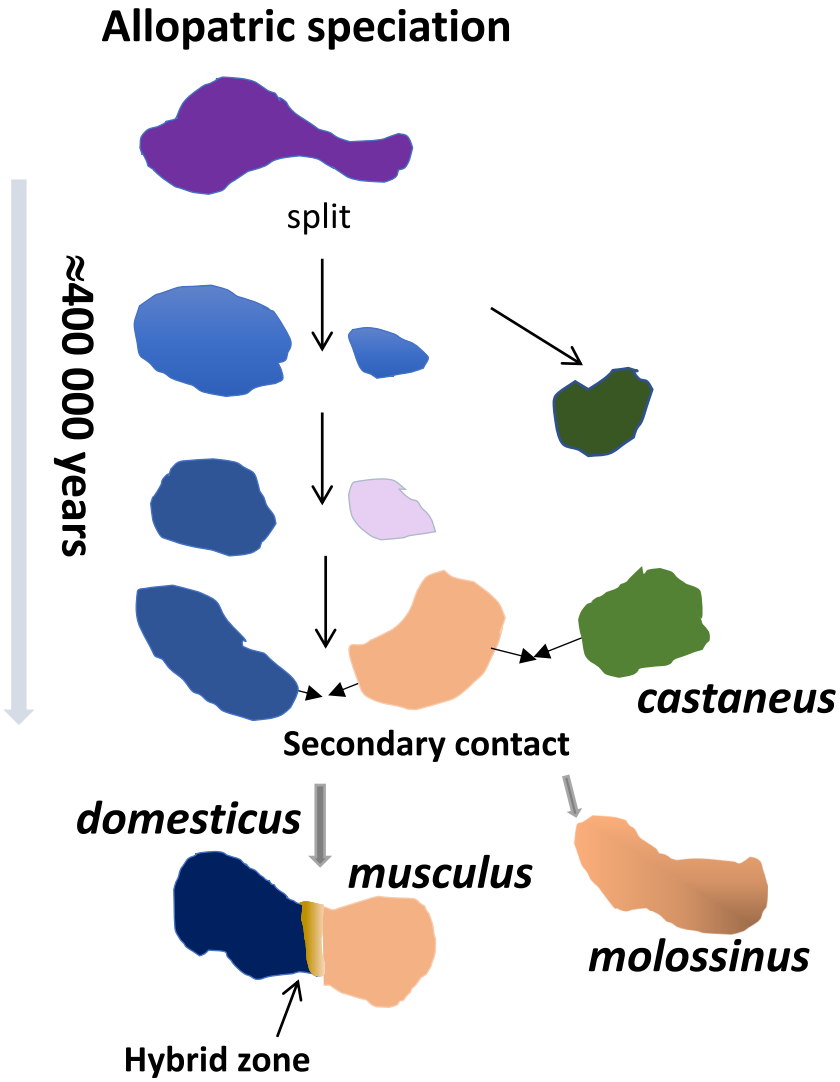

FIG. 1. Simplified scheme of allopatric speciation on the model of the European *Mus musculus* species. The ancestors of today's subspecies *Mus musculus musculus*, *Mus musculus domesticus*, and *Mus musculus castaneus* separated from a common predecessor about 400 thousand years ago (KYA) and independently accumulated mutations until their secondary contact a few thousand years ago (Boursot et al. 1996; Duvaux et al. 2011; Phifer-Rixey and Nachman 2015). In Europe, the *musculus* and *domesticus* subspecies developed incomplete reproductive isolation manifested by a narrow hybrid zone (Baird and Macholan 2012), while *musculus* and *castaneus* were less differentiated and generated in Japan the hybrid subspecies, *Mus musculus molossinus*, predominantly of *musculus* origin (Suzuki and Aplin 2012).

intragenomic conflict as proposed by mouse and *Drosophila* models (Cocquet et al. 2012; Parhad et al. 2017)? Although ~70 years have passed since the Modern Synthesis, the answers are still inconclusive. The main difficulty lies in the repeatedly documented complexity of genetic control of the hybrid incompatibilities, which prevents identifying its individual components (Maheshwari and Barbash 2011; Morgan et al. 2020; Presgraves and Meiklejohn 2021; Chou et al. 2022). Here we propose that part of this genetic complexity can be explained by impaired mutual recognition of the evolutionarily diverged homologs resulting in infertility of interspecific hybrids.

Mutual recognition, pairing, and synapsis of homologous chromosomes are unique features of gametogenesis associated with the onset of meiosis of all sexually reproducing species (fig. 2A). Pairing of homologs can occur before the beginning of meiosis in mitotically dividing gonocytes (Boateng et al. 2013; Rubin et al. 2022; Sole et al. 2022). In many organisms, synapsis of homologous chromosomes occurs at the molecular level during the first meiotic prophase and is stabilized by a proteinaceous scaffold, the synaptonemal complex. Failure to complete synaptonemal complex formation can activate the pachytene (meiotic) checkpoint and lead to delay or cell death of primary spermatocytes (Subramanian and Hochwagen 2014).

In many species, the synapsis of paternal and maternal copies of chromosomes is interlinked with homologous recombination. The process begins with the induction of programmed DNA double-strand breaks (DSBs) followed by a homology search in the DNA molecules (Lam and Keeney 2014). Fine-tuning of homologous template recognition may represent a critical step in DNA repair; if too rigorous, some DSBs will remain unrepaired due to natural polymorphisms and cause cell death. If too relaxed, nonallelic homologous recombination (NAHR) could lead to aneuploidy and genome instability. Appropriate control of homolog recognition, dependent or independent of recombination, is monitored by multiple meiotic checkpoints, antirecombination mismatch repair checkpoint (Rayssiguier et al. 1989; Radman 2022), DNA DSB repair checkpoint (Pacheco et al. 2015), or pachytene checkpoint (Li et al. 2009). We speculate that as the evolutionary divergence of homologous sequences increases, the efficiency of homology search in inter(sub)specific F1

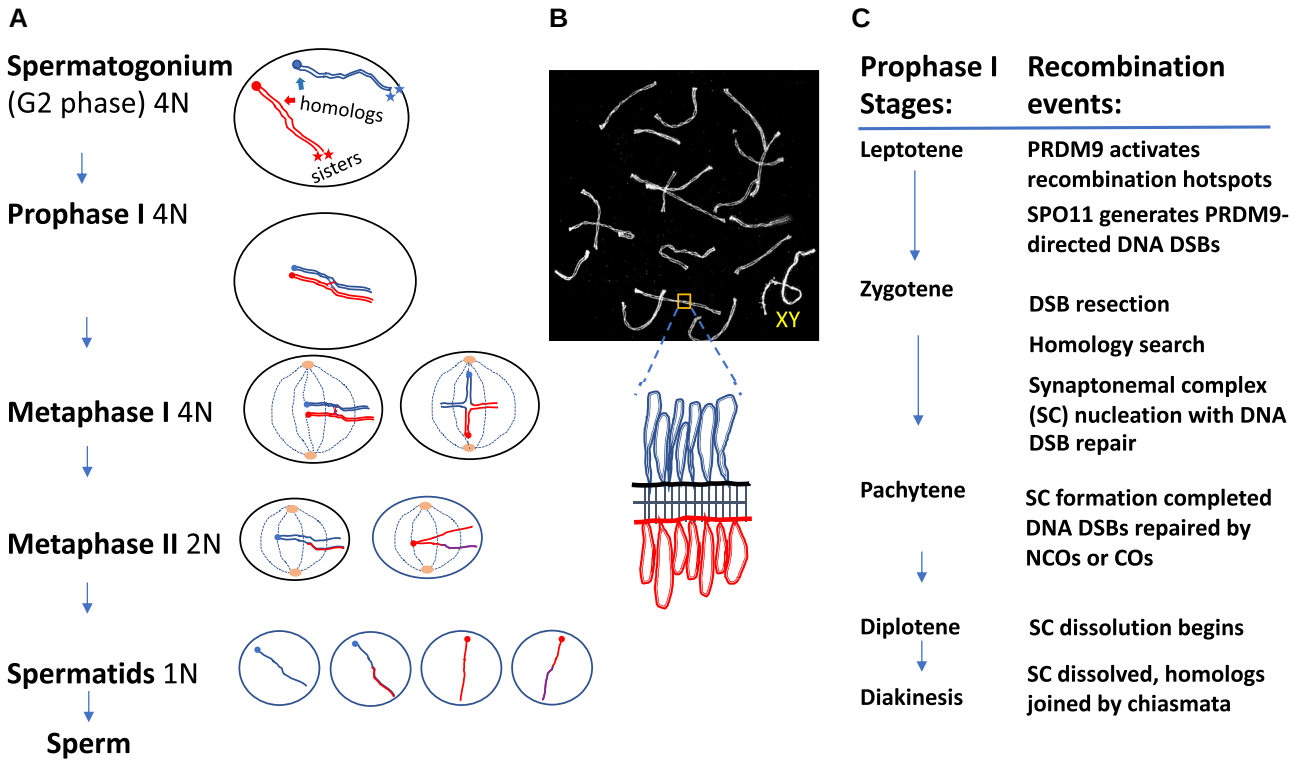

Fig. 2. Recombination-dependent meiotic progression. (*A*) Two meiotic divisions produce four haploid sperm from one diploid spermatogonium. For clarity, only one pair of homologous chromosomes is shown, each presented as two sister chromatids (paternal—blue, maternal—red). (*B*) Synapsis of homologous chromosomes occurs in prophase I. SYCP3 protein immunostaining reveals 19 autosomal synaptonemal complexes (SCs) and XY bivalent in the mouse pachytene spermatocyte. Schematic of an enlarged SC section showing DNA loops of two paternal and two maternal chromatids anchored in the lateral elements of the SC interconnected by transverse filaments. (*C*) Homologous recombination between paternal and maternal chromosomes occurs during the five stages of prophase I. The pachynema is the only cell type in the organism where homologous DNA sequences pair at the molecular level.

hybrids deteriorates, creating an early barrier between emerging species. Pre-meiotic and/or meiotic recognition of evolutionarily diverged homologs may thus represent an underappreciated component of the genetic architecture of hybrid sterility.

Searching for Mechanisms of Meiotic Homology Search

Mutual recognition of paternal and maternal copies of each chromosome is an obligatory step for their proper disjunction at meiosis I and for the segregation of individual chromatids into haploid gametes (Zickler and Kleckner 1999; Hunter 2015). Each chromosome must carry information to distinguish its homolog during gametogenesis. The homolog recognition and pairing can occur before meiosis in mitotically dividing gonocytes and during the first meiotic prophase. Homolog synapsis can be recombination independent as in *Drosophila* or *Caenorhabditis elegans*, or dependent as in mice or budding yeast, and can participate in hybrid sterility by different mechanisms. Unfortunately, cellular and molecular mechanisms of homolog recognition in higher eukaryotes are still poorly understood (Zickler and Kleckner 2016; Sybenga 2020; Addo Nyarko and Mason 2022), even in model organisms.

Drosophila

In male gametogenesis, homolog centromeres, decorated by synaptonemal complex proteins C (3) G and Corona, progressively pair during four rounds of premeiotic divisions, before entering meiotic prophase (Joyce et al. 2013; Rubin et al. 2022). *Drosophila* male meiosis is atypical in that homologous chromosomes associate in meiotic prophase without forming synaptonemal complexes and recombining. The mechanism of homolog recognition is associated with chromosome territory formation (Sun et al. 2019; Vernizzi and Lehner 2022) and requires four proteins, three of them, MNM, SNM, and UNO necessary for X–Y chromosome pairing, and one (TEF) for maintaining autosomal bivalents (Thomas et al. 2005; Arya et al. 2006; Weber et al. 2020). The *Drosophila* female meiosis is preceded by centromere pairing during premeiotic mitotic cycles as in males. However, in female meiosis, the complete synaptonemal complex is formed independently of DNA DSB repair, to support the homolog's synapsis (Hughes et al. 2018).

Caenorhabditis elegans

Homolog recognition, pairing, and synapsis in *C. elegans* do not depend on meiotic recombination. A pairing center composed of a repetitive DNA near one end of each

chromosome (Hawley and Gilliland 2009) forms a nucleoprotein complex with one of four C2H2 zinc-finger proteins (ZIM-1, ZIM-2, ZIM-3, or HIM-8), which interact with a nuclear envelope LINC protein complex composed of SUN-1 and ZYG-12 proteins. LINC (linker of nucleoskeleton and cytoskeleton) interactions with chromosomes result in rigorous movement of chromosome ends and appear to contribute to homolog recognition and synapsis (Hillers et al. 2017). Though pairing centers are necessary for homolog pairing and synapsis, they are not sufficient (Nabeshima et al. 2011). Recently, MJL-1 (MAJIN-like-1) has been shown essential not only for interaction between pairing centers and LINC, but more importantly for active chromosome movements ensuring homolog pairing and synapsis (Kim et al. 2023).

Budding Yeast and Mouse

Facilitation of homology search in yeast and mice is secured by homolog pairing preceding programmed DNA cleavage and recombination (Peoples et al. 2002; Boateng et al. 2013; Sole et al. 2022) as documented by the persistent homolog pairing in the absence of SPO11-induced DNA double-strand breaks (DSBs) in mouse meiotic prophase I (Ishiguro et al. 2014). In fission yeast, the recognition and subsequent recombination-independent pairing of homologs are ensured by long noncoding RNA-protein complexes (Ding et al. 2019). The pairing and synapsis are further supported by telomere clustering ("bouquet" formation) at the inner nuclear envelope by zygotene cilium (Mytlis et al. 2022) and SUN1 protein (Ding et al. 2007).

In most eukaryotes, including yeast and mouse, synapsis of homologs is linked to the repair of developmentally programmed DNA DSBs at the leptotene stage of the first meiotic prophase. After DSB formation by SPO11 topoisomerase VI-like complex and DSB 5′end resection, the resulting ssDNA 3′ nucleoprotein filament loaded by RAD51 and DMC1 recombinases begins homology search to copy the missing sequence from the homologous template (Lam and Keeney 2014). The length of these ssDNA filaments is ∼1,100 bp in mice and ∼820 bp in yeast (Yamada et al. 2020). The sites of DSB repair predetermine nucleation of chromosome synapsis by the formation of the synaptonemal complex, a tripartite protein structure stabilizing the synapsis (Zickler and Kleckner 2015; Dubois et al. 2019) (fig. 2*B*). The necessity of DSBs repair for proper homolog synapsis is indisputable in these species; however, the molecular mechanism of homolog's mutual recognition is still unclear. In higher eukaryotes, the complexity of the genome is several orders of magnitude higher than in yeast, and mechanisms other than ssDNA filaments homology search may be involved (Weiner et al. 2009; Sybenga 2020).

In mice, humans (Baudat et al. 2010; Myers et al. 2010; Parvanov et al. 2010), and many other vertebrates (Baker et al. 2017; Cavassim et al. 2022; Damm et al. 2022), the genomic localizations of SPO11-driven DSBs are predetermined by the PRDM9 histone methyl transferase catalytic activity (fig. 2C). The PRDM9 zinc finger array binds to the allele-specific genomic sites, and trimethylates histones 3 at lysine 4 and lysine 36 (H3K4me3 and H3K36me3) (Baudat et al. 2010; Parvanov et al. 2010; Diagouraga et al. 2018). The activated PRDM9 binding sites occupy narrow ∼1,000 bp intervals called hotspots (Paigen and Petkov 2018; Tock and Henderson 2018). The PRDM9 hotspots localized on 250–500 kb long DNA loops of leptotene spermatocytes (Grey and de Massy 2021) are pulled down to the chromosome axis to be processed by the SPO11 DNA DSB machinery. Recently the ZCWPW1 protein, a reader of the H3K4 and H3K36 methylation, and the CXXC1 protein were proposed to participate in this step (Parvanov et al. 2017; Wells et al. 2020; Cavassim et al. 2022; Yuan et al. 2022). However, the regulation of these processes on a genome-wide level still needs to be clarified. Approximately 4,700 PRDM9 binding sites are trimethylated per mouse leptotene spermatocyte (Baker et al. 2014), of which ∼ 200–300 are converted into developmentally programmed DNA DSBs by SPO11 (Baudat et al. 2000; Romanienko and Camerini-Otero 2000). Of them, ∼90% are repaired as noncrossovers and 10% as crossovers (Li et al. 2019; Gergelits et al. 2021). Most likely, the choice is preferentially focused on the sites bound with PRDM9 on both alleles (Hinch et al. 2019; Li et al. 2019). If confirmed, the complexity of the homology search could be fundamentally reduced just to biallelically marked PRDM9 hotspots.

PRDM9 hotspots are characterized by their continuous evolutionary erosion. In a leptonema heterozygous for a weaker and stronger PRDM9 binding site, the chance is higher that the stronger motif will be bound by PRDM9, then disrupted by the SPO11-generated DNA DSB, and repaired using the weaker site as a template. At the evolutionary scale, the rapid replacement of active PRDM9 hotspots by inactive allelic sites is counterbalanced by mutations under positive selection, changing the PRDM9 zinc-finger arrays, which recognize new PRDM9 binding motifs (Baudat et al. 2013; Pratto et al. 2014; Baker et al. 2015; Smagulova et al. 2016). Thus, recombination hotspots persist despite continuous erosion, the phenomenon known as the hotspot paradox (Boulton et al. 1997; Baker et al. 2015). The heterozygosity for erased PRDM9 binding sites in sterile F1 hybrids between closely related mouse subspecies forms a basis for the hotspot asymmetry hypothesis of the *Prdm9*-controlled HS (see below).

HS in Model Species

In the initial genetic studies of HS, failure of homologous chromosomes to pair in meiosis was considered as one of the possible causes (Dobzhansky 1933; Stebbins 1958; White 1969). Later, chromosomal HS, represented at that time only by large chromosome rearrangements such as reciprocal translocations or inversions, was reclassified as an unlikely mechanism of speciation (reviewed in (Coyne and Orr 2004) due to the strong selection against deleterious, underdominant, rearrangement heterozygosity and

low probability of their fixation. An analogous argument against the underdominant effect (interallelic incompatibility) of heterozygous genes in the case of genic HS was overcome by the Dobzhansky–Muller incompatibility model (DMI) (Dobzhansky 1951). In this model, reproductive isolation does not require interallelic incompatibility because impaired epistatic interaction can occur between two or more mutually interacting genes that evolve independently in closely related taxa.

HS in Drosophila

Most of our knowledge on the genetic control of HS comes from the studies of hybrids between closely related *Drosophila* species. Quantitative trait locus (QTL) genetic mapping in interspecific backcrosses or introgressions of small chromosome segments from one species into the genome of another species (Lienard et al. 2016) was the most used approach. The main conclusions include the complex architecture of hybrid sterility, including a large number of gene incompatibilities in Drosophilidae hybrids (Coyne and Orr 2004; Maheshwari and Barbash 2011; Presgraves and Meiklejohn 2021). Thus, about 140 hybrid sterility genetic factors are estimated to operate in hybrids between *Drosophila simulans* and *Drosophila mauritiana*, of which one hundred are on chromosome X (Tao et al. 2003).

There are several caveats to this model of the HS genetic architecture. First, the time since divergence from a common ancestor among *Drosophila* species is relatively long, compared to *Mus musculus* subspecies, 240 thousand years (240 Kya) for *D. simulans*/*D. mauritiana* (Garrigan et al. 2012) or 100 Kya subspecies *Drosophila pseudobscura pseudobscura* and *Drosophila pseudobscura bogotana* (Russo et al. 1995). This means that many, perhaps the majority of incompatibilities in the extant species, arose after reproductive barrier had been established. Thus, a significant part of the identified genetic architecture of HS could be a consequence, rather than the cause of speciation (Lewontin 1974; Coyne and Orr 2004). Another concern is about the physical nature of HS polygenes. *Drosophila melanogaster* genome has been sequenced and coding sequences annotated; still, it is unclear which of the identified genes could fit the description of interchangeable HS polygenes (see below and Presgraves and Meiklejohn 2021).

Despite the complexity of genetic control, three major HS genes were identified by their genomic sequence, usually by interspecific introgression of a small chromosomal fragment, namely *OdsH—Ods-site homeobox* (Ting et al. 1998; Bayes and Malik 2009), *JYalpha* (Masly et al. 2006), and *Ovd* (Phadnis and Orr 2009; Go and Civetta 2022). Except for *JYalpha*, which is transposed between autosomes of *D. melanogaster* and *D. simulans* species and not sensu stricto reproductive isolation gene, the remaining two are fast-evolving genes under positive selection.

Until recently, reports on the pairing of homologous chromosomes during spermatogenesis of sterile F1 *Drosophila* males were lacking. The first such analysis showed nondisjunction in meiosis I as a likely cause of male infertility in three interspecific *Drosophila* species pairs. The authors concluded that failure of proper chromosome disjunction at meiosis I, which, in the absence of the pachytene checkpoint can indicate failure of homologs' recognition at prophase I, "may be a general phenomenon underlying *Drosophila* male sterility" (Kanippayoor et al. 2020). Further studies in this direction could renew the ideas of the chromosomal contribution to mechanisms of hybrid sterility in *Drosophila*.

HS in Saccharomyces

Studies of budding yeast *Saccharomyces cerevisiae* have provided the fundamental basis of our knowledge of meiotic chromosome organization and recombination (Hunter 2015) However, less is known about HS in yeast-interspecific hybrids. The leading causes of HS include mismatch repair (MMR) controlled antirecombination (Hunter et al. 1996; Bozdag et al. 2021) and genic incompatibilities (Delneri et al. 2003). Chromosomal rearrangements may contribute but are not crucial to initiating the reproductive isolation (Ono and Greig 2020). *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* show a 12% genome sequence divergence, and their sterile interspecific hybrids form inviable spores which cannot germinate because over 90% of them are aneuploid (Rogers et al. 2018). Deleting the MMR gene *MSH2* increased spore viability approximately 10-fold (Hunter et al. 1996; Greig et al. 2003), but simultaneous meiosis-specific suppression of *MSH2* and the DNA helicase *SGS1* restored euploidy and increased fertility 70-fold, to the levels comparable to intraspecific hybrids. *MSH2* forms a complex with other MMR proteins, identifying positions on the DNA heteroduplex with mismatched bases. The other function is to inhibit recombination in case of scanning diverged sequences (antirecombination). *SGS1* DNA helicase, an ortholog of the human Bloom syndrome protein, unwinds nascent recombination intermediates in case of increasing nonhomology (Myung et al. 2001; Bozdag et al. 2021). These results show that MMR-controlled antirecombination determines a significant part of the HS barrier between *S. cerevisiae* and *S. paradoxus* (Rogers et al. 2018; Bozdag et al. 2021).

HS in *Mus musculus*

House mouse, *Mus musculus*, encompasses three major subspecies, *Mus m. musculus*, *Mus m. domesticus*, and *Mus m. castaneus* (henceforth *musculus*, *domesticus*, and *castaneus*) that diverged from a common ancestor 350–500 Kya (Geraldes et al. 2008; Duvaux et al. 2011) and showed incomplete reproductive isolation (fig. 1) (Geraldes et al. 2011; Janousek et al. 2012; Turner et al. 2012). The former two subspecies formed a hybrid zone of limited gene flow in Central Europe 1–5 Kya (Baird and Macholan 2012; Turner and Harr 2014). Genome-wide mapping in mice from the hybrid zone revealed complex multigenic control of male fertility with a disproportional contribution of the X chromosome (Janousek et al. 2012; Turner et al. 2014; Morgan et al. 2020). Backcrosses and F2 laboratory crosses of *musculus*

and *domesticus* wild-derived mouse strains showed, contrary to most studies in *Drosophila* and other species, intrasubpecific polymorphism of HS factors (Forejt and Ivanyi 1974; Good et al. 2008; Vyskocilova et al. 2009; White et al. 2011; Mukaj et al. 2020). The complexity of the genetic control of HS was significantly reduced in testcrosses where only intrasubspecific variants segregated, which enabled identification of the *Prdm9* (*Hst1*) and the *Hstx1/2* HS genes/loci (Storchova et al. 2004; Mihola et al. 2009; Forejt et al. 2012; Bhattacharyya et al. 2014).

The Trans-Acting *Prdm9* Gene Determines the *Cis*-Acting Chromosomal HS

Besides the role in meiotic recombination, *Prdm9* controls the fertility of *musculus* × *domesticus* F1 hybrid males [(Mihola et al. 2009); for review, see Forejt et al. 2021]. Heterosubspecific heterozygosity for particular *Prdm9* alleles (*domesticus: Prdm9*^{dom2} or *Prdm9*^{dom3}; *musculus: Prdm9*^{msc1}, *Prdm9*^{msc2} or *Prdm9*^{msc5}) and the presence of *musculus* (PWD) allele of the X-linked *Hstx2* locus ensures complete or partial (*Prdm9*^{msc5}) HS of (*musculus* × *domesticus*)F1 hybrid males (Bhattacharyya et al. 2013, 2014; Lustyk et al. 2019; Mukaj et al. 2020; Forejt et al. 2021; Valiskova et al. 2022). The sterility supporting alleles *Prdm9*^{dom2} of *domesticus* B6 strain and *Prdm9*^{msc1} of *musculus* PWD strain shows the highest level of evolutionary erasure of their hotspots (Davies et al. 2016, 2021; Smagulova et al. 2016) and their asymmetric erasure in (PWD × B6)F1 hybrids causes the preponderance of the DNA DSBs of *musculus* origin on *domesticus* autosomes and vice versa, majority of the DNA DSBs of *domesticus* origin on *musculus* chromosomes (fig. 3). Davies and colleagues proposed (Davies et al. 2016), that these asymmetric DSBs will be difficult or impossible to repair, will prevent proper homolog synapsis, and may cause male sterility. Asynapsis of diverged homologs activates meiotic silencing of unsynapsed chromatin (MSUC) and impairs the male sex chromosome inactivation (MSCI) (Baarends et al. 2005; Turner 2015). Together with the DNA DSB repair checkpoint, these mechanisms could result in cell death and male sterility (fig. 3) (Forejt et al. 2021). The alternative, nonexclusive hypotheses posit the deleterious effect of default, PRDM9-independent DSB hotspots (Smagulova et al. 2016), or the involvement of PRDM9 binding motifs in retroelements independently dispersed between both subspecies (Yamada et al. 2017).

Until recently, *Prdm9*-driven HS was studied using hybrids of PWD (*musculus*) and B6 (*domesticus*) inbred strains, making the significance of this laboratory model for reproduction isolation between both subspecies unclear. To answer the question, hybrids of wild-derived mouse strains originating from 16 localities on both sides of the European Hybrid Zone (Mukaj et al. 2020) were tested for fertility and *Prdm9* allelic variants. Ten different *Prdm9* alleles were identified in these mice, of which only the *Prdm9*^{msc1}/*Prdm9*^{dom3} allelic combinations resulted in complete F1 HS irrespective of genetic background (with one exception, see Mukaj et al. 2020). The central role of *Prdm9* was further confirmed by the partial rescue of male fertility of interspecific hybrids between *Mus spretus* and *Mus m. domesticus* (Davies et al. 2021). Partial fertility was restored after substituting the mouse with a human zinc-finger domain in the PRDM9 molecule, which resulted in the elimination of PRDM9 hotspot asymmetry. The preponderance of *Prdm9* in the genetic control of HS was further documented in crosses of three mouse subspecies, where the male hybrids segregated *Mus m. castaneus* and *Mus m. musculus* chromosomes on the *Mus m. domesticus* background (Valiskova et al. 2022).

The *Hstx2* Locus on the X Chromosome Modulates *Prdm9*-Driven Chromosome Pairing, HS, and the Meiotic Recombination Rate

The hybrids of *domesticus*^{B6} × *musculus*^{PWD} with *domesticus* as a female parent show the incomplete arrest of spermatogenesis contrary to the completely sterile reciprocal *musculus*^{PWD} × *domesticus*^{B6} F1 hybrid males. Of the three possible explanations, the effect of an imprinted gene, mitochondrial inheritance, or an X-linked modifier, the last one proved valid (Storchova et al. 2004; Dzur-Gejdosova et al. 2012; Bhattacharyya et al. 2014). The difference between reciprocal F1 hybrids is controlled by the *Hstx2* locus (Hybrid sterility X Chromosome 2 QTL) delimited to 2,700 kbp interval of X chromosome (X: 66.51–69.21 Mbp, mm10 genome) (Lustyk et al. 2019). The *Hstx2*^{PWD} allele ensures a complete meiotic arrest in contrast to the partial rescue allowed by the *Hstx2*^{B6} allele.

In contrast, the B6.PWD-ChrX1s *domesticus* males carrying the proximal part of *musculus* chromosome X, including the *Hstx2*^{PWD}, show normal synapsis at the pachytene stage, but a high percentage of abnormal sperm, consistent with the definition of the *Hstx1* locus (Storchova et al. 2004; Bhattacharyya et al. 2014; Lustyk et al. 2019). Clearly, to understand the molecular mechanism of the *Hstx2 -Prdm9* interaction, the genes responsible for the *Hstx2*/*Hstx1* phenotypes need to be identified.

Suppression of recombination between genes responsible for reproductive isolation plays a significant role in the early stages of speciation (Ortiz-Barrientos et al. 2016; Schluter and Rieseberg 2022). The meiotic recombination 1, *Meir1*, the third genetic factor situated within the *Hstx2* locus (Balcova et al. 2016) and located in a cold spot of recombination (Lustyk et al. 2019), acts as the most robust genome-wide suppressor of the meiotic recombination rate in *musculus*^{PWD} × *domesticus*^{B6} F1 hybrid males. However, the relationship between *Hstx2* and *Meir1* is unclear because it is unknown which of the 20 predicted genes, two clusters of microRNA genes, and a few more ncRNA genes localized within 2,7 Mb *Hstx2* interval are responsible for recombination suppression and for meiotic arrest. Among the candidates for the *Hstx2* gene, the microRNA *mir465* cluster is differentially duplicated and overexpressed in *musculus*^{PWD} and *domesticus*^{B6} (Bhattacharyya et al. 2014; Lustyk et al. 2019). Recently, 12 genes of the *Hstx2* locus polymorphic

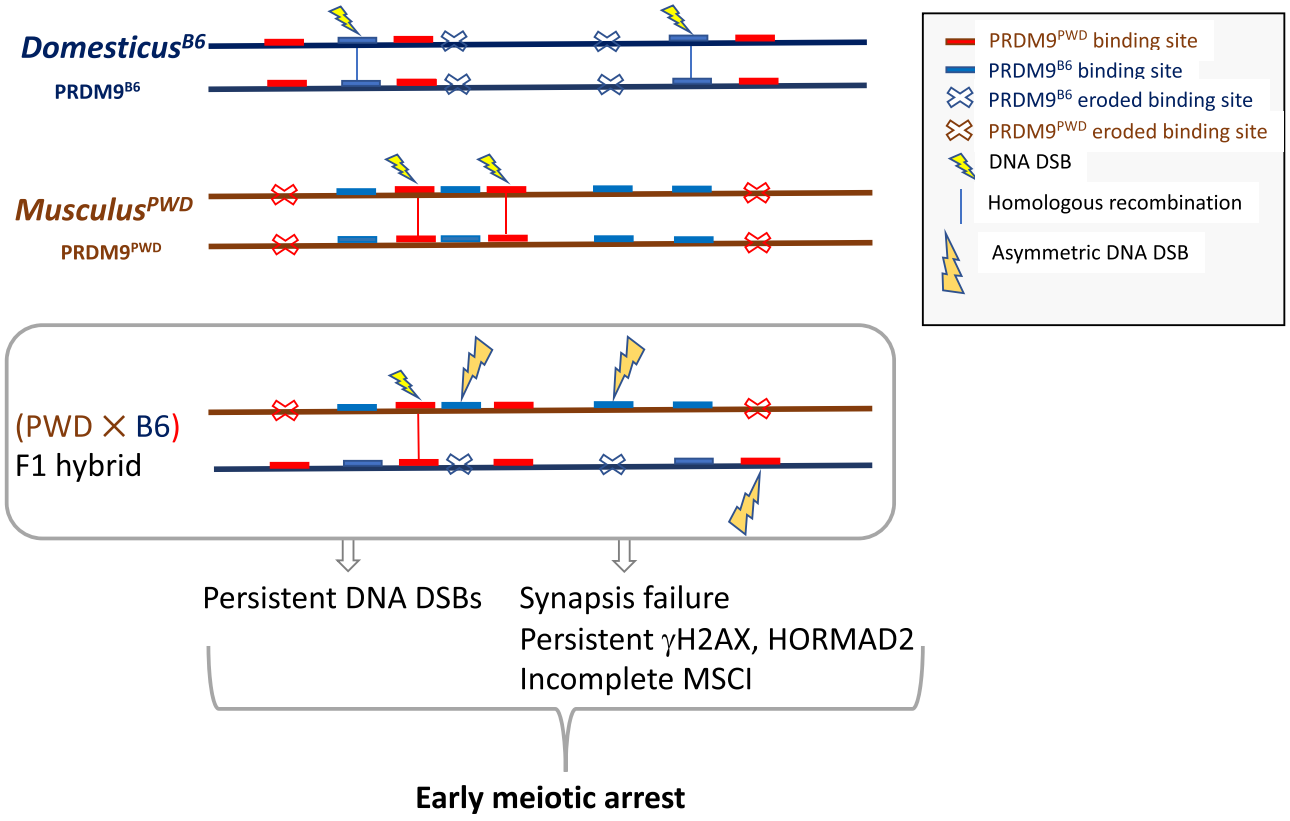

FIG. 3. Enhanced evolutionary divergence of PRDM9 binding motifs as a molecular mechanism of impaired homolog's recognition in HS. PRDM9^{PWD} and PRDM9^{B6} binding sites are present in both subspecies, but their erased forms occur predominantly in their own subspecies, PRDM9^{PWD} sites in *musculus*, and PRDM9^{B6} in *domesticus*. Heterozygosity for an intact and erased PRDM9 binding motif in sterile hybrids results in DNA DSBs, which are difficult to repair and may cause problems with homolog recognition. Failure of DSB repair or the repair via sister chromatid can activate the DSB checkpoint and/or prevent homolog synapsis, resulting in the meiotic arrest and arrest of spermatogenic differentiation.

between *musculus* and *domesticus* subspecies were shown to be dispensable for spermatogenesis on the *musculus*^{B6} background (Morimoto et al. 2020). However, the absence of a phenotype within *domesticus* subspecies does not preclude a role of any of them in a Dobzhansky–Muller-type of intergenic incompatibility (Dobzhansky 1951) in *musculus* × *domesticus* hybrids.

The most apparent epigenetic difference between male and female meiosis is the male sex chromosome inactivation (MSCI), first proposed as a mechanism to explain male-specific sterility associated with chromosomal translocations in *Drosophila* and mice (Lifschytz and Lindsley 1972; Forejt 1984) and later shown as a chromosome-wide transcriptional silencing of X and Y chromosomes (Turner 2007). All testis-expressed genes within the X-linked *Hstx2* locus, including miRNA gene clusters, are silenced by MSCI in pachynemas of fertile males (Royo et al. 2015), but their silencing is disturbed in sterile F1 hybrid males (Bhattacharyya et al. 2013; Campbell et al. 2013; Larson et al. 2022). Furthermore, the adverse effect of continuous expression of the *Hstx2* locus-linked miRNA genes on spermatogenesis was shown after their continuous expression from the autosome-integrated transgenes (Royo et al. 2015).

The role of MSCI as a component of postzygotic reproductive isolation between species does not need to be restricted to mammals. For example, the evidence for silencing the X-linked genes in the first meiotic division comparable to MSCI was reported in *C. elegans* (Kelly et al. 2002), and transient silencing of the W chromosome was shown in female meiosis of chicken (Schoenmakers et al. 2009). In *Drosophila*, *MSCI* has been considered controversial (Vibranovski et al. 2009; Meiklejohn et al. 2011; Meiklejohn and Presgraves 2012). Recently, however, a combination of single-cell RNA sequencing and fluorescence in situ hybridization (FISH) revealed transcriptional silencing of the X chromosome localized to a distinct territory of *Drosophila* primary spermatocyte nuclei. Unlike mammalian MSCI, the Y chromosome is transcriptionally active, and the X-derived diploid minichromosome 4 is silenced in *Drosophila* spermatocytes (Mahadevaraju et al. 2021).

The Role of Homolog Recognition in HS. A Hypothesis

We hypothesize that some of the observed complexity of HS genetic architecture can be of chromosomal origin,

namely that recognition between evolutionarily diverged homologs may function as a chromosomal checkpoint. The mechanism of this type of chromosomal HS may differ in different species, operating either premeiotically or during the first meiotic prophase. A significant cytological landmark of homolog recognition failure may be the occurrence of incomplete homolog pairing in meiotic prophase I, leading to an early meiotic arrest or later to the formation of aneuploid spermatids of sterile F1 hybrid males. Such a homolog's recognition-dependent mechanism may have been difficult to distinguish from that of polygenic control of HS without concurrent examination of meiotic pairing. Besides asynapsis in *musculus* × *domesticus* hybrids reviewed here, the incomplete meiotic synapsis and potential involvement of homologs mutual recognition were found in sterile hybrids of *Mus m. domesticus* and *Mus spretus* (Hale et al. 1993), in sterile mules and hinnies (Chandley et al. 1975), in interspecific hybrids of *Bos taurus* and *Bos grunniens* (Tumennasan et al. 1997), house musk shrews (Borodin et al. 1998), and gray voles (Torgasheva and Borodin 2016; Bikchurina et al. 2021). Failure of regular chromosome pairing was also shown in plant hybrids (Lee et al. 2011; Addo Nyarko and Mason 2022).

Surprisingly scarce is the information on meiotic homolog recognition in *Drosophila* hybrids, the paradigm of HS genetics. Dobzhansky reported univalents and other meiotic pairing disorders in the diakinesis of sterile hybrid males of *Drosophila pseudoobscura* (Dobzhansky 1933). In later studies, the studied phenotype of interspecific hybrid infertility was mainly limited to tests of sperm motility and the ability to produce offspring (Coyne 1984; Orr and Irving 2005; Moehring et al. 2006). In a classical genetic experiment that could suggest homologs' incompatibility, *Drosophila koepferae* chromosome segments were introgressed into the *Drosophila buzzati* genome. Male sterility occurred when >40% of a chromosome length was transferred but never occurred if less than 30% was involved, regardless of the chromosomal location of exchange or the autosome tested (Naveira and Fondevila 1991; Naveira and Fontdevila 1991; Naveira and Maside 1998). Based on this and similar experiments, the genetic architecture of HS has been characterized as an epistatic interaction of interchangeable HS polygenes, a hundred or more per genome, that activate the mechanism of HS after exceeding a certain threshold (Davis and Wu 1996; Presgraves and Meiklejohn 2021). An alternative explanation pointing to insufficient homolog recognition and subsequent sperm aneuploidy, reminiscent of the effect of consubspecific intervals on homologous synapsis and infertility in mouse hybrids (Gregorova et al. 2018), could be tested by examining the meiotic pairing of chromosomes with heterospecific introgressions on interspecific F1 hybrid background.

Admittedly, failure of meiotic synapsis is a relatively common phenotype associated with the dysfunction of many testis-expressed genes, hence distinguishing between a *trans*-acting genic cause of asynapsis and a *cis*-acting chromosomal incompatibility in interspecific hybrids is not a trivial task. Nevertheless, the first unequivocal evidence of a chromosome-autonomous failure of the homologous synapsis in sterile F1 hybrids was provided by chromosome substitutions between the genomes of *musculus* and *domesticus* subspecies (Bhattacharyya et al. 2013; Gregorova et al. 2018).

Engagement of the Mismatch Repair Mechanism in Homolog Recognition of Sterile Hybrids

The proposed chromosomal mechanism of HS anticipates decreasing efficiency of homolog's mutual recognition with their increasing evolutionary divergence. However, little is known about the molecular mechanism monitoring the level of sequence divergence. In the prokaryotic model and budding yeast, the homology checkpoint operates by the antirecombination mechanism of the mismatch repair machinery (MMR). The 10^{-4}-fold inhibition of prokaryotic recombination between *Escherichia* and *Salmonella* was reversed in a receiver strain deficient in MutS or MutL MMR function (Rayssiguier et al. 1989). In this and the following experiments (see Radman 2022 for review), the deletion of the antirecombination function of MMR overcame 20% of sequence divergence between the two bacterial genera. Antirecombination is also the major checkpoint of HS in hybrids between *S. cerevisiae* and *S. paradoxus*, as shown above.

How universal the antirecombination MMR mechanism is as a reproductive barrier in other species remains unclear. The restriction of antirecombination to small genomes, as in the case of *S. cerevisiae* (12 Mbp), seems unlikely because the allohexaploid wheat (1,700 Mbp) carries three homeologous genomes whose mixing by meiotic recombination between homeologous chromosomes is prevented by the *Ph1* and *Ph2* loci (for review Bomblies 2022). Recently, the *Ph2* (pairing homeologous 2) locus was identified with the MHS7, a paralog of the eukaryotic MMR gene MSH6 (Serra et al. 2021) and like in *S. cerevisiae*, deletion of MSH7 in wheat/Aegilops hybrids resulted in a 5-fold increase in the homeologous recombination (Serra et al. 2021), most probably by promoting dissociation of the invading strand from a homeologous template by heteroduplex rejection (Chakraborty and Alani 2016).

The role of MMR in inhibiting homeologous recombination thus resembles the heteroduplex rejection mechanism responsible for the sterility of interspecific *Saccharomyces* hybrids. However, the role of MMR in mammalian HS, particularly in protecting against degenerate PRDM9 binding motifs, remains unknown. The MSH2 antirecombination mechanism strictly reduced homologous recombination between polymorphic sites in mammalian somatic cells (Smith et al. 2007; Larocque and Jasin 2010), but did not distinguish between the polymorphic and nonpolymorphic variants of two meiotic recombination hotspots in an intrasubspecific (*Mus m. domesticus*) cross. (Peterson et al. 2020). The authors conclude that in mammals, the sequence divergence threshold for MMR-directed heteroduplex rejection may be higher in meiotic than somatic cells.

Conclusions and Future Directions

The nongenic effect of DNA divergence as a reproductive barrier between species was first described by Radman in recombination suppression between prokaryotes (Matic et al. 1996) and later documented as a primary cause of HS in budding yeast species (Hunter et al. 1996; Ono et al. 2020; Bozdag and Ono 2022). Here, we present allelic incompatibility of PRDM9 binding sites in *Mus musculus* subspecies hybrids as the best-documented example of homolog recognition failure due to evolutionary divergence of noncoding DNA and discuss indications of this form of chromosomal HS in *Drosophila* hybrids. In addition, other mutually nonexclusive forms of chromosome-based HS have been proposed, such as the possible role of ncRNA in the homology search (Ding et al. 2019; Sybenga 2020).

The complexity of the genetic architecture of HS may have been artificially increased by rather broad definitions of the HS phenotype, such as the percentage of live and dead sperm cells in *Drosophila* hybrids, the percentage of inviable spores in budding yeast, or the relative testes weight and sperm count in mouse hybrids. Including the cytological and molecular phenotypes of meiotic (premeiotic) pairing may contribute to the genetic analysis of HS and help validate the role of nongenic effect of DNA divergence in HS in *Drosophila* and house mouse models.

Acknowledgments

We thank Jiri Forejt's laboratory for the discussions and the three reviewers' valuable comments. This work was supported by the Czech Science Foundation grant No.:22-29928S.

Data Availability

There are no new data to report in this review.

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