Published in final edited form as:
Evolution. 2008 January ; 62(1): 50–65. doi:10.1111/j.1558-5646.2007.00257.x.

ASYMMETRY AND POLYMORPHISM OF HYBRID MALE STERILITY DURING THE EARLY STAGES OF SPECIATION IN HOUSE MICE

Jeffrey M. Good[1,2], **Mary Ann Handel**[3,4], and **Michael W. Nachman**[1,5]

[1] Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

[3] The Jackson Laboratory, Bar Harbor, Maine 04609

Abstract

House mice offer a powerful system for dissecting the genetic basis of phenotypes that isolate species in the early stages of speciation. We used a series of reciprocal crosses between wild-derived strains of *Mus musculus* and *M. domesticus* to examine F_1 hybrid male sterility, one of the primary phenotypes thought to isolate these species. We report four main results. First, we found significantly smaller testes and fewer sperm in hybrid male progeny of most crosses. Second, in some crosses hybrid male sterility was asymmetric and depended on the species origin of the X chromosome. These observations confirm and extend previous findings, underscoring the central role that the *M. musculus* X chromosome plays in reproductive isolation. Third, comparisons among reciprocal crosses revealed polymorphism at one or more hybrid incompatibilities within *M. musculus*. Fourth, the spermatogenic phenotype of this polymorphic interaction appears distinct from previously described hybrid incompatibilities between these species. These data build on previous studies of speciation in house mice and show that the genetic basis of hybrid male sterility is fairly complex, even at this early stage of divergence.

Keywords

Hybridization; polymorphism; reproductive isolation; speciation

Identifying the specific mutations that isolate incipient species in nature remains a central problem in evolutionary biology. In animals, one common form of isolation occurs when reproduction between two populations results in hybrid offspring with reduced fertility or viability independent of external factors (i.e., intrinsic postzygotic isolation). According to the simple genetic model developed by Bateson (Bateson 1909), Dobzhansky (Dobzhansky 1937), and Muller (Muller 1942), intrinsic postzygotic isolation often evolves due to incompatible mutations at interacting genes (i.e., Dobzhansky-Muller [D–M] incompatibilities). For example, consider two populations with alternative genotypes fixed at two interacting loci, *AAbb* and *aaBB*, where *aabb* was the ancestral genotype. Reproduction between these two populations will produce an *AaBb* F_1 hybrid genotype that may have reduced fitness because *A* and *B* have never been tested together by natural selection. There is now considerable empirical support for this model (Coyne and Orr 2004), including multiple studies that have implicated specific genes involved in hybrid sterility

[2]jgood@email.arizona.edu
[4]maryann.handel@jax.org
[5]nachman@email.arizona.edu

(Ting et al. 1998) or inviability (Wittbrodt et al. 1989; Barbash et al. 2003; Presgraves et al. 2003; Brideau et al. 2006; Harrison and Burton 2006).

Despite these fundamental insights, we still know little about the genetic details of reproductive isolation during the earliest stages of speciation. This is because many genetic studies of speciation have relied upon crosses between divergent species that do not hybridize in nature (e.g., Barbash et al. 2003; Presgraves et al. 2003; Brideau et al. 2006). In such cases it is impossible to determine if phenotypes contributing to isolation in the laboratory were ever relevant to gene flow between natural populations (Harrison 1990; Orr and Presgraves 2000). Furthermore, several of the predictions of the D–M model depend critically upon the degree of functional divergence between two hybridizing genomes. For example, under one common model of speciation the number of D–M incompatibilities is expected to increase much faster than linearly with time (Orr 1995). When considering very divergent species, it is likely that many incompatible mutations arose long after the completion of reproductive isolation in nature (Orr 1995). Whether the genetic details of postisolation incompatibilities are representative of the processes that directly contributed to speciation remains an open question.

During the early stages of speciation, reproductive isolation might exhibit patterns not seen at later stages of divergence. For example, consider the general result that F_1 hybrid sterility or inviability usually manifests in the heterogametic sex first (i.e., Haldane's rule; Haldane 1922; Coyne and Orr 1997; Laurie 1997; Orr 1997). A primary explanation for Haldane's rule is the exposure of epistatic interactions between recessive sex-linked and dominant autosomal D–M incompatibilities in the heterogametic sex (i.e., the dominance theory; Turelli and Orr 1995, 2000). For taxa with heterogametic males, including mammals and *Drosophila*, this typically involves X-autosome interactions. Even at low levels of divergence Haldane's rule is often observed regardless of the direction of the intercross (Coyne and Orr 1989a, 1997), presumably because X-linked incompatibilities have accumulated independently in both lineages. However, if the early stages of isolation involve X-linked incompatibilities in one lineage only, then F_1 hybrid male dysfunction may initially be asymmetric and depend upon the maternal origin of the X.

The strength of reproductive isolation may also depend on whether individual incompatibilities are fixed between diverging populations. Most theoretical treatments of the D–M model assume the instantaneous fixation of incompatible mutations between populations (e.g., Orr 1995;Turelli and Orr 1995;Orr and Orr 1996). On the other hand, several examples of intraspecific variation in the degree of postzygotic isolation between plant (e.g., Stebbins 1958;Christie and Macnair 1987;Sweigart et al. 2007) and animal species (e.g., Gordon 1927;Patterson and Stone 1952;Forejt and Iványi 1975;Wade and Johnson 1994;Reed and Markow 2004;Kopp and Frank 2005;Shuker et al. 2005;Vyskocilová et al. 2005;Demuth and Wade 2007) have been described. Although only a few studies have examined natural variation in the context of specific loci (Forejt and Iványi 1975;Christie and Macnair 1987;Vyskocilová et al. 2005;Sweigart et al. 2007), all of these data are consistent with polymorphism of D–M incompatibilities.

House mice provide a particularly powerful system for studying the early stages of speciation, with considerable genetic and genomic resources (Dietrich et al. 1996; Mouse Genome Sequencing Consortium 2002; Su et al. 2004; Shifman et al. 2006). House mice are comprised of at least three closely related lineages (*Mus domesticus*, *M. musculus*, and *M. castaneus*) that have diverged from a common ancestor within the last 0.5 million years ago (MYA; She et al. 1990; Boursot et al. 1993). This recent divergence is reflected in the literature by the alternative taxonomic treatment of the three lineages as subspecies of *M. musculus* (i.e., *M. m. domesticus*, *M. m. musculus*, and *M. m. castaneus*). The most studied

pair from the standpoint of speciation is *M. domesticus* and *M. musculus*. The native range of *M. domesticus* occurs in Western Europe, North Africa, and the Middle East, whereas *M. musculus* is found in Eastern Europe and Northern Asia (Boursot et al. 1993). The two species form a narrow hybrid zone along ~ 2000 km in Europe (Boursot et al. 1993; Sage et al. 1993). Although the exact phenotypes governing reproductive isolation across the hybrid zone are unresolved, some hybrid populations have elevated parasite loads (Sage et al. 1986; Moulia et al. 1991, 1993) and reduced testis size (Britton-Davidian et al. 2005). In the hybrid zone, the X chromosome shows reduced introgression relative to autosomal loci across multiple transects (Tucker et al. 1992; Dod et al. 1993; Munclinger et al. 2002; Macholán et al. 2007), suggesting a large contribution of the X chromosome to reproductive isolation.

Complementary to hybrid zone studies, several laboratory experiments support the notion that *M. domesticus* and *M. musculus* are isolated by hybrid male sterility (Table 1). Two sets of D–M incompatibilities have been described between *M. domesticus* and *M. musculus*: one set of dominant autosomal epistatic interactions (≥ 3 major sterility factors) that include one or more tightly linked loci on chromosome 17 (*Hybrid sterility 1* or *Hst1*; Forejt and Iványi 1975;Forejt et al. 1991;Vyskocilová et al. 2005) and one set of X-autosome interactions (Storchová et al. 2004;Britton-Davidian et al. 2005; see also Oka et al. 2004,2007). Interestingly, some of the underlying D–M incompatibilities involved in hybrid male sterility, including *Hst1*, are apparently not fixed within these species (Forejt and Iványi 1975;Vyskocilová et al. 2005). However, many of these studies are compromised by the use of classic laboratory inbred strains to represent *M. domesticus* (Table 1). Although primarily of *M. domesticus* origin (> 80%), the genomes of most classic inbred strains include substantial genetic contributions from both *M. musculus* and *M. castaneus* (Wade et al. 2002;Frazer et al., 2007;Yang et al., 2007). The result is a collection of introgressed hybrid genomes (Wade and Daly 2005) shaped by epistatic selection against D–M incompatibilities (Payseur and Hoekstra 2005) and strong artificial laboratory selection (Petkov et al. 2005). Thus, the species origin, existence of natural polymorphism, and biological relevance of D–M incompatibilities remain ambiguous in crosses involving classic inbred strains.

Data from crosses involving exclusively wild-derived mice are more limited, and most of these have used outbred mice. In some cases, crosses between the two species produce fully fertile hybrid males (Vanlerberghe et al. 1986), whereas others show reduced hybrid male fertility with variation in both the strength and direction of sterility (Alibert et al. 1997; Britton-Davidian et al. 2005). The genetic basis of this variation is very difficult to discern with outbred wild mice. The most comprehensive study of hybrid male sterility using wild mice was performed by Britton-Davidian et al. (2005), who used both outbred and inbred strains (crosses 8–11, Table 1). The inbred strains consisted of one from each species (PWK representing *M. musculus* and WLA representing *M. domesticus*). In crosses between these strains, they found asymmetric F_1 male sterility consistent with an X-linked locus in *M. musculus* interacting with one or more autosomal loci in *M. domesticus*. Britton-Davidian et al. (2005) did not cross PWK to other strains and thus could not assess the generality of this pattern. Asymmetric sterility has not been observed broadly in other experiments using wild mice (Vanlerberghe et al. 1986; Alibert et al. 1997; Britton-Davidian et al. 2005) and/or classic inbred strains (Vyskocilová et al. 2005).

Here, we examine patterns of hybrid male fertility between *M. musculus* and *M. domesticus* using a series of reciprocal crosses between multiple wild-derived inbred and outbred strains of mice. Our goals are to test the generality of several previous findings across different genetic backgrounds, to describe the number of different sets of interacting loci, and to develop these findings in the context of specific wild-derived inbred strains that can subsequently be used for mapping of genes involved in mouse speciation. We address four

primary questions: (1) Do F_1 hybrid males show reduced fertility in crosses involving wild-derived inbred lines? (2) Is hybrid male sterility asymmetric? (3) Are D–M incompatibilities polymorphic within a single species? (4) At what developmental stage does hybrid sterility arise?

Materials and Methods

ANIMALS AND HUSBANDRY

Breeding colonies for each of four wild-derived inbred strains were established from individuals purchased from the Jackson Laboratory (Bar Harbor, ME). Within *M. domesticus* we used the strains *M. domesticus*$^{LEWES/EiJ}$ and *M. domesticus*$^{WSB/EiJ}$ (hereafter *domesticus*^{LEWES} and *domesticus*^{WSB}). Both were isolated from natural populations in eastern North America (Delaware and Maryland, USA, respectively) and represent a recent range expansion of *M. domesticus* associated with human migration from Western Europe. Within *M. musculus* we used two strains isolated from different localities outside of the hybrid zone within the Czech Republic, *M. musculus*$^{CZECHII/EiJ}$ and *M. musculus*$^{PWK/PhJ}$ (hereafter *musculus*^{CZECH} and *musculus*^{PWK}). All four strains have the standard house mouse karyotype ($2n = 40$). Detailed information on the known history of each strain is available from the Jackson Laboratory (www.jax.org). In addition, we founded an outbred colony of *M. musculus* derived from mice collected by J. Piálek in the eastern portion of the Czech Republic during 2003–2004 (vicinity of Studenec, 49°11′N, 16°03′E). Starting with eight initial founding pairs, we maintained four breeding pairs per generation using a crossing scheme designed to maximize inbreeding avoidance (Wright 1921). All crosses using outbred mice involved males from the fourth or fifth laboratory generation since collection. The expected inbreeding coefficient after five generations of breeding under our scheme is $f = 0.0625$, assuming the founding mice were unrelated. Because some of the founders were collected from the same locality, the actual inbreeding coefficient may be higher. Mice were maintained at the University of Arizona Central Animal Facility in accordance with IACUC regulations. Breeding pairs were housed two per cage and pregnant females were isolated and caged individually prior to giving birth.

EXPERIMENTAL DESIGN

We conducted two kinds of crosses. First, we performed control crosses between the two wild-derived inbred strains within each species to establish null expectations for patterns of F_1 male fertility in each species. These controls are important for removing the effects of inbreeding depression. For *M. musculus* we crossed female *musculus*^{PWK} with male *musculus*^{CZECH}, and for *M. domesticus* we crossed female *domesticus*^{LEWES} with male *domesticus*^{WSB}. Second, we performed 11 different types of interspecific crosses. With two strains per species a total of eight pairwise combinations are possible (Fig. 1A, B). We performed two additional crosses involving inbred females from each species (*musculus*^{PWK}, *domesticus*^{LEWES}) mated to intraspecific F_1 males to evaluate the segregation of hybrid sterility including the potential role of the Y chromosome (Fig. 1C). Finally, to extend the generality of our findings, we performed a series of interspecific crosses between inbred *domesticus*^{LEWES} females and outbred *M. musculus* males.

Interspecific crosses involving *musculus*^{PWK} are potentially informative regarding the presence of the *Hst1* sterility system in the *domesticus*^{LEWES} and *domesticus*^{WSB} strains. *Hst1*-related sterility was described based on crosses between classic inbred strains and wild-caught *M. musculus* (Table 1). Sterility is caused by interactions between one or more tightly linked loci on chromosome 17 (*Hst1*; Forejt and Iványi 1975;Forejt et al. 1991) and several other loci (as yet uncharacterized). The *Hst* locus contains alleles in both classic inbred strains (sterile allele, *Hst1*^{s}; fertile allele, *Hst1*^{f}) and wild-derived *M. musculus*

(sterile allele, Hst^{ws}; fertile allele, Hst^{wf}). Sterility occurs in male F_1 hybrid mice that are heterozygous $Hst1^{s}/Hst^{ws}$. The *musculus*^{PWK} strain is derived from wild-caught *M. musculus* mice and is thought to be fixed for the sterility-ensuring Hst^{ws} allele (Forejt 1981). Crosses between male *musculus*^{PWK} and some classic inbred strains (e.g., C57BL/10, BALB/c) yield sterile males characterized by spermatogenic arrest at the pachytene spermatocyte stage (Forejt 1981;Chubb and Nolan 1987). Thus, if the $Hst1^{s}$ allele and related interacting loci are present in *domesticus*^{LEWES} or *domesticus*^{WSB}, then sterile sons with early meiotic arrest should result when crossed with male *musculus*^{PWK}. This assumes that the *musculus*^{PWK} strain in our experiment retained *Hst1*-related sterility described in earlier experiments (Forejt 1981;Chubb and Nolan 1987).

A minimum of four crosses was attempted per experimental treatment. Male offspring were weaned at approximately 20 days postpartum and housed in sibling groups of up to four. To reduce potential variance in male fertility associated with social dominance interactions, males were separated into individual cages at 40 days and maintained in isolation until being sacrificed.

QUANTIFICATION OF MALE FERTILITY PARAMETERS

We considered multiple male reproductive phenotypes including testis weight, sperm count, sperm motility, and seminal vesicle weight. In mice, testis weight is highly correlated with sperm count and provides a good measure of the overall reproductive status whereas seminal vesicle weight is highly sensitive to serological levels of testosterone (Forejt and Iványi 1975). Throughout we use the terms “sterility” and “fertility” to reflect general patterns in multiple fertility-related phenotypes. Nevertheless, hybrid male sterility is a complex phenotype and in some cases males with dramatically reduced gamete production will nevertheless be capable of siring some offspring (see below).

All males were sacrificed at an age of 60 days unless noted. In mice, spermatogenesis starts at birth (Eddy 2002), weaning occurs ~20 days postpartum (Pelikán 1981), and males are capable of inseminating females by the age of 50 days (J. M. Good, pers. obs.; L. Drickamer, unpubl. data). For each male, testes and seminal vesicles were immediately dissected and placed in sealed Eppendorf tubes until they could be weighed. We also collected body weight and standard size measurements including length of total body, tail, right hind foot, and right ear.

We evaluated sperm motility and counts using a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) and a light microscope at 200× magnification. Sperm suspensions were created by placing the caudal epididymides in a prewarmed (37°C) watch glass with 1 mL of modified Phosphate Buffered Saline (Modified Dulbecco’s solution; www.jax.org/cryo/media.html). The paired caudal epididymides were cut into pieces with a razor blade, covered with parafilm, and allowed to incubate at 37°C for 10 m. Following incubation the epididymides were removed with forceps and the solution was gently mixed with a 200 mL pipettor using a wide-bore tip. To estimate sperm motility, we transferred 5 μl of sperm suspension to the Makler chamber and immediately counted the number of motile and immotile sperm. Overlaid onto the cover slip of the Makler chamber is a 1 mm^2 10 × 10 grid. We arbitrarily chose one row of 10 squares, recording the total number of motile sperm summed across squares with a 10 sec observation time per square. We then recorded the total number of nonmotile sperm per row and calculated the proportion of motile sperm. To estimate overall sperm counts, 200 mL of the incubated sperm suspension was transferred to a 0.6-mL tube and heat shocked at 60°C for 5 min to stop motility. This aliquot was then gently mixed and 5 μl was transferred to the Makler chamber. The average number of sperm heads was determined across five rows where each row represents an estimate of sperm concentration in million per 1 mL. In general, these methods were

characterized by high precision. For example, we found relatively low variance among 10 independent estimates of sperm count from the same male (mean = 16.5×10^6 sperm, SD = 1.1).

For a subset of males we examined fecundity based on litter size following pairing with *musculus*^{PWK} females. F_1 males (> 60 days postpartum) were caged with a single female and each male was paired successively with two separate females. Females were separated when pregnant or after 20 days. Litter size was recorded within 5 days postpartum. We chose *musculus*^{PWK} females for this assay because they consistently produce larger litters than the other three inbred strains.

TESTIS HISTOLOGY

Testis histological cross-sections were examined in four intraspecific males ($N = 2$, ♀ *musculus*^{PWK} × ♂ *musculus*^{CZECH}; $N = 2$, ♀ *domesticus*^{LEWES} × ♂ *domesticus*^{WSB}) and 10 interspecific males ($N = 2$, ♀ *musculus*^{PWK} × ♂ *domesticus*^{LEWES}; $N = 2$, ♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK}; $N = 1$, ♀ *musculus*^{CZECH} × ♂ *domesticus*^{LEWES}; $N = 1$, ♀ *domesticus*^{LEWES} × ♂ *musculus*^{CZECH}; $N = 2$, ♀ *musculus*^{CZECH} × ♂ *domesticus*^{WSB}; $N = 2$, ♀ *domesticus*^{WSB} × ♂ *musculus*^{CZECH}). For each male, we transferred a single whole testis into Bouin's solution (Ricca Chemical) immediately following sacrifice. Following 24 h of fixation in Bouin's solution, testes were progressively dehydrated with 30-min ethanol washes (×2 25%, ×2 50%, ×4 70%, ×2 100%). Dehydrated testes were embedded in paraffin, cut into 5-μm cross-sections, stained using periodic acid-Schiff (PAS) according to standard protocols. Histological cross-sections were examined and classified as normal or abnormal based on standard criteria for the progression of mouse spermatogenesis (Russell et al. 1990). All specimens were analyzed blind with respect to their genotype and sample classifications were independently verified.

MOLECULAR ANALYSES

Male fertility within both *M. domesticus* and *M. musculus* can be strongly influenced by the *t* allele (Silver 1985). To detect the presence of the *t* bearing haplotype in crosses involving outbred *M. musculus* males we performed a length polymorphism PCR assay using a previously developed protocol for the *Tcp-1* locus (Planchart et al. 2000). This assay is diagnostic of *t* haplotypes versus wild-type alleles but does not discriminate among different *t* haplotypes. DNA was extracted from fresh tissue using a Purgene DNA isolation kit (Gentra Systems, Minneapolis, MN).

Results

INTRASPECIFIC COMPARISONS

We collected data on several characters related to male fertility for a total of 136 inter- and intraspecific F_1 males (all 60-day old) from 59 litters. We first considered F_1 males from crosses between two inbred strains within each species (Table 2). Consistent with known species differences, *M. domesticus* males were significantly larger than *M. musculus* males for both body (Wilcoxon $P = 0.0091$) and testis weight (Wilcoxon $P = 0.0140$). For our combined intraspecific data we found a strong positive association between body weight and testis weight ($r^2 = 0.58$, $F_{1,18} = 24.94$, $P < 0.0001$), seminal vesicle weight ($r^2 = 0.56$, $F_{1,18} = 22.76$, $P = 0.0002$), and sperm count ($r^2 = 0.34$, $F_{1,18} = 9.46$, $P = 0.0065$) but a weak negative association between body weight and sperm motility ($r^2 = 0.23$, $F_{1,18} = 5.47$, $P < 0.0311$). To correct for size-related differences, we also report testis and seminal vesicle weight (mg) relative to body weight (g) (i.e., Relative Testis Weight, RTW; Relative Seminal Vesicle Weight, RSVW).

INTERSPECIFIC COMPARISONS

Most interspecific F_1 hybrid males had strongly reduced male reproductive parameters. Half of the crosses between *M. domesticus* and *M. musculus* produced males that were significantly larger than males from intraspecific control crosses (Table 2). In contrast, six of the eight interspecific crosses produced males with severely reduced testis weights and sperm counts. Both crosses involving a male *musculus*^{PWK} produced reproductively normal hybrid males and will be discussed in more detail below. In the six crosses yielding sterile males the average hybrid RTW ranged between 38% (♀ *domesticus*^{WSB} × ♂ *musculus*^{CZECH}) and 62% (♀ *musculus*^{PWK} × ♂ *domesticus*^{WSB}) of the average for the combined intraspecific control set. Likewise, approximately 40% (22/54) of the males from these six crosses contained no sperm in their caudal epididymides. Because sperm numbers were low or absent, reliable estimates of sperm motility in progeny from these crosses were not possible. General qualitative differences between groups given in Table 2 remained consistent when considering older males (> 60-day old). For example, the strong asymmetrical reduction in testis weights and sperm counts observed in the reciprocal cross between *musculus*^{PWK} and *domesticus*^{LEWES} was still apparent in males 110 days and older ($N = 44$, data not shown). We observed approximately equal sex ratios for interspecific crosses (39 litters, 198 offspring, 51.0% male) with no significant heterogeneity across cross types ($\chi^2 = 7.82$, $P = 0.55$, df = 9).

Hybrid male sterility was partially asymmetric—Crosses involving female *musculus*^{PWK} and male *M. domesticus* (*domesticus*^{WSB} or *domesticus*^{LEWES}) produced male progeny with significantly reduced testis weights and sperm counts (Fig. 2B, Table 2). In contrast, the reciprocal crosses involving a male *musculus*^{PWK} produced reproductively normal hybrid males (Fig. 2C, Table 2). Males sired from reciprocal crosses share the same autosomal genotype but differ in the species origin of the sex chromosomes (Fig. 1B). In these crosses, reduced fertility was observed when hybrid males had a *M. domesticus* Y chromosome and a *M. musculus* X chromosome. Thus, at least one set of hybrid incompatibilities involved one or both of the sex chromosomes. As part of an ongoing research program, we have introgressed the *musculus*^{PWK} X chromosome onto the *domesticus*^{LEWES} autosomal background over several backcross generations. In these backcrosses, male sterility segregates with the *M. musculus* X chromosome (J. M. Good, unpubl. data).

To test if hybrid sterility was due to a common set of incompatibilities that are fixed across strains of *M. domesticus*, we crossed *musculus*^{PWK} females to *M. domesticus*$^{LEWES×WSB}$ F_1 males. Consistent with a common set of incompatibilities, all 13 males demonstrated significantly reduced reproductive parameters (Table 3). However, given the sample size we cannot exclude a more complicated scenario involving multiple independent loci within each strain.

Asymmetric fertility was also apparent in patterns of fecundity. Five crosses between ♀ *musculus*^{PWK} and F_1 males from the ♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK} cross produced an average of 6.4 offspring per litter (± 1.7 SD) whereas five crosses between ♀ *musculus*^{PWK} and F_1 males from the female ♀ *musculus*^{PWK} × ♂ *domesticus*^{WSB} yielded an average of 2.4 offspring per litter (± 2.9 SD). Although small sample sizes preclude significance (Wilcoxon $P = 0.0555$), this result is qualitatively consistent with our expectations given the differences in testis weight and sperm count between these groups (Table 2).

One or more sterility factors were polymorphic in *M. musculus*—Hybrid fertility was dramatically reduced in interspecific crosses involving either male or female

musculus^{CZECH} (i.e., a symmetric reduction in reproductive parameters), whereas normal fertility occurred in intercrosses involving a male *musculus*^{PWK} (i.e., an asymmetric reduction in reproductive parameters; Table 2; Fig. 2C). At a minimum, symmetric F_1 sterility requires either multiple sets of sex-linked incompatibilities or a single set of exclusively autosomal interactions. Given the existence of X-linked incompatibilities in crosses involving female *musculus*^{PWK}, these *musculus*^{CZECH} data indicate that at least two different sets of incompatibilities underlie hybrid male sterility in our crosses. Further, one or more incompatibilities involved in one set were not present in *musculus*^{PWK} and thus are polymorphic within *M. musculus*.

In principle, F_1 male sterility in crosses between female *M. domesticus* and male *musculus*^{CZECH} could be due to either autosomal or Y-linked *M. musculus* incompatibilities. To explore this in more detail, we crossed *domesticus*^{LEWES} females to *M. musculus*$^{PWK \times CZECH}$ F_1 males. Hybrid males from these crosses will have a *musculus*^{CZECH} Y, a *domesticus*^{LEWES} X, and a heterozygous autosomal background with *domesticus*^{LEWES} alleles combined with a random complement of *musculus*^{CZECH} or *musculus*^{PWK} alleles (Fig. 1C). If F_1 sterility maps to the *musculus*^{CZECH} Y chromosome and has a fairly simple genetic basis, we would expect all males from this cross to continue to have significantly reduced fertility. Contrary to this, we observed considerable variance in male reproductive parameters with five of 10 males showing at least partial recovery of fertility, defined as testis weights and sperm counts all exceeding the range observed in the sterile ♀ *domesticus*^{LEWES} × ♂ *musculus*^{CZECH} cross (Table 3). These data are consistent with one or more sterility factors on the *musculus*^{CZECH} autosomal background.

The dramatic difference in hybrid fertility observed between interspecific crosses involving the two different *M. musculus* genotypes raises the question of the status of this apparent polymorphism in natural populations of *M. musculus*. To begin to address this issue we analyzed 20 hybrid males descended from crosses between female *domesticus*^{LEWES} and a total of seven out-bred *M. musculus* sires derived from wild mice collected in eastern Czech Republic. Six sires were from the fifth generation of our outbred colony whereas the seventh sire was from the fourth generation. Many of the hybrid males had reproductive parameters within the “normal” range of parameters characteristic of the control intraspecific crosses and the ♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK} interspecific cross, whereas a subset showed fairly severe reductions in RTW and/or sperm counts (Fig. 2D). Eighteen of the 20 hybrid males we considered were heterozygous for *t* haplotypes based on the *Tcp-1* PCR diagnostic (Planchart et al. 2000). In contrast, *t* alleles were completely absent from all crosses involving wild-derived inbred strains (as expected). Two observations argue that segregation of *t* haplotypes does not explain the reproductive variance among hybrid males we observed. First, of the two wild-type males, one was reproductively normal whereas the other had reduced testis weight and low sperm counts. Second, we observed both normal and sterile *t* bearing individuals segregating within the same pedigree (i.e., with the same *t* allele) arguing against the potential influence of different variants of the *t* locus. These data are thus consistent with results from crosses involving wild-derived inbred lines in suggesting that hybrid sterility factors are polymorphic within natural populations of *M. musculus*.

Reduced hybrid fertility involved primarily postmeiotic disruption of spermatogenesis—PAS-stained histological cross-sections were assessed to determine the extent of spermatogenesis and any abnormal features (Fig. 3). Spermatogenesis appeared normal in all four intraspecific males (*N* = 2, ♀ *domesticus*^{LEWES} × ♂ *domesticus*^{WSB}; *N* = 2, ♀ *musculus*^{PWK} × ♂ *musculus*^{CZECH}; Fig. 3A) and the two interspecific males from a ♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK} cross (Fig. 3B), consistent with the observation of normal testis weights and sperm counts in these mice (Table 2). In contrast, reduction in spermatogenesis was evident in the remaining eight males analyzed from interspecific

crosses (*N* = 2, ♀ *musculus*^{PWK} × ♂ *domesticus*^{LEWES}; *N* = 1, ♀ *musculus*^{CZECH} × ♂ *domesticus*^{LEWES}; *N* = 1, ♀ *domesticus*^{LEWES} × ♂ *musculus*^{CZECH}; *N* = 2, ♀ *musculus*^{CZECH} × ♂ *domesticus*^{WSB}; *N* = 2, ♀ *domesticus*^{WSB} × ♂ *musculus*^{CZECH}). In these eight males we observed no gross abnormalities in the structure or frequency of primary spermatocytes and many normal-appearing round spermatids with intact acrosomes. However, disruptions in postmeiotic spermiogenesis were present in all eight samples, including fewer than normal elongating and condensing spermatids, abnormal spermatid head morphology and clusters of abnormally swollen and pycnotic cells consistent with necrotic or apoptotic cell death and phagocytosis by Sertoli cells (see Fig. 3C, D for examples). We observed some variation in the degree of disruption among individuals of the same genotype. For example, we observed variation in the degree of spermatogenic disruption for the two males from a cross between ♀ *musculus*^{PWK} × ♂ *domesticus*^{LEWES}. One male was characterized by a strong reduction in the number of elongating and condensing spermatids and the occurrence of abnormal head morphology. Small clusters of abnormally pycnotic cells were also observed in multiple tubule lumens of this male (Fig. 3C). For the other male, spermatogenesis appeared more normal albeit with diminished numbers of maturing spermatids. We also observed variation in the degree of disruption across tubules within individual interspecific males. In these instances, all tubules appeared abnormal but some were characterized by more advanced stages of normal development. One of the hybrid males (♀ *domesticus*^{LEWES} × ♂ *musculus*^{CZECH}) with severely abnormal histology was 90-day old, again confirming that the spermatogenic abnormalities we observed were not strongly age dependent.

Discussion

Using a series of reciprocal crosses between wild-derived inbred strains of *M. musculus* and *M. domesticus*, we found evidence for multiple genetic factors underlying hybrid male sterility. Many hybrid males showed reduced fertility. Reduced hybrid fertility was asymmetric in some crosses, consistent with a large effect of the *M. musculus* X chromosome. Reciprocal crosses also revealed polymorphism within *M. musculus* for one or more loci necessary for reduced hybrid male fertility. Hybrid male sterility was due primarily to postmeiotic disruptions of spermatogenesis. When combined with previous results, these data suggest a fairly complex genetic basis to hybrid male sterility in this system. Below we discuss our findings in relation to other studies on hybrid male sterility in house mice. We also discuss the broader significance of asymmetry and polymorphism in the evolution of reproductive isolation.

ASYMMETRY, X-LINKED STERILITY, AND THE INITIATION OF HALDANE'S RULE

The X chromosome often plays a central role in the evolution of postzygotic reproductive isolation (i.e., the large X-effect; Dobzhansky 1936; Coyne and Orr 1989b; Tao et al. 2003). One of the most striking aspects of our data was the strong asymmetry in hybrid male sterility in reciprocal crosses between *musculus*^{PWK} and either *M. domesticus* strain (Table 2, Fig. 2), suggesting hybrid incompatibilities originate on one or both of the sex chromosomes. Asymmetric F_1 sterility has been shown in one other cross, also involving *musculus*^{PWK} (Britton-Davidian et al. 2005), and our results demonstrate that this pattern is consistent across multiple wild-derived *M. domesticus* genotypes. Two lines of evidence suggest that the observed asymmetric reduction in hybrid fertility was due to interactions involving the *M. musculus* × chromosome. First, we have introgressed the *musculus*^{PWK} X chromosome onto the *domesticus*^{LEWES} autosomal background as part of an ongoing research program to map X-linked hybrid male sterility genes. In these backcrosses, male sterility segregates with the *M. musculus* X chromosome (J. M. Good, unpubl. data; see also Britton-Davidian et al. 2005). Second, hybrid male sterility is caused by substitution of a

wild-derived *M. musculus* X chromosome onto the genomic background of the classic inbred strain C57BL/6 (Storchová et al. 2004). It remains to be seen if the underlying genetic architecture across these examples is the same.

In the hybrid zone, the sex chromosomes show significantly reduced cline widths (i.e., reduced introgression) relative to the autosomes in most transects (Vanlerberghe et al. 1986; Tucker et al. 1992; Dod et al. 1993; Munclinger et al. 2002; Macholán et al. 2007). Significantly reduced cline widths for the X and Y chromosomes are commonly assumed to reflect selection against sex-linked hybrid incompatibilities in the hybrid zone (Tucker et al. 1992; Dod et al. 1993; Payseur et al. 2004; Macholán et al. 2007) and our data on hybrid male sterility are consistent with this interpretation for the X chromosome. Interestingly, genomic regions with very narrow cline widths are also often highly asymmetric in cline shape (Payseur et al. 2004). However, asymmetric patterns of gene flow across the hybrid zone are seen at many X-linked and autosomal loci and are probably strongly influenced by demography (Macholán et al. 2007; Teeter et al., in press). It is unclear whether some of these asymmetries in the hybrid zone are also caused by epistatic interactions.

Dominance theory posits that Haldane's rule reflects the exposure of interactions between recessive X-linked and dominant autosomal incompatibilities in the heterogametic sex (Muller 1942; Turelli and Orr 1995, 2000). X-autosome incompatibilities in F_1 hybrids are different from dominant interactions between autosomal loci in that they depend on the direction of the cross (Fig. 1B). If the initiation of sterility or inviability involves an X-autosome incompatibility, the first mutation in the evolution of F_1 postzygotic isolation will necessarily present asymmetric problems in hybrid males. Thus, asymmetry in the reciprocal crosses between *musculus*^{PWK} and either *M. domesticus* strain (Table 2, Fig. 2) likely reflects the relatively recent divergence of these species.

Haldane's rule for sterility evolves quite rapidly relative to inviability (Wu 1992;True et al. 1996;Coyne and Orr 1997;Sawamura et al. 2000;Tao et al. 2003) and likely has multiple causes in species in which males are the heterogametic sex (Wu and Davis 1993;Wu et al. 1996;Presgraves and Orr 1998). For example, the rapid evolution of hybrid male sterility may occur as a byproduct of intense sexual selection on male reproductive genes and/or an inherent developmental sensitivity of spermatogenesis (Wu and Davis 1993). Both processes may contribute to the evolution of hybrid male sterility in mice. Multiple mating is common in mice, creating a potential arena for intense postcopulatory sexual selection in the form of sperm competition (Dean et al. 2006). Consistent with this there is abundant evidence that genes involved in male reproduction evolve rapidly in mice (Mouse Genome Sequencing Consortium 2002;Winter et al. 2004;Torgerson et al. 2005) and are frequently the target of positive selection (Torgerson et al. 2002;Good and Nachman 2005;Torgerson and Singh 2006). Moreover, gene knockout and mutagenesis models disproportionately cause male sterility suggesting mouse spermatogenesis is exceptionally sensitive to genetic disruptions (Escalier 2001;Handel et al. 2006).

POLYMORPHISM OF DOBZHANSKY–MULLER INCOMPATIBILITIES

The large amount of variation in the degree of hybrid male sterility observed across a range of crosses has long suggested polymorphism within mice for hybrid sterility factors (Table 1). In an important set of experiments, Forejt and colleagues used a series of crosses between classic inbred females and wild-derived *M. musculus* males to describe the *Hst1* sterility system (Forejt and Iváanyi 1975;Forejt 1981;Forejt et al. 1991;Trachtulec et al. 1994,2005). In these crosses, epistatic interactions between three or more loci caused early meiotic arrest in hybrid males (Forejt and Iványi 1975;Forejt 1981). Polymorphism for sterility factors was observed in both species and was shown to represent allelic variation in both classic inbred strains (sterile allele, *Hst1*^{s}; fertile allele, *Hst1*^{f}) and wild-derived *M.*

musculus (sterile allele, Hst^{ws}; fertile allele, Hst^{wf}). The Hst^{ws} and Hst^{wf} alleles have since been shown to be polymorphic in multiple populations of *M. musculus* (Forejt and Iványi 1975;Vyskocilová et al. 2005). We also found evidence of polymorphism within *M. musculus* at one or more hybrid sterility factors; however, the variation we observe is likely distinct from *Hst1* (see below for a detailed discussion).

Understanding how D–M incompatibilities are fixed within natural populations is central to determining which evolutionary forces are most important for the evolution of reproductive isolation (Schluter 2000; Coyne and Orr 2004; Funk et al. 2006). Data on polymorphic male sterility in house mice add to a growing list of species that show intraspecific variation in the strength of intrinsic postzygotic isolation in both plants (Stebbins 1958; Christie and Macnair 1987; Sweigart et al. 2007) and animals (Gordon 1927; Patterson and Stone 1952; Forejt and Iványi 1975; Wade and Johnson 1994; Reed and Markow 2004; Kopp and Frank 2005; Shuker et al. 2005; Vyskocilová et al. 2005). Transient polymorphism is a necessary state of mutations fixed by genetic drift or positive selection; therefore, the documentation of polymorphic reproductive isolation in and of itself does not speak to the prevalence of either force during speciation. Nevertheless, because targets of positive directional selection are less likely to be sampled during the polymorphic state, the frequent occurrence of polymorphic D–M incompatibilities could suggest that nondirectional evolutionary forces play an important role early in speciation (e.g., genetic drift or balancing selection).

Some examples of polymorphic reproductive isolation, including house mice, involve genetic variation for hybrid sterility or inviability segregating within single populations (Forejt and Iványi 1975; Reed and Markow 2004; Kopp and Frank 2005; Shuker et al. 2005; Vyskocilová et al. 2005; Sweigart et al. 2007). The *Hst* sterile and fertile alleles (Hst^{ws} and Hst^{wf}) are polymorphic within multiple localities of *M. musculus* in the Czech Republic and appear to segregate at intermediate frequencies (Forejt and Iványi 1975; Vyskocilová et al. 2005). These data remain one of the clearest examples of naturally segregating variation at a specific D–M incompatibility locus. Likewise, sterility factors segregating between $musculus^{CZECH}$ and $musculus^{PWK}$ reflect variation in reproductive isolation sampled over a small geographic scale. Our intercrosses involving outbred *M. musculus* males from a third locality in the Czech Republic also yielded hybrid male offspring with fertility ranging from normal to severely reduced (Fig. 2D). Although the frequency and overall geographic spread of the underlying D–M incompatibilities remains to be determined, polymorphism at loci involved in reproductive isolations seems to be a common phenomenon in house mice.

Two very different processes could generate polymorphism of D–M incompatibilities in mice. First, D–M incompatibilities could be polymorphic within either mouse species because of interspecific introgression of previously fixed loci. Reduced interspecific gene flow is considered a hallmark of loci directly involved in hybrid incompatibilities (Rieseberg et al. 1999; Payseur et al. 2004) but recombination within a hybrid zone may break up D–M incompatibilities and enable introgression of underlying loci (Virdee and Hewitt 1994). Polymorphism introduced via interspecific gene flow may extend well beyond the boundaries of the mouse hybrid zone due to human-mediated dispersal (Macholán et al. 2007) and could occur in any wild-derived strains of mice. Indeed, a recent genomic scan of SNP variation among multiple wild-derived inbred strains (including WSB) found multiple putative cases of interspecific introgression (Yang et al., 2007).

Alternatively, polymorphism may reflect naturally segregating variation within species. Genetic drift likely plays an important role in the local fixation of Robertsonian chromosomal rearrangements within some populations of *M. domesticus* (Nachman and Searle 1995). In many instances these rearrangements appear weakly under-dominant (Searle 1993) and result in reduced fertility in hybrids between populations fixed for

alternative karyotypes (Piálek et al. 2001). However, drift seems a less plausible explanation for the maintenance of polymorphic D–M incompatibilities in mice, given the apparent frequency and geographic scale at which polymorphism has been observed (Forejt and Iványi 1975; Vyskocilová et al. 2005). In general, determining the frequency and geographic spread of polymorphic D–M incompatibilities will be critical in evaluating the relative importance of natural selection versus genetic drift in the evolution of hybrid male sterility between *M. musculus* and *M. domesticus*.

A COMPLEX GENETIC BASIS TO F $_1$ REPRODUCTIVE ISOLATION IN MICE

The results presented here suggest that at least three sets of F_1 epistatic D–M incompatibilities occur between *M. musculus* and *M. domesticus* (Table 4). It is difficult to speculate on the genetic basis of the sterility polymorphism within *M. musculus* without a direct linkage analysis; however, it is unlikely that the incompatibilities we observed between ♀ *M. domesticus* (both strains) and ♂ *musculus*^{CZECH} involve *Hst1* for the following reasons. First, we observed primarily postmeiotic problems in all impaired hybrid males (Fig. 3) and this general phenotype is distinct from the early meiotic arrest generated by *Hst1* and interacting loci (Forejt 1981;Chubb and Nolan 1987). Second, as described previously, the *musculus*^{PWK} strain has been described to carry the sterility-ensuring *Hst*^{ws} allele (Forejt 1981;Chubb and Nolan 1987), yet the male progeny produced from crossing female *domesticus*^{LEWES} or *domesticus*^{WSB} to male *musculus*^{PWK} were normal. If the strain of *musculus*^{PWK} that we used has retained the *Hst*^{ws} allele described in these previous experiments (Forejt 1981;Chubb and Nolan 1987), then both *domesticus*^{LEWES} and *domesticus*^{WSB} appear to be missing some component of this epistatic interaction necessary for sterility. Consequently, it follows that the male sterility involved in crosses of female *domesticus*^{LEWES} or *domesticus*^{WSB} to male *musculus*^{CZECH} appears distinct from both the *Hst1* and the *musculus* X chromosome D–M incompatibility systems.

These arguments suggest that a third set of F_1 epistatic D–M incompatibilities occur between *M. musculus* and *M. domesticus*. In this set of D–M incompatibilities, one or more of the underlying sterility factors is polymorphic in *M. musculus* (present in *musculus*^{CZECH}, absent in *musculus*^{PWK}). In a recent geographic survey of the *Hst1* incompatibility system, Vyskocilová et al. (2005) described three pedigrees of mice (out of seven) where hybrid male sterility was polymorphic and did not segregate with variation at the *Hst1* locus. Their experiment involved outbred wild *M. musculus*. Therefore, reciprocal crosses controlled for genotype could not be produced. It is unclear whether the polymorphism they described involved loci interacting with *Hst1*, the X-autosome incompatibility system, or a new set of incompatibilities. It is possible that the polymorphism observed in their study was tracking the same sterility factors that are polymorphic between *musculus*^{CZECH} and *musculus*^{PWK}.

Overall, hybrid male sterility in house mice appears more complicated than previously thought (Forejt 1996). Currently, we have little information on the total number or independence of loci involved in each of the three proposed set of epistatic interactions that underlie F_1 hybrid male sterility between *M. musculus* and *M. domesticus*. If the interacting partners in these three putative sets of epistatic interactions were partially nonindependent then the total number of incompatibilities would be reduced. However, the number of hybrid incompatibilities typically increases as a function of experimental resolution (Coyne and Orr 2004) and thus Table 4 likely represents a severe underestimate. Regardless, the observation of polymorphism at multiple loci that have a large impact on F_1 hybrid fertility suggests that resolving the overall genetic architecture of hybrid male sterility may require considerable population-level sampling within both *M. musculus* and *M. domesticus* (Vyskocilová et al. 2005). The unexpected combination of genetic complexity and polymorphism during incipient mouse speciation highlights the utility of this system for studying the early stages of speciation.

Although our approach has been to focus exclusively on hybrid male fertility, it is likely that other phenotypes also play an important role in reproductive isolation between these species. For example, increased hybrid susceptibility to parasites (Sage et al. 1986; Moulia et al. 1991, 1993), female sterility (Britton-Davidian et al. 2005), and assortative mating have all been described (Smadja and Ganem 2002; Smadja et al. 2004; Ganem et al. 2005). None of these phenotypes appear as strong or as consistent across studies as hybrid male sterility but nevertheless may contribute to overall reproductive isolation between *M. musculus* and *M. domesticus*. The existence of several potential isolating mechanisms combined with our increasing appreciation of the genetic complexity underlying male sterility is consistent with recent estimates suggesting many genes are involved in reproductive isolation across the hybrid zone (Macholán et al. 2007).

CONCLUSIONS AND FUTURE DIRECTIONS

The true power of studying speciation in a model genetic system is the potential to experimentally dissect the genetic basis of reproductive isolation. In mice, this goal has been impaired by a lack of genetic studies using inbred strains derived from natural populations. We have shown that F_1 hybrid male sterility in mice involves both polymorphic and X-linked loci, broadly consistent with previous results. At least one set of D–M incompatibilities described here appears distinct from previously described X-linked or *Hst1*-related interactions. Importantly, although reproductive isolation in mice appears to have a fairly complex and variable genetic basis, a surprising amount of genetic complexity in F_1 hybrid male sterility was captured by a small subset of wild-derived inbred strains. These strains are widely available and should facilitate the fine-scale genetic dissection of reproductive isolation in this system without reliance on classic laboratory strains.

Acknowledgments

We are grateful to J. Piálek for collecting and providing access to wild *M. musculus* individuals. We thank S. Bornstein for assistance with histological analyses. C. W. Birky, M. Dean, A. Doyle, and T. Quill provided useful advice on experimental design and methodology. We thank members of the Nachman lab, J. Piálek, B. Payseur, A. Sweigart, J. Feder, R. Storchová, nd two anonymous reviewers for providing critical comments on early versions of the manuscript. We thank B. Gibson, M. Longhi, K. Smith, M. Dean, Dr. M. Rand, and the staff of the University of Arizona Central Animal Facility for assistance with mouse husbandry. This research was supported by an NSF IGERT grant Genomics Initiative (#DGE0114420, JMG), an NSF grant (#DEB0213013, MWN), and two NIH grants (1 RO1 GM074245-01A1, MWN; HD48998, MAH).

LITERATURE CITED

Alibert P, Fel-Clair F, Manolakou K, Britton-Davidian J, Auffray JC. Developmental stability, fitness, and trait size in laboratory hybrids between European subspecies of the house mouse. Evolution 1997;51:1284–1295.

Barbash DA, Siino DF, Tarone AM, Roote J. A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. Proc Natl Acad Sci USA 2003;100:5302–5307. [PubMed: 12695567]

Bateson, W. Heredity and variation in modern lights. In: Seward, AC., editor. Darwin and modern science. Cambridge Univ. Press; Cambridge. UK: 1909. p. 85-101.

Boursot P, Auffray JC, Britton-Davidian J, Bonhomme F. The evolution of house mice. Ann Rev Ecol Syst 1993;24:119–152.

Brideau NJ, Flores HA, Wang J, Maheshwari S, Wang X, Barbash DA. Two Dobzhansky–Muller genes interact to cause hybrid lethality in *Drosophila*. Science 2006;314:1292–1295. [PubMed: 17124320]

Britton-Davidian J, Fel-Clair F, Lopez J, Alibert P, Boursot P. Postzygotic isolation between two European subspecies of the house mouse: estimates from fertility patterns in wild and laboratory-bred hybrids. Biol J Linn Soc 2005;84:379–393.

Christie P, Macnair MR. The distribution of postmating reproductive isolating genes in populations of the yellow monkey flower, *Mimulus guttatus*. Evolution 1987;41:571–578.

Chubb C, Nolan C. Mouse hybrid sterility and testicular function. Biol Reprod 1987;36:1343–1348. [PubMed: 3620567]

Coyne JA, Orr HA. Patterns of speciation in *Drosophila*. Evolution 1989a;43:362–381.

Coyne, JA.; Orr, HA. Two rules of speciation. In: Otte, D.; Endler, J., editors. Speciation and its consequences. Sinauer Associates; Sunderland, MA: 1989b. p. 180-207.

Coyne JA, Orr HA. Patterns of speciation in *Drosophila* revisited. Evolution 1997;51:295–303.

Coyne, JA.; Orr, HA. Speciation. Sinauer Associates, Inc; Sunderland, MA: 2004.

Dean MD, Ardlie KG, Nachman MW. The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus*). Mol Ecol 2006;15:4141–4151. [PubMed: 17054508]

Demuth JP, Wade MJ. Population differentiation in the beetle *Tribolium castaneum*. II. Haldane's rule and incipient speciation. Evolution 2007;61:694–699. [PubMed: 17348932]

Dietrich WF, Miller J, Steen R, Merchant MA, Damron-Boles D, Husain Z, Dredge R, Daly MJ, Ingalls KA, O'Connor TJ, Evans CA, DeAngelis MM, Levinson DM, Kruglyak L, Goodman N, Copeland NG, Jenkins NA, Hawkins TL, Stein L, Page DC, Lander ES. A comprehensive genetic map of the mouse genome. Nature 1996;380:149–152. [PubMed: 8600386]

Dobzhansky T. Studies on hybrid sterility. II. Localization of sterility factors of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 1936;21:113–135. [PubMed: 17246786]

Dobzhansky, T. Genetics and the origin of species. Columbia Univ. Press; New York: 1937.

Dod B, Jermiin LS, Boursot P, Chapman VH, Nielsen JT, Bonhomme F. Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. J Evol Biol 1993;6:529–546.

Eddy EM. Male germ cell gene expression. Recent Prog Horm Res 2002;57:103–128. [PubMed: 12017539]

Escalier D. Impact of genetic engineering on the understanding of spermatogenesis. Hum Reprod Update 2001;7:191–210. [PubMed: 11284662]

Forejt, J. Hybrid sterility gene located in T/t-H-2 supergene on chromosome. In: Reisfeld, R.; Ferrone, S., editors. Current trends in histocompatibility. Immunogenetic and molecular profiles. Vol. 17. Plenum Press; London: 1981. p. 103-131.

Forejt J. Hybrid sterility in the mouse. Trends Genet 1996;12:412–417. [PubMed: 8909138]

Forejt J, Iványi P. Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). Genet Res 1975;24:189–206. [PubMed: 4452481]

Forejt J, Vincek V, Klein J, Lehrach H, Loudová-Micková M. Genetic mapping of the *t*-complex region on mouse chromosome 17 including the Hybrid sterility-1 gene. Mammal Genome 1991;1:84–91.

Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, Beilharz EJ, Gupta RV, Montgomery J, Morenzoni MM, Nilsen GB, et al. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. Nature 2007;448:1050–1053. [PubMed: 17660834]

Funk DJ, Nosil P, Etges WJ. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. Proc Natl Acad Sci USA 2006;103:3209–3213. [PubMed: 16492742]

Ganem G, Ginane C, Ostrowski MF, Orth A. Assessment of mate preference in the house mouse with reference to investigations on assortative mating. Biol J Linn Soc 2005;84:461–471.

Good JM, Nachman MW. Rates of protein evolution are positively correlated with developmental timing of expression during mouse spermatogenesis. Mol Biol Evol 2005;22:1044–1052. [PubMed: 15647515]

Gordon M. The genetics of a viviparous top-minnow *Platypoecilus*; the inheritance of two kinds of melanophores. Genetics 1927;12:253–283. [PubMed: 17246524]

Haldane JBS. Sex ratio and unisexual sterility in animal hybrids. J Genet 1922;12:101–109.

Handel MA, Lessard C, Reinholdt L, Schimenti J, Eppig JJ. Mutagenesis as an unbiased approach to identify novel contraceptive targets. Mol Cell Endo 2006;250:201–205.

Harrison RG. Hybrid zones: windows on evolutionary process. Oxf Surv Evol Biol 1990;7:69–128.

Harrison JS, Burton RS. Tracing hybrid incompatibilities to single amino acid substitutions. Mol Biol Evol 2006;23:559–564. [PubMed: 16280539]

Kopp A, Frank AK. Speciation in progress? A continuum of reproductive isolation in *Drosophila bipectinata*. Genetica 2005;125:55–68. [PubMed: 16175455]

Laurie CC. The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics 1997;147:937–951. [PubMed: 9383043]

Macholán M, Munclinger P, Sugerková M, Dufková P, Bímová B, Bozíková E, Zima J, Piálek J. Genetic analysis of autosomal and X-linked markers across a mouse hybrid zone. Evolution 2007;61:746–771. [PubMed: 17439609]

Moulia C, Aussel JP, Bonhomme F, Boursot P, Nielsen JT, Renaud F. Wormy mice in a hybrid zone: a genetic control of susceptibility to parasite infection. J Evol Biol 1991;4:679–687.

Moulia C, Lebrun N, Dallas J, Orth A, Renaud F. Experimental evidence of genetic determinism in high susceptibility to intestinal pinworm infection in mice: a hybrid zone model. Parasitology 1993;106:387–393. [PubMed: 8316436]

Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520–562. [PubMed: 12466850]

Muller HJ. Isolating mechanisms, evolution, and temperature. Biol Symp 1942;6:71–125.

Munclinger P, Boziková E, Sugerková M, Piálek J, Macholán M. Genetic variation in house mice (*Mus*, Muridae, Rodentia) from the Czech and Slovak Republics. Folia Zool 2002;51:81–92.

Nachman MW, Searle JB. Why is the house mouse karyotype so variable? Trends Ecol Evol 1995;10:397–402.

Oka A, Mita A, Sakurai-Yamatani N, Yamamoto A, Takagi N, Takano-Shimizu T, Toshimori K, Moriwaki K, Shiroishi T. Hybrid breakdown caused by substitution of the X chromosome between two mouse subspecies. Genetics 2004;166:913–924. [PubMed: 15020476]

Oka A, Aoto T, Totsuka Y, Takahashi R, Ueda M, Mita A, Sakurai-Yamatani N, Yamamoto H, Kuriki S, Takagi N, Moriwaki K, Shiroishi T. Disruption of genetic interaction between two autosomal regions and the X chromosome causes reproductive isolation between mouse strains derived from different subspecies. Genetics 2007;175:185–197. [PubMed: 17057240]

Orr HA. The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics 1995;139:1805–1813. [PubMed: 7789779]

Orr HA. Haldane's rule. Ann Rev Ecol Syst 1997;28:195–218.

Orr HA, Orr LH. Waiting for speciation: the effect of population subdivision on the time to speciation. Evolution 1996;50:1742–1749.

Orr HA, Presgraves DC. Speciation by postzygotic isolation: forces, genes and molecules. BioEssays 2000;22:1085–1094. [PubMed: 11084624]

Patterson, JT.; Stone, WS. Evolution in the genus *Drosophila*. Macmillan and Co; New York: 1952.

Payseur BA, Hoekstra HE. Signatures of reproductive isolation in patterns of single nucleotide diversity across inbred strains of mice. Genetics 2005;171:1905–1916. [PubMed: 16143616]

Payseur BA, Krenz JG, Nachman MW. Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. Evolution 2004;58:2064–2078. [PubMed: 15521462]

Pelikán, J. Patterns of reproduction in the house mouse. In: Berry, RJ., editor. Biology of the house mouse. Academic Press; New York: 1981. p. 205-229.

Petkov PM, Graber JH, Churchill GA, DiPetrillo K, King BL, Paigen K. Evidence of a large-scale functional organization of mammalian chromosomes. PLoS Genet 2005;1:312–322.

Piálek J, Hauffe HC, Rodriguez-Clark KM, Searle JB. Raciation and speciation in house mice from the Alps: the role of chromosomes. Mol Ecol 2001;10:613–625. [PubMed: 11298973]

Planchart A, You Y, Schimenti JC. Physical mapping male fertility and meiotic drive quantitative trait loci in the mouse *t* complex using chromosome deficiencies. Genetics 2000;155:803–812. [PubMed: 10835401]

Presgraves DC, Orr HA. Haldane's rule in taxa lacking a hemizygous X. Science 1998;282:952–954. [PubMed: 9794768]

Presgraves DC, Balagopalan L, Abmayr SM, Orr HA. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. Nature 2003;423:715–719. [PubMed: 12802326]

Reed LK, Markow TA. Early events in speciation: polymorphism for hybrid male sterility in *Drosophila*. Proc Natl Acad Sci USA 2004;101:9009–9012. [PubMed: 15184657]

Rieseberg LH, Whitton J, Gardner K. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics 1999;152:713–727. [PubMed: 10353912]

Russell, LD.; Ettlin, RA.; Sinha, AP.; Hikin; Clegg, ED. Histological and histopathological evaluation of the testis. Cache River Press; Clearwater, FL: 1990.

Sage RD, Atchley WR, Capanna E. House mice as models in systematic biology. Syst Biol 1993;42:523–561.

Sage RD, Heyneman D, Lim KC, Wilson AC. Wormy mice in a hybrid zone. Nature 1986;324:60–63. [PubMed: 12356091]

Sawamura K, Davis AW, Wu C-I. Genetic analysis of speciation by means of introgression into *Drosophila melanogaster*. Proc Natl Acad Sci USA 2000;97:2652–2655. [PubMed: 10706624]

Schluter, D. The ecology of adaptive radiation. Oxford Univ. Press; New York: 2000.

Searle, JB. Chromosomal hybrid zones in eutherian mammals. In: Harrison, RG., editor. Hybrid zones and the evolutionary process. Oxford Univ. Press; New York: 1993. p. 309-353.

She JX, Bonhomme F, Boursot P, Thaler L, Catzeflis F. Molecular phylogenies in the genus *Mus*: comparative analysis of electrophoretic, scnDNA hybridization, and mtDNA RFLP Data. Biol J Linn Soc 1990;41:83–103.

Shifman S, Bell JT, Copley RR, Taylor MS, Williams RW, Mott R, Flint J. A high-resolution single nucleotide polymorphism genetic map of the mouse genome. PLoS Biol 2006;4:2227–2237.

Shuker DM, Underwood K, King TM, Butlin RK. Patterns of male sterility in a grasshopper hybrid zone imply accumulation of hybrid incompatibilities without selection. Proc R Soc Lond B 2005;272:2491–2497.

Silver LM. Mouse *t* haplotypes. Ann Rev Genet 1985;19:179–208. [PubMed: 3909941]

Smadja C, Ganem G. Subspecies recognition in the house mouse: a study of two populations from the border of a hybrid zone. Behav Ecol 2002;13:312–320.

Smadja C, Catalan J, Ganem G. Strong premating divergence in a unimodal hybrid zone between two subspecies of the house mouse. J Evol Biol 2004;17:165–176. [PubMed: 15000659]

Stebbins GL. The inviability, weakness, and sterility of interspecific hybrids. Adv Genet 1958;9:147–215. [PubMed: 13520442]

Storchová R, Gregorová S, Buckiová D, Kyselová V, Divina P, Forejt J. Genetic analysis of X-linked hybrid sterility in the house mouse. Mammal Genome 2004;15:515–524.

Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci USA 2004;101:6062–6067. [PubMed: 15075390]

Sweigart AL, Mason AR, Willis JH. Natural variation for a hybrid incompatibility between two species of *Mimulus*. Evolution 2007;61:141–151. [PubMed: 17300433]

Tao Y, Xhen SN, Hartl DL, Laurie CC. Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. I. Differential accumulation of hybrid male sterility effects on the x and autosomes. Genetics 2003;164:1383–1397. [PubMed: 12930747]

Teeter KC, Payseur BA, Harris LW, Bakewell MA, Thibodeau LM, O'Brien JE, Krenz JG, Sans-Fuentes M, Nachman MW, Tucker PK. Genome-wide patterns of gene flow across a house mouse hybrid zone. Genome Res. In press.

Ting CT, Tsaur SC, Wu ML, Wu CI. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 1998;282:1501–1504. [PubMed: 9822383]

Torgerson DG, Kulathinal RJ, Singh RS. Mammalian sperm proteins are rapidly evolving: evidence of positive selection in functionally diverse genes. Mol Biol Evol 2002;19:1973–1980. [PubMed: 12411606]

Torgerson DG, Singh RS. Enhanced adaptive evolution of sperm-expressed genes on the mammalian X chromosome. Heredity 2006;96:39–44. [PubMed: 16175195]

Torgerson DG, Whitty BR, Singh RS. Sex-specific functional specialization and the evolutionary rates of essential fertility genes. J Mol Evol 2005;61:650–658. [PubMed: 16245009]

Trachtulec Z, Vincek V, Hamvas RMJ, Forejt J, Lehrach H, Klein J. Physical map of mouse chromosome 17 in the region relevant for positional cloning of the Hybrid Sterility-1 gene. Genomics 1994;23:132–137. [PubMed: 7829061]

Trachtulec Z, Mihola O, Vlcek C, Himmelbauer H, Paces V, Forejt J. Positional cloning of the Hybrid sterility 1 gene: fine genetic mapping and evaluation of two candidate genes. Biol J Linn Soc 2005;84:637–641.

True JR, Weir BS, Laurie CC. A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. Genetics 1996;142:819–837. [PubMed: 8849890]

Tucker PK, Sage RD, Warner J, Wilson AC, Eicher EM. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution 1992;46:1146–1163.

Turelli M, Orr HA. The dominance theory of Haldane's rule. Genetics 1995;140:389–402. [PubMed: 7635302]

Turelli M, Orr HA. Dominance, epistasis and the genetics of postzygotic isolation. Genetics 2000;154:1663–1679. [PubMed: 10747061]

Vanlerberghe F, Dod B, Boursot P, Bellis M, Bonhomme F. Absence of Y chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. Genet Res 1986;48:191–197. [PubMed: 3569903]

Virdee SR, Hewitt GM. Clines for hybrid dysfunction in a grasshopper hybrid zone. Evolution 1994;48:392–407.

Vyskocilová M, Trachtulec Z, Forejt J, Piálek J. Does geography matter in hybrid sterility in house mice? Biol J Linn Soc 2005;84:663–674.

Wade CM, Daly MJ. Genetic variation in laboratory mice. Nat Genet 2005;37:1175–1180. [PubMed: 16254563]

Wade MJ, Johnson NA. Reproductive isolation between two species of flour beetles, *Tribolium castaneum* and *T. freemani*: variation within and among geographical populations of T. castaneum. Heredity 1994;72:155–162. [PubMed: 8150655]

Wade CM, Kulbokas EJ, Kirby AW, Zody MC, Mullikin JC, Lander ES, Lindblad-Toh K, Daly MJ. The mosaic structure of variation in the laboratory mouse genome. Nature 2002;420:574–578. [PubMed: 12466852]

Winter EE, Goodstadt L, Ponting CP. Elevated rates of protein secretion, evolution, and disease among tissue-specific genes. Genome Res 2004;14:54–61. [PubMed: 14707169]

Wittbrodt J, Adam D, Malitschek B, Maueler W, Raulf F, Telling A, Robertson SM, Schartl M. Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. Nature 1989;341:415–421. [PubMed: 2797166]

Wright S. Systems of mating. II. The effects of inbreeding on the genetic composition of a population. Genetics 1921;6:124–143. [PubMed: 17245959]

Wu C-I. A note on Haldane's rule: hybrid inviability versus hybrid sterility. Evolution 1992;46:1584–1587.

Wu C-I, Davis AW. Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. Am Nat 1993;142:187–212. [PubMed: 19425975]

Wu C-I, Johnson NA, Palopoli MF. Haldane's rule and its legacy: why are there so many sterile males? Trends Ecol Evol 1996;11:281–284.

Yang H, Bell TA, Churchill GA, Pardo-Manuel deVillena F. On the subspecific origin of the laboratory mouse. Nat Genet 2007;39:1100–1107. [PubMed: 17660819]

Yonekawa H, Moriwaki K, Gotoh O, Miyashita N, Matsushima Y, Shi LM, Cho WS, Zhen XL, Tagashira Y. Hybrid origin of Japanese mice *Mus musculus molossinus*: evidence from restriction analysis of mitochondrial DNA. Mol Biol Evol 1988;5:63–78. [PubMed: 2833677]

Yoshiki A, Moriwaki K, Sakakura T, Kusakabe M. Histological studies on male sterility of hybrids between laboratory and wild mouse strains. Dev Growth Diff 1993;35:271–281.

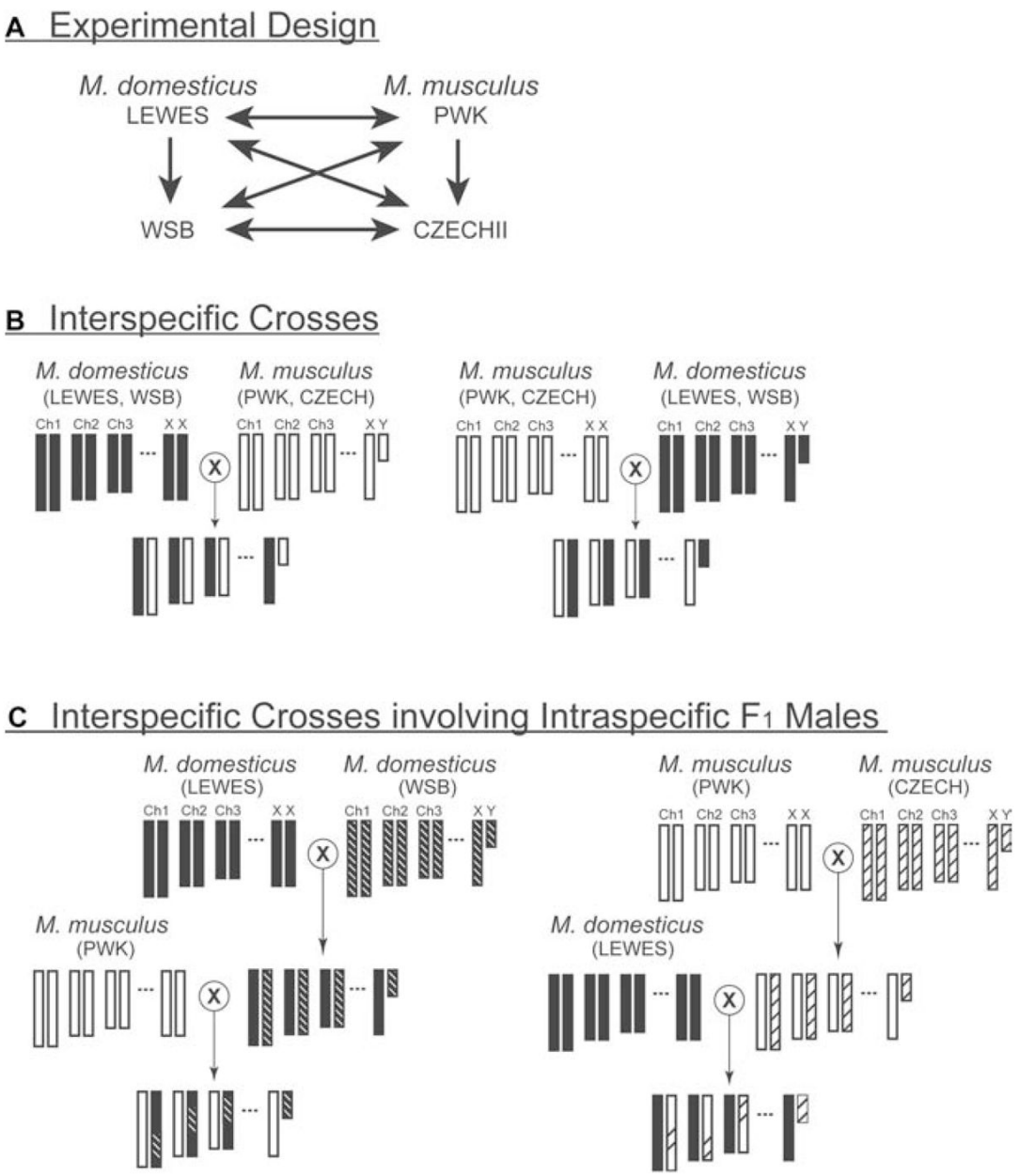

Figure 1.

Experimental crossing scheme and genetic composition of F_1 hybrid males. (A) Inter- and intraspecific crossing design, reciprocal crosses are indicated with double headed arrows. (B) Schematic of reciprocal interspecific crosses with *M. musculus* chromosomes colored white and *M. domesticus* chromosomes colored black. (C) Interspecific crosses involving an inbred female crossed to an intraspecific F_1 male. Recombinant genotypes for *M. domesticus* (LEWES and WSB) and *M. musculus* (PWK and CZECH) are distinguished with crosshatch shading.

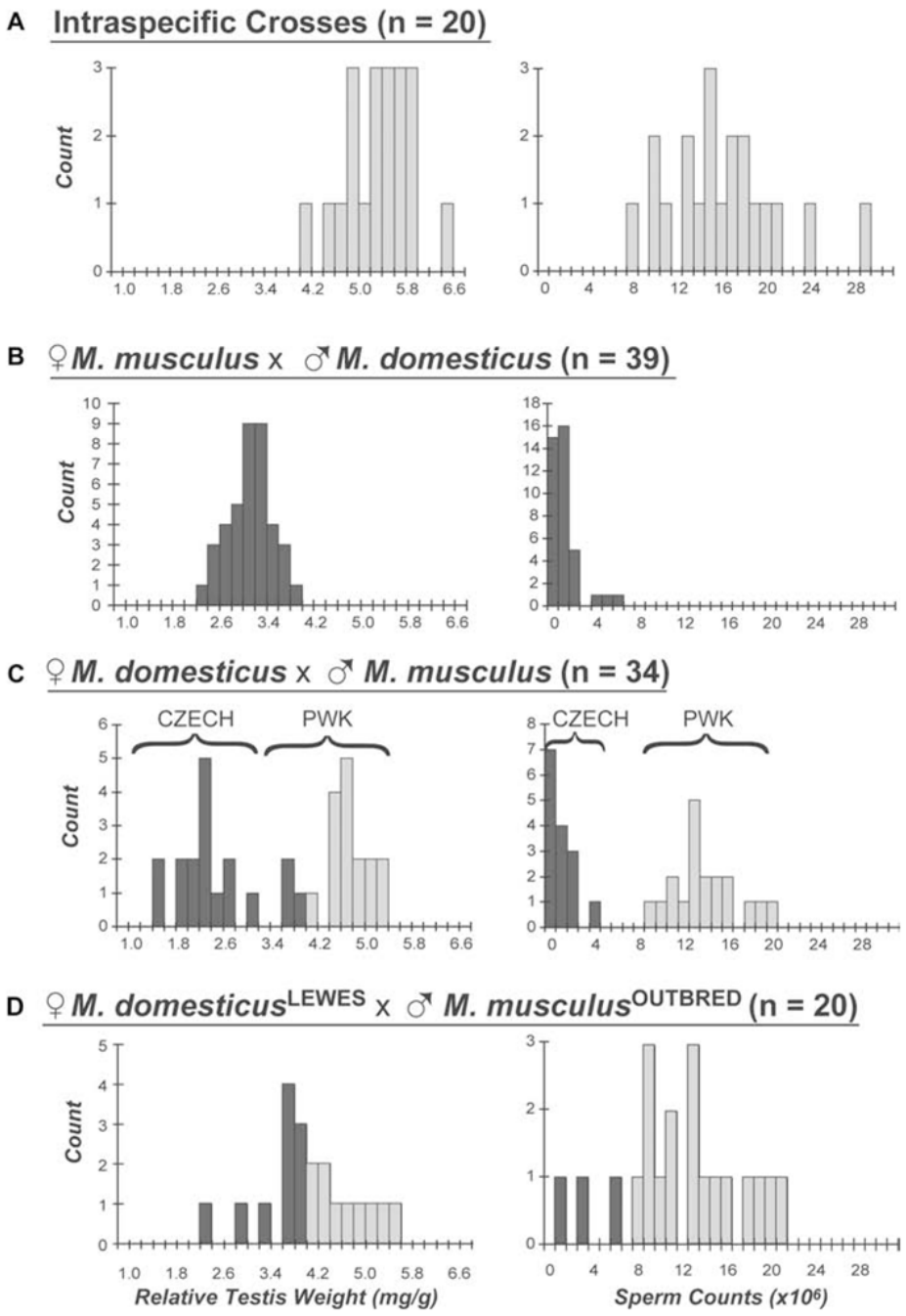

Figure 2.

Distributions of relative testis weights (RTW) and sperm counts for male progeny from (A) the combined intraspecific crosses, (B) the interspecific crosses between female *M. musculus* and male *M. domesticus*, (C) the interspecific crosses between female *M. domesticus* and male *M. musculus* with the strain of the sire indicated (PWK or CZECH), and (D) the interspecific crosses between female *M. domesticus*^{LEWES} and seven outbred *M. musculus* males. Shading in (B), (C), and (D) indicate values within the observed range of the combined intraspecific crosses (light) or values outside of these distributions (dark).

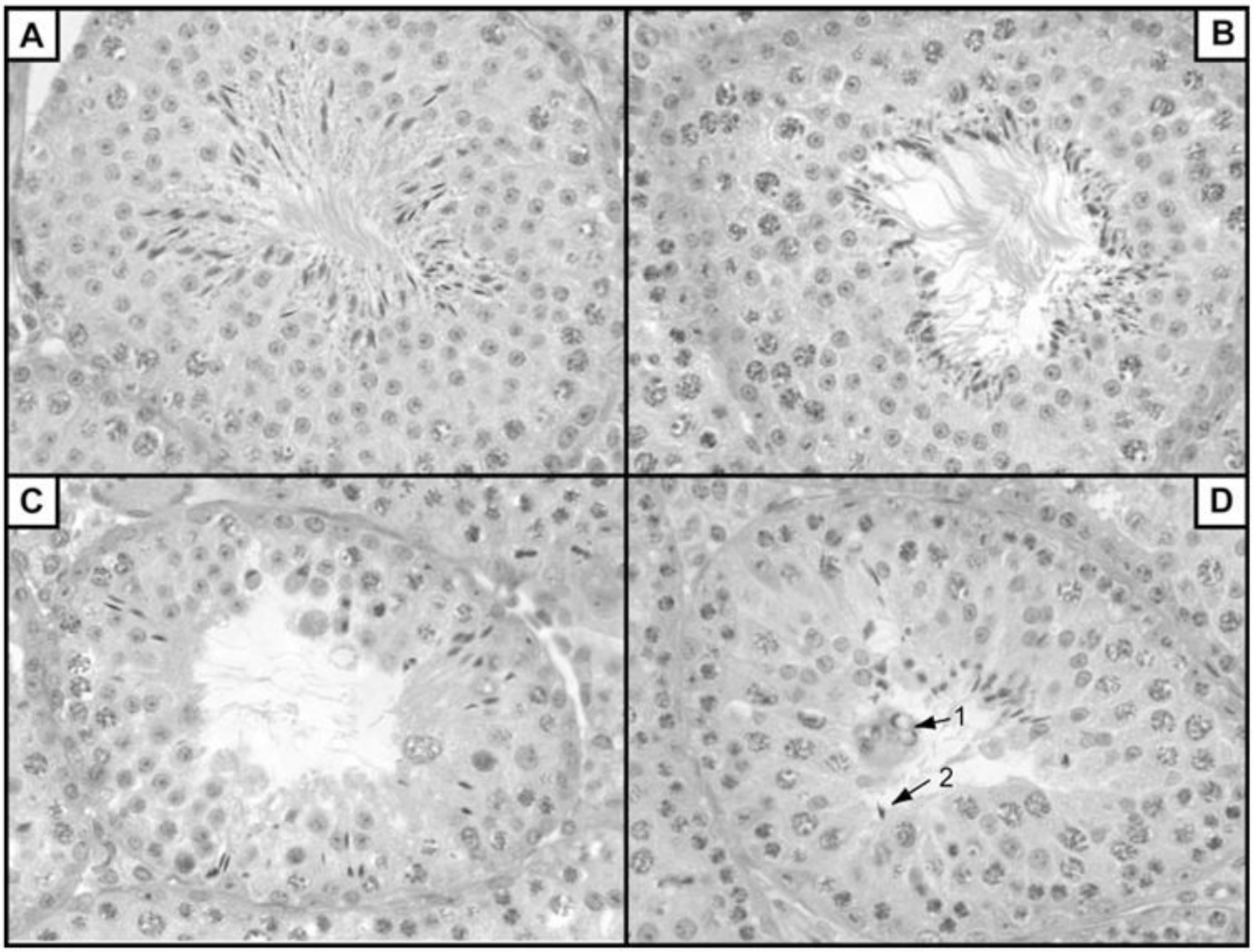

Figure 3.

Histological cross-sections from testes. Panels A and B show seminiferous tubules from males with normal spermatogenesis: (A) *M. musculus* F_1 male (♀ *musculus*^{PWK} × ♂ *musculus*^{CZECH}), (B) interspecific F_1 male (♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK}). Panels C and D are of seminiferous tubules from interspecific F_1 males with disrupted spermatogenesis: (C) ♀ *musculus*^{PWK} × ♂ *domesticus*^{LEWES} and (D) ♀ *domesticus*^{LEWES} × ♂ *musculus*^{CZECH}. In panels C and D, note the diminished numbers of germ cells overall, and poor organization of the seminiferous epithelium with clumps of vesiculated cells (D1) and abnormal sperm head morphology (D2).

Table 1

Summary of experiments on F_1 hybrid male fertility in house mice.

No.	Experiment type		HMS[1]	Polymorph.[2]	References
Classic inbred×wild-derived (directional)					
1	♀Classic inbreds (C57BL/10, A/Ph, BALB/c, DBA/1, AKR/J, C3H/Di, CBA/J, P/J, I/St, F/st)	× ♂*M. musculus* (wild isolates)	Yes	Maternal and paternal	(Forejt and Iványi 1975)
2	♀Classic inbred (C57BL/10)	× ♂*M. musculus* (PWD, PWK, PWB)	Yes	No	(Forejt 1981)
3	♀Classic inbred (BALB/c)	× ♂*M. musculus* (PWD, PWK)	Yes	No	(Chubb and Nolan 1987)
4	♀Classic inbred (A/J, BALB, C57BL/6, AKR, 129, CBA, GRS, SM, C3H)	× ♂*M. musculus* (NJL)	Yes	Maternal	(Yoshiki et al. 1993)
5	♀Classic inbred (C57BL/6)	× ♂*M. m. molossinus*[3] (MSM/Ms)	No	N/A	(Oka et al. 2004)
Classic inbred×wild-derived (reciprocal)					
6	♀Classic inbred (C57BL/10)	× ♂*M. musculus* (wild isolates)	Yes	Paternal	(Vyskocilová et al. 2005)
7	♀*M. musculus* (wild isolates)	× ♂classic inbred (C57BL/10)	Yes	Maternal	(Vyskocilová et al. 2005)
Wild-derived×wild-derived (reciprocal)					
8	♀*M. musculus* (PWK)	× ♂*M. domesticus* (WLA)	Yes	No	(Britton-Davidian et al. 2005)
9	♀*M. domesticus* (WLA)	× ♂*M. musculus* (PWK)	No	N/A	(Britton-Davidian et al. 2005)
10[4]	♀*M. musculus* (MDH)	× ♂*M. domesticus* (DDO)	Yes	Yes – unresolved	(Alibert et al. 1997; Britton-Davidian et al. 2005)
11[4]	♀*M. domesticus* (DDO)	× ♂*M. musculus* (MDH)	Yes	Yes – unresolved	(Alibert et al. 1997; Britton-Davidian et al. 2005)
12	♀*M. musculus* (wild isolates)	× ♂*M. domesticus* (wild isolates)	No	N/A	(Vanlerberghe et al. 1986)
13	♀*M. domesticus* (wild isolates)	× ♂*M. musculus* (wild isolates)	No	N/A	(Vanlerberghe et al. 1986)

[1] Hybrid male sterility for F_1 males for a given cross, based on some observed reduction male fertility-related parameters.

[2] Indicates if polymorphism in F_1 HMS was observed and could be traced to the maternal or paternal line.

[3] *M. musculus molossinus* (MSM/Ms) is a wild-derived inbred strain from natural populations in Japan that are of hybrid origin (*M. musculus*×*M. castaneus*; Yonekawa et al. 1988).

[4] MDH and DDO are wild-derived outbred strains with low levels of natural introgression. DDO is fixed for a nonstandard karyotype ($2n = 34$).

Table 2

Mean reproductive parameters for *M. musculus*, *M. domesticus*, and their F_1 hybrids.

Cross	Male progeny							
	No. of males	No. of litters	Body weight g (SD)	Testis weight[1] mg (SD)	RTW[2] mg/g (SD)	RSVW[3] mg/g (SD)	Motility % (SD)	Sperm Count 1×10^6 (SD)
Intraspecific (control) crosses								
♀ musculus^{PWK}× ♂ musculus^{CZECH}	10	5	13.2 (0.9)	67.8 (8.3)	5.1 (0.6)	4.3 (0.8)	92.5 (4.0)	14.3 (3.2)
♀ domesticus^{LEWES}× domesticus^{WSB}	10	6	14.7 (1.3)	81.1 (11.3)	5.5 (0.5)	4.6 (0.6)	87.3 (6.7)	17.3 (6.0)
Combined intraspecific crosses	20	11	13.9 (1.3)	74.4 (11.8)	5.3 (0.6)	4.5 (0.7)	89.9 (6.0)	15.8 (4.9)
Interspecific crosses								
♀ musculus^{CZECH}× ♂ domesticus^{LEWES}	9	4	14.0 (0.8)	39.6 (4.8)▼	2.8 (0.2)▼	4.7 (1.1)	n.a.	2.1 (2.6)▼
♀ musculus^{CZECH}× ♂ domesticus^{WSB}	8	4	17.6 (1.8)▲	51.5 (3.0)▼	2.9 (0.3)▼	5.1 (0.7)	n.a.	0.1 (0.4)▼
♀ musculus^{PWK}× ♂ domesticus^{LEWES}	12	5	15.1 (1.4)	49.5 (5.3)▼	3.3 (0.2)▼	4.6 (0.7)	n.a.	1.4 (2.0)▼
♀ musculus^{PWK}× ♂ domesticus^{WSB}	10	4	15.1 (1.2)	50.1 (7.5)▼	3.3 (0.4)▼	4.0 (0.8)	n.a.	0.9 (0.6)▼
♀ domesticus^{LEWES}× ♂ musculus^{CZECH}	7	4	15.4 (1.1)	39.3 (4.2)▼	2.6 (0.3)▼	5.0 (1.7)	n.a.	1.4 (0.9)▼
♀ domesticus^{LEWES}× ♂ musculus^{PWK}	11	4	17.1 (0.9)▲	83.4 (2.3)▼	4.9 (0.3)	5.9 (0.8)▲	87.0 (5.7)	12.8 (2.6)
♀ domesticus^{WSB}× ♂ musculus^{CZECH}	8	3	17.7 (1.4)▲	34.5 (5.8)▼	2.0 (0.3)▼	5.6 (0.9)▲	n.a.	0.0 (0.1)▼
♀ domesticus^{WSB}× ♂ musculus^{PWK}	8	4	16.3 (0.6)▲	68.0 (5.8)	4.2 (0.4)▼	5.1 (0.8)	91.9 (2.9)	14.3 (3.4)

▲ or ▼, significantly larger or smaller versus the combined intraspecific crosses based on a Wilcoxon rank sum test and a Bonferroni-corrected $\alpha = 0.0063$.

[1] Single average testis weight in milligrams.

[2] Relative testis weight in milligrams of testis per gram of body weight.

[3] Relative seminal vesicle weight in milligrams of seminal vesicle per gram of body weight.

Table 3

Mean reproductive parameters for interspecific crosses involving females of each species mated to intraspecific F_1 males.

Cross	Progeny				
	No. of males	No. of litters	RTW[1] mg/g (SD)	Sperm count 1×10^6 (SD)	No. of recovered males
♀ *musculus*^{PWK}× ♂ *domesticus*$^{LEWES\times WSB}$	13	3	2.9 (0.8)	1.8 (2.8)	0[2]
♀ *domesticus*^{LEWES} × ♂ *musculus*$^{PWK\times CZECH}$	10	3	3.2 (0.8)	6.4 (4.3)	5[3]

[1] Relative testis weight in milligrams of testis per gram of body weight.

[2] Males with testis weight, RTW, and sperm counts all higher than the range of values observed for male progeny from crosses between ♀ *musculus*^{PWK}× ♂ *domesticus*^{LEWES} and ♀ *musculus*^{PWK}× ♂ *domesticus*^{WSB}.

[3] Males with testis weight, RTW, and sperm counts all higher than the range of values observed for male progeny from crosses between ♀ *domesticus*^{LEWES}× ♂ *musculus*^{CZECH}.

Evolution. Author manuscript; available in PMC 2010 July 21.

Table 4

Inferred genetic linkage and number of loci involved in three sets of hybrid incompatibilities involved in F_1 male sterility between *M. musculus* and *M. domesticus*.

***musculus*[1]**	***domesticus***	**Polymorphic[2]**	**No. of loci[3]**	**Supporting crosses[4]**
Auto (*Hst*^{ws})	Auto (*Hst1*)	M, D[5]	≥3	1–4, 6–7, 10, 11
X (*Hstx1*)	Auto	Unresolved	≥3	CW, 8[6]
Auto/Y	Auto/X	M	≥2[7]	CW

[1] Genetic linkage of incompatibilities by species, Auto, autosomal; X, X chromosome.

[2] Evidence of polymorphism in *musculus* (M) or *domesticus* (D).

[3] Estimated number of sterility factors.

[4] Crosses consistent with inferred incompatibilities. CW, current work, and numbers correspond to crosses in Table 1.

[5] Evidence of polymorphism in *domesticus* is based on variation among classic inbred strains.

[6] See also Storchová et al. 2004.

[7] Minimum estimate assuming a D–M epistatic incompatibility model.