

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

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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 F₁ 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.

KEY WORDS: 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 pro-

duce an *AaBb* F₁ 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 (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 F₁ 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 F₁ 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

Table 1. Summary of experiments on F₁ 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)	× ♂ <i>M. musculus</i> (wild isolates)	Yes	Maternal and paternal	(Forejt and Iványi 1975)
2	♀ Classic inbred (C57BL/10)	× ♂ <i>M. musculus</i> (PWD, PWK, PWB)	Yes	No	(Forejt 1981)
3	♀ Classic inbred (BALB/c)	× ♂ <i>M. musculus</i> (PWD, PWK)	Yes	No	(Chubb and Nolan 1987)
4	♀ Classic inbred (A/J, BALB, C57BL/6, AKR, 129, CBA, GRS, SM, C3H)	× ♂ <i>M. musculus</i> (NJL)	Yes	Maternal	(Yoshiki et al. 1993)
5	♀ Classic inbred (C57BL/6)	× ♂ <i>M. m. molossinus</i> ³ (MSM/Ms)	No	N/A	(Oka et al. 2004)
Classic inbred × wild-derived (reciprocal)					
6	♀ Classic inbred (C57BL/10)	× ♂ <i>M. musculus</i> (wild isolates)	Yes	Paternal	(Vyskocilová et al. 2005)
7	♀ <i>M. musculus</i> (wild isolates)	× ♂ classic inbred (C57BL/10)	Yes	Maternal	(Vyskocilová et al. 2005)
Wild-derived × wild-derived (reciprocal)					
8	♀ <i>M. musculus</i> (PWK)	× ♂ <i>M. domesticus</i> (WLA)	Yes	No	(Britton-Davidian et al. 2005)
9	♀ <i>M. domesticus</i> (WLA)	× ♂ <i>M. musculus</i> (PWK)	No	N/A	(Britton-Davidian et al. 2005)
10 ⁴	♀ <i>M. musculus</i> (MDH)	× ♂ <i>M. domesticus</i> (DDO)	Yes	Yes – unresolved	(Alibert et al. 1997; Britton-Davidian et al. 2005)
11 ⁴	♀ <i>M. domesticus</i> (DDO)	× ♂ <i>M. musculus</i> (MDH)	Yes	Yes – unresolved	(Alibert et al. 1997; Britton-Davidian et al. 2005)
12	♀ <i>M. musculus</i> (wild isolates)	× ♂ <i>M. domesticus</i> (wild isolates)	No	N/A	(Vanlerberghe et al. 1986)
13	♀ <i>M. domesticus</i> (wild isolates)	× ♂ <i>M. musculus</i> (wild isolates)	No	N/A	(Vanlerberghe et al. 1986)

¹Hybrid male sterility for F₁ males for a given cross, based on some observed reduction male fertility-related parameters.

²Indicates if polymorphism in F₁ 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).

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

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 F₁ 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 F₁ 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*^{CZECH/EIJ} and *M. musculus*^{PWK/PIJ} (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 F₁ 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 F₁ 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.

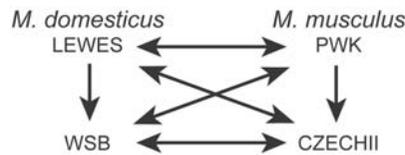
Interspecific crosses involving *musculus*^{PWK} are potentially informative regarding the presence of the *Hst1* sterility system in the *domesticus*^{LEWES} and *domesticus*^{WSB} strains. *Hst1*-related sterility was described based on crosses between classic inbred strains and wild-caught *M. musculus* (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 F₁ 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.

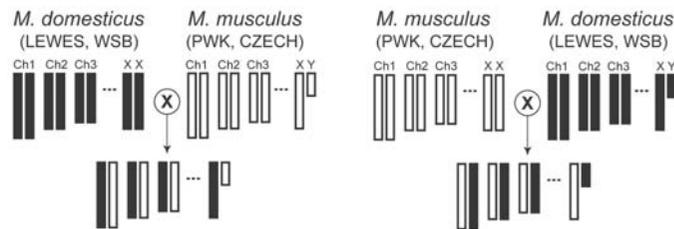
QUANTIFICATION OF MALE FERTILITY PARAMETERS

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

A Experimental Design



B Interspecific Crosses



C Interspecific Crosses Involving Intraspecific F₁ Males

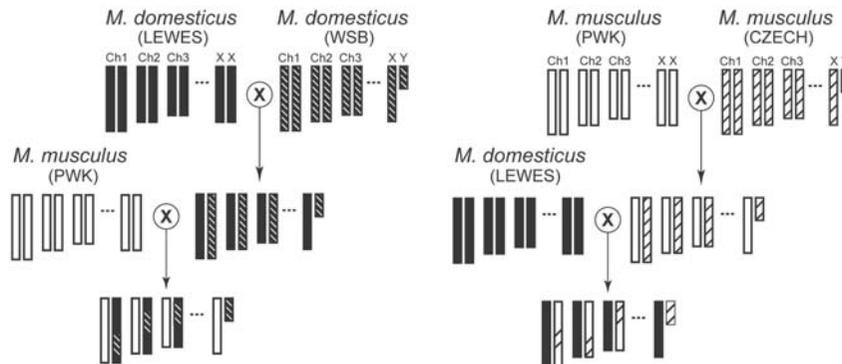


Figure 1. Experimental crossing scheme and genetic composition of F₁ hybrid males. (A) Inter- and intraspecific crossing design, reciprocal crosses are indicated with double headed arrows. (B) Schematic of reciprocal interspecific crosses with *M. musculus* chromosomes colored white and *M. domesticus* chromosomes colored black. (C) Interspecific crosses involving an inbred female crossed to an intraspecific F₁ male. Recombinant genotypes for *M. domesticus* (LEWES and WSB) and *M. musculus* (PWK and CZECH) are distinguished with crosshatch shading.

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 µL 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 mm² 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 × 10⁶ sperm, SD = 1.1).

For a subset of males we examined fecundity based on litter size following pairing with *musculus*^{PWK} females. F₁ 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 F₁ males (all 60-day old) from 59 litters. We first considered F₁ 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 F₁ 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

Table 2. Mean reproductive parameters for *M. musculus*, *M. domesticus*, and their F₁ 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								
♀ <i>musculus</i> ^{PWK} × ♂ <i>musculus</i> ^{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)
♀ <i>domesticus</i> ^{LEWES} × ♂ <i>domesticus</i> ^{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								
♀ <i>musculus</i> ^{CZECH} × ♂ <i>domesticus</i> ^{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)▼
♀ <i>musculus</i> ^{CZECH} × ♂ <i>domesticus</i> ^{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)▼
♀ <i>musculus</i> ^{PWK} × ♂ <i>domesticus</i> ^{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)▼
♀ <i>musculus</i> ^{PWK} × ♂ <i>domesticus</i> ^{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)▼
♀ <i>domesticus</i> ^{LEWES} × ♂ <i>musculus</i> ^{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)▼
♀ <i>domesticus</i> ^{LEWES} × ♂ <i>musculus</i> ^{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)
♀ <i>domesticus</i> ^{WSB} × ♂ <i>musculus</i> ^{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)▼
♀ <i>domesticus</i> ^{WSB} × ♂ <i>musculus</i> ^{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 $\alpha = 0.0063$.

¹Single average testis weight in milligrams.

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

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

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* F₁ 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 F₁ 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 F₁ 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

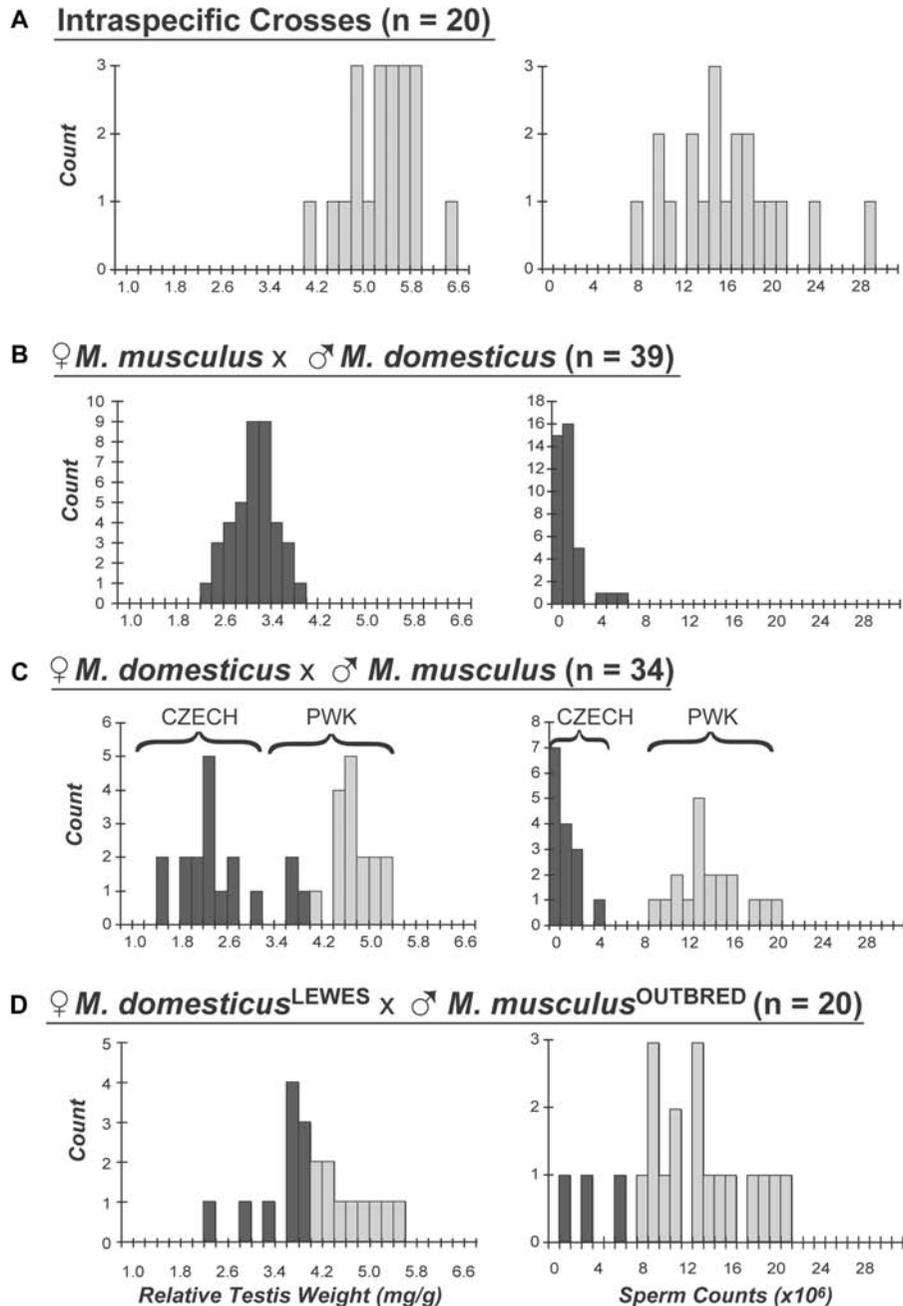


Figure 2. Distributions of relative testis weights (RTW) and sperm counts for male progeny from (A) the combined intraspecific crosses, (B) the interspecific crosses between female *M. musculus* and male *M. domesticus*, (C) the interspecific crosses between female *M. domesticus* and male *M. musculus* 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).

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 symmet-

ric 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 F₁ 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

Table 3. Mean reproductive parameters for interspecific crosses involving females of each species mated to intraspecific F₁ males.

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

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

²Males 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}.

³Males 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}.

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, F₁ 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} × CZECH F₁ 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 F₁ 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 outbred *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

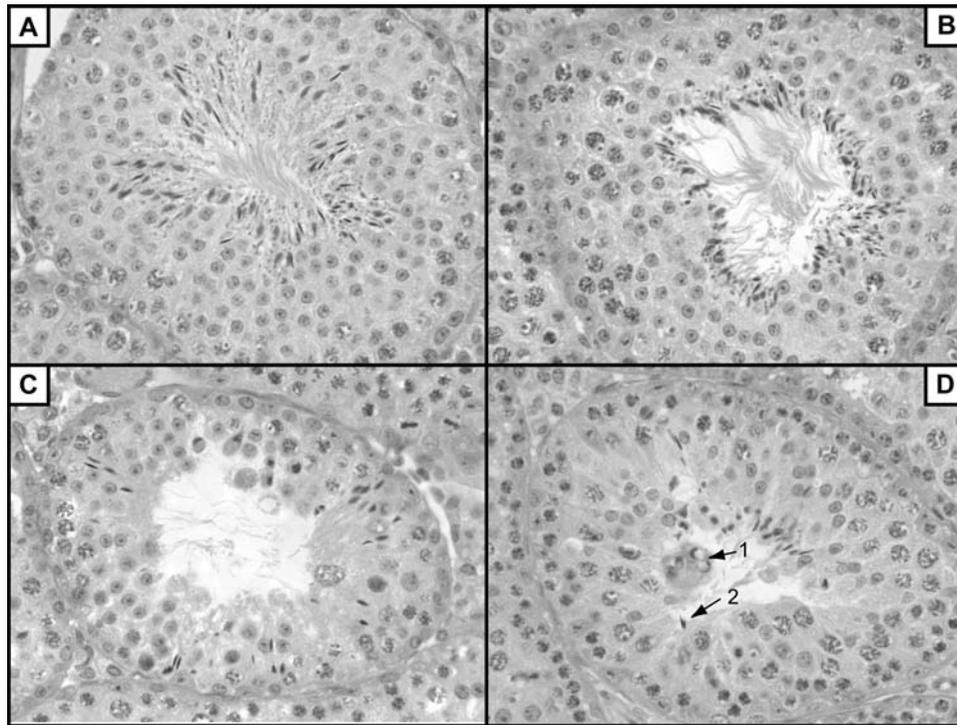


Figure 3. Histological cross-sections from testes. Panels A and B show seminiferous tubules from males with normal spermatogenesis: (A) *M. musculus* F₁ male (♀ *musculus*^{PWK} × ♂ *musculus*^{CZECH}), (B) interspecific F₁ male (♀ *domesticus*^{LEWES} × ♂ *musculus*^{PWK}). Panels C and D are of seminiferous tubules from interspecific F₁ 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).

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 F₁ 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 F₁ 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 F₁ 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 by-product 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 Iványi 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 F₁ REPRODUCTIVE ISOLATION IN MICE

The results presented here suggest that at least three sets of F₁ 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

Table 4. Inferred genetic linkage and number of loci involved in three sets of hybrid incompatibilities involved in F₁ male sterility between *M. musculus* and *M. domesticus*.

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

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

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

³Estimated number of sterility factors.

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

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

⁶See also Storchová et al. 2004.

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

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 F₁ 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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.

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