The Contribution of the Y Chromosome to Hybrid Male Sterility in House Mice

Polly Campbell,*,[1] Jeffrey M. Good,[†] Matthew D. Dean,[‡] Priscilla K. Tucker,[§] and Michael W. Nachman*
*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, [†]Division of Biological Sciences, University of Montana, Missoula, Montana 59812, [‡]Molecular and Computational Biology, University of Southern California, Los Angeles, California 90089, and [§]Department of Ecology and Evolutionary Biology and Museum of Zoology, University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT Hybrid sterility in the heterogametic sex is a common feature of speciation in animals. In house mice, the contribution of the *Mus musculus musculus* X chromosome to hybrid male sterility is large. It is not known, however, whether F_1 male sterility is caused by X–Y or X-autosome incompatibilities or a combination of both. We investigated the contribution of the *M. musculus domesticus* Y chromosome to hybrid male sterility in a cross between wild-derived strains in which males with a *M. m. musculus* X chromosome and *M. m. domesticus* Y chromosome are partially sterile, while males from the reciprocal cross are reproductively normal. We used eight X introgression lines to combine different X chromosome genotypes with different Y chromosomes on an F_1 autosomal background, and we measured a suite of male reproductive traits. Reproductive deficits were observed in most F_1 males, regardless of Y chromosome genotype. Nonetheless, we found evidence for a negative interaction between the *M. m. domesticus* Y and an interval on the *M. m. musculus* X that resulted in abnormal sperm morphology. Therefore, although F_1 male sterility appears to be caused mainly by X-autosome incompatibilities, X–Y incompatibilities contribute to some aspects of sterility.

THE large contribution of the sex chromosomes to the evolution of postzygotic isolation is a common feature of the early stages of speciation in animals, and provides the basis for the two "rules of speciation" (Coyne and Orr 1989; Presgraves 2008). First, when F_1 hybrids experience sex-biased sterility or inviability, most obey Haldane's rule: deficits are pronounced in the heterogametic sex (Haldane 1922; Laurie 1997; Presgraves 2002; Price and Bouvier 2002; Coyne and Orr 2004). Second, in taxa with XY males (*e.g.*, *Drosophila* and mammals), the contribution of the X chromosome to hybrid male sterility is typically disproportionately large relative to that of the autosomes (the "large X effect" Coyne and Orr 1989; Coyne 1992; Masly and Presgraves 2007). While Y-linked effects are less prevalent, the Y chromosome is responsible for male sterility in multiple crosses between *Drosophila* species pairs (Coyne 1985; Sweigart 2010; reviewed in Turelli and Orr 2000; Coyne and Orr 2004). In mammals, however, the potential contribution of the Y to hybrid male sterility has received considerably less attention (but see Eicher *et al.* 1982; Geraldes *et al.* 2008).

House mice in the *Mus musculus* species complex hybridize in nature and exhibit partial reproductive isolation when crossed in the lab. They are thus an excellent mammalian model for studying the genetic details of the early stages of speciation. The best-studied subspecies pair, *M. m. musculus* and *M. m. domesticus*, diverged ~350,000 years ago (Geraldes *et al.* 2011) and came into secondary contact along a hybrid zone that extends from Denmark to Bulgaria. The genetic architecture of reproductive barriers between *M. m. musculus* and *M. m. domesticus* conforms to the Bateson–Dobzhansky–Muller model for the evolution of intrinsic postzygotic isolation, in which incompatibilities in hybrids are caused by disrupted epistasis between allelic combinations that function well in parental backgrounds (Bateson 1909; Dobzhansky 1937; Muller 1942). Consistent with Haldane's rule, F_1 sterility is prevalent in males while females are typically fertile (Forejt and Iványi 1974; Storchová *et al.* 2004; Good *et al.* 2008a; Oka *et al.* 2010; but see Britton-Davidian *et al.* 2005). A large contribution of the X chromosome to reproductive barriers between the subspecies is evident in both laboratory crosses and hybrid zone studies (Tucker *et al.* 1992a; Payseur *et al.* 2004; Storchová

Copyright © 2012 by the Genetics Society of America
doi: 10.1534/genetics.112.141804
Manuscript received March 3, 2012; accepted for publication May 10, 2012
[1]Corresponding author: Department of Ecology and Evolutionary Biology, Biosciences West 333, 1041 E Lowell St., University of Arizona, Tucson, AZ 85721. E-mail: pollyc@email.arizona.edu

et al. 2004; Macholán *et al.* 2007; Good *et al.* 2008b; Teeter *et al.* 2010; White *et al.* 2011).

In many laboratory crosses, hybrid male sterility is X-linked and asymmetric: males with all or part of a *musculus*-derived X exhibit a range of reproductive deficits, whereas males with a *domesticus*-derived X are fertile (Storchová *et al.* 2004; Britton-Davidian *et al.* 2005; Good *et al.* 2008a,b; White *et al.* 2011). Thus, hybrid male sterility in these crosses may be explained by negative epistasis between loci on the *M. m. musculus* X chromosome and loci elsewhere in the *M. m. domesticus* genome.

Whether the *M. m. domesticus* Y chromosome is important for hybrid male sterility is an open question. In crosses in which *M. m. musculus* is represented by the wild-derived inbred strain PWD/PhJ, X-linked sterility does not require a *domesticus*-derived Y (Storchová *et al.* 2004; White *et al.* 2011). However, most males in these mapping studies carried a *musculus*-derived Y (Storchová *et al.* 2004; White *et al.* 2011). Therefore, the potential for negative interactions between the *M. m. domesticus* Y and intervals on the *M. m. musculus* X was not assessed. In addition, the genetic basis of hybrid male sterility in house mice is polymorphic (Forejt 1996; Good *et al.* 2008a; Vyskočilová *et al.* 2005, 2009). For example, *Prdm9*, the only known hybrid male sterility gene in vertebrates, segregates "sterile" and "fertile" alleles in *M. m. domesticus* (Forejt and Iványi 1974; Forejt 1996; Mihola *et al.* 2009). Multiple polymorphic hybrid sterility factors have also been detected in natural populations of *M. m. musculus* (Forejt 1996; Vyskočilová *et al.* 2005, 2009). It is therefore important to evaluate X–Y incompatibilities in more than one cross. Moreover, minimal introgression of both X- and Y-linked markers across several hybrid zone transects (Vanlerberghe *et al.* 1986; Tucker *et al.* 1992a; Prager *et al.* 1997; Teeter *et al.* 2010; but see Macholán *et al.* 2008) suggests that loci underlying hybrid incompatibilities are present on both sex chromosomes in natural populations.

Additional evidence that X–Y interactions may be important in hybrid male sterility comes from recent work on multicopy genes (Cocquet *et al.* 2009, 2010; Ellis *et al.* 2011). In house mice, both the X and Y chromosomes are enriched for multicopy genes, such as *Sly* and *Slx*, and copy numbers differ between the subspecies (Mueller *et al.* 2008; Scavetta and Tautz 2010; Ellis *et al.* 2011). In reproductively normal males, the X and Y chromosomes are transcriptionally silenced midway through meiosis I (McKee and Handel 1993; Turner 2007) and remain repressed in haploid spermatids (postmeiotic sex chromatin repression, PSCR) (Namekawa *et al.* 2006; Turner *et al.* 2006). However, *Sly*, along with several other multicopy genes on both sex chromosomes, escapes PSCR and is thought to be essential for proper transcriptional regulation of the X and Y during sperm differentiation (Mueller *et al.* 2008; Cocquet *et al.* 2009; Reynard and Turner 2009; Reynard *et al.* 2009). Notably, PSCR is disrupted in *Sly*-deficient laboratory mice, resulting in the upregulation of X- and Y-linked postmeiotic genes and sperm head abnormalities (Cocquet *et al.* 2009). Similar phenotypes have been observed in sterile F_1 hybrids; in a cross between wild-derived inbred strains of *M. m. musculus* (*musculus*^{PWK}) and *M. m. domesticus* (*domesticus*^{LEWES}), sterility is asymmetric, strongly X-linked, and not associated with known sterility variants of *Prdm9* (Good *et al.* 2008a,b, 2010). Severe reproductive problems and X chromosome overexpression are observed in F_1 males with a *M. m. musculus* X chromosome, while hybrid males with a *M. m. domesticus* X are normal (Good *et al.* 2008a, 2010). Ellis *et al.* (2011) speculated that sterility in this cross is caused by mismatch between the *M. m. musculus* X and the *M. m. domesticus* Y, which has fewer copies of *Sly* than the *M. m. musculus* Y.

Here, we evaluate the contribution of X–Y *vs.* X-autosome interactions to F_1 male sterility in the cross between *musculus*^{PWK} and *domesticus*^{LEWES}. In previous studies using these strains, the *M. m. musculus* X chromosome was always paired with the *M. m. domesticus* Y (Good *et al.* 2008a,b). Therefore, it was not possible to determine whether sterility was due to X–Y or X-autosome incompatibilities or a combination of both. We quantified reproductive phenotypes in the male progeny of reciprocal crosses between X introgression line females and pure heterosubspecific males (Figure 1). Hybrid males in this experiment share the same heterozygous F_1 autosomal genome but differ in the origin of the Y and the size and location of the *M. m. musculus* X introgression. We asked three main questions. (1) Is the *M. m. domesticus* Y essential for hybrid male sterility? If so, then males with a *M. m. domesticus* father should exhibit significantly greater reproductive deficits than males from the reciprocal crosses. (2) Is there evidence for negative epistasis between the *M. m. domesticus* Y and specific intervals on the *M. m. musculus* X? We addressed this question by mapping reproductive QTL on the X and conditioning on Y chromosome genotype. If X–Y incompatibilities contribute to hybrid male sterility, then some sterility-associated QTL on the *M. m. musculus* X should be unique to males with a *M. m. domesticus* Y. (3) How does the genetic architecture of X-autosome incompatibilities differ between F_1 and largely homozygous late-backcross backgrounds? We evaluated this question by comparing the location of X-linked sterility QTL identified in this study to those mapped in an earlier study, in which regions of the *M. m. musculus* X were introgressed onto a *M. m. domesticus* background (Good *et al.* 2008b).

Materials and Methods

Animals

Breeding colonies of wild-derived inbred strains purchased from the Jackson Laboratory (http://www.jax.org) were maintained at the University of Arizona Central Animal Facility. *M. m. domesticus* was represented by the LEWES/EiJ strain, originally isolated from a natural population in Lewes, Delaware. *M. m. musculus* was represented by the PWK/PhJ strain, derived from Lohtka in the central region of the Czech Republic

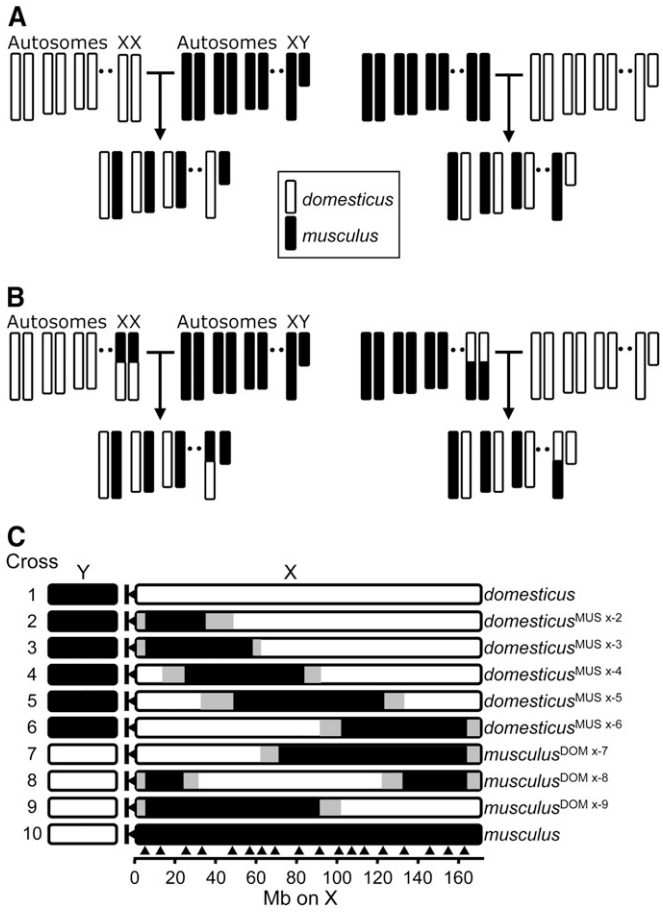

Figure 1 Crossing design and genotypes of experimental males. (A) Reciprocal F_1 crosses. F_1 males with a *domesticus*^{LEWES} X chromosome (open) are fertile; F_1 males with a *musculus*^{PWK} X chromosome (solid) have severe reproductive problems, including complete sterility. (B) Example of reciprocal X introgression F_1 cross. (C) Sex chromosome genotypes of all experimental males. Regions of uncertainty between *domesticus*^{LEWES} (open) and *musculus*^{PWK} (solid) recombination break points on the X are shaded. Genotypes are named according to maternal autosomal background, superscript denotes origin of X introgression (MUS, *musculus*; DOM, *domesticus*) and cross number. See Table 1 for complete list of crosses. Triangles indicate approximate locations of markers used to establish X chromosome genotypes (Good *et al.* 2008b).

(Gregorová and Forejt 2000). The WSB/EiJ (*domesticus*^{WSB}) and CZECHII/EiJ (*musculus*^{CZECHII}) strains were used in control crosses. Mice were maintained in accordance with the University of Arizona Animal Care and Use Committee regulations.

Experimental design

Females from two pure and eight X chromosome introgression lines were used in experimental crosses (Figure 1). Each introgression line was homozygous for defined regions of either the *musculus*^{PWK} or *domesticus*^{LEWES} X chromosome on the background of the other subspecies (Figure 1B). Construction of six of these X introgression lines is described in detail in Good *et al.* (2008b). Briefly, female progeny of a cross between female *musculus*^{PWK} and male *domesticus*^{LEWES} were backcrossed to either *musculus*^{PWK} or *domesticus*^{LEWES} for a minimum of 10 generations. The X chromosome was divided into overlapping proximal (7.2–56.7 Mb, Ensembl Sept. 2011 update of NCBIM37), central (49.0–126.9 Mb), and distal regions (101.3–163.7 Mb). In each generation, females were genotyped for 18 subspecies-specific microsatellite markers on the X chromosome, and individuals carrying the targeted region of the *musculus*^{PWK} or *domesticus*^{LEWES} X were selected for breeding (Good *et al.* 2008b). In the current study, we included two additional X introgression lines with *musculus*^{PWK} introgressions from 7.2 to 38.2 Mb, and 33.7 to 82.8 Mb. All X introgression lines carry the *musculus*^{PWK} mitochondrial haplotype.

X introgression females were crossed to pure heterosubspecific males, such that F_1 male progeny were heterozygous at all autosomal loci with varying degrees of mismatch between the X and Y chromosomes (Figure 1, B and C). Genotypes in Figure 1C are named according to maternal autosomal background with superscript denoting the origin of the X introgression (MUS, *musculus*; DOM, *domesticus*) and cross number. Details of these crosses are provided in Table 1. X chromosome genotypes were reconfirmed in a subset of males from each cross using the 18 microsatellite markers described in Good *et al.* (2008b). While the four possible combinations of sex chromosome genotypes are represented at the scale of these markers (triangles in Figure 1C), introgressed segments were large (~27–88 Mb) and identical reciprocal X introgressions were not available for this study.

To eliminate the effects of inbreeding depression in controls we generated F_1 males from intrasubspecific crosses: *domesticus*^{WSB} × *domesticus*^{LEWES} and *musculus*^{CZECHII} × *musculus*^{PWK} (Table 1). All litters were weaned at 21 days. Male progeny were maintained in cages containing a maximum of three same-sex sibs until 50 days, after which they were caged singly for 20 days and killed at 70 days.

Quantification of reproductive phenotypes

Males were weighed to the nearest 0.01 g and three reproductive parameters were assessed: testis weight, sperm count, and sperm head morphology. Detailed methods are provided in Good *et al.* (2008a,b). Testes were dissected and weighed to the nearest 0.1 mg. Mature spermatozoa were collected by macerating caudal epididymides in modified Dulbecco's medium, prewarmed to 37°. Following a 10-min incubation at 37°, 200 μl of sperm suspension was heat shocked for 5 min at 60°. Sperm counts were made using a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel) and a light microscope at ×200 magnification. The number of sperm heads in each of five chamber columns was counted and averaged. The capacity of each column is 1×10^{-6} ml. Thus, the average number of sperm heads per column provides an estimate of sperm concentration in millions per milliliter.

Sperm head morphology was evaluated for a minimum of 100 sperms per male using a phase contrast microscope with ×400 magnification. Heat-shocked sperm suspension was

Table 1 Mean reproductive parameters for experimental and control males

Experimental crosses[a]	*n*	RTW[b] (SD)	Sperm count[c] (SD)	% normal sperm (SD)
1 ♀ *domesticus* × ♂ *musculus*	14	4.6** (0.3)	15.0* (4.2)	95.2 (4.1)
2 ♀ *domesticus*$^{\text{MUS X-2}}$ × ♂ *musculus*	14	4.0** (0.2)	8.6 ** (2.7)	90.0 (9.6)
3 ♀ *domesticus*$^{\text{MUS X-3}}$ × ♂ *musculus*	12	3.9** (0.2)	11.3* (5.1)	63.1** (24.3)
4 ♀ *domesticus*$^{\text{MUS X-4}}$ × ♂ *musculus*	14	3.3** (0.1)	4.0** (2.3)	28.3** (19.6)
5 ♀ *domesticus*$^{\text{MUS X-5}}$ × ♂ *musculus*	13	4.3** (0.2)	11.6** (8.5)	90.2 (16.2)
6 ♀ *domesticus*$^{\text{MUS X-6}}$ × ♂ *musculus*	14	5.0 (0.4)	15.2* (3.7)	94.4 (9.4)
7 ♀ *musculus*$^{\text{DOM X-7}}$ × ♂ *domesticus*	14	5.5 (0.3)	19.6 (6.6)	82.7 (19.8)
8 ♀ *musculus*$^{\text{DOM X-8}}$ × ♂ *domesticus*	14	4.7* (0.3)	18.2 (7.5)	73.0* (20.8)
9 ♀ *musculus*$^{\text{DOM X-9}}$ × ♂ *domesticus*	12	4.1** (0.2)	9.5 (5.0)	23.0** (12.7)
10 ♀ *musculus* × ♂ *domesticus*	14	3.5** (0.3)	3.5** (1.9)	2.5** (2.9)
Control crosses				
♀ *musculus*$^{\text{CZECHII}}$ × ♂ *musculus*$^{\text{PWK}}$	14	5.1 (0.6)	19.9 (7.6)	88.8 (11.1)
♀ *domesticus*$^{\text{WSB}}$ × ♂ *domesticus*$^{\text{LEWES}}$	14	5.4 (0.4)	30.8 (10.6)	95.9 (3.7)

* Wilcoxon $P < 0.005$, $^{**}P \leq 0.0001$ *vs.* intrasubspecific controls; Bonferroni-corrected $\alpha = 0.005$.

[a] Crosses are numbered 1–10 as in Figs. 1–3; *domesticus* strain is LEWES and *musculus* strain is PWK; superscript following maternal autosomal genotype denotes origin of X introgression (MUS, *musculus*; DOM, *domesticus*) and cross number.

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

[c] $\times 10^6$ per ml.

spread on a microscope slide, air-dried, fixed in 1% acetic acid in 95% ethanol, stained with 1% eosin yellow (Sigma), rinsed in 70% ethanol, and mounted with Permount (Fisher). We scored four classes of head morphology as in Good *et al.* (2008b): (1) normal, characterized by a rounded head and a strongly curved apical hook (Russell *et al.* 1990), (2) moderately abnormal, characterized by a flattened head and shortened hook, (3) abnormal, characterized by a shortened head and a hook reduced to a short point, and (4) severely abnormal, characterized by a small, asymmetrical head lacking a hook. Sperm were scored blind to genotype.

Analysis of reproductive phenotypes

Across experimental genotypes, all reproductive measures were significantly correlated with body weight (all $P < 0.0001$). If this relationship were purely isometric we would expect the same positive scaling within as between genotypes and would expect to observe the same effect in control genotypes. Although most within-genotype correlations were nonsignificant, there was a positive association between body weight and testis weight (all genotypes) and body weight and sperm count (controls and 5/10 experimental genotypes; results not shown). In analyses involving pairwise comparisons between genotypes, we used relative testis weight (milligrams of testis per gram of body weight) to correct for the effect of body size. For QTL analyses we used the residuals from least-square regressions of testis weight and sperm count on body weight. Sperm head morphology was scored as the percentage of normal sperm in all analyses.

All reproductive measures deviated from a normal distribution (Shapiro–Wilk *W* test, all $P < 0.003$) and none of the transformations applied significantly improved the normal fit. To account for these distributions we used nonparametric tests when feasible. Significance thresholds for multiple tests were Bonferroni corrected.

QTL analysis

We mapped associations between genotype and reproductive phenotypes in 107 F_1 males with eight different recombinant X chromosomes. X genotypes were scored using 18 microsatellite makers as described (Good *et al.* 2008b). Composite interval mapping (CIM) on the X was implemented in WinQTLCart (v 2.5_009, Wang *et al.* 2011) with a window size of 10 cM and a walk speed of 1 cM. Significance of additive effects was evaluated using the likelihood ratio statistic with critical values for $\alpha = 0.05$ determined by 1000 permutations. To look specifically for X–Y interactions, we performed composite interval mapping on the X chromosome with samples split by Y chromosome (*musculus*$^{\text{PWK}}$ Y, $n = 67$; *domesticus*$^{\text{LEWES}}$ Y, $n = 40$) and asked whether X-linked QTL were influenced by Y

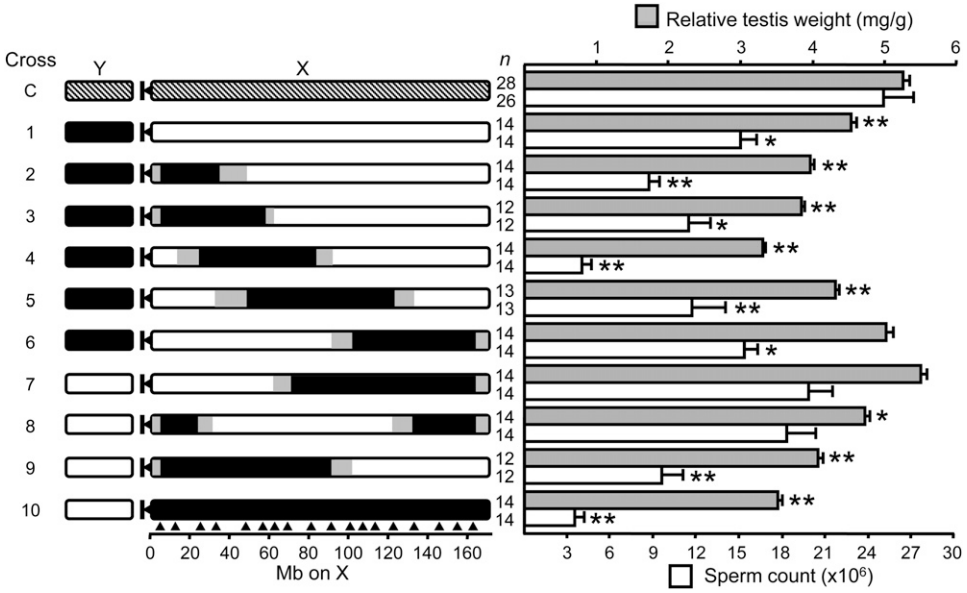

Figure 2 Sex chromosome genotypes, relative testis weight and sperm count in experimental *vs.*. control males. All experimental males share the same F_1 autosomal background. Hatched chromosomes represent combined intrasubspecific controls (C, *domesticus*^{WSB} × *domesticus*^{LEWES} and *musculus*^{CZECHII} × *musculus*^{PWK}). Numbers on far left correspond to cross numbers in Table 1. Regions of uncertainty between *domesticus*^{LEWES} (open) and *musculus*^{PWK} (solid) recombination break points on the X are shaded. Triangles indicate approximate locations of markers used to establish X chromosome genotypes (Good *et al.* 2008b). Bars represent genotypic means for relative testis weight (shaded) and sperm count (open); error bars are +1 SE. Sample sizes (*n*) for each genotype are along the vertical axis. Significance based on Wilcoxon pairwise comparisons *vs.* intrasubspecific controls, Bonferroni-corrected $\alpha = 0.005$: $^*P < 0.005$, $^{**}P \leq 0.0001$.

chromosome genotype. This procedure is similar to that used by White *et al.* (2011), in which X-autosome interactions were mapped by conditioning on X chromosome genotype. Some QTL in both analyses had a nonnegative effect on reproductive phenotypes. We refer to these as "positive" QTL.

Results

Pervasive reproductive deficits in hybrid males

In pairwise comparisons with intrasubspecific controls, relative testis weight (RTW) and sperm count were significantly reduced in seven of the eight X introgression genotypes (Figure 2, Table 1). Four X introgression genotypes exhibited a significant reduction in the percentage of normal sperm (Figure 3, Table 1).

Previous studies have demonstrated that male sterility segregates with the *musculus*^{PWK} X chromosome (Good *et al.* 2008a,b). As expected, RTW, sperm count, and the percentage of normal sperm were severely reduced in pure F_1 males with a *musculus*^{PWK}-derived X chromosome (cross 10). However, RTW and sperm count were also reduced, albeit to a lesser degree, in the reciprocal F_1 (cross 1, RTW, $P < 0.0001$; sperm count, $P = 0.0008$), a genotype that was not statistically different from controls in an earlier comparison of F_1 males from this cross (Good *et al.* 2008a). This difference between studies may be explained by larger sample sizes in the present study. Importantly, the absolute difference in mean sperm count between the reciprocal F_1 hybrids was consistent across studies (11.5×10^6/ml, Table 1; 11.4×10^6/ml, Good *et al.* 2008a, Table 2).

The domesticus Y chromosome is not required for sterility

A key motivation for this study was to ask whether the *domesticus* Y chromosome is necessary for F_1 male sterility in

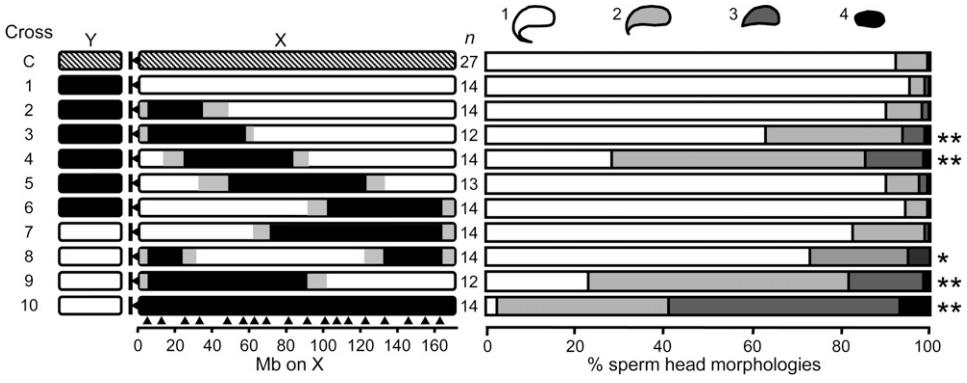

Figure 3 Percentage of sperm head morphologies in experimental *vs.* control males. Hatched chromosomes represent combined intrasubspecific controls (C, *domesticus*^{WSB} × *domesticus*^{LEWES} and *musculus*^{CZECHII} × *musculus*^{PWK}). Numbers on far left correspond to cross numbers in Table 1. Regions of uncertainty between *domesticus*^{LEWES} (open) and *musculus*^{PWK} (solid) recombination break points on the X are shaded. Triangles indicate approximate locations of markers used to establish X chromosome genotypes (Good *et al.* 2008b). Sample sizes (*n*) for each genotype are along vertical axis. Sperm heads were classified into four classes ranging from normal to severely abnormal: (1) normal (open), (2) flattened head and shortened hook (light shading), (3) shortened head and hook reduced to short point (dark shading), and (4) small, asymmetrical head without hook (solid). Bars represent genotypic mean percentages for each of the four classes. Significant reduction in the percentage of normal sperm was assessed with Wilcoxon pairwise comparisons *vs.* intrasubspecific controls, Bonferroni-corrected $\alpha = 0.005$: $^*P < 0.005$, $^{**}P \leq 0.0001$.

Table 2 Reproductive QTL on the *musculus*^{PWK} X chromosome in X introgression F_1 males

Trait[a]	QTL position (CI)[b]	Phenotypic contribution (%)[c]
Testis weight	25.0 (24.5–31.5)	−8.0
	32.5 (31.5–32.8)	−6.0
	47.5 (47.0–51.6)	33.0
	60.5 (59.5–60.5)	33.0
Sperm head morphology	20.5 (18.3–22.6)	−32.0
	58.0 (53.3–59.1)	29.0
Sperm count	12.6 (11.8–14.8)	14.0
	60.5 (59.5–60.6)	17.0

[a] Measured in 107 males.
[b] Position in centimorgans estimated using composite interval mapping (CIM). CI, 2-LOD confidence interval.
[c] Estimate of R^2 in CIM model expressed as percent. Negative values indicate a negative effect of *musculus*^{PWK} genotype.

crosses between *musculus*^{PWK} and *domesticus*^{LEWES}. In Figures 2 and 3, males from crosses 1–6 carry a *musculus* Y chromosome, whereas males from crosses 7–10 carry a *domesticus* Y chromosome. Severe reproductive deficits are seen in both sets of crosses, demonstrating that the *domesticus* Y is not an essential component of sterility in this cross.

QTL mapping on the X

We detected four QTL associated with variation in testis weight and two QTL associated with variation in sperm head morphology (Figure 4). For both phenotypes, the *musculus*^{PWK} genotype was associated with negative effects on the proximal half of the X and with positive effects on the distal half (Table 2).

On the proximal X, LOD scores for testis weight exceeded the critical value (LOD = 1.6, α = 0.05) in the interval between 24.5 and 34.7 cM, with peaks estimated at 25.0 cM (LOD = 6.7) and 32.5 cM (LOD = 6.2). LOD scores for sperm head morphology were significant between 14.1 and 29.5 cM, with a single peak at 20.5 cM (LOD = 15.7). On the distal X, the interval between 43.6 and 62.2 cM was significant for testis weight, with peaks at 47.5 cM (LOD = 21.9) and 60.5 cM (LOD = 21.4). For sperm head morphology, the interval between 48.5 and 60.0 cM was significant, with a single peak at 58.0 cM (LOD = 15.5).

We detected two smaller QTL for which the *musculus*^{PWK} genotype was associated with a positive effect on sperm count (Table 2). The first peak was at 12.6 cM (LOD = 3.1). The second peak overlaid the distalmost QTL for testis weight at 60.5 cM (LOD = 4.9).

Evidence that X–Y incompatibilities contribute to sperm abnormality

While the distribution of reproductive deficits across genotypes with different Y chromosomes indicates that the *domesticus*^{LEWES} Y is not essential for hybrid male sterility, it does not rule out a contribution of X–Y interactions. We therefore asked whether sterility-associated QTL on the X were dependent on Y genotype.

Y genotype had a large effect on QTL for sperm head morphology. The negative effect of *musculus*^{PWK} X genotypes in the proximal interval was larger when combined with the *domesticus*^{LEWES} Y, (LOD = 7.7 and 7.3, combined R^2 = 0.91; Figure 5A, Table 3) than with the *musculus*^{PWK} Y (LOD = 4.9, R^2 = 0.20; Figure 5B). Whereas the estimated location of the QTL peak for *musculus*^{PWK} Y genotypes was the same as that in the full data set (20.5 cM, Tables 2 and 3), peaks for *domesticus*^{LEWES} Y genotypes were estimated at 24.0 and 31.5 cM. Overlap in 2-LOD confidence intervals (CIs) suggests that these peaks represent a single QTL (Table 3). Notably, the entire interval between 14.1 and 34.7 cM exceeded critical values (Figure 5A, Table 3). Likewise, the positive effect of the distal interval remained highly significant for *musculus*^{PWK} Y genotypes (LOD = 14.5, R^2 = 0.44;

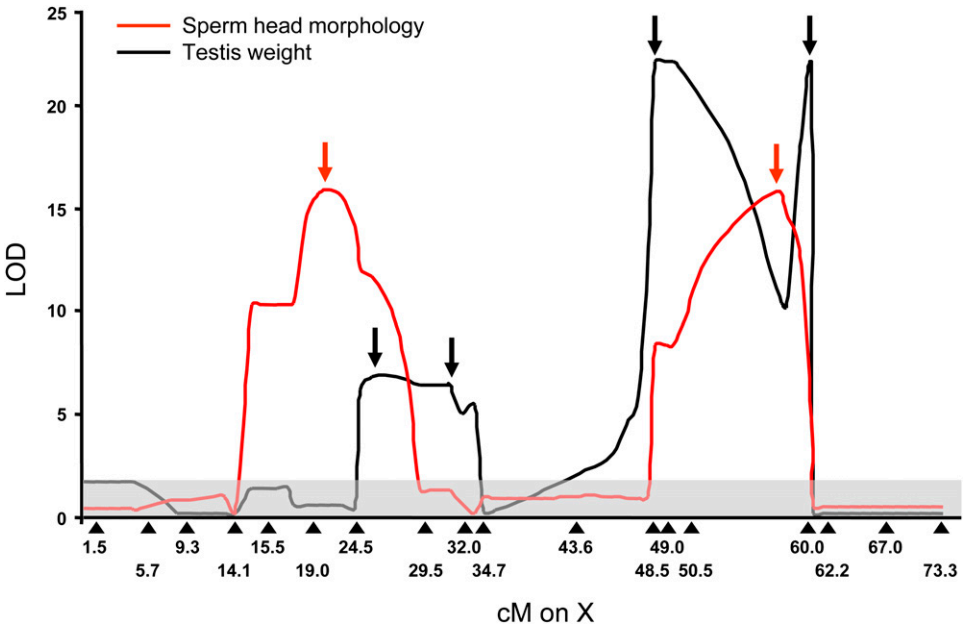

Figure 4 Results of composite interval mapping on the X chromosome for two reproductive traits in F_1 males with recombinant X chromosomes. Trait values for sperm head morphology (red) are the proportion of normal sperm. Testis weight (black) is the residual trait score of testis weight regressed on body weight. Significance of additive effects was evaluated using the likelihood ratio statistic with critical values determined using 1000 permutations for α = 0.05 (gray shading, LOD ≥ 1.6). Arrows indicate QTL peaks listed in Table 2. Triangles along the X axis represent the approximate locations of markers used to establish X chromosome genotypes with genetic positions in centimorgans (cM).

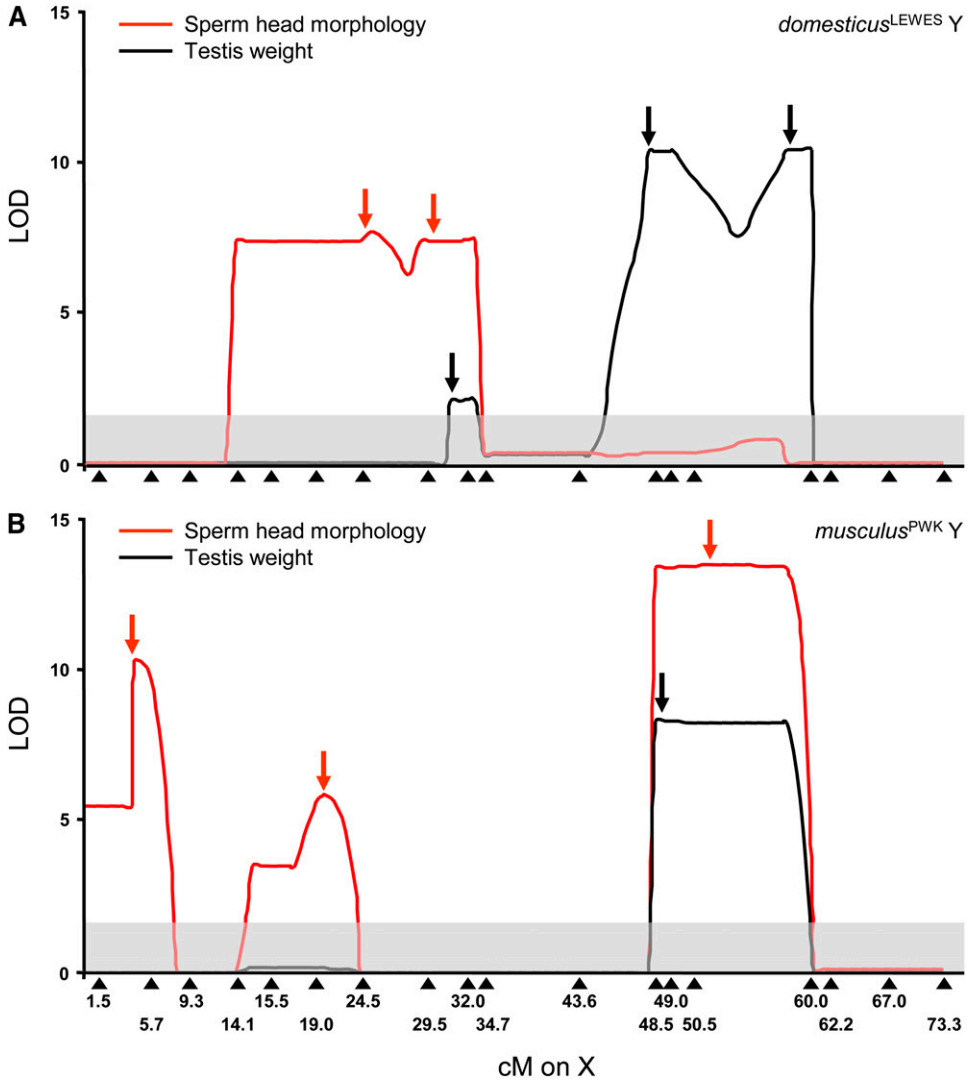

Figure 5 Results of composite interval mapping on the X chromosome for the proportion of normal sperm (red) and testis weight (black) in F_1 males with Y chromosomes from (A) *domesticus*^{LEWES} or (B) *musculus*^{PWK}. Significance of additive effects was evaluated using the likelihood ratio statistic with critical values determined using 1000 permutations for $\alpha = 0.05$ (gray shading, LOD $\geq$ 1.6). Arrows indicate QTL peaks listed in Table 3. Triangles along the X axis represent the approximate locations of markers used to establish X chromosome genotypes with genetic positions in centimorgans (cM).

Figure 5B) but was absent for *domesticus*^{LEWES} Y genotypes (Figure 5A). Finally, an interval proximal to 7.2 cM with a large positive effect on sperm head morphology was unique to *musculus*^{PWK} Y genotypes (LOD = 10.3, $R^2 = 0.37$; Figure 5B, Table 3).

The role of the *domesticus*^{LEWES} Y in sperm head morphology is further supported by an analysis in which all data (all X and Y genotypes) were considered together in a single model using CIM. In this analysis, the *domesticus*^{LEWES} Y was associated with a small but significant negative effect on sperm head morphology (LOD = 1.65; $R^2 = 0.02$).

Y genotype had little effect on QTL for testis weight. QTL at 25.0 and 32.5 cM for which there was a negative effect of *musculus*^{PWK} X genotype were absent for *musculus*^{PWK} Y genotypes (Figure 5B, Table 3), but a small interval between 32.0 and 34.7 cM remained marginally significant for *domesticus*^{LEWES} Y genotypes (LOD = 2.1, $R^2 = 0.07$; Figure 5A, Table 3). Y genotype did not influence the location of distal QTL for which the *musculus*^{PWK} X genotype had a positive effect on testis weight: the interval between 48.5 and 62.2 cM remained significant regardless of Y genotype.

No evidence for X-autosome incompatibilities unique to F_1 males

The pervasive deficits in testis mass and sperm count shown in Figure 2 demonstrate that X-autosome interactions play a major role in hybrid male sterility. However, these results do not indicate whether X-autosome incompatibilities in F_1's persist on other genetic backgrounds. For example, while pairwise incompatibilities between X-linked loci and autosomal-dominant loci can act in both F_1 and late-backcross backgrounds, X-autosome incompatibilities involving underdominant alleles are unique to the F_1 background. We evaluated the potential for F_1-specific incompatibilities by comparing the location of 2-LOD CIs for X-linked sterility QTL identified in this study to those mapped in an earlier study, in which regions of the *M. m. musculus* X were introgressed onto a *M. m. domesticus* background (Good *et al.* 2008b).

We found no evidence of QTL specific to F_1 males. QTL analysis in the N_{6-8} progeny of the backcross to *domesticus*^{LEWES} identified intervals of large negative effect on testis weight,

Table 3 Reproductive QTL on the *musculus*^{PWK} X chromosome in X introgression F_1 males split by Y chromosome genotype

	domesticus^{LEWES} Y (n = 40)		*musculus*^{PWK} Y (n = 67)	
Trait	QTL position (CI)[a]	Phenotypic contribution (%)[b]	QTL position (CI)	Phenotypic contribution (%)
Testis weight	30.5 (30.0–37.2)	−7.0	—	—
	47.0 (46.1–53.0)	3.0	47.5 (47.0–59.3)	19.0
	58.5 (55.5–60.5)	54.0	—	—
Sperm head morphology	—	—	4.2 (4.0–6.1)	37.0
	24.0 (12.4–27.0)	−50.0	20.5 (17.8–22.1)	−20.0
	31.5 (27.0–32.7)	−41.0	—	—
	—	—	54.0 (47.4–59.5)	44.0

[a] Position in centimorgans estimated using composite interval mapping (CIM). CI, 2-LOD confidence interval; —, no overlap in QTL CI across Y chromosome genotypes.
[b] Estimate of R^2 in CIM model expressed as percent. Negative values indicate a negative effect of *musculus*^{PWK} X genotype.

sperm count, and sperm head morphology on the *musculus*^{PWK} X proximal to 29.5 cM and intervals of smaller negative effect distal to 49.0 cM (Good *et al.* 2008b). In F_1 males, QTL with negative effects on reproductive phenotypes were all proximal to 34.7 cM and were only detected for sperm head morphology and testis weight. The 2-LOD CI for the sperm head morphology QTL peak on the proximal X (18.3–22.6 cM; Figure 4, Table 3) was almost completely contained within the 2-LOD CI for the QTL with the largest negative effect on that phenotype in late-backcross males (18.5–24.5 cM; Good *et al.* 2008b). Similarly, the CI for the first of two QTL with modest negative effects on testis mass in F_1 males (24.5–31.5 cM) overlapped that for a QTL with a large negative effect on testis mass in late-backcross males (15.1–25.5 cM; Good *et al.* 2008b). In contrast, many sterility-associated QTL detected on a *domesticus*^{LEWES} background were missing in F_1's. These included two QTL for testis weight at (13.3 and 59.5 cM), two QTL for sperm count (15.1 and 67.0 cM), and five QTL for sperm head morphology (2.5, 10.3, 25.5, 49.0, and 73.3 cM) (Good *et al.* 2008b).

Discussion

We used an F_1 cross between *M. m. musculus* and *M. m. domesticus* to evaluate the contribution of the *M. m. domesticus* Y chromosome to X-linked male sterility. Sterility did not depend on Y chromosome genotype: most genotypes had reduced testis weight and sperm count relative to controls, regardless of the origin of the Y. However, we found evidence for a negative interaction between the *M. m. domesticus* Y and an interval on the proximal region of the *M. m. musculus* X that was specific to sperm head morphology. Finally, there was considerable overlap between X-linked sterility QTL in F_1 males and those mapped in X introgression males from the same cross. We discuss these results in light of the role of Y-linked genes in spermatogenesis, previous studies of post-zygotic isolation in house mice, and theoretical expectations for the genetic architecture of reproductive incompatibilities during the early stages of speciation.

The contribution of the Y chromosome to hybrid male sterility

Previous work on the strains used in this study demonstrated a large negative effect of the *M. m. musculus* X in both F_1 and *M. m. domesticus* autosomal backgrounds (Good *et al.* 2008a,b). The present study rules out the possibility that this effect is due solely to incompatibilities between the *M. m. musculus* X and *M. m. domesticus* Y. This result is consistent with a recent F_2 study using different wild-derived inbred strains, in which large sterility QTL on the *M. m. musculus* X were detected in a mapping population with mainly *M. m. musculus* Y genotypes (White *et al.* 2011). Similar results were obtained in mapping studies in which *M. m. musculus* or *M. m. molossinus* (a *M. m. musculus–M. m. castaneus* hybrid) were crossed to the largely *M. m. domesticus*-derived laboratory strain, C57BL/6J (B6), which carries a *M. m. musculus*-derived Y chromosome (Tucker *et al.* 1992b; Storchová *et al.* 2004; Oka *et al.* 2007; Yang *et al.* 2011). We did, however, find evidence for a negative effect of the *M. m. domesticus* Y in males with *M. m. musculus* introgressions on the proximal X chromosome.

Sex chromosome genotypes in this study were heterogeneous in that each Y was paired with different regions of the *M. m. musculus* X. Therefore, we are cautious in our interpretation of statistical evidence for negative interactions between the X and Y. However, despite loss of power in the QTL analyses split by Y genotype, we detected a large negative effect of the *domesticus*^{LEWES} Y on sperm head morphology. This result suggests that negative epistasis between the *domesticus*^{LEWES} Y and *musculus*^{PWK} X contributes to sperm abnormalities. Crosses to introgress the *domesticus*^{LEWES} Y onto a *musculus*^{PWK} background are underway and will allow us to directly test this hypothesis.

Although the mammalian Y chromosome contains few genes, there is no shortage of candidates for contribution to hybrid sperm abnormality. Most genes on the Y are expressed predominantly or exclusively in the testes, and several are known to be essential for male reproduction. These include the testis determinant *Sry*, spermatogonial proliferation factor *Eif2s3y*, *Zfy2*, which regulates meiotic check points, and a cluster of multicopy genes on the male-specific region of the long arm of the Y (MSYq), which are implicated in postmeiotic spermiogenesis (Mazeyrat *et al.* 2001; Touré *et al.* 2004; Ferguson *et al.* 2009; Royo *et al.* 2010; Vernet *et al.* 2011). Among the latter group, *Sly* is required for the maintenance of PMSR and normal sperm differentiation in mice (Cocquet

et al. 2009). *Sly* copy number imbalance between *M. m. domesticus* and *M. m. musculus* was recently put forth as the primary cause of sterility in males with a *musculus*^{PWK} X and *domesticus*^{LEWES} Y (Ellis *et al.* 2011). The results presented here clearly refute this hypothesis. Indeed, the introgression line that causes the most severe effects on male reproduction has a *musculus*^{PWK} Y (cross 4, Figure 2). However, these findings do not rule out a *Sly*-linked effect on sperm abnormality.

In *Drosophila*, the Y chromosome influences the expression of a large number of X-linked and autosomal genes (Jiang *et al.* 2010; Lemos *et al.* 2010; Sackton *et al.* 2011). While there is currently no evidence for a genome-wide effect of the Y on expression in house mice, F_1 *musculus*^{PWK} × *domesticus*^{LEWES} males and males with a *D. sechellia* Y on a *D. simulans* background share several sterility phenotypes, including misexpression of postmeiotic genes and low quality sperm (Good *et al.* 2010; Sackton *et al.* 2011). As proposed by Ellis *et al.* (2011), this raises the intriguing possibility that there is a Y-linked effect on X overexpression during the later stages of spermatogenesis in F_1 males (Good *et al.* 2010), and that this causes abnormal sperm morphology. However, it is important to note that the disruption of X-linked gene expression may be a common consequence of diverse incompatibilities that disrupt the later stages of spermatogenesis (Homolka *et al.* 2007; Mihola *et al.* 2009; Good *et al.* 2010).

Given the lack of evidence for an essential role of the Y chromosome in hybrid male sterility in the laboratory, the comparably steep clines for X and Y chromosome markers in several transects across the *M. m. domesticus–M. m. musculus* European hybrid zone (*e.g.*, Tucker *et al.* 1992a) are somewhat puzzling. One explanation is that the fitness costs of X–Y incompatibilities are high enough to eliminate Y introgression across the hybrid zone. Males in the hybrid zone exhibit a variety of reproductive deficits that include abnormal sperm (Turner *et al.* 2012); it would be interesting to determine the contribution of Y genotype to sperm phenotypes in these males. It is also possible that Y chromosomes from wild-derived inbred strains are not representative of Y chromosomes in nature.

The genetic architecture of male sterility

The preferential sterility or inviability of heterogametic F_1 hybrids is one of the most consistent patterns in speciation genetics (Haldane 1922; Coyne and Orr 2004). While the causes of F_1 male sterility have been studied for the better part of a century (*e.g.*, Dobzhansky 1936; Oka *et al.* 2010) the underlying genetic architecture is difficult to map, and the assumption that loci that cause deficits in F_1's are among those mapped in backcross or F_2 backgrounds is rarely tested (Coyne and Orr 2004).

In this study, we found no convincing evidence for X-linked sterility QTL that were unique to F_1 males. Instead, QTL detected in this study were a subset of those detected on a largely homozygous background (Good *et al.* 2008b). This observation suggests that X-autosome incompatibilities in late backcross hybrids include those seen in F_1's as well as others, likely involving autosomal-recessive mutations.

There are several caveats to this conclusion. First, with 11 recombination breakpoints on the X our study was underpowered to detect QTL. Therefore, we cannot exclude the existence of X-linked sterility QTL whose autosomal interaction partners are unique to a heterozygous background. Second, overlap between sterility QTL in F_1 and late-backcross males suggests, but does not demonstrate, the same genetic basis. Fine-scale mapping in recombinant genetic backgrounds will be required to test this hypothesis. Third, moderate reductions in testis mass and sperm count in most hybrid genotypes, including the F_1 with a complete *domesticus*^{LEWES} X, suggests that autosomal incompatibilities that do not involve the X might contribute to reproductive deficits in F_1 males. This could explain the partial recovery of testis weight in some N_2 progeny of backcrosses to *domesticus*^{LEWES} (Good *et al.* 2008b) or B6 (Storchová *et al.* 2004).

On average, loci contributing to hybrid sterility or inviability are expected to be partially recessive (Orr 1993; Turelli and Orr 1995; Turelli and Orr 2000). The exposure of recessive incompatibility loci on the hemizygous X is among the best-supported explanations for Haldane's rule (Coyne and Orr 2004), and empirical work in *Drosophila* and house mice suggests that autosomal recessive incompatibilities outnumber autosomal dominants (Presgraves 2003; Tao and Hartl 2003; Masly and Presgraves 2007; White *et al.* 2011; but see Orr and Irving 2001). In this study, the absence of several large X-linked QTL in F_1 *vs.* late-backcross males indicates that X-autosomal recessive interactions contribute to hybrid male sterility. Nonetheless, F_1 males with a *musculus*^{PWK} X are partially sterile and the results of this study demonstrate that X-autosomal dominant incompatibilities are essential to this phenotype. If the inference that the same X-autosome incompatibilities persist on a homozygous background is correct, then the minimum number of incompatibilities required for reproductive isolation may be considerably smaller than the total number of loci that *can* cause hybrid deficits on backgrounds in which autosomal recessive incompatibilities are exposed.

Acknowledgments

This manuscript was significantly improved by the comments of D. Begun and three anonymous reviewers. We are grateful to M. Phifer-Rixey and R. Reifova for insightful comments and discussion and to B. Walsh for advice on statistical analyses. We thank N. Bittner, T. Suzuki, and the staff of the University of Arizona Central Animal Care Facility for assistance with mouse husbandry. C. W. Birky provided equipment used during the course of the experiment. P.C. is supported by a G. G. Simpson Postdoctoral Fellowship from the University of Arizona. This work was funded by National Science Foundation grants to M.W.N. and P.K.T., and a National Institutes of Health grant to M.W.N.

Literature Cited

Bateson, W., 1909 Heredity and variation in modern lights, pp. 85–101 in *Darwin and Modern Science*, edited by A. C. Seward. Cambridge University Press, Cambridge, UK.

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

Cocquet, J., P. J. I. Ellis, Y. Yamauchi, S. K. Mahadevaiah, N. A. Affara *et al.*, 2009 The multicopy gene *Sly* repressed the sex chromosomes in the male mouse germline after meiosis. PLoS Biol. 7: e1000244.

Cocquet, J., P. J. I. Ellis, Y. Yamauchi, J. M. Riel, T. P. S. Karacs *et al.*, 2010 Deficiency in the multicopy *Sycp3*-like X-linked genes *Slx* and *Slxl1* causes major defects in spermatid differentiation. Mol. Biol. Cell 21: 3497–3505.

Coyne, J. A., 1985 The genetic basis of Haldane's rule. Nature 314: 736–738.

Coyne, J. A., 1992 Genetics and speciation. Nature 355: 511–515.

Coyne, J. A., and H. A. Orr, 1989 Two rules of speciation, pp. 180–207 in *Speciation and Its Consequences*, edited by D. Otte and J. Endler. Sinauer Associates, Sunderland, MA.

Coyne, J. A., and H. A. Orr, 2004 *Speciation*, Sinauer Associates, Sunderland, MA.

Dobzhansky, T., 1936 Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 21: 113–135.

Dobzhansky, T., 1937 *Genetics and the Origin of Species*, Columbia University Press, New York.

Eicher, E. M., L. W. Washburn, J. B. Whitney III. and K. E. Morrow, 1982 *Mus poschiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. Science 217: 535–537.

Ellis, P. J. I., J. Bacon, and N. A. Affara, 2011 Association of *Sly* with sex-linked gene amplification during mouse evolution: a side effect of genomic conflict in spermatids? Hum. Mol. Genet. 20: 3010–3021.

Ferguson, L., P. J. I. Ellis, and N. A. Affara, 2009 Two novel mouse genes mapped to chromosome Yp are expressed specifically in spermatids. Mamm. Genome 20: 193–206.

Forejt, J., 1996 Hybrid sterility in the mouse. Trends Genet. 12: 412–417.

Forejt, J., and P. Iványi, 1974 Genetic studies on male sterility of hybrids between laboratory and wild mice (*Mus musculus* L.). Genet. Res. 24: 189–206.

Geraldes, A., M. Carneiro, M. Delibes-Mateos, R. Villafuerte, M. W. Nachman *et al.*, 2008 Reduced introgression of the Y chromosome between subspecies of the European rabbit (*Oryctolagus cuniculus*) in the Iberian Peninsula. Mol. Ecol. 17: 4489–4499.

Geraldes, A., P. Basset, K. L. Smith, and M. W. Nachman, 2011 Higher differentiation among subspecies of the house mouse (*Mus musculus*) in genomic regions with low recombination. Mol. Ecol. 20: 4722–4736.

Good, J. M., M. A. Handel, and M. W. Nachman, 2008a Asymmetry and polymorphism of hybrid male sterility during the early stages of speciation in house mice. Evolution 62: 50–65.

Good, J. M., M. D. Dean, and M. W. Nachman, 2008b A complex genetic basis to X- linked hybrid male sterility between two species of house mice. Genetics 179: 2213–2228.

Good, J. M., T. Giger, M. D. Dean, and M. W. Nachman, 2010 Widespread over-expression of the X chromosome in sterile F_1 hybrid mice. PLoS Genet. 6: e1001148.

Gregorová, S., and J. Forejt, 2000 PWD/Ph and PWK/Ph inbred mouse strains of *Mus m. musculus* subspecies: a valuable resource of phenotypic variations and genomic polymorphisms. Folia Biol. 46: 31–41.

Haldane, J. B. S., 1922 Sex ratio and unisexual sterility in animal hybrids. J. Genet. 12: 101–109.

Homolka, D., R. Ivanek, J. Capkova, P. Jansa, and J. Forejt, 2007 Chromosomal rearrangement interferes with meiotic X chromosome inactivation. Genome Res. 17: 1431–1437.

Jiang, P.-P., D. L. Hartl, and B. Lemos, 2010 Y not a dead end: epistatic interactions between Y-linked regulatory polymorphisms and genetic background affect global gene expression in *Drosophila melanogaster*. Genetics 186: 109–118.

Laurie, C. C., 1997 The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics 147: 937–951.

Lemos, B., A. T. Branco, and D. L. Hartl, 2010 Epigenetic effects of polymorphic Y chromosomes modulate chromatin components, immune response, and sexual conflict. Proc. Natl. Acad. Sci. USA 107: 15826–15831.

Macholán, M., P. Munclinger, M. Šugerková, P. Dufková, B. Bímová *et al.*, 2007 Genetic analysis of autosomal and X-linked markers across a mouse hybrid zone. Evolution 61: 746–771.

Macholán, M., S. J. E. Baird, P. Munclinger, P. Dufková, B. Bímová *et al.*, 2008 Genetic conflict outweighs heterogametic incompatibility in the mouse hybrid zone? BMC Evol. Biol. 8: 271.

Masly, J. P., and D. C. Presgraves, 2007 High-resolution genome-wide dissection of the two rules of speciation in *Drosophila*. PLoS Biol. 5: e243.

Mazeyrat, S., N. Saut, V. Grigoriev, S. K. M. Mahadevaiah, and O. A. Ojarikre, 2001 A Y-encoded subunit of the translation initiation factor Eif2 is essential for mouse spermatogenesis. Nat. Genet. 29: 49–53.

McKee, B. D., and M. A. Handel, 1993 Sex chromosomes, recombination, and chromatin conformation. Chromosoma 102: 71–80.

Mihola, O., Z. Trachtulec, C. Vlcek, J. C. Schimenti, and J. Forejt, 2009 A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science 323: 373–375.

Mueller, J. L., S. K. Mahadevaiah, P. J. Park, P. E. Warburton, D. C. Page *et al.*, 2008 The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat. Genet. 40: 794–799.

Muller, H. J., 1942 Isolating mechanisms, evolution and temperature. Biol. Symposia 6: 71–125.

Namekawa, S. H., P. J. Park, L. F. Zhang, J. E. Shima, J. R. McCarrey *et al.*, 2006 Postmeiotic sex chromatin in the male germline of mice. Curr. Biol. 16: 660–667.

Oka, A., T. Aoto, Y. Totsuka, R. Takahashi, M. Ueda *et al.*, 2007 Disruption of genetic interaction between two autosomal regions and the X chromosome causes reproductive isolation between mouse strains derived from different subspecies. Genetics 175: 185–197.

Oka, A., A. Mita, Y. Takada, H. Koseki, and T. Shiroishi, 2010 Reproductive isolation in hybrid mice due to spermatogenesis defects at three meiotic stages. Genetics 186: 339–351.

Orr, H. A., 1993 A mathematical model of Haldane's rule. Evolution 47: 1606–1611.

Orr, H. A., and S. Irving, 2001 Complex epistasis and the genetic basis of hybrid sterility in the *Drosophila pseudoobscura* Bogota-USA hybridization. Genetics 158: 1089–1100.

Payseur, B. A., J. G. Krenz, and M. W. Nachman, 2004 Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. Evolution 58: 2064–2078.

Prager, E. M., P. Boursot, and R. D. Sage, 1997 New assays for Y chromosome a p53 pseudogene clines among East Holstein mice. Mamm. Genome 8: 279–281.

Presgraves, D. C., 2002 Patterns of postzygotic isolation in Lepidoptera. Evolution 56: 1168–1183.

Presgraves, D. C., 2003 A fine-scale genetic analysis of hybrid incompatibilities in *Drosophila*. Genetics 163: 955–972.

Presgraves, D. C., 2008 Sex chromosomes and speciation in *Drosophila*. Trends Genet. 24: 336–343.

Price, T. D., and M. M. Bouvier, 2002 The evolution of postzygotic incompatibilities in birds. Evolution 56: 2083–2089.
Reynard, L. N., and J. M. A. Turner, 2009 Increased sex chromosome expression and epigenetic abnormalities in spermatids from male mice with Y chromosome deletions. J. Cell Sci. 122: 4239–4248.
Reynard, L. N., J. Cocquet, and P. S. Burgoyne, 2009 The multi-copy mouse gene *Sycp3*-Like Y-linked (*Sly*) encodes an abundant spermatid protein that interacts with a histone acetyltransferase and an acrosomal protein. Biol. Reprod. 81: 250–257.
Royo, H., G. Polikiewicz, S. K. Mahadevaiah, H. Prosser, M. Mitchell *et al.*, 2010 Evidence that meiotic sex chromosome inactivation is essential for male fertility. Curr. Biol. 20: 2117–2123.
Russell, L. D., R. A. Ettlin, A. P. Sinha Hikin, and E. D. Clegg, 1990 *Histological and Histopathological Evaluation of the Testis*, Cache River Press, Clearwater, FL.
Sackton, T. B., H. Montenegro, D. L. Hartl, and B. Lemos, 2011 Interspecific Y chromosome introgressions disrupt testis-specific gene expression and male reproductive phenotypes in *Drosophila*. Proc. Natl. Acad. Sci. USA 108: 17046–17051.
Scavetta, R. J., and D. Tautz, 2010 Copy number changes of CNV regions in intersubspecific crosses of the house mouse. Mol. Biol. Evol. 27: 1845–1856.
Storchová, R., S. Gregorová, D. Buckiová, V. Kyselová, P. Divina *et al.*, 2004 Genetic analysis of X-linked hybrid sterility in the house mouse. Mamm. Genome 15: 515–524.
Sweigart, A. L., 2010 Simple Y-autosomal incompatibilities cause hybrid male sterility in reciprocal crosses between *Drosophila virilis* and *D. americana*. Genetics 184: 779–787.
Tao, Y., and D. L. Hartl, 2003 Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *Drosophila mauritiana*. III. Heterogeneous accumulation of hybrid incompatibilities, degree of dominance and implications for Haldane's rule. Evolution 57: 2580–2598.
Teeter, K. C., L. M. Thibodeau, Z. Gompert, C. A. Buerkle, M. W. Nachman *et al.*, 2010 The variable genomic architecture of isolation between hybridizing species of house mice. Evolution 64: 472–485.
Touré, A., M. Szot, S. K. Mahadevaiah, Á. Rattigan, O. A. Ojarikre *et al.*, 2004 A new deletion of the mouse Y chromosome long arm associated with the loss of *Ssty* expression, abnormal sperm development and sterility. Genetics 166: 901–912.
Tucker, P. K., R. D. Sage, J. Warner, A. C. Wilson, and E. M. Eicher, 1992a Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution 46: 1146–1163.
Tucker, P. K., B. K. Lee, B. L. Lundrigan, and E. M. Eicher, 1992b Geographic origin of the Y chromosome in "old" inbred strains. Mamm. Genome 3: 254–261.
Turelli, M., and H. A. Orr, 1995 The dominance theory of Haldane's rule. Genetics 140: 389–402.
Turelli, M., and H. A. Orr, 2000 Dominance, epistasis and the genetics of postzygotic reproductive isolation. Genetics 154: 1663–1679.
Turner, J. M. A., 2007 Meiotic sex chromosome inactivation. Development 10: 1823–1831.
Turner, J. M. A., S. K. Mahadevaiah, P. J. I. Ellis, M. J. Mitchell, and P. S. Burgoyne, 2006 Pachytene asynapsis drives meiotic sex chromosome inactivation and leads to substantial postmeiotic repression in spermatids. Dev. Cell 10: 521–529.
Turner, L. M., D. J. Schwahn, and B. Harr, 2012 Reduced male fertility is common but highly variable in form and severity in a natural house mouse hybrid zone. Evolution 66: 443–458.
Vanlerberghe, F., B. Dod, P. Boursot, M. Bellis, and F. Bonhomme, 1986 Absence of Y chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. Genet. Res. Camb. 48: 191–197.
Vernet, N., S. K. Mahadevaiah, O. A. Ojarikre, G. Longepied, H. M. Prosser *et al.*, 2011 The Y-encoded gene *Zfy2* acts to remove cells with unpaired chromosomes at the first meiotic metaphase in male mice. Curr. Biol. 21: 787–793.
Vyskočilová, M., Z. Trachtulec, J. Forejt, and J. Piálek, 2005 Does geography matter in hybrid sterility in house mice? Biol. J. Linn. Soc. Lond. 84: 663–674.
Vyskočilová, M., G. Pražanová, and J. Piálek, 2009 Polymorphism in hybrid male sterility in wild-derived *Mus musculus musculus* strains on proximal chromosome 17. Mamm. Genome 20: 83–91.
Wang, S., C. J. Basten, and Z.-B. Zeng, 2011 *Windows QTL Cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, NC, http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.
White, M. A., B. Steffy, T. Wiltshire, and B. A. Payseur, 2011 Genetic dissection of a key reproductive barrier between nascent species of house mice. Genetics 189: 289–304.
Yang, H., J. R. Wang, J. P. Didion, R. J. Buus, T. A. Bell *et al.*, 2011 Subspecific origin and haplotype diversity in the laboratory mouse. Nat. Genet. : 648–655.

Communicating editor: D. Begun