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

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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 F1 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 F1 autosomal background, and we measured a suite of male reproductive traits. Reproductive deficits were observed in most F1 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 F1 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 F1 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, F1 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...
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 multilocus genes (Cocquet et al. 2009, 2010; Ellis et al. 2011). In house mice, both the X and Y chromosomes are enriched for multilocus genes, such as Sly and Sli, and copy numbers differ between the subspecies (Mueller et al. 2008; Scavetta and Tautz 2010; Ellis et al. 2011). In reproducively 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 multilocus 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 F1 hybrids; in a cross between wild-derived inbred strains of M. m. musculus (musculusPWK) and M. m. domesticus (domesticusLEWES), 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 F1 males with a M. m. musculus X chromosome, while hybrid males with a M. m. domesticus X are normal (Good et al. 2008a,b). 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 F1 male sterility in the cross between musculusPWK and domesticusLEWES. 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 F1 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 F1 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/EJ 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
y effects on F1 male sterility in mice

(Gregorová and Forejt 2000). The WSB/EiJ (domesticusWSB) and CZECHII/EiJ (musculusCZECHIII) 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 musculusPWK or domesticusLEWES 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 musculusPWK and male domesticusLEWES were backcrossed to either musculusPWK or domesticusLEWES 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 musculusPWK or domesticusLEWES X were selected for breeding (Good et al. 2008b).

In the current study, we included two additional X introgression lines with musculusPWK introgressions from 7.2 to 38.2 Mb, and 33.7 to 82.8 Mb. All X introgression lines carry the musculusPWK mitochondrial haplotype.

X introgression females were crossed to pure heterosubspecific males, such that F1 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 F1 males from intrasubspecific crosses: domesticusWSB × domesticusLEWES and musculusCZECHIII × musculusPWK (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 Dulbeco’s medium, prewarmed to 37°C. Following a 10-min incubation at 37°C, 200 μl of sperm suspension was heat shocked for 5 min at 60°C. 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
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 small, asymmetrical 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.

Table 1 Mean reproductive parameters for experimental and control males

<table>
<thead>
<tr>
<th>Experimental crosses</th>
<th>n</th>
<th>RTW (SD)</th>
<th>Sperm count (SD)</th>
<th>% normal sperm (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ♀ domesticus × ♂ musculus</td>
<td>14</td>
<td>4.6** (0.3)</td>
<td>15.0* (4.2)</td>
<td>95.2 (4.1)</td>
</tr>
<tr>
<td>2 ♀ domesticus*MUS x-2 × ♂ musculus</td>
<td>14</td>
<td>4.0** (0.2)</td>
<td>8.6** (2.7)</td>
<td>90.0 (9.6)</td>
</tr>
<tr>
<td>3 ♀ domesticus*MUS x-3 × ♂ musculus</td>
<td>12</td>
<td>3.9** (0.2)</td>
<td>11.3* (5.1)</td>
<td>63.1** (24.3)</td>
</tr>
<tr>
<td>4 ♀ domesticus*MUS x-4 × ♂ musculus</td>
<td>14</td>
<td>3.3** (0.1)</td>
<td>4.0** (2.3)</td>
<td>28.3** (19.6)</td>
</tr>
<tr>
<td>5 ♀ domesticus*MUS x-5 × ♂ musculus</td>
<td>13</td>
<td>4.3** (0.2)</td>
<td>11.6** (8.5)</td>
<td>90.2 (16.2)</td>
</tr>
<tr>
<td>6 ♀ domesticus*MUS x-6 × ♂ musculus</td>
<td>14</td>
<td>5.0 (0.4)</td>
<td>15.2* (3.7)</td>
<td>94.4 (9.4)</td>
</tr>
<tr>
<td>7 ♀ musculus*DOM X-7 × ♂ domesticus</td>
<td>14</td>
<td>5.5 (0.3)</td>
<td>19.6 (6.6)</td>
<td>82.7 (19.8)</td>
</tr>
<tr>
<td>8 ♀ musculus*DOM X-8 × ♂ domesticus</td>
<td>14</td>
<td>4.7* (0.3)</td>
<td>18.2 (7.5)</td>
<td>73.0* (20.8)</td>
</tr>
<tr>
<td>9 ♀ musculus*DOM X-9 × ♂ domesticus</td>
<td>12</td>
<td>4.1** (0.2)</td>
<td>9.5 (5.0)</td>
<td>23.0** (12.7)</td>
</tr>
<tr>
<td>10 ♀ musculus × ♂ domesticus</td>
<td>14</td>
<td>3.5** (0.3)</td>
<td>3.5** (1.9)</td>
<td>2.5** (2.9)</td>
</tr>
</tbody>
</table>

Control crosses

| ♀ musculus*CZECH × ♂ musculus*PWK | 14 | 5.1 (0.6) | 19.9 (7.6) | 88.8 (11.1) |
| ♀ domesticus*VSR × ♂ domesticus*LEWES | 14 | 5.4 (0.4) | 30.8 (10.6) | 95.9 (3.7) |

* Wilcoxon $P < 0.005$, **$P < 0.0001$ 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 tests weight in milligrams per gram of body weight.

c $\times 10^6$ per ml.

All reproductive measures deviated from a normal distribution (Shapiro–Wilk $W$ test, all $P < 0.0003$) 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 F1 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*PWK Y, $n = 67$; domesticus*LEWES Y, $n = 40$) and asked whether X-linked QTL were influenced by Y.
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 F1 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 F1 (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 F1 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 F1 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 F1 male sterility in...
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, \(a = 0.05\)) 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 5B) but was absent for *domesticus*\textsubscript{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*\textsubscript{PWK} Y genotypes (LOD = 10.3, $R^2 = 0.37$; Figure 5B, Table 3).

The role of the *domesticus*\textsubscript{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*\textsubscript{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*\textsubscript{PWK} X genotype were absent for *musculus*\textsubscript{PWK} Y genotypes (Figure 5B, Table 3), but a small interval between 32.0 and 34.7 cM remained marginally significant for *domesticus*\textsubscript{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*\textsubscript{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 F1 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 F1's persist on other genetic backgrounds. For example, while pairwise incompatibilities between X-linked loci and autosomal-dominant loci can act in both F1 and late-backcross backgrounds, X-autosome incompatibilities involving underdominant alleles are unique to the F1 background. We evaluated the potential for F1-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 F1 males. QTL analysis in the N\textsubscript{6.8} progeny of the backcross to *domesticus*\textsubscript{LEWES} identified intervals of large negative effect on testis weight,
sperm count, and sperm head morphology on the Musculus\textsuperscript{PWK} X proximal to 29.5 cM and intervals of smaller negative effect distal to 49.0 cM (Good et al. 2008b). In F1 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 F1 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 Musculus\textsuperscript{LEWES} background were missing in F1’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 F1 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 F1 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 postzygotic 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 F1 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 F2 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\textsuperscript{LEWES} Y on sperm head morphology. This result suggests that negative epistasis between the domesticus\textsuperscript{LEWES} Y and musculus\textsuperscript{PWK} X contributes to sperm abnormalities. Crosses to introgress the domesticus\textsuperscript{LEWES} Y onto a musculus\textsuperscript{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 (Mazurey 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).
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 musculusPWK X and domesticusLEWES 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 musculusPWK 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, F1 musculusPWK × domesticusLEWES 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 F1 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 chromosome in hybrid male sterility in the laboratory, the

The genetic architecture of male sterility

The preferential sterility or inviability of heterogametic F1 hybrids is one of the most consistent patterns in speciation genetics (Haldane 1922; Coyne and Orr 2004). While the causes of F1 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 F1’s are among those mapped in backcross or F2 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 F1 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 F1’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 F1 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 F1 with a complete domesticusLEWES X, suggests that autosomal incompatibilities that do not involve the X might contribute to reproductive deficits in F1 males. This could explain the partial recovery of testis weight in some N2 progeny of backcrosses to domesticusLEWES (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 F1 vs. late-backcross males indicates that X-autosomal recessive interactions contribute to hybrid male sterility. Nonetheless, F1 males with a musculusPWK 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.

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Literature Cited


