Letter to the Editor

Does Hybrid Lethality Depend on Sex or Genotype?

H. Allen Orr

Department of Biology, University of Rochester, Rochester, New York 14627

A recent spate of articles in this and other journals has highlighted a pattern that appears to characterize the genetics of speciation: the genes causing hybrid inviability typically kill both sexes, while the genes causing hybrid sterility typically sterilize one sex only (Orr 1993; Wu and Davis 1993; Turelli and Orr 1995; Hollocher and Wu 1996; True et al. 1996). An analogous pattern clearly holds within species: mutagenesis experiments in Drosophila conclusively show that lethal mutations usually kill both sexes, whereas sterile mutations usually sterilize one sex only, although exceptions do exist (reviewed in Ashburner 1989).

This apparent parallelism between and within species is important for several reasons. First, it strongly suggests that speciation typically involves “ordinary” genes having normal within-species functions. (Such parallelism would be remarkable if speciation routinely involved unusual genetic processes, not normal genes.) Second, the fact that hybrid steriles, like those within species, affect one sex only suggests that substitutions affecting, say, hybrid males might accumulate faster during evolution than those affecting hybrid females. Indeed it appears that such “faster male” evolution, possibly driven by sexual selection, plays an important role in speciation (Wu and Davis 1993; True et al. 1996; Presgraves and Orr 1998; Turelli 1998). Last, the fact that hybrid lethals affect both sexes implies that Haldane’s rule for inviability [the preferential lethality of heterogametic (X/Y) hybrids] cannot be due to faster accumulation of hybrid male than female lethals. This rule must instead have some other cause, probably that the genes causing hybrid inviability usually act recessively in hybrids (Turelli and Orr 1995 and below).

Given the importance of this apparent parallelism, we require more and better information about the sex specificity of hybrid lethals and steriles. (Surely no more information about within-species expression is needed.) To this end, I have performed a simple but novel test of the claim that hybrid lethals typically affect both sexes.

To see the logic of the test, consider the simplest case in which a single genetic incompatibility causes hybrid inviability. One can imagine two scenarios. In the first, a hybrid lethal is expressed in both sexes. If so, hybrid fitness depends solely on genotype: if hybrid males and females have the same genotype, they will both be viable or both be inviable. In the second case, a hybrid lethal is expressed in one sex only (say, males). In this case, hybrid fitness depends solely on somatic sex: if a hybrid of given genotype expresses male genes, it will die; if a hybrid of identical genotype expresses female genes, it will live.

Here I use genetic tools from Drosophila melanogaster to distinguish these possibilities. In particular, I use a mutation at the transformer (tra) locus, part of the Drosophila sex determination pathway, to transform the somatic sex of D. melanogaster-D. simulans species hybrids. Tra, which acts immediately downstream of the master switch gene, Sex-lethal, is the first gene in the sex determination cascade that affects sex determination alone, not dosage compensation (Cline 1985). Tra is active in genetically normal females (X:A ratio = 2:2) and inactive in genetically normal males (X:A = 1:2), where activity reflects alternative splicing of tra transcripts (for details, see McKeown et al. 1988). The hstra mutation is a P-element construct bearing female-specific tra cDNA fused to an hsp70 promoter (McKeown et al. 1988) that causes essentially complete somatic transformation of genetic males into females (a male-specific muscle remains untransformed). By introducing hstra into hybrids, we can test the viability of hybrids of identical genotype but different somatic sex. Because we must transform the sex of species hybrids—which are necessarily heterozygous for D. melanogaster mutations—we require dominant alleles. Fortunately, hstra overrides wild-type tra when in single dose (McKeown et al. 1988). (No dominant mutation allowing the reverse transformation of genetic females into phenotypic males is available.)

The cross of D. melanogaster females × D. simulans males normally produces viable female but inviable male hybrids (Sturtevant 1929), a result that we confirmed (Table 1, line 1). Genetic analysis has shown that this lethality, which occurs at the larval/pupal transition, does not involve the Y or the cytoplasm (Hutter et al. 1990; Yamamoto 1992). Instead, it appears that a gene(s) on the D. melanogaster X is incompatible with autosomal factors from D. simulans (Sturtevant 1929; Hutter et al. 1990).

Two explanations of the sex-limited effects of these
strongly support the first hypothesis \( X_{mel} \) females to genetically female. In fact, the \( X_{mel} \)-linked hybrid lethal(s) might be expressed in both males but be recessive. Thus hemizygous \( X_{mel}/Y_{sim} \) males die, while heterozygous \( X_{mel}/X_{sim} \) females live. Alternatively, the \( X_{mel} \)-linked hybrid lethal(s) might be expressed in males only. Although attached- \( X \) experiments strongly support the first hypothesis \([X_{ned}/X_{ned} \) females who are otherwise hybrid die (reviewed in Hutter et al. 1990)], \( hstra \) allows an independent and, as we will see, more informative test of these possibilities: by introducing \( hstra \) into hybrids, we produce hybrids of identical sex chromosome genotype that differ only in somatic sex.

We crossed \( D. melanogaster \) y w; \( K_{i}, hstra/ T M 68, T b, H u, e \) females to \( D. simulans \) st e (III-34, -61) males (for crossing details, see Table 1 legend). If viable, somatically transformed \( X/Y \) hybrids would appear as phenotypically yellow, white, forked, Kinked, non-ebony “females” (see Figure 1). No such individuals appeared, although over 1400 hybrids were recovered (Table 1, line 2). Further, no hybrid males appeared, as expected. Instead markers proved that all surviving flies were genetically female.

There are, however, two artifactual reasons why \( X_{ned}/Y_{sim} \): \( hstra \) somatic females might not appear. First, the \( hstra \) chromosome may simply be lethal in species hybrids. We can, however, rule out this possibility: we recovered abundant \( 885 \) \( X_{ned}/X_{sim} \); \( hstra \) genetically female hybrids, as Table 1 (line 2) shows. Second, and more seriously, \( hstra \) may not function in hybrids. Our failure to recover \( X_{ned}/Y_{sim} \); \( hstra \) “females” might, in other words, simply reflect \( hstra \)’s inability to transform sex in a hybrid genetic background. Given the rapid evolution of at least some sex-determining factors (e.g., deBono and Hodgkin 1996), this possibility must be taken seriously.

Fortunately, we can test this hypothesis. We repeated the cross of \( D. melanogaster \) y w; \( hstra/ T M 68, T b, H u, e \) females to \( D. simulans \) males, but the \( D. simulans \) males now carried a hybrid viability rescue mutation, \( L h r \). This allele restores the viability of the normally inviable hybrid males (Watanabe 1979). If \( hstra \) functions properly in hybrids we should now recover normally inviable males as well as somatically transformed \( X_{ned}/Y_{sim} \) phenotypic females. This is precisely what occurred. As Table 1 (line 3) shows, \( L h r \) successfully rescued 33 normally inviable hybrid males and 282 yellow, white, forked, Kinked \( X_{ned}/Y_{sim} \); \( hstra \) somatic females. This finding shows that \( hstra \) functions in hybrids and thus shows that our earlier failure to

### Table 1

<table>
<thead>
<tr>
<th>Cross</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. mel w × sim Islomanada</td>
<td>1034</td>
<td>0</td>
</tr>
<tr>
<td>2. mel y w f; hstra/ TM 68 × sim st e</td>
<td>885</td>
<td>0</td>
</tr>
<tr>
<td>3. mel y w f; hstra/ TM 68 × sim Lhr</td>
<td>355</td>
<td>228</td>
</tr>
</tbody>
</table>

\( mel, D. melanogaster; sim, D. simulans; hstra/ TM 68, P [hstra], Df[3L]tra, K_{i}, roe, p/y \) TM 68, Tb, Hu, e. In practice, somatic transformation in this stock occurs even at room temperature and heat shock is unnecessary (B. Taylor, personal communication; our results). Females are listed first in crosses. All crosses were performed at 22\(^\circ\). All somatic males appearing in the last cross possessed morphologically intermediate hybrid genitalia and were sterile.

\(^1\) 7 y\(^+\) w\(^+\) f\(^+\); TM 68 males were recovered. X-linked markers show that these males were nondisjunctional exceptions who carried the (viable) \( D. simulans \) X, not the (inviable) \( D. melanogaster \) X.

\(^2\) 1 y\(^+\) w\(^+\) f\(^+\); TM 68 male was also recovered. X-linked markers again show that this male was a nondisjunctional exception who carried the \( D. simulans \) X.

Figure 1.—Species cross resulting in transformation of hybrid sex. \( D. melanogaster \) chromosomes are shown in white, \( D. simulans \) in black. For each individual, the X chromosome genotype is shown at the left and the third chromosome genotype at the right. Among F1 hybrids, genotypic females are shown in the left column and genotypic males in the right. The somatic sex of each hybrid genotype is given.
In short, the lethality of D. melanogaster-D. simulans hybrids is a function of hybrid sex chromosome genotype, not somatic sex. While these findings confirm previous ones (Hutter et al. 1990), they also point to three new conclusions. First, although the primary hybrid incompatibility—that responsible for the complete absence of F1 males—depends on sex chromosome genotype, there is clearly some effect of somatic sex: the 1 hr cross shows that it is far easier to rescue individuals who are somatically female than male (228 somatic females vs. 33 somatic males), despite the fact that these individuals are all of X\textsubscript{mel} \textit{Y} \textsubscript{sim} sex chromosome genotype. This order of magnitude effect does not reflect an inherent difference in viability between the hstra and TM 6B chromosomes as no such large difference appears in genetic females (Table 1, line 3). Thus while expression of an X\textsubscript{mel} \textit{Y} \textsubscript{sim} genotype is sufficient to cause complete lethality in somatic males and females, somatic males appear “further gone” than females, suggesting that some of the factors affecting hybrid viability have more severe effects in one sex. This effect could not be readily detected in previous experiments because rescued X\textsubscript{mel} \textit{Y} \textsubscript{sim} males and X\textsubscript{mel} X\textsubscript{sim} attached-X females do not segregate together in the same cross.

Second, the fact that hstra functions across species boundaries suggests that primary sex determination mechanisms have not diverged at extraordinarily rapid rates, despite some evidence for rapid change in the worm Caenorhabditis (deBono and Hodgkin 1996) as well as evidence for selection at the tra locus itself (Civetta and Singh 1998). [D. melanogaster and D. simulans are fairly distantly related, showing 4–8% nucleotide differentiation (Begun and Aquadro 1994, 1995) and an allozyme genetic distance of D = 0.55 (Coyne and Orr 1989).] Functional conservation of sex determination genes within Drosophila is also supported by recent work by Erickson and Cline (1998). Third, our results suggest that hybrid lethality itself does not result from a disruption of sex determination. Although some evidence suggests that X chromosome number elements (which cells count to determine the number of X’s present and thus to determine sex) may evolve rapidly (Cline 1998), and although species hybrids sometimes suffer intersexuality (Sturtevant 1946), the lethality studied here is not caused by a collapse of sex determination, at least upstream of douplex activity: hstra ensures strict female somatic differentiation in X \textit{Y} individuals regardless of any ambiguity in upstream signals in hybrids (e.g., diverged number elements). Nonetheless, these strictly female X\textsubscript{mel} \textit{Y} \textsubscript{sim} hstra hybrids remain lethal. D. melanogaster-D. simulans hybrids do not, therefore, appear to suffer disruptions in either sex determination or dosage compensation (Orr 1989).

I thank A. Clark, J. A. Coyne, C. D. Jones, D. C. Presgraves, B. Taylor, M. Turelli, and two anonymous reviewers for conversations, criticisms, and comments. I especially thank S. Irving for expert technical assistance. This work was supported by National Institutes of Health grant GM-51932 and by the David and Lucile Packard Foundation.

LITERATURE CITED

Cline, T. W., 1988 Evidence that sisterless-α and sisterless-β are two of several discrete “numerator elements” of the X/α sex determination signal in Drosophila that switch Sxl between two alternative stable expression states. Genetics 119: 829–862.
Hutter, P., and J. R. Taylor, 1996 The genetics of reproductive isolation in the Drosophila simulans clade: X vs. autosomal effects and male vs. female effects. Genetics 143: 1243–1255.

Communicating editor: A. G. Clark