Letter to the Editor

Does Hybrid Lethality Depend on Sex or Genotype?

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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 hs-*tra* 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 *hs-tra* 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, hs-*tra* 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

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TABLE 1

Results of species crosses

Cross	Females		Males	
1. mel $w \times$ sim Islamorada	1034		0	
	Females		Males	
Cross	*X/X*; hs-*tra*	*X/X*; *tra*$^+$	*X/Y*; hs*tra*	*X/Y*; *tra*$^+$
2. mel *y w f*; hs-*tra*/*TM6B* $\times$ sim *st e*	885	589	0	0[a]
3. mel *y w f*; hs-*tra*/*TM6B* $\times$ sim *Lhr*	355	322	228	33[b]

mel, *D. melanogaster*; sim, *D. simulans*; hs-*tra*/*TM6B*, *P*[hs-*tra*], *Df(3L)tra*, *Ki*, *roe*, p^p/TM6B, *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°. All somatic males appearing in the last cross possessed morphologically intermediate hybrid genitalia and were sterile.

[a] 7 $y^+ w^+ f^+$; *TM6B* males were recovered. *X*-linked markers show that these males were nondisjunctional exceptions who caried the (viable) *D. simulans X*, not the (inviable) *D. melanogaster X*.

[b] 1 $y^+ w^+ f^+$; *TM6B* male was also recovered. *X*-linked markers again show that this male was a nondisjunctional exception who carried the *D. simulans X*.

hybrid lethals are possible. First, the *D. melanogaster X*-linked hybrid lethal(s) might be expressed in both sexes but be recessive. Thus hemizygous (X_{mel}/Y_{sim}) males die, while heterozygous (X_{mel}/X_{sim}) females live. Alternatively, the *X*-linked hybrid lethal(s) might be expressed in males only. Although attached-*X* experiments strongly support the first hypothesis [X_{mel}/X_{mel} females who are otherwise hybrid die (reviewed in Hutter *et al.* 1990)], hs-*tra* allows an independent and, as we will see, more informative test of these possibilities: by introducing hs-*tra* into hybrids, we produce hybrids of *identical* sex chromosome genotype that differ only in somatic sex.

We crossed *D. melanogaster y w f; Ki*, hs-*tra*/*TM6B, Tb, Hu, 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_{mel}/Y_{sim}; hs-*tra* somatic females might not appear. First, the hs-*tra* chromosome may simply be lethal in species hybrids. We can, however, rule out this possibility: we recovered abundant (885) X_{mel}/X_{sim}; hs-*tra* genetically *female* hybrids, as Table 1 (line 2) shows. Second, and more seriously, hs-*tra* may not function in hybrids. Our failure to recover X_{mel}/Y_{sim}; hs-*tra* "females" might, in other words, simply reflect hs-*tra*'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 f; hs-tra*/*TM6B, Tb, Hu, e* females to *D. simulans* males, but the *D. simulans* males now carried a hybrid viability rescue mutation, *Lethal hybrid rescue* (*Lhr* II-95). This allele restores the viability of the normally inviable hybrid males (Watanabe 1979). If hs-*tra* functions properly in hybrids we should now recover normally inviable males *as well as* somatically transformed X_{mel}/Y_{sim}; phenotypic females. This is precisely what occurred. As Table 1 (line 3) shows, *Lhr* successfully rescued 33 normally inviable hybrid males and 228 yellow, white, forked, Kinked X_{mel}/Y_{sim}; hs-*tra* somatic females. This finding shows that hs-*tra* functions in hybrids and thus shows that our earlier failure to

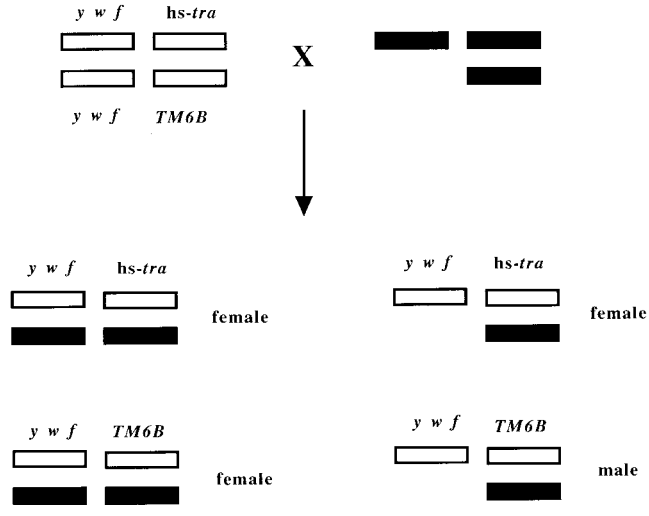

Figure 1.—Species cross resulting in transformation of hybrid sex. *D. melanogaster* chromosomes are shown in white, *D. simulans* in black. For each individual, the *X* chromosome genotype is shown at the left and the third chromosome genotype at the right. Among F_1 hybrids, genotypic females are shown in the left column and genotypic males in the right. The somatic sex of each hybrid genotype is given.

recover X_{mel}/Y_{sim}; hs-*tra* females reflected the lethality of this genotype.

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 F_1 males—depends on sex chromosome genotype, there is clearly *some* effect of somatic sex: the *Lhr* 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_{mel}/Y_{sim} sex chromosome genotype. This order of magnitude effect does not reflect an inherent difference in viability between the hs-*tra* and *TM6B* chromosomes as no such large difference appears in genetic females (Table 1, line 3). Thus while expression of an X_{mel}/Y_{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_{mel}/Y_{sim} males and X_{mel}/X_{mel} attached-*X* females do not segregate together in the same cross.

Second, the fact that hs-*tra* 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 divergence (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 numerator elements (which cells count to determine the number of *X*'s present and thus to determine sex) may evolve rapidly (Cline 1988), 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 *doublesex* activity: hs-*tra* ensures strict female somatic differentiation in *X*/*Y* individuals regardless of any ambiguity in upstream signals in hybrids (*e.g.*, diverged numerator elements). Nonetheless, these strictly female X_{mel}/Y_{sim}; hs-*tra* hybrids remain lethal. *D. melanogaster-D. simulans* hybrids do not, therefore, appear to suffer disruptions in either sex determination or dosage compensation (Orr 1989).

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