Does Postzygotic Isolation Result From Improper Dosage Compensation?

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ABSTRACT

The *X* chromosome invariably has the largest effect on postzygotic isolation between animal species. One explanation of this pattern is that inviability and sterility result from a breakdown in the dosage compensation of *X*-linked genes in hybrids. In Drosophila, such breakdown could result from divergence of the genes used to assess the *X*/autosomal (*X/A*) ratio, and thus the sex, of an individual. I test this hypothesis by introducing mutant alleles of the *Sex-lethal* locus into *Drosophila melanogaster-Drosophila simulans* hybrids. These mutants "ignore" any perceived anomalous *X/A* ratio and thus can be used to ensure proper dosage compensation in hybrids. These mutants do not rescue hybrid viability or fertility, implying that postzygotic isolation in this hybridization does not result from a disruption of dosage compensation caused by divergence of the *X/A* counting system.

RECENT studies have shown that the *X* chromosome plays a large role in postzygotic isolation in animals: the *X* has the greatest effect on hybrid male and female sterility in all 13 Drosophila hybridizations yet analyzed. Large *X*-effects have also been found in the four non-Drosophila hybridizations that have been studied [most of these involve insect species; see COYNE and ORR (1989) for review]. Several theories have been proposed to account for this pattern. Perhaps the simplest hypothesis argues that the *X*-effect reflects a breakdown in dosage compensation in hybrids. As *X*-linked genes occur in different doses in the two sexes, mechanisms have evolved to equalize the dose of *X*-linked gene products in the two sexes. If dosage compensation were disrupted in hybrids, hybrid inviability or sterility could result. Genetic analysis would then reveal a large effect of the *X* on postzygotic isolation.

In Drosophila, dosage compensation is achieved by doubling the rate of transcription of the *X* in males (LUCCHESI 1977). The transcription rate is apparently controlled on a locus-by-locus basis via *cis*-acting sequences scattered along the *X* (JAFFE and LAIRD 1986); genes near such sequences can be transcribed at twice the basal rate. However, it is the ratio of *X* to autosomes that determines whether such "hyperactivation" of the *X* will occur (*X/A* = 1 in normal females, and 0.5 in normal males). This ratio is read by the *Sex-lethal* (*Sxl*) locus, the master-switch for compensation, which either allows or blocks hyperactivation of the *X* (CLINE 1988). Individuals assess their *X/A* ratio by summing the number of *X*-linked and autosomal "counter genes" present; two *X*-linked counter genes, *sisterless-a* and *sisterless-b*, have been found to date (CLINE 1988).

As COYNE and ORR (1989) note, dosage compensation in species hybrids could be disrupted in two ways. First, the *cis*-acting sequences near most *X*-linked genes could diverge between two species. Thus, *X*-linked genes from one species would not be "recognized" by the regulatory signals produced in part by the other species, disrupting compensation. Recent work, however, shows that this scenario is very unlikely. JAFFE and LAIRD (1986) have found that these *cis*-acting sequences are conserved between even very distantly related Drosophila species: the *X*-linked Hsp82 gene from *Drosophila pseudoobscura* remains dosage compensated when it and flanking DNA are transformed into several autosomal sites in *Drosophila melanogaster*. *D. melanogaster*'s dosage compensation system must therefore still recognize the *cis*-acting sequence from *D. pseudoobscura* which labels Hsp82 as an *X*-linked gene in need of compensation.

Dosage compensation could, however, break down in another way: the *X/A* counter genes in two related species could diverge to such an extent that *X* counters from species *A* do not recognize autosomal counters from *B*; similarly, the number of *X* and autosomal counter genes could diverge between two species. In either case, hybrids would appear to have an abnormal *X/A* ratio: as a result, hyperactivation of the *X* could occur in females or fail to occur in males, causing inviability or sterility. This hypothesis has not been tested. Moreover, it is strengthened by the recent finding that the *X/A* counter system appears to evolve rapidly: even *populations* of *D. melanogaster* show differences in their *X/A* signaling systems (CLINE 1988).

Here I test this explanation of the large *X*-effect by introducing *Sxl* mutant alleles that effectively "ignore"

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the *X/A* ratio into *D. melanogaster-Drosophila simulans* hybrids. These mutants can be used to ensure proper dosage compensation in both male and female hybrids even if these hybrids appear to have an anomalous *X/A* ratio. If postzygotic isolation between these species results from a breakdown in dosage compensation, these mutants should rescue hybrid viability/fertility.

All hybrids between *D. melanogaster* and *D. simulans* are either inviable or sterile (STURTEVANT 1920): the *D. melanogaster* female *X D. simulans* male cross normally produces only hybrid females, who are sterile; the reciprocal hybridization produces only males, who are sterile. Male inviability is known to involve a large effect of the *D. melanogaster X* chromosome (PONTECORVO 1943a). The *X* is also involved in *D. melanogaster-D. simulans* hybrid male and female sterility (PONTECORVO 1943b).

Two *Sxl* mutants were introduced into hybrids. The Sxl^{Ml} mutant is a dominant gain-of-function allele that ensures a female-level of *X*-activity, apparently by preventing hyperactivation *regardless* of the *X/A* ratio (CLINE 1979, 1983). Sxl^{Ml} is therefore lethal to normal males. By introducing Sxl^{Ml} into hybrid females, one can ensure that the *X* chromosomes are transcribed at a level appropriate to females. Fertility should be restored if hybrid female sterility results from inappropriate hyperactivation of the *X* in some tissues. Sxl^{fl} is a recessive loss-of-function mutation that causes hyperactivation of the *X regardless* of the perceived *X/A* ratio (LUCCHESI and SKRIPSKY 1981; CLINE 1983); it is, therefore, lethal to normal females. Obviously, hybrid males carrying this allele should be viable and fertile if inviability/sterility results from lack of dosage compensation.

MATERIALS AND METHODS

All morphological markers used are described by LINDSLEY and GRELL (1968). The *D. simulans* Lethal hybrid rescue (*Lhr* 11-95) mutation, which allows rescue of the normally inviable males produced in the *D. melanogaster* female-*D. simulans* male hybridization, is described by WATANABE (1979).

Sxl^{Ml} was introduced into hybrid females by crossing *D. melanogaster y Hw* Sxl^{Ml} *sn B/Binsinscy, y w* sn^{x2} *B* females to *D. simulans Lhr* males. The resulting *Hw* hybrid females carry Sxl^{Ml}, while the Hw^{+} females carry Sxl^{+}. Female fertility was tested by mass mating females to *D. melanogaster* Bellows Falls and *D. simulans* Belmont wild-type males. Females producing no larvae were checked for insemination at day 10 [see ORR (1987) for details].

Sxl^{fl} was introduced into hybrid males by the following crosses: *D. melanogaster y Hw* Sxl^{Ml} *sn B/Binsinscy, y w* sn^{x2} *B* females were crossed to *cm* Sxl^{fl} ct^{6} males. The resulting *cm* Sxl^{fl} ct^{6}*/Binsinscy, y w* sn^{x2} *B* females were selected and crossed to *D. simulans Lhr* males, yielding carmine-eye, cut-wing hybrid males carrying Sxl^{fl}. Fertility of 4-day-old hybrid males was tested by COYNE's (1984) method, which scores the presence or absence of motile sperm. All flies were reared at 24°.

TABLE 1

Fertility of male and female hybrids between *D. melanogaster* and *D. simulans* carrying different alleles at the *Sxl* locus

Genotype	Fertile	Sterile
Females		
y Hw Sxl^{Ml} *sn* B/X_{sim}	0	82
Binsinscy, y w Sxl^{+} sn^{x2} B/X_{sim}	0	66
Males		
cm Sxl^{fl} ct^{6}/Y_{sim}	0	191
Binsinscy, y w Sxl^{+} sn^{x2} B/Y_{sim}	0	22

The small number of Sxl^{+} males scored reflects difficulty in recovering this genotype (see text). *sim* = *D. simulans*.

RESULTS AND DISCUSSION

Hybrid female fertility: All hybrid females are sterile, whether or not they carry Sxl^{Ml} (Table 1). There are two possible interpretations of this result: first, the *Sxl* locus may not function properly on a hybrid genetic background (*i.e.*, Sxl^{Ml} may not prevent *X*-hyperactivation in hybrids). Alternatively, Sxl^{Ml} may be unable to rescue hybrid fertility, despite normal functioning. We can rule out the first possibility: for as expected given normal *Sxl* function, no hybrid males carrying Sxl^{Ml} were recovered, although 58 males carrying Sxl^{+} (white-eyed) were obtained. Sxl^{Ml} thus remains male-lethal on a hybrid background, demonstrating that it prevents hyperactivation in hybrids, as desired. Sxl^{Ml} is simply unable to restore hybrid female fertility. Hybrid female sterility is not, therefore, due to improper hyperactivation of the *X*.

Hybrid male fertility and viability: All F_1 hybrid males are sterile, whether or not they carry Sxl^{fl} (Table 1); these males had atrophied testes and lacked mature sperm. Interestingly, however, hybrid males carrying Sxl^{fl} (*cm* Sxl^{fl} ct^{6}) were recovered over six times more frequently than males carrying Sxl^{+} (*Binsinscy, y w* Sxl^{+} sn^{x2} *B*) (Table 2, cross 1). This ratio significantly differs from the 1:1 expected under Mendelian segregation ($\chi^2 = 139.92$, 1 d.f., $P < 0.0001$). All males also carry the autosomal *D. simulans* Lethal hybrid rescue gene. It is thus possible that, while Sxl^{fl} has no effect on male *fertility*, it does help rescue hybrid male *viability* above the partial rescue afforded by *Lhr*. Alternatively, this result could reflect the deleterious pleiotropic effects of the many morphological markers segregating with Sxl^{+}.

To distinguish between these possibilities, I crossed wild-type *D. melanogaster* Bellows Falls females to *cm* Sxl^{fl} ct^{6} males. The resulting females were crossed to *D. simulans Lhr* males, yielding two genotypes of hybrid males, *cm* Sxl^{fl} ct^{6} and cm^{+} Sxl^{+} ct^{+}. If the preferential recovery of males carrying the Sxl^{fl} allele observed in cross 1 reflects marker effects, no Sxl^{fl} "rescue" should be observed in the present hybridization because Sxl^{+} does not segregate with any mutant marker. As Table 2 (cross 4) shows, Sxl^{+} and Sxl^{fl}

TABLE 2

Tests of effect of Sxl^{fl} allele on hybrid male viability

Cross	Males: *cm* Sxl^{fl} ct^6/Y_{sim}	Males: *y w* Sxl^+ sn^{x2} B/Y_{sim}	Females
1	224	34	797
2	0	0	197
3	0	0	203
	cm Sxl^{fl} ct^6/Y_{sim}	cm^+ Sxl^+ ct^+/Y_{sim}	
4	128	135	440

Values represent number of males and females of each genotype recovered in cross. See text for description of each cross. *sim* = *D. simulans*.

males are recovered at equal frequencies ($\chi^2 = 0.19$, 1 d.f., $P > 0.65$). [This result would be trivial if *Lhr* itself *completely* rescued males as it would then be impossible to discern any additional rescue by Sxl^{fl}. However, *Lhr* did not fully rescue males: males were recovered only 60% as often as female hybrids (Table 2, cross 4). Any additional rescue by Sxl^{fl} could have been easily detected.] Other crosses (data not shown) also demonstrate that the markers segregating with the Sxl^+ allele reduce viability even within *D. melanogaster*. Thus the "rescue" effect observed in "cross 1" (Table 2) was apparently an artifact of the mutant markers employed.

An additional test of the effect of Sxl^{fl} on male viability was performed to determine whether Sxl^{fl} can rescue hybrid males in the absence of *Lhr*. *D. melanogaster cm* Sxl^{fl} ct^6*/Binsinscy, y w* sn^{x2} *B* females were crossed to wild-type *D. simulans* Belmont. As low temperature is known to facilitate recovery of these hybrid males (STURTEVANT 1920), crosses were performed and progeny reared at both 18°C and 24°C (Table 2, crosses 2 and 3, respectively). No males were recovered at 18°C. A single male was recovered at 24°; this male, however, was wild-type, instead of the expected cut or white phenotype, and was thus almost surely a result of non-disjunction in the mother (nullo-X egg fertilized by X-bearing sperm; such males are viable in this hybridization) (STURTEVANT 1929). Sxl^{fl} clearly does not rescue hybrid males.

I therefore find no evidence that sterility or inviability of *D. melanogaster-D. simulans* hybrids results from a breakdown in dosage compensation due to divergence of *X/A* counters: hybrids carrying *Sxl* alleles which ensure a proper rate of transcription of the *X*—regardless of the perceived *X/A* ratio—remain completely inviable or sterile. Strickly speaking, this result does not necessarily show that dosage compensation proceeds perfectly normally in hybrids; rather, it demonstrates that hybrids *remain* inviable or sterile even if any hypothetical irregularity in dosage compensation is corrected, *i.e.*, postzygotic isolation does not result from a disruption of dosage compensation due to divergence of the *X/A* counter system. LAKHOTIA, MISHRA and SINHA (1981), however, provided evidence which suggests that dosage compensation does proceed normally in *D. melanogaster-D. simulans* hybrids (these workers assessed the rate of *X* transcription by measuring the density of radioactively-labelled uridine on polytene chromosome preparations). Unfortunately, their study suffered from two problems: first, the rate of *X*-relative-to-autosomal transcription in single-*X* nuclei was 1.6 times—not the expected two times—greater than in diplo-*X* nuclei. More important, the male-like *XO* nuclei examined contained a *D. simulans*—not a *D. melanogaster*—*X* chromosome. However, hybrid males carrying a *D. simulans X* are viable; it is far more important to determine whether the *inviable* male hybrids who carry a *D. melanogaster X* show normal dosage compensation.

There are, however, several other lines of evidence which suggest that postzygotic isolation does not result from a breakdown in dosage compensation. First, as COYNE and ORR (1989) note, taxa with dosage compensation (diptera and mammals) and taxa apparently lacking dosage compensation (birds and butterflies) obey HALDANE's rule: when only one hybrid sex is sterile or inviable, it is the heterogametic sex (HALDANE 1922). This similarity is very difficult to explain if postzygotic isolation results from a disruption of dosage compensation in the former but not the latter taxa. Similarly, a large *X*-effect on hybrid fitness has been found in the only butterfly hybridization that has been genetically analyzed (GRULA and TAYLOR 1980); again, this is difficult to explain if the large *X*-effect typically reflects a breakdown in dosage compensation.

All this evidence suggests that postzygotic isolation does not result from a breakdown in dosage compensation. One must, therefore, consider alternative explanations of the cause of postzygotic isolation and of the large effect of the *X* [see COYNE and ORR (1989) for a review of these alternatives].

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