Genetic testing of the hypothesis that hybrid male lethality results from a failure in dosage compensation

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ABSTRACT
Several recent studies have suggested that F1 hybrid male lethality in crosses between Drosophila melanogaster and D. simulans is due to a failure in dosage compensation, caused by incompatibilities between D. simulans dosage compensation proteins and the D. melanogaster X chromosome. Contrary to the predictions of this hypothesis, mutations in four essential D. melanogaster dosage compensation genes are shown here to moderately increase rather than decrease hybrid male viability.

Crosses between Drosophila melanogaster females and males from its sibling species D. simulans produce semi-viable F1 daughters and lethal F1 sons. This lethality is specific to F1 hybrid males carrying the D. melanogaster X chromosome (referred to henceforth as X_{mel}), because patroclinous exceptional sons inheriting the D. simulans X (X_{sim}) are viable (STURTEVANT 1920). F1 hybrid males carrying X_{mel} are delayed in development and die as larvae or pseudopupae (STURTEVANT 1929; BOLKAN et al. 2007) and their X chromosome has an aberrant condensation state in polytenized salivary glands (HUTTER et al. 1990; CHATTERJEE et al. 2007), but the mechanistic basis of the lethality remains unknown.

Dosage compensation is a regulatory process whereby transcription of X-linked genes is equalized between the two sexes (reviewed in LUCCHESI et al. 2005). In Drosophila, dosage compensation is achieved by binding of a multi-subunit dosage compensation complex (DCC) to the male X chromosome, and mutations in genes encoding DCC subunits result in male-specific lethality. Dosage compensation is controlled by the master sex determination gene Sex-lethal (Sxl). Sxl is active only in females, where it represses the DCC gene msl-2, while in males the absence of Sxl-mediated repression allows for activation of dosage compensation (reviewed in CLINE and MEYER 1996). Males mutant for DCC genes develop slowly and die as larvae or prepupae (BELOTE and LUCCHESI 1980). The similarity of this phenotype to that observed in lethal hybrid males and the apparent male-specificity of both lethalities might suggest that hybrid male lethality is caused by a failure in dosage compensation.

However, the proposal that dosage compensation defects are the primary cause of hybrid male lethality seems implausible because hybrid lethality is not in fact male-specific but rather X_{mel}-specific. This idea was first suggested by STURTEVANT (1920), who
generated viable $X_{sim}$-carrying exceptional F1 hybrid sons but no corresponding viable $X_{mel}/X_{mel}$ exceptional F1 daughters. Lethality of this genotype of hybrid daughters was later confirmed using compound-X chromosomes (Biddle 1932). Furthermore, Hutter et al. (1990) demonstrated that the genetic basis of lethality in $X_{mel}/Y_{sim}$ male and $X_{mel}/X_{mel}$ female hybrids is the same, because both are rescued by mutations in Hybrid male rescue ($Hmr$). In addition, $X_{mel}/X_{sim}$ females are lethal when raised at high temperature, and this lethality also is suppressed by $Hmr$ mutations (Barbash et al. 2000). Since dosage compensation is a male-specific process, it is difficult to envision how dosage compensation defects could be the underlying cause of male and female hybrid lethalities that have the same genetic basis. It is possible, however, that hybrid lethality in both males and females could involve interactions with some of the DCC genes that are expressed in both sexes.

One test of the dosage compensation hypothesis found that $Sxl$ mutants do not rescue hybrid males (Orr 1989). This experiment demonstrated that hybrid males do not die due to inappropriate activation of $Sxl$, but left open the possibility that hybrid males might die due to a failure in dosage compensation that is downstream of $Sxl$ regulation. A subsequent study found that $Sxl$ mutations increase larval viability of hybrid males but do not suppress adult lethality, even when other DCC components ($rox$ RNAs) are overexpressed (Palandra et al. 2006).

A genome-wide microarray comparison of mRNA abundance between lethal ($Hmr^+$) and viable ($Hmr^-$) hybrids found that a lower than expected number of genes down-regulated in lethal hybrid males are located on the X chromosome (Barbash and Lorigan 2007). If lethal hybrids fail to execute dosage compensation then one would expect the opposite pattern of increased numbers of X-linked genes having significantly reduced expression. This study therefore indicates that lethal hybrids do not suffer from a general failure in dosage compensation, though it remains possible that a small subset of dosage compensated X-linked genes are misregulated in hybrids.

A number of recent studies have discovered aberrations in DCC localization and dosage compensation in hybrids, as well as interesting evolutionary patterns of DCC genes. PalBhadra et al. (2006) discovered that several DCC proteins fail to localize to the $D. melanogaster$ X chromosome in dying hybrid males, but do localize correctly when lethality is suppressed using the Lethal hybrid rescue$^+$ ($Lhr^+$) mutation. Chatterjee et al. (2007)
observed reduced transcription on \(X_{mel}\) in lethal hybrid males compared to rescued hybrid males and pure-species controls. In addition, two population genetic studies of DCC genes in \(D. melanogaster\) and \(D. simulans\) found that four of them (\(msl-1, msl-2, msl-3\) and \(mof\)) show evidence of having diverged under positive selection along the lineage leading to \(D. melanogaster\), likely due to excessive nonsynonymous (amino-acid changing) substitutions (LEVINE et al. 2007; RODRIGUEZ et al. 2007). Finally, population genetic analyses also found evidence for positive selection acting in \(D. melanogaster\) on several X chromosome entry sites to which the DCC binds (BACHTROG 2008).

Following from these reports several groups have suggested that defects in dosage compensation contribute to or cause hybrid male lethality (CHATTERJEE et al. 2007; RODRIGUEZ et al. 2007; BACHTROG 2008). The most explicit model was provided by RODRIGUEZ et al. (2007). They proposed that DCC proteins and their corresponding binding sites evolved on the \(D. melanogaster\) lineage, such that DCCs composed of either \(D. simulans\) proteins or a heterospecific mix of \(D. melanogaster\) and \(D. simulans\) proteins would be unable to bind to \(X_{mel}\) in F1 hybrid males. F1 hybrid males carrying \(X_{sim}\), however, would survive because this X chromosome corresponds to the ancestral state and could be bound by either homospecific or heterospecific DCCs.

This model makes the clear prediction that \(X_{mel}/Y_{sim}\) F1 hybrid males heterozygous for mutations in the \(D. melanogaster\) orthologs of DCC genes should be lethal (or perhaps have reduced viability), because they will be unable to assemble a DCC that is composed only of \(D. melanogaster\) subunits. Here I tested these predictions for four DCC genes in hybrids that are rescued from lethality by \(D. simulans\) stocks homozygous for either the \(Lhr^1\) or \(Lhr^2\) mutations (WATANABE 1979; BRIDEAU et al. 2006). All DCC mutants are loss-of-function alleles that cause male lethality (TWEEDIE et al. 2009).

Not all DCC mutant stocks mated successfully to both \(D. simulans\) stocks, and in some crosses few or no hybrid sons were produced. Low and variable penetrance of hybrid male rescue by \(Lhr^1\) has been previously observed (PRESGRAVES 2003). But among informative crosses, all produced results inconsistent with the above hypothesis. F1 hybrids heterozygous for DCC mutations either had equivalent or higher viability compared to their balancer reference brothers (Table 1). This result was not observed in a previous screen that tested several multi-locus deficiencies predicted to remove \(mle\) in cytological region 42A6 or
msl-1 in cytological region 36F11-37A1 (Presgraves 2003). In none of those crosses were Deficiency/+ F1 males more viable than Balancer/+ controls. The reason for this difference from the results presented here might be explained if the deleted regions contain recessive hybrid inviability genes that decrease rather than increase viability when hemizygous.

Surprisingly, a significant viability increase was observed here in at least one cross for all four DCC genes tested (Table 1). Viability of rescued hybrid males is highly sensitive to temperature (Hutter and Ashburner 1987; Aruna et al. 2009). In several crosses, such as with mle¹, mle⁰ and msl-3¹, a significant enhancement was observed at <25°, but few or no hybrid males were produced at 25°. This result suggests that these DCC mutations are acting as minor modifiers of hybrid viability rather than as major-effect hybrid rescue mutations comparable to Hmr and Lhr. Nevertheless, the positive effect on viability among all DCC genes is intriguing.

I suggest that hybrid lethality is caused by a defect in X_mel chromatin state or structure, one consequence of which is that the DCC fails to properly localize in male hybrids as described by Pal Bhadra et al. (2006). This failure of DCC localization certainly must further reduce hybrid male fitness but it does not appear to be the primary cause of the lethality. Several genes encoding heterochromatin proteins such as HP1 and SU(VAR)3-7 are required in both sexes, but also show preferential effects in males on viability and X chromosome structure (Liu et al. 2005; Spierer et al. 2005). Su(var)3-7 shows particularly intriguing interactions, with both reduced and increased dosage leading to defects in DCC localization (Spierer et al. 2008). Increased dosage of Su(var)3-7 also induces changes in X chromosome morphology and enrichment of SU(VAR)3-7, HP1 and the post-translational histone modification H3K9me2 on the X chromosome. Strikingly, these alterations require a functional DCC, even in females where the DCC is not normally assembled.

LHR (also called HP3) interacts with HP1 and its localization to heterochromatin depends on HP1 (Brudeau et al. 2006; Greil et al. 2007). One possibility is that Lhr-dependent hybrid lethality is caused by increased localization of HP1 and other heterochromatin proteins to the X chromosome, which induces an improper level of X chromosome heterochromatin formation. By analogy to the Su(var)3-7 overexpression phenotype, mutations in DCC genes may reduce this X chromosome enrichment of heterochromatin proteins in hybrid males and thus enhance their viability.
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LITERATURE CITED

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Genetics. 122: 891-894.


Table 1. Testing DCC mutants for suppression or enhancement of hybrid viability.

<table>
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<th>Temp.</th>
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*D. melanogaster* females heterozygous for the indicated alleles were crossed to *D. simulans* males homozygous for either *Lhr¹* or *Lhr²*. For most crosses two replicates were performed containing ~15-25 female and ~20-30 male parents. Eclosion was the criterion for viability. Full genotypes of *D. melanogaster* mutant stocks were: 1) *y¹ w¹ N¹¹¹¹/Dp(1;Y)B¹ ; mle⁰/SM¹, 2) mle⁰ cn¹ bw¹/CyO, 3) ms*l-¹²¹⁶ cn¹ bw¹/CyO, 4) w; msl² cn/CyO, 5) w; msl²⁰²²⁷ bw¹/CyO, 6) msl-3° red¹/TM³, Sb¹ Ser¹. All mutant alleles, including molecular information when known, are described on FlyBase (Tweedie *et al.* 2009).

*Unscoreable males were mostly progeny that had died and become stuck in the media, and thus could not be scored for the Cy marker on the balancer chromosome. A negligible number of females were unscoreable (between 0 and 4 in all crosses) and are not shown.*

*Inhomogeneity in the numbers of the 4 classes of progeny was tested by Fisher’s Exact Test, unless otherwise indicted.*

*Significance tested using a chi-squared test due to the large sample size.*