Revisiting the Role of the Male-Specific Lethal Complex in the Sex Determination Decision of *Drosophila*

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PREVIOUSLY, we suggested that before female sexual identity in *Drosophila* is fully established, the default state of expression is male and X chromosome genes are expressed at a higher, twofold male level (Gladstein *et al.* 2010). This enhanced expression, driven by the *male-specific lethal* (*msls*) genes, which target the X chromosome for hypertranscription [reviewed in Lucchesi and Kuroda (2015)], was proposed to consolidate female sex determination, given that the primary sex determination genes and their target, the *Sex-lethal* (*Sxl*) master-switch, all reside on the X. However, a recent publication has claimed that the *Drosophila* sex determination process does not utilize the MSL complex, as it found no female-lethal genetic interactions between the *msls* and *Sxl*, or the primary sex determination genes (Erickson 2016). Erickson did not address the molecular data, which included demonstrating reduced transcription at both the initiation (*Sxl_{pe}*) and maintenance (*Sxl_{pm}*) promoters of *Sxl* in several MSL complex mutant backgrounds.

The genetics tested for synergism in female lethality from combining reduced *msl* dose with mutations in sex determination genes:

1. Reducing *Sxl* dose when mothers are homozygous mutant for *msls* was found to reduce female viability in some *msl* backgrounds by Gladstein *et al.* (2010), but Erickson, using different *msl* alleles, did not observe this. The exception was *msl-3¹*, where the results appeared similar.
2. With two defective primary sex determination genes [or X signal elements (XSE)], again with homozygous mutant *msl* mothers, Gladstein *et al.* (2010) observed strong female lethality but Erickson, using common alleles, did not.

Since sex determination is dose-sensitive, Erickson also tested the heterozygous condition over balancers, and found no differences in female-lethal synergism between homozygous and heterozygous *msl* mothers.

3. Erickson demonstrated that the original *msl-3¹* background has an additional mutation that strongly synergizes with mutations in the sex determination pathway.

We concur with Erickson on (3) above. However, more work is needed to resolve the differences between the two publications, particularly for (2) where the crosses used common alleles. Close examination, including replicating some crosses, suggests explanations for some of these disparities, discussed below.

Double-Mutant XSE Stock Does Not Behave as Expected

The double-mutant XSE stock Erickson used does not appear to be intact, since control classes show female viabilities up to 57% at the nonpermissive temperature of 29°, where female viability is expected to be well below 5% (Cline 1988; Duffy and Gergen 1991; Kappes *et al.* 2011). This may explain the disparity for crosses that should have been comparable [(2) above]. The XSE combination does not suffer from significant background effects; reconstructing it from the individual mutations gives consistent genetic results.

Balancers Are a Problem

An additional difference between the studies is the presence of balancers. In testing for *msl* dose effects [(2) above], Erickson had the heterozygotes over balancers. Balancers generally adversely affect female viability; *In(2LR)Bc Gla* in particular, which is in the majority of tests involving the second chromosome (for *mle*, *msl-1*, and *msl-2*), is very strongly female negative. In an XSE double-mutant cross, it alone lowers female viability to 2.5%, more than

Table 1 Outcrossing *mle* and *msl-2* stocks reveals background effects that favor females

Cross ♀♀ X <i>sis-b^{sc3-1}</i> , <i>w</i> , <i>sis-a¹</i> , <i>m/Y</i> ♂♂				
Maternal genotype	Zygotic autosomal genotype	Female viability (%) compared to brothers	Number of female progeny	Number of male progeny
<i>w⁺; mle¹</i> (2010)	<i>mle¹/+</i>	1.2	11	923
<i>w⁻; mle¹</i> (2017)	<i>mle¹/+</i>	86.6	214	247
<i>w⁺; mle¹</i> (\$17; 2017)	<i>mle¹/+</i>	14.4	40	278
<i>w⁺; mle¹</i> (\$33; 2017)	<i>mle¹/+</i>	3.9	8	205
<i>w⁻; msl-2¹</i> (2010)	<i>+/+</i>	2.9	24	825
<i>w⁻; msl-2¹</i> (2017)	<i>msl-2¹/+</i>	46.3	152	328
<i>w⁻; msl-2¹</i> (outcrossed)	<i>msl-2¹/+</i>	17.9	67	374
<i>w⁻; msl-2¹</i> (outcrossed)/Df (2L)	<i>msl-2¹</i> or Df/+	9.9	36	364

All crosses done at 25°. Homozygous female parents obtained from stocks: *pr¹ mle¹/CyO* or *w¹¹¹⁸; pr¹ mle¹/CyO*; *w*; *msl-2¹ cn /CyO*. Df (2L) is from *w¹¹¹⁸; Df(2L)Exel 7016*/CyO and uncovers *msl-2*. *sis-b^{sc3-1}* and *sis-a¹* are the two X signal elements, *sis-b^{sc3-1}* being the temperature-sensitive mutation in this combination (Cline 1988). Crosses from Gladstein *et al.* (2010) are in bold.

ninefold lower than nonbalancer sisters (unpublished observation). Thus, *msl* homozygous to heterozygous comparisons for dose-sensitive interactions with sex determination genes appear “negative,” as the effects are masked by balancer lethality.

Accumulation of Modifiers

Repeating some of our crosses revealed that recently outcrossed stocks gave much stronger genetic interactions than nonoutcrossed stocks. The *mle¹* to XSE cross in Gladstein *et al.* (2010) combined two newly outcrossed lines (\$17 and \$33), which gave 1.4 and 1.1% female viability, respectively. These lines maintained the original mutation as judged by male lethality when homozygous or over an *mle* deletion [*Df(2R)ED1484*]. The same lines ~7 years later gave viabilities of 14.4 and 3.9%, respectively, while the original, nonoutcrossed stock from which they were derived gave an astounding 86.6% (Table 1). The original *msl-2¹* background ~7 years later also showed elevated female viability, from 2.91 to 46.3%. Limited outcrossing dropped it to 17.9%, which was further reduced to 9.9% over an *msl-2* Df [*Df(2L)Exel 7016*]. That the outcrossed chromosome still carries *msl-2¹* was verified by male-specific lethality when homozygous or over *Df(2L)Exel 7016*. These data suggest that modifiers are slowly accumulating. If Erickson did not recently outcross his stocks, he likely would not observe strong genetic interactions. The background modifiers also suggest that reducing *msls* introduces a negative load on females; over time, this load is counteracted by mutations which favor them. As MSL complex components impact chromatin, these effects may reflect generalized chromatin changes. Support for background changes also comes from the observation that some of our *msl* stocks (*e.g.*, *msl-2²²⁷* and *msl-1^{γ216}*) remain homozygous female viable but the females no longer breed.

Erickson questioned, “The model postulates that the MSL complex normally elevates expression of the XSEs twofold, effectively providing females with a 4X and males a 2X concentration of XSE proteins... how can it be that *msl* mutant females activate *Sxl^{Pe}* and wild-type males do not, when both should have equivalent 2X levels of XSE proteins?” To answer: (1) not all X chromosome genes are dosage compensated, including some of the primary sex determination genes (*e.g.*, *run*;

Gergen 1987; Smith *et al.* 2001); (2) males have a Y chromosome that is a sink for chromatin proteins, particularly heterochromatin protein 1a (HP1), which is required for robust female *Sxl^{Pe}* expression (Li *et al.* 2011), so its presence will affect the ease by which males can activate the promoter; and (3) having two X chromosomes gives females the option of pairing them, which can affect gene expression. It is not known if pairing influences sex determination, but two X chromosomes make nondosage-compensating females constitutionally different from males.

The foregoing provides the basis for many of the genetic differences, and identifies the need to examine *msl-3* in a background without additional mutations. Additionally, chromatin immunoprecipitation (ChIP) experiments on MSL components in early embryos should clarify their contribution to *Sxl* expression. ChIP-sequencing data from S2 cells has shown that *Sxl^{Pm}* is an MSL target, behaving like other dosage-compensated promoters with bound MSL-1, MSL-2, MOF, and MLE (Straub *et al.* 2013).

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