Incompatibility Between X Chromosome Factor and Pericentric Heterochromatic Region Causes Lethality in Hybrids Between *Drosophila melanogaster* and Its Sibling Species

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ABSTRACT The Dobzhansky–Muller model posits that postzygotic reproductive isolation results from the evolution of incompatible epistatic interactions between species: alleles that function in the genetic background of one species can cause sterility or lethality in the genetic background of another species. Progress in identifying and characterizing factors involved in postzygotic isolation in *Drosophila* has remained slow, mainly because *Drosophila melanogaster*, with all of its genetic tools, forms dead or sterile hybrids when crossed to its sister species, *D. simulans*, *D. sechellia*, and *D. mauritiana*. To circumvent this problem, we used chromosome deletions and duplications from *D. melanogaster* to map two hybrid incompatibility loci in F₁ hybrids with its sister species. We mapped a recessive factor to the pericentromeric heterochromatin of the X chromosome in *D. simulans* and *D. mauritiana*, which we call *heterochromatin hybrid lethal* (*hhl*), which causes lethality in F₁ hybrid females with *D. melanogaster*. As F₁ hybrid males hemizygous for a *D. mauritiana* (or *D. simulans*) X chromosome are viable, the lethality of deficiency hybrid females implies that a dominant incompatible partner locus exists on the *D. melanogaster* X. Using small segments of the *D. melanogaster* X chromosome duplicated onto the Y chromosome, we mapped a dominant factor that causes hybrid lethality to a small 24-gene region of the *D. melanogaster* X. We provide evidence suggesting that it interacts with *hhl*^{mau}. The location of *hhl* is consistent with the emerging theme that hybrid incompatibilities in *Drosophila* involve heterochromatic regions and factors that interact with the heterochromatin.

MORE than 70 years ago, Dobzhansky (1937) and Muller (1942) proposed a simple two-locus model in which postzygotic isolation arises as a byproduct of divergence between effectively allopatric populations. Genes that function well in one species' genetic background might not be functional in a hybrid genetic background, rendering hybrids dead or sterile. Despite early acceptance of the Dobzhansky–Muller model, progress in identifying and characterizing factors involved in hybrid incompatibilities has remained slow. One of the reasons for the slow progress is that *Drosophila melanogaster*, with its many genetic tools, forms largely dead or sterile hybrids when crossed to its

sister species *D. simulans*, *D. sechellia*, and *D. mauritiana* (Sturtevant 1920, 1929; Lachaise *et al.* 1986; Provine 1991; Barbash 2010).

In general, three methods have been used to circumvent this problem. First, alleles that suppress postzygotic isolation, so-called hybrid rescue mutations, have been used to characterize hybrid inviability between *D. melanogaster* and its three sister species in the *D. simulans* complex. When *D. melanogaster* females are crossed to sibling species males, only hybrid females are produced while males die as larvae (Sturtevant 1920; Lachaise *et al.* 1986). These males are rescued by the *D. melanogaster* mutant allele of *Hybrid male rescue* (*Hmr*) (Hutter and Ashburner 1987; Barbash *et al.* 2003) or by the *D. simulans* mutant allele of *Lethal hybrid rescue* (*Lhr*) (Watanabe 1979; Brideau *et al.* 2006). The wild-type alleles of *Hmr* and *Lhr*, along with one or more uncharacterized factors, cause F₁ hybrid male lethality (Barbash *et al.* 2003; Brideau *et al.* 2006). LHR (also known as Heterochromatin Protein 3, HP3) interacts with HP1 and localizes to heterochromatin. In the reciprocal cross between *D. simulans*

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females and *D. melanogaster* males, hybrid males are produced while females usually die as embryos (Sturtevant 1920, 1921; Hadorn 1961). These normally lethal hybrid females are rescued by the *D. simulans* mutant allele of *maternal hybrid rescue* (*mhr*) (Sawamura *et al.* 1993; see also Orr 1996; Carracedo *et al.* 2000; Gérard and Presgraves 2012) or by the *D. melanogaster* mutant allele of *Zygotic hybrid rescue*, *Zhr* (Sawamura and Yamamoto 1997). The incompatible wild-type allele of *Zhr* is thought to correspond to a large block of 359-bp repetitive satellite DNA from *D. melanogaster*, and F₁ hybrid females die as a consequence of widespread mitotic defects (Ferree and Barbash 2009).

Second, mutations that weakly rescue the fertility of normally sterile F₁ hybrid females have been used to introgress *D. simulans* genetic material into an otherwise *D. melanogaster* background to study hybrid inviability and hybrid sterility between these species (Davis *et al.* 1996; Sawamura *et al.* 2000). Once in a *D. melanogaster* background, genetic tools can be used to map and characterize factors involved in hybrid incompatibilities (e.g., Sawamura *et al.* 2004, 2010; Masly *et al.* 2006).

Third, deficiency mapping, which involves chromosome deletions with known physical breakpoints, has been used to map recessive hybrid incompatibilities in F₁ hybrids (Coyne *et al.* 1998; Presgraves 2003; Presgraves *et al.* 2003; Tang and Presgraves 2009; Matute *et al.* 2010). In one study, Coyne *et al.* (1998) used a collection of deficiencies from *D. melanogaster* to uncover recessive *D. simulans* factors that cause lethality in F₁ hybrid females. Screening ~50% of the *D. simulans* genome revealed four regions that significantly decreased viability in hybrids. Deficiencies that proved lethal in *D. melanogaster*/*D. simulans* hybrid females were then tested in hybrids with the two sister species in the *D. simulans* clade, *D. sechellia* and *D. mauritiana*. One hybrid lethal region at the base of the X chromosome (cytological region 20A–20F) proved strongest in *D. melanogaster*/*D. mauritiana* hybrid females but appeared absent in *D. melanogaster*/*D. sechellia* hybrids. In later work, some of the same deficiencies revealed a recessive factor at the base of the X chromosome of *D. santomea* that causes hybrid inviability in F₁ hybrids with *D. melanogaster* (Matute *et al.* 2010).

The existence of hybrid lethal factors at the heterochromatic base of the X chromosome is consistent with an emerging theme in which hybrid incompatibilities in *Drosophila* involve heterochromatin (e.g., *Zhr*) (Sawamura and Yamamoto 1997; Ferree and Barbash 2009) or genes whose products interact with heterochromatin (e.g., *Hmr*, *Lhr*; Brideau *et al.* 2006 and *OdsH*; Bayes and Malik 2009). Loci involved in hybrid incompatibilities map to the pericentromeric heterochromatin of the X chromosome between other species pairs as well (Moehring *et al.* 2006; Cattani and Presgraves 2009). Cattani and Presgraves (2009), for instance, showed that the *D. mauritiana* allele of *hybrid lethal on the X* (*hlx*), which maps to the pericentromeric heterochromatin, is incompatible with an autosomal factor, *Su(hlx)*, in both sister

species, *D. simulans* and *D. sechellia*. Together, these mapping results raise the possibility that the heterochromatin is a hotspot for hybrid lethality and, in particular, that *Zhr*, the regions uncovered by Coyne *et al.* (1998), Matute *et al.* (2010), and *hlx* could all involve the repeated evolution of interspecific incompatibilities at the same genomic locus.

The discovery of a recessive X-linked factor from *D. simulans* (*sim*) and *D. mauritiana* (*mau*) that causes lethality in *D. melanogaster* (*mel*) hybrid females (Coyne *et al.* 1998) seems puzzling at first, as F₁ hybrid males that are hemizygous for the *D. simulans* or *D. mauritiana* X chromosome (X^{sim}/Y^{mel} or X^{mau}/Y^{mel} , respectively) are viable. The only genotypic difference between, e.g., X^{sim}/Y^{mel} males and the inviable Df^{mel}/X^{sim} hybrid females is that the latter have a *D. melanogaster* X chromosome (except for the region deleted). The lethality of *Df*-bearing hybrid females therefore implies that a recessive factor(s) from *D. simulans* and *D. mauritiana* must be incompatible with a dominant factor elsewhere on the *D. melanogaster* X chromosome.

In this report, we address two issues. First, to test whether the region mapped by Coyne *et al.* (1998) and *hlx* map to the same or different loci, we refine the initial mapping of the former using a larger collection of *D. melanogaster* deficiencies. Second, we test whether a dominant hybrid lethal region of the *D. melanogaster* X exists using a large collection of small duplications spanning most of the X chromosome. Our results show that a hemizygous factor in the pericentromeric heterochromatin of the *D. mauritiana* and *D. simulans* X appears to interact with a dominant factor on the *D. melanogaster* X resulting in hybrid lethality.

Materials and Methods

Crossing scheme

Following Coyne *et al.* (1998), we used *D. melanogaster* stocks carrying chromosomal deficiencies (*Df*) on the X chromosome kept heterozygous over a balancer chromosome bearing the dominant visible marker, *Bar* (*B*). Using replicated mass matings, we crossed ~20 virgin heterozygous (*Df/B*) females that had been aged for 1 day to ~20 males from one of the sibling species, *D. simulans*, *D. mauritiana*, or *D. sechellia* (Figure 1). Hybrid males from these crosses typically die as late-stage larvae (Figure 1, c and d; Hutter *et al.* 1990). Hybrid females, on the other hand, inherit the balancer chromosome (*B* females) or the deficiency chromosome (*Df* females). For most crosses we expect *Df/sim* females to be ~50% of the total number of females (= *Df/sim* + *B/sim*). We arbitrarily define viable deficiencies as those in which the percentage of *Df/sim* females is >33%; semilethal deficiencies are those in which the percentage of *Df/sim* females is between 10 and 33% (and significantly different from 50%); lethal deficiencies as those in which the percentage of *Df/sim* females is <10% (and significantly different from 50%). We tested 20 deficiency stocks that delete material at the base of the X chromosome,

Figure 1 Crossing scheme to map the cause of hybrid lethality at the base of the X chromosome in *D. mauritiana*, *D. simulans*, and *D. sechellia*. *D. melanogaster* females heterozygous for a deficiency over a balancer chromosome (*Df/Bal*) were crossed to males from *D. mauritiana*, *D. simulans*, and *D. sechellia*. F₁ hybrid females inherit either (A) the deficiency (*Df/mau*), exposing a small hemizygous region of *D. mauritiana* or (B) the balancer chromosome (*Bal/mau*). F₁ hybrid males die due to (C) lacking a piece of the X and/or (D) *Hmr^{mel}*-mediated hybrid lethality. White and gray rectangles represent *D. melanogaster* and sibling species' X chromosomes, respectively. The short rectangle with a hook represents the Y chromosome.

between cytological positions 19A and the heterochromatic region h32, all obtained from the Bloomington Stock Center at Indiana University. We assayed variation for hybrid lethality within and among species by crossing hybrid lethal deficiencies to four lines of *D. mauritiana*—*D. mauritiana w* (*mau*), *D. mauritiana* Synthetic (*mau* Syn), *D. mauritiana* 14021.0241.01 (*mau* 0241.01), and *D. mauritiana y w* (*mau y w*)—and to *D. simulans w^{XD1}* (*sim*) and *D. sechellia w* (*sech*) males. In all cases, flies were reared on standard cornmeal-agarose medium in *Drosophila* vials at room temperature (23°–25°) with a 12-hour light cycle. The number of crosses for each experiment is reported below; adults were flipped once to new food after 4 days and the vials were hydrated with a solution of 0.5% propionic acid.

Determination of molecular breakpoints of chromosomal deficiencies

For many of the deficiencies used, the breakpoints were previously estimated only from complementation tests or cytologically. For some deficiencies, we refined breakpoints by genotyping SNPs in *Df/mau* females. If *Df/mau* females were heterozygous for a SNP, that SNP must be present on the *Df* chromosome (i.e., not deleted); conversely, if *Df/mau* females were hemizygous for a SNP, we inferred that site to be deleted.

To genotype SNP differences, we used two approaches. For some deficiencies (*Df(1)mal10/Binsn*, *Df(1)16-3-22/FM6*, *Df(1)17-257/Binsn*, and *Df(1)ED7664/FM7h*) we used TILLING (Till *et al.* 2006) following the protocols of the Transgenomic SURVEYOR Mutation Detection kit (Transgenomic). This approach involves a DNA endonuclease that cleaves mismatches in heteroduplex DNA. Briefly, to geno-

type individual flies using TILLING, we PCR amplified a marker region of 400–700 bp, formed heteroduplex DNA, cut the heteroduplex DNA with SURVEYOR endonuclease, and then visualized the digestion products on a 2% agarose gel. After heteroduplex formation, we treated 30 μ l of the sample with 2 μ l of SURVEYOR enhancer and 2 μ l of SURVEYOR endonuclease and incubated the reaction for 30 min at 42°. We stopped the reaction with 1/10 stop solution and froze the samples at –20° until ready for loading in a 2% agarose gel. TILLING reactions were performed simultaneously with positive controls involving heteroduplexed DNA between *D. melanogaster* and *D. mauritiana* and negative controls involving pure species DNA from a single line (*D. melanogaster* Ore-R or *D. mauritiana w*).

For the rest of the deficiencies we PCR amplified a marker or gene of interest and sequenced the products using standard Sanger sequencing. Briefly, we PCR amplified a 500- to 700-bp region and sequenced the products using ABI prism BigDye Chemistry on an automated ABI sequencer. As above, if *Df/mau* females were heterozygous, SNPs appear as double peaks in the chromatograms. For any product we used at least two independent SNPs to corroborate the result. However, if *Df/mau* females were hemizygous at that marker, SNPs would be absent.

Test for a dominant hybrid lethal on the *D. melanogaster* X chromosome

To test whether hybrid lethality involves an interaction between a recessive factor on the *D. mauritiana* X chromosome and a dominant factor on the *D. melanogaster* X chromosome, we took advantage of 28 *Dp(1;Y)* chromosomes that together span most of the *D. melanogaster* X chromosome (Cook *et al.* 2010). These stocks have small segments of the *D. melanogaster* X chromosome duplicated onto the Y chromosome. We crossed *D. mauritiana w* and/or *D. simulans* females (from various stocks) to *Dp(1;Y)* males but were unable to obtain offspring due to strong prezygotic isolation in this direction of the cross. To circumvent this problem, we first crossed *C(1)RM, y v/0* *D. melanogaster* females to males from the 28 *Dp(1;Y)* stocks to generate *C(1)RM, y v/Dp(1;Y)* females (these females were all viable and fertile). We then crossed these *C(1)RM, y v/Dp(1;Y)* females to *D. mauritiana w* males and scored the hybrid offspring. The only surviving offspring will be *X^{mau}/Dp(1;Y)*; *mau/mel* males unless the *Dp(1;Y)* segment contains a dominant hybrid lethal locus from the *D. melanogaster* X (Figure 2).

Results

A hybrid lethal factor maps to the sibling species X chromosome heterochromatin

We crossed heterozygous *Df(1)JC4/FM7c* *D. melanogaster* females to *D. mauritiana w* (*mau*) males and, separately, to *D. simulans w^{XD1}* (*sim*) males. As Table 1 shows, *Df(1)*

Figure 2 Genetic screen for dominant *D. melanogaster* X factors incompatible with recessive factors on the *D. mauritiana* X. *C(1)RM, y v D. melanogaster* females were first crossed to *Dp(1;Y)* stocks to generate *C(1)RM, y v/Dp(1;Y)* females. These *D. melanogaster* females were then crossed to *D. mauritiana w* to generate: (A) $X^{mau}/Dp(1;Y); mau/mel$ F₁ hybrid males. (B) *C(1)RM, y v/Y^{mau}; mel/mau* hybrid females die as larvae; (C) *C(1)RM, y v/X^{mau}* triplo-X hybrid females are lethal; and (D) *Dp(1;Y)/Y^{mau}* flies, which lack an X chromosome, are inviable. White rectangles represent *D. melanogaster* genetic material; gray rectangles represent *D. mauritiana* genetic material. Small rectangle with a hook represents Y chromosome; two rectangles joined by two lines represent attached-X; small hooked rectangle joined to another small rectangle by a single line represents *Dp(1;Y)* chromosome.

JC4/mau and *Df(1)JC4/sim* hybrid females are completely inviable (or very nearly so) relative to *FM7c*-bearing sisters, consistent with Coyne *et al.* (1998). We tested for within-species variation in the strength of hybrid lethality by crossing *Df(1)JC4/FM7c* females to males of other *D. mauritiana* stocks. Table 1 shows that the viability of the *Df/mau* females ranges from 0 to 32.9%, depending on the line used. *Df(1)JC4/sech* females also show significantly reduced viability, although offspring numbers are small due to the difficulty of the cross. As a control, we crossed *Df(1)JC4/FM7c* females to *D. melanogaster* Ore-R males and saw no significant difference in the viability of *Df/mel* vs. *FM7c/mel* females (Table 1). These crosses suggest that at least one recessive hybrid lethal factor resides in cytological region 20A–20F, at the base of the X chromosome of *D. mauritiana*, *D. simulans*, and *D. sechellia*, and that it varies in strength among *D. mauritiana* lines.

To further map the 20A–20F region, we used 19 additional stocks with deletions of the 19F–h32 region of the *D. melanogaster* X. We crossed heterozygous *D. melanogaster* *Df/B* females from these 19 stocks to *D. mauritiana w* males and scored the viability of the *Df/mau* females (Table 2). We found evidence that the 20A–20F region contains at least two separate factors that cause hybrid lethality. First, we found evidence for one hybrid lethal region uncovered by the *Df(1)JC77/FM7c* deficiency (line 7 in Table 2) in

Table 1 Hybrid daughters from *D. melanogaster Df(1)JC4/FM7c* females crossed to sibling species males reveal hybrid lethality in cytological region 20A–20F

Sibling sp.	n	Df/sib	B/sib	% Df	χ^2 test
<i>sim w^{XD1}</i>	2	8	172	4.44	**
<i>mau w</i>	4	1	613	0.16	**
<i>mau Syn</i>	4	0	877	0	**
<i>mau 0241.01</i>	4	448	912	32.94	**
<i>mau yw</i>	4	86	705	10.87	**
<i>sech w</i>	4	10	29	25.64	*
<i>mel Ore-R (control)</i>	2	198	210	48.53	

n = number of replicate crosses. * χ^2 test, $P < 0.05$; ** χ^2 test, $P < 0.001$.

cytological region 19F6–20A3. However, using other overlapping deficiencies, we obtained complex results (Table 2). Three other overlapping deficiencies, *Df(1)C74*, *Df(1)S54*, *Df(1)16-3-22*, together spanning region 19D1–20A4 are all semilethal (lines 4–6 in Table 2), whereas another deficiency, *Df(1)BSC588*, which eliminates 19F3–20A4, is viable (line 8 in Table 2). We confirmed the overlap of these deficiencies by molecularly characterizing the breakpoints of *Df(1)S54* and *Df(1)16-3-22* (Supporting Information, Table S2). These results suggest the existence of a hybrid lethal factor in the region uncovered by the *Df(1)JC77* deficiency in 19F6–20A3, a relatively small (~120 kb) interval spanning no more than nine genes, but the strength of the hybrid lethality varies depending on the genetic background of *D. melanogaster*: a *D. mauritiana* factor uncovered by *Df(1)JC77/FM7c* may be lethal only in the presence of a factor in the *Df(1)JC77/FM7c* deficiency background but not in the *Df(1)BSC588/Binsinsky* background.

Second, we found strong evidence for the existence of a hybrid lethal factor, hereafter called *heterochromatin hybrid lethal (hhl)*. Three overlapping hybrid lethal deficiencies (lines 17–19 in Table 2) and seven viable deficiencies (lines 9–15 in Table 2) support the existence of *hhl*. *Df(1)JC4* is lethal in hybrids, but we cannot distinguish the effects of two hybrid lethals: the same hybrid lethal factor uncovered by *Df(1)JC77* in 19F6–20A3 or the hybrid lethal factor in the heterochromatin around h26 (the exact determination of this breakpoint is uncertain given its position in the heterochromatin). Together, these results show that a hybrid lethal factor resides in cytological region 20A3–h32. As the longest viable deficiency extends to region h26, we conclude that the lethal factor must lie between h26 and h32, in the pericentromeric heterochromatin. Molecular characterization of the deficiency breakpoints suggests that *hhl* lies proximal to the predicted gene *CR41602*, on *Xhet* (in cytological region h26), a heterochromatic scaffold that is not contiguous with the euchromatic assembly of *D. melanogaster* (Hoskins *et al.* 2007).

***hhl*-mediated hybrid lethality depends on genetic background and temperature**

To assay genetic variation in the strength of *hhl* in different *D. mauritiana* hybrid backgrounds, we also crossed lethal deficiencies *Df(1)GA90*, *Df(1)A209*, and *Df(1)17-87* to males from *mau Syn*, *mau 0241.01*, and *mau y w* (Table 3). The

Table 2 Deficiency mapping hybrid lethality in cytological region 19A–h32

Line	<i>D. melanogaster</i> genotype	Cytological region	<i>n</i>	<i>Df/mau</i>	<i>B/mau</i>	% <i>Df</i>	χ^2 test
1	<i>Df(1)mal10/Binsn</i>	19A5–19E1	2	92	77	54.44	
2	<i>Df(1)17-257/Binsn</i>	19F2–20A2	2	153	186	45.13	
3	<i>Df(1)GA104/FM7c</i>	19F2–19F6	2	141	140	50.18	
4	<i>Df(1)C74/FM6</i>	19F1–20A4	2	49	186	20.85	*
5	<i>Df(1)S54/FM6</i>	19E8–20A2	2	48	126	27.59	*
6	<i>Df(1)16-3-22/FM6</i>	19D1–20A2	2	22	73	23.16	*
7	<i>Df(1)JC77/FM7c</i>	19F6–20A3	2	6	132	4.35	*
8	<i>Df(1)BSC588/Binsinscy</i>	19F3–20A4	2	117	116	50.21	
9	<i>Df(1)ED7664/FM7h</i>	19F1–19F6	2	109	94	53.69	
10	<i>Df(1)BSC824/Binsinscy</i>	20C1–20F3	4	31	25	55.36	
11	<i>Df(1)Exel6255/FM7c</i>	20A1–20C1	4	756	1118	40.34	*
12	<i>Df(1)ED14021/FM7h</i>	20C3–20F1	4	931	878	51.46	
13	<i>Df(1)R8A/FM7c</i>	20B–h26	4	635	559	53.18	
14	<i>Df(1)17-148/Binsn</i>	20C–h26	4	756	635	54.35	
15	<i>Df(1)n23/Binsn</i>	20E	4	720	676	53.49	
16	<i>Df(1)16-185/Binsn</i>	20E–h26	4	592	523	53.09	
17	<i>Df(1)17-87/FM0</i>	20C–h32	4	9	503	1.76	*
18	<i>Df(1)GA90/FM6</i>	20C–h32	4	0	630	0	*
19	<i>Df(1)A209/FM7a</i>	20A3–h32	4	1	776	0.13	*
20	<i>Df(1)JC4/FM7c</i>	20A1–h26	4	17	305	5.28	*

n = number of replicate crosses. * χ^2 test, $P < 0.001$.

results show that the strength of hybrid lethality varies among *D. mauritiana* stocks. Hybrid lethality is strongest in *D. mauritiana w* background, and the viability of *Df*-bearing hybrid females for the other three *D. mauritiana* stocks ranges from 12.8 to 49.1%.

We also tested the temperature dependence of hybrid lethality. We crossed deficiencies *Df(1)A209*, *Df(1)17-87*, and *Df(1)GA90* to males from *mau w*, *mau Syn*, *mau* 0241.01, and *mau y w* at 18° (Table 3). Most have significantly increased viability at lower temperatures. The tem-

perature dependence of the lethality seems also to be contingent on genetic background: hybrid lethality remains strong in *Df(1)A209/mau w* and *Df(1)GA90/mau w* females (lines 1 and 13 in Table 3) but decreases when the two deficiencies are heterozygous over *mau Syn*, *mau* 0241.01, or *mau y w* backgrounds (lines 2, 4, and 14–16 in Table 3). Finally, we tested whether *hhl* is also lethal in *D. simulans* and *D. sechellia* hybrid backgrounds by crossing *Df(1)A209*, *Df(1)17-87*, and *Df(1)GA90* to *sim* and *sech* males. *hhl* is lethal in a *sim w* background (lines 5, 11, and 17 in Table 3)

Table 3 The strength of *hhl* hybrid lethality depends on temperature and genetic background

Line	<i>D. melanogaster</i> genotype	Sibling sp.	<i>n</i>	22–23°			<i>n</i>	18°			FET <i>P</i> -value
				<i>Df/sib</i>	<i>Ballsib</i>	% <i>Df</i>		<i>Df/sib</i>	<i>Ballsib</i>	% <i>Df</i>	
1	<i>Df(1)A209/FM7a</i>	<i>mau w</i>	4	1	773	0.13*	4	27	267	9.18*	<0.0001
2	(20A3–h32)	<i>mau Syn</i>	4	119	804	12.89*	4	122	442	21.63*	<0.0001
3		<i>mau</i> 0241.01	4	281	759	27.02*	4	134	421	24.14*	0.19
4		<i>mau yw</i>	4	346	770	31*	4	119	200	37.30**	0.04
5		<i>sim w</i> ^{XD1}	2	14	143	8.91*	2	10	92	9.80*	0.83
6		<i>sech w</i>	4	—	—	—	4	2	7	22.22	
7	<i>Df(1)17-87/FM0</i>	<i>mau w</i>	4	9	503	1.76*	4	111	137	44.76	<0.0001
8	(20C–h32)	<i>mau Syn</i>	4	56	58	49.12	4	31	37	45.59	0.65
9		<i>mau</i> 0241.01	4	39	89	30.47**	4	54	64	45.76	0.02
10		<i>mau yw</i>	4	22	81	21.36*	4	64	113	36.16**	0.13
11		<i>sim w</i> ^{XD1}	2	11	242	4.35*	2	19	152	11.11*	0.01
12		<i>sech w</i>	4	—	—	—	4	7	8	46.67	
13	<i>Df(1)GA90/FM6</i>	<i>mau w</i>	4	0	630	0*	4	50	534	8.56*	<0.0001
14	(20C–h32)	<i>mau Syn</i>	4	98	656	12.99*	4	170	413	29.16*	<0.0001
15		<i>mau</i> 0241.01	4	303	617	32.93*	4	312	533	36.92*	0.08
16		<i>mau yw</i>	4	177	743	19.24*	4	200	267	42.83***	<0.0001
17		<i>sim w</i> ^{XD1}	2	9	169	5.05*	2	8	113	6.61*	0.62
18		<i>sech w</i>	4	—	—	—	4	25	16	60.97	

n = number of replicate crosses. * χ^2 test, $P < 0.001$; ** χ^2 test, $P < 0.01$; *** χ^2 test, $P < 0.05$.

but not, it seems, in a *sech* background (lines 6, 12, and 18 in Table 3). We found little evidence of an effect of temperature on hybrid lethality for *D. simulans* *w*.

A dominant hybrid lethal on the *D. melanogaster* X chromosome

The hybrid lethality of the *D. simulans* and *D. mauritiana* allele of *hhl* suggests the existence of a dominant partner on the *D. melanogaster* X chromosome. Therefore, to screen for dominant hybrid lethals on the *D. melanogaster* X, we used a collection of 18 *Dp(1;Y)* stocks, i.e., stocks that have a small segment of the *D. melanogaster* X duplicated onto the Y chromosome. Together, these 18 duplication *Dp(1;Y)* stocks span ~80% of the *D. melanogaster* X chromosome. Initially, we crossed *D. mauritiana w* females and/or *D. simulans* females (from several strains) to *Dp(1;Y)* *D. melanogaster* males but were unable to obtain any offspring due to the strong sexual isolation in this direction of the cross. To get around this problem, we generated *C(1)RM, y v/Dp(1;Y)* females and then crossed them to *mau* males (Figure 2). We expect three hybrid genotypes from this cross to be lethal. First, *Dp(1;Y)/Y^{mau}* zygotes, which lack most of the X chromosome, are inviable. Second, *C(1)RM, y v/X^{mau}* triplo-X hybrid females are also inviable. Third, *C(1)RM, y v/Y^{mau}* hybrid females die as larvae, presumably due to the effects of *Hmr^{mel}* on the compound *D. melanogaster* X (Hutter *et al.* 1990). We are, however, interested in the remaining genotype, the *X^{mau}/Dp(1;Y)* hybrid males. These males are genetically equivalent to viable F₁ *X^{mau}/Y^{mel}* hybrid males, except that they carry a small additional segment from the *D. melanogaster* X chromosome. Therefore, unless a particular *Dp(1;Y)* carries a factor incompatible with *hhl^{mau}*—or some other recessive hybrid lethal on the *D. mauritiana* X—hybrid males should be viable.

As Table 4 shows, 17 of the 18 *Dp(1;Y)* chromosomes are viable in hybrid males. One, however, is not: *Dp(1;Y)BSC220*, which spans cytological region 9A4–9B1;10B14 produces no *X^{mau}/Dp(1;Y)* males; we do, however, observe dead embryos (corresponding to *Dp(1;Y)/Y^{mau}* zygotes and to triplo-X females), dead larvae (corresponding to *C(1)RM, y v/Y^{mau}* females), and dead pupae (probably corresponding to the missing *X^{mau}/Dp(1;Y)* males). The duplicated segment in *Dp(1;Y)BSC220* spans ~1.4 Mb and contains ~161 genes (including *Hmr*). To confirm this hybrid lethality, and to narrow down the region further, we tested 10 additional overlapping *Dp(1;Y)*, *B* stocks spanning the 9A4–9B1;10B14 region. Eight of the 10 *Dp(1;Y)*, *B* chromosomes are viable in hybrid males, while two, *Dp(1;Y)BSC221* and *Dp(1;Y)BSC263*, are not (Table 5). From the overlap of these duplications, we infer that a hybrid lethal factor lies in cytological region 9F12–10A6 of the *D. melanogaster* X chromosome. This region is ~0.2 Mb and contains ~24 genes (but not *Hmr*).

The hybrid lethal effect of this ~24-gene region from *D. melanogaster* could result from a specific incompatibility with *hhl^{mau}* or, alternatively, from an interaction with some

other recessive factor on the *D. mauritiana* X. To distinguish these possibilities, we crossed *C(1)RM, y v/Dp(1;Y)BSC263* females to males from *D. mauritiana* lines for which *hhl^{mau}* hybrid lethality is incomplete: *mau* Syn, *mau* 0241.01, and *mau y w*. If the hybrid lethality of the 24-gene region is due to its incompatibility with *hhl^{mau}*, then the lethality of the *D. melanogaster* *Dp(1;Y)BSC263* chromosomes in F₁ hybrid males from these lines should be correspondingly incomplete. However, if the hybrid lethality of the 24-gene region is not specific to *hhl^{mau}* but instead due to some other incompatibility locus, then complete lethality of *D. melanogaster* *Dp(1;Y)BSC263* might show no correspondence with *hhl^{mau}* lethality. We find that *Dp(1;Y)BSC263* chromosomes are viable in F₁ hybrid males from all three *D. mauritiana* stocks (Table 6). The hybrid lethality of the 24-gene *D. melanogaster* region is therefore consistent with a specific incompatibility with *hhl^{mau}*.

Discussion

Our findings show that an X-linked recessive factor, *hhl*, in *D. simulans* and segregating in *D. mauritiana* causes lethality when hemizygous in *Df*-bearing F₁ hybrid females with *D. melanogaster* (see also Coyne *et al.* 1998). F₁ hybrid males are genotypically similar to *Df*-bearing F₁ hybrid females, being hemizygous for *hhl*, and having the same autosomal genotype. *X^{sim}/Y^{mel}* and *X^{mau}/Y^{mel}* F₁ hybrid males are, however, viable. There are two genotypic differences between the viable F₁ hybrid males and the lethal *Df*-bearing F₁ hybrid females: F₁ hybrid males carry the *D. melanogaster* Y chromosome, and *Df*-bearing F₁ hybrid females carry most of the *D. melanogaster* X chromosome (i.e., all but the region removed by the deficiency). We can rule out the possibility that the *D. melanogaster* Y suppresses *hhl*-mediated hybrid lethality, as hybrid females homozygous for the *D. simulans* X and lacking a *D. melanogaster* Y are viable (Sawamura *et al.* 1993). We therefore infer that *hhl* in *D. simulans* and (some) *D. mauritiana* is incompatible with a dominant factor(s) on the *D. melanogaster* X chromosome—a recessive-by-dominant X–X hybrid incompatibility.

This conclusion led us to screen for dominant X-linked factors from *D. melanogaster* that cause lethality in F₁ hybrids with *D. mauritiana*. Using a panel of *D. melanogaster* *Dp(1;Y)* duplications, we identified a single small ~24-gene region that causes dominant lethality when introduced into F₁ hybrid males. F₁ hybrid females (produced in the reciprocal cross) are genotypically similar to these lethal *Dp(1;Y)*-bearing F₁ hybrid males, being heterozygous for the same X-linked region and heterozygous for the autosomes, but are viable. The difference is that lethal *Dp(1;Y)*-bearing F₁ hybrid males are hemizygous for most of the *D. mauritiana* X chromosome (i.e., all but the region duplicated) whereas viable F₁ hybrid females are not. We therefore infer that a dominant factor in the duplicated region of the *D. melanogaster* X is incompatible with a recessive factor on the *D. mauritiana* X chromosome—a recessive-by-dominant X–X

Table 4 Screen for dominant hybrid lethal on the *D. melanogaster* X chromosome

<i>D. melanogaster</i> genotype	Duplicated region	<i>n</i>	$X^{\text{mau}}/Dp(1;Y)$ F ₁ males
<i>C(1)RM, y, v / Dp(1;Y)BSC296</i>	2C1–2C8	2	93
<i>C(1)RM, y, v / Dp(1;Y)BSC74</i>	2B16–2C1;3E4	2	84
<i>C(1)RM, y, v / Dp(1;Y)BSC158</i>	3F3–3F9;4D7	2	24
<i>C(1)RM, y, v / Dp(1;Y)BSC91</i>	4D1–4D1;5D1	3	205
<i>C(1)RM, y, v / Dp(1;Y)BSC172</i>	7A3–7B1;7D18	3	163
<i>C(1)RM, y, v / Dp(1;Y)BSC33</i>	7D12–7D18;8C3	3	151
<i>C(1)RM, y, v / Dp(1;Y)BSC171</i>	8A2–8A2;8F9	3	70
<i>C(1)RM, y, v / Dp(1;Y)BSC220</i>	9A4–9B1;10B14	3	0
<i>C(1)RM, y, v / Dp(1;Y)BSC47</i>	10B3;11A1	3	172
<i>C(1)RM, y, v / Dp(1;Y)BSC185</i>	12A4–12A9;12F4	2	50
<i>C(1)RM, y, v / Dp(1;Y)BSC240</i>	14A1–14A5;15A8	2	32
<i>C(1)RM, y, v / Dp(1;Y)BSC201</i>	15A1–15A3;16C1	3	175
<i>C(1)RM, y, v / Dp(1;Y)BSC207</i>	15F4–15F9;16C1	3	212
<i>C(1)RM, y, v / Dp(1;Y)BSC67</i>	15F4–15F9;17C1	3	241
<i>C(1)RM, y, v / Dp(1;Y)BSC68</i>	16C1–16E1;17C1	2	45
<i>C(1)RM, y, v / Dp(1;Y)BSC11</i>	16F6–17A1;18A7	3	200
<i>C(1)RM, y, v / Dp(1;Y)BSC133</i>	18A3–18A6	3	209
<i>C(1)RM, y, v / Dp(1;Y)BSC31</i>	18A3–18A6;18A7	2	68

n = number of replicate crosses.

hybrid incompatibility. Taken together, our findings suggest two possibilities. We may have identified two different recessive *D. mauritiana* X-by-dominant *D. melanogaster* X hybrid incompatibilities, each with an unmapped partner locus. Alternatively, our finding that a dominant factor in region 9F12–10A6 of the *D. melanogaster* X chromosome only kills hybrid males that have lethal alleles of *hhl* from *D. mauritiana* (Table 6) suggests that we have instead identified loci involved in a single recessive *D. mauritiana* X-by-dominant *D. melanogaster* X hybrid incompatibility.

Location of *hhl*

The genetic analyses presented here show that a hybrid lethal factor, *hhl*, maps to the pericentromeric heterochromatin of the X chromosome of *D. mauritiana* and *D. simulans* w^{XD1} , proximal to the predicted gene *CR41602* in cytological region h26, and thus proximal to any known protein coding gene in *D. melanogaster*. Only one viability-essential gene, the ribosomal DNA locus (*rDNA*), resides in the pericentric heterochromatin (Lindsley *et al.* 1960; Hilliker and Appels 1982), and although the region of Xh distal to the rDNA locus is not required for viability, studies have shown

that it affects the stability (Parry and Sandler 1974) and endoreduplication (Procunier and Tartof 1978) of the rDNA locus. *hhl* causes hybrid lethality when hemizygous in an otherwise heterozygous *D. melanogaster/D. mauritiana* or *D. melanogaster/D. simulans* hybrid genetic background, and the strength of the lethality depends on both temperature and genetic background. This is not the first hybrid lethality factor mapped to the pericentromeric heterochromatin of the X chromosome: *Zhr* from *D. melanogaster* (Sawamura *et al.* 1995; Sawamura and Yamamoto 1997); a factor from *D. santomea* (Matute *et al.* 2010); and *hlx* from *D. mauritiana* (Cattani and Presgraves 2009) all localize to this region.

We cannot exclude the possibility that *Zhr*, *hlx*, and *hhl* involve the same locus at the heterochromatic base of the X chromosome. We can, however, exclude the possibility that *Zhr*, *hlx*, and *hhl* are involved in the same incompatibility, as *Zhr*^{mel} is incompatible with a maternal factor(s) from *D. simulans* (Sawamura *et al.* 1993; Carracedo *et al.* 2000; Gérard and Presgraves 2012); *hlx*^{mau} is incompatible with a recessive, autosomal *Su(hlx)* from both *D. simulans* and *D. sechellia* (Cattani and Presgraves 2009); and *hhl*^{mau} is incompatible with a dominant, X-linked factor from *D. melanogaster*. The

Table 5 Evidence for dominant hybrid lethal in *D. melanogaster* cytological region 9F12–10B14

<i>D. melanogaster</i> genotype	Duplicated region	<i>N</i>	$X^{\text{mau}}/Dp(1;Y)$ F ₁ males
<i>C(1)RM, y, v / Dp(1;Y)BSC58</i>	8D9–8E4;9E2	2	45
<i>C(1)RM, y, v / Dp(1;Y)BSC59</i>	9B1–9B4;9E2	3	267
<i>C(1)RM, y, v / Dp(1;Y)BSC62</i>	9C4–9D4;9E2	3	245
<i>C(1)RM, y, v / Dp(1;Y)BSC61</i>	9B7–9B14;9E2	2	86
<i>C(1)RM, y, v / Dp(1;Y)BSC60</i>	9B7–9B14;9E2	3	206
<i>C(1)RM, y, v / Dp(1;Y)BSC63</i>	9C4–9D4;9E2	3	270
<i>C(1)RM, y, v / Dp(1;Y)BSC221</i>	9C4–9D4;10B14	2	0
<i>C(1)RM, y, v / Dp(1;Y)BSC263</i>	9F12–10A4;10B14	3	0
<i>C(1)RM, y, v / Dp(1;Y)BSC264</i>	10A6–10A10;10B14	3	329
<i>C(1)RM, y, v / Dp(1;Y)BSC48</i>	10B3–10B10;11A1	3	210

n = number of replicate crosses.

Table 6 *D. melanogaster* duplication *Dp(1;Y)BSC263* is incompatible with *hhl*^{mau}

<i>D. melanogaster</i> genotype	<i>D. mauritiana</i> stock	<i>N</i>	<i>X</i> ^{mau} / <i>Dp(1;Y)</i> F ₁ males
<i>C(1)RM, y, v / Dp(1;Y)BSC263</i>	Syn	1	18
<i>C(1)RM, y, v / Dp(1;Y)BSC263</i>	241.01	1	36
<i>C(1)RM, y, v / Dp(1;Y)BSC263</i>	<i>y w</i>	1	29
<i>C(1)RM, y, v / Dp(1;Y)BSC263</i>	<i>w</i>	3	0 ^a

n = number of replicate crosses.

^a From Table 5, for comparison.

mapping of *Zhr*, *hlx*, and now *hhl* to the heterochromatin is consistent with an emerging theme that *Drosophila* hybrid incompatibilities often involve functional divergence of heterochromatin (Sawamura *et al.* 1995; Sawamura and Yamamoto 1997; Ferree and Barbash 2009) and genes whose products interact with heterochromatin (Barbash *et al.* 2003; Brideau *et al.* 2006; Bayes and Malik 2009).

Pericentric heterochromatin is involved in functions such as meiotic and mitotic chromosome segregation (Karpen *et al.* 1996) and the transcriptional regulation of flanking sequences like rDNA loci (Hilliker and Appels 1982; Karpen *et al.* 1988). However, the amount and composition of satellite DNA is highly variable among species (Lohe and Roberts 1988; Platero *et al.* 1998). There is considerable variation in composition and quantity of satellite sequences between *D. melanogaster* and *D. simulans* (Lohe and Burtlag 1987; Ferree and Barbash 2009) as well as between the younger species of the *D. simulans* clade (Lohe and Roberts 1988; Platero *et al.* 1998). Given that the *D. simulans* clade species split from one another just ~250,000 years ago (McDermott and Kliman 2008), the evolution of satellite DNAs occurs quickly.

Large differences in the amount and identity of repetitive satellite sequences between closely related species can result from nearly neutral processes (Charlesworth *et al.* 1994). The rapid evolution of satellite DNAs might also result from genetic conflicts such as meiotic drive, particularly in female meiosis (Zwick *et al.* 1999; Henikoff *et al.* 2001; Ferree and Barbash 2009). During oogenesis, there is opportunity for competition among chromatids as only one of the four meiotic products becomes the primary oocyte. Selfish centromeres can exploit this opportunity to gain preferential access to the egg, spurring a coevolutionary arms race with chromatin-binding proteins that evolve to suppress drive (Malik and Henikoff 2001; Fishman and Saunders 2008). Species-specific centromeric satellites can thus evolve rapidly, and, consequently, chromatin-binding proteins fixed in one species might become incompatible with independently evolving centromeric satellite DNAs of another species (Henikoff *et al.* 2001).

A dominant X-linked factor from *D. melanogaster* is incompatible with a recessive X-linked factor from *D. mauritiana*

X^{mau}/*Y*^{mel} hybrid males are viable but *Df/mau* hybrid females are lethal, suggesting that, in the latter, a recessive factor on the *D. mauritiana* X is incompatible with a dominant factor on the *D. melanogaster* X. By surveying ~80% of the *D. melanogaster* X chromosome, we identified a ~24-gene interval in

cytological region 9F12–10A6 that causes lethality when introduced into *X*^{mau}/*Y*^{mel} hybrid males. The hybrid lethality of this region is consistent with its specific incompatibility with *hhl*^{mau}: *Dp(1;Y)BSC263* chromosomes are viable in F₁ hybrid males from the three *D. mauritiana* stocks with incompletely hybrid lethal *hhl* alleles (or genetic backgrounds). If the incompatibility is specific, we might expect the dominant X-linked factor from *D. melanogaster* to interact with heterochromatin, to be rapidly evolving, or both. Of the 24 genes, 11 have known functions, none of which involves chromatin binding. Of the 13 with no known functions, *CG11160* contains a predicted SET domain, a protein domain that catalyzes histone methylation (Dillon *et al.* 2005). SET proteins are involved in transcription regulation and in heterochromatin formation (Shilatfard 2006). Given the location of *hhl*^{mau} in the heterochromatin, *CG11160* is a possible candidate for the dominant hybrid lethal partner, although *CG11160* does not show evidence of particularly rapid sequence evolution between *D. melanogaster* and *D. simulans* (*K_a/K_s* = 0.074). The 24 genes have *K_a/K_s* ranging from 0 (*CG1552*) to 0.6 (*Rab9Fa*) (Begun *et al.* 2007).

***hhl*-mediated hybrid lethality depends on temperature and genetic background**

The temperature dependence of *hhl*-mediated hybrid lethality is consistent with previous work on hybrid lethality between *D. melanogaster* and its sibling species: for most factors and species pairs, the viability of hybrids improves at lower temperatures (*e.g.*, Sturtevant 1929; Watanabe *et al.* 1977; Lee 1978; Coyne *et al.* 1998; Barbash *et al.* 2000). Our finding that the strength of lethality depends on genetic background is also consistent with previous work. Considerable variation in lethality among stocks exists for *D. melanogaster/D. simulans* hybrids (Watanabe *et al.* 1977; Lee 1978; Orr 1996; Barbash *et al.* 2000; Presgraves 2003), and variation in the strength of postzygotic isolation exists between other pairs of *Drosophila* species (*e.g.*, Macknight 1939; Crow 1942; Mainland 1942; Patterson and Stone 1952; Ayala *et al.* 1974; Kimura 1987; Kopp and Frank 2005; Orr and Irving 2005; Reed *et al.* 2008), non-*Drosophila* animal species (Forejt and Ivány 1975; Wade and Johnson 1994; Thomson *et al.* 1995; Shuker *et al.* 2005; Vyskocilová *et al.* 2005; Good *et al.* 2008; Kozłowska *et al.* 2012), and plant species (Stebbins 1958; Christie and Macnair 1987; Sweigart *et al.* 2007). Together, these studies suggest that polymorphism in Dobzhansky–Muller incompatibilities is common.

Caveats and conclusions

One caveat of using chromosomal deficiencies to map hybrid lethals is that the hemizygous regions uncovered by deficiencies may not identify true hybrid lethals but instead regions that are haploinsufficient specifically in hybrids (Barbash 2011; Maheshwari and Barbash 2011). The best tests of the hybrid-specific haploinsufficiency hypothesis would involve reagents or genotypes not presently available. First, to test whether hemizygosity for pericentric heterochromatic regions is, in general, problematic for hybrid females, we would like to assay the viability of F_1 *Df/mel* hybrid females using deficiencies originating from the sibling species; these deficiencies do not however exist. Second, ideally we would forgo hemizygous genotypes altogether and directly assay the viability of F_1 hybrid females that are otherwise homozygous for the pericentric heterochromatin of the *D. mauritiana* (or *D. simulans*) X chromosome; this F_2 -like genotype cannot however be constructed due to the prohibitive hybrid sterility separating these species. A definitive test of hybrid-specific haploinsufficiency is thus not immediately available.

We suggest however that there are at least three currently available observations that militate against hybrid-specific haploinsufficiency in the present case. First, while it has been argued that hybrids might be more sensitive to haploinsufficiency than pure species (Barbash 2011), there is no evidence that deficiencies in the *hhl* region are lethal or weak *within* species (Table 1). It is not the case, then, that sick deletions are made worse in “sensitized” hybrid female genetic backgrounds. Second, Tables 1 and 3 show that the strength of *hhl* lethality varies within and among the different species’ genetic backgrounds. If the *hhl* region deficiencies were generally haploinsufficient in hybrids, we might expect them to have the greatest effect on the weakest hybrids. Previous work has shown that *D. melanogaster*–*D. sechellia* hybrid females are especially weak (Barbash *et al.* 2000), and yet deficiencies in the *hhl* region are not lethal in crosses involving *D. sechellia* (Tables 1 and 3). Finally, if *hhl* kills hybrid females by hybrid-specific haploinsufficiency, we would not necessarily expect to find a dominant lethal interactor on the *D. melanogaster* X chromosome. Instead, our *Dp* (1;Y) screen identified a single region a dominant *D. melanogaster* X-linked factor in a 24-gene interval in cytological region 9F12–10A6 that causes lethality when introduced into $X^{\text{mau}}/Y^{\text{mel}}$ hybrid males. These data provide strong evidence that *hhl* and a factor(s) in the 9F12–10A6 region together constitute a previously unknown *bona fide* hybrid incompatibility.

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Supporting Information

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Incompatibility Between X Chromosome Factor and Pericentric Heterochromatic Region Causes Lethality in Hybrids Between *Drosophila melanogaster* and Its Sibling Species

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Table S1 Markers used to narrow down deficiency breakpoints in Xh and Xhet

Deficiency	Marker						
	Xh				Xhet		
	<i>CG17598</i>	<i>CG17599</i>	<i>CG42346</i>	<i>FucTC</i>	<i>CG12061</i>	<i>su(f)</i>	<i>CR41602</i>
<i>Df(1)JC4</i>	-	-	-	-	-	-	+
<i>Df(1)A209</i>	-	-	-	-	-	-	-
<i>Df(1)GA90</i>	+	+	+	+	-	-	-
<i>Df(1)17-87</i>	+	+	+	+	-	-	-
<i>Df(1)16-185</i>	+	+	+	-	-	-	+
<i>Df(1)n23</i>	+	+	+	-	-	-	+
<i>Df(1)17-148</i>	+	+	-	-	-	-	+
<i>Df(1)R8A</i>	+	+	+	-	-	-	+

- = gene is deleted in deficiency; + = gene is not deleted in the deficiency

Table S2 Markers used to narrow down deficiency breakpoints in cytological region 19D-20C.

	Marker											
	19D			19E		19F			20A		20C	
Deficiency	<i>mal</i>	<i>Tak1</i>	<i>run</i>	<i>CG15450</i>	<i>unc</i>	<i>CG1718</i>	<i>sol</i>	<i>Hlc</i>	<i>Ir20a</i>	<i>Cyp6t1</i>	<i>DIP1</i>	<i>tilB</i>
<i>DF(1)16-3-22</i>	+	-	-	-	-	-	-	-	-	-	+	+
<i>Df(1)S54</i>	+	+	+	+	-	-	-	-	-	+	+	+
<i>Df(1)GA104</i>	+	n/a	+	+	+	+	-	-	+	+	+	+
<i>Df(1)17-257</i>	+	+	+	+	+	+	-	-	+	+	n/a	+

-= gene is deleted in the deficiency; += gene is not deleted in the deficiency; n/a= not available.