The *Drosophila melanogaster Hybrid male rescue* Gene Causes Inviability in Male and Female Species Hybrids

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Manuscript received September 20, 1999
Accepted for publication December 29, 1999

ABSTRACT

The *Drosophila melanogaster* mutation *Hmr* rescues inviable hybrid sons from the cross of *D. melanogaster* females to males of its sibling species *D. mauritiana*, *D. simulans*, and *D. sechellia*. We have extended previous observations that hybrid daughters from this cross are poorly viable at high temperatures and have shown that this female lethality is suppressed by *Hmr* and the rescue mutations *In(1)AB* and *D. simulans Lhr*. Deficiencies defined here as Hmr^- also suppressed lethality, demonstrating that reducing Hmr^+ activity can rescue otherwise inviable hybrids. An Hmr^+ duplication had the opposite effect of reducing the viability of female and sibling *X*-male hybrid progeny. Similar dose-dependent viability effects of *Hmr* were observed in the reciprocal cross of *D. simulans* females to *D. melanogaster* males. Finally, *Lhr* and Hmr^+ were shown to have mutually antagonistic effects on hybrid viability. These data suggest a model where the interaction of sibling species Lhr^+ and *D. melanogaster* Hmr^+ causes lethality in both sexes of species hybrids and in both directions of crossing. Our results further suggest that a twofold difference in Hmr^+ dosage accounts in part for the differential viability of male and female hybrid progeny, but also that additional, unidentified genes must be invoked to account for the invariant lethality of hybrid sons of *D. melanogaster* mothers. Implications of our findings for understanding Haldane's rule—the observation that hybrid breakdown is often specific to the heterogametic sex—are also discussed.

THE sterility and lethality of species hybrids is a defining characteristic of species (Mayr 1942), but little is known about why hybrids are unfit or what allelic changes are responsible (Wu and Palopoli 1994; Coyne and Orr 1998). Without such information, it is not possible to determine whether there are general patterns among the genes and alleles that cause hybrid breakdown or to understand the evolutionary forces that lead to allelic divergence between species.

Lack of progress cannot be attributed to the lack of a model for explaining hybrid breakdown. Dobzhanksy (1937) and Muller (1940) proposed that hybrid breakdown results from interactions between alleles that have evolved independently in the parental species. This theory remains compelling because of its simplicity and generality, but the supporting evidence is largely indirect (Coyne and Orr 1998). The lack of more direct evidence is due to the great difficulties in finding species groups that both display hybrid breakdown and are amenable to the identification and experimental manipulation of incompatibility alleles.

When Sturtevant (1919) discovered *Drosophila simulans* and its close relationship to *D. melanogaster*, he quickly realized its potential for investigating questions of species divergence (Provine 1991). *D. simulans* is now known to form a three-member clade with *D. mauritiana* and *D. sechellia*; we refer collectively to these three species as the "siblings" of *D. melanogaster*. Hybrids between *D. melanogaster* and its sibling species generally show the same pattern of viability as described for *D. melanogaster*/*D. simulans* hybrids (Sturtevant 1920; reviewed in Ashburner 1989; Sawamura *et al.* 1993b; Hutter 1997; Sawamura 2000). *D. melanogaster* females crossed to sibling species males produce viable but sterile hybrid daughters and lethal sons, while hybrid progeny of sibling species mothers include viable but sterile sons and poorly viable daughters. These sibling species are more closely related to one another than to *D. melanogaster* because they produce viable hybrids of both sexes, with the daughters being fertile; their greater evolutionary distance from *D. melanogaster* is also supported by cytological and molecular data (Lemeunier *et al.* 1986; Caccone *et al.* 1996). The genetics of hybrid breakdown among the sibling species has been characterized extensively (Hollocher and Wu 1996; True *et al.* 1996; Joly *et al.* 1997; Maside *et al.* 1998; Ting *et al.* 1998).

The complete sterility of *D. melanogaster* hybrids has been the primary obstacle to identifying the genes that distinguish *D. melanogaster* from its siblings. The recent discovery of *D. simulans* strains that produce fertile F_1 female hybrids with *D. melanogaster* provides reason for optimism (Davis *et al.* 1996), but it remains to be determined whether hybrid incompatibility genes can be identified in *D. melanogaster* by backcross analysis or introgression (Wu and Palopoli 1994), let alone by direct

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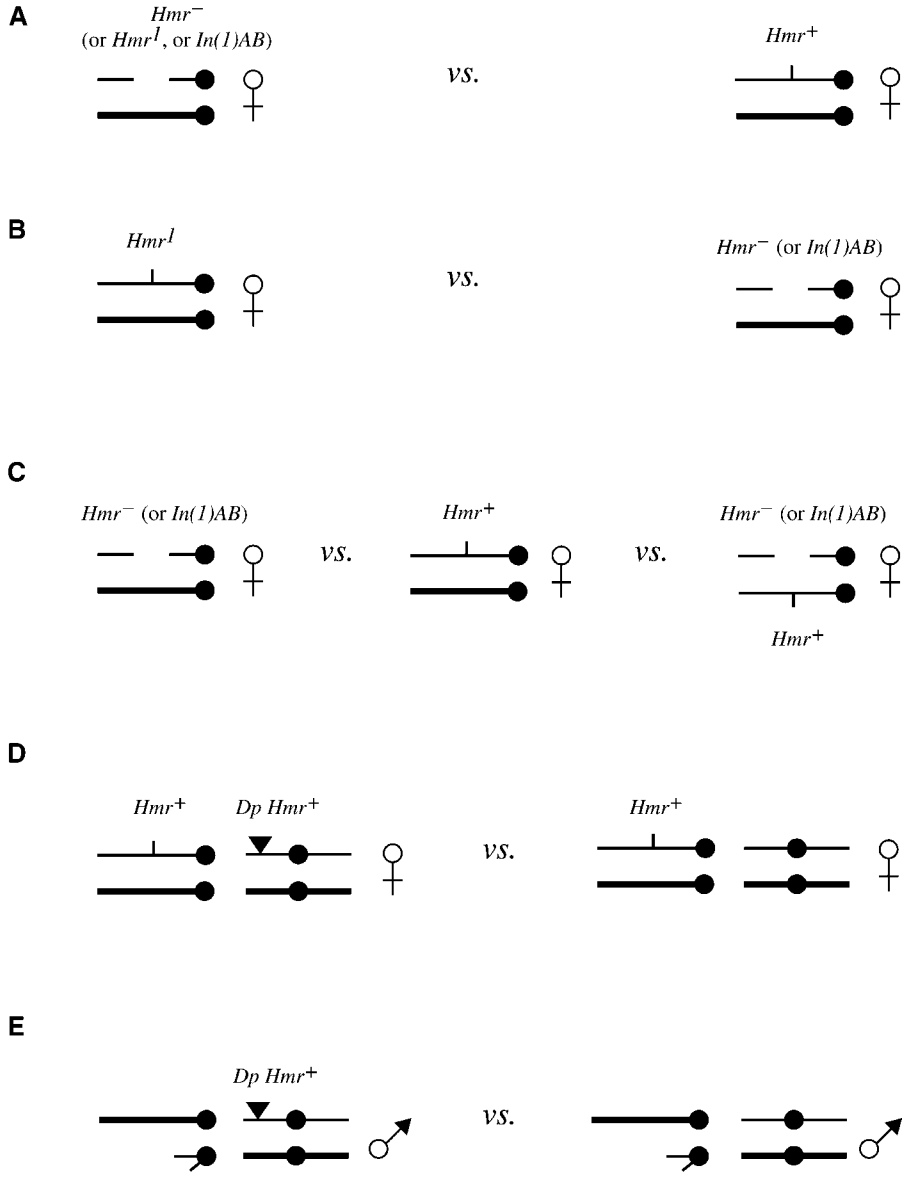

Figure 1.—Schematic of viability comparisons performed in this study. Genotypes within each section are hybrid siblings (with the exception of section F, see below). *D. melanogaster* chromosomes are represented as thin lines, and sibling species chromosomes as thick lines. Only those chromosomes relevant to each experiment are shown; the X, Y, and autosomes are indicated as rod-shaped acrocentric, J-shaped submetacentric, and metacentric chromosomes, respectively. (A) Comparison of female hybrids heterozygous for deletions in the 9D region [or for Hmr^1 or *In(1)AB*; left] with wild-type females (right) to assay aberrations and rescue mutations for dominant suppression of female lethality (Tables 3 and 4). (B) Comparison of female hybrids heterozygous for Hmr^1 (left) with females heterozygous for *In(1)AB* or for Hmr^- deletions (right) to determine whether Hmr^1 is a null mutation (Table 5). (C) Comparison of matroclinous exceptional female hybrids (right) with sibling females of the same genotypes as in A. See Table 6 and Figure 6 for details of the method used to generate these hybrids. (D) Comparison of females carrying a duplication of Hmr^+ (left) with wild-type female siblings (right) to determine whether additional doses of Hmr^+ reduce hybrid viability (Table 7, B and C). (E) Comparison of X_{sib} male progeny of $C(1)_{mel}$ mothers to determine whether a duplication of Hmr^+ reduces hybrid male viability and whether the deleterious effect of Hmr^+ occurs even in the absence of X_{mel} (Table 8). (F and G) Crosses to determine whether *Hmr*-dependent lethality occurs in progeny of *D. simulans* mothers. (F) Comparison of females heterozygous for Hmr^1 (top left) with wild-type females (bottom left). Viabilities of these females were determined in separate crosses, relative to their X_{sim} brothers (right; see Table 9). (G) Comparison of females heterozygous for Hmr^- deletions (top right) relative to siblings with a wild-type dosage of Hmr^+ (top left). This cross (see Table 10) also allows the comparison of X_{sim} males carrying a duplication of Hmr^+ (bottom left), relative to nonduplication brothers (bottom right).

selection through mutagenesis. Researchers have therefore searched natural populations or laboratory stocks for alleles that suppress the inviability of F_1 hybrids. Three mutations that rescue lethal hybrid sons of *D. melanogaster* mothers have been discovered: *D. simulans Lethal hybrid rescue* (*Lhr*; Watanabe 1979) and *D. melanogaster Hybrid male rescue* (*Hmr*) and *In(1)AB* (Hutter and Ashburner 1987; Hutter *et al.* 1990). Two mutations that rescue subviable daughters from the reciprocal cross to sibling species females have also been discovered: *D. simulans maternal hybrid rescue* (*mhr*; Sawamura *et al.* 1993a) and *D. melanogaster Zygotic hybrid rescue* (*Zhr*; Sawamura *et al.* 1993c). The existence of these distinct sets of rescuing mutations suggests that two independent mechanisms of lethality exist in *D. melanogaster* hybrids (Sawamura *et al.* 1993b).

It is not unreasonable to suppose that these rescue mutations are alleles of genes that actually cause hybrid lethality, but it is also possible that they are mutations that suppress lethal interactions between other unknown genes (Coyne 1992; Wu and Palopoli 1994). Distinguishing between these possibilities requires the ability to manipulate the wild-type alleles of the rescue mutations in hybrids. This has been convincingly achieved only for the *Zhr* locus. Zhr^+ appears to cause hybrid lethality, because deletions of the locus mimic *Zhr* rescue activity, while Zhr^+ duplications reduce hybrid viability (Sawamura and Yamamoto 1993). Less is known about *Hmr*. Hutter *et al.* (1990) showed that an Hmr^+ duplication suppresses *Hmr*-dependent male rescue, but it is unclear whether Hmr^+ itself is deleterious to hybrids. Because *Hmr* is *X* linked, deletions of

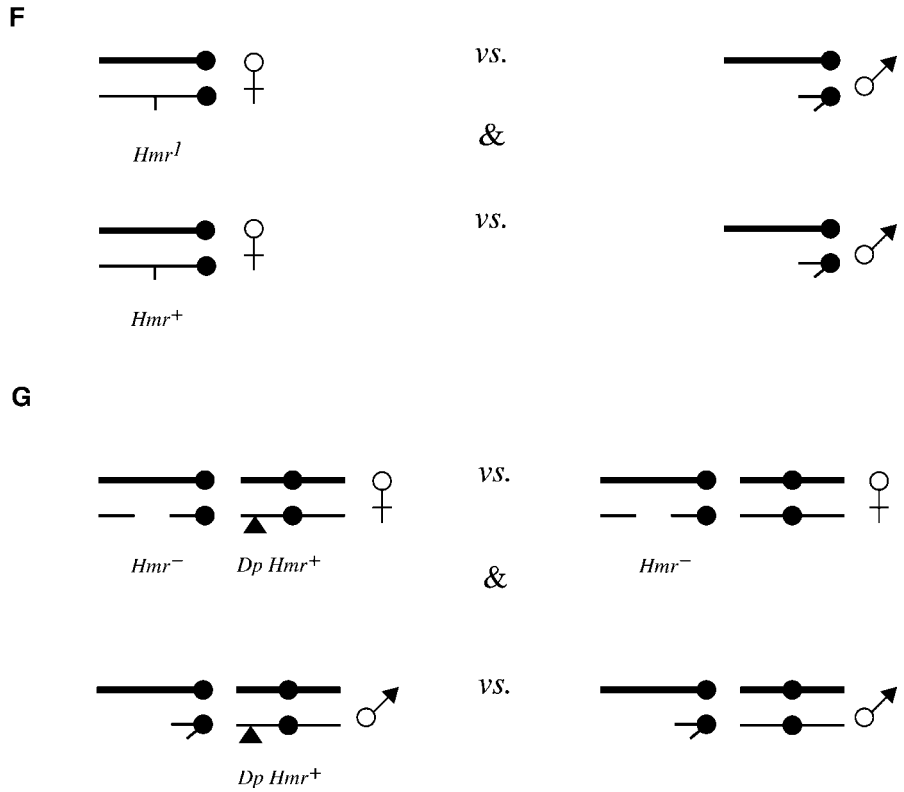

Figure 1.—*Continued.*

the *Hmr* region are lethal to hemizygous males and therefore cannot be assayed in hybrid males. This limitation, together with the absence of an *Hmr* phenotype within *D. melanogaster*, has impeded the characterization and isolation of *Hmr*.

We have looked, therefore, for possible phenotypes of *Hmr* in hybrid females. It has long been known that *D. melanogaster*/*D. simulans* hybrid females are fully viable only at low temperatures (Sturtevant 1929; Kerkis 1933b). We have found that high-temperature lethality is even stronger in *D. sechellia* hybrids and, more importantly, that it is suppressed by *Lhr*, *In(1)AB*, *Hmr*, and by deletions that we define here as being Hmr^-. We have used the suppression of high-temperature female lethality, as well as other assays, to investigate in greater detail the relationship between *Hmr* and hybrid viability. Our results suggest that Hmr^+ gene dosage is a major factor in determining the viability of *D. melanogaster* interspecific hybrids.

MATERIALS AND METHODS

Culture conditions: All crosses were done at 25°. Progeny were collected for 1 or 2 days; after removing the parents, cultures were immediately shifted to the temperatures indicated in each table, with the following two exceptions. Crosses with *D. simulans mhr* mothers (see Tables 9 and 10) were kept at 25° for ~24 hr after removing the parents and then shifted to the appropriate temperature. Progeny for the temperature-shift experiments shown in Figure 4 were collected at 25° for 6–9 hr (29° to 18° shifts) or 12–14 hr (18° to 29° shifts); shorter collections were used for the 29 to 18° shifts because we found the viability of these cultures to be particularly sensitive to overcrowding. After removing the parents, cultures were placed immediately at the appropriate starting temperature and then shifted at the times indicated in Figure 4.

For some experiments it was informative to count and score dead pharate and eclosed adults. We defined pharate adults as those stages where sex and eye color (*w* or w^a *vs.* w^+) could be scored easily; this corresponds approximately to stage P10 of Bainbridge and Bownes (1981; cited in Ashburner 1989, pp. 181–187) until eclosion.

Stocks: Sibling species marker stocks were from the Drosophila Species Center (DSC, Bowling Green, OH, or Cambridge, United Kingdom) stock collections, with the exception of *D. sechellia f*, which was obtained from J. Coyne. Wild-type stocks were as follows:

D. mauritiana: C164.1 was collected in Riviere Noire, Mauritius, and is identical to stock S7 used in Hutter *et al.* (1990); Iso 152 and Iso 197 are iso-female stocks obtained from the DSC.
D. simulans: Tsimbazaza (Gif 247.1) and Ethiopia (Gif 225.1) are described in Lachaise *et al.* (1986); C167.4 was collected in Kenya and reported in Davis *et al.* (1996).
D. sechellia: Gif 228.1 is described in Lachaise *et al.* (1986); Iso 4 and Iso 24 are isofemales lines from the DSC.
D. melanogaster: Nguruman-4 was obtained from the Umeå (Sweden) stock center; Oregon-R was originally obtained from the National Institute of Genetics (Mishima, Japan).

D. melanogaster deficiency and duplication stocks were obtained from the Bloomington or Umeå stock centers. Their breakpoints are shown in Figure 3; we verified the published cytologies (with the exception of *Df(1)ras-v17*) by analyzing orcein-stained squashes of polytene chromosomes. All *D. melanogaster* marker mutations and aberrations are described in Lindsley and Zimm (1992) and in FlyBase (1999).

Nomenclature: Chromosomes from the *melanogaster* complex species *D. melanogaster*, *D. mauritiana*, *D. sechellia*, and *D. simulans* are indicated by the subscripts *mel*, *mau*, *sec*, or *sim*,

TABLE 1

Temperature-dependent viability of hybrids from wild-type and marker stocks

Female parent (All *D. melanogaster*)	Male parent	Number hybrid females (males in parentheses)[a] Temp.	Alive	Dead, eclosed	Dead, pharate	Total	Viability[b] (%)
A.	*D. mauritiana*						
Oregon-R	*w*	18°	76	3	2	81	93.8
		25°	325	9	10	344	94.5
		29°	315	16	8	339	92.9
Oregon-R	C164.1	25°	286	10	12	308	92.9
		29°	333	30	12	375	88.8
Oregon-R	Iso 152	25°	62	5	1	68	91.2
		29°	330	13	5	348	94.8
Nguruman-4	Iso 152	18°	94	8	7	109	86.2
		25°	42	5	11	58	72.4
		29°	65	5	30	100	65.0
Oregon-R	Iso 197	25°	198	1	6	205	96.6
		29°	55	171	15	241	22.8
Nguruman-4	Iso 197	18°	118	9	6	133	88.7
		25°	44	18	24	86	51.2
		29°	37	43	103	183	20.2
B.	*D. simulans*						
Oregon-R	w^{501}	25°	32	23	0	55	58.2
Nguruman-4	w^{501}	25°	19	22	16	57	33.3
		29°	0	1	26	27	0
Oregon-R	*v*	18°	181	11	39	231	78.4
		25°	163	95	23	281	58.0
		29°	0	15	173	188	0
Oregon-R	ry^{i83}	18°	126	2	4	132	95.5
		25°	1	147	47	195	0.5
		29°	0	3	214	217	0
Nguruman-4	ry^{i83}	18°	98	0	0	98	100.0
		25°	26	34	5	65	40.0
		29°	0	3	72	75	0
Oregon-R	$v\ f^{2}$	18°	396	7	19	422	93.8
		25°	321	12	4	337	95.3
		29°	13	156	72	241	5.4
Oregon-R	$v\ f^{2}; +/ry^{i83}$ [c]	25°	321	195	165	681	47.1
Oregon-R	$+; ry^{i83}/+$ [d]	25°	195	173	67	435	44.8
Oregon-R	C167.4	18°	244	23	17	284	85.9
		25°	23	240	44	307	7.5
		29°	1	16	100	117	0.9
Nguruman-4	C167.4	18°	68	2	1	71	95.8
		25°	58	20	3	81	71.6
Oregon-R	Tsimbazaza	18°	224	6	6	236	94.9
		25°	13	149	62	224	5.8
		29°	0	2	217	219	0
Nguruman-4	Tsimbazaza	18°	44	1	2	47	93.6
		25°	10	33	30	73	13.7
		29°	0	0	51	51	0
Oregon-R	Ethiopia	18°	236	6	25	267	88.4
		25°	142	216	52	410	34.6
		29°	0	5	45	50	0
Nguruman-4	Ethiopia	18°	72	0	7	79	91.1
		25°	100	0	2	102	98.0
		29°	3	19	58	80	3.8
Oregon-R	*Lhr*	18°	69	1	3	73	94.5
			(92)	(1)	(0)	(93)	(98.9)
		25°	41	1	6	48	85.4
			(51)	(4)	(5)	(60)	(85.0)
		29°	104	8	11	123	84.6
			(3)	(2)	(42)	(47)	(6.4)

(*continued*)

TABLE 1

(Continued)

Female parent (All *D. melanogaster*)	Male parent	Number hybrid females (males in parentheses)[a] Temp.	Alive	Dead, eclosed	Dead, pharate	Total	Viability[b] (%)
C.	*D. sechellia*						
Oregon-R	*w*	18°	69	56	25	150	46.0
		25°	0	2	94	96	0
Nguruman-4	*w*	18°	118	1	10	129	91.5
		25°	2	16	81	99	2.0
Oregon-R	*v*	18°	29	118	152	299	9.7
		25°	0	0	42	42	0
Nguruman-4	*v*	18°	86	5	20	111	77.5
		25°	1	24	51	76	1.3
Oregon-R	228.1	18°	78	64	75	217	35.9
		25°	0	1	27	28	0
Nguruman-4	228.1	25°	0	2	5	7	0
Oregon-R	Iso 24	18°	13	12	105	130	10.0
		25°	0	1	55	56	0
Nguruman-4	Iso 24	18°	22	0	3	25	88.0
		25°	0	21	37	58	0
Oregon-R	Iso 4	25°	0	5	70	75	0

[a] Excluding crosses with *D. simulans Lhr* males, some crosses produced a small number of live males (no more than four) that were assumed to be exceptional and are not shown. The crosses of Nguruman-4 females to Iso 152 and Iso 197 *D. mauritiana* males at 18° produced 4 and 12 dead pharate males, respectively, that may be (nonexceptional) hybrids carrying the *D. melanogaster X* chromosome (see text).

[b] Viability equals the number of live animals divided by total animals.

[c] *D. simulans* F_1 sons from the cross $v\ f^2$ females to ry^{i83} males.

[d] *D. simulans* F_1 sons from the cross ry^{i83} females to $v\ f^2$ males.

respectively. The latter three species are referred to collectively as siblings, abbreviated as *sib*. For clarity we use the designation Hmr^1 to refer explicitly to the rescue allele described in Hutter and Ashburner (1987); this remains the only known allele of *Hmr*.

Experimental design: Most of the experimental crosses involved comparisons of sibling hybrids of different genotypes with respect to *Hmr*. A summary diagram is shown in Figure 1.

RESULTS

High-temperature lethality in female hybrids: *D. simulans* hybrid daughters from *D. melanogaster* mothers vary in their viability at 25°, depending on the stocks used (Watanabe *et al.* 1977; Lee 1978). To investigate whether female hybrids with *D. mauritiana* and *D. sechellia* show similar properties, we measured hybrid viability at three temperatures with a small number of stocks from each species (Table 1). We used two *D. melanogaster* stocks, Oregon-R and Nguruman-4, and found in most cases that hybrids with Oregon-R had viability lower than that of Nguruman-4 hybrids. Quite unintentionally, we used the same Oregon-R stock used previously by Lee (1978) to measure viability in *D. simulans* female hybrids, who also found it to be strongly biased against hybrid viability. We placed hybrids into three classes—viable, dead eclosed, and dead pharate (see materials and methods). The calculated viability will therefore be an overestimate of the true viability, if there is significant prepharate lethality. This may be the case for *D. sechellia* hybrids, because a cursory examination of the hybrid cultures often revealed a large number of dead embryos and young larvae; we did not, however, determine the sex of these dead early-stage animals.

Our results with *D. simulans* hybrids were consistent with previous studies: all female hybrids were at least 78% viable at 18° but varied from fully viable to fully lethal at 25°. Viable escapers at 25° often displayed morphological defects including crinkled wings, rough eyes, and multiple necrotic patches similar to those shown in Figure 2B.

To investigate the genetic basis of the variation among *D. simulans* stocks, we made reciprocal crosses between a stock that produced fully viable female hybrids with Oregon-R at 25° ($v\ f^2$) and a second stock (ry^{i83}) that produced lethal female hybrids at 25° and crossed the resulting F_1 males to Oregon-R (Table 1B). Female hybrids from both crosses had ~50% viability, suggesting that the difference in hybrid viability between the *D. simulans* ry^{i83} and $v\ f^2$ stocks is caused by an autosomal gene (or genes). This result contrasts with the report of Lee (1978), which implicated the *X* chromosome

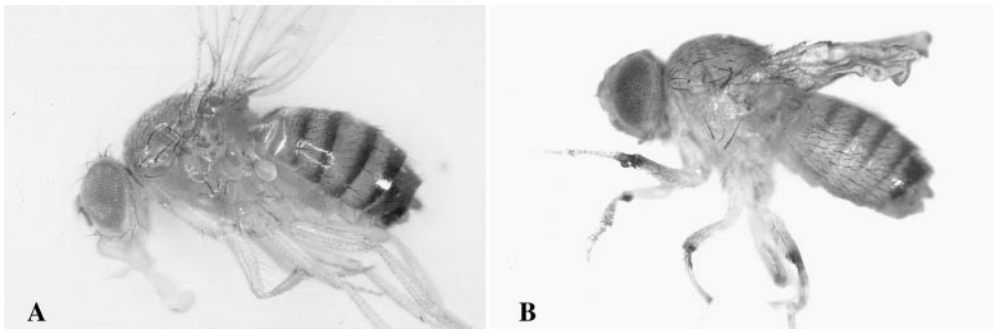

Figure 2.—Hybrid daughters from the cross of *D. melanogaster Df(1)N110/FM6* females to *D. simulans* ry^{i83} males at 25°. (A) A *Df(1)N110*, Hmr^{-}/X_{sim} animal. (B) An *FM6*, Hmr^{+}/X_{sim} sibling. Note the rough eyes, necrotic tissue patches, and malformed wings. At least one such necrotic patch was observed in 93% (n = 115) of $FM6/X_{sim}$ females that eclosed, with none observed in $Df(1)N110/X_{sim}$ siblings (n = 182).

as being largely responsible for differences in hybrid viability between *D. simulans* stocks (based on less direct measurements with different *D. simulans* stocks). Determining whether or not this discrepancy reflects the existence of distinct systems of hybrid viability modifiers within *D. simulans* will require more extensive mapping efforts.

All *D. simulans* hybrids were essentially lethal at 29°, with rare escapers being quite sickly (Table 1B). Intriguingly, the one exception occurred in hybrids between Oregon-R and a stock homozygous for the mutation *Lhr* (Watanabe 1979). Female hybrids were 85% viable at 29° and showed none of the morphological abnormalities characteristic of hybrid escapers. Male hybrids were poorly rescued at 29° (3 live males *vs.* 104 females) but fully rescued at 18° and 25°. Several attempts at mating Nguruman-4 females to *Lhr* males failed.

D. mauritiana hybrid females had significantly higher viabilities than *D. simulans* hybrids. Of four stocks tested, three produced female hybrids with Oregon-R at both 25° and 29° that were ≥89% viable (Table 1A). Hybrids from the fourth stock, Iso 197, however, were only 23 and 20% viable at 29° with Oregon-R and Nguruman-4, respectively. Escapers from these crosses had morphological defects similar to those seen in *D. simulans* hybrid escapers, albeit at a reduced frequency and intensity. At 18° a small number of pharate males were found in some crosses (see Table 1, footnote a). These animals typically had extreme morphological defects including split and malformed nota and greatly reduced eyes. Because patroclinous males (X_{mau}) are viable (Hutter *et al.* 1990; see also Table 6 below), these malformed pharate males may be nonexceptional hybrid "escapers" (*i.e.*, X_{mel}). The observation of these rare pharate escapers does not contradict the accepted fact that X_{mel}/Y_{mau} hybrid males are invariably lethal; they do serve to emphasize a point revealed by the results presented below, namely that hybrid males and females exist on a single *Hmr*-dependent continuum of viability.

Female hybrids with *D. sechellia* had much lower viability than *D. mauritiana* or *D. simulans* hybrids (Table 1C). Female hybrids at 25° were essentially lethal with all five *D. sechellia* stocks tested; rare escapers were severely necrotic. Even at 18° female hybrids between two different stocks (*v* and Iso 24) and Oregon-R were only 10% viable, and many of the surviving adults had rough eyes.

The data presented in Table 1 are derived from a small number of strains and therefore may not be repre-

TABLE 2

Mapping suppression by Hmr^{1} of male and female hybrid lethality, relative to a 9D marker

	Male progeny		Female progeny	
Temp.	Relative viability ry^{+} [a] (%)	No. of ry brothers for reference	Relative viability ry^{+} [a] (%)	No. of ry sisters for reference
18°	< 0.8	127[b]	114.3	643
27°	—[c]	0	0.2[d]	1086

Progeny from the cross of *y* Hmr^{1} *v/+ P{ry*$^{+t10.7}$ *= hsP}22 +; ry* females to *D. simulans* ry^{i83} males. A control cross of *P{ry*$^{+t10.7}$ *= hsP}22/FM7* females to *D. simulans* w^{501} males showed that the *P*-element insertion had no effect on hybrid viability. At 25°, 170 *P{ry*$^{+t10.7}$ *= hsP}22*/X_{sim} and 142 *FM7*/X_{sim} hybrid females were obtained; many animals of both classes had morphological defects similar to those shown in Figure 2B.

[a] This value is equivalent to the map distance between *Hmr* and the *P*-element marker when the Hmr^{+} progeny (Hmr^{+}/Y_{sim} males or Hmr^{+}/X_{sim} females) are fully lethal.

[b] Composed of 85 y v, 41 y^{+} v, and 1 y v^{+} males.

[c] No male progeny were recovered at 27°.

[d] Composed of two ry^{+} females, one of which was necrotic.

TABLE 3

Assaying *Hmr*^{1} and 9D/9E aberrations for suppression of hybrid female lethality

Female parent (deduced *Hmr* genotype)	Temp.	Hybrid female progeny (*Hmr*^{1} or *In(1)AB* males in parentheses)			
		$+/X_{sim}$	Hmr^{1}/X_{sim}, *In(1)AB*/X_{sim}, or *Df*/X_{sim}		Total females (or males)[c]
		Relative viability[a] (%)	Number alive for reference	Estimated viability[b] (%)	
w Hmr^{1}/*FM6* (Hmr^{-})	18°	111.0	82 (1)	84.1 (1.2)	195 (138)
	25°	0	110 (0)	108.9 (0)	202 (5)
In(1)AB, w/*FM6* (Hmr^{-})	18°	79.7	79 (28)	84.9 (16.8)	186 (167)
	25°	0	92 (33)	86.0 (37.0)	214 (89)
	29°	0	99 (0)	85.0 (0)	233 (99)
Df(1)ras203,y v /*FM7c* (Hmr^{-})	18°	109.7	154[d]	77.4	398
	25°	0.7	137	97.9	280
	29°	0	100	88.5	226
Df(1)N110,w/*FM6* (Hmr^{-})	18°	107.1	113	89.0	254
	25°	0	279	100.9	553
	29°	0	103	104.6	197
Df(1)HC133/*FM6* (Hmr^{-})	18°	114.0	86	80.8	213
	25°	43.0[e]	142	77.4	367
	29°	0.6	173	91.1	380
Df(1)ras-v17/*ct oc* (Hmr^{-})	18°	100.0	40	96.4	83
	25°	1.2	81	95.9	169
	29°	0	67	97.1	138
Df(1)v-L11,v^{-}/*FM6* (Hmr^{-})	18°	73.9	153	100.7	304
	25°	2.4	126	98.8	255
	29°	0.5	187	96.9	386
Df(1)v-L15,v^{-}/*FM7c* (Hmr^{-})	25°	18.5	65[f]	106.6	122
	29°	0	214	98.6	434
Df(1)RJ7, v f/*FM6* (Hmr^{-})	18°	83.0	247	107.6	459
	25°	15.3	202	92.4	437
	29°	0	135	117.4	230
Df(1)B13/*FM7c* (Hmr^{-})	18°	40.0	25	135.1	37
	25°	9.5	168	106.7	315
	29°	0	89	140.2	127
Df(1)CH6/*FM7c* (Hmr^{+})	18°	92.8	139	96.9	287
	25°	68.2	22	21.9	201
	29°	—	0	0	152
Df(1)C52/*FM6* (Hmr^{+})	18°	229.2	48	50.3	191
	25°	100.0	4	6.5	123
	29°	—	0	0	62
Df(1)AC2^{L}*AB*^{R}/*FM7c* (Hmr^{+})	18°	70.2	57	114.0	100
	25°	—	0	0	363
	29°	—	0	0	233
Df(1)v-64f/*FM7c* (Hmr^{+})	18°	87.7	203	105.2	386
	25°	40.0	15	6.9	435
	29°	—	0	0	239

All crosses were *D. melanogaster* females of the genotype indicated in the first column crossed to *D. simulans ry*^{i83} males.

[a] Calculated as the number of alive $+/X_{sim}$ females relative to the number of alive reference siblings.

[b] Estimated viability for females is calculated as the number alive divided by one-half the total number of females; for males, as the number alive divided by the total number of males. This calculation assumes that rescued males and both classes of females are fully viable up to the pharate adult stage and that nonrescued males are fully lethal before the pharate adult stage.

[c] Total number equals live, dead eclosed, and dead pharate animals. The lethal phase of hybrid females was predominantly posteclosion at 25° and pharate adult at 29°.

[d] An additional two live, six dead eclosed, and seven dead pharate exceptional females (y^{2} v B/B^{+} phenotype) were observed.

[e] This cross also included 36 phenotypically wild-type males (presumed to be patroclinous exceptions), suggesting that there was a high rate of nondisjunction. Some of these females thus may also be exceptional (*Df(1)HC133*/*FM6*/Y_{sim}) but would be phenotypically indistinguishable from nonexceptional (*FM6*/X_{sim}) siblings. We observed 13 and 29 phenotypically wild-type males at 18° and 29°, respectively.

[f] An additional single live exceptional female (v B/B^{+} phenotype) was observed.

sentative of the range of variability within each species. However, the relative designations of *D. mauritiana*, *D. simulans*, and *D. sechellia* female hybrids as having high, intermediate, and low viabilities, respectively, seems to be a justified generalization. We note the striking observation that these qualitative descriptions for each species are the same for the strength of suppression of hybrid male lethality by Hmr^1 (Hutter and Ashburner 1987).

Suppression of female lethality by Hmr^1: We began our investigation of temperature-sensitive hybrid female lethality after obtaining the unexpected mapping results shown in Table 2. On the basis of its rescue of male hybrids, *Hmr* was mapped distally to *ras* (*1*-32.41) and estimated to be in cytological region 9D1-9E4 (Hutter *et al.* 1990). Here we have mapped Hmr^1 relative to a ry^+-marked homozygous, viable *P* element inserted at 9D; this insertion had no effect on hybrid female viability (see Table 2). Hybrids were generated using the *D. simulans* stock ry^{i83}, which showed strong female lethality effects (Table 1). At 18°, all hybrid males were ry, demonstrating the expected close linkage to 9D, while both ry and ry^+ females were obtained in roughly equal proportions. At 27°, no hybrid males survived, a result consistent with the known temperature sensitivity of male rescue (Hutter and Ashburner 1987). Surprisingly, however, only 2 ry^+ females survived compared with >1000 ry siblings (which appeared generally wild type in morphology). One of these ry^+ females was necrotic and had rough eyes, suggesting that it was an escaper, while the other was wild type in appearance and, thus, may have been either an escaper or a recombinant between *Hmr* and the marker. At 29° the cultures contained a large number of dead pharate and eclosed females, and the relatively small number of viable females were all ry (data not shown). We propose that the closely linked suppressors of male and female hybrid lethality are both in fact Hmr^1.

Suppression by *In(1)AB* and deficiencies: In Table 3 we show that *In(1)AB* also suppressed the lethality of female hybrids with the *D. simulans* ry^{i83} stock, by comparing the viability of $In(1)AB/X_{sim}$ females relative to their $FM6/X_{sim}$ sisters (Figure 1A). As in Table 1, we have also estimated the viability of $In(1)AB/X_{sim}$ females by scoring the total number of females that reached pharate adulthood and beyond. At 18° there was little difference in viability between the sibling classes, but at 25° and 29° only $In(1)AB/X_{sim}$ hybrids survived; they were of normal morphology. We note that a similar cross using $Hmr^1/FM6$ mothers also produced many viable female hybrids at 29°, and these were probably of genotype Hmr^1/X_{sim}, but many had misshapen eyes and, thus, we could not unambiguously distinguish them from their $FM6/X_{sim}$ (B/B^+) siblings (data not shown). Hmr^1/X_{sim} daughters of this cross did appear to be fully viable at 18° and 25° (Table 3).

Because this rescue of hybrid female lethality is dominant, we could determine whether homozygous lethal deletions in the *Hmr* region have similar rescuing properties. In crosses with 12 different deletions, $+/X_{sim}$ females generally had similar viabilities relative to Df/X_{sim} siblings at 18° (Table 3). At 25° and particularly at 29°, however, the crosses fell into two discrete classes, those that included viable Df/X_{sim} females of normal morphology and lethal $+/X_{sim}$ siblings and those that produced only occasional highly necrotic escapers of both genotypes (see Figure 2). We define the first class as being Hmr^-; these deficiencies all delete cytological region 9D and place *Hmr* between the distal 9D1 breakpoint of *Df(1)ras203*, *Df(1)B13*, and *Df(1)ras-v17* and the proximal 9D3-4 breakpoint of *Df(1)N110* (Figure 3). The second class of nonrescuing deficiencies does not delete 9D. It is worth mentioning that most of the rescuing deficiencies were generated independently in unrelated screens, including *Df(1)ras-v17*, which was induced on the balancer chromosome *Binsc* (Lindsley and Zimm 1992).

Our results are consistent with previous mapping of *Hmr* to region 9D1-9E4 (Hutter *et al.* 1990), as well as mapping based on duplications (see *Effects of an Hmr^+ duplication in female hybrids* below). We also note that there was no correlation between rescue of hybrid female lethality and complementation of *sesB* (Figure 3), which agrees with the conclusion that *Hmr* and *sesB/Ant2* are distinct loci (Zhang *et al.* 1999).

Quantification of pharate and posteclosion lethality: The viabilities calculated in Table 3 assumed that Hmr^-/X_{sim} and $+/X_{sim}$ hybrid females are equally viable up to the pharate adult stage, with $+/X_{sim}$ hybrids dying at high temperature as adults. To test this assumption, we performed crosses where dead hybrid females could be genotyped readily on the basis of whether they had wild-type (red) or white eyes (Table 4). This allowed us to measure both viability within each sibling class as well as relative viability between classes. In crosses to a *D. simulans w* strain, very few $+/X_{sim}$ females survived at 29°, in contrast to their $In(1)AB/X_{sim}$ or $Df(1)N110/X_{sim}$ siblings. The majority (83 and 78%, respectively) of the absent $+/X_{sim}$ hybrids, though, could be found among the dead adults.

Similar results were obtained with *D. sechellia* hybrids at 25°, where ≥75% of the relative viability difference was due to pharate adult and posteclosion lethality. At 29°, however, unrescued *D. sechellia* hybrids suffered from extensive prepharate lethality, as only 23–58% of $+/X_{sec}$ hybrids reached the pharate adult stage relative to their rescued siblings. In some cases there was also significant lethality within the rescued class. For example, only 27% of the $Df(1)N110/X_{sec}$ hybrids that reached the pharate adult stage were viable (Table 4C). These data suggest that *D. sechellia* female hybrids suffer from both *Hmr*-dependent and *Hmr*-independent lethality at high temperatures.

All five genotypes shown in Table 4 were crossed to *D. mauritiana w* males at 18°, 25°, and 29° (data not shown). No significant viability effects were found within

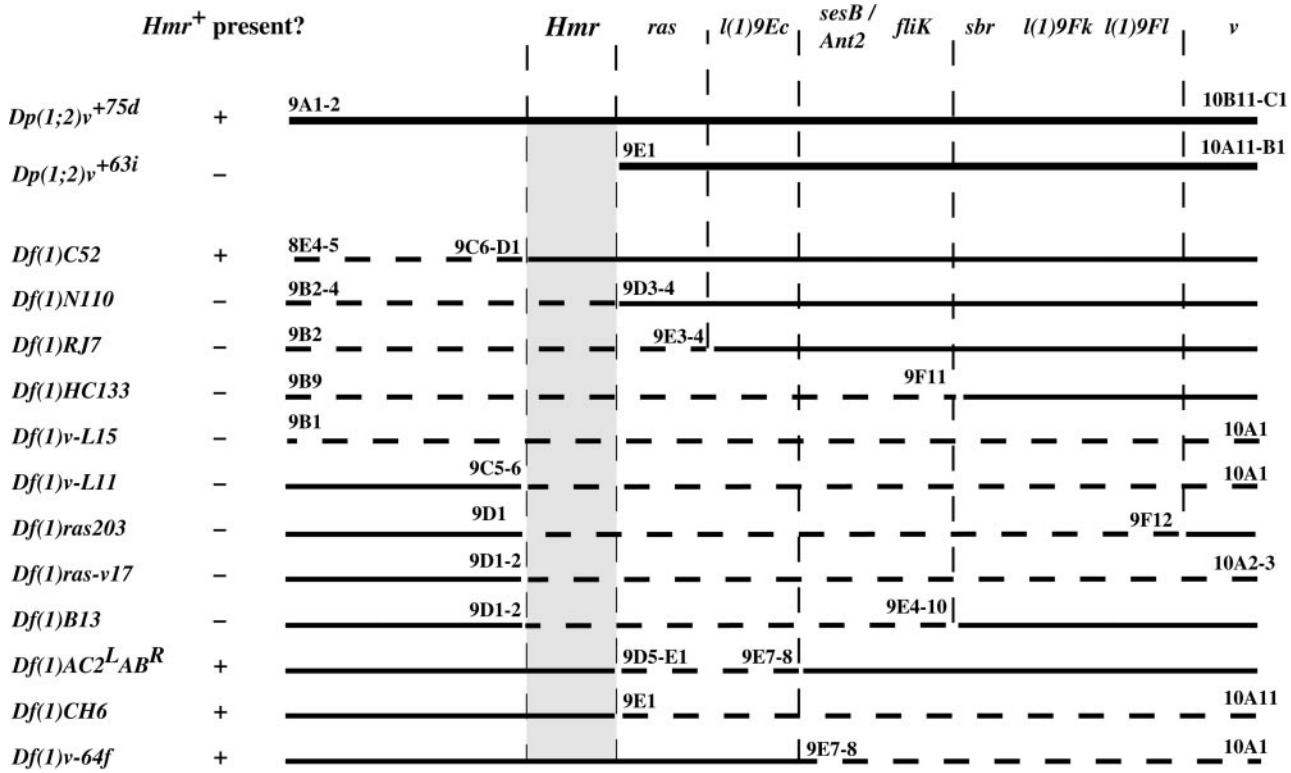

Figure 3.—Genetic map of the *Hmr* region. Duplicated regions are indicated by solid, thick lines and deleted regions are represented by dashed lines. Presence or absence of Hmr^+ was determined from Table 3 for deficiencies, and from Table 7 and Hutter *et al.* (1990) for duplications. Breakpoints relative to *sesB*/*Ant2* and other genes shown are from Zhang *et al.* (1999) and references therein.

or between sibling classes. At 29° the $+/X_{mau}$ hybrids were ≥82% viable relative to reference siblings, and the maximum pharate/posteclosion lethality was 24%.

The lethal phase of hybrid females: Hybrid females die predominantly as pharate adults or after eclosion at high temperatures, but the time of death does not reveal at what stage(s) development is disrupted. We therefore performed reciprocal temperature shifts of unrescued *FM6*/X_{sim} *D. melanogaster*/*D. simulans* hybrid females and compared their viability relative to *Df(1)N110*/X_{sim} siblings (Figure 4). *FM6*/X_{sim} hybrids grown at 18° until approximately the mid-third instar larval stage (L3) and then shifted to 29° had high viability, while cultures shifted before L3 were poorly viable or lethal. There was little apparent difference between siblings in time of development, even in crosses where *FM6*/X_{sim} females were poorly viable. Escaper females often had rough eyes and necrotic leg patches, as seen in nontemperature shifted escapers (Figure 2B).

In the reciprocal shift from 29° to 18°, however, *FM6*/X_{sim} females were delayed in development by ~1–2 days relative to their *Df(1)N110*/X_{sim} siblings. We did not determine the precise phase of this developmental delay, but it was apparent in cultures shifted from 29° at 76 hr after egg laying (AEL), where *FM6*/X_{sim} hybrids were fully viable (but often had rough eyes). In cultures shifted from 29° at 96 hr AEL or later, the *FM6*/X_{sim} hybrids that eclosed first often had extreme morphological defects, including severely misshapen eyes, missing ocelli, and disarrayed notal microchaetes. Their wings were typically normal in length but reduced in width, with absent or incomplete crossveins (Figure 5B); Sturtevant (1920) also noted crossvein defects in hybrid females (that were not temperature shifted). Later eclosing *FM6*/X_{sim} females from the same cultures generally were more normal in morphology.

These results suggest that culture at high temperature causes a general delay and disruption of development beginning in L2 or early L3 larvae that can be alleviated by transfer to low temperature, with the severity of lethality and morphological defects depending on how far development proceeds before the temperature shift. Both the general time course of viability and the developmental delay at high temperature are comparable to that observed for Hmr^1-dependent rescue of *D. melanogaster*/*D. mauritiana* hybrid males (Hutter and Ashburner 1987).

Comparison of Hmr^1, *In(1)AB*, and deficiencies: In crosses to the *D. simulans* ry^{i83} stock described in Tables 2 and 3, Hmr^1 only weakly suppressed the lethality of female hybrids at 29° (data not shown), but Hmr^- deficiencies fully suppressed lethality at 29° (Table 3). In contrast, the crosses with the *D. sechellia w* stock in Table 4 showed little difference between Hmr^1 and deficienc-

TABLE 4

Quantification of postpupal lethality in hybrid females

Cross	Genotype of progeny	Temp.	Number of hybrid progeny				Viability[a] (%)	Relative viability of total + / X_{sib} (to total Df/X_{sib}, $In(1)AB/X_{sib}$ or Hmr^1/X_{sib}) (%)
			Alive	Dead, eclosed	Dead, pharate	Total		
A	Hmr^1/X_{sec}	18°	121	6	24	151	80.1	
	$+/X_{sec}$		53	31	62	146	36.3	96.7
	Hmr^1/X_{sec}	25°	94	14	28	136	69.1	
	$+/X_{sec}$		0	24	78	102	0	75.0
	Hmr^1/X_{sec}	29°	133	18	77	228	58.3	
	$+/X_{sec}$		0	0	102	102	0	44.7
B	$In(1)AB/X_{sim}$	18°	68	1	1	70	97.1	
	$+/X_{sim}$		49	3	7	59	83.1	84.3
	$In(1)AB/X_{sim}$	25°	84	5	1	90	93.3	
	$+/X_{sim}$		39	40	9	88	44.3	97.8
	$In(1)AB/X_{sim}$	29°	22	1	0	23	95.7	
	$+/X_{sim}$		0	12	7	19	0	82.6
	$In(1)AB/X_{sec}$	18°	61	6	13	80	76.3	
	$+/X_{sec}$		56	8	10	74	75.7	92.5
	$In(1)AB/X_{sec}$	25°	75	2	10	87	86.2	
	$+/X_{sec}$		3	33	33	69	4.3	79.3
	$In(1)AB/X_{sec}$	29°	62	4	52	118	52.5	
	$+/X_{sec}$		0	0	34	34	0	28.8
C	$Df(1)N110/X_{sim}$	18°	168	6	6	180	93.3	
	$+/X_{sim}$		167	3	15	185	90.3	102.8
	$Df(1)N110/X_{sim}$	25°	345	26	24	395	87.3	
	$+/X_{sim}$		178	105	15	298	59.7	75.4
	$Df(1)N110/X_{sim}$	29°	269	9	12	290	92.8	
	$+/X_{sim}$		9	162	56	227	4.0	78.3
	$Df(1)N110/X_{sec}$	18°	249	18	56	323	77.1	
	$+/X_{sec}$		255	32	121	408	62.5	126.3
	$Df(1)N110/X_{sec}$	25°	168	21	124	313	53.7	
	$+/X_{sec}$		7	82	176	265	2.6	84.7
	$Df(1)N110/X_{sec}$	29°	77	10	201	288	26.7	
	$+/X_{sec}$		0	1	166	167	0	58.0
D	$Df(1)ras203/X_{sec}$	18°	67	0	1	68	98.5	
	$+/X_{sec}$		33	0	4	37	89.2	54.4
	$Df(1)ras203/X_{sec}$	25°	178	19	17	214	83.2	
	$+/X_{sec}$		1	37	136	174	0.6	81.3
	$Df(1)ras203/X_{sec}$	29°	124	2	2	128	96.9	
	$+/X_{sec}$		0	2	28	30	0	23.4
E	$Df(1)CH6/X_{sec}$	18°	40	0	8	48	83.3	
	$+/X_{sec}$		35	0	16	51	68.6	106.3
	$Df(1)CH6/X_{sec}$	25°	2	37	158	197	1.0	
	$+/X_{sec}$		0	39	154	193	0	98.0
	$Df(1)CH6/X_{sec}$	29°	0	1	72	73	0	
	$+/X_{sec}$		0	3	36	39	0	53.4

Full genotypes of females crossed to w^{501}/Y *D. simulans* or *w*/*Y D. sechellia* males: (A) *w* $Hmr^1/FM6$; (B) *In(1)AB, w*/*FM6*; (C) *Df(1)N110, w*/*FM6*; (D) *Df(1)ras203, y v*/*FM7c,* w^a; (E) *Df(1)CH6*/*FM7c,* w^a.

[a] Viability equals number alive divided by total.

ies for high-temperature rescue. These discrepancies could reflect a difference between hybrid rescue in *D. simulans* and *D. sechellia* or could merely be a consequence of comparing results from different genetic backgrounds. To determine more directly whether or not rescue by Hmr^1 and deletions are equivalent, we compared the viabilities of hybrids heterozygous for Hmr^1, *In(1)AB*, and Hmr^- deficiencies as sibling progeny of the same mothers (Table 5; Figure 1B). Hybrids were made with the *D. sechellia v* stock, which produced poorly

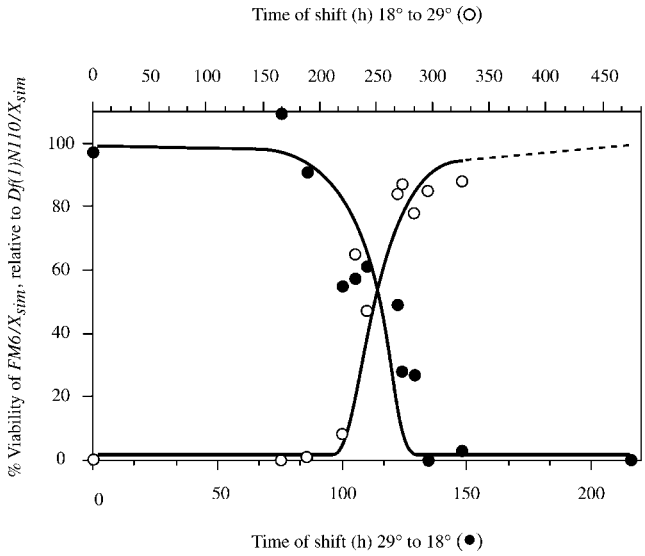

Figure 4.—Viability of female *D. melanogaster*/*D. simulans* hybrids shifted between 18° and 29° during development. Percentage viability was calculated as the number of $FM6/X_{sim}$ hybrids relative to $Df(1)N110/X_{sim}$ siblings, from the cross of *D. melanogaster* $Df(1)N110/FM6$ females to *D. simulans* ry^{i83} males. Cultures were shifted from 18° to 29° (open circles) or 29° to 18° (solid circles) at the times indicated; the curves were drawn by hand. Developmental times correspond to $Df(1)N110/X_{sim}$ hybrids; $FM6/X_{sim}$ hybrids grown at 29° were delayed in development by ~1–2 days. A minimum of 183 $Df(1)N110/X_{sim}$ animals were scored for each data point (mean 372). See materials and methods for further experimental details.

viable hybrids with Oregon-R (Table 1). At 25° all pairwise comparisons had similar viabilities. At 29°, Hmr^1/X_{sec} hybrids were ~50% viable compared to Df/X_{sec} siblings, and many had wing defects (Table 5, A and B). Hmr^1/X_{sec} hybrids were only 9% viable compared to $In(1)AB/X_{sec}$ siblings; this cross used a different Hmr^1 stock than the deficiency-containing crosses (Table 5C). $In(1)AB/X_{sec}$ and $Df(1)v\text{-}L11/X_{sec}$ hybrids were equally viable at 29°.

We conclude that Hmr^1 is a somewhat weaker dominant suppressor of hybrid female lethality than *In(1)AB* or deficiencies. It is important to note, however, that the partial rescue by Hmr^1 of *D. melanogaster*/*D. sechellia* female hybrids at 29° stands in marked contrast to its rescue of *D. sechellia* hybrid males, which is low at 18° and absent at higher temperatures (Hutter and Ashburner 1987; our unpublished data).

Dominant rescue of exceptional female hybrids: *D. mauritiana* and *D. simulans* hybrid females carrying two X_{mel} chromosomes are lethal (Sturtevant 1920; Biddle 1932; Kerkis 1933a; Hutter *et al.* 1990), but can be rescued by *Lhr* (Takamura and Watanabe 1980) or by Hmr^1 and *In(1)AB* (Hutter *et al.* 1990). In several crosses with Hmr^- deficiencies, we observed occasional matroclinous exceptional hybrids (X_{mel}, Hmr^-/X_{mel}, Hmr^+; see footnotes d–f in Table 3). To generate exceptional hybrid females at high frequency, we took advantage of the fact that females carrying a normal sequence *X* chromosome, an inverted *X* chromosome, and a *Y* chromosome produce *X-X* nondisjunctional progeny at much greater frequencies compared to the wild type (Sturtevant and Beadle 1936). Besides being less laborious than constructing compound chromosomes, this technique allowed us to compare the viabilities of exceptional and nonexceptional sibling progeny from a single cross (Figure 1C); the expected progeny of such crosses are shown in Figure 6.

We constructed marked-*Y* stocks with *In(1)AB*/*FM7*, *Df(1)ras203*/*FM7*, and *Df(1)N110*/*FM4* (see Table 6 for complete genotypes). Because *Df(1)ras-v17* is present on the balancer chromosome *Binsc*, we constructed a marked-*Y* stock with *Df(1)ras-v17* and the normal sequence *X* chromosome *y v f*. Control crosses with *D. melanogaster* males demonstrated the success of this technique in generating exceptional progeny. The *In(1)AB*, *Df(1)ras203*, and *Df(1)ras-v17* marked-*Y* stocks produced exceptional females at between 57 and 72% the rate of nonexceptional *In(1)AB*/+ or *Df*/+ siblings (Table 6, A, C, and D), while exceptional females from the *Df(1)N110* stock were twice as frequent as nonexceptional siblings (Table 6B). Because these frequencies varied among the different stocks, the viabilities of exceptional female hybrids relative to regular siblings must be evaluated in comparison to the intraspecific control for each cross.

One caveat in the interpretation of these crosses is that Y_{mel} or its markers might have effects on hybrid viability unrelated to *Hmr*. The $Dp(1;Y)B^s$ chromosome

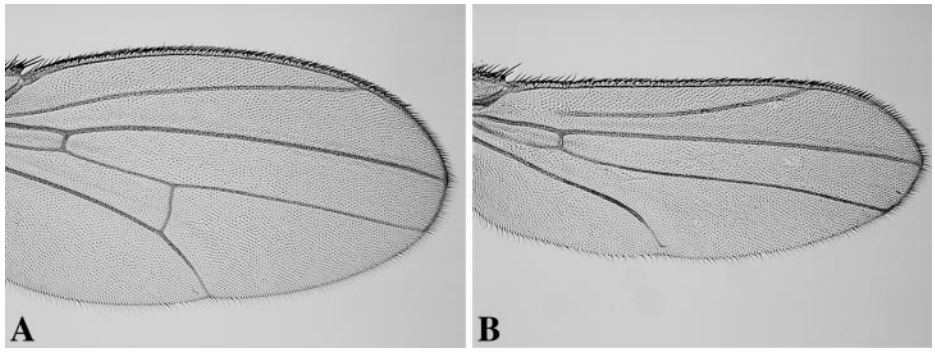

Figure 5.—Wings from *D. melanogaster*/*D. simulans* hybrid females grown at 29° for 96–104 hr and then shifted to 18° as described in Figure 4. (A) Wing from a $Df(1)N110/X_{sim}$ animal. (B) Wing from an $FM6/X_{sim}$ animal. Note the missing posterior crossvein, incomplete longitudinal veins, and substantial reduction in width. Wings from less severely affected animals ranged from having incomplete posterior crossveins but normal shape to being comparable to $Df(1)N110/X_{sim}$ siblings as in A.

present in crosses with *Df(1)N110/FM4* could be scored in all progeny (Table 6B). Hybrid females with this Y_{mel} were ≥65% viable relative to siblings without it (*e.g.*, 106 *Df(1)N110/FM4/Dp(1;Y)B*^{s} *vs.* 162 *Df(1)N110/FM4*, in the cross to *D. simulans v f*^{2} at 18°); the high viability of exceptional X_{sib}/Y_{mel} sons also suggests that this marked *Y* chromosome did not have significant viability effects. *Dp(1;Y), y*$^{+}$ and especially *Dp(1;Y), y*$^{+}$ *v*$^{+}$ present in the *Df(1)ras-v17/y v f* stock (Table 6D) did appear to reduce X_{sib}/Y_{mel} viability compared to the intraspecific controls, presumably due to the duplicated material. Such effects should be less severe in females (because of the absence of dosage compensation), and only one-half of the nonexceptional females will carry Y_{mel}; nonetheless, any Y_{mel}-induced viability reduction of these females would increase inappropriately the relative viabilities calculated for exceptional females.

The partial rescue of *In(1)AB/FM7 D. mauritiana* female hybrids at 18° confirms the discovery of Hutter *et al.* (1990), who used a compound-*X* chromosome. In the cross to *D. mauritiana* Iso 197 males at 25°, these hybrids were fully rescued (Table 6A). *In(1)AB/FM7* hybrids were 71.0% viable relative to their $In(1)AB/X_{mau}$ sisters, which is essentially identical to the 71.6% viability of *In(1)AB/FM7* females relative to their $In(1)AB/X_{mel}$ sisters in the intraspecific control. At 29° exceptional female hybrids with Iso 197 were partially rescued; most (10 of 11 scored) had rough eyes but were not necrotic, while 11 of 38 of their $FM7/X_{mau}$ sisters had necrotic thoracic patches (but were fully viable relative to $In(1)AB/X_{mau}$ siblings). Surprisingly, the *In(1)AB/FM7* exceptional females appeared to have equivalent or greater viability at 25° and 29° than $In(1)AB/Y_{mau}$ sibling males.

All three deficiencies also produced exceptional female hybrids with *D. mauritiana*; in some cases relative viability again approached that seen in intraspecific controls. The relative viability of exceptional females could not be precisely measured in crosses with *Df(1)N110/FM4/Dp(1;Y)B*^{s} (see Table 6, footnote *a*), but we believe the higher estimated limits shown in Table 6B for *D. mauritiana* hybrids are more accurate for two reasons: first, the nonexceptional hybrids are likely to be half $Df(1)N110/X_{mau}$ and half $FM4/X_{mau}$ on the basis of results with other deficiencies in Table 6 and other results described above, and second, the higher estimates are more consistent with the number of exceptional males observed.

Several unexpected features concerning rescue of exceptional *D. simulans* female hybrids deserve comment. First, viability at 25° was always similar to or even higher than viability at 18°, which is the opposite temperature profile consistently observed for nonexceptional X_{mel}/X_{sib} hybrids throughout this study. This phenomenon is most clearly demonstrated in the cross of *Df(1)ras203/FM7/Dp(1;Y), y*$^{+}$ to *D. simulans ry*^{i83} males (Table 6C). The exceptional *Df(1)ras203/FM7* females were not only significantly more viable at 25° than at 18°, but they were also more viable than their nonexceptional $FM7/X_{sim}$ sisters. The same appeared to be true for hybrids between *Df(1)N110/FM4* and *D. simulans ry*^{i83} (Table 6B). For this cross, we suspect that the higher relative viability estimate of exceptional females is more accurate at 18° (29%) and the lower is more accurate at 25° (55%) and 29° (4%), for reasons analogous to those noted above for the *D. mauritiana* crosses. An independent estimate of viability at 25° was provided by using the ectopic mesopleural hair phenotype associated with the *sc*^{8} marker of *FM4*. This phenotype was 50% penetrant in *Df(1)N110/FM4* exceptional females and was

TABLE 5

Direct comparison of *Hmr*^{1}, *In(1)AB*, and *Hmr*$^{-}$ deletions for suppression of female lethality in *D. sechellia* hybrids

		Hybrid female progeny			
Cross	Temp.	Genotype 1	Genotype 2	Relative viability of genotype 1 (%)	Number of genotype 2 for reference
A	25°	Hmr^{1}/X_{sec}	$Df(1)ras203/X_{sec}$	116.9	77
	29°			46.8[a]	267[a]
B	25°	Hmr^{1}/X_{sec}	$Df(1)v\text{-}L11/X_{sec}$	83.1	71
	29°			50.6	160
C	25°	Hmr^{1}/X_{sec}	$In(1)AB/X_{sec}$	106.3	48
	29°			9.5	95
D	25°	$In(1)AB/X_{sec}$	$Df(1)v\text{-}L11/X_{sec}$	71.4	133
	29°			95.2	62

Full genotypes of females (all crossed to *D. sechellia v* males): (A) $Hmr^{1}/Df(1)ras203$, *v*; (B) $Hmr^{1}/Df(1)v\text{-}L11$, v^{-}; (C) *y* Hmr^{1} *v/In(1)AB, w*; (D) *In(1)AB, w/Df(1)v-L11,* v^{-}.

[a] In cross A at 29°, 81% of the Hmr^{1}/X_{sec} and 2% of the $Df(1)ras203/X_{sec}$ progeny had wing defects (curled or notched).

	Maternal gametes \ Paternal gametes	X_{sib}	Y_{sib}
a	*Df(1)Hmr⁻*	++	– –
b	X_{mel}/Y_{mel}	+	–
b	*Df(1)Hmr⁻*/Y_{mel}	++	– –
c	X_{mel}	+	–
c	*Df(1)Hmr⁻*/X_{mel}	– –	?
	Y_{mel}	++	– –

Figure 6.—Expected progeny from a hybrid cross of *Df(1)Hmr*$^{-}$/X_{mel}/Y_{mel} females and X_{sib}/Y_{sib} males. To reflect their similarity to the products of nondisjunction typically observed in *XX* females, we refer to the products of maternal *X-Y* nondisjunction (a and b) as regular or nonexceptional progeny and the products of maternal *X-X* nondisjunction (c) as exceptional progeny. Note that the frequency of *X-X* nondisjunctional maternal gametes varies depending on the specific stocks used, as shown by control crosses to *D. melanogaster* males in Table 6. Rare progeny of *XXY* eggs or *XY* sperm are not shown. Viability designations are as follows: ++, viable; +, semiviable due to *Hmr*$^{+}$-dependent hybrid lethality; – –, lethal due to aneuploidy; –, lethal due to *Hmr*$^{+}$-dependent hybrid lethality; ?, the experimental class. The +, –, and ? classes are viable in control crosses to *D. melanogaster* males. Crosses were also performed with *In(1)AB* instead of *Df(1)Hmr*$^{-}$; hybrid sons carrying *In(1)AB* are semiviable. *Df(1)Hmr*$^{-}$/X_{mel}/X_{mau} metafemale (3*X*; 2A) hybrids with *D. mauritiana* also appear to be semiviable (see Table 6, footnote *b*).

observed in 8% of the nonexceptional females, allowing us to estimate that 16% of the X_{mel}/X_{sim} females in this cross were *FM4*/X_{sim}. We therefore estimate that the 238 total nonexceptional females included 38 (16%) *FM4*/X_{sim} and 200 (84%) *Df(1)N110*/X_{sim}; the viability of *Df(1)N110*/*FM4* exceptions relative to *Df(1)N110*/X_{sim} would then be 65.5% (131/200).

A second intriguing result involves the genetic background difference between the *ry*^{i83} and *v f*^{2} *D. simulans* stocks. In these crosses and others (Table 1), the *ry*^{i83} stock caused much greater lethality to X_{mel}/X_{sim} hybrids at 25° and 29° than the *v f*^{2} stock; recall that the apparent variation appeared to be entirely autosomal (Table 1B). Yet both *Df(1)N110*/*FM4* and *Df(1)ras203*/*FM7* exceptional hybrids were more viable at 25° with *D. simulans ry*^{i83} than with the *v f*^{2} stock. These dissimilar genetic background and temperature effects on X_{mel}/X_{mel} *vs.* X_{mel}/X_{sim} hybrids suggest that *X*-linked factors other than *Hmr* may influence hybrid viability (see discussion).

No exceptional female *D. sechellia* hybrids were recovered at either 18° or 25° with *Df(1)N110* (crossed to *D. sechellia v* and *f* stocks) or with *Df(1)ras203* and *Df(1)ras-v17* crossed to the *D. sechellia w* stock (data not shown). We also generated exceptional females heterozygous for *Hmr*^{1} with a stock of *y Hmr*^{1} *v*/*FM7*/*Dp(1;Y), y*$^{+}$. A high frequency of exceptional females was produced in control crosses to *D. melanogaster* males, but none were observed in crosses to *D. mauritiana w* or Iso 197 males at either 18° or 25° (data not shown). This negative result is consistent with the data of Hutter *et al.* (1990), who found that *Hmr*^{1} only rescues compound-X_{mel} hybrid females with *D. mauritiana* when homozygous.

Effects of an *Hmr*$^{+}$ duplication in female hybrids: The above results show that *Hmr*^{1} and deficiencies in the 9D region are qualitatively equivalent in suppressing hybrid lethality and thus imply that the wild-type *Hmr*$^{+}$ product is deleterious to hybrids. We therefore tested whether increasing the dosage of *Hmr*$^{+}$ would decrease the viability of hybrids, using the *Hmr*$^{+}$ duplication *Dp(1;2)v*$^{+75d}$ (Table 7).

Hutter *et al.* (1990) showed that *Dp(1;2)v*$^{+75d}$ causes a modest decrease in viability of *D. mauritiana* female hybrids at 18° (as well as a developmental delay). In Table 7 we have extended this analysis to all three sibling species at a range of temperatures. We first did a series of control crosses to generate *Df(1)HC133*/X_{sib}; *Dp(1;2)v*$^{+75d}$/2_{sib} and *Df(1)HC133*/X_{sib}; $2_{mel}/2_{sib}$ sibling female hybrids (Table 7A). If *Hmr* is the only gene affecting hybrid viability within these aneuploid segments, then the hybrids should be equivalent in viability to +/X_{sib} and *Hmr*$^{-}$/X_{sib} hybrids, respectively, and display the same temperature-sensitive viability profile described above (Tables 1, 3, 4, and 6). The control crosses suggest that this assumption is correct: *Df(1)HC133*/X_{sib}; *Dp(1;2)v*$^{+75d}$/2_{sib} females had high viability at all temperatures in *D. mauritiana* hybrids, but reduced viability at 29° in *D. simulans* hybrids and at 25° in *D. sechellia* hybrids. *Lhr* suppressed female lethality but failed to rescue males at 29°, as was also observed earlier (Table 1B).

We used two different stocks to assay the effect of *Dp(1;2)v*$^{+75d}$ in the presence of an *Hmr*$^{+}$ X_{mel} (diagrammed in Figure 1D). The second stock used (Table 7C) generally produced stronger lethal effects than the first (Table 7B), but qualitatively, the results were similar. At 25°, *Dp(1;2)v*$^{+75d}$ reduced the viability of *D. mauritiana* hybrids and, more strongly, that of *D. simulans* hybrids. *D. sechellia* female hybrids carrying *Dp(1;2)v*$^{+75d}$ were lethal at both 18° and 25° (Table 7B).

The one notable exception involved the *D. simulans Lhr* stock (Table 7, B and C). *Dp(1;2)v*$^{+75d}$ had no significant effect on female viability in crosses to *Lhr* males, suggesting that *Lhr* suppressed the deleterious effect of

TABLE 6

***Hmr*$^{-}$ deletions partially suppress lethality of exceptional female hybrids**

Female parent (all *D. mel*)	Male parent	Temp.	Regular female progeny (subset carrying marked-*Y* chromosome in parentheses, where scoreable)			Exceptional progeny		Viability of $X_{mel}/X_{mel}/Y_{sib}$ exceptional females, relative to Df/X_{sib} or $In(1)AB/X_{sib}$ regular females (%)
			Relative viability $+/X_{sib}$ (%)	Number Df/X_{sib} or $In(1)AB/X_{sib}$ for reference [*In(1)AB* males in brackets]	Total	No. of $X_{mel}/X_{mel}/Y_{sib}$ females	No. of X_{sib}/Y_{mel} males	
(A) *In(1)AB*	*D. mel. y w* (control)	25°	89.0	109 (52) [54 (21)]	206 (82)	78	74	71.6
	D. mau. Iso 197	18°	103.1	64 [46 (17)]	130	28	83	43.8
		25°	82.6	69 [22 (5)]	126	49	74	71.0
		29°	102.7	37 [6 (0)]	75	15	42	40.5
	D. sim. ry^{i83}	18°	98.8	80 [9 (4)]	159	4	63	5.0
		25°	4.6	109 [16 (0)]	114	2	80	1.8
		29°	0	45 [0]	45	0	58	0
(B) *Df(1)N110*	*D. mel. y v f; ry* (control)	25°	76.3	59 (31)	104 (51)	118	109	200.0
	D. mau. C164.1	18°	—[a]	—[a]	57 (31)	42	68	73.7–147.4[a]
		25°	—	—	137 (69)	66	106	48.2–96.4
		29°	—	—	250 (108)	90	215	36.0–72.0
	D. mau. Iso 197	18°	—	—	192 (104)	145	136	75.5–151.0
		25°	—	—	222 (106)	172	173	77.5–155.0
		29°	—	—	60 (31)	44	43	73.3–146.7
	D. sim. v f^{2}	18°	81.0	268 (106)	485 (198)	18	182	6.7
		25°	81.0	210 (97)	380 (178)	31	204	14.8
		29°	64.1	103 (42)	169 (83)	8	108	7.8
	D. sim. ry^{i83}	18°	—[a]	—[a]	547 (250)	78	199	14.3–28.5[a]
		25°	—	—	238 (150)	131	333	55.0–110.1
		29°	—	—	76 (39)	3	110	3.9–7.9
(C) *Df(1)ras203*	*D. mel. y w* (control)	25°	78.7	155 (66)	277 (109)	102	129	65.8
	D. mau. w	18°	70.8	120	205[b]	40	72	33.3
		25°	119.2	78	171[b]	8	100	10.3
		29°	90.0	20	38	0	14	0.0
	D. mau. Iso 197	18°	105.9	118	243	40	126	33.9
		25°	105.8	69	142	32	68	46.4
		29°	59.7	62	99	11	40	17.7
	D. sim. ry^{i83}	18°	84.5	168	310	4	33	2.4
		25°	9.4	117	128	20	46	17.1
		29°	0.0	90	90	0	53	0.0
	D. sim. v f^{2}	18°	74.0	77	134	2	21	2.6
		25°	96.0	99	194	1	45	1.0

(*continued*)

TABLE 6

Continued

Female parent (all *D. mel*)	Male parent	Temp.	Regular female progeny (subset carrying marked-*Y* chromosome in parentheses, where scoreable): Relative viability $+/X_{sib}$ (%)	Number Df/X_{sib} or $In(1)AB/X_{sib}$ for reference [*In(1)AB* males in brackets]	Total	Exceptional progeny: No. of $X_{mel}/X_{mel}/Y_{sib}$ females	Exceptional progeny: Number of X_{sib}/Y_{mel} males	Viability of $X_{mel}/X_{mel}/Y_{sib}$ exceptional females, relative to Df/X_{sib} or $In(1)AB/X_{sib}$ regular females (%)
(D) *Df(1)ras-v17*	*D. mel. y w* (control)	25°	120.9	148	327	85	54	57.4
	D. mau. w	18°	66.2	68	113	35	1	51.5
		25°	72.1	43	74	20	4	46.5
		29°	116.7	6	13	2	0	33.3
	D. mau. Iso 197	18°	70.0	20	34	8	3	40.0
		25°	130.2	43	99	12	7	27.9
	D. sim. ry^{i83}	18°	82.7	98	179	9	0	9.2
		25°	61.1	90	145	5	3	5.6

Full genotypes of females (crossed to males of genotype indicated in column 2): (A) *In(1)AB, y cv f mal/FM7c, y* sc^8 w^a *sn v g B/Dp(1;Y)* y^+; (B) *Df(1)N110, w/FM4, y* sc^8 *w f/Dp(1;Y)* B^s; (C) *Df(1)ras203, y v/FM7c, y* sc^8 w^a *sn v g B/Dp(1;Y)* y^+; (D) *Df(1)ras-v17,* sc^8 v^- *B/y v f/Dp(1;Y)* y^+ v^+.

[a] *Df(1)N110*/X_{sib} and *FM4*/X_{sib} regular females could not be distinguished in these crosses. The lower limit of the relative viability calculations for exceptional females corresponds to the *Df(1)N110*/X_{sib} and *FM4*/X_{sib} classes being equally viable, while the upper limit corresponds to complete lethality of the *FM4*/X_{sib} class.

[b] An additional 3 (18°) and 11 (25°) females of phenotype y^+ w^+ v^+ B/B^+ were observed. Most had rough and distorted eyes and some had malformed wings. These are likely to be *Df(1)ras203/FM7c*/X_{mau}; *2A* metafemales. $X_{mel}/X_{mel}/X_{sim}$; $2_{mel}/2_{sim}$, *Lhr* metafemale hybrids are also semiviable (Takamura and Watanabe 1980).

duplicating Hmr^+. The opposite effect was observed in male progeny, where $Dp(1;2)v^{+75d}$ appeared to strongly suppress the rescue activity of *Lhr*. Duplication-containing sons were ≤7% viable relative to nonduplication brothers at all temperatures. These reciprocal effects support a model where the Hmr^+ and Lhr^+ loci form a pair of interacting genes that causes hybrid lethality (see discussion).

The distal breakpoint of $Dp(1;2)v^{+63i}$ is at 9E, which is very close to the proximal limit of the *Hmr* region defined by deficiencies (Figure 3). Hutter *et al.* (1990) suggested that this duplication does not carry Hmr^+, but the sole evidence was the absence of effects in *D. mauritiana* hybrid females at low temperature. In contrast to the suppression by $Dp(1;2)v^{+75d}$ described above, we found that $Dp(1;2)v^{+63i}$ did not suppress rescue of hybrid males by *Lhr* (Table 7D). It also had little effect on male rescue by Hmr^1, even at 25°, where nonduplication males were only 12% viable (relative to their nonduplication sisters; Table 7E). We also conclude that $Dp(1;2)v^{+63i}$ does not carry Hmr^+.

Effects of an Hmr^+ duplication in male hybrids: The results described above show that an extra copy of Hmr^+ reduces the viability of X_{mel}/X_{sib} *D. simulans* and *D. mauritiana* hybrid females, but does not cause unconditional lethality. Yet X_{mel}/Y_{sib} hybrid males, which have an equivalent Hmr^+ dosage, are invariably lethal. Does this discrepancy reflect a sex-specific effect of Hmr^+, or the fact that hybrid males are hemizygous for all X_{mel} genes, including Hmr^+? One way to address this question is to measure the effect of the Hmr^+ duplication on X_{sib}/Y_{mel} hybrids derived from compound-*X D. melanogaster* mothers. Assuming that the duplication is fully dosage compensated, these males will have the same dosage of Hmr^+ as X_{mel} hybrid males. In Table 8 we have compared the viability of X_{sib}/Y_{mel}; $Dp(1;2)v^{+75d}$, $Hmr^+/2_{sib}$ hybrids with their nonduplication-carrying brothers (diagrammed in Figure 1E).

Control crosses with *D. melanogaster* showed that nonhybrid males heterozygous for $Dp(1;2)v^{+75d}$ have reduced viability; this result was not unexpected considering the large size of the duplication. X_{sim}/Y_{mel}; $Dp(1;2)v^{+75d}/2_{sim}$ hybrid males showed little reduction in viability at 18° and 25° but were essentially lethal at 29°. X_{sec}/Y_{mel}; $Dp(1;2)v^{+75d}/2_{sec}$ males were completely lethal at both 18° and 25°; scoring of dead animals showed that most of the lethality must have occurred before the pharate adult stage (Table 8, footnotes *d* and *e*). Attempts to make hybrids with a *D. mauritiana v* stock failed to produce any progeny. The results with *D. simulans* hybrids in Tables 7 and 8 show that Hmr^+ reduces the viability of both male and female hybrids, but also that unconditional lethality requires hemizygosity of X_{mel}. While a male-like dosage of Hmr^+ appears to be sufficient to kill both male and female *D. sechellia* hybrids, other results suggest that additional X_{mel} genes also influence viability (see *Dose dependence of* Hmr^+ in discussion).

Dominant effects of Hmr^+ in hybrids from *D. simulans*

TABLE 7

An *Hmr*$^{+}$ duplication reduces hybrid female viability and interacts with *Lhr*

Female parent (all *D. mel.*)	Male parent	Hybrid female progeny (male progeny in parentheses)[a] 18° Relative viability $Dp/2_{sib}$ (%)	18° No. $+/2_{sib}$ for reference	25° Relative viability $Dp/2_{sib}$ (%)	25° No. $+/2_{sib}$ for reference	29° Relative viability $Dp/2_{sib}$ (%)	29° No. $+/2_{sib}$ for reference
(A) *Df(1)HC133; Dp(1;2)v*$^{+75d}$/+	*D. mel.* Oregon-R (control)	62.8 (103.3)[b]	242	102.2 (80.6)[b]	369	117.3 (74.8)[b]	139
	D. mau. Iso 197	73.9	69	96.0	99	105.6	125
	D. mau. C164.1	83.1	172	88.8	322	80.0	275
	D. sim. v	89.0	246	100.0	204	5.5	110
	D. sim. C167.4	82.7	75	90.1	161		n.d.
	D. sim. Tsimbazaza	105.2	115	63.3	496	0.6	169
	D. sim. Lhr	89.7 (31.1)[b]	165	88.5 (15.9)[b]	78	89.8 (0)[b]	118
	D. sech. w	107.2	263	12.5	287	1.4	138
	D. sech. v	119.0	279	1.5	399		n.d.
(B) *ras v; Dp(1;2)v*$^{+75d}$/+	*D. mel.* Oregon-R (control)		n.d.	146.8 (97.2)	156 (176)	76.5 (50.0)	68 (68)
	D. mau. Iso 197	103.0	66	21.4	28	5.7	53
	D. mau. C164.1	53.6	151	55.6	459	12.7	316
	D. sim. v	140.8	98	30.4	523		n.d.
	D. sim. Tsimbazaza	81.0	121	21.8	110		n.d.
	D. sim. Lhr	113.9 (4.4)	237 (204)	84.8 (6.7)	269 (225)	89.8 (0)	401 (108)
	D. sech. w	0.24	412	0	18		n.d.
	D. sech. v	0	142	0	194		n.d.
(C) *Dp(1;2)v*$^{+75d}$/+	*D. mau.* C164.1	52.6	173	11.4	201		n.d.
	D. sim. v	54.9	173	0	176		n.d.
	D. sim. C167.4	42.0	143	5.7	176		n.d.
	D. sim. Tsimbazaza	178.8	104	4.8	126		n.d.
	D. sim. Lhr	106.8 (3.1)	118 (97)	119.6 (0)	46 (61)		n.d.
(D) *Dp(1;2)v*$^{+63i}$/+	*D. sim. y; Lhr*		n.d.	82.7 (85.3)	335 (312)		n.d.
(E) *Hmr*^{1}; *Dp(1;2)v*$^{+63i}$/+	*D. mau.* C164.1	128.9 (56.6)	152 (99)	93.0 (75.0)	200 (24)		n.d.

Full genotypes of females crossed to males in column 2: (A) *Df(1)HC133, Hmr*$^{-}$*; Dp(1;2)v*$^{+75d}$*/CyO*; (B) *ras v; Dp(1;2)v*$^{+75d}$*/CyO*; (C) *Dp(1;2)v*$^{+75d}$*/CyO*; (D) *Dp(1;2)v*$^{+63i}$*/CyO*; (E) *y Hmr*^{1} *v*; *Dp(1;2)v*$^{+63i}$*/CyO*. n.d., not determined.

[a]Excluding crosses with Oregon-R or *D. simulans Lhr* males, a small number of male progeny were obtained in some crosses and are not shown. These were presumed to be exceptional males; all displayed the expected *X*-linked markers if present in the cross.

[b]Calculated as number of *Df(1)HC133/Y; Dp(1;2)v*$^{+75d}$/+ males relative to *Df(1)HC133/+; Dp(1;2)v*$^{+75d}$/+ female siblings.

mothers: The reciprocal cross of *D. simulans* females to *D. melanogaster* males produces viable sons but poorly viable daughters. The lethality of these female hybrids is embryonic and can be rescued by the *D. melanogaster* mutation *Zhr* and the *D. simulans* mutation *mhr*, but not by *Hmr*^{1}. These rescued hybrids are also sensitive to pupal and posteclosion lethality at 23°, but not at 18° (Sawamura *et al.* 1993a,c). Because this late lethality seemed similar to the *Hmr*-dependent lethality of female hybrids from *D. melanogaster* mothers that we have described above, we decided to investigate further the potential role of *Hmr* in hybrids derived from *D. simulans* mothers.

The viability of female hybrids from *D. simulans* mothers is highly dependent on genetic background variation (Sawamura and Yamamoto 1993; Sawamura *et al.* 1993a; Davis *et al.* 1996; Orr 1996), which complicates the effort to determine whether *Hmr* might influence pupal but not embryonic lethality in this cross. We therefore performed two different schemes of parallel crosses to *mhr* females using *Hmr*^{1} and *Hmr*$^{+}$ sibling brothers (Table 9; diagrammed in Figure 1F). The first scheme

TABLE 8

An Hmr^+ duplication reduces the viability of X_{sib}/Y_{mel} hybrid sons of compound-X_{mel} mothers

Male parent	Temp.	X_{sib}/Y_{mel} hybrid male progeny: No. *Dp* $Hmr^+/2_{sib}$ (% relative viability)	X_{sib}/Y_{mel} hybrid male progeny: No. $+/2_{sib}$ for reference
D. mel. y v f (control)	18°	44 (53.7)	82
	25°	85 (64.4)	132
	29°	57 (81.4)	70
D. sim. v	18°	54 (37.8)[a,b]	143[a,b]
	25°	58 (43.3)	134
	29°	2 (1.1)[a,c]	188[a,c]
D. sech. v	18°	0[a,d]	99[a,d]
	25°	0[a,e]	124[a,e]

Full genotype of crosses: *C(1)M4/Y; Dp(1;2)v*$^{+75d}$, Hmr^+ $v^+/+$ females crossed to males indicated in column 1.
[a] Dead males were scored in these crosses; duplication and nonduplication genotypes could not be distinguished.
[b] 14 dead eclosed, 40 dead pharate.
[c] 7 dead eclosed, 88 dead pharate.
[d] 0 dead eclosed, 13 dead pharate.
[e] 1 dead eclosed, 3 dead pharate.

utilized the close linkage of *Hmr* and *v* (<2 cM; Hutter *et al.* 1990) to distinguish Hmr^1 and Hmr^+ males (Table 9A). When postembryonic cultures were grown at 18°, females derived from Hmr^+ and Hmr^1 fathers were 44 and 32% viable, respectively, relative to their X_{sim}/Y_{mel} brothers. At 25°, hybrid daughters from Hmr^1 fathers had similar viability (29%), but those from Hmr^+ fathers were essentially lethal (<2% viability). When dead pharate and eclosed adults (many of which were necrotic) are included, the proportion of females in the latter cross rose to 18%, indicating that much of the lethality was postpupal stage (Table 9A, footnotes *c* and *d*).

TABLE 9

Hmr^1 suppresses postembryonic lethality in *mhr*-rescued hybrid females

Crossing scheme	Temp.[a]	Male parent (deduced *Hmr* genotype)	Hybrid progeny: No. of females (% relative viability)	Hybrid progeny: No. of X_{sim}/Y_{mel} brothers for reference
A	18°	v^+ (Hmr^+)	37 (43.5)	85
		v (Hmr^1)	25 (31.6)	79
	25°	v^+ (Hmr^+)	2 (1.5)[b,c]	130[b,c]
		v (Hmr^1)	29 (28.5)[b,d]	102[b,d]
B	18°	w^+ (Hmr^+)	29 (24.6)	118
		w (Hmr^1)	31 (12.3)	253
	25°	w^+ (Hmr^+)	0	442
		w (Hmr^1)	8 (3.0)	263
	25°	w^+ (Hmr^+)	1 (0.30)[b,e]	329[b,e]
		w (Hmr^1)	27 (9.5)[b,f]	284[b,f]
	29°	w^+ (Hmr^+)	0	168
		w (Hmr^1)	2 (0.8)	251

Crossing schemes: (A) Hmr^1 *v/FM6* females were crossed to Nguruman-4 males; nonbalancer virgin daughters (Hmr^1 *v*/++) were crossed again to Nguruman-4 males. F_2 v^+ (presumed Hmr^+) and *v* (presumed Hmr^1) sons were crossed separately to *y w f; mhr D. simulans* females. (B) *w* Hmr^1 *v/FM6* females were crossed to *w P{w*$^{+mC}$ *= EP}EP1093* males; nonbalancer virgin daughters ($Hmr^1/P\{w^+\}$) were crossed to *y w* males. F_2 w^+ (presumed Hmr^+) and *w* (presumed Hmr^1) sons were crossed separately to *y w f; mhr D. simulans* females.
[a] Crosses were kept at 25° for 24 hr after removing the parents and then shifted to the indicated temperature.
[b] Dead hybrid progeny were scored in these crosses.
[c] 15 dead eclosed, 9 dead pharate females; 1 dead eclosed, 14 dead pharate males.
[d] 0 dead eclosed, 2 dead pharate females; 0 dead eclosed, 5 dead pharate males.
[e] 14 dead eclosed, 26 dead pharate females; 4 dead eclosed, 2 dead pharate males.
[f] 2 dead eclosed, 6 dead pharate females; 3 dead eclosed, 45 dead pharate males.

TABLE 10

***Hmr*⁻ deficiencies suppress postembryonic lethality in *mhr*-rescued hybrid females**

		Hybrid progeny				
			Dp Hmr$^+$/2_{sib}	+/2_{sib}	*Dp Hmr*$^+$/2_{sib} and +/2_{sib} [a]	
Male parent	Temp.[b]	Sex of progeny	No. alive (% relative viability)	No. alive for reference	No. dead, eclosed	No. dead, pharate
(A) *Df(1)N110*	25°	F (*Df*/X_{sim})	0	19	n.d.[c]	n.d.
		M (X_{sim}/Y_{mel})	0	25	n.d.	n.d.
	25°	F (*Df*/X_{sim})	0	12	10	9
		M (X_{sim}/Y_{mel})	1 (5.3)	19	1	11
(B) *Df(1)HC133*	25°	F (*Df*/X_{sim})	19 (25.7)	74	41	24
		M (X_{sim}/Y_{mel})	13 (22.0)	59	17	37
	29°	F (*Df*/X_{sim})	1 (3.8)	26	2	24
		M (X_{sim}/Y_{mel})	0	50	1	18

Full genotypes of males crossed to *y w f; mhr D. simulans* females: (A) *Df(1)N110; Dp(1;2)v*$^{+75d}$*/CyO* males; (B) *Df(1)HC133; Dp(1;2)v*$^{+75d}$*/CyO* males.

[a] Duplication and nonduplication genotypes could not be distinguished among dead animals.

[b] Crosses were kept at 25° for 24 hr after removing the parents and then shifted to the indicated temperature.

[c] Not determined.

A second crossing scheme (Table 9B) used a homozygous viable w^+ *P* element inserted in 9E to distinguish *Hmr*$^+$ from *Hmr*^{1} males. Female viability was lower than in scheme A and there was also substantial variation among different cultures of identical genotypes. For example, female viability in crosses from *Hmr*^{1} fathers ranged from 3 to 10% at 25°. As in scheme A, however, temperature-dependent late lethality of female hybrids was observed and only in female progeny of *Hmr*$^+$ sons. At 25° these hybrids were essentially lethal with ~12% of the females dying as pharate adults or posteclosion (Table 9B, footnotes *e* and *f*).

Effects of Hmr⁻ deletions: We also assayed two *Hmr*⁻ deficiencies for their ability to suppress late lethality in daughters of *mhr* mothers. X_{sim}/*Df(1)Hmr*⁻; 2_{sim}/2_{mel} females were compared with their X_{sim}/*Df(1)Hmr*⁻; 2_{sim}/*Dp(1;2)v*$^{+75d}$, *Hmr*$^+$ siblings (Table 10, diagrammed in Figure 1G). At 25°, X_{sim}/*Df(1)N110;* 2_{sim}/*Dp(1;2)v*$^{+75d}$ females were lethal, while X_{sim}/*Df(1)HC133;* 2_{sim}/*Dp(1;2)v*$^{+75d}$ females were nearly lethal at 29° and had reduced viability at 25° compared to their nonduplication siblings. Although dead animals were not genotyped for whether or not they carried the duplication, the number of dead females in each cross was in approximate correspondence to the expected number of missing X_{sim}/*Df(1)Hmr*⁻; 2_{sim}/*Dp(1;2)v*$^{+75d}$ females. In combination with the data in Table 9, the results of Table 10 suggest that *Hmr*^{1} and *Hmr*⁻ deficiencies are dominant suppressors of late lethality in female hybrids of *D. simulans* mothers and *D. melanogaster* fathers.

X_{sim}/Y_{mel}; 2_{sim}/*Dp(1;2)v*$^{+75d}$ sons of *mhr* mothers also were less viable than their nonduplication brothers at 25°, with the number of dead animals again suggesting that the missing duplication-carrying males were dying after the pupal stage (Table 10). Together with the results of Table 8, these data suggest that X_{sib} males are sensitive to *Hmr*$^+$ dosage, regardless of the direction of crossing. Deleterious effects of *Dp(1;2)v*$^{+75d}$ on hybrid male viability have also been observed independently by H. A. Orr and S. Irving (personal communication).

DISCUSSION

Temperature-sensitive pupal lethality in hybrids: Hybrid X_{mel} sons and X_{mel}/X_{mel} daughters of *D. melanogaster* mothers die as larvae or pseudopupae (Sturtevant 1920, 1929; Hutter *et al.* 1990), and much effort has been made to understand the genetic and developmental basis of this lethality. Less is known about the temperature-sensitive lethality of X_{mel}/X_{sib} females, first noted by Sturtevant (1929) in *D. melanogaster*/*D. simulans* hybrids. After Sawamura *et al.* (1993b), we refer to the lethality of X_{mel} male and X_{mel}/X_{mel} female hybrids as larval lethality and that of X_{mel}/X_{sib} females as pupal lethality (although many females in fact survive until eclosion). Investigating this female lethality can potentially overcome the limitations associated with assaying *X*-linked alleles in hemizygous males.

We quantified female viability in hybrids between *D. melanogaster* and its three sibling species at three temperatures (Table 1). *D. mauritiana* hybrids had the highest viability, followed by *D. simulans* and finally by *D. sechellia* hybrids, which were not fully viable even at 18°. The genetic basis of these species-specific differences in hybrid viability is unknown. Although F_1 hybrid males of the sibling species are sterile, interspecific heterozygous introgressions created by repeated backcrossing of hybrid females are often male fertile (Hollocher and

Wu 1996; True *et al.* 1996). Such introgressions could be used to map the genetic differences responsible for this variation in hybrid viability.

Even with the small number of strains sampled, there was substantial variation in hybrid lethality among different stocks of each species, as observed in previous studies of *D. melanogaster*/*D. simulans* hybrids (Watanabe *et al.* 1977; Lee 1978). While it appears from Table 1 that variability within *D. mauritiana* and *D. sechellia* is somewhat less than that observed among *D. simulans* stocks, this result may merely reflect the fact that *D. melanogaster*/*D. simulans* viability falls in the middle of the phenotypic range detectable by our assay. Alternatively, the insular species *D. mauritiana* and *D. sechellia* may in fact harbor less variation for hybrid lethality than the cosmopolitan *D. simulans*.

The substantial genetic background variation observed can complicate the analysis of hybrid viability to the point where meaningful conclusions about particular genotypes cannot be easily reached from any single cross. On a more positive note, however, understanding the evolutionary forces responsible for the origin and maintenance of this type of variation is relevant to understanding the process of speciation. Several other studies have identified intraspecific variation for traits that cause hybrid breakdown and reproductive isolation. Wade *et al.* (1997) discovered substantial population-level variation in beetles for inviability and morphological defects of interspecific hybrids, while Takano (1998) has found that loss of macrochaetes in *D. melanogaster*/*D. simulans* hybrids is highly dependent on variation within *D. simulans* stocks.

The wild-type *Hmr*$^+$ causes hybrid lethality: One important question raised by the discovery of rescue alleles such as *Lhr* and *Hmr*^{1} is whether the wild-type allele of the rescue gene causes hybrid lethality. Addressing this requires the ability to manipulate the wild-type gene in hybrids. We have done so for *Hmr* and found that *Hmr*$^-$ deficiencies and an *Hmr*$^+$ duplication have reciprocal effects on hybrid viability. The qualitatively similar activities of *Hmr*^{1} and *Hmr*$^-$ deficiencies further suggest that *Hmr*^{1} rescues hybrids by reducing the level of *Hmr*$^+$, as proposed by Hutter *et al.* (1990). In other words, hybrid rescue does not require a mutation that switches Hmr^+_{mel} to an Hmr^+_{sib}-like allele.

Two previous studies failed to detect any effect on hybrid female viability of deletions that we have defined here as being *Hmr*$^-$. This discrepancy probably reflects the fact that viability was assayed under conditions less stringent than used in this study: Hutter *et al.* (1990) looked only in *D. mauritiana* hybrids while Coyne *et al.* (1998) examined *D. simulans* hybrids at 24°.

On the basis of his pioneering analysis of *D. melanogaster*/*D. simulans* hybrids, Sturtevant (1929) proposed that hybrid lethality is caused by either the presence of the *D. simulans Y* chromosome or the absence of the *D. simulans X* chromosome. The first hypothesis was ruled out by Yamamoto (1992), who used a *D. simulans C(1;Y)* chromosome to generate X_{mel}/O hybrid males and found that they remain inviable. So is X_{sim} (and more generally X_{sib}) required for hybrid viability? Although not explicitly stated by Sturtevant, his second hypothesis, that hybrids require X_{sib}, implies that hybrid lethality results from a gene (or genes) on X_{mel} that fails to function in hybrids or, alternatively, that X_{sib} provides a function that counteracts a deleterious effect of X_{mel} (Sawamura *et al.* 1993a).

These predictions are in contrast to our suggestion that the activity of *Hmr*$^+$ causes hybrid lethality and that reducing its function is sufficient to rescue hybrids. The most direct demonstration of this point is that simply removing one copy of *Hmr*$^+$ partially rescued exceptional X_{mel}/X_{mel} female hybrids (Table 6)—X_{sib} is clearly not absolutely required for hybrid viability. The rescue of hybrid males by *Lhr*, *Hmr*^{1}, and *In(1)AB* first suggested that X_{sib} is not required for hybrid viability, but was subject to the reservation that the precise nature of these alleles is unknown. Although it remains possible that X_{sib} may have some positive effect on hybrid viability (see *Dose dependence of Hmr* below), in accordance with Sturtevant's second hypothesis, our results strongly support a third, alternative hypothesis that hybrid lethality results from the presence of X_{mel} and, more specifically, *Hmr*$^+$.

Are larval and pupal lethality caused by the same mechanism? If we wish to use the temperature-sensitive pupal lethality of hybrid females as a new assay for investigating *Hmr*, it is important to consider whether this lethality is caused by the same mechanism that causes larval lethality. Orr *et al.* (1997) have proposed that hybrid larval lethality may be due to a mitotic defect. The rough eyes, malformed wings, and necrotic tissue found in hybrid female escapers are consistent with this hypothesis, as similar phenotypes also occur in certain hypomorphic cell cycle alleles (White-Cooper *et al.* 1996; Secombe *et al.* 1998). However, this syndrome of defects is also reminiscent of phenotypes associated with mutations in pleiotropic signaling molecules such as Notch (Artavanis-Tsakonas *et al.* 1999) and epidermal growth factor (Freeman 1998). These possibilities are not mutually exclusive and can be addressed by detailed examination of rescued and unrescued female hybrids. The temperature dependence of unrescued female hybrids and the use of temperature shifts at different developmental stages will be particularly useful for identifying the most direct consequences of *Hmr*$^+$ activity in hybrids.

The strongest available evidence that larval and pupal lethality are caused by the same mechanism is that both are suppressed by the rescue mutations *Hmr*^{1}, *In(1)AB*, and *Lhr*. Using similar logic, Sawamura *et al.* (1993a,c) have convincingly argued that the embryonic lethality of hybrid daughters of sibling species mothers and *D. melanogaster* fathers is mechanistically unrelated to larval

lethality because it is rescued by a distinct set of mutations. The relative degree of lethality with the different sibling species is a second common character. As noted above for pupal lethality, larval lethality appears to be strongest with *D. sechellia*, intermediate with *D. simulans*, and weakest with *D. mauritiana*, with strength of lethality measured by its inverse correlation to strength of rescue of exceptional females by Hmr^- deletions (Table 6) and of hybrid males by Hmr^1 (Hutter and Ashburner 1987). This ranking of the sibling species also holds for the effects of the Hmr^+ duplication on both female (Table 7) and X_{sib} male (Table 8) hybrids.

However, the patterns of conditional variability for larval and pupal lethality are not entirely equivalent. First, larval lethality was more severe with the *D. simulans* $v\ f^2$ stock than with the ry^{i83} stock, while the opposite was true for pupal lethality (Tables 1 and 6; note that larval lethality here refers to that found in X_{mel}/X_{mel} exceptional females). Second, pupal lethality is clearly temperature sensitive, with little or no lethality detected at 18° and increasing lethality at higher temperatures. Larval lethality, however, appears to be temperature insensitive (below 29°) or even somewhat cold sensitive. Rescue was generally equivalent or lower at 18° than at 25° for *In(1)AB* males (Table 3; see also Hutter *et al.* 1990) and for X_{mel}, Hmr^-/X_{mel} females (Table 6). [The temperature profile of Hmr^1 is more complicated. Rescue of male lethality is most effective at 18° (Hutter and Ashburner 1987; our unpublished data). If larval lethality is not itself a temperature-sensitive trait, as suggested by our results with deficiencies in females, then the preferential rescue at cold temperatures by Hmr^1 may mean that it is a cold-sensitive loss-of-function allele.]

Unknown gene(s) on the *X* chromosome are probably responsible for these differences between larval and pupal lethality, since the autosomal component is identical in all classes of hybrids, but whether X_{mel}, X_{sib}, or both are involved is unknown. Our interpretation of the possible role of X_{sib} differs from Sawamura *et al.* (1993b), who suggested that temperature-sensitive pupal lethality is caused by X_{sib} and is distinct from larval lethality, which they associated with hybrids that do not carry X_{sib}. We propose instead that the primary cause of both larval and pupal hybrid lethality is X_{mel} and, more specifically, Hmr^+, with X_{sib} possibly functioning as a modifier of hybrid lethality. Results presented in Table 6 showed that X_{mel}, Hmr^-/X_{mel}, Hmr^+ hybrids are, in some crosses, more viable than X_{mel}, Hmr^+/X_{sib} siblings, suggesting that X_{sib} may have a deleterious effect on hybrids. Such an effect would have to involve an interaction with X_{mel}, since X_{sib}/Y_{mel} hybrid males are viable at all temperatures (Tables 6 and 8).

Hmr^+ causes lethality in both directions of crossing: Hybrid daughters of sibling mothers that are rescued from embryonic lethality die as pupae or young adults if cultured at high temperature (Sawamura *et al.* 1993a,c). We have shown that Hmr^1 and Hmr^- deficiencies suppress this lethality (Tables 9 and 10), just as Hmr^1 suppresses larval lethality of X_{mel} hybrid sons from sibling species mothers (Hutter *et al.* 1990; Sawamura *et al.* 1993a,c). Likewise, we also found that $Dp(1;2)v^{+75d}$ causes pupal lethality to both male and female hybrids in both directions of crossing (Tables 7, 8, and 10). Our results do not contradict the hypothesis of Sawamura *et al.* (1993b) that embryonic and larval lethality have distinct causes, because the *Hmr*-dependent effects we observed were clearly postembryonic. Particular care must be taken when attempting to distinguish between these systems, however, because the penetrance of embryonic lethality in hybrids from *D. simulans* mothers appears to be at least as variable as we have found for *Hmr*-dependent lethality (Sawamura and Yamamoto 1993; Sawamura *et al.* 1993a; Davis *et al.* 1996; Orr 1996).

Has *Hmr* diverged in the *melanogaster* complex? It is important to emphasize that none of the available data prove that the different effects of X_{mel} and X_{sib} in hybrids are caused by species-specific differences at the *Hmr* locus itself. An alternative possibility, first raised by Hutter *et al.* (1990), is that *Hmr* is identical in the *melanogaster* complex species, with hybrids being sensitive to Hmr^+ dosage due to allelic differences at other *X*-linked gene(s). Without the ability to manipulate the dosage of Hmr_{sib} alleles in hybrids, we see no way to distinguish between these hypotheses by genetic means.

Modeling hybrid viability: Our conclusions regarding the relationship between Hmr^+ dosage and hybrid viability rest on several assumptions. First, we assume that the effects of Hmr^1, $Dp(1;2)v^{+75d}$, and the deficiencies defined as Hmr^- (Figure 3) reflect the activity of a single gene in region 9D. Although we will discuss the rescue activity of the *In(1)AB* chromosome in comparison to Hmr^1, there is no evidence that they are in fact allelic. We do know that neither breakpoint of *In(1)AB* (9E7-8; 13E1-2) is itself likely to cause hybrid rescue. The distal breakpoint of *In(1)AB* is very close to the genes *sesB*/*Ant2* (Hutter and Karch 1994), which have no apparent effect on hybrid viability (Zhang *et al.* 1999). We can also rule out a role for the proximal breakpoint because it is absent in $Dp(1;1)AB^LAC2^R$, which retains male rescue (J. Roote, unpublished observations), and present in $Df(1)AC2^LAB^R$, which does not retain female rescue (Table 3).

A second caveat is that $Dp(1;2)v^{+75d}$ is the only Hmr^+ duplication available. Our model assumes that it contains full Hmr^+ activity and is fully dosage compensated in hybrid males.

Our final assumption, that the viability differences between male and female hybrids are due to their different composition of sex chromosomes, and not their sexual phenotype *per se*, is supported by several findings. Sturtevant (1920) first noted that $X_{mel}/X_{mel}/Y_{sim}$ hybrid females are lethal, and this was confirmed with

compound-X_{mel} chromosomes (Sturtevant 1929; Biddle 1932; Kerkis 1933a). More direct evidence is that the lethal phase of $C(1)_{mel}$ female hybrids is similar to that of hybrid males, and both sexes show comparable levels of rescue when homozygous or hemizygous for Hmr^1 (Hutter *et al.* 1990). *Lhr* also rescues both male and $C(1)_{mel}$ female hybrids (Takamura and Watanabe 1980). Additional evidence is that increasing Hmr^+ dosage is deleterious to both sexes. $Dp(1;2)v^{+75d}$ was lethal to both X_{mel}/X_{sib} female and X_{sib} male hybrids, at 25° or 29° with *D. simulans*, and at 18° with *D. sechellia* (Tables 7 and 8).

It remains possible, however, that sexual phenotype may have some influence on hybrid viability. Orr (1999) has recently suggested that *Lhr*-dependent rescue of hybrid males is enhanced if they are feminized by constitutive expression of the sex-determining gene *transformer* (*tra*). A similar effect of sexual phenotype might explain the puzzling fact that *In(1)AB* appeared to rescue both exceptional female and regular male *D. mauritiana* hybrids to a comparable extent (Table 6). Further experiments are necessary to evaluate this question, as neither our experiments nor Orr's excluded the possibility that the balancer chromosomes used might influence hybrid rescue.

Dose dependence of Hmr^+: In Table 11 we have summarized the range of viabilities observed in different genotypes of *D. melanogaster*/*D. simulans* hybrids. The ordering of genotypes (other than those involving *Lhr*) can also generally be applied to hybrids with *D. mauritiana* and *D. sechellia*, provided that one shifts the viability designations upward and downward, respectively. This observation suggests that the same general mechanism of lethality exists in all three species hybrids, with uncharacterized species-specific modifiers affecting the penetrance of lethality.

Complete lethality of hybrids requires two conditions: two doses of *D. melanogaster* Hmr^+ and two "doses" of X_{mel}. Full viability, in turn, requires either a strong reduction in or removal of one of these conditions. The ranking in Table 11 of intermediate cases such as X_{mel}/X_{sim} females is somewhat problematic because their viability tended to be highly variable, depending on genetic background and temperature.

It is clear, however, that two doses of Hmr^+ are not sufficient to account fully for the unconditional lethality of X_{mel}/Y_{sib} and $X_{mel}/X_{mel}/Y_{sib}$ hybrids because hybrids carrying $Dp(1;2)v^{+75d}$ were not invariably lethal (Tables 7 and 8) and exceptional females heterozygous for Hmr^- deficiencies were not fully rescued (Table 6), even at low temperatures where pupal lethality is not observed. The "remaining" lethality must result from the activity of additional dosage-sensitive deleterious gene(s) on X_{mel}, the loss of activity of essential X_{mel} genes (and thus the absence of X_{sib}), or both. A positive effect on hybrid viability of the wild-type Hmr_{sib} is one possible explanation of the hypothetical X_{sib} effect. Whatever the mechanism of these additional hypothetical *X*-linked alleles may be, their effects on hybrid viability are difficult to predict, other than to suggest that they are likely to be synergistic with Hmr^+. Since at present we can detect their phenotypic effects only in the context of manipulating the entire *X*, it seems premature to make any detailed mechanistic speculations.

Recall that while $Dp(1;2)v^{+75d}$-induced lethality was fully penetrant in *D. sechellia* hybrids, no rescue was observed in the exceptional female assay (see results). These data suggest that while two doses of Hmr^+ are sufficient to cause complete lethality, even in the absence of X_{mel}, the effects of the additional X_{mel} genes proposed above can also be observed in *D. sechellia* hybrids.

Hmr^1 retains ≥50% of the activity of Hmr^+: The direct comparison of Hmr^1 and deficiencies for dominant rescue in X_{mel}/X_{sec} hybrid females (Table 5) suggests that Hmr^1 is a hypomorphic mutation, as proposed by Hutter *et al.* (1990). A more stringent test is to measure rescue in hybrids homozygous or hemizygous for X_{mel}. The relevant genotypes to compare are Hmr^1 hybrid males and X_{mel}/X_{mel} exceptional females heterozygous for Hmr^- deficiencies [note that Hmr^1 rescues exceptional females when homozygous, but not when heterozygous (Hutter *et al.* 1990; see also *Dominant rescue of exceptional female hybrids* in results)]. Several factors complicate this comparison. First, exceptional female hybrids vary greatly in viability (Table 6). Likewise, Hmr^1 was originally reported to fully rescue *D. mauritiana* and *D. simulans* hybrid males at 18° (Hutter and Ashburner 1987), but subsequent experiments have shown lower levels of rescue, especially with *D. simulans* (Tables 2 and 3; D. A. Barbash and J. Roote, unpublished observations; see also Table 6 of Hutter *et al.* 1990; Orr *et al.* 1997). A second complication is that Hmr^1 male rescue is strongest at 18°, while rescue of exceptional females is strongest at 25°. Considering all the available data, it nevertheless seems reasonable to generalize that Hmr^1 males are not more viable than X_{mel}, Hmr^-/X_{mel}, Hmr^+ hybrid females. In other words, Hmr^1 appears to retain ≥50% of the function of Hmr^+.

Using similar arguments, *In(1)AB* is a stronger loss-of-function allele than Hmr^1. *In(1)AB* rescues male hybrids better than does Hmr^1 (Table 3; Hutter *et al.* 1990) and is equivalent to deficiencies in high-temperature female rescue (Table 5). However, *In(1)AB* strongly rescued exceptional female hybrids with *D. mauritiana*, but only weakly with *D. simulans* (Table 6), suggesting that *In(1)AB* may not be amorphic. We conclude that *In(1)AB* has somewhere between 0 and 50% the activity of Hmr^+.

***Hmr* and *Lhr* interact:** The Dobzhansky/Muller model of hybrid lethality and sterility states that hybrid incompatibilities must be caused by a minimum of two interacting genes, one from each species. The second chromosome *D. simulans Lhr* allele rescues hybrid males and has been proposed to correspond to a gene that inter-

TABLE 11

Summary of viabilities of hybrid progeny from *D. melanogaster* females and *D. simulans* males

Genotype	Dosage of *D. mel. Hmr*$^{+}$ [a]	Dosage of other X_{mel} genes [a]	Viability [b]	References
X_{mel}, *Hmr*$^{-}$/X_{sim} females	0	1	High	Tables 3–5
X_{mel}, *In(1)AB*/X_{sim} females	<0.5	1		Tables 3–5
X_{mel}/X_{sim} ; +/*Lhr* females	1	1		Tables 1, 7
X_{sim} males	0	0		Sturtevant (1920); Tables 6, 8
X_{mel}, *Hmr*^{1}/X_{sim} females	≥0.5	1	High, <29°	Tables 2, 3, 5
X_{mel}; +/*Lhr* males	2	2	Intermediate[c]	Watanabe (1979); Tables 1, 7
X_{mel}, *In(1)AB* males	<1	2		Hutter *et al.* (1990); Table 3
X_{sim}; *Dp Hmr*$^{+}$ males	2	0		Tables 8, 10
X_{mel}/X_{sim} females	1	1		Sturtevant (1929), Kerkis (1933b), Watanabe *et al.* (1977), Lee (1978); Table 1
X_{mel}/X_{sim}; *Dp Hmr*$^{+}$ females	2	1	Low[c]	Table 7
X_{mel}, *Hmr*$^{-}$/X_{mel} females	1	2		Table 6
X_{mel}, *In(1)AB*/X_{mel} females	<1.5	2		Hutter *et al.* (1990); Table 6
X_{mel}, *Hmr*^{1} males	≥1	2		Hutter and Ashburner (1987); Tables 2, 3
X_{mel}, *Hmr*^{1}/X_{mel}, *Hmr*$^{+}$ females	≥1.5	2	Lethal	Hutter *et al.* (1990); see also *Dominant rescue of exceptional female hybrids* in results.
X_{mel}/X_{mel} females	2	2		Sturtevant (1920)
X_{mel} males	2	2		Sturtevant (1920)

[a] Dosage calculations assume that *X*-linked genes are fully dosage compensated in hybrid males. See *Hmr*^{1} *retains ≥50% of the activity of Hmr*$^{+}$ in discussion for the estimation of *Hmr*$^{+}$ dosage for the *Hmr*^{1} and *In(1)AB* rescue alleles.

[b] The order of genotypes listed within each viability class is not significant.

[c] Viabilities of genotypes in these classes were often highly variable. See references for details.

acts with *Hmr* to cause hybrid lethality (Hutter *et al.* 1990; Sawamura *et al.* 1993b). Our results provide the first experimental evidence in support of this hypothesis. We found that *Lhr* suppressed *Hmr*$^{+}$-dependent high-temperature female lethality (Table 1). Data in Table 7 also showed that *Hmr*$^{+}$ and *Lhr* have antagonistic effects on hybrid viability. *Lhr* suppressed the deleterious effect of *Dp(1;2)v*$^{+75d}$ on female hybrids, while *Dp(1;2)v*$^{+75d}$ suppressed the male rescue activity of *Lhr*. If *Lhr* is a loss-of-function allele of the sibling *Lhr*$^{+}$ locus, then these data suggest that the *D. melanogaster Hmr*$^{+}$ and the sibling *Lhr*$^{+}$ loci interact to cause lethality in hybrids. This hypothesis is consistent with data from "partial" hybrids obtained by mating triploid *D. melanogaster* females to heavily irradiated *D. simulans* males (Pontecorvo 1943). Pontecorvo obtained several X_{mel}/X_{mel}; 2_{mel}/2_{mel}; 3_{mel}/3_{sim} hybrids; in terms of the *Hmr-Lhr* model their viability would be due to the absence of *D. simulans Lhr*$^{+}$. Sawamura (2000) has noted that an analogous third chromosome locus may exist, as several X_{mel}/X_{mel}; 2_{mel}/2_{sim}; 3_{mel}/3_{mel} hybrids were also obtained. Although Pontecorvo recovered only X_{mel} males with the autosomal genotype 2_{mel}/2_{sim}; 3_{mel}/3_{mel}, and not with 2_{mel}/2_{mel}; 3_{mel}/3_{sim}, a small number of both autosomal classes of X_{mel} males were obtained by Coyne (1983) from compound-chromosome rather than triploid *D. melanogaster* females.

These data from partial hybrids suggest that hybrid lethality may result from an interaction involving (at least) three loci, and furthermore, that removing any one of the three causal alleles is sufficient to suppress lethality. A study using interspecific introgression between *D. buzzatii* and *D. koepferae* has also found evidence for a system of hybrid lethality involving three loci (Carvajal *et al.* 1996). Although Pontecorvo (1943) invoked a total of nine alleles to explain the lethality of *D. melanogaster*/*D. simulans* hybrids, the small number of partial hybrids obtained, as well as other potential complications, makes this conclusion somewhat uncertain (Coyne *et al.* 1998; Sawamura 2000).

How many genes cause hybrid lethality? We have discussed three lines of evidence that suggest that additional unknown gene(s) on both the *X* and third chromosomes contribute to larval and pupal hybrid lethality:

(1) the existence of distinct systems of variation that modify larval and pupal lethality; (2) the incomplete penetrance of lethality and rescue associated with an Hmr^+ duplication and Hmr^- deficiencies, respectively; and (3) data from experiments with partial hybrids. Two reports have recently surveyed the literature of hybrid genetics and concluded that hybrid inviability in *D. melanogaster* (and in other Drosophila as well) is likely to be caused by a relatively small number of genes (Hutter 1997; Coyne *et al.* 1998). Considering the available data, we do not disagree with this conclusion, but it is important to recognize that the evidence remains largely indirect.

The only systematic search for inviability genes in *D. melanogaster* hybrids is that of Coyne *et al.* (1998), who sampled approximately half of the genome using *D. melanogaster* deficiencies in female hybrids with *D. simulans.* Their study was designed to detect *D. simulans* genes that fail to function in female hybrids, that is to say loss-of-function *D. simulans* alleles that are normally complemented by the homologous *D. melanogaster* allele. Two deficiencies that reduced greatly the viability of hybrids made with several different *D. simulans* stocks as well as with *D. mauritiana* and/or *D. sechellia* were found. These lethal effects are opposite to the rescue we observed with Hmr^- deficiencies, but the magnitude of the viability differences relative to control siblings were comparable. Whether these two deficiencies are uncovering single loci with large effects on hybrid viability remains to be investigated.

The screen of Coyne *et al.* (1998) was unlikely to detect alleles like Hmr^+ that cause lethality because temperatures of 24° or lower were used, and in fact the Hmr^- deficiency *Df(1)v-L15* showed no viability difference compared to a reference balancer chromosome. A similar deficiency screen to look for suppressors of high-temperature female lethality will be needed to determine whether or not additional *Hmr*-like genes exist in *D. melanogaster*.

***Hmr* and Haldane's rule:** A widespread pattern of hybrid breakdown is described by Haldane's rule. Haldane (1922) observed that if one sex of hybrids suffers from sterility or inviability, it is most commonly the heterogametic sex (for simplicity we refer to this sex as being male, as in Drosophila, but it is also valid in taxa with *ZZ*/*ZW* sex chromosomes). Haldane's rule holds in many taxa including insects, mammals, and birds and therefore has been studied intensively, with the expectation that it will have general implications for understanding the genetics of reproductive isolation (reviewed in Coyne 1992; Wu and Davis 1993; Wu *et al.* 1996; Laurie 1997; Orr 1997).

Haldane's rule for hybrid lethality appears to be best explained by the *X:A* imbalance of hybrid males (Wu and Davis 1993; Hollocher and Wu 1996; True *et al.* 1996; Coyne *et al.* 1998). This model proposes that hybrid males will be inviable more often than hybrid females because they are genetically imbalanced: their *X* chromosome derives from one species but their autosomes are from both, while hybrid females have a balanced set of both *X* chromosomes and autosomes (Muller 1940). This model can be tested by constructing female hybrids carrying both *X* chromosomes from one of the parental species, with the expectation that these unbalanced females will be as unfit as male hybrids (Coyne 1985; Orr 1993a). This prediction holds for the *D. melanogaster* female/sibling male cross and is further supported by the fact that both unbalanced sexes are rescued by *Lhr*, Hmr^1, and *In(1)AB* (discussed above). The *X:A* imbalance model is falsified only if hybrid lethality is specific to the heterogametic sex because of its sexual phenotype rather than chromosomal constitution or because of deleterious *X-Y* or *Z-W* interactions.

A model dubbed the "dominance theory" has been developed to quantify the conditions under which *X:A* imbalances will lead to Haldane's rule (Orr 1993b; Turelli and Orr 1995). The dominance theory concludes that Haldane's rule will result when the deleterious contributions of *X*-linked alleles in females are, on average, less than one-half those in males; such alleles are defined as recessive.

The genetic properties of *Hmr* are consistent with both the *X:A* imbalance model and the dominance theory. We emphasize, however, that our data suggest that female viability is not due to the heterospecific *X* "preventing" or "masking" the deleterious effect of Hmr^+, as recessive hybrid lethals are often described (Muller 1942; Turelli and Orr 1995). Rather, we propose that Haldane's rule in *D. melanogaster* hybrids depends on the lower dosage of Hmr^+ in females *vs.* males and, more importantly, the nonlinear relationship between Hmr^+ dosage and hybrid fitness.

We also note that while the fitness effects of Hmr^+ can be described accurately as recessive at low temperatures, where Haldane's rule holds, Hmr^+ is a dominant lethal at high temperatures. The conditional nature of dominance properties with respect to genetic background and environmental variation is not unexpected in hybrids (Wu and Davis 1993). As a general and noncontingent description of Hmr^+ we suggest the term "dosage sensitive," as opposed to "additive," to avoid the implication that the fitness effects of Hmr^+ as a function of gene dosage are likely to be either linear or continuous. The dosage-sensitive nature of Hmr^+ is apparent in the developmental delay and morphological defects of $+/X_{sib}$ females that occur even when they are fully viable compared to Hmr^-/X_{sib} siblings. Likewise, we propose that the earlier larval lethal phase of hybrid males compared to the later pharate/posteclosion lethality of females also results from differential Hmr^+ dosage.

Like the results presented here, the hybrid lethality effects reported by Coyne *et al.* (1998) were also highly dependent on temperature. As these authors noted,

most other reports of hybrid lethals have not investigated whether the phenotypic effects observed might be similarly conditional. Therefore, while several studies have shown that hybrid lethals in Drosophila can act recessively under fixed conditions (Carvajal *et al.* 1996; Hollocher and Wu 1996; True *et al.* 1996), it remains uncertain whether phenotypic recessivity will be a general characteristic of hybrid lethals.

Implications of *Hmr*-like effects: Considering the limited data available from other species, the potential generality of conclusions drawn from the study of *Hmr* and *D. melanogaster* hybrids is unknown. But it is instructive to consider the implications if other examples of hybrid lethality are caused by similar alleles of large effect. Surveys of the literature on hybrid breakdown suggest that examples of Haldane's rule for inviability are infrequent (in male-heterogametic species), compared to examples of hybrid sterility (Wu and Davis 1993; Laurie 1997; Orr 1997; Turelli and Begun 1997; Presgraves and Orr 1998). This may be because sex-limited lethality requires the existence of *X*-linked hybrid lethality alleles with a viability threshold that occurs between the dosage of females and males. More common outcomes would be either both sexes lethal (viability threshold lower than female dosage or presence of strong autosomal alleles) or both sexes viable (no major effect lethal alleles). It seems remarkable that by simply adjusting culture temperature and varying Hmr^{+} dosage by twofold, both of these outcomes can be obtained in a hybridization that otherwise conforms to Haldane's rule.

We thank the Drosophila Species Center, the Bloomington and Umeå stock centers, and Jerry Coyne for fly stocks. We gratefully acknowledge Ben Yudkin for preliminary experiments done as a Part II Genetics student and Terri Morley and Glynnis Johnson for technical assistance. We thank Allen Orr for sharing unpublished data and Andrew Davis, Pierre Hutter, Allen Orr, Kyoichi Sawamura, and Michael Turelli for helpful comments on the manuscript. We were supported by a grant from the UK Medical Research Council to M.A., D. Gubb, and S. R. H. Russell and a National Science Foundation and Alfred P. Sloan Foundation Fellowship to D.A.B.

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Communicating editor: C.-I Wu