A Genetic Basis for the Inviability of Hybrids Between Sibling Species of *Drosophila*

Pierre Hutter,* John Roote† and Michael Ashburner†

**Laboratoire de Génétique, Université de Genève, CH-1224 Chêne-Bougeries, Geneva, Switzerland, and †Department of Genetics, University of Cambridge, Cambridge, England CB2 3EH*

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ABSTRACT

A mutation of *Drosophila melanogaster* whose only known effect is the rescue of otherwise lethal interspecific hybrids has been characterized. This mutation, *Hmr*, maps to *1*-31.84 (9D1-9E4). *Hmr* may be the consequence of a *P* element insertion. It rescues hybrid males from the cross of *D. melanogaster* females to males of its three sibling species, *D. simulans*, *D. mauritiana* and *D. sechellia*. This rescue is recessive, since hybrid males that carry both *Hmr* and a duplication expected to be Hmr^+ are not rescued. *Hmr* also rescues the otherwise inviable female hybrids from the cross of compound-*X D. melanogaster* females to males of its sibling species. This rescue is also recessive, since a compound-*X* heterozygous for *Hmr* does not rescue. Another mutation, discovered on the *In(1)AB* chromosome of *D. melanogaster*, is also found to rescue normally inviable species hybrids: unlike *Hmr*, however, *In(1)AB* rescues hybrid females from the cross of *In(1)AB/Y* males to sibling females, as well as hybrid males from the cross of *In(1)AB* females to sibling males. These data are interpreted on the basis of a model for the genetic basis of hybrid inviability of complementary genes.

Nature has one last trump card **(MAYR 1942)**

THE reproductive isolation between closely related species is generally considered to be due to the "building up of systems of complementary genes," rather than to "single mutational steps" (DOBZHANSKY 1951, p. 203; following MULLER 1940). Unfortunately genetic studies of reproductive isolating mechanisms, be they premating or postmating, are not as plentiful as one would wish to put DOBZHANSKY's statement on a firm footing. Indeed, there are several examples of single mutations that can override isolating mechanisms, either premating (*e.g.*, in *Chrysopa*, TAUBER, TAUBER and NECHOLS 1977) or postmating. A dramatic instance of a mutation that can override hybrid inviability, a not uncommon component of postmating isolating systems (HALDANE 1922; HERTWIG 1936; COYNE and ORR 1989), was characterized by HOLLINGSHEAD in *Crepis* (Compositae). Hybrids between *Crepis capillaris* and *C. tectorum* are normally inviable, the seedlings die at the stage of two cotyledons. Some isolates of *C. tectorum*, however, gave only viable hybrids with *C. capillaris* while some other isolates gave 50% viable and 50% lethal hybrids. HOLLINGSHEAD (1930) showed this to be due to a genetic polymorphism in *C. tectorum* for a pair of alleles, *l* and *L*, which had no discernible effect within this species. Any species hybrid that carried the *L* allele lived, any that carried the *l* allele died. Not dissimilar cases are known in some Graminae and in cotton (*e.g.*, STEPHENS 1950; GERSTEL 1954). Simple complementary lethal systems are also not uncommon in plants, and may not necessarily be fixed; an example within a species, *Mimulus guttatus* (Scrophulariaceae), has been analyzed by CHRISTIE and MACNAIR (1984, 1987). In such cases one species (or population) can be thought to have the genotype *Aa bb* and the other *aa Bb*, all genotypes other than those which combine an *A* and *B* allele are viable, those that do are lethal.

Within the genus *Drosophila* about one-fifth of the instances of interspecific hybridization listed by BOCK (1984) gave inviable progeny of one or both sexes (see also COYNE and ORR 1989). These include examples within the *D. melanogaster* species subgroup. *D. melanogaster* has three very close relatives, *D. simulans*, *D. mauritiana* and *D. sechellia*. The first of these was discovered by STURTEVANT (1919) just 70 years ago and has become, like *D. melanogaster*, a cosmopolitan species. Both *D. mauritiana* and *D. sechellia* are endemic species, known only from particular islands in the Indian Ocean (TSACAS and DAVID 1974; TSACAS and BÄCHLI 1981). All four species are morphologically very similar to each other, close inspection of their male genitalia being the only rigorous way to distinguish between them as adult flies. *D. simulans*, *D. mauritiana* and *D. sechellia* (which we will call "the siblings" when we do not need to distinguish among them) have homosequential polytene chromosome banding patterns differing from those of *D. melanogaster* by one long inversion on chromosome arm *3R*

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and a few much smaller inversions (LEMEUNIER and ASHBURNER 1976, 1984). Female interspecific hybrids between the sibling species are usually fertile (DAVID *et al.* 1974; LACHAISE *et al.* 1986). These data indicate a closer genetic relationship between these three species than of any one of them to *D. melanogaster* since, when they are crossed to this species, all hybrids are sterile; moreover, only one sex of the hybrids usually survives to adulthood. STURTEVANT (1920, 1921a, b, 1929) noted that the hybrid males from the cross of *D. melanogaster* females to *D. simulans* males die as larvae or early pupae, a result subsequently confirmed by several authors. By contrast, the hybrid females from the reciprocal cross die as embryos (HADORN 1961). A summary of the outcomes of crosses between *D. melanogaster* and its sibling species is given in Table 1.

The genetic causes of hybrid inviability in the *melanogaster* species complex clearly include interactions between the zygotic genotype and maternal factors. Indeed, as ORR (1989a) has pointed out "strong maternal effects on postzygotic isolation" are a common feature within the genus *Drosophila* (see also the discussion of KAUFMANN 1940). In this species subgroup these maternal effects can be readily seen by the fact that X^{mel}/X^{sim} hybrid zygotes (where the superscripts indicate the origin of the sex-chromosomes) are viable if the mother is *melanogaster* but die as embryos if she is *simulans*. A maternal effect is not, however, a general rule as X^{sim}/Y^{mel} zygotes are viable regardless of whether their mother was *melanogaster* or *simulans*. What is clear is that the causes of hybrid inviability can be overridden by mutations of single genes in either parent. This does not imply that single mutations are the original cause of hybrid inviability (see DISCUSSION). Variations in the outcome of crosses between *D. melanogaster* and *D. simulans* were noted by STURTEVANT (1929) but were not pursued by him or anybody else for 50 years. Then, WATANABE (1979; also TAKAMURA and WATANABE 1980) discovered a strain of *D. simulans* that gave viable adult hybrids of both sexes when crossed to *D. melanogaster*. Genetic analysis identified a mutation on chromosome arm *2R* of this strain which, when heterozygous in a hybrid zygote, "rescued" an otherwise lethal hybrid. WATANABE called this mutation *Lhr* (*L*ethal *h*ybrid *r*escue) and this strain (K18) has been widely distributed and its properties confirmed (*e.g.*, HUTTER and ASHBURNER 1987).

The study to be described in this paper began with the idea of discovering the homologous mutation to *Lhr* in *D. melanogaster* (see DISCUSSION). To this end over 60 different strains of *D. melanogaster* were gathered from all over the world and systematically crossed, as the female parent, to a panel of *D. simulans* strains. The combined progeny from crosses of 62 of these strains of *D. melanogaster* to *D. simulans* males included 23,686 hybrid females and 9 hybrid males. One exceptional strain, collected in Uman (Ukraine, USSR) gave about 5% hybrid male progeny. This was readily shown to be due to the presence at low frequency in this strain of a "rescuing" mutation. When a pure-breeding stock had been obtained by pair-mating, the sex ratio of the hybrid progeny was found to be normal with 2380 females, 2331 males. The males were sterile. To our surprise the mutation responsible for rescue was not homologous to *Lhr*–by virtue of the fact that it mapped to the *X* chromosome and not to *2R*. The mutation extracted from the Uman strain was called *Hmr* (*H*ybrid *m*ale *r*escue) (HUTTER and ASHBURNER 1987).

Hmr rescues hybrid males from the cross of *Hmr* females to males of all three sibling species. The rescue is zygotic and not maternal, since from *Hmr*/+ mothers only *Hmr*/*Y* sons, and not sons carrying the Hmr^+ homolog, are rescued. The effects of *Hmr* are temperature sensitive, rescue is more effective at 18° than at 25° and the temperature sensitive period is in the early larval stage. Rescue is most effective with *D. mauritiana* as the male parent, less so with *D. simulans* and least so with *D. sechellia*. In contrast to *Lhr*, the *Hmr* mutation does not rescue the inviable hybrid females from the cross of sibling females to *D. melanogaster* males. It must be said, however, that the rescue of hybrid X^{mel}/X^{sim} females from *D. simulans* mothers by *Lhr* is far from perfect, WATANABE (1979) recovered only 16% of the females expected were rescue to be complete.

In this paper we continue the genetic analysis of

TABLE 1

A summary of the viabilities of the interspecific hybrids

Zygotes	Mother: [*mel*]	Mother: [*sib*]
Female zygotes		
X^{mel}/X^{mel}	l-p[a,b]	—[c]
X^{mel}/X^{sib}	v	e[b]
X^{sib}/X^{sib}	—	v
Male zygotes		
X^{mel}	l-p[a,b]	l[a,b]
X^{sib}	v	v

The viabilities of various hybrid genotypes, partitioned with respect to the origin of their *X* chromosome and with respect to whether their mothers were *D. melanogaster* [*mel*] or a sibling species [*sib*] are shown. The *Y* chromosome has been ignored. Although MORGAN (1929) speculated that Y^{sim} may be the basis of hybrid inviability PONTECORVO (1943) showed that this cannot be so. v, viable; e, embryonic lethal; l-p, larval-pupal lethal; l, lethal, time of death not determined.

[a] Rescued if homozygous or hemizygous *Hmr*.

[b] Rescued if *In(1)AB* or *simulans Lhr*. Whether or not *Lhr* will rescue X^{mel} males from *sib* mothers has not been tested. It could be done, by crossing *D. simulans* compound-*X* females carrying *Lhr* to *D. melanogaster* males.

[c] — = no data.

Hmr in an effort to understand the reason for its curious phenotype. While doing so we have quite serendipitously discovered another *X*-linked mutation in *D. melanogaster* that rescues interspecific hybrids. This mutation may be of the same gene as that of *Hmr*, although its phenotypic effects differ. Surprisingly, this newly identified mutation may have been in laboratory stock collections since 1935.

MATERIALS AND METHODS

Stocks: The *Hmr* chromosome was originally isolated from a wild population of flies collected in Uman, Ukraine, USSR, in 1979. After the establishment of a homozygous *Hmr* stock this chromosome has been marked with various *X*-linked mutations; most commonly used in these experiments were y^2 *Hmr* and y^1 *Hmrv* chromosomes. Other stocks of *D. melanogaster* that have been used carry aberrations or mutations as described by LINDSLEY and GRELL (1968) or by LINDSLEY and ZIMM (1985–1987). Two different strains of *D. simulans* have been used—these strains were derived from wild-caught flies in Islamorada, Florida (obtained from the Bowling Green Stock Center), and in Dietikon, Switzerland (in 1982). The *S7* wild-strain of *D. mauritiana* was used. For *D. sechellia* we used strain number 228 of the Gif-sur-Yvette stock collection. Mutant strains of *D. melanogaster* were from the Bowling Green, Bloomington or Cambridge stock collections.

Crosses: Crosses were usually done with ten 1-day-old virgin females and 15 males aged for 5 days as virgins. Crosses between species (and some intraspecific crosses that were their controls) were set up at 25° (for 1–2 days) followed by culture at 18–19° (LEE 1978), at least until the first pupae appeared; cultures were then usually transferred to room temperature (20–21°). Most crosses were done on yeast-glucose medium; for the interspecific crosses this medium was seeded with live yeast.

Care was taken to ensure that all of the progeny from a cross emerged and were scored. This is particularly important for hybrids since, for example, at 18° *melanogaster*/*mauritiana*, *Hmr*/*Y* rescued males are delayed in their development, compared to their sisters, by 22–30 hr.

Irradiation: X-irradiation was at a dose rate of 300 R/min (220 kV, 15 mA, 1-mm Al and 0.5-mm Cu filtration).

Construction of *C(1)Hmr* chromosomes: To construct a compound-*X* homozygous for *Hmr*, y^2 *Hmr*/*Y* males were irradiated (4,200 R) and crossed to *C(1)M4*, y^2/y^+Y females. Seven wild-type females were recovered from 24,160 daughters. Of five tested, one proved to carry a new compound-*X*. The polytene cytology of this chromosome shows no chromosome aberration–it is presumably *C(1)RM*, y^2 *Hmr*. Two spontaneous free-*X* breakdowns of this chromosome were recovered (from 1,944 progeny) by exchange between *C(1)RM*, y^2 *Hmr* and a *Y* chromosome. These detachments are presumably $y^2 Hmr \cdot Y^{arm}$ chromosomes. To synthesize a balanced compound-*X* heterozygous for *Hmr*, y^2 $Hmr \cdot Y^{arm}/FM6$ females were irradiated (4,500 R) and crossed to phenotypically wild-type males. Eight new heterozygous compounds were recovered from 23,528 progeny. Spontaneous breakdowns of two of these new heterozygous compounds were selected, as exceptional *yellow* sons, and their *X* chromosomes were verified as being *Hmr*.

Cytology: Conventional temporary propionic-orcein-carmine squash preparations were made of larval salivary gland chromosomes. All of the chromosomes synthesized from *Hmr*, that is the compounds and their breakdown products, were checked for cytologically detectable changes in polytene chromosomes. All were either wild type or had the structure expected from their synthesis. *In situ* hybridizations to polytene chromosomes were done with biotinylated probes detected by the horseradish peroxidase reaction (ASHBURNER 1989). The *P* element probe used was the internal *Hin*dIII restriction enzyme fragment.

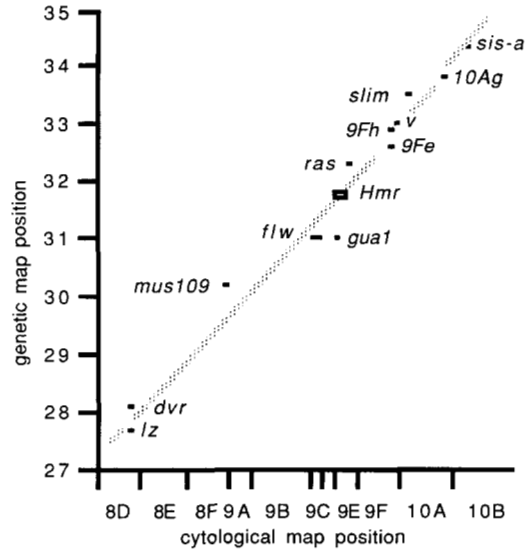

FIGURE 1.—The relationship between genetic and cytological map positions in the middle region of the *X* chromosome of *D. melanogaster*. The data are from LINDSLEY and ZIMM (1985–1987) and other sources. The spacings of the regions on the abscissa are proportional to their lengths on the revised polytene chromosome map (BRIDGES 1938). The line, which is drawn by eye, allows an estimate of 9D1 to 9E4 as the most likely cytological location of a mutation mapping to *1*-31.84.

Nomenclature: The symbols *mel*, *sim*, *sec* and *mau* will be used to designate chromosomes from *D. melanogaster*, *D. simulans*, *D. sechellia* and *D. mauritiana*, respectively. The abbreviation *sib* will be used for the sibling species collectively. Where it is convenient to specify the maternal origin of a zygote (that is of the maternal species) we will use the convention [*mel*], [*sim*], etc. Thus a hybrid X^{mel}/X^{sim} [*mel*] comes from a *melanogaster* mother while X^{mel}/X^{sim} [*sim*] comes from a *simulans* mother.

RESULTS

Mapping *Hmr*: The interpretation of some of the experiments to be described depends upon an accurate cytological location for *Hmr*. In the absence of any breakpoints known to affect this gene this can only be determined indirectly. HUTTER and ASHBURNER (1987) meiotically mapped *Hmr* to *1*-31.84 (the 95% confidence limits of this estimate are 31.58–32.10) with respect to the linked markers *oc* (*1*-23.1; 8A1.2), *lz* (*1*-27.7; 8D8.9) and *v* (*1*-33.0; 10A1.2). In a small-scale experiment crossovers between *Hmr* and *ras* (*1*-32.41; 9E3.4) showed *Hmr* to be distal to *ras*. We show, in Figure 1, a correlation between cytogenetic and meiotic map positions in the middle region of the *X*-chromosome, allowing a reasonably confident estimate of 9D1-9E4 as the location of *Hmr* on the polytene chromosome map.

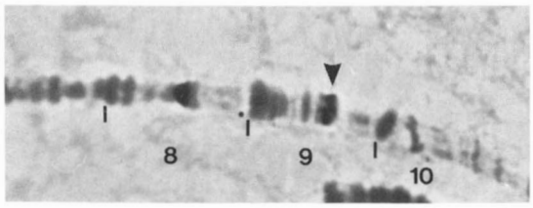

FIGURE 2.—*In situ* hybridization of a *P* element probe to the *X* chromosome of an *Hmr* stock. The weak signal of hybridization at 9E1.2 is indicated by the arrow. This signal appears to be either along the distal margin of the 9E1.2 doublet or in the fine bands 9D3.4.

Both the y^2 *Hmr* and y^1 *Hmr v* chromosomes carry *P* elements by the criterion of *in situ* hybridization with a biotinylated *P* element probe. The numbers of sites on the *X* chromosomes vary both within and between these stocks (from 4 to 10). A weakly hybridizing site at 9D3.4 or 9E1.2 is, however, seen in almost all individuals (in 10/12 y^1 *Hmr v* and 4/4 y^2 *Hmr* chromosomes analyzed). The weakness of this signal (Figure 2) may account for it not being seen in some chromosomes. This site is also labeled in all other chromosomes that carry *Hmr*, *i.e.*, the compound-*X* chromosomes that we constructed and their breakdown products. In the compound-*X* chromosome that is heterozygous for *Hmr* and *FM6* the signal is seen only on the *Hmr* homolog.

Phenotype of *Hmr D. melanogaster*: *Hmr*/*Y* males, Hmr/*Hmr* and *Hmr*/*Df(1)HC133* females of *D. melanogaster* are phenotypically indistinguishable from Hmr^+ flies in their appearance, viability and fertility. From its cytology *Df(1)HC133* (= Df(1)9B;9EF) should include *Hmr*. It does include *ras*, a locus proximal to *Hmr* (ZHIMULEV *et al.* 1982).

The rescue of female hybrids: The cross between female *D. melanogaster* carrying a compound-*X* chromosome and male *D. simulans* gives only male adult hybrids (BIDDLE 1932). This has been confirmed for four different compound-*X* chromosomes and, with one exception, extended to the crosses with male *D. mauritiana* (Table 2). The result with *C(1)DX* (Table 2) was not unexpected, since this compound-*X* is deficient for rRNA genes, and *C(1)DX* females will only survive if this deficiency is complemented, *e.g.*, by a *Y* chromosome. The *Y* chromosome of *D. simulans*, at least, is bb^- (STURTEVANT 1929) and has no functional rRNA genes (ROBERTS and LOHE 1989) and would not, therefore, complement *C(1)DX*. The only exception is the balanced compound-*X*, *C(1)M4*, y^2–when *C(1)M4* y^2 females are crossed to *D. mauritiana* males about one-quarter of the hybrid progeny are female. This curious result was shown to be due to the *X* chromosome of this stock, and not to its autosomes: when *C(1)M4*, y^2; *CyO*, *Cy*/+; *TM3*, *Ser*/+ females (the wild-type autosomes being from the *C(1)M4*, y^2 stock) were crossed to *D. mauritiana* males the rescued female hybrids were of all four possible autosomal genotypes, *i.e.*, 43 wild type, 34 Curly, 24 Serrate and 16 Curly, Serrate. These data were confirmed by crosses of *C(1)M4*, y^2; *CyO*/+ and *C(1)M4*, y^2; *TM3 Ser*/+ females to *D. mauritiana* males. The fourth chromosome was not studied. The components of *C(1)M4*, y^2 are $In(1)w^{m4}$+*In(1)AB* and the balancer *In(1)FM7* (CRAYMER 1974). The stock we used is not bb^- (see CRAYMER 1974), since females with this compound-*X* and no *Y* chromosome are viable (J. ROOTE, unpublished observations). Neither *In(1)FM7* nor $In(1)w^{m4}$ rescues male hybrids when free-*X* stocks are crossed to male *D. mauritiana* (Table 3, see Table 9 for *FM7* data). The $In(1)w^{m4}$+*In(1)AB* chromosome does, however, rescue hybrid males. From crosses of either homozygous or heterozygous $In(1)w^{m4}$+*AB*, y^2 females to males of the sibling species hybrid males are rescued; hybrid males from mothers that were heterozygous for this chromosome and *Basc* are invariably Bar^+ (Table 3).

TABLE 2

Lethality of hybrids with a compound-*X* chromosome

	Male parent			
	D. simulans		*D. mauritiana*	
Compound	♂♂	♀♀	♂♂	♀♀
C(1)RM, *y*/*Y*	512	0	239	0
C(1)A, *y*/*Y*	370	0	328	0
C(1)M4, y^2/y^+Y	831	0	856	282
C(1)DX, *y f*/y^+Y	519	0	886	0

Data for *D. simulans* pooled from crosses to both Islamorada and Dietikon stocks. Data for *D. mauritiana* from *S7* stock. All progeny were raised at 18°.

Hybrid rescue by $In(1)w^{m4}$+*AB* is due to its *AB* component, since a cross of *In(1)AB*/*Basc* females to sibling males gives hybrid males (Table 3). All of the hybrid males were wild type with respect to *Bar*, that is, like *Hmr* the *In(1)AB* chromosome has no maternal effect. Cytologically *In(1)AB* has breaks between 9E1.2 and 9E3.4 and between 13E1.2 and 13E3.4. The distal breakpoint is tantalizingly close to the predicted locus of *Hmr*. However, the mutation (or mutations) on the *In(1)AB* chromosome responsible for hybrid rescue and *Hmr* are not identical. Unlike *Hmr* the *In(1)AB* chromosome rescues female hybrids from crosses of *In(1)AB*/*Y* males to females of the sibling species (Table 3). Furthermore, the rescue of hybrid females seen in crosses of *C(1)M4*, y^2 to *D. mauritiana* males implies that the mutation on *In(1)AB* is partially dominant in its rescuing effect. This conclusion is strengthened by the observation that a different compound-*X* chromosome heterozygous for *In(1)AB*, *C(1)RA*, *In(1)AB*–$In(1)sc^8$ also rescues hybrid females: from a cross of *C(1)RA*, *In(1)AB*, *y*–$In(1)sc^8$, $sc^8/YL \cdot sc^{S1}$ females to *D. mauritiana* males there were 141 rescued daughters and 248 hybrid

TABLE 3

Rescue of interspecific hybrids by *In(1)AB*

Cross	°C	Progeny ♂♂[a]	♂♂[b]	♀♀
(a) Rescue of hybrid males:				
w^{m4} + *AB*/w^{m4} + *AB* ♀ × *sim* ♂	18	60	0	420
	25	117	1	429
w^{m4} + *AB*/w^{m4} + *AB* ♀ × *mau* ♂	18	375	2	954
w^{m4} + *AB*/w^{m4} + *AB* ♀ × *sec* ♂	18	28	1	200
w^{m4}/*Basc* ♀ × *mau* ♂	18	0	20	491[c]
AB/*Basc* ♀ × *sim* ♂	18	224	0	538
	25	429	0	987
AB/*Basc* ♀ × *mau* ♂	18	187	0	293
w^{m4} + *AB*/*Basc* ♀ × *sec* ♂	18	74	0	441
w^{m4} + *AB*/*Basc* ♀ × *mau* ♂	25	305	0	1094
w^{m4} + *AB*/*Basc* ♀ × *sim* ♂	18	97	5	339
	25	171	0	402
(b) Rescue of hybrid females:				
sim ♀ × w^{m4} + *AB*/*Y* ♂	18	111	0	87
	25	816	0	63
mau ♀ × w^{m4} + *AB*/*Y* ♂	25	189	0	150

w^{m4} + *AB* = *In(1)*w^{m4} + *In(1)AB*, y^2 w^{m4}; w^{m4} = *In(1)*w^{m4}, w^{m4}; *AB* = *In(1)AB*.

[a] All males Bar^+ if from heterozygous *Basc* mothers and *y w*-mottled if from *In(1)*w^{m4} + *In(1)AB* mothers.

[b] Nondisjunctional progeny.

[c] In some of these crosses the females were heterozygous for third chromosome balancer chromosomes, hence the high frequency of nondisjunctional progeny.

TABLE 4

Rescue of female species hybrids by compound-*X* chromosomes

Compound	Fathers: *D. mauritiana* ♂♂	*D. mauritiana* ♀♀	*D. simulans* ♂♂	*D. simulans* ♀♀
C(1)RM, y^2 *Hmr*	459	426	426	118
C(1), *Hmr–FM6~1*	770	2[a]	485	8[a]
C(1), *Hmr–FM6~2*	485	8[a]	425	5[a]

C(1)RM, y^2 *Hmr* is a compound that is homozygous for *Hmr*, *C(1)*, *Hmr–FM6~1* and *C(1)*, *Hmr–FM6~2* are two independently synthesized compounds heterozygous for *Hmr*. All progeny were raised at 18°.

[a] Interpreted as inheriting breakdown products of the compound-*X* chromosomes, all were heterozygous *Bar*.

sons. As shown below, *Hmr* behaves as a recessive allele by this criterion. Lastly, rescue of hybrids by *Hmr* is very temperature sensitive (HUTTER and ASHBURNER 1987). We have compared the rescue of hybrids by *In(1)AB* at both 18° and 25° and find no consistent differences, indeed, if anything, rescue is usually poorer when the hybrids are grown at the lower temperature (Table 3).

The time of death of *C(1)* hybrid females: The lethal female hybrids from a cross of *D. melanogaster* males to *D. simulans* females die as embryos (HADORN 1961). We have confirmed this observation for hybrids with both *D. simulans* and *D. mauritiana*. By contrast the hybrid males from the reciprocal cross, with free-*X D. melanogaster* females, die as third instar larvae or "pseudopupae." It was of interest, therefore, to determine when *C(1)* hybrid females, from crosses of *C(1)*, *melanogaster* to *D. mauritiana* males, died. Embryos from crosses of *C(1)RM* and *C(1)A* females to *D. mauritiana* were followed throughout their development–death occurred as third instar larvae or "pseudopupae," indicating that these hybrid females resemble hybrid males in their time of death.

***Hmr* rescues *C(1)* hybrid females when it is homozygous:** Females carrying the compound-*X* homozygous for the y^2 *Hmr* chromosome (and carrying a *melanogaster Y* chromosome) were crossed to both *D. simulans* and *D. mauritiana*. The results of these crosses (Table 4) show that good rescue of otherwise inviable females is achieved with *D. mauritiana* and partial rescue with *D. simulans*. The females that fail to eclose from the cross of *C(1)RM*, y^2*Hmr* females to *D. simulans* males die as pharate adults. These hybrid females are sterile and, on dissection, are seen to have ovaries that are very reduced in their size. Thus, when homozygous *Hmr* can rescue otherwise inviable females.

***Hmr* is recessive in *C(1)* females:** What is the nature of the mutational difference between *Hmr* and Hmr^+? As one step toward an answer to this question we have determined the dominance relationship of *Hmr* and Hmr^+ under two conditions. Since hybrid females homozygous for *Hmr* are rescued we constructed six compound-*X* chromosomes that were necessarily heterozygous for this mutation, and crossed females carrying these to *D. mauritiana* males. In all crosses only males survived to adulthood–*i.e.*, heterozygous *Hmr* females were not rescued. More extensive tests with two of the compounds confirmed these data (Table 4). To verify the genotype of these compounds both the free-*X* chromosomes from which they were derived and free-*X* chromosomes derived by spontaneous breakdown from them were crossed, in females heterozygous with *Basc*, to *D. mauritiana* males. All gave wild-type and heterozygous *Bar* hybrid females and y^2 hybrid males.

Hmr^+ is also dominant to two copies of *Hmr* with respect to the survival of *C(1)* hybrid females to adulthood. *C(1)RM*, y^2 *Hmr/Y* females carrying $Dp(1;2)v^{+75d}$ were crossed to *D. mauritiana* males. The hybrid females that carry the duplication, rather than its balancer homolog, are very poorly rescued (Table 5). Indeed, *C(1)RM*, y^2*Hmr*/Y^{mau}; $Dp(1;2)v^{+75d}$ Hmr^+/2^{mau} [*mel*] females die as embryos, rather than as third instar larvae.

The evidence that $Dp(1;2)v^{+75d}$ indeed carries Hmr^+ is indirect but convincing. First this duplication (9A2-10C2) extends far to both sides of 9DE, the predicted locus of *Hmr*. Secondly, as we will show below, *Hmr*/Y^{mau}; $Dp(1;2)v^{+75d}$/2^{mau} [*mel*] males die as embryos but

TABLE 5

One copy of *Hmr*$^+$ is dominant to two of *Hmr* in hybrid females

Progeny	Number
C(1)RM, y^2 Hmr/Y^{mau}; Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♀♀	2
C(1)RM, y^2 Hmr/Y^{mau}; Gla/2^{mau} ♀♀	49
X^{mau}/Y^{mel}; Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♂♂	28
X^{mau}/Y^{mel}; Gla/2^{mau} ♂♂	86

C(1)RM, y^2 Hmr/Y; Dp(1;2)v^{+75d}/Gla females were mated to *D. mauritiana* males and the progeny raised at 18°.

In addition to these flies, 70–80 dead embryos were seen. These are presumed to be the *Dp*-bearing female and *YY* zygotes. No dying larvae or pupae were observed.

TABLE 6

Is *Hmr* recessive in hybrid males?

Progeny genotype	Number
y^2 Hmr/+; Dp(1;2)v^{+75d}/Gla females	
Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♀♀	300
Gla/2^{mau} ♀♀	333
Hmr/Y^{mau}; Gla/2^{mau} ♂♂	23[a]
Hmr/Y^{mau}; Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♂♂	0
y^1 Hmr v/y^1 Hmr v; Dp(1;2)v^{+75d}/CyO females	
Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♀♀	551
CyO/2^{mau} ♀♀	644
Hmr/Y^{mau}; CyO/2^{mau} ♂♂	324
Hmr/Y^{mau}; Dp(1;2)v^{+75d}, Hmr$^+$/2^{mau} ♂♂	6[b]

Data from crosses to *D. mauritiana* males, progeny raised at 18°.

[a] Also 3 *y$^+$ Gla* males; the reason for the low rescue of these males is not known.

[b] About 1100–1200 dead embryos observed; also 11 *y$^+$ v$^+$ Cy* males.

Df(1)HC133/Y^{mau}; Dp(1;2)v^{+75d}/2^{mau} [*mel*] die as third instar larvae or pseudopupae: from *Df(1)HC133/+; Dp(1;2)v^{+75d}/CyO* females crossed to *D. mauritiana* males there emerged 62 Curly females and 50 non-Curly females and about 60 zygotes died as embryos (presumably *Df(1)HC133/Y^{mau}; CyO/2^{mau}*) and about 50 as third instar larvae or prepupae (presumably *Df(1)HC133/Y^{mau}; Dp (1;2)v^{+75d}/2^{mau}*).

Is *Hmr* recessive in hybrid males? To test the dominance relationship of *Hmr* and its wild-type allele in males we constructed male *D. melanogaster/D. mauritiana* hybrids carrying *Hmr* on their *X* chromosome and *Hmr*$^+$, carried by a duplication, on chromosome 2. *Dp(1;2)*$^{+v75d}$ was used for these experiments. The results from two experiments (Table 6) show that *Hmr/Y^{mau}; Dp(1;2)v^{+75d}/2^{mau}* [*mel*] are not rescued–indeed they die, not as third instar larvae or pseudopupae, but as embryos.

Does a duplication for *Hmr*$^+$ rescue hybrids? One possible basis for the *Hmr* mutation is that it is a hypermorphic allele of *Hmr*$^+$. If this were so, then hybrid males carrying two doses of *Hmr*$^+$ might be rescued to adulthood. Three different insertional duplications of the *X*-chromosome have been used to test this hypothesis. One of these duplications, *Dp(1;2)*$^{+v75d}$, is expected (see above) to carry *Hmr*: the other two were used as controls; at least *Dp(1;2)v^{65b}* (= Dp(1;2)10A1-11A7.8) should not include *Hmr*$^+$, in view of its cytological extent. No similar case can be made for *Dp(1;2)v^{+63i}* (= Dp(1;2)9E1-10A11), although the evidence (see below) suggests that it does not carry *Hmr*$^+$ (this duplication does include *ras*$^+$). All three duplications were introduced to hybrids by crossing females with wild-type *X*-chromosomes, heterozygous for the duplication and a *CyO* balancer chromosome, to *D. mauritiana* males. Were two doses of *Hmr*$^+$ sufficient for the viability of male hybrids then non-*Cy* hybrid males would survive to adulthood. Hybrid males carrying *Dp(1;2)v^{+75d}*, *Hmr*$^+$ are not rescued, indeed they die as embryos (Table 7). There are two features of the *Dp(1;2)v^{+75d}* data that warrant comment: the first is that there is a marked reduction in viability of female hybrids carrying this duplication chromosome, as compared to those carrying its *Curly* homolog (or, for that matter, either of the other two duplications). The second feature is that from this cross, and not from those with the other duplications, the duplication bearing female hybrids emerged after their Curly sisters: for the crosses with *Dp(1;2)v^{+63i}* and *Dp(1;2)v^{65b}* the duplication bearing females emerged before their Curly sisters. Since these data may result from some trivial feature of *Dp(1;2)v^{+75d}* similar crosses were done to *D. melanogaster* males (Table 7). From these crosses at least as many duplication-bearing females as Curly sibs were produced in all cases and the *Dp*-bearing females always emerged first. It does seem, however, as though *melanogaster* males carrying two doses of *Hmr*$^+$ have a reduced viability; moreover these males sometimes have abnormal external genitalia.

Hybrid females from *D. melanogaster* mothers that carry a duplication for *Hmr*$^+$ are delayed in their development and have a slightly lowered relative viability (Table 7). We tested whether or not hybrid females that were heterozygous for a deletion of *Hmr* showed any phenotype, by crossing *Df/Hmr*$^+$ *D. melanogaster* females to *D. mauritiana* males. Two deletions, were tested; at least one (*Df(1)HC133*, see above), and perhaps both, of these should include the locus of *Hmr*. The data (Table 8) show that hybrid females heterozygous for either deficiency are as viable as their balancer chromosome bearing sibs. Both classes of female developed at the same rate.

***Hmr* rescues otherwise inviable males from *simulans* mothers:** ORR (1989b) has shown that when *D. simulans* females that carry a compound-*X* chromosome are crossed to *melanogaster* males the only viable adult progeny are female, the *X^{mel}/Y^{sim}* [*sim*] males die. We have confirmed this observation (Table 9). These males are partially rescued by an *Hmr*-carrying *melanogaster X* chromosome (Table 9), confirming ORR's preliminary data. The small numbers in these experiments reflect the great difficulty in achieving

TABLE 7

Duplication for *Hmr*$^+$ does not rescue hybrid males

Duplication	Region	Progeny				Notes
		Dp ♂♂	*CyO* ♂♂	*Dp* ♀♀	*CyO* ♀♀	
(a) *D. mauritiana* males						
Dp(1;2)v^{+75d}, *Hmr*$^+$	9A2-10C2	0	3	190	294	*Dp* ♀♀ emerge last
Dp(1;2)v^{+63i}	9E1-10A11	2	0	534	481	*Dp* ♀♀ emerge first
Dp(1;2)v^{65b}	10A1-11A7.8	4	2	434	417	*Dp* ♀♀ emerge first
(b) *D. melanogaster* Canton-S males						
Dp(1;2)v^{+75d}, *Hmr*$^+$	9A2-10C2	385	463	515	508	*Dp* ♀♀ emerge first
Dp(1;2)v^{65b}	10A1-11A7.8	336	188	342	253	*Dp* ♀♀ emerge first
(c) *D. melanogaster y*^{2} *Hmr* males						
Dp(1;2)v^{+75d}, *Hmr*$^+$	9A2-10C2	353	502	513	511	*Dp* ♀♀ emerge first

Data from crosses of *Hmr*$^+$; *Dp/CyO* females to (a) *D. mauritiana* and (b, c) *D. melanogaster* males at 18°.

Progeny of all crosses to *D. mauritiana* males included substantial numbers of lethal third instar larvae and pseudopupae (*i.e.*, 492 pseudopupae in the cross with *Dp(1;2)v*$^{+63i}$, 379 pseudopupae with *Dp(1;2)v*^{65b}). The cross with *Dp(1;2)v*$^{+75d}$ gave, in addition, a large number (>400) of lethal embryos, presumably including the *Dp*-bearing males. The *CyO* chromosome in all of the females came from the same stock.

TABLE 8

Effects of deletions for *Hmr* on hybrid females

Mother	Region	Progeny			
		+ ♂♂	*Bal* ♂♂	*Df*/+ ♀♀ or +/+ ♀♀	*Bal*/+ ♀♀
D. mauritiana males					
Canton-S/*FM6*	—	14[a]	7[b]	304	265
Df(1)HC133/FM7c	9B9.10-9EF	4[a]	5[b]	356	347
Df(1)N110/FM6	9B3.4-9D1.2	5[a]	4[b]	491	435
D. melanogaster Canton-S males					
Canton-S/*FM6*	—	446	118	416	409
Df(1)HC133/FM7c	9B9.10-9EF	0	197	239	241
Df(1)N110/FM6	9B3.4-9D1.2	2[a]	449	514	474

[a] Presumably primary nondisjunctional exceptions.

[b] These males all carried *Bar* but not all of the recessive markers expected from the balancer chromosomes; those from the *Df(1)HC133/FM7c* females were Hairy-wing (but not yellow, white or singed), those from the *Df(1)N110/FM6* females were Hairy-wing, but not yellow. Similar anomalous *B* progeny have been seen in other crosses with *FM6*, *FM7* but not with *FM1* or *Basc* females to *D. mauritiana* and *D. simulans* males by us and others (P. HUTTER 1990; M. STEINMANN-ZWICKY, personal communication). The salivary gland chromosomes of about 40 non-y male larvae from a cross of *FM6/y*^{1} *v f D. melanogaster* females to *y w/Y simulans* males were all clearly from hybrid larvae (*i.e.*, they were heterozygous for the *simulans* inversion on chromosome arm *3R*; their *X* chromosomes had a wild-type, and not balancer, sequence).

crosses between *melanogaster* males and compound-*X* *D. simulans* females.

Hybrid males are sterile even if they carry a *melanogaster Y* chromosome: Hybrid males from the cross of *D. melanogaster Hmr* females to either *D. simulans* or *D. mauritiana* males are sterile (HUTTER and ASHBURNER 1987). These hybrids carry, of course, a *melanogaster X* chromosome and a *Y* chromosome from the sibling species. These males have reduced testes, accessory glands and ejaculatory ducts. These testes appear to be aspermic by light microscopy. Would these males be fertile if they also carried a *Y* chromosome from *D. melanogaster*? Although unlikely (but see COYNE 1985 and VIGNEAULT and ZOUROS 1986), this was so easy to test that it seemed worthwhile to find out. By exchange between *ct*^{n} *oc Hmr v* and a compound-*XY* chromosome with the *X* in normal sequence and all the male-fertility factors of the *Y* appended proximally (*f B YS·YL*, from J. R. MERRIAM), a recombinant chromosome that was *ct*^{n} *oc Hmr v f B YS·YL* was obtained. *ct*^{n} *oc Hmr v f B YS·YL/* + *D. melanogaster* females were crossed to *D. mauritiana* males. Over 800 hybrid males (*ct*^{n} *oc Hmr v f B YS·YL/Y*^{mau}) were tested for their fertility by crossing to *D. melanogaster* Canton-S females: all were sterile, although *ct*^{n} *oc Hmr v f B YS·YL/Y*^{mel} males were fertile.

DISCUSSION

Hybrid lethal phenotypes: Hybrid males from the cross of *D. melanogaster* females to sibling males die as third-instar larvae or pseudopupae. In fact, at temperatures above 25° even hybrid females are inviable and die during metamorphosis or soon after eclosion (STURTEVANT 1929; KERKIS 1933; WATANABE *et al.* 1977). LEE (1978) has documented the very considerable variation seen in the viability of *X*^{mel}/*X*^{sim} [*mel*]

TABLE 9

The rescue of X^{mel}/Y^{sim} [*sim*] males by *Hmr*

Father	Progeny ♀♀	Progeny ♂♂
IHmr$^+$/*Y*	59	0
y Hmr v/*Y*	46	17
Hmr/*Y*	14	7

Data from crosses of *D. simulans C(1)RM, y w* females to *D. melanogaster* males.

The *Hmr*$^+$ *X* chromosome was from the Canton-S wild-type stock.

hybrid females, and shown that this variation is due, in major part, to the paternal *simulans X* chromosome. The causes of hybrid death are unknown. X^{mel}/Y^{sib} [*mel*] hybrid males may live for several days after their sisters have pupariated, but remain very sluggish. On dissection they are seen to have a reduced fat body and other internal organs, *e.g.*, salivary glands (personal observations and SEILER and NÖTHIGER 1974). Their salivary gland chromosomes are thin and their *X* chromosome unusually contracted (M. ASHBURNER, unpublished observations). Whether these symptoms reflect the direct cause of death or, more probably, are simply a secondary consequence of an earlier dysfunction, is not known. It is probable that the immediate reason for the failure of metamorphosis is the absence of the correct hormonal stimuli, since imaginal discs from *D. melanogaster*/*D. mauritiana* hybrids will metamorphose on transplantation into a suitable host (SÁNCHEZ and DÜBENDORFER 1983).

Although the difference in lethal phenotype between X^{mel}/X^{sib} [*sib*] zygotes (embryos) and X^{mel}/Y^{sib} [*mel*] or $X^{mel}/X^{mel}/Y^{sib}$ [*mel*] zygotes (third-instar larvae or pseudopupae) may discourage attempts to find a unitary cause of hybrid inviability the genetic data argue that such a unitary event may exist. Both *In(1)AB* of *D. melanogaster* and *Lhr* of *D. simulans* rescue all of these genotypes. *Hmr* does not, yet X^{mel}/Y^{sib} [*mel*] males, be they *Hmr* or *Hmr*$^+$, that carry a duplication for *Hmr*$^+$ die as embryos. These data suggest that embryonic and larval death are not as fundamentally different as they might appear and that the *Hmr* gene product can influence the developmental stage achieved by hybrids.

The relationship between *Hmr* and the *In(1)AB* chromosome: The discovery that the *In(1)AB* chromosome can rescue otherwise lethal interspecific hybrids was quite fortuitous. The mutation carried by this chromosome is clearly different in its phenotypic effects from *Hmr*. It rescues X^{mel}/X^{sib} [*sib*] females and its rescue of $X^{mel}/X^{mel}/Y^{sib}$ [*mel*] females is dominant and not clearly temperature-sensitive. One possibility is that *Hmr* and the mutation carried by *In(1)AB* are genetically unrelated, another is that they are different alleles of the same gene. The second of these possibilities is attractive in view of the fact that the distal breakpoint of this inversion is so close to the cytological position predicted for *Hmr*. Although we have not mapped the rescuing gene (or genes) on *In(1)AB*, it is presumably closely linked to the inversion, since it is retained by the *In(1)*w^{m4}+*AB* chromosome. Unfortunately the origin of *In(1)AB* is lost in the mists of time: STONE and THOMAS (1935) simply say that it was "found by Miss ELSIE BODEMAN"–whether spontaneous or X-ray induced is not stated, but we know that the Austin laboratory was very active in inducing new chromosome aberrations with X-rays in the 1930s. The question of the allelism between *Hmr* and the mutation carried by *In(1)AB* will best be settled by molecular evidence (P. HUTTER and F. KARCH, in progress). (We should add that the *In(1)AB* stocks are free of *P* elements by the criterion of *in situ* hybridization.)

Similar examples of variation in hybrid viability from Drosophila: The examples of genetic rescue of hybrid viability seen in the *melanogaster* species complex are by no means unique. Indeed the first example was described over 40 years ago by CROW (1942) for the sibling species pair *D. mulleri* and *D. aldrichi*. Hybrid progeny of these species, from the cross of female *D. mulleri* to male *D. aldrichi*, are usually of both sexes. One strain of *D. aldrichi* was found to differ since, when crossed to *D. mulleri* females, all the hybrids were male. This was probably due to an X-linked allele in this strain; if so this would be analogous to *Hmr*$^+$ of *D. melanogaster*, the majority of *D. aldrichi* strains being "*Hmr.*"

A second, and particularly instructive, example has been analyzed in the *D. virilis* species group by PATTERSON and GRIFFEN (1944). A cross of female *D. montana* by male *D. americana texana* gives only adult males; the X^{mon}/X^{tex} [*mon*] zygotes die as embryos (KINSEY 1967). From the reciprocal cross both males and females develop to adulthood. PATTERSON and GRIFFEN took advantage of the facts that *D. virilis*/*D. americana texana* hybrids are fertile and that the *X* chromosome of *D. virilis* is well marked in order to map the *americana texana* gene(s) responsible for hybrid inviability. From *virilis*/*americana texana* hybrids heterozygous for a marked *virilis X* chromosome, *y ec cv v sn dy g* (we use the *melanogaster* genetic notation), various recombinant chromosomes were recovered and put onto a *virilis* genetic background by six generations of backcrossing. Males carrying these recombinants were then crossed to *D. montana* females. Only when the *X* chromosome carried the *ec–cv* interval from *americana texana* were the female hybrids inviable; *X* chromosomes carrying other regions from *americana texana* gave both male and female adult hybrids. *D. americana texana* would thus seem to carry an analog of the *D. melanogaster Hmr*$^+$ allele. Its sibling

species, *D. americana americana* carries an analog of *Hmr*, since both *D. montana* × *D. americana americana* crosses give a 1:1 hybrid sex ratio. In fact, the parallels between this example and those in the *D. melanogaster* complex go further, since some strains of *D. americana texana* give some adult hybrid females with *D. montana* (between 7 and 20% of the progeny, but of course these wild strains may well have been polymorphic for a rescuing allele). Too much should probably not be made of the fact that the *cv* gene of *D. virilis* is closely linked (within 0.5 map unit) to *v* (ALEXANDER 1976).

There is one other example, also from the *virilis* species group, that deserves notice, because it gives evidence for autosomal loci that can affect hybrid viability in a species other than *D. simulans*. *D. virilis* and *D. lummei* produce hybrids with a normal sex ratio when crossed together in either direction. However, backcross hybrids that carry certain combinations of *virilis* and *lummei* chromosomes give all male progeny when crossed to *D. lummei* males, but bisexual progeny when crossed to *D. virilis* males. This phenomenon is temperature sensitive, the females die at 25° but not at 17°. The major effect maps to the second chromosome of *D. virilis* but there is an enhancer on chromosome *3* and a suppressor on chromosome *5*. Thus, backcross flies of the genotype *vir/vir*; *vir/vir*; *vir/lum*; *vir/lum*; *vir/lum*; *vir/lum* [*vir*], when crossed to *D. lummei* males at 25°, give predominantly male offspring. When crossed to *D. virilis* males the sex ratio of the progeny is normal (MITROFANOV and SIDOROVA 1981).

These parallels encourage the view that the examples of genetic rescue of interspecific hybrids seen in the *D. melanogaster* species complex are not peculiarities of these species, but reflect more general genetic phenomena, at least within *Drosophila*.

The nature of the *Hmr* mutation: The viabilities of interspecific hybrids between *D. melanogaster* and its sibling species are summarized in Table 10. HUTTER and ASHBURNER (1987) posed the question of the nature of the *Hmr* mutation, whether, for example, it was a loss- or gain-of-function allele. The data we have described suggest that it is a loss-of-function allele, but a hypomorph rather than an amorph. *Hmr* acts as a recessive mutation–Hmr/Y^{mau}; $Dp(1;2)Hmr^{+}$ [*mel*] males are not rescued nor are $Hmr/Hmr^{+}/Y^{mau}$ [*mel*] females. The data that suggest that *Hmr* is a hypomorph are that $Df(1)Hmr/Y^{mau}$; $Dp(1;2)Hmr^{+}$ [*mel*] hybrids die as third-instar larvae but Hmr/Y^{mau} $Dp(1;2)Hmr^{+}$ [*mel*] hybrids die as embryos. That is to say *Hmr* and a deletion for *Hmr* are not equivalent. These data might be interpreted to mean that *Hmr* is an antimorphic allele. This is unlikely, in view of the observation that Hmr^{+}/Y^{mau}; $DpHmr^{+}$ [*mel*] hybrids (with two doses of Hmr^{+}) die as embryos, suggesting that it might be the *amount* of *Hmr* gene product, rather than its quality, which determines the time of death of these animals.

TABLE 10

The dominance relationships of *Hmr* and Hmr^{+}

Genotype	Viability
Females:	
Hmr^{+}/Hmr^{+}	Larval/pupal lethal
Hmr^{+}/Hmr	Lethal[a]
Hmr/Hmr	Viable
Hmr/Hmr; Hmr^{+}	Embryonic lethal
Males:	
Hmr^{+}/Y	Larval/pupal lethal
Hmr/Y	Viable
Hmr/Y; Hmr^{+}	Embryonic lethal
Hmr^{-}/Y; Hmr^{+}	Larval/pupal lethal

The viabilities of *melanogaster/mauritiana* hybrids from crosses of *D. melanogaster* females to *D. mauritiana* males. All the hybrids are, therefore, [*mel*] and carry only *D. melanogaster X* chromosomes; the males carry Y^{mau}. The duplication for Hmr^{+} is that carried by $Dp(1;2)v^{+75d}$.

[a] Probably larval/prepupal lethal (D. CROMPTON, unpublished observations).

The rôle of the Y^{sib} chromosome: STURTEVANT (1929) contrasted two hypotheses to explain the patterns of viability and lethality seen in *melanogaster/simulans* hybrids–either the survival of the hybrids depended on their having an X^{sim} chromosome or their inviability resulted from their having a Y^{sim} chromosome. He pointed out that these possibilities could be distinguished if the fate of patroclinous sons from the cross of *D. simulans* females to *D. melanogaster* males was known, since these males will be X^{mel}/O: "on the first interpretation these should die, having no *simulans X*; on the second interpretation they should live, having no *simulans Y*." The available data indicate that they die, certainly none were found in STURTEVANT's experiments (which gave 4204 regular sons) and, to our knowledge, none have been found since. The problem, of course, is that the frequency of exceptional sons is expected to be very low, between 0.05 and 0.1% of regular sons (assuming the frequency of primary nondisjunction in *D. simulans* to be similar to that in *D. melanogaster*, see STURTEVANT 1921a).

More critical data come from the survival of "partial" hybrids, derived from crossing 3*n* *D. melanogaster* to irradiated *D. simulans* males. MULLER and PONTECORVO (1940) recovered a viable (and fertile) X^{mel}/Y^{sim}; 2^{mel}; 3^{mel}; $4^{mel}/4^{sim}$ [*mel*] male–showing that the combination of a *melanogaster X* chromosome with a *simulans Y* chromosome on a *melanogaster* cytoplasm is not lethal. Many other viable (but sterile) combinations of X^{mel} with Y^{sim} were recovered by PONTECORVO (1943) and KOSKE-WESTPHAL (1964), as "partial" hybrids. We cannot do better than repeat PONTECORVO's conclusion: "It seems highly improbable that Y^{sim} could have a lethal action in hybrids with one set of

autosomes of its own species, whilst giving no effect in (partial) hybrids with part or all chromosomes of the foreign species" (PONTECORVO 1943).

The genetic basis of hybrid inviability: The inviability of hybrids between *D. melanogaster* and its sibling species can be rescued by mutations at at least two, and possibly three, loci: *Hmr* and that on the *In(1)AB* chromosome of *D. melanogaster* and *Lhr* of *D. simulans*. These observations are, at first sight, in contrast with the conclusion of PONTECORVO (1943), from an analysis of the viabilities of "partial" *D. melanogaster/D. simulans* [*mel*] hybrids (see above). The conclusion was that at least nine genes are concerned with hybrid viability. In PONTECORVO's scheme these genes formed complementary groups of recessive lethals and their recessive suppressors, so that any hybrid which was homozygous, or hemizygous, for a lethal, but only heterozygous for its suppressor, would die.

The discovery of single mutations that can override the genetic basis of hybrid inviability does not contradict these conclusions. For example, PONTECORVO suggested that male X^{mel}/Y^{sim} [*mel*] and female $X^{mel}/X^{mel}/Y^{sim}$ [*mel*] hybrids die because of a lethal mutation on their *X* chromosome, normally suppressed within *D. melanogaster* itself by recessive suppressor alleles on chromosome *2* or *3*. On this model the genotype of *D. melanogaster* would be l^+; $su(l^+)^+$ and that of *D. simulans l*; $su(l^+)$. A hybrid male from the cross of *D. melanogaster* females to *D. simulans* males would die, because it would be hemizygous for l^+ but only heterozygous for $su(l^+)^+$. Hybrid females from a cross of compound-*X D. melanogaster* females would die because they would be homozygous for l^+ and also heterozygous for $su(l^+)^+$. Hybrid males from this cross would live, since they would carry the *D. simulans*, *l* allele. Similarly hybrid females from the cross of compound *X D. simulans* would live (being *l/l*) but their brothers would die (being l^+/Y; $su(l^+)/su(l^+)^+$). X^{mel}/X^{sim} females live if their mother was *D. melanogaster* but die if she was *D. simulans*. The most obvious, although *ad hoc*, explanation of this difference is that the $su(l^+)^+$ product must be maternally inherited if the zygote is l^+/l.

Consider the consequences of a *D. simulans* mutation from $su(l^+)$ to $su(l^+)^+$–then any hybrid would be homozygous for $su(l^+)^+$ and would live. *Lhr* is just such a mutation. The partial rescue of X^{mel}/X^{sim} [*sim*] hybrid females by *Lhr* (WATANABE 1979) can be interpreted as a maternal effect of $su(l^+)^+$. Consider too the consequences of a *D. melanogaster* mutation from l^+ to *l* on the simple assumption that the l^+ allele is functional and the *l* allele non-functional. This would rescue hybrids but would be recessive to l^+ since the product of the l^+ allele would lead to death. *Hmr* is just such a mutation, and we can write Hmr^+ for l^+ and $su(Hmr^+)^+$ for $su(l^+)^+$. We realize that this is only one possible hypothesis. An alternative would be that the sibling species have not diverged at the *Hmr* and *Lhr* loci, but have done so at loci that respond to these genes.

The consequences of this hypothesis are that a mutation of the *D. melanogaster* $su(Hmr^+)^+$ allele (*i.e.*, the homolog of the *D. simulans Lhr* mutation) will be lethal, unless Hmr^+ is also mutant. That is to say *Hmr* will act as a specific suppressor of an autosomal mutation of a gene that is the homolog of the *D. simulans Lhr* gene. Our original strategy to discover this gene in *D. melanogaster* (see Introduction) was clearly wrong.

We have shown that duplications for Hmr^+ (carried by $Dp(1;2)^{+v75d}$) have an adverse effect on the viabilities of hybrids. For example X^{mau}/X^{mel}; $Dp(1;2)^{+v75d}$ [*mel*] females are delayed in their development and have a somewhat reduced viability, with respect to their nonduplication carrying sibs. Moreover hybrids that carry both *Hmr* and Hmr^+ die as embryos, rather than third instar larvae. These data, which were at first very puzzling, are readily interpreted within the model we suggest: since *Hmr* is a hypomorphic allele (see above) then the ratio of the *Hmr* gene's product to that of the $su(Hmr^+)^+$ gene is increased in duplication genotypes. It is no surprise, therefore, that these should show a more mutant phenotype. It is difficult to be more precise than this, because we have no knowledge of the level at which the interaction between the *X*-linked lethal and its autosomal suppressor occurs.

The relationship between *Hmr* and the mutation carried by *In(1)AB* remains to be defined. We have already discussed the two alternatives: either these are allelic or they are not. The most striking difference between them is the almost complete rescue of X^{mel}/X^{mau} [*mau*] females by *In(1)AB* and the circumstantial evidence (from the behavior of compound-*X* chromosomes heterozygous for *In(1)AB*) that the mutation on this inversion is dominant, with respect to rescue, in X^{mel}/X^{mel} [*mel*] hybrids. If an allele of *Hmr*, then the mutation on *In(1)AB* must be more extreme (*i.e.*, less functional) than that of *Hmr* itself. An implication of this conclusion is that the *Hmr* allele of the sibling species may not be an amorph, but that of the *In(1)AB* chromosome is. An alternative formal explanation is that the mutation carried by *In(1)AB* is non-allelic to *Hmr* but that it is a dominant enhancer of the autosomal suppressor, $su(Hmr^+)^+$.

We conclude by stating that we have, by an analysis of single mutations whose sole phenotype is the rescue of otherwise lethal interspecific hybrids, a formal genetic model for the basis of hybrid lethality. This model is clearly derived, but different, from that of PONTECORVO (1943) and is consistent with earlier

ideas that hybrid inviability arises as a consequence of complementary mutations in the isolated species.

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