Hybrid Lethal Systems in the *Drosophila melanogaster* Species Complex. I. The *maternal hybrid rescue* (*mhr*) Gene of *Drosophila simulans*

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ABSTRACT

Hybrid females from *Drosophila simulans* females × *Drosophila melanogaster* males die as embryos while hybrid males from the reciprocal cross die as late larvae. The other two classes are sterile adults. Letting *C*, *X*, and *Y* designate egg cytoplasm, *X*, and *Y* chromosomes, respectively, and subscripts *m* and *s* stand for *melanogaster* and *simulans*, $C_mX_mY_s$ males are lethal in the larval stage and are rescued by the previously reported genes, *Lhr* (*Lethal hybrid rescue*) in *simulans* or *Hmr* (*Hybrid male rescue*) in *melanogaster*. We report here another rescue gene located on the second chromosome of *simulans*, *mhr* (*maternal hybrid rescue*) that, when present in the mother, rescues $C_sX_mX_s$ females from embryonic lethality. It has been postulated that the hybrids not carrying the X_s like $C_mX_mY_s$ males are larval lethal and that the hybrids carrying both the C_s and the X_m like $C_sX_mX_s$ females are embryonic lethal. According to these postulates $C_sX_mY_s$ males (obtained by mating attached-*X simulans* females to *melanogaster* males) should be doubly lethal, at both embryo and larval stages. When both rescuing genes are present, *Hmr* in the father and *mhr* in the mother, males of this genotype are fully viable, as predicted.

REPRODUCTIVE isolation, especially lethality and sterility in interspecific hybrids, has been amply documented for various *Drosophila* species combinations (PATTERSON and STONE 1952; BOCK 1984, for reviews), but the genetic mechanisms are yet to be elucidated. DOBZHANSKY (1936) pioneered in the analysis of hybrid male sterility in crosses between *Drosophila pseudoobscura* and *Drosophila persimilis*, and many subsequent studies have extended his analysis [see COYNE and ORR (1989) for a recent review]. In these studies the genes for hybrid male sterility were mapped by backcrossing the fertile hybrid females to males of the parental species. The method, however, has many limitations (WU *et al.* 1992). Neither can it be applied to the analysis of the sex-specific hybrid lethality in the *Drosophila melanogaster-Drosophila simulans* combination, because surviving hybrids are sterile. But if genes that rescue the hybrids from lethality or sterility are available, we have an opportunity to analyze the genetic mechanisms of reproductive isolation. In fact, several rescue genes for such hybrids have already been discovered (see below).

In the cross between *melanogaster* females and *simulans* males, only sterile hybrid females eclose, while males develop slowly and die at the third-instar larval to prepupal stages (larval lethality) (Figure 1A) (STURTEVANT 1920, 1929; HADORN 1961; DAVID *et al.* 1974). In the reciprocal cross, the surviving hybrids are sterile males, while hybrid females die before the eggs hatch (embryonic lethality) (Figure 1B) (HADORN 1961; DAVID *et al.* 1974). Figure 1C and D show the results of crosses when attached-*X* females are used.

Hybrids always have heterospecific autosomes, while their cytoplasms and sex chromosomes differ in reciprocal crosses. For brevity of description, hybrids may be expressed as, for example, $C_mX_mY_s$, where *C* stands for the maternally generated egg cytoplasm, *X* and *Y* for the sex chromosomes, and subscripts *m* and *s* for *melanogaster* and *simulans*. Since $C_mX_mX_s$ females (coming from the cross between *melanogaster* females and *simulans* males, Figure 1A) are viable while $C_sX_mX_s$ females (from the reciprocal cross, Figure 1B) are embryonic lethal, it has been supposed that the cytoplasm or a maternal effect gene(s) is involved (STURTEVANT 1920, 1929; PONTECORVO 1943; HUTTER and ASHBURNER 1987; HUTTER, ROOTE and ASHBURNER 1990).

On the other hand, $C_mX_mX_s$ males (Figure 1A) and $C_m\widehat{X_mX_m}Y_s$ females (Figure 1C) die as larvae (WATANABE 1979; TAKAMURA and WATANABE 1980; HUTTER, ROOTE and ASHBURNER 1990). It has been assumed that the hybrids not carrying X_s or carrying Y_s are larval lethal (STURTEVANT 1920, 1929; PONTECORVO 1943; WATANABE 1979; TAKAMURA and WATANABE 1980; HUTTER and ASHBURNER 1987; HUTTER, ROOTE and ASHBURNER 1990). Recently, however, YAMAMOTO (1993) demonstrated that the absence of X_s results in the lethality but not the presence of Y_s by

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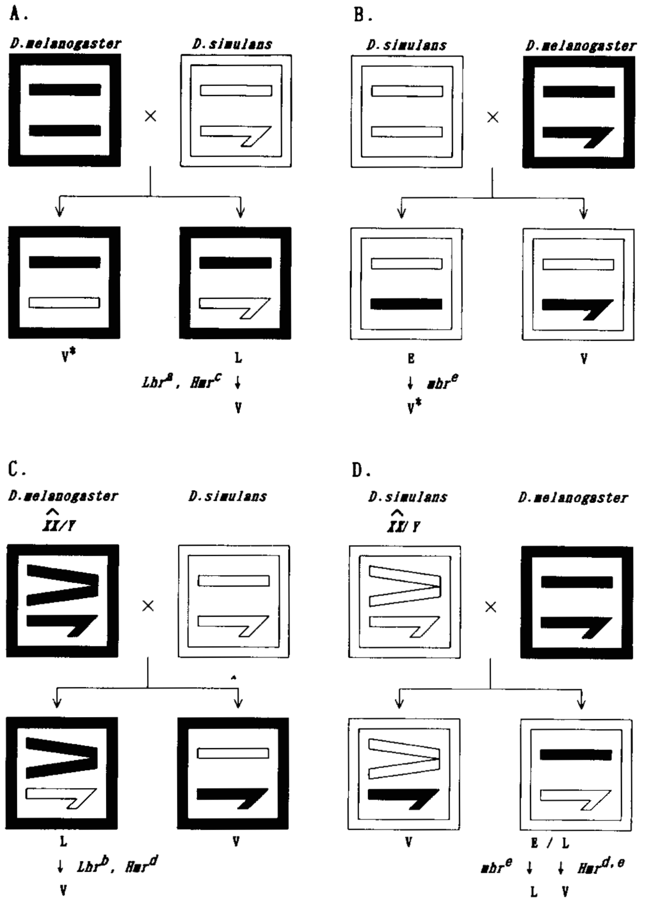

FIGURE 1.—Four types of interspecific crosses between *D. melanogaster* and *D. simulans*. Since all hybrids have heterospecific autosomes, only cytoplasms (boxes), *X* chromosomes [bars (attached-*X*'s are represented by attached bars)], and *Y* chromosomes (J shaped) are shown. V: viable; E: embryonic lethal; L: larval lethal; *: low viability at high temperature. Arrows mean that they are rescued by the genes shown ([a]WATANABE 1979; [b]TAKAMURA and WATANABE 1980; [c]HUTTER and ASHBURNER 1987; [d]HUTTER, ROOTE and ASHBURNER 1990; [e] present paper).

using a compound-*XY* chromosome of *simulans*. We tentatively describe that the hybrids not carrying X_s are larval lethal in this paper (see DISCUSSION).

Two rescue genes for such hybrids have been identified and two additional strains with rescue action have also been recovered. These are: (1) the *Lethal hybrid rescue* (*Lhr*,*2*-95) gene which was found in the K18 strain of *simulans* from Kokura, Japan, and which rescues $C_m\widehat{X_m}Y_s$ hybrid males or $C_m\widehat{X_m}X_mY_s$ hybrid females from larval lethality (Figure 1, A and C) (WATANABE 1979; TAKAMURA and WATANABE 1980), (2) the *Hybrid male rescue* (*Hmr*, *1*-31.84) gene which was found in the U79 strain of *melanogaster* from Uman, Ukraine, and which rescues from the same type of lethality (Figure 1, A and C) (HUTTER and ASHBURNER 1987; HUTTER, ROOTE and ASHBURNER 1990), (3) a strain of *simulans* from Florida, which rescues $C_sX_mX_s$ hybrid females from embryonic lethality (Figure 1B) (BOCQUET and TSACAS 1969), and (4) the Gif 255.1 strain of *melanogaster* from Taï, Ivory Coast, which rescues from the same type of lethality as (3) above (LACHAISE *et al.* 1986). Further details from the last two have not been reported.

We have searched for a gene(s) in *simulans* that rescues the hybrids from embryonic lethality in $C_sX_mX_s$ females. Two strains with the expected effect were found and the factor(s) responsible for such an effect was located on the second chromosome. Although the finer mapping required to rule out a linked cluster has not yet been done, we named it *maternal hybrid rescue* (*mhr*).

We first hypothesized that the hybrids carrying both C_s and X_m, like $C_sX_mX_s$ females (Figure 1B), would be embryonic lethal and that this lethality could be prevented by *mhr*. We also hypothesized that the hybrids not carrying X_s, like $C_mX_mY_s$ males (Figure 1A) and $C_m\widehat{X_m}X_mY_s$ females (Figure 1C), would be larval lethal and that this lethality could be prevented by *Hmr* of *melanogaster* and by *Lhr* of *simulans*. We thus expected that $C_m\widehat{X_m}X_mY_s$ females (Figure 1D) would be viable while $C_sX_mY_s$ males (Figure 1D) would be doubly lethal, once at the embryonic stage because of the presence of C_s and X_m, and once at the larval stage because of the absence of X_s. Finally, we expected that the $C_sX_mY_s$ males would be rescued from embryonic lethality by *mhr* and that they would be further rescued from larval lethality by *Hmr*. We show that these expectations were met in the experiments presented below.

MATERIALS AND METHODS

Strains: Five *D. melanogaster* stocks were employed. These were (1) Oregon-R, (2) Canton-S, (3) *bw*; *st*, (4) y^2 *w*, and (5) *Hmr* (provided by M. ASHBURNER). For descriptions of the original *Hmr* see HUTTER and ASHBURNER (1987), and of the others see LINDSLEY and GRELL (1968).

The following *D. simulans* stocks were examined or employed [10 laboratory strains: (1)–(10), and three natural population strains: (11)–(13)]. (1) y^{NC} (*yellow*-North Carolina), (2) *y* pn^2, (3) *y w f* (S10430, the stock number at the National Institute of Genetics), (4) *y w f* (S10440), (5) *net b py sd pm*, (6) b^2 bw^{K25} (synthesized by crossing *black*-2 and *brown*-Kokura25), (7) *y f*; *bw*; *st*, (8) *w*; *net*; *e*, (9) *Lhr* (K18), (10) *C(1)RM,y w/w m/Y* (attached-*X* strain), (11) Florida (provided by J. R. DAVID), (12) Islamorada, Florida, (13) Oita, Japan (O9). For descriptions of the original *Lhr* see WATANABE (1979), and of the other mutants see STURTEVANT (1929).

Matings: Virgin females were collected without anesthesia within 8 hr after eclosion, and were mated immediately. Males were collected with anesthesia, and 10–20 were kept without females for 5–7 days before mating. All matings were performed under constant light. If not mentioned otherwise, 8–10 females and 10–13 males were used for each mass mating.

Culture conditions: A standard cornmeal, agar, sugar, and yeast medium was used. Interspecific matings were performed at 23°, and as soon as larvae appeared, parental flies were transferred into new vials every 3–4 days. The first brood was raised at 23°, and the later broods at 18°.

TABLE 1

Numbers of hybrids from crosses of *D. simulans* ♀ × *D. melanogaster* ♂ using various strains

	D. melanogaster ♂											
	Oregon-R						Canton-S					
	23°			18°			23°			18°		
D. simulans ♀	♂	♀	Relative viability[a]	♂	♀	Relative viability	♂	♂	Relative viability	♂	♀	Relative viability
y w f (S10440)	793	18[b]	0.023	230	147	0.639	983	311[b]	0.316	83	75	0.904
y pn^{2}	161	0	0	81	21	0.259	33	2[b,c]	0.061	28	56	>1
Others[d]	2,513	0	0	1,011	18	0.018	368	12[b]	0.033	58	9	0.155

[a] Viability relative to males, assuming equal zygotic frequencies.

[b] Females died immediately after eclosion (temperature sensitive). Exceptions were nine individuals in the *y*^{NC} ♀ × Canton-S♂ cross.

[c] One was a gynandromorph.

[d] Data from nine strains [six laboratory strains–*y*^{NC}, *y w f* (S10430), *net b py sd pm*, *b*^{2} *bw*^{K25}, *y f*; *bw*; *st*, and *w*; *net*; *e*, and three natural population strains—Florida, Islamorada, and O9].

RESULTS

A maternal effect gene in *simulans* that prevents embryonic lethality in females from the cross between *simulans* females and *melanogaster* males: In the search for a gene(s) that rescues the hybrid females from embryonic lethality, females from a total of 11 *D. simulans* strains (eight laboratory strains and three natural population strains) were separately crossed with males of Oregon-R and Canton-S strains of *D. melanogaster* (Table 1). Although not mentioned in previous publications, escaper females occasionally appear, but die immediately after eclosion when flies are raised at 23°. Since the flies survive at 18°, we raised broods at two temperatures, 23° and 18°. Two out of 11 *simulus* strains [*y w f* (S10440) and *y pn*^{2}] produced a large number of hybrid females at 18°, especially when they were crossed to Canton-S males. These rescued hybrid females died at the pupal stage or immediately after eclosion when they were raised at 23°.

The results suggest three things: (1) there is a gene(s) in the two strains of *simulans* that rescues the lethal hybrid females, the point that will be dealt with in the present paper, (2) there probably exists a gene(s) in *melanogaster* that rescues the same lethal hybrid females [further evidence for such a gene is presented in a companion study by SAWAMURA, YAMAMOTO and WATANABE (1993)], and (3) the temperature-sensitive lethality at the pupal stage or immediately after eclosion in the rescued hybrid females and in the rare escaper hybrid females is probably caused by a separate gene(s). Analysis of this last aspect will be dealt with elsewhere (SAWAMURA 1993).

It may be noted in Table 1 that some crosses are represented by a small number of progeny flies. This is because the *simulans*♀ × *melanogaster*♂ matings often do not occur (pre-mating isolation: STURTEVANT 1920, 1929; WATANABE and KAWANISHI 1979; LEE and WATANABE 1987) and even when matings are successful oviposition often fails.

Assuming that there is a gene(s) with the lethal-hybrid rescue action in the two *simulans* strains, *y w f* (S10440) and *y pn*^{2}, we examined (1) whether the gene is recessive or dominant, (2) whether the gene(s) carried by the two strains occupy the same locus, and (3) on which chromosome the gene is located. Since the female viability appeared to depend partly on the paternal strains (Table 1), Oregon-R was used to minimize the background variations in the experiments presented below if not specially mentioned.

First, we made *simulans* intraspecific crosses between strains with and without the rescue action and, employing the resulting progeny females, made interspecific crosses with *melanogaster* males (Table 2, A and B). Except for one case, heterozygous *simulans* females did not suppress the hybrid female lethality irrespective of their cytoplasm. Therefore, the rescue action is not due to a cytoplasmically encoded factor but is due to a recessive gene(s). Females homozygous for the rescuing gene should produce 100% heterozygous hybrid females, while females heterozygous for the rescuing gene would produce 50% heterozygous hybrid females. Since the former females rescued hybrid females while the latter females did not, the rescue gene is not acting zygotically but maternally. Females from *y pn*^{2} (rescue strain)♀ × O9 (non-rescue strain)♂ produced a large number of hybrid females. There are two possibilities. First, female lethality in such hybrids is incompletely penetrant. Second, there may exist other genes in O9 that affect the rescue behavior. When we made the same cross later, no hybrid females were emerged (only 27 males). Table 2C shows the results when females from matings between two rescuing strains were employed. It is apparent that the two strains, *y w f* (S10440) and *y pn*^{2}, have allelic rescue gene(s). It is concluded that there is a recessive, maternal-effect gene(s) in *simulans*

TABLE 2

Hybrid female rescuing effect of *D. simulans* females heterozygous or homozygous for the rescuing gene when crossed with *D. melanogaster* Oregon-R males

D. simultans ♀ derived from		No. of hybrids		Relative viability
		♂	♀	
A. Rescue strain ♀	× Non-rescue strain ♂			
y w f (S10440) ♀	× *y f; bw; st* ♂	132	0	0
y w f (S10440) ♀	× *y w f* (S10430) ♂	374	8[a]	0.021
y w f (S10440) ♀	× O9 ♂	22	0	0
y w f (S10440) ♀	× Florida ♂	74	2	0.027
y pn^2 ♀	× *y w f* (S10430) ♂	164	15[b]	0.091
y pn^2 ♀	× O9 ♂	383	146	0.381
B. Non-rescue strain ♀	× Rescue strain ♂			
y f; bw; st ♀	× *y w f* (S10440) ♂	64	0	0
y w f (S10430) ♀	× *y w f* (S10440) ♂	271	19	0.070
O9 ♀	× *y w f* (S10440) ♂	65	0	0
O9 ♀	× *y* pn^2 ♂	9	0	0
C. Rescue strain ♀	× Rescue strain ♂			
y w f (S10440) ♀	× *y* pn^2 ♂	564	353	0.626
y pn^2 ♀	× *y w f* (S10440) ♂	405	360	0.889

Data at 18°.

[a] One female was *XXY* from nondisjunctional diplo-*X* egg. Her phenotype was y w f.

[b] One was a gynandromorph.

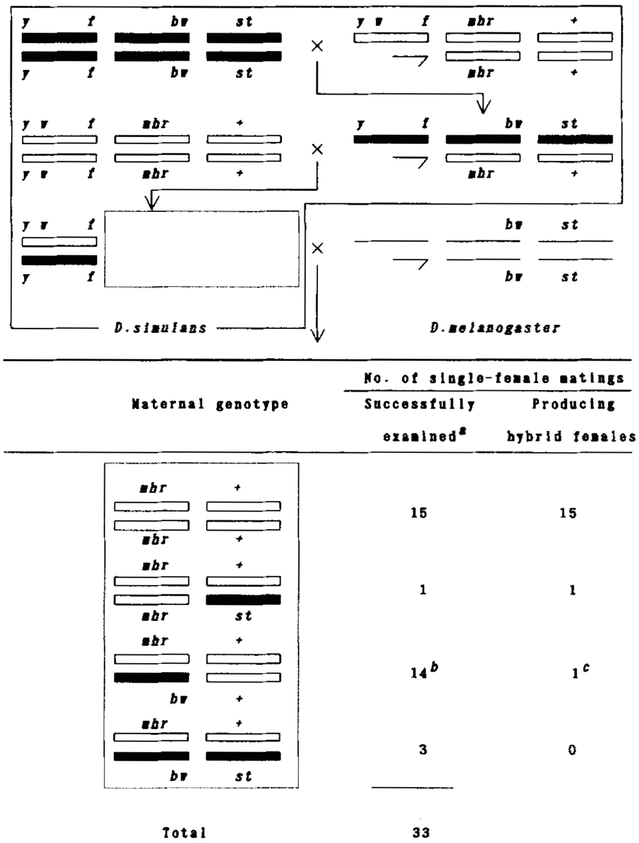

FIGURE 2.—Location of *mhr*. The chromosomes derived from the non-rescue strain [*y f; bw; st* (mhr^+/mhr^+)] are represented by solid bars, while the chromosomes derived from the rescue strain [*y w f* (S10440) (*mhr*/*mhr*)] are open. The *X*, the second, and the third chromosomes are shown. ([a] See text. [b]Although one mating produced only 14 progeny flies of which one was female (relative viability, 0.077) at 18°, we regard it as an escaper. [c]See text for the details.)

which rescues hybrid females from embryonic lethality. The gene is named *maternal hybrid rescue* (*mhr*).

In a separate experiment, male progeny from crosses between the rescuing and non-rescuing strains of *simulans* were backcrossed to females of the rescuing strain. The resulting females were individually crossed to *melanogaster* males. Some crosses produced hybrid females, while the other crosses did not (data not shown). These results confirm the conclusion that *mhr* is a recessive, maternal-effect gene. These results also indicate that *mhr* is not *X*-linked but resides in the autosome(s).

The autosomal location of *mhr* was examined in two experiments employing stocks in which the *X* and the two major autosomes, *2* and *3*, each carried a marker mutation(s). First, *y w f*/*y w f* (*mhr*/*mhr*) (S10440) females were crossed with *y f*/*Y*; *bw*/*bw*; *st*/*st* (mhr^+/mhr^+) males, and the male progeny, *y w f*/*Y*; *bw*/+; *st*/+ (*mhr*/mhr^+), were backcrossed to *y w f*/*y w f* (*mhr*/*mhr*) females. Female progeny are all *y w f*/*y w f*, but they carry autosomes in various combinations, (*bw* or +)/+; (*st* or +)/+. They were then crossed, in mass, to +/*Y*; *bw*/*bw*; *st*/*st melanogaster* males, and produced 196 +, 2 bw, 63 st, and 0 bw; st females in addition to 402 males at 18°. This result suggests that *mhr* is located on the second chromosome. Two bw females possibly represent escapers.

In the other experiment, a slightly modified mating scheme was employed as shown in Figure 2. Here interspecific crosses were made with individual females mated to two or three males (single-female matings). Out of more than 1,000 such matings only 36 were successful and 33 produced sufficient progeny to identify the genotype of their mothers. The results (Figure 2) confirm the conclusion above that *mhr* is located on the second chromosome. The remaining three mothers classified into unidentified genotype produced neither bw males nor wild-type females. Since they produced few progenies and the progenies were raised only at 23°, we could not determine the mothers were *mhr*/*mhr* or *mhr*/*bw*. One exceptional case contradicting the conclusion was observed where we obtained only 32 progeny flies of which five were females (relative female viability, 0.185) at 18°. These females may be escapers. Figure 2 also shows the difficulty in these interspecific crosses, especially in single-female matings. The deviation from the expected 1:1:1:1 ratio for the four genotypes of mothers most probably reflect genetic variations in crossabilities.

Next, we examined if *mhr* is a single gene or polygenes scattered along the second chromosome. *simulans* females heterozygous for the second chromosome bearing *mhr* were backcrossed to males of the rescuing strain which were homozygous for *mhr*-bearing chromosomes. Then the recombinant daughters were individually crossed to *melanogaster* males. Ninety-two crosses produced more than 10 hybrids at 18°. They were classified into three groups depending on the viability of hybrid females relative to the brothers. Forty-three percent of the crosses produced very few viable hybrid females (relative female viability was less than 0.1), suggesting that the mothers were heterozygous for the rescuing gene(s). For 36% of the crosses, relative female viability was higher than 0.3, indicating that the mothers were homozygous for the gene(s). For the final 21% of the crosses, however, relative female viability was intermediate between 0.1 and 0.3, perhaps as a consequence of the genetic background which might depend on minor genes or modifiers functioning to rescue the hybrids. Nevertheless we regard them as the rescuing because 20% of the interspecific crosses produced hybrid females in such a degree when non-recombinant mothers obtained by backcrossing *mhr*/+ males to *mhr*/*mhr* females were used. The ratio of the rescuing mothers to the non-rescuing mothers was about 1:1. Thus, we conclude that *mhr* is a single gene unless several genes clustering in a small region on the second chromosome.

To see if *mhr* has anything to do with the larval lethality of $C_mX_mY_s$ hybrid males, we crossed *melanogaster* y^2 *w* females with *simulans mhr*/*mhr* males. As shown in Table 3 hybrid males were not rescued. Only the exceptional C_mX_sO hybrid males derived from maternally nondisjunctional nullo-*X* survived as previously observed (WATANABE 1979). Thus, *mhr* rescues $C_sX_mX_s$ hybrid females from embryonic lethality, but not $C_mX_mY_s$ hybrid males from larval lethality. It may be noted that $C_mX_mX_s$ hybrid females also die at the pupal stage or immediately after eclosion at higher temperature than 23° as previously shown (STURTEVANT 1920, 1929; HADORN 1961; WATANABE *et al.* 1977; LEE 1978). This aspect along with the temperature-sensitive late lethality in the reciprocal cross ($C_sX_mX_s$: Table 1) is to be dealt with elsewhere (SAWAMURA 1993).

It has been supposed that *Lhr* not only rescues $C_mX_mY_s$ males completely from larval lethality but also $C_sX_mX_s$ females partially from embryonic lethality (WATANABE 1979). If this were so, *Lhr* itself would be acting as a weak *mhr*-like gene; however, this is not the case. Thirty inbred sublines from the original K18 strain carrying *Lhr* were crossed separately to Oregon-R males. Six lines produced no females, while the remaining 24 lines produced some females. We conclude that the original K18 strain used by WATANABE carried a polymorphic gene(s), separable from *Lhr*, that is responsible for the hybrid female rescue. Whether this gene is the same as *mhr* remains to be examined.

TABLE 3

The absence of rescuing effect for hybrid male larval lethality by *mhr*

	No. of hybrids			
	23°		18°	
D. simulans ♂	♀	♂	♀	♂
y w f (S10440) (*mhr*/*mhr*)	114[a]	0	1,119	1[b]
y pn^2 (*mhr*/*mhr*)	70[a]	0	219	2[b]
y f; *bw*; *st* (mhr^+/mhr^+)	353[a]	0	72	0

y^2 *w melanogaster* females were used.

[a] Females died immediately after eclosion (temperature sensitive).

[b] XO males from nondisjunctional nullo-*X* eggs. They showed paternal phenotypes.

$C_sX_mY_s$ hybrid males are doubly lethal once at the embryonic stage and once at the larval stage: Earlier we have hypothesized that the hybrids carrying both C_s and X_m like $C_sX_mX_s$ hybrid females (Figure 1B) are embryonic lethal, and we have shown above that the incompatibility can be negated by the *simulans* maternal-effect *mhr* gene. We have also hypothesized that the hybrids not carrying X_s like $C_mX_mY_s$ males (Figure 1A) are larval lethal. This lethality has been shown to be rescuable by the *Lhr* mutation in *simulans* and the *Hmr* mutation in *melanogaster* (WATANABE 1979; HUTTER and ASHBURNER 1987). If the two hypotheses are correct, $C_sX_mY_s$ males derived from the cross between *simulans* $\widehat{XX}/Y$ females and *melanogaster* X/Y males (Figure 1D), which are known to be lethal (HUTTER, ROOTE and ASHBURNER 1990), should be doubly lethal at both the embryonic and the larval stages.

Table 4 summarizes the results of single-female matings between *simulans* $\widehat{XX}/Y$ females and *melanogaster* X/Y (Oregon-R) males. First, out of more than 500 matings, 27 were successful employing $\widehat{XX}/Y$; mhr^+/mhr^+ mothers (Table 4A). In 23 matings a sufficient number of adult females was produced (each more than eight), but no males appeared. In fact $C_sX_mY_s$ male larvae (identifiable by the marker mutations) were not observed in any of the 23 matings. We conclude that these males are embryonic lethal. We then crossed $\widehat{XX}/Y$; mhr^+/mhr^+ females and X/Y; *mhr*/*mhr* (S10440) males. $\widehat{XX}/Y$; mhr/mhr^+ females thus obtained did not produce any male larvae when crossed to *melanogaster* males. $\widehat{XX}/Y$; mhr/mhr^+ females were backcrossed to X/Y; *mhr*/*mhr* (S10440) males. From these crosses $\widehat{XX}/Y$; *mhr*/*mhr* and $\widehat{XX}/Y$; mhr/mhr^+ females should be produced. Sixteen single-female matings were successful between these females and *melanogaster* males (Table 4B). Fourteen matings produced a sufficient number of females but no adult males. Seven out of 14, however, produced many hybrid male larvae. This is consistent with the 1:1

TABLE 4

Rescuing effect for hybrid male embyronic lethality by *mhr* in single-female matings of *D. simulans* $\widehat{XX}/Y$ ♀ × *D. melanogaster X/Y* (Oregon-R) ♂

	No. of single-female matings		Total No. of adult hybrids[a]		
D. simulans ♀	Successfully examined[b]	Producing $C_sX_mY_s$ larvae[c]	$C_s\widehat{X_sX_s}Y_m$	$C_sX_mY_s$	Exc.[d]
A. $\widehat{XX}^e/Y$; *mhr*⁺/*mhr*⁺	23	0	641	0	0
B. $\widehat{XX}/Y$; *mhr*/(*mhr* or *mhr*⁺)	14	7	630	0	1♂
C. $\widehat{XX}/Y$; *mhr*/*mhr*	25	25	795	0	2♂

[a] Since no temperature-sensitive viability effects are observed, pooled data at 23° and 18° are presented (see MATERIALS AND METHODS).

[b] See text.

[c] Hybrid males have black mouth hooks and denticle bands (yellow⁺) and yellow Malpighian tubles (white⁺), while hybrid females have yellow mouth hooks and denticle bands (y) and white Malpighian tubles (w). Hybrid males remained as third-instar larvae of small size when hybrid females eclosed to adults, similar to the lethal $C_mX_mY_s$ hybrids from the cross of *melanogaster* ♀ × *simulans* ♂.

[d] Produced by the detachments of $\widehat{XX}$. Males were y w.

[e] *C(1)RM, y w*, an attached-*X* chromosome marked with *y* and *w*.

TABLE 5

Rescuing effect for hybrid male larval lethality by *Hmr* in crosses of *D. simulans* $\widehat{XX}/Y$ ♀ × *D. melanogaster Hmr/Y* ♂

		No. of hybrids			
D. simulans ♀	Temp.	$C_m\widehat{X_sX_s}Y_m$	$C_sX_mY_s$	Relative viability[a]	Exc.[b]
A. $\widehat{XX}^c/Y$; *mhr*⁺/*mhr*⁺	23°	316	0	0	1♀1♂
	18°	381	56	0.147	1♂
B. $\widehat{XX}/Y$; *mhr*/*mhr*	23°	464	10	0.022	1♀8♂
	18°	188	206	>1	0

[a] Viability relative to $C_s\widehat{X_sX_s}Y_m$ females.

[b] Produced by the detachments of *XX*. Females were wild type, and males were y w.

[c] *C(1)RM, y w*, an attached-*X* chromosome marked with *y* and *w*.

ratio for rescuing females to nonrescuing females, which is expected when *mhr* is a single gene or clustering genes. It was possible to establish the $\widehat{XX}/Y$; *mhr*/*mhr* strains by further backcrossing $\widehat{XX}/Y$; *mhr*/(*mhr* or *mhr*⁺) females individually to *X/Y*; *mhr*/*mhr* (S10440) males. One such example when crossed to *melanogaster* males is shown in Table 4C in which 31 single-female interspecific matings were successful. Twenty-five matings produced a sufficient number of females but no adult males, and in all of these matings hybrid male larvae were detected. We conclude that $C_sX_mY_s$ hybrid males were rescued from embryonic lethality by the maternal effect of *mhr*, but died as larvae.

Whether these lethal larvae can be rescued further was examined by employing the *Hybrid male rescue* (*Hmr*) gene of *melanogaster* (Table 5). *Hmr* alone does not significantly rescue $C_sX_mY_s$ males (Table 5A). However, these males when rescued from the embryonic lethality by *mhr*, are now further rescued by *Hmr* and eclose as adults (Table 5B). Leaving aside the temperature sensitivity in rescued males [probably caused by the temperature sensitivity of the *Hmr* gene itself as was shown to be the case in the cross of *melanogaster*♀ × *simulans*♂ (HUTTER and ASHBURNER 1987)] we note that the combined *mhr-Hmr* rescue action is complete. Although it appears that *Hmr* also prevented the embryonic lethality of hybrid males in a small fraction of cases, it is possible that some yet unidentified factors are involved (*e.g.*, strain-dependent escapability from embryonic lethality, see Table 1).

DISCUSSION

The hybrid lethalities in the *D. melanogaster-D. simulans* interspecific crosses have been examined in four types of crosses (Figure 1). We identified and characterized a novel genetic factor in *simulans* that prevents the embryonic lethality in the hybrid females from the cross of *simulans* females and *melanogaster* males. The recessive, maternal-effect gene, *maternal hybrid rescue* (*mhr*), is located on the second chromosome.

The role of maternal-effect genes in post-mating isolation is not unique to the *melanogaster-simulans* combination, nor is it rare among other *Drosophila* species combinations. For example, lethality in hybrid females from the cross of *Drosophila montana* females and *Drosophila americana texana* males is caused by an interaction between a recessive, maternal-effect gene(s) of *montana* and an *X*-linked gene of *texana* (PATTERSON and GRIFFEN 1944). The hybrid females die at the embryonic stage (KINSEY 1967), which is a common phenotype of maternal-effect mutations.

In the four types of crosses (Figure 1) we have also summarized the previous and the present results concerning the rescue genes for lethal hybrids. Interspecific hybrids die either at the embryonic stage or at the larval stage. In the case of the lethality seen in $C_sX_mY_s$ males from the cross of *simulans* $\widehat{XX}/Y$ females and *melanogaster X/Y* males (Figure 1D) we have established that the hybrid males are doubly lethal at both the embryonic and the larval stages and that these lethalities are independently prevented by the *mhr* gene of *simulans* and the *Hmr* gene of *melanogaster*, at least in the major fraction of the cases. A possibility remains that *Hmr* can sometimes rescue from embryonic lethality. HUTTER, ROOTE and ASHBURNER (1990) conclude that somewhat more than a small fraction of $C_sX_mY_s$ hybrid males are rescued by *Hmr*. It seems that more rigorous examinations are needed for the action of *Hmr*.

We have identified two categories in the hybrid lethalities seen in the *melanogaster-simulans* interspecific crosses: (1) the hybrids carrying both C_s and X_m are embryonic lethal (C_s-X_m incompatibility) and (2) the hybrids not carrying X_s (or carrying Y_s) are larval lethal. YAMAMOTO (1993) recently showed that the hybrid larval lethality was independent of the presence of Y_s but that the hybrids not carrying X_s were larval lethal. The hybrid larval lethality may be attributed to X_m-A_s incompatibility, which is weakened by the presence of X_s (in other words, X_s is behaving like an antimorph).

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LITERATURE CITED

BOCK, I. R., 1984 Interspecific hybridization in the genus *Drosophila*. Evol. Biol. **18:** 41–70.

BOCQUET, C., and L. TSACAS, 1969 Tests of interspecific crosses between different stocks of *Drosophila simulans* and *D. melanogaster*. Drosophila Inform. Serv. **44:** 192.

COYNE, J. A., and H. A. ORR, 1989 Two rules of speciation, pp. 180–207 in *Speciation and Its Consequences*, edited by D. OTTE and J. ENDLER. Sinauer, Sunderland, Mass.

DAVID, J. R., F. LEMEUNIER, L. TSACAS and C. BOCQUET, 1974 Hybridation d'une nouvelle espèce, *Drosophila mauritiana* avec *D. melanogaster* et *D. simulans*. Ann. Genet. **17:** 235–241.

DOBZHANSKY, TH., 1936 Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics **21:** 113–135.

HADORN, E., 1961 Zur Autonomie und Phasenspezifität der Latalität von Bastarden zwischen *Drosophila melanogaster* und *Drosophila simulans*. Rev. Suisse Zool. **68:** 197–207.

HUTTER, P., and M. ASHBURNER, 1987 Genetic rescue of inviable hybrids between *Drosophila melanogaster* and its sibling species. Nature **327:** 331–333

HUTTER, P., J. ROOTE and M. ASHBURNER, 1990 A genetic basis for the inviability of hybrids between sibling species of *Drosophila*. Genetics **124:** 909–920.

KINSEY, J. D., 1967 Studies on an embryonic lethal hybrid in *Drosophila*. J. Embryol. Exp. Morphol. **17:** 405–423.

LACHAISE, D., J. R. DAVID, F. LEMEUNIER, L. TSACAS, and M. ASHBURNER, 1986 The reproductive relationships of *Drosophila sechellia* with *D. mauritiana*, *D. simulans*, and *D. melanogaster* from the Afrotropical region. Evolution **40:** 262–271.

LEE, W. H., 1978 Temperature sensitive viability of hybrid between *Drosophila melanogaster* and *D. simulans*. Jpn. J. Genet. **53:** 339–344.

LEE, W. H., and T. K. WATANABE, 1987 Evolutionary relationships based on matings, hybrids and proteins. Jpn. J. Genet. **62:** 225–239.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **627**.

PATTERSON, J. T., and A. B. GRIFFEN, 1944 A genetic mechanism underlying species isolation. Univ. Texas Publ. **4445:** 212–223.

PATTERSON, J. T., and W. S. STONE, 1952 *Evolution in the Genus Drosophila*. Macmillan, New York.

PONTECORVO, G., 1943 Viability interactions between chromosomes of *Drosophila melanogaster* and *Drosophila simulans*. J. Genet. **45:** 51–66.

SAWAMURA, K., 1993 Temperature-sensitive pupal lethality in hybrids between *Drosophila melanogaster* and its sibling species. Jpn. J. Genet. (in press).

SAWAMURA, K., M.-T. YAMAMOTO and T. K. Watanabe, 1993 Hybrid lethal systems in the *Drosophila melanogaster* species complex. II. The *Zygotic hybrid rescue* (*Zhr*) gene of *D. melanogaster*. Genetics **133:** 307–313.

STURTEVANT, A. H., 1920 Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics **5:** 488–500.

STURTEVANT, A. H., 1929 The genetics of *Drosophila simulans*. Carnegie Inst. Wash. Publ. **399:** 1–62.

TAKAMURA, T., and T. K. WATANABE, 1980 Further studies on the *Lethal hybrid rescue* (*Lhr*) gene of *Drosophila simulans*. Jpn. J. Genet. **55:** 405–408.

WATANABE, T. K., 1979 A gene that rescues the lethal hybrids between *Drosophila melanogaster* and *D. simulans*. Jpn. J. Genet. **54:** 325–331.

WATANABE, T. K., and M. KAWANISHI, 1979 Mating preference and the direction of evolution in *Drosophila*. Science **205:** 906–907.

WATANABE, T. K., W. H. LEE, Y. INOUE and M. KAWANISHI, 1977 Genetic variation of the hybrid crossability between *Drosophila melanogaster* and *D. simulans*. Jpn J. Genet. **52:** 1–8.

WU, C.-I., D. E. PEREZ, A. W. DAVIS, N. A. JOHNSON, E. L. PALOPOLI and M.-L. WU, 1992 Molecular genetic studies of postmating reproductive isolation in *Drosophila*, in *Molecular Paleo-population Biology*, edited by N. TAKAHATA and A. G. CLARK. Springer-Verlag (in press).

YAMAMOTO, M-T., 1993 Inviability of hybrids between *D. melanogaster* and *D. simulans* results from the absence of *simulans* *X* not the presence of *simulans Y* chromosome. Genetica (in press).

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