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The unexpected recovery of hybrids in a *Drosophila* species cross: a genetic analysis

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Summary

The species cross between *Drosophila melanogaster* and *D. simulans* was first described by Sturtevant in the 1920s. According to his description, the hybridization of *D. simulans* females and *D. melanogaster* males produces only (or almost only) male progeny. Female hybrids are embryonic lethal. Here it is shown that these traditional results no longer hold. Instead, *D. simulans* is polymorphic for factor(s) that qualitatively affect the outcome of species crosses to *D. melanogaster*. Remarkably, many, if not most, strains of *D. simulans* produce abundant female hybrids when crossed to *D. melanogaster* males. Genetic analysis of the difference between *D. simulans* strains that produce many versus few hybrid females shows that recovery of hybrid females depends on autosomal, maternally acting gene(s).

1. Introduction

The *D. melanogaster–D. simulans* hybridization is perhaps the best-studied of all species crosses in *Drosophila*. First described by Sturtevant (1920), this hybridization has been repeatedly studied over the last 75 years (e.g. Sturtevant, 1929; Muller & Pontecorvo, 1940; Pontecorvo, 1943; Lee, 1978; Watanabe, 1979; Hutter & Ashburner, 1987; Orr, 1991; Sawamura *et al.* 1993*a b*), and the results of the cross are well-known. When *D. melanogaster* females are crossed to *D. simulans* males, only female hybrids appear. Male hybrids die as third instar larvae (Hutter *et al.* 1990). The reciprocal cross produces only (or almost only) male hybrids (Sturtevant, 1920). Female hybrids die as embryos, although a few 'escaper' females sometimes appear (Hadorn, 1961).

Because all hybrids remain completely sterile, genetic analysis of reproductive isolation between *D. melanogaster* and *D. simulans* has proved difficult. Some progress has been made, however, along two fronts. First, various 'trick' crosses have been devised, allowing rough mapping of the factors causing hybrid inviability and sterility, e.g. use of attached-X, attached-XY, compound autosome, and triploid stocks (reviewed in Ashburner, 1989). Second, several mutations that 'rescue' normally inviable hybrids have been recovered (Watanabe, 1979; Hutter & Ashburner, 1987; Hutter *et al.* 1990; Sawamura *et al.* 1993*a, b*). These mutations provide some information about the developmental and genetic basis of hybrid inviability between these species. Unfortunately, all rescued hybrids remain sterile.

Recently, I reported that the traditional description of crossing results between *D. melanogaster* and *D. simulans* may be wrong (Orr, 1993). In particular, I found that several strains of *D. simulans* produce abundant female hybrids when crossed to *D. melanogaster* males, despite traditional claims that these females are embryonic lethals. These unexpected females were not rare escapers, as in some species crosses, up to 67% of hybrids were female. Although there have been sporadic reports of recovery of females from this cross (see Discussion), no one has systematically determined if such rescue is common; moreover, we know nothing about the genetic basis of this rescue.

Here I report new results showing that many, if not most, strains of *D. simulans* – including those newly established from the wild – produce abundant hybrid females when crossed to *D. melanogaster* males. Two of the strains tested, however, behave as traditionally described. All strains behave consistently through time. Thus, natural populations of *D. simulans* are polymorphic for a factor or factors that qualitatively affects the outcome of species crosses with *D. melanogaster*. The most common, 'wild-type', factor(s) no longer behaves as traditionally described. I investigate the genetic basis of this unexpected polymorphism. The results show that recovery of

female hybrids involves maternally acting nuclear factor(s) in *D. simulans*. The 'rescue' factor(s) is dominant to that causing inviability of female hybrids. Mapping experiments suggest that the difference between rescue and non-rescue strains may have a fairly simple genetic basis: recovery of hybrid females depends on gene(s) that are restricted to the second chromosome.

2. Materials and methods

(i) *Stocks and crosses*

The following stocks were used (information on the age and geographical origin of stocks is included where known).

D. melanogaster

Australia Fairfield-2 (wild-type line collected in Melbourne, Australia in 1980).

Bellows Falls (wild-type isofemale line collected by J. Coyne in Bellows Falls, Vermont in August, 1984).

Ives (wild-type stock established from 400 flies collected in Amherst, Massachusetts in August, 1975).

Napa isofemale 1 (wild-type line collected in Napa, California in December, 1991).

Okinawa NP280 (wild-type line collected in Okinawa by T. Yamazaki in the mid-1980s).

Oregon-R (standard wild-type line descended from flies collected by D. E. Lancefield in Roseburg, Oregon in or before 1925; this 'copy' of Oregon-R from C. H. Langley).

D. simulans

Cy^{NC}/Cy^{NC} (collected in North Carolina by T. K. Watanabe in the early 1980s).

v^C (a spontaneous mutation recovered by J. Coyne from the wild 'Florida City' stock in 1985–6).

v m (origin unclear; the *vermilion* allele is not, however, identical to v^C described above).

w^{2C} (X-ray induced in a wild stock by M. M. Green in the early 1980s).

Ro (from T. K. Watanabe; early 1980s).

Ubx^m/Dl (originally from A. H. Sturtevant's Caltech stock collection; *Dl* was isolated by C. Bridges in 1920).

Davis lines 1, 2, 3 (wild-type isofemale lines collected by T. Prout in Davis, California in August, 1991).

Florida City (wild-type isofemale line collected by J. Coyne in Florida City, Florida in June, 1985).

Islamorada (wild-type line from A. H. Sturtevant's Caltech stock collection; collected in Islamorada, Florida).

Solway-Hochman (wild-type line from A. H. Sturtevant's Caltech stock collection).

D. mauritiana

Synthetic (a mixture of six isofemale lines collected by O. Kitagawa on Mauritius in 1981; lines were pooled in 1983).

w (a spontaneous mutation recovered in a wild-type isofemale line by R. C. Woodruff; homologous to white of *D. melanogaster*).

D. sechellia

Robertson (wild-type isofemale line collected from the Seychelles archipelago in approximately 1980).

Unless otherwise indicated, species crosses were performed at 22° on a 12L:12D light cycle. While the cross of *D. melanogaster* females to *D. simulans* males is easy, the reciprocal cross is often extremely difficult (Sturtevant, 1920). The main difficulty appears to be sexual isolation – *D. simulans* females are very reluctant to accept *D. melanogaster* males (Sturtevant, 1929). I typically aged females and males in isolation for several days prior to crossing in an effort to improve the rate of hybridization. Nonetheless, in some cases, only tens of hybrids could be obtained after many weeks or months of crossing.

Hybrid female fertility was tested by setting up females with males from the parental stocks of both *D. melanogaster* and *D. simulans*. Hybrid male fertility was tested by microscopically examining testis squashes using dark field optics and scoring for the presence of motile sperm (Coyne, 1984). All χ^2 values were continuity-corrected to give more conservative tests.

(ii) *PCR assay*

Drosophila strains were tested for *Wolbachia* infection by performing polymerase chain reactions (PCR) or DNA extracted from whole flies (using single flies). Two sets of primers were used. First, the '76-99 forward' and '1012-994 reverse' primers described in O'Neill *et al.* (1992) were used to amplify the variable V1 and V6 regions of 16S rRNA genes from *Wolbachia*. If *Wolbachia* is present, PCR amplification yields a 0·9 kb product of 16S rDNA. Second, primers that are specific for insect mitochondrial 12S rRNA (see O'Neill *et al.* 1992) were simultaneously used as a control for DNA extraction and PCR failure.

The role of non-*Wolbachia* endosymbionts was tested by rearing flies on tetracycline, following the protocol of Hoffmann *et al.* (1986) for *D. simulans*.

3. Results

(i) *The phenomenon*

Unexpected female hybrids were first observed when females from three strains of *D. simulans* (v^C, *v m*, and Florida City) were crossed to males from three strains of *D. melanogaster* (Oregon-R, Bellows Falls, and

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Table 1. *Results of all possible species crosses between three strains of* D. melanogaster *and three strains of* D. simulans

Cross	Females	Males	% Females
sim *vm* × mel Oregon-R	186	471	28·3
sim *vm* × mel Bellows Falls	142	526	21·3
sim *vm* × mel Ives	55	256	17·7
sim Fla. City × mel Oregon-R	3	8	27·3
sim Fla. City × mel Bellows Falls	62	43	59·0
sim Fla. City × mel Ives	2	12	14·3
sim v^C × mel Oregon-R	150	90	62·5
sim v^C × mel Bellows Falls	201	202	49·9
sim v^C × mel Ives	62	45	57·9
mel Oregon-R × sim *vm*	297	0	100·0
mel Bellows Falls × sim *vm*	425	1*	99·8
mel Ives × sim *vm*	347	0	100·0
mel Oregon-R × sim Fla. City	429	0	100·0
mel Ives × sim Fla. City	382	0	100·0
mel Bellows Falls × sim Fla. City	190	0	100·0
mel Oregon-R × sim v^C	87	0	100·0
mel Bellows Falls × sim v^C	46	0	100·0
mel Ives × sim v^C	36	0	100·0

The top half of the Table shows results from crosses between *D. simulans* females and *D. melanogaster* males. Bottom half of the Table shows results from the reciprocal crosses. The small sample sizes in several crosses reflect the difficulty of the hybridizations.
* Male was *vm* and thus a product of maternal non-disjunction.

Ives). Preliminary data were reported in Orr (1993). All possible species crosses between these strains (and their reciprocals) have now been made. *All* of these *D. simulans* (hereafter 'sim') females × *D. melanogaster* (hereafter 'mel') males crosses produce hybrid females at surprisingly high frequencies (Table 1): 14% in the lowest case to 62% in the highest. Across strains, an average of 38% of hybrids are female. Although not statistically significant, it is worth noting that the number of female hybrids appears to vary with the strain of sim used, e.g. crosses using sim *vm* tend to produce about 20% hybrid females (no matter what mel stock is used), while those using sim v^C tend to produce about 50% hybrid females (no matter what mel stock is used). Stronger evidence that recovery of hybrid females depends on sim genotype will be presented later.

Unfortunately, all of these unexpected females were sterile (no eggs were observed). In all cases, the reciprocal crosses (mel female × sim male) behave as expected: only female hybrids appear (Table 1).

To see if recovery of female hybrids is typical of sim female × mel male crosses, a fairly random collection of sim strains was crossed to a random set of mel

Table 2. *Results of crosses between various wild-type and mutant of* D. simulans *and* D. melanogaster

Cross	Females	Males	% Females
sim v^C × mel Okinawa (NP280)	40	34	54·1
sim v^C × mel Australia (Fairfield 2)	37	43	46·3
sim w^{2C} × mel Napa (iso-f 1)	105	111	48·6
sim Islamorada × mel Oregon-R	46	51	47·4
sim Cy^{NC}/Cy^{NC} × mel Oregon-R	7	30	18·9
sim Ubx^M/Dl × mel Oregon-R	28	25	52·8
sim Davis 1 × mel Davis 1	41	125	24·7
sim Davis 2 × mel Davis 2	55	80	40·7
sim Davis 3 × mel Davis 3	3	6	33·3
sim *Ro*/+ × mel Oregon-R	27	735	3·5
sim *Ro*/+ × mel B. Falls	3	192	1·5
sim Solway-Hochman × mel Oregon-R	39	521	6·9
sim Solway-Hochman × mel B. Falls	12	110	9·8

strains (these strains were a sampling of those available at the University of California, Davis when these experiments began: see Materials and methods for the origins of strains). Remarkably, almost all sim female × mel male crosses produced abundant, but sterile, hybrid females (Table 2). As expected, the reciprocal mel female × sim male cross produced only females (not shown).

(ii) *Two 'non-rescue' strains of* D. simulans

Two sim strains were found, however, that more or less behave as traditionally described. As Table 2 (bottom) shows, sim *Ro*/+ and sim Solway-Hochman (a wild strain) consistently produce almost all male hybrids no matter which mel strains they are crossed to. These stocks will be referred to as 'non-rescue' strains to denote that female hybrids are not often recovered when crossed to mel males. The strains discussed earlier will be referred to as 'rescue' strains.

Isolation of non-rescue strains shows that recovery of females depends on the identity of the sim strain. The cross sim *Ro*/+ × mel Oregon-R, for instance, produces only 4% hybrid females, while the cross of sim v^C to the same mel strain produces 62% hybrid females. Similarly, sim Solway-Hochman × mel Oregon-R produces 7% females, while sim v^C × mel Oregon-R produces 50% females. All of these crosses were repeated several times over the course of 2 years and the results were very consistent: non-rescue strains of sim never produced more than a few hybrid females while rescue strains always produced many.

This rescue phenomenon does not appear to extend to other species that are closely related to *D. simulans*:

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Table 3. *Tests for female rescue using sibling species of D. simulans*

Cross	Females	Males	% Females
maur Synthetic × mel Oregon-R	0	485	0·0
maur *white* × mel Oregon-R	0	20	0·0
sech Robertson × mel Oregon-R	0	100	0·0

maur = *D. mauritiana* and sech = *D. sechellia*.

as Table 3 shows, neither *D. mauritiana* nor *D. sechellia* females produce hybrid females when crossed to *D. melanogaster*.

(iii) *Possible environmental effects*

It seemed likely that the unexpected recovery of hybrid females reflected some unusual environmental effect: hybrid females were consistently appearing after 70 years of study because the species cross was being performed on an unusual *Drosophila* medium, or at an unusual temperature or humidity, etc. Crosses showed, however, that rescue sim strains always yield abundant hybrid females whether crosses are performed on Davis, Rochester, or Instant medium, or in several different incubators or buildings.

The only environmental factor that influenced recovery of hybrid females was one already known to affect mel-sim species results: with some sim strains, hybrid females – just like hybrid males (Lee, 1978) – are less viable at higher temperatures. For instance, the cross sim Fla. City × mel Bellows Falls produces 45% females at 22° ($n = 111$ hybrids), but 5·3% females at 25° ($n = 56$), ($\chi^2 = 25{\cdot}26$, $P < 0{\cdot}001$).

Cooler temperatures do not, however, appear to explain why hybrid females were rarely recovered in the past as *Drosophila* species crosses are often performed at lower temperatures. More importantly, some sim strains produce abundant hybrid females even at room temperature: sim v^c × mel Oregon-R produces 45% females at 22° ($n = 137$) and 50% females at 25° ($n = 58$) ($\chi^2 = 0{\cdot}20$, $P = 0{\cdot}65$). But because temperature can affect the percent females recovered with at least some strains, all of the crosses that follow were performed at standard conditions of 22° and 12L:12D.

(iv) *Possible role of endosymbionts*

Mutations are known that restore the viability of normally inviable mel-sim hybrids (reviewed by Sawamura *et al.* 1993*b*). The fact that virtually all sim strains tested produce hybrid females would seem, however, to rule out the involvement of such mutations: it is very unlikely that all of the strains used above happen to carry a hybrid rescue mutation.

Table 4. *PCR tests for the presence of the endosymbiont* Wolbachia*-specific DNA sequences (16S rDNA)*

Strain	PCR amplification
sim v^c	–
sim *vm*	–
sim Fla. City	–
sim *Ro*/+	–
sim Solway-Hochman	–
mel Oregon-R	–
mel Ives	+
mel Bellows Falls	+

+ = infected with *Wolbachia*. – = not infected with *Wolbachia*. Most strains tested twice (results were always identical). Control PCR reaction with insect mitochondrial 12S rRNA primers always simultaneously run.

Instead, recovery of females from almost all crosses suggests that these stocks may have been fortuitously 'cured' of some factor that normally causes hybrid female inviability. Curable cellular endosymbionts that cause incompatibilities between different strains or species of insects are well known, and curable cytoplasmic incompatibility has been found in at least five orders of insects (Hoffmann *et al.* 1986). Most, if not all, of these cases involve the rickettsia *Wolbachia* (O'Neill *et al.* 1992). Hybrid inviability typically results when females from uninfected strains are crossed to males from infected strains. If males are 'cured' of *Wolbachia* with antibiotics, they become compatible with uninfected females. Although no cases are yet known where endosymbionts cause sex-specific lethality, it seemed possible that sim-mel hybrid female inviability might involve a curable endosymbiont. In particular, it seemed possible that the mel stocks used above were fortuitously cured of an endosymbiont that normally kills hybrid females (fortuitous curing could, for instance, occur through accidental heat stock). Alternatively, rescue sim strains may harbor *Wolbachia*, while non-rescue do not.

I tested this possibility in several ways. First, using PCR, I tested for the presence of *Wolbachia*-specific DNA sequences (16S rDNA) in a sample of mel and sim stocks (both rescue and non-rescue). Table 4 shows that hybrid females are recovered whether a sim strain (e.g. v^c) is crossed to infected (Bellows Falls, Ives) or uninfected (Oregon-R) mel males. Conversely, despite the fact that all of the sim strains – rescue and non-rescue – are uninfected, they give qualitatively different results when crossed to a standard mel strain (e.g. Oregon-R). The presence or absence of *Wolbachia* does not, therefore, account for the presence or absence of hybrid females.

The primers used above were designed to be specific for *Wolbachia* 16S sequence (O'Neill *et al.* 1992); they

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Table 5. *Tetracycline treatment of rescue and non-rescue strains of* D. simulans

Cross	Females	Males	χ^2
sim *Ro*/+ (tet) × mel Oregon-R (tet)	7	162	0·00
sim *Ro*/+ (con) mel Oregon-R (con)	23	487	
sim v^C (tet) × mel Oregon-R (tet)	12	7	0·16
sim v^C (con) × mel Oregon-R (con)	12	11	

tet = tetracycline treated. con = non-tetracycline treated controls.

almost certainly would not detect the presence of other, unrelated, endosymbionts. To test if a non-*Wolbachia* bacterial endosymbiont plays a role in sim rescue, I tetracycline-treated a rescue sim strain, a non-rescue sim strain and a standard mel strain. To ensure that tetracycline treatments were effective, I simultaneously treated the W and R strains of *D. simulans* (kindly provided by M. Turelli). The R strain harbors a curable *Wolbachia*, while the W strain is uninfected. W female × R males crosses are incompatible, with low egg hatch (Hoffmann *et al.* 1986). These controls confirmed that W female × R male (both lines untreated) progeny have very low egg hatch rates. When both strains were reared on tetracycline for one generation, the W (tet) female × R (tet) male progeny showed very high egg hatch rates (not shown).

Tetracycline treatment did not, however, affect the results of sim-mel species crosses. Species crosses using a non-rescue sim strain (*Ro*/+) produce very few females (~ 4%) regardless of whether the sim and mel strains had been reared for one generation on tetracycline-treated medium or on standard medium (Table 5; $\chi^2 = 0{\cdot}00$, $P = 0{\cdot}99$). Similarly, species crosses using a rescue sim strain (v^C) produce many females (~ 50–60%) whether or not the strains were treated with tetracycline (Table 5; $\chi^2 = 0{\cdot}16$, $P = 0{\cdot}69$). All hybrid males and females from the 'cured' crosses were scored for fertility; all were completely sterile. Thus hybrid sterility between these species also does not involve a curable endosymbiont.

Perhaps the clearest evidence against the fortuitous-laboratory-curing hypothesis is shown in Table 2. Flies were collected from the wild in Davis, California and several iso-female lines of each species were established. Species crosses revealed that even newly established wild lines produce abundant hybrid females (table 2). The cross of sim Davis 1 × mel Davis 1 is of particular interest: these strains were never exposed to our standard medium – instead, they were caught, maintained and hybridized on Instant Drosophila food.

Thus the recovery of abundant hybrid females is neither an artifact of some unusual environmental condition nor a result of accidental laboratory curing. Rather, *D. simulans* is clearly polymorphic for some factor(s) that determines the fate of hybrid females. Remarkably, the rescue type appears to be most common (10 of 12 sim strains tested), while the 'traditional' non-rescue type is rarer (2 of 12). Our main task is to explain the genetic basis of this polymorphism.

(v) *The genetic basis of sim rescue*

Reciprocal crosses were made between the rescue stock sim v^C and each of the two non-rescue stocks, sim *Ro*/+ and sim Solway-Hochman. F_1 females from these crosses were then crossed to mel oregon-R. Several important observations emerge from these species crosses (table 6). First, rescue does not depend on the origin of the cytoplasm: although there is some variation between reciprocal crosses, F_1 females produce many more hybrid females than do non-rescue stocks whether F_1 females carry cytoplasm ultimately derived from a rescue or a non-rescue stock. (Too much should not be made of the differences between reciprocal crosses: in the case of *Ro*/+, F_1 females with non-rescue mothers produce more hybrid females, while in the case of Solway-Hochman, F_1 females with rescue mothers produce more hybrid females. In any case, the results of these hybridizations tend to vary somewhat even when repeating the same cross.) Second, rescue is dominant to non-rescue: in all cases, F_1 females between the rescue and non-rescue stocks produce many hybrid females (Table 6). Although there is variation, the percent hybrid females recovered is far higher than that seen with non-rescue stocks.

Third, Table 6 shows that the gene(s) causing rescue is maternally acting. If rescue were due to a single

Table 6. *Rescue involves a dominant, maternally acting factor(s) from* D. simulans

Cross	+Females	+Males	v Males	% Females
sim (*Ro*/+ × v^C) × mel Oregon-R	269	146	147	47·9
sim (v^C × *Ro*/+) × mel Oregon-R	106	105	84	35·9
sim (S-H × v^C) × mel Oregon-R	65	42	35	45·8
sim (v^C × S-H) × mel Oregon-R	172	55	44	63·5

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Table 7. *Mapping of gene(s) causing hybrid female rescue. sim females having the genotype shown at the left were crossed to mel males and the sex ratio of hybrid progeny scored*

Maternal genotype	Females	Males	χ^2
X_nX_n†	143	608	3·85
X_rX_n	50	306	
2_n2_n	14	370	9·29**
2_r2_n	19	162	
3_n3_n	52	602	3·39
3_r3_n	20	139	

* $P < 0{\cdot}05$, ** $P < 0{\cdot}005$.
† n = chromosome derived from a non-rescue stock (sim *Ro*/+); r = chromosome derived from rescue stock (Cy^{NC} or *Dl*).
To obtain backcross mothers differing in second chromosome genotype the following crosses were made: sim Cy^{NC}/Cy^{NC} females × sim *Ro*/+ males. F_1 $Cy^{NC}/+$ males were then backcrossed to *Ro*/+ females, yielding two genotypes of females: $Cy^{NC}/+$ and +/+. These females were separately crossed to mel Oregon-R males and the number of resulting hybrid females and males scored. Unfortunately, we have little power to detect second chromosome rescue factors as the Cy^{NC}/Cy^{NC} marker stock is a poor rescuer, yielding only 18% hybrid females (see Table 2). For the third chromosome, analogous crosses were made except that the third was marked with *Dl*.

zygotically acting factor, only half of all hybrid females in Table 6 would inherit the v^C rescue allele. Thus, rescue could only be half as great as that seen with pure sim v^C mothers. In fact, F_1 (sim v^C × non-rescue) females produce about as many hybrid daughters as pure v^C females (in different trials, the sim v^C × mel Oregon-R cross produced 63% ($n = 240$) and 45% ($n = 137$) females). The rescue allele(s) must, therefore, be mostly dominant and nuclear, but maternally acting.

(vi) *Mapping of rescue factor(s)*

Because rescue is dominant, mapping of the gene(s) involved requires backcrossing to a non-rescue strain, yielding two genotypes of sim mothers – 'rescue/non-rescue' and 'non-rescue/non-rescue'. These sim females can then be crossed separately to mel males to determine which chromosome(s) harbor maternally-acting rescue factors. Because almost all sim strains tested show rescue, this design requires use of dominantly marked rescue strains. Dominant visible rescue stocks are available for each of the major autosomes (no marker is required for the X as backcross female genotype can be controlled by backcrossing either through F_1 males carrying a rescue stock X or through reciprocal F_1 males carrying a non-rescue stock X).

Separate backcross analyses were performed for the X, second and third chromosomes (for details, see Table 7). The dot fourth, which represents 1–2% of the genome, was not studied. In all cases, backcrossing was performed through F_1 males to ensure that visible mutations mark the species origin of entire (unrecombined) chromosomes.

Table 8. *Addition of the In(1)AB chromosome does not cause any additional hybrid female rescue*

Cross	Females	Males	% Females
mel *FM4,B*/*In(1)AB* × sim Fla. City	177	76*	70·0
sim *vm* × mel Oregon-R	137	226	37·4
sim *vm* × mel *In(1)AB*	41	102	28·7
sim v^C × mel Oregon-R	111	69	61·7
sim v^C × mel *In(1)AB*	9	10	47·4

* All rescued males were non-*Bar*, and thus carried the *In(1)AB* chromosome, as expected.

Data from the first backcross (Table 7) show that the genes involved are not X-linked: sim females who carry an X from a rescue stock produce no more hybrid daughters than females who carry both X's from a non-rescue stock (although the χ^2 statistic has borderline significance [$P = 0{\cdot}05$], the effect is in the wrong direction). Thus the genes causing rescue are autosomal. Indeed, females who carry a second chromosome from a rescue stock produce significantly more hybrid daughters then females who carry both second chromosomes from a non-rescue stock (Table 7, $P = 0{\cdot}002$). The third chromosome, on the other hand, has no discernible effect on rescue (Table 7, $P = 0{\cdot}09$), although I cannot rule out the presence of minor factors.

In sum, the difference between the rescue and non-rescue strains of sim seems to have a fairly simple genetic basis: the gene(s) involved appear to be limited to chromosome 2.

(vii) *Is rescue complete?*

We know that some sim rescue strains do not cause complete rescue (see Table 1). When sim *vm*, for instance, is crossed to mel, only about 20% of hybrid progeny are female. Will the addition of a hybrid female rescue mutation to such hybrids cause any 'additional' hybrid rescue? The crosses reported in Table 8 test the effect of introduction of the *D. melanogaster* rescue mutation *In(1)AB* into hybrids. The *In(1)AB* chromosome causes partial rescue of hybrid males and females (Hutter *et al.* 1990); it is unclear whether these effects are due to one or two X-linked rescue mutations (Sawamura *et al.* 1993*a*). Although control crosses show that *In(1)AB* rescues normally inviable hybrids (Table 8, first line), *In(1)AB* does not improve hybrid female recovery when crossed to the 'weak' rescue strain, sim *vm* ($\chi^2 = 3{\cdot}31$, $P = 0{\cdot}07$).

This result suggests (but certainly does not prove) that *In(1)AB* and the rescue due to *vm* involve the

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same development pathway. If *In(1)AB* and *v m* acted on independent (additive) pathways, the addition of *In(1)AB* to a sim *v m* cross should improve recovery of hybrid females.

4. Discussion

The most important result of these experiments is the simplest: the *D. simulans* female × *D. melanogaster* male species cross does not behave as traditionally described. Instead, many *D. simulans* strains produce abundant hybrid females when crossed to *D. melanogaster* males.

Most of the experiments reported here attempted to determine what distinguishes 'rescue' from the less-common 'non-rescue' strains of *D. simulans*. The results clearly show what does not cause rescue. Recovery of hybrid females is not an artifact of accidental laboratory 'curing' of strains: even strains newly-established from the wild produce abundant hybrid daughters when crossed to mel (Table 2). Moreover, the presence versus absence of the cellular endosymbiont *Wolbachia* does not account for the rescue polymorphism (Table 4). Indeed, rescue/non-rescue behavior has nothing to do with any tetracycline curable endosymbiont (Table 5). Further tests show that neither hybrid inviability nor sterility in the *D. simulans–D. melanogaster* hybridization is caused by curable endosymbionts.

Instead, crosses show that rescue is due to nuclear, but maternally acting, factor(s). The rescue alleles are dominant to the non-rescue alleles (Table 6). Mapping experiments suggest that the difference between the rescue and non-rescue sim stocks may have a fairly simple genetic basis: the gene or genes causing recovery of hybrid females appear to be limited to the second chromosome (Table 7).

It is possible that the rescue found here involves alleles of previously discovered hybrid rescue genes. In some ways, for instance, the behavior of the sim rescue stocks mimics that of *maternal hybrid rescue* (*mhr*) from *D. simulans*. Sawamura *et al.* (1993*a*) found two marker strains of sim that, when crossed to mel males, produced many hybrid females. They showed that this rescue mutation was maternally acting and linked to chromosome 2. In other ways, however, our results differ: while *mhr* is recessive (Sawamura *et al.* 1993*a*, the rescue characterized here is almost completely dominant. Last, while *mhr*-rescued females die at temperatures above 22° (Sawamura *et al.* 1993*a*), some of the present strains produce abundant hybrid females even at room temperature. Alternatively, the rescue reported here could involve a maternally acting allele of the *Lethal hybrid rescue* (*Lhr*) locus, which also resides on chromosome 2 of sim (Watanabe, 1979). Although the original *Lhr* allele has no known maternal effect, Hutter *et al.*'s (1990) model of the genetic basis of hybrid inviability posits some maternal effect of *Lhr*. Unfortunately, this matter cannot be settled with certainty as complementation tests with *mhr* and *Lhr* are not possible due to the dominance of the present rescue.

The important issue is not, however, whether the rescue observed here involves factors that may turn out to be allelic with known rescue mutations. Rather, the important point is that recovery of hybrid females is not due to some rare rescue 'mutation' that segregates at low frequencies in natural populations (Watanabe, 1979; Hutter & Ashburner, 1987) or that must be induced by mutagenesis (Hutter *et al.* 1990). Instead, recovery of female hybrids appears to be common and perhaps typical (Tables 1 and 2). Whatever locus or loci are involved, *D. simulans* is clearly polymorphic for factors that qualitatively affect the fate of species hybrids, and the rescue variant segregates at a very high frequency. Indeed, the present results suggest that hybrid female rescue is 'wild-type'.

Because the *D. melanogaster–D. simulans* species cross was well-studied by Sturtevant (1920, 1929) and co-workers early in the century, it is tempting to speculate that the crossing behavior of these species has changed within the last 70 years. This is not as implausible as it may at first seem: rapid invasion of cellular endosymbionts (cf. Hoffmann *et al.* 1986) could, for example, cause rapid changes in crossing relations between taxa. Although we have ruled out any direct role for tetracycline-sensitive endosymbionts (including *Wolbachia*), related scenarios involving rapid hitchhiking of some mutant allele with a tetracycline-resistant cellular endosymbiont remain formally possible.

All such scenarios are, however, mere speculation: we simply to not know if such historical changes occurred or if 'modern' crosses are merely performed in some way that subtly differs from traditional crosses. Indeed, hybrid female viability may be an extremely 'touchy' threshold character: under certain conditions, all sim strains may produce no female hybrids, while under slightly different conditions, some strains – but not those that slightly differ genetically – may produce abundant females. This threshold model might also explain why the sim-mel cross differs from the maur-mel and sech-mel crosses (Table 3), despite the fact that hybrid inviability appears to have a similar genetic basis in all three hybridizations (e.g. the rescue mutation *Hmr* rescues hybrids from all three species crosses [Hutter *et al.* 1990]). Alternatively, the late discovery of abundant hybrid females may reflect the sheer difficulty of the hybridization: while the cross of *D. melanogaster* females to *D. simulans* males is easy (and thus well-studied), the reciprocal cross analysed here is extremely difficult (and, so, less well-studied).

In any case, it is interesting to note that isolated appearances of abundant hybrid females in this species cross have been reported previously. Lachaise *et al.*

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(1986), for instance, found two *D. simulans* strains that produced many hybrid daughters when crossed to *D. melanogaster* males, although rescue appeared to depend on crossing these strains to one particular *D. melanogaster* line. Moreover, Sturtevant (1929, p. 8) himself, in his classic monograph on *D. simulans*, noted that '[o]ne large series of matings consistently gave females when the *simulans* mother came from one particular shock, and no females when she came from a certain other stock; but more recent experiments, using the first of these stocks and various other stocks, have given from 1 to 5 per cent of females in every series. This difference in behavior remains unexplained.'

The present results show that this 'exceptional' behavior may now be typical of *D. simulans*.

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