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The Effects of Interspecific *Y* Chromosome Replacements on Hybrid Sterility Within the *Drosophila simulans* Clade

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ABSTRACT

We attempted to introgress *Y* chromosomes between three sibling species of *Drosophila*: *D. simulans*, *D. sechellia* and *D. mauritiana*. Four *D. sechellia Y* chromosomes were introgressed into *D. simulans* without loss of fertility whereas the four reciprocal introgressions (*D. simulans Y* introgressed into *D. sechellia*) all result in sterility. Both reciprocal *Y* introgressions of *D. simulans* and *D. mauritiana* (four of each) also result in sterility. Compared with *D. simulans* males, the males with the *D. sechellia Y* chromosome in *D. simulans* background had lower productivity but only after multiple matings with virgin females. These males also were inferior compared with pure species males in sperm displacement and/or remating ability. The two different *Y* genotype males, however, were comparable in viability, longevity and mating success in female choice tests. We also use our results to estimate the effective number of autosomal loci interacting with *X*-linked genes to produce hybrid male sterility.

IN most species of *Drosophila*, including those of the *melanogaster* species subgroup, *X*O males are fully viable and somatically normal but invariably sterile because of *Y*-linked male fertility genes. In *Drosophila melanogaster*, at least six *Y*-linked genes are each required for fertility [reviewed in WILLIAMSON (1976) and GATTI and PIMPINELLI (1983)]. These fertility factors have been linked to lampbrush loops in *Drosophila hydei* [reviewed in HESS (1976) and HENNIG *et al.* (1989) and in *D. melanogaster* (BONACCORSI *et al.* (1988)]. In *Drosophila affinis* and a few other exceptional species, *X*O males are fully fertile (VOELKER and KOIJMA 1971; ASHBURNER 1989).

Within the genus, certain regions of the *Y* chromosome have the potential for rapid evolution. VOGT *et al.* (1986) find that the *ay1* family of *Y*-specific repeated sequence associated with the lampbrush loops in *D. hydei* is found in only two other species closely related to *D. hydei*. In another example, when probed with the *D. melanogaster Stellate*, the *Y*-linked repetitive sequence, *Suppressor of Stellate*, is detected with great intensity in *D. melanogaster* and *Drosophila simulans*, with less intensity in *Drosophila sechellia* and *Drosophila mauritiana*, and apparently not at all in *Drosophila yakuba* (LIVAK 1984, 1990; JOHNSON *et al.* 1992). Whether differences in the actual copy number or differences in the sequence similarity to the *D. melanogaster* probe are being detected, it is clear that *Suppressor of Stellate* sequences diverge rapidly, as all of the above species are in the *melanogaster* species subgroup (see also BALAKIREVA *et al.* 1992). LOHE and ROBERTS (1990) found that the *Y* of *D. simulans*, unlike that of *D. melanogaster*, possessed few, if any, functional *rRNA* genes but had an amplification of non-transcribed spacer elements. There is also ample evidence for rapid cytological evolution of the *Y* chromosome (see DOBZHANSKY 1935; MILLER and ROY 1964; STEINEMANN 1982; ASHBURNER 1989, Ch. 20).

Because the *Y* chromosome is required for male fertility and it can diverge rapidly, the *Y* could be expected to play a large role in the sterility in interspecific hybrids. Interactions between heterospecific *X* and *Y* chromosomes have been proposed (HALDANE 1932) to explain HALDANE's (1922) rule, namely, in cases of unisexual hybrid inviability and sterility, it is the heterogametic sex that is most affected. The empirical evidence for a *Y* effect and/or *X-Y* interactions in hybrid sterility in Drosophila is mixed (DOBZHANSKY 1936; HENNIG 1977; COYNE 1985; ORR 1987, 1989; PANTIZIDIS and ZOUROS 1989; PANTIZIDIS, GALOUOPOULES and ZOUROS 1993; JOHNSON and WU 1992; JOHNSON *et al.* 1992; ZENG and SINGH 1993). It is clear, however, that the *Y* chromosome in some interspecific crosses is involved in hybrid sterility.

COYNE (1985) concluded that *X-Y* interactions were a major cause of the sterility observed in *D. simulans*/*D. mauritiana* hybrids. In his study, males with an intact *X* from *D. simulans*, a *Y* from *D. mauritiana*, and a mixed autosomal background were seldom fertile, whereas males with a recombinant *X* (both *D. simulans* and *D. mauritiana* in origin) and the same *Y* and autosomal background were more often fertile. A *Y* chromosome sterility effect is quite evident from the data. It is still possible, however, that there is no incompatibility between the *D. simulans X* and the *D. mauritiana Y* as the sterility could be due to *X*-autosome and *Y*-autosome interactions.

Previously (JOHNSON and WU 1992; JOHNSON *et al.* 1992), we reported the introgression of a *D. sechellia* *Y* chromosome (Y_{sec}) into *D. simulans* background. Molecular probes were used to confirm the identity of the *Y* chromosomes. The males with the interspecific *Y* are as fertile as the pure species males but other fitness components were not examined in the previous studies. The reciprocal introgression (Y_{sim} in *D. sechellia* background) results in sterility (JOHNSON *et al.* 1992) but the Y_{sim} does not interact with any of the *X*-linked hybrid sterility factors that were tested. ZENG and SINGH (1993) have also independently introgressed a Y_{sec} chromosome into *D. simulans* using a different approach.

An asymmetric relationship of the effects on hybrid fitness caused by genetic interactions is predicted by certain models of the evolution of postmating reproductive isolation (WU and BECKENBACH 1983; ZOUROS 1986; ZENG and SINGH 1993). These models, extending the ideas of DOBZHANSKY (1937) and MULLER (1942), assume that changes in at least two loci are required for reproductive isolation to evolve and that these changes arise independently. With this independence, it would not be expected that the same loci which cause reproductive isolation in one direction of the cross would also cause reproductive isolation in the other direction of the cross; hence, the interactions should be asymmetric. Furthermore, in order for the reproductive isolation to evolve, the loci causing the reduction of fitness in hybrid backgrounds should not reduce fitness in their normal background. An examination of the components of fitness of Y_{sec} introgressions would determine the degree of the asymmetry of the fitness reduction caused by introgressions of heterospecific *Y* chromosomes.

The previous studies on the *Y* effect have each considered only a single *Y* chromosome and background for each species. Thus, it is not known whether there exists intraspecific variation for *Y*-linked hybrid sterility. In fact, except for the studies on hybrid inviability rescue (WATANABE 1979; HUTTER, ROOTE and ASHBURNER 1990; SAWAMURA, TAIRA and WATANABE 1993; SAWAMURA, YAMAMOTTO and WATANABE 1993), there have been few studies which directly address intraspecific polymorphism for hybrid sterility/inviability in Drosophila. The amount of polymorphism present is important for developing models attempting to explain the origin of reproductive isolation. In this particular case, the theoretical models and experimental studies of *Y*-linked fitness within natural populations (CLARK 1987a,b, 1990) predict that there will be little *Y*-linked polymorphism within a species for hybrid fitness except under special conditions such as frequency-dependent selection and environmental heterogeneity.

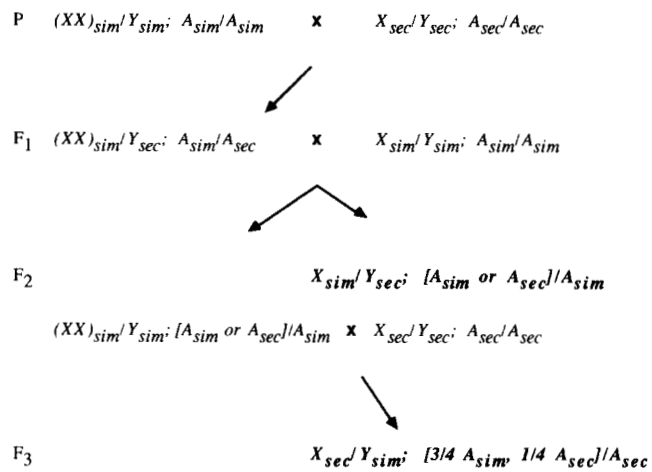

FIGURE 1.—Crosses used to generate *Y* chromosome introgressions. (*XX*) is the *D. simulans* attached-*X* (C(1)RM) chromosome. *X*, *Y*, and *A* denote the *X* chromosome, *Y* chromosome, and autosomes respectively. Genetic material derived from *D. simulans* is denoted by *sim*. The genetic material derived from the island species (*D. sechellia* or *D. mauritiana*, depending on the cross) is denoted by *sec*. The F_2 backcross males are backcrossed to *D. simulans* females for many generations to purify the autosomal background. The F_3 backcross males are backcrossed to the island species females to purify the background. See the text and JOHNSON and WU (1992, Figure 1) for details.

MATERIALS AND METHODS

Stocks used for *Y* introgressions: We used the method described in JOHNSON and WU (1992, Figure 1) to introgress the *Y* chromosomes. Below we present a summary of this approach (see Figure 1). F_1 females (parents *D. simulans* female and *D. sechellia* male) which had a *D. simulans* attached-*X* chromosome (C(1)RM) were mated to *D. sechellia* males. The resulting males (F_2 backcross) were backcrossed to *D. simulans* females several times to purify the autosomal background. This provided us with Y_{sec} introgressions into *D. simulans*. In JOHNSON and WU, the Y_{sec} introgressed was from the IF stock (obtained from JERRY COYNE) Here, *Y* chromosomes from three additional *D. sechellia* isofemale lines (lines 4, 21 and 81; also obtained from JERRY COYNE) were introgressed into *D. simulans yvf* (*yellow, vermillion* and *forked*) background. We used the same approach to introgress *Y* chromosomes from *D. mauritiana* (Y_{mau}) into *D. simulans* (substituting *mauritiana* for *sechellia* in the crosses above). Here, *Y* chromosomes from four different *D. mauritiana* stocks (ST, G102, G122 and G284; obtained from DANIEL HARTL) were introgressed into *D. simulans yvf* background.

We also performed the reciprocal introgressions (Y_{sim} into *D. sechellia* or *D. mauritiana* backgrounds) following the procedure of JOHNSON and WU (1992). In this set of crosses, the F_2 backcross females of the cross in the previous paragraph (the brothers of the F_2 backcross males) were mated with either *D. sechellia* or *D. mauritiana* males (depending on the initial cross). The resulting F_3 backcross progeny were backcrossed for several generations to either *D. sechellia* or *D. mauritiana* to purify the autosomes. Here the *Y* chromosomes were derived from four different *D. simulans* strains (*yvf*, Morro Bay, Lima, and South Africa) but were introgressed into a single *D. sechellia* (IF) or *D. mauritiana* (ST) background.

Determination of fertility: We examined the fertility for each class of the F_2 backcross males (F_3 for the reciprocal introgressions). Three different methods were employed.

The first criterion was sperm motility. In this test, following the protocol of COYNE (1984), the testes of individual males were dissected and examined under light microscopy. The presence of at least one motile sperm was required for the classification of fertile. If a class of males had a high (greater than 20%) fertility in the sperm motility test, males of that class were individually mated to two virgin *D. simulans yw* females. In each replicate, the male and females were confined in a vial for 6 days, then transferred to another vial for 6 more days. If progeny emerged from either vial, the male was classified as fertile. If, in the sperm motility test, the proportion of fertile males was low, the males were mass mated with their attached-*X* sisters. Typically 9–11 males and 6–9 females were in each vial. We corrected for the possibility of multiple fertile males in a single vial by a poisson adjustment: $X = (-\log_e S)/n$, where X is the adjusted proportion of fertile males, S is the proportion of vials with no offspring (sterile), and n is the mean number of males per vial.

Molecular identification of the *Y* genotypes: The *Stellate* probe from *D. melanogaster* hybridizes with *Y*-linked repetitive sequences (*Suppressor of Stellate*); these hybridization patterns of these sequences after restriction digests are species-specific (LIVAK 1984, 1990; JOHNSON *et al.* 1992). We used this probe to confirm the species identities of introgressed *Y* chromosomes. Molecular analysis was performed according to the protocols outlined in JOHNSON *et al.* (1992).

Productivity: In all of the measurements of the components of fitness, the same Y_{sec}, introgressed from the *D. sechellia* IF stock, was used. To test for differential male productivity between the *Y* genotypes in *D. simulans* background, we crossed individual males (less than 1 day old) of each *Y* genotype (Y_{sec} and Y_{sim}) to two 2–4-day-old virgin *D. simulans* females every other day for 8 days. We took care to ensure that the females given to each male genotype were approximately the same age and size. The four different pairs of females are designated in chronological order as A, B, C and D. Males were anesthetized briefly (less than 60 sec) with CO_2 when transferred. The females were transferred to a new vial after 4 days and were cleared 4 days later. Females were anesthetized only when establishing the first mating. The progeny from both vials were scored and sexed at two day intervals until 19 days after the vial was established. Two trials were performed within 3 months of each other. In trial 1, the Y_{sec} was from the F_{10} of the backcross into *D. simulans*, hence the autosomes are expected to be 1/1024 *D. sechellia*. In trial 2, the Y_{sec} had been backcrossed to *D. simulans* three more times (autosomes expected to be 1/8192 *D. sechellia*). All of these flies possessed the *yellow*, *vermillion* and *forked* (*yvf*) genetic markers. In both trials, all flies were maintained at 25° with a light:dark schedule of 16:8 hr and were raised on standard cornmeal media.

Viability: This design for male productivity also provides an test of male viability. Since the daughters of the Y_{sec} and the Y_{sim} males should be genetically identical, the relative viabilities of the *Y* genotypes can be determined by comparing the sex ratios of the progeny of Y_{sec} and the Y_{sim} males. The viability of the Y_{sec} males (V) relative to the pure species is: V = (secm)(simf)/(secf)(simm), where secm and secf are, respectively, the numbers of males and females produced by the Y_{sec} males and simm and simf are the numbers of males and females produced by the Y_{sim} males. Note that in this test any apparent differences in viability could actually be due to slight segregation biases.

Longevity: Male longevity was also determined with this design. In trial 2, after the males had completed their final mating, they were anesthetized briefly and transferred to individual fresh vials. Every other day, the males were examined and the dead flies were recorded and removed. Every 6 days, the males were transferred, without anesthesia, to individual vials with fresh medium.

Negative control: The effects of the *Y* genotype on male productivity, longevity, and viability (segregation) were also measured under the condition where different Y_{sec} chromosomes were in *D. sechellia* background. The attempted introgression of Y_{sim} into *D. sechellia* background was performed by the procedure outlined above and was continued until the F_{10} generation (autosomes 3/1024 *D. simulans*). Molecular probing of this line established that this *Y* chromosome was actually Y_{sec} (see RESULTS). This chromosome will be identified as Y_i. The productivity test in this background was the same as the one in the *simulans* background with two exceptions. First, a small piece of tissue paper was placed in each vial four days after it was established, as tissue paper in the vial substantially improves egg to adult viability and development time in *D. sechellia* (N. JOHNSON, personal observation). Second, the vials were scored at 2-day intervals until 20 days after they were established.

This test allows us to determine whether there were systematic biases in our analysis of fitness components and whether the process of the introgression itself had an effect on fitness.

Sperm displacement/remating ability: The differential ability of the *Y* genotypes in the *D. simulans* background to displace sperm was also measured. To this end, we constructed stocks of Y_{sec} and Y_{sim} in *simulans* background which were marked with *yellow* and *white* (*yw*) (previously they were marked with *yvf*). Virgin 2-day-old *yvf/yvf* females were individually mated with males (less than 1 day old) of either *Y* genotype marked with *yw*. After 4 days, the *yw* males were removed and the females were transferred to fresh individual vials and mated with *yvf* males (less than 1 day old) of the opposite *Y* genotype. Thus, if the female first mated with Y_{sec}, her second mating was with Y_{sim} and vice versa. Both the males and females were anesthetized only when they were isolated. From an analysis of the female offspring produced in the second vial, one can determine the relative contributions of the two males. Females resulting from sperm received from the first male are genotypically *yvf/yw* and thus phenotypically wild type for *vermilion* and *forked*, while those resulting from the second male's sperm are *yvf/yvf*. Progeny were scored as in the productivity test in *D. simulans* background. Only females which produced progeny in both vials were counted in this experiment. As different ability to remate may confound the results of this test, we refer to this test as sperm displacement/remating ability.

Mating test: The above marked stocks were used to test mating ability. Virgin males of each of four genotypes (*yvf/*Y_{sim} *yvf/*Y_{sec}, *yw/*Y_{sim}, and *yw/*Y_{sec}) and virgin females (*yvf/yvf*) were collected, placed in individual small vials (1 dram), and aged for 3 days. After this, individual *yw/*Y_{sec} and *yvf/*Y_{sim} males were placed together in small vials (without anesthesia) and individual *yvf/*Y_{sec} and *yw/*Y_{sim} were treated similarly. These vials were placed in a mating board, a device which allows one to observe many vials at once. Each set of two males and one female was observed for 40 min or until one male copulated with the female.

RESULTS

***Y* introgressions:** All four Y_{sec} chromosomes tested were successfully introgressed into *D. simulans yvf/yvf*

TABLE 1

Proportions of fertile males in the F_2 backcross from different introgressions of Y_{sec}

Strain	Method	Date	Percent fertile (*n*)
IF	Sperm motility	9/90	65.7 (166)
IF	Sperm motility	10/90	59.1 (137)
IF	Ind. mating	11/90	50.0 (120)[a]
Line 4	Ind. mating	10/91	29.4 (34)
Line 21	Ind. mating	10/91	40.0 (30)
Line 81	Ind. mating	10/91	38.1 (42)

All introgressions are into *D. simulans* yvf. There is no significant difference in the fertilities of all of the lines tested by the individual (Ind.) mating test as determined by a *G*-test on a 2 × 4 contingency table ($G = 5.55$, 3 d.f., $P > 0.10$).

[a] Data presented in JOHNSON and WU (1992).

TABLE 2

Fertility of F_3 backcross males from different Y_{sim} introgressions into *D. sechellia*

Strain	Method	Date	Percent fertile (*n*)
yvf	Sperm motility	10/90	0.00 (67)
yvf	Mass mating	10/90	0.00 (556)[a]
yvf	Mass mating	12/90	0.00 (1484)[a]
yvf	Mass mating	2/91	0.12 (1760)[a]
South Africa	Mass mating	9/92	0.31 (328)
Lima	Mass mating	9/92	0.00 (599)
Morro Bay	Mass mating	9/92	0.00 (377)

[a] Data presented in JOHNSON *et al.* (1992).

background. In fact, there is no statistically significant difference in the proportion of fertile F_2 backcross males (Table 1, *G* test, $G = 5.55$, 3 d.f., $P > 0.10$) (SOKAL and ROHLF 1981; ROHLF and SOKAL 1981). The sex ratio of the progeny of these males does not significantly differ from 50% female (data presented in JOHNSON 1992).

In six of the seven lines checked, molecular probing confirmed the identity of the Y_{sec} chromosomes; however, one of the putatively introgressed Y_{sec} chromosomes (line 21–12) was actually Y_{sim}, probably the result of non-disjunction (see JOHNSON *et al.* 1992). Thus even in cases where the fertility of the F_2 backcross males is high, molecular probing should still be employed.

The Y_{sim} cannot be introgressed into *D. sechellia* for any of the four *D. simulans Y* chromosomes tested. In Table 2, the fertility of the F_3 backcross males is presented; in no case was the fertility greater than 0.5%. In JOHNSON *et al.* (1992), we reported that both of the 2 (of 3800) fertile introgressions into *D. simulans yvf* background were actually Y_{sec}. In the introgressions into other *D. simulans* backgrounds, we obtained only one other fertile male which, unfortunately, could not be maintained in a line (and hence was unavailable for molecular probing). See the DISCUSSION for further comments about the non-introgressibility of this *Y* chromosome.

TABLE 3

Proportion of fertile F_2 backcross males from introgressions of Y_{mau} into *D. simulans* yvf

Strain	Method	Date	Percent fertile (*n*)
ST	Sperm motility	9/90	5.77 (156)
ST	Sperm motility	11/90	2.15 (139)
ST	Mass mating	9/90	1.45 (973)
ST	Mass mating	11/90	0.77 (877)
ST	Mass mating	4/92	0.43 (471)
G102	Mass mating	4/92	0.63 (330)
G122	Mass mating	4/92	0.56 (369)
G284	Mass mating	4/92	0.24 (831)

TABLE 4

Fertility of F_3 males from different Y_{sim} introgressions into *D. mauritiana*

Strain	Method	Date	Percent fertility (*n*)
yvf	Sperm motility	10/90	0.00 (86)
yvf	Mass mating	10/90	0.11 (877)
yvf	Mass mating	12/90	0.42 (2209)
South Africa	Mass mating	9/92	0.00 (775)
Lima	Mass mating	9/92	0.00 (752)
Morro Bay	Mass mating	9/92	0.00 (984)

TABLE 5

Fertilities of introgressed interspecific *Y* chromosomes

	X and autosomes		
Y chromosome	sim	sec	mau
sim (4 tested)	1	0	0[a]
sec (4 tested)	(1)	1	NT
mau (4 tested)	0	NT	1

Key: 1 = normal fertility and fitness (for that species), (1) = near normal fitness and 0 = sterile. NT = not tested; neither *D. mauritiana* nor *D. sechellia* has an attached-*X* chromosome required for the introgressions.

[a] Three *Y* chromosomes definitely result in sterility when introgressed, one *Y* chromosome may result in sterility when introgressed (see DISCUSSION).

The Y_{mau} cannot be introgressed into *D. simulans*. The fertility of the F_2 males (presented in Table 3) varies depending upon time and strain tested and the method used, but is usually less than 5%. Six lines (three ST, two G112 and one G284) were maintained for molecular analysis; in all cases, the introgressions were actually Y_{sim} and not Y_{mau}.

In the reciprocal introgression (Y_{sim} into *D. mauritiana*), we use a statistical argument to claim that it is unlikely that this *Y* chromosome can be introgressed without loss of fertility (see DISCUSSION). Though the fertility of these F_3 backcross males was consistently less than 0.5% (see Table 4), 10 males (all from the *yvf* cross) were fertile. Unfortunately, none of these lines could be maintained for molecular analysis.

Table 5 is a summary of the fertilities of each category of *Y* introgression.

Fitness effects: *Productivity, viability and longevity tests in D. simulans background:* Productivity was defined as the number of adult offspring produced by the male and was calculated separately for each transfer (A–D). The mean productivities and their standard errors for each *Y* chromosome genotype in *D. simulans* background during each transfer for each trial are presented in Table 6. The patterns observed in both trials are quite consistent despite the rather different mean values between trials. In the first transfer (A), there is little or no difference in the mean productivities of the *Y* genotypes. In subsequent transfers (particularly C and D), the mean productivity of Y_{sec} is demonstrably lower than that of Y_{sim} (relative productivity 0.5–0.7). This difference occurs because although the productivity of Y_{sim} declines with each transfer, the productivity of Y_{sec} declines even more steeply. In both trials there were no significant differences (by a *t*-test) between the *Y* genotypes in sets A and B and highly significant differences in set D. For set C the difference approached significance in the first trial but was highly significant in the second; thus by the Fisher-Pearson test (SOKAL and ROHLF 1981), the overall significance for both sets C and D was high ($P < 0.002$ and $P < 0.001$ respectively) when the two trials are combined. Note that the *P* values have not been adjusted for multiple tests. Because the data sets are non-normal (see Figure 2 for the distribution in trial 1), we also used the non-parametric Kruskal-Wallis test on the productivity data and obtained *P* values similar to those found with the *t*-test (data not shown).

The viability data for both trials in *D. simulans* background are presented in Table 7. A *G*-test on a 2 × 2 contingency table was performed on the total numbers of males and females produced by the two *Y* genotypes (transfers A-D combined). In both trials, there is no significant difference in the sex ratios of the progeny and thus in male viability. The relative viabilities of Y_{sec} were 1.033 and 0.991 for trials 1 and 2, respectively.

In *D. simulans* background, the Y_{sec} males lived a average of 27.8 days (SE = 1.240, $n = 51$) while the Y_{sim} males lived an average of 24.3 days (SE = 1.569, $n = 52$). This difference approaches significance if tested with either a *t*-test ($t = 1.793$, $0.05 < P < 0.10$) or a Kruskal-Wallis test.

Negative control: The productivity data for the test in *D. sechellia* background are presented in Table 8. Here there are no consistent nor significant differences between the *Y* genotypes. Unlike both of the trials in the *D. simulans* background, there is no decline in the mean productivities of either *Y* genotype with successive transfers (there may even be a slight increase between sets A and B). There is a slight but significant difference in the viabilities of the *Y* genotypes in *D. sechellia* background (data in JOHNSON 1992). The viability of the Y_i relative to that of Y_{sec} is 0.930 ($G = 9.02$, $P < 0.01$). The differential viability is due to the pure species (Y_{sec}) males producing a slightly but statistically significant male-biased sex ratio (48.46% female, $G = 10.94$, $P < 0.001$); the progeny from Y_i have a sex ratio not significantly different from 50% female. The two classes of males in the control had equivalent longevities but their average longevity was somewhat greater (34 days) than that of males of either *Y* genotype with the *D. simulans* background.

The results of the negative control strongly suggest that the differences found between Y_{sec} and Y_{sim} in *D. simulans* background are not due to the introgression process itself nor systematic differences in the measurement of fitness components.

Sperm displacement/remating ability: There is a substantial difference in the proportion of progeny produced by each of the *Y* genotypes (see Figure 3). When the second male is Y_{sim}, over 20% of the female progeny in the second vial are from the second male in 16 of 29 replicates. In only 4 of 32 replicates where the second male is Y_{sec}, does he contribute to over 20% of the female progeny. The overall difference in distri-

TABLE 6

Productivity of *Y* genotypes in *D. simulans* background as measured by number of offspring produced

Y genotype	Time period (transfer #) A	B	C	D
Trial 1				
simulans				
Mean	88.05	73.70	59.59	63.19
SE	5.219	6.761	6.122	6.184
n	43	43	43	42
sechellia				
Mean	86.10	70.52	43.29	30.71
SE	4.802	5.956	6.567	6.600
n	42	42	41	41
Relative productivity	0.978	0.957	0.727	0.486
t value	0.275	0.353	1.819*	3.599***
Trial 2				
simulans				
Mean	55.39	45.46	41.17	37.40
SE	3.289	3.271	3.367	3.798
n	51	52	52	52
sechellia				
Mean	53.80	38.55	26.82	25.29
SE	2.789	3.283	3.192	3.670
n	50	51	50	52
Relative productivity	0.971	0.848	0.651	0.676
t value	0.369	1.492	3.089**	2.294**

Relative productivity defined as the mean of the introgressed line divided by the mean of the pure species (here, *sechellia* divided by *simulans*).

* $0.10 < P < 0.05$ (all *P* values for two-tailed test); ** $0.05 < P < 0.001$; *** $P < 0.001$.

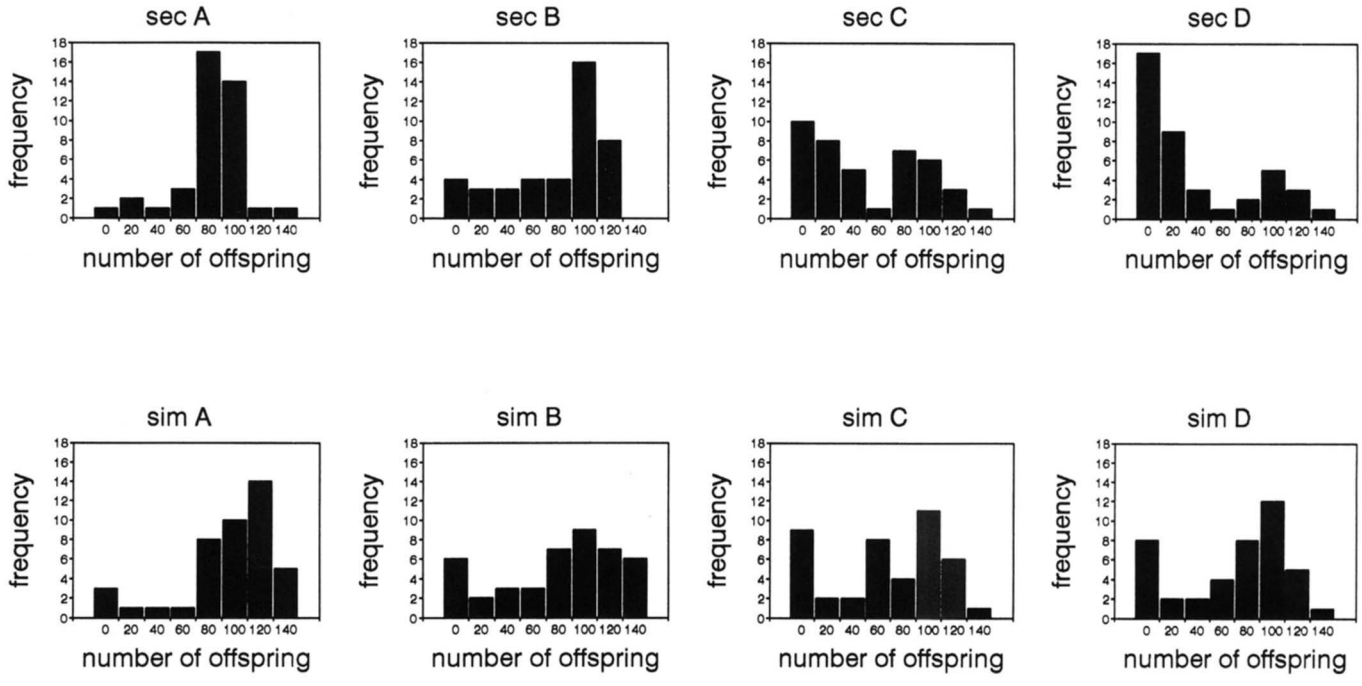

FIGURE 2.—Frequency histograms of the distributions of productivity of Y_{sec} and Y_{sim} males. sim refers to the Y_{sim} males with *D. simulans* genetic background. sec refers to the Y_{sec} males with *D. simulans* genetic background. A, B, C and D are the first, second, third and fourth sets of matings, respectively. The *x*-axis is the number of offspring produced; the number listed is the highest number of offspring in that class; *e.g.*, 20 = 1–20 offspring produced. See text for details.

TABLE 7

Viability test of *Y* genotypes in *D. simulans* background

Group	No. of females	No. of males	Percent females
Trial 1			
sechellia A	1812	1787	50.35
sechellia B	1507	1438	51.17
sechellia C	912	856	51.16
sechellia D	610	640	48.80
sec total	4841	4721	50.63
simulans A	1935	1833	51.35
simulans B	1629	1532	51.53
simulans C	1282	1266	50.31
simulans D	1390	1255	52.55
sim total	6266	5886	51.44
Relative viability of Y_{sec} = 1.038; G = 1.424 (NS)			
Trial 2			
sechellia A	1340	1335	50.19
sechellia B	1013	939	51.90
sechellia C	689	646	51.61
sechellia D	648	659	49.58
sec total	3690	3579	50.76
simulans A	1379	1428	49.13
simulans B	1177	1172	50.11
simulans C	1080	1041	50.92
simulans D	1018	913	52.72
sim total	4654	4554	50.54
Relative viability of Y_{sec} = 0.991; G = 0.080 (NS)			

bution is highly significant when tested with a Kruskal-Wallis test (H = 9.783, 0.001 < P < 0.002). Even if the females who produced no offspring from the second male are excluded (which could be due to a failure to remate), the distributions are still significantly different.

TABLE 8

Productivity of *Y* genotypes in *D. sechellia* background as measured by number of offspring produced

	Time period (transfer #)			
Y genotype	A	B	C	D
Y_i				
Mean	42.55	65.58	48.48	68.42
SE	5.534	6.401	6.614	7.084
n	51	50	50	50
Y_{sec}				
Mean	49.53	59.92	59.41	58.06
SE	6.066	5.733	6.829	6.976
n	51	51	51	50
Relative productivity	0.859	1.094	0.816	1.178
t value	0.850	0.655	1.149	1.042

Relative productivity defined as the mean of Y_i divided by the mean of Y_{sec}. Y_i is the Y_{sec} chromosome that was reextracted into *D. sechellia* during the attempted introgression of Y_{sim} (see text for details).

* 0.10 > P > 0.05 (all P values two-tailed); ** 0.05 > P > 0.001; *** P < 0.001.

Mating test: A difference in the mating abilities of the *Y* genotypes, if any, is obscured by a large marker effect. Of the males copulating, 17 of 34 were Y_{sec}. In 28 of 34 matings, however, the *yvf* male was copulating (G = 15.446, P < 0.001).

DISCUSSION

Criteria for determining a *Y* sterility effect: Failure to introgress the *Y* chromosome into the genome

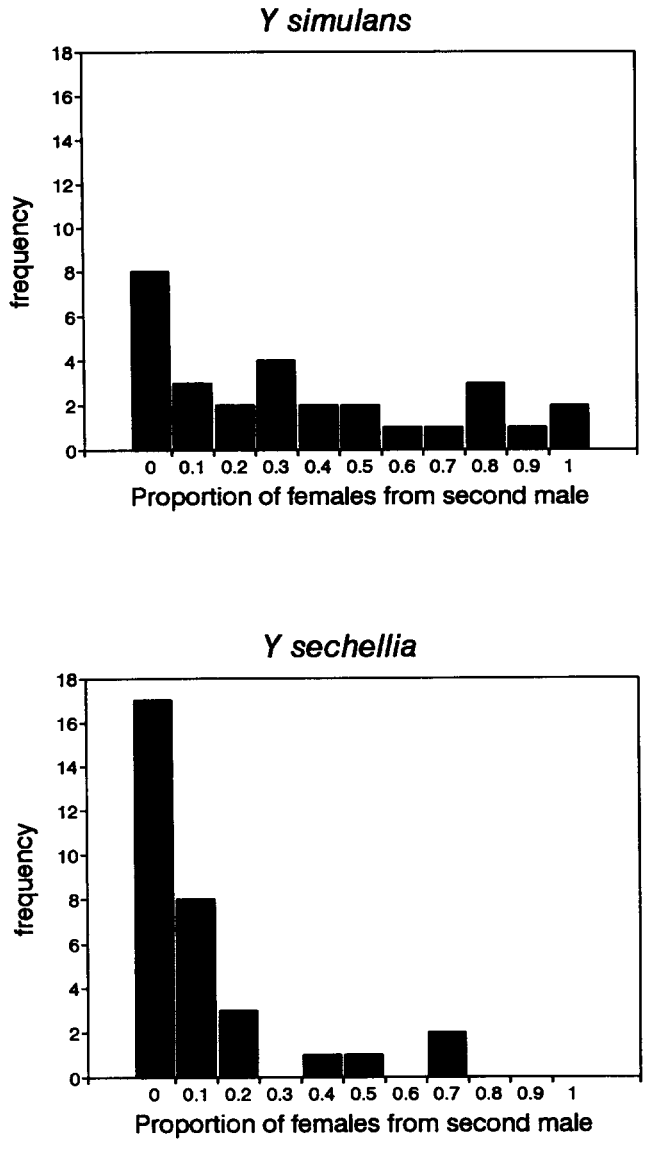

FIGURE 3.—Frequency histograms of the proportion of female offspring sired by the second male in the sperm displacement experiments. *Y simulans* refers to experiment in which the male with Y_{sim} in *D. simulans* background was the second male. *Y sechellia* refers to the experiment in which the male with Y_{sec} in *D. simulans* background was the second male. The numbers listed on the *x*-axis refer to the highest proportion in that class; thus 0.1 = 0.001–0.1 of the offspring are sired by the second male.

of another species could be due to either a *Y* effect or *X*-autosome interactions. In JOHNSON *et al.* (1992), we determined when a failure to introgress the *Y* can be interpreted as a *Y* effect. We were able to test the fertility of 3800 males whose genotypes were either X_{sec}/Y_{sim}; (1/4 A_{sec}, 3/4 A_{sim})/A_{sec} or X_{sec}/Y_{sec}; (1/4 A_{sec}, 3/4 A_{sim})/A_{sec} and estimated the frequency of the two types to be 50:1 (the rare Y_{sec} males are produced by attached-*X* females with two *Y* chromosomes ultimately resulting from non-disjunction). Only two males were fertile and both are Y_{sec} when tested by molecular probes. Thus if Y_{sim} has no sterility effect, the chance of observing two fertile males both Y_{sec} is $(0.02)^2$ or much less than 0.01 (JOHNSON *et al.* 1992). A similar argument can be made for the cases in the present study wherein Y_{mau} chromosomes were introgressed into *D. simulans* background. Six of the fertile lines of males were checked and all had Y_{sim}. Thus it is extremely unlikely (probability on the order of $(0.02)^6$) that Y_{mau} has no sterility effect when introgressed into *D. simulans* background (see also COYNE 1985).

If no fertile males are produced in the cross (and thus none are available for molecular analysis), it is still possible to determine the confidence one has in claiming a *Y* sterility effect by estimating the number of males which should be free of background (*X* autosome) using the methods and results of JOHNSON *et al.* (1992). We had estimated that 2% of the 3800 males tested in that study were X_{sec}/Y_{sec}, of which only two were fertile. Since these males have Y_{sec} and hence should not have a *Y* sterility effect, the sterility of the other approximately 74 of 76 males is due to "background interactions." Thus 2.6% (2 of 76) of the X_{sec}/Y_{sim} males are expected to be free of background sterility. Using binomial sampling, we estimate the lower (more conservative) bound of the 95% confidence interval for the figure to be 0.87%. We also note that the frequency of attached-*X* females with two *Y* chromosomes appears to vary across time as evident by the variation in the fertility of F_2 backcross males in the Y_{mau} introgressions (Table 3).

In our introgressions of other Y_{sim} chromosomes into *D. sechellia* background (Table 2), we observed a single fertile male which produced only two progeny. Based on our conservative estimate of 0.87–2.63% males free of background sterility, we would expect between 11 and 34 fertile males if the Y_{sim} had no sterility effect. ZENG and SINGH (1993), using a different approach, tested 149 males with the Y_{sim} introgression. All were sterile, leading them to assume a *Y* sterility effect. Based on our calculations above, in ZENG and SINGH'S (1993) experiment, one to four males are expected to be free of background sterility.

In the case of Y_{sim} introgressions into *D. mauritiana*, we do not have any lines which were maintained for molecular analysis nor do we know the extent of background sterility. We do note that the proportion of fertile males differed between introgressions using *yvf* as the source of Y_{sim} and subsequent introgressions using South Africa, Lima, and Morro Bay as the source of Y_{sim}. In the introgressions into *yvf*, 10 of 3081 males are fertile by the mass mating test. In the subsequent introgressions, none of 2511 males are fertile. This difference is highly significant (*G*-test with correction for continuity; $G = 8.754$, 1 d.f., $P < 0.005$).

There are two possible explanations for this discrepancy. One is that in both sets of introgressions,

the Y_{sim} can not be introgressed into *D. mauritiana* background and that the difference is due to differences in the frequency of attached-*X* females harboring two *Y* chromosomes. The alternative is that the Y_{sim} from *yvf* can be introgressed into *D. mauritiana*. If this is the case, it is unlikely that the other Y_{sim} chromosomes can be introgressed. In either case, failure of the subsequent introgressions appears to be due to a *Y* chromosome sterility effect. Whether the Y_{sim} chromosomes from *yvf* has a sterility effect is unknown. ZENG and SINGH (1993) have also found no fertile males of this class. The amount of background sterility in this case is again unknown.

Fitness effects study: The difference in the productivity among the *Y* genotypes in *D. simulans* background in the later transfers could be due to a number of physiological causes including sperm depletion, seminal fluid depletion, and sperm dysfunction; wherein sperm produced early are normal, but sperm produced later are of lesser quality. We have not ruled out the possibility that Y_{sec} males reach reproductive senescence sooner than Y_{sim} males and that the difference among these males is not due to exhaustion but merely age.

Regardless of the physiological cause, the differential productivity is consistent. It is not due to the effects of the residual *D. sechellia* in the autosomes because trials 1 and 2 are rather similar (Table 6). If the autosomes were involved, the difference in productivity would be far less in trial 2 in which the Y_{sec} had been backcrossed to *D. simulans* three more times. The difference must then be due to negative interactions between the *sechellia Y* and the rest of the genome, as the reciprocal introgression (Y_{sim} in *D. sechellia* background) causes sterility. The results of the sperm displacement/remating ability experiment are consistent with those of the productivity test: in *D. simulans* background, Y_{sec} males have a lower reproductive capacity than do the Y_{sim} males. The results of the sperm displacement test may have been confounded by differential mating ability of the *Y* genotypes but this is unlikely since a mate choice experiment failed to find any differences between the genotypes.

No viability differences between the *Y* genotypes in the *D. simulans* background can be detected despite the large sample size. In the control (*D. sechellia* background), the difference is statistically significant but rather small. The sex-ratio data for the progeny of the *Y* introgression and the F_2 backcross males, in conjunction with the results of JOHNSON and WU (1992) and COYNE and ORR (1993), argue against mutual meiotic drive, as envisioned by HURST and POMIANKOWSKI (1991), as being a major cause of hybrid sterility and specifically Haldane's rule in Drosophila. See also FRANK (1991a,b), COYNE, CHARLESWORTH and ORR (1991), CHARLESWORTH, COYNE and ORR (1993) and POMIANKOWSKI and HURST (1993) for discussions of this controversy.

The marginally significant increase in longevity of the Y_{sec} males in *D. simulans* background may be due not to an inherently longer life span of these males but rather to a tradeoff between male productivity and longevity (see ROSE 1991). This tradeoff may be due to antagonistic pleiotropy (*sensu* WILLIAMS 1957) or mating activity having negative effects on longevity (PARTRIDGE and FARHAQUHAR 1981). It is intriguing that the *Y* from *sechellia* increases longevity slightly and that *D. sechellia* males live longer than their *D. simulans* counterparts, though we caution that these experiments were done at different times and have not been repeated.

The findings that the only appreciable effects of the Y_{sec} replacements are a decrease in male productivity and sperm displacement (and/or remating ability) in one background appears to contrast with some of the results known for intraspecific *Y* introgressions. CLARK (1990) had found little effect on male productivity from intraspecific replacements within *D. melanogaster* but his test was somewhat different (male given 10 virgin females at once) and in a different species. With the same lines, CLARK (1987a) did find small but significant variation in the segregation ratios (viability) in his intraspecific replacements. It then appears that the variations in intraspecific and interspecific replacements may be different.

In contrast, HOLLOCHER (1991) found differences in fitness components under natural conditions between males with different *Y* chromosome genotypes in a population of *D. mercatorum* near Kamuela, Hawaii. In her study, males with *abnormal abdomen* linked to the *Y* had delayed sexual development and decreased mating success but increased longevity compared to wild-type males. No difference in egg to adult development time or viability was detected. Thus these intraspecific results seem to agree with our interspecific findings. A full and complete comparison of intraspecific and interspecific fitness differences would thus require a systematic study of fitness components of both types of replacements of the *Y* chromosome using the same overall experimental design.

Another comparison to the *Y* introgression fitness effects, is the fitness effects resulting from *X*-linked introgressions which contain hybrid male sterility factors. JOHNSON and WU (1993) found that these introgressions, while causing complete male sterility and substantial reductions in female productivity, had little or no effect on the viability of either sex.

We have observed differences between the *Y* genotypes in productivity under exhaustive conditions and sperm displacement/remating ability. Whether these

differences will translate into an actual fitness difference will depend upon the breeding system of the organism, and more specifically the frequency of multiple mating [see WU (1983) for an explicit model]. It is possible that there is no fitness difference due to the *Y* introgression, if the frequency of multiple mating is low. Many species of Drosophila, however, do quite often multiply mate in nature (ANDERSON 1974; MILKMAN and ZEITLER 1974; GROMKO, SHEEHAN and RICHMOND 1980; HOLLOCHER 1991). Thus it is likely that males with the Y_{sec} would be selected against in nature.

Asymmetries: Regardless of the exact fitness value of the Y_{sec} introgression in the *D. simulans* background, the fitness is relatively close to normal as it requires exhaustive conditions to detect a difference between the *Y* genotypes. In comparison, introgressions of Y_{sim} into *D. sechellia* invariably result in sterility. Thus there is a large asymmetry in the fitnesses of *Y* introgressions in the reciprocal directions of the *D. simulans/D. sechellia* (see Table 5). In contrast, both directions of *Y* introgressions in the *D. simulans/D. mauritiana* cross appear to result in sterility and thus the effects are symmetrical. We note that although the effects are symmetrical, this does not imply that the same loci are involved in reciprocal introgressions. Although the species phylogeny appears to be a trichotomy (COYNE and KREITMAN 1986; J. COYNE and M. KREITMAN, personal communication; CACCONE, AMATO and POWELL 1988; KLIMAN and HEY 1993), hybrid male sterility appears to have evolved more rapidly between *D. simulans* and *D. mauritiana* than it has between *D. simulans* and *D. sechellia* (WU *et al.* 1993; PEREZ *et al.* 1993). These findings are consistent with models of postmating isolation which predict initial asymmetry followed by symmetrical sterility (WU and BECKENBACH 1983; ZENG and SINGH 1993).

The nature of the interactions: Because the hybrid sterility factors on the X_{sim} [see WU *et al.* (1993) for a review] do not interact with the Y_{sec}, they must either interact with other *X*-linked factors or the autosomes. As the F_1 males and the F_2 backcross males from the attached-*X* cross receive a complete *X* chromosome from *D. simulans*, at least some of the interactions must be *X*-autosome (JOHNSON *et al.* 1992). From the fertility data of the F_2 backcross males, we can estimate the number of effective factors on the *D. sechellia* autosomes involved in the interaction. These males have one set of autosomes from *D. simulans* and the other set is on average 1/2 *D. sechellia* and 1/2 *D. simulans*. Thus the fertility of the F_2 backcross males (F) should be $(1/2)^n$ where n is the number of effective autosomal loci interacting with the *X* and therefore: $n = -\log_2(F)$. As the fertility of these males ranges from 30 to 65% (depending on method and strain used, see Table 1), we estimate that in this cross there are between 0.6 and 1.7 effective factors on the autosomes which are involved in sterility interactions with the *X* when heterozygous. There are probably more autosomal loci that are involved in sterility interactions when they are homozygous. Furthermore, this number is based on the assumption of completely penetrant, independently assorting loci.

We can use similar calculations to estimate the number of effective *D. simulans* autosomal loci that interact with the X_{sec}. Our calculations (see above) suggest that 2.63% of the F_3 backcross males in the introgression of Y_{sim} into *D. sechellia* are free of background sterility and would thus be fertile (F) if Y_{sim} did not have a sterility effect. These males have one complete set of autosomes from *D. sechellia* and the other set is 1/4 *D. sechellia* and 3/4 *D. simulans*. Thus by the reasoning in the previous paragraph, $n = -\log_4(F)$. As F is 0.026, we estimate there are 2.6 effective autosomal factors in this cross.

In JOHNSON *et al.* (1992), we stated that the sterility due to Y_{sim} introgressions into *D. sechellia* background was probably not due to *X-Y* interactions but more likely due to *Y*-autosome interactions. Our evidence was that co-introgressions of Y_{sec} do not rescue the sterility (or spermatogenic phenotype) associated with three different *X*-linked introgressions of *D. sechellia* into *D. simulans*. Unfortunately, because both reciprocal *Y* introgressions between *D. simulans* and *D. mauritiana* cause sterility, we can not determine whether the *Y* effect in crosses between these species is due to *X-Y* or *Y* autosome interactions.

Evolution of reproductive isolation and the quest for major genes: Recently, there has been a debate as to whether hybrid sterility in Drosophila involves genes of major effect or the cumulation of many genes, each with small effect (COYNE and CHARLESWORTH 1986, 1989; NAVEIRA and FONTDEVILLA 1986, 1991; NAVEIRA 1992; ORR 1992; PEREZ *et al.* 1993). PEREZ *et al.* (1993) have mapped a factor (originally localized by COYNE and CHARLESWORTH 1986), which causes sterility when introgressed from *D. mauritiana* into *D. simulans*, to a small cytological interval.

We propose that the sterility due to the interspecific *Y* chromosome replacements may also be due to changes in major genes. Between *D. simulans* and *D. sechellia*, the effect of *Y* replacements is minor in one direction (Y_{sec} in *D. simulans* background). In the other direction, the *Y* introgressions result in complete sterility. Introgressions of the *Y* of *D. mauritiana*, however, appear to invariably result in sterility (COYNE 1985; ZENG and SINGH 1993; this study). The difference of the phylogenetic distances between *D. simulans* and *D. mauritiana* and *D. simulans* and *D. sechellia* is not large (in fact, the species phylogeny is still unresolved, COYNE and KREITMAN 1986; J. COYNE

and M. KREITMAN, personal communication; CACCONE, AMATO and POWELL 1988, KLIMAN and HEY 1993), yet the difference in the effects of *Y* chromosome replacements is quite large. If *Y*-linked hybrid sterility evolved by the accumulation of many genes each with small effect, then one would expect less difference in the amount of fertility reduction caused by the different reciprocal *Y* chromosome introgressions. Thus our data suggests (though not conclusively) that the sterility interactions involving the *Y* are due to major genes.

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