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Genetics of sexual isolation in male hybrids of *Drosophila simulans* and *D. mauritiana*

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Summary

Sexual isolation between the sibling species *D. simulans* and *D. mauritiana* is due largely to the rejection of *D. simulans* males by *D. mauritiana* females. Genetic analysis shows that genes on the X and third chromosomes contribute to the differences between males causing sexual isolation, while the Y chromosome, second chromosome and cytoplasm have no effect. These chromosome effects differ from those observed in a previous analysis of sexual isolation in hybrid females, implying that different genes cause sexual isolation in the two sexes.

1. Introduction

Closely related animal species can often produce fertile hybrids in captivity despite their failure to do so where they co-occur in nature. In many of these cases, the absence of natural hybrids probably reflects strong sexual isolation (mate discrimination) between the species. Such sexual isolation is especially likely to be important in animal speciation because it may be the byproduct of sexual selection operating in geographically isolated populations (Lande, 1981; Iwasa & Pomiankowski, 1995). In birds, for example, the degree of sexual dimorphism of species within a clade is correlated with its rate of speciation, indicating a possible relationship between the two factors (Barraclough *et al.*, 1995). Moreover, in some cases natural selection can, through a process called 'reinforcement', quickly increase the level of sexual isolation between sympatric, hybridizing taxa that produce unfit hybrids (Liou & Price, 1994; Kelly & Noor, 1996). Among sympatric *Drosophila* species, prezygotic isolation evolves much more rapidly than postzygotic isolation (Coyne & Orr, 1989), perhaps because of reinforcement.

Despite its potential importance as a primary isolating mechanism, we know far less about the genetics of sexual (prezygotic) isolation than about the genetics of *postzygotic* isolation (hybrid sterility and inviability), which fills an extensive literature (Coyne, 1992*a*, Wu & Palopoli, 1994). The paucity of work on the genetics of mate discrimination is explained by its onerous requirement for large samples of hybrids whose sexual behaviour can be observed under controlled conditions. In addition, sexual isolation is generally more sensitive than hybrid sterility and inviability to environmental conditions, and hence more difficult to measure accurately. There have in fact been only six genetic analyses of sexual isolation that examined large portions of the genome, all but one of these in *Drosophila* (Tan, 1946; Ehrman, 1961; Zouros, 1981; Roelofs *et al.*, 1987, Coyne, 1989, 1992). Among these, moreover, only the studies of Ehrman (1961), Zouros (1981) and Roelofs *et al.* (1987) conducted genetic analysis on both male and female hybrids.

Here I present a genetic analysis of sexual isolation in male hybrids of the sibling species *D. mauritiana* and *D. simulans*. These results complement my previous study of sexual isolation in hybrid females of the same two species (Coyne, 1989), allowing a comparison of the effects of the different chromosomes on sexual isolation in the two sexes. Such a comparison can address several theories that predict that the same genes or chromosome regions will affect sexual isolation in males and females (see Discussion).

Drosophila simulans, like its relative *D. melanogaster*, is a cosmopolitan species largely associated with humans. *D. mauritiana*, on the other hand, is restricted to the oceanic island of Mauritius, 800 km east of Madagascar. *D. simulans* does not occur on Mauritius (Tsacas & David, 1974). Phylogenies of the *melanogaster* subgroup show that *D. simulans* and *D. mauritiana* are sister species, more closely related to each other than to the outgroup species *D. melanogaster* (Cariou, 1988; Kliman & Hey, 1993; Caccone *et al.*, 1996). *D. mauritiana* probably arose after a

colonization of Mauritius by its common ancestor with either *D. simulans* or that of another close relative, *D. sechellia*.

The close relationship between *D. simulans* and *D. mauritiana* is underscored by their identical banding pattern in polytene chromosomes (Lemeunier & Ashburner, 1976) and the indistinguishable morphology of females (Tsacas & David, 1974; Lemeunier & Ashburner, 1976). Males, however, can be distinguished by diagnostic differences in the shape of their genital arch (Tsacas & David, 1974) and the colour of the testes (Coyne, 1985); there are also significant but non-diagnostic differences in the number of teeth in the foretarsal 'sex combs' (Coyne, 1985).

Despite their phenotypic similarity, *D. simulans* and *D. mauritiana* show several forms of reproductive isolation when tested in the laboratory. Male hybrids are sterile, but females are fertile and can be backcrossed to either species (David *et al.*, 1974), allowing genetic analysis of sterility, other reproductive isolating mechanisms and morphological differences among the species (Coyne, 1984, 1993; Coyne & Charlesworth, 1989; Davis *et al.*, 1994; True *et al.*, 1996*a*,*b*).

The sexual isolation between these species is strong but asymmetrical. *D. mauritiana* males mate readily with *D. simulans* females. *D. mauritiana* females, however, appear to discriminate strongly against *D. simulans* males, who court them ardently but rarely achieve copulation (David *et al.*, 1974; Watanabe & Kawanishi, 1979; Robertson, 1983; Cobb *et al.*, 1988; Coyne, 1989). As with all sexual isolation, this pattern suggests that there are genetic differences between the species affecting both females (one species of females discriminates and the other does not) and males (*D. mauritiana* females can recognize differences between males of the two species).

In our previous work, we showed that the differences between females in their willingness to copulate with *D. simulans* males were due to evolutionary change at a minimum of three loci, with at least two genes on the second chromosome and at least one on the third, while the X chromosome had little or no effect (Coyne, 1989, 1992*a*). The present analysis of males enables us to compare the genetic architecture of sexual isolation in the two sexes.

2. Materials and methods

(i) *Stocks*

Our crosses employed the following strains (abbreviations of the strain names are given in parentheses).

(*a*) *D. simulans*

Florida City (FC): an isofemale line collected in Florida City, Florida in 1985. This strain was used in our previous analysis of sexual isolation in female hybrids (Coyne, 1989).

C(1)RM, yw, Y females, +males [*C(1)RM*]: A stock in which females have attached-X chromosomes carrying the recessive mutations *yellow* (1–0·0) and *white* (1–1·5), as well as a free Y chromosome. The free X chromosome in males carries the wild-type alleles at both loci. Before this stock was used, its females were crossed to males from the FC stock for three successive generations to give females of these two strains similar autosomal backgrounds.

C(1)RM, yw, 0 females, T(*1; Y*)*A13* males: A stock produced by Yamamoto (1992) by irradiating the previous stock. The females of this stock also have compound X chromosomes carrying the *yellow* and white markers, but do not carry a Y chromosome. In males of this stock, the X and Y chromosomes have become attached by a translocation. The attached-X, O females were used to generate interspecific F_1 hybrids lacking a Y chromosome.

(*b*) *D. mauritiana*

Synthetic (syn): A mixture of six isofemale lines collected on Mauritius in 1981 and combined in 1983. This stock was used in our earlier study of sexual isolation in female hybrids (Coyne, 1989).

All three of the following single-mutant stocks were extracted from one original stock – *sn; j; irr* – which carried a recessive mutation on each of the three major chromosomes. This stock was crossed to the synthetic stock of *D. mauritiana* and the individual mutants extracted in the F_2 generation. This cycle of outcrossing and re-extraction was repeated three times for each mutation, ensuring that all three single-mutant stocks, described below, would have genetic backgrounds containing substantial genetic material from the *synthetic* stock (depending on the cross and chromosome, this material will constitute about 50–95% of the genome in the final mutant strain).

singed (*sn*): A stock homozygous for *singed* (bristles small and misshapen), whose locus is near the middle of the X chromosome (*singed* is located at 1–21 in *D. simulans*:Sturtevant, 1929).

jaunty (*j*): A stock homozygous for *jaunty* (wings curled up at tips), a mutation located roughly in the middle of the second chromosome. In the sibling species *D. melanogaster*, *jaunty* is at position 48·7 (Lindsley & Zimm, 1992), which puts it at about map position 43 in *D. simulans* (Sturtevant, 1929). Its position in *D. mauritiana* may differ somewhat from that in *D. simulans* because of difference in map length between the two species (True *et al.*, 1996*b*).

irregular (*ir*): A stock homozygous for the third-chromosome mutation *irregular* (eye facets not arranged in regular rows). The site of this gene has not been determined because of the near-absence of mutant markers on the third chromosome.

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Due to a lack of appropriate stocks, we did not study the effect of the tiny fourth chromosome, which comprises about 1–2% of the genome.

(ii) *Crosses*

All flies were reared on cornmeal/yeast/Karo syrup/agar food and kept in incubators at 24 °C on a 12h light/dark cycle. All crosses involved 8–10 pairs of flies in an 8-dram vial.

As the sexual isolation in this species pair operates primarily between *D. mauritiana* females and *D. simulans* males, we tested all hybrid and pure-species males against pure *D. mauritiana* females. We made two types of interspecific crosses for our genetic analysis: F_1 crosses, which allowed us to estimate the effect of entire sex chromosomes, and backcrosses, which allowed us to estimate the effects of the chromosomal segments linked to the three marker alleles.

For the analysis of F_1 hybrids, we made three pairs of crosses. The first comprised reciprocal crosses between the *D. simulans* FC and *D. mauritiana* syn strains. (Because of mating discrimination by *D. mauritiana* females, the cross to *D. simulans* males was very difficult, and many crosses were required to secure adequate numbers of F_1 males.) Hybrid males from the two reciprocal crosses have identical autosomes but differ in their X chromosome, Y chromosome and cytoplasm.

To control for cytoplasmic effects, we made a second pair of hybridizations. The first of these involved *C(1)RM, yw, Y D. simulans* females and *D. mauritiana* syn males, and the second involved *D. simulans* FC females and *D. mauritiana* syn males (this latter cross was also used in the reciprocal F_1 crosses described above, but was re-made so that the male offspring could be tested simultaneously with those from the attached-X cross). Male offspring of these two crosses share cytoplasm from the *D. simulans* mothers, but differ in their X and Y chromosomes.

The third pair of hybridizations was made to examine the possible effect of the Y chromosome on sexual isolation. This chromosome is largely genetically inert in *Drosophila*, appearing to carry only loci affecting spermatogenesis and ribosomal RNA, and there is little evidence that it affects other quantitative or behavioural characters (Williamson, 1976). However, Zouros (1981) reported an effect of this chromosome on male sexual isolation in *D. mojavensis/D. arizonae* hybrids. It is not possible in our hybridization to produce two male genotypes differing only in the species origin of the Y chromosome. We therefore produced two F_1 hybrid genotypes, both possessing a *D. mauritiana* X chromosome but one of them carrying a *D. simulans* Y chromosome and the other no Y chromosome at all. The first genotype was the offspring of *D. mauritiana* syn males and *C(1)RM, Y, yw* females, and the second of *D. mauritiana* syn males and *C(1)RM, yw*, O females.

To study the effects of the individual chromosomes on a randomized genetic background, we backcrossed F_1 hybrid females to pure-species males. Because previous work on another species showed that multiple phenotypic markers can severely reduce the viability of hybrid males (Dobzhansky, 1936; M. Noor, unpublished data), our backcrosses involved the segregation only of single markers. There were thus three backcrosses, one for each chromosome. While this method enabled us to estimate the main effects of each chromosome segment segregating against a heterogeneous genetic background, we could not study interactions among the chromosomes. (We used an identical protocol in our earlier work on hybrid females: Coyne, 1992*b*, 1993.)

In preliminary backcrosses of F_1 hybrid females to wild-type males from both species, we found that male offspring from the backcross to *D. simulans* mated only rarely with *D. mauritiana* females, making this cross of little value for discriminating among chromosome effects. All our backcrosses hence used F_1 hybrid females backcrossed to *D. mauritiana* males.

To make the backcrosses, *D. mauritiana* males homo- or hemizygous for a recessive marker were first crossed to *D. simulans* synthetic females. Their F_1 female offspring were then backcrossed to the *D. mauritiana* marker males. The offspring of this backcross were of two genotypes: those showing the marker allele, and hence either homozygous or hemizygous for a linked segment of *D. mauritiana* genome, and those that were wild-type and therefore heterozygous for autosomal segments from both species or (in the case of the X chromosome) hemizygous for the *D. simulans* segment. Depending on its location on the chromosome arm, each marker in such a backcross is non-randomly associated with between 40 and 90 centimorgans of conspecific genome – at least an entire chromosome arm (Naveira & Barbadilla, 1992). Equal numbers of marked and non-marked males were scored simultaneously for their sexual isolation from pure *D. mauritiana* females (see below).

It is possible that any differences in courtship or copulation frequency in these crosses might be due not to species-specific differences in 'sexual isolation genes', but to pleiotropic effects of the markers themselves on behaviour and copulation frequency. We assessed this possibility by testing each marker's effect on mating behaviour when it was segregating in a pure *D. mauritiana* background. In these tests, males from the three *D. mauritiana* marker stocks were crossed to *D. mauritiana* syn females, and the F_1 females backcrossed to males from the marker stock. Each such backcross produces two classes of male offspring which differ in their possession of the marker alleles but were of pure *D. mauritiana* genome. These two classes of males were presented to virgin *D.*

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mauritiana females and scored in the same way as were males from the interspecific backcrosses.

(iii) *Scoring courtship*

Because hybrid males are completely sterile in the F_1 and largely sterile in the backcrosses, we could not use insemination rate as an index of copulation frequency (Coyne, 1989). Instead, we observed courtship and mating directly. Males and females were collected as virgins and held separately until mating observations were made on the morning of the fourth day after eclosion, 1 h after the incubator lights came on. Observations were conducted at room temperature, which varied between 20·5 and 23 °C.

Each set of observations involved two genotypes differing in the species origin of either an entire chromosome (the X chromosome in the F_1) or a chromosome segment (all backcrosses). Males of both genotypes were collected and observed simultaneously. For each of the two genotypes being compared, three or four vials were watched at once, each vial containing a single *D. mauritiana* syn female and two males of identical marker genotype. (We used two males to increase the probability of courtship.) Males were aspirated into the vial and the trio of flies observed for 20 min. Each scoring run involved 6–10 vials of each of the two male genotypes.

We scored the frequency of copulation as well as two other behaviours: courtship latency, defined as the time from the beginning of observations to the first occurrence of male courtship (the characteristic wing vibration), and copulation latency, defined as the time from the beginning of observations to the onset of copulation. Copulations were scored only if they lasted longer than 30 s, as a male occasionally became disconnected immediately after mounting.

(iv) *Statistics*

To assess the differences among genotypes, we used the non-parametric Mann–Whitney *U*-test to compare courtship and copulation latencies, and Fisher's exact test to compare the number of courtships or copulations. Although we held the *a priori* hypothesis that males with more genome from *D. mauritiana* would court and copulate more readily with *D. mauritiana* females, we calculated conservative two-tailed probabilities. In addition, we used a sequential Bonferroni correction (Rice, 1989) to adjust the significance levels, dividing the threshold probability of 0·05 by 3 for the three reciprocal F_1 crosses that tested X-chromosome effects and by 6 for the control and experimental backcrosses, each of which contained six genotypes.

3. Results

Table 1 gives all the results, with data from each pair of genotypes shown on successive lines. All statistical comparisons were made between genotypes of a pair.

(i) *Pure species*

Genotypes 1 and 2 in Table 1 (the two pure-species males) demonstrate the sexual isolation between the species: *D. mauritiana* females mate readily with conspecific males but not at all with *D. simulans* males. As seen in previous work (Robertson, 1983; Cobb *et al.*, 1985; Coyne, 1989), this isolation is due largely to rejection of *D. simulans* males by *D. mauritiana* females, as males of both species court these females ardently. (Indiscriminate courtship by males is expected here, as females from both species have nearly identical profiles of the cuticular pheromones that induce male courtship: Jallon & David, 1987; Cobb & Jallon, 1990; Coyne, 1995.) In both hybridizations there was ample courtship: the fraction of vials in which males courted the *D. mauritiana* female was 98% or greater, and males of the two species did not differ in courtship latency. Although we did not count or estimate the duration of courtship behaviours, there was no obvious difference in the courtship intensity of the two types of males: *D. simulans* males, though refused by *D. mauritiana* females, courted them persistently, often through the entire 20 min observation period. Their repeated attempts to mount the female were, however, always rebuffed. This intensity and asymmetry of sexual isolation have now been seen in several different strains of both species (David *et al.*, 1974; Cobb *et al.*, 1988; Coyne, 1993), and must therefore be species-specific phenomena that are not limited to certain pairs of strains.

(ii) *F_1 hybrids*

Two paired comparisons of F_1 hybrid males (Table 1, genotypes 3 *v.* 4 and 5 *v.* 6) allowed us to estimate the effects of the X chromosomes on sexual isolation. Both sets of comparisons show that *D. mauritiana* females accept F_1 males having a *D. simulans* X chromosome more often than they accept pure *D. simulans* males, and they accept F_1 males having a *D. mauritiana* X chromosome less often than they accept pure *D. mauritiana* males. This shows that at least some of the genes causing sexual isolation of males reside on the autosomes, and that these autosomal genes are not completely recessive in hybrids.

Both comparisons also imply a substantial effect of the X chromosome on copulation frequency and possibly a weaker effect on courtship latency. (The effect of the X chromosome on courtship *latency* of F_1 males is somewhat problematic, as males of the two

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Table 1. *Sexual isolation of male genotypes tested with* D. mauritiana *females*

Male genotypes	Females tested	Courtships				Copulation			
		n	Fraction	Mean latency (s)	S.E.	*n*	Mean fraction	Latency (s)	S.E.
Pure species									
1 mau	137	136	0·99	241	10	121 (< 0·0001)*	0·88	449	19
2 sim	137	134	0·98	277	17	0	0	—	—
F_1 hybrids									
3 X^mY^s	154	148	0·96	299 (0·012)*	18	75 (< 0·001)*	0·49	509 (0·006)*	28
4 X^sY^m	154	146	0·95	366	20	31	0·20	688	48
5 X^mY^s	69	69	1·00	220 (0·012)*	16	29 (0·0096)*	0·42	556	52
6 X^sY^m	69	64	0·93	290	22	14	0·20	697	67
7 X^mY^s	140	138	0·99	262	14	62	0·44	540	31
8 X^mO	140	140	1·00	247	14	60	0·43	523	34
Interspecific backcross									
9 *sn*/+	180	171	0·95	326	16	107 (0·0043)*	0·59	601	25
10 +/+	180	166	0·92	344	17	79	0·44	601	31
11 *j*/+	140	131	0·94	350 (0·0025)*	18	83	0·59	590	28
12 +/+	140	133	0·95	289	16	96	0·69	537	25
13 *ir*/+	144	143	0·99	253	14	116 (0·0024)*	0·81	520	23
14 +/+	144	144	1·00	220	10	92	0·64	489	26
Control (intraspecific) backcross									
15 *sn*/+	139	136	0·98	251	14	112	0·81	470	24
16 +/+	139	136	0·98	274	16	120	0·86	431	20
17 *j*/+	149	142	0·95	347 (0·037)	18	125	0·84	540	23
18 +/+	149	148	0·99	293	13	133	0·89	503	20
19 *ir*/+	140	140	1·00	226	11	132	0·94	437	17
20 +/+	140	140	1·00	215	8	127	0·91	418	19

Each test involved a comparison of two genotypes, as indicated by the spacing between lines. (See Results for description of genotypes and for distinction between the three comparisons of F_1 males). Probabilities lower than 0·05 are given in parentheses between the estimates that were compared. Probabilities that were significant under the sequential Bonferroni test are indicated with an asterisk. Superscripts 'm' and 's' indicate chromosomes from *D. mauritiana* and *D. simulans* respectively. Standard errors (S.E.) are given for courtship and copulation latencies.

pure species did not differ significantly in this character (Table 1, genotypes 1 *v.* 2), and the X chromosome did not significantly affect courtship latency in the backcross (see below).) Because of these disparities, we do not consider this character further.

Genotypes 3 and 4 were the males from the two reciprocal F_1 crosses. As expected, these did not differ in courtship frequency: in both cases at least 95% of the females were courted. There was, however, a highly significant difference in copulation frequency, with males carrying the *D. mauritiana* X chromosome copulating 29% more often than males with the *D. simulans* X.

This difference in copulation frequency could in principle be due either to X-linked genes or to the cytoplasm, which segregate together in F_1 males. (the Y chromosome, on the other hand, is heterospecific to the X chromosome and cytoplasm, and so would act to reduce their effects if it carried 'sexual isolation' genes.) To separate X from cytoplasmic effects, we compared the sexual isolation of male genotypes 5 and 6 (Table 1), which, while differing in their X chromosomes, both have cytoplasm from *D. simulans* (see Materials and Methods). This comparison also shows a significant difference in copulation frequency in the same direction seen in genotypes 3 *v.* 4. Moreover, in both paired comparisons the differences are of similar magnitude: the 22% effect of the X chromosome in comparison 5 *v.* 6 does not differ significantly from the 29% effect seen when comparing genotypes 3 *v.* 4. The X chromosome, then, appears to carry genes having a substantial effect on sexual isolation between these species. This effect is confirmed in the backcrosses described below, in which males have both identical cytoplasm and identical Y chromosomes.

Cobb *et al.* (1988) also observed a difference in copulation frequency when testing the two reciprocal F_1 males with *D. mauritiana* females. Because their samples were small, this difference was not significant (a Fisher's exact test applied to data in their table 3 gives $P = 0·16$), but its magnitude was strikingly

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similar to ours (their reciprocal F_1 genotypes differed in copulation frequency by 0·25, ours by 0·29).

Finally, one pair of crosses (Table 1, genotypes 7 *v.* 8) examined the possible effect of the Y chromosome on sexual isolation. Genotype 7 consisted of F_1 hybrid males with a *D. mauritiana* X chromosome and a *D. simulans* Y chromosomes; genotype 8 was identical to this but completely lacked a Y chromosome. These two classes of males showed nearly identical levels of sexual isolation from *D. mauritiana* females (Table 1), levels which were also similar to that seen in $X^{mau}Y^{stm}$ males from the other F_1 comparisons (genotypes 3 and 5). Strictly speaking, this comparison does not test for interspecific differences on the Y chromosome, but instead compares the effects of having foreign Y chromosome with having no Y chromosome at all. It is possible that, compared with a hypothetical $X^{mau}Y^{mau}$ F_1 male (which cannot be produced), replacing the *D. mauritiana* Y with a *D. simulans* Y would increase sexual isolation to exactly the same degree as eliminating the Y chromosome entirely, so that our comparison would miss true interspecific differences on the Y. It seems more parsimonious, however, to assume that the Y chromosome simply has no effect on sexual isolation. Analysis of the backcrosses (see below) also implies that the Y chromosome does not have a large effect on sexual isolation.

(iii) *Backcross hybrids*

(*a*) *X chromosome*

As shown in comparing genotypes 9 and 10 from the backcross (Table 1), the X-chromosome segment linked to the *singed* marker has a significant effect on copulation frequency, with males hemizygous for the *D. mauritiana* segment mating 15% more frequently than males carrying the segment from *D. simulans*. (This effect is smaller than that seen in the comparisons between F_1 males, but this is expected: F_1 males differ by the entire X chromosome, while the two classes of backcross males differ only by the chromosome segment linked to *singed*, which may recombine away from X-linked genes affecting sexual isolation.)

The difference between genotypes 9 and 10 cannot be simply an artifact of the *sn* marker itself, as one can see from the control backcross in *D. mauritiana* (Table 1, genotypes 15 *v.* 16). In this latter comparison, the effects of the *singed* marker are not statistically significant and are in fact in the direction opposite to that seen in the interspecific backcross. The 95% confidence interval for the difference in proportion of copulations between the two genotypes ($sn/sn - sn/+$) is $0{\cdot}155 \pm 0{\cdot}075$) for the interspecific comparison and $-0{\cdot}057 \pm 0{\cdot}054$ for the control intraspecific comparison. These confidence intervals are non-overlapping.

We note that the sizes of the X-effects on copulation frequency in the reciprocal-F_1 versus backcross males argue against a large effect of the Y chromosome on sexual isolation. The F_1 males always have their X and Y chromosomes from different species, while all backcross males have Y chromosomes from *D. mauritiana*. If there were an effect of the Y chromosome on sexual isolation, it would act in an antagonistic fashion to the X in F_1 males, reducing the effects of the X chromosomes estimated from comparing the reciprocal F_1 males. In the backcrosses, however, the 'X-effect' is not reduced by an antagonistic Y, though it is reduced by undetected recombination between the *singed* marker and 'sexual isolation' genes. Obviously, since the estimated X-effects were lower in backcross males than in F_1 males, any effect of the Y was not large enough to counteract the effect of undetected recombination in the backcross. This is further evidence that the Y does not play a large role in sexual isolation.

The effect of the X chromosome on courtship latency observed in the F_1 hybrids was not observed in this comparison. Although crosses 9 and 10 show a slight but non-significant difference in this character in the same direction seen in the F_1 crosses, an almost identical difference in courtship latency is observed in the control intraspecific cross (Table 1, genotypes 15 *v.* 16), and so could be an effect of the *singed* marker itself.

(*b*) *Second chromosome*

Comparing genotypes 11 *v.* 12 in Table 1, one finds no difference between these second-chromosome genotypes in courtship frequency, copulation frequency or copulation latency, but an apparent effect on courtship latency. An effect of identical direction and similar magnitude was, however, also seen in the intraspecific control crosses (Table 1, genotypes 17 *v.* 18). The 95% confidence interval around the difference between j/j and $j/+$ in the experimental cross is 61 ± 48 s, which overlaps broadly with the confidence interval for the difference in the control cross (52 ± 42 s). A *t*-test comparing these two differences (Snedecor & Cochran, 1967) shows that they are homogeneous ($t = 0{\cdot}216$, 558 D.F.). The effect of the *jaunty* segment on courtship latency is therefore probably a marker effect and not a species-specific difference.

The second chromosome thus has no effect on sexual isolation. Moreover, the statistical power of our tests rules out the possibility of this chromosome having a substantial but undetected effect: the upper 95% confidence limit for its effect on copulation frequency (calculated from the difference between genotypes 9 *v.* 10) is only 2%.

(*c*) *Third chromosome*

Because the map position of *irregular* is not known, the amount of *D. mauritiana* genome associated with

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it in the backcross is also unclear, but must lie between about 42 and 85 cM (Naveira & Barbadilla, 1992). The interspecific crosses (Table 1, genotypes 13 *v.* 14) show a significant effect of this segment on copulation frequency: males homozygous for the *D. mauritiana* segment (*irr/irr*) copulate 17% more often with *D. mauritiana* females than do males heterozygous for one segment from each species (*irr/+*). The control cross (Table 1, genotypes 19 *v.* 20), however, also shows a slight effect of the marker (3%) in the same direction, so we must compare the relative magnitude of these effects. Calculating the 95% confidence intervals of the effects of this segment on copulation frequency (*ir/ir − ir/+*), one obtains $0{\cdot}167 \pm 0{\cdot}061$ for the interspecific cross and $0{\cdot}036 \pm 0{\cdot}043$ for the intraspecific control cross. These confidence intervals are non-overlapping, indicating that there is a significantly larger effect of the marked segment in the interspecific than in the control cross, i.e. this third-chromosome segment almost certainly carries genes affecting the probability that a male will be accepted by *D. mauritiana* females.

In sum, at least two genes, one on the X and one on the third chromosome, affect the sexual isolation between *D. simulans* males and *D. mauritiana* females, while the second and Y chromosomes have no detectable effect.

4. Discussion

At least two genetic changes are responsible for the difference between *D. simulans* and *D. mauritiana* males that affects their probability of copulating with *D. mauritiana* females. This is of course a minimum estimate of the number of genes, for we used only three markers, one of which (*irregular*) could be linked to genes on only one chromosome arm. The relative effects of chromosomes imply that sexual isolation in males is probably not caused by many genes spread evenly throughout the genome. If this were the case, the small X chromosome would have an effect either equal to or half of that produced by either autosome (depending on dominance and dosage compensation) and the effects of the two larger autosomes would be roughly equal. The absence of a detectable effect of one autosome, however, may imply that there are relatively few genes involved in sexual isolation among males in this hybridization. This conclusion, of course, must await fine-structure mapping, which will require molecular markers.

We offer one caveat: our study involved 'no-choice' mating observations, in which females were tested against only one type of male. It is possible that we would have seen different results had we used so-called choice experiments, in which females are presented with more than one type of male. (We do not know, of course, which of these two designs is a more realistic model of *Drosophila* mating in nature.) However, Cobb *et al.* (1988, table IV) used both no-choice and 'female choice' experiments to compare the two reciprocal F_1 hybrid males, and found no effect of experimental design on the degree of sexual isolation.

Large X-chromosome effects in males have been conjectured to result from adaptive natural or sexual selection acting on that sex. As Charlesworth *et al.* (1987) have shown, advantageous new mutations that are completely or partly recessive will accumulate more rapidly on the X chromosome than on an autosome, while dominants and semidominants will be more evenly distributed among all chromosomes. However, large X-effects have rarely been found in the few existing genetic studies of sexual isolation. Although Kawanishi & Watanabe (1981) found an X-effect in sexual isolation between *Drosophila melanogaster* and *D. simulans*, and Grula & Taylor (1979, 1980) found the same for female pheromones and mate-choice in *Colias* butterflies (females are heterogametic in Lepidoptera), large X-effects were not observed in hybrids between races of *D. mojavensis* (Krebs, 1990), subspecies of *D. paulistorum* (Ehrman, 1961), or in the sibling species *D. pseudoobscura* and *D. persimilis* (M. Noor, personal communication). Moreover, the X does not show a disproportionately large effect on species differences in other male-limited morphological characters in *Drosophila*, such as sex combs and male genitalia (Coyne, 1983, 1985; Coyne & Kreitman, 1986).

It is worthwhile to compare the effects of chromosomes in this study with those seen in the previous analysis of sexual isolation in females (Coyne, 1989), as the only previous study examining the effects of major chromosomes on sexual isolation in both sexes was that of Zouros (1981). (The studies of Roelofs *et al.* (1987) and Löfstedt *et al.* (1989) in races of corn borers did not examine the effects of all chromosomes, but this was unnecessary because sexual isolation resulted from changes at only three unlinked loci, two in males and one in females.)

Two theories predict that genetic correlations between the sexes will produce a similarity of chromosome effects on sexual isolation in the two sexes (see Butlin & Ritchie (1989) for a fuller discussion). The first is that such correlations are caused by pleiotropy: the same genes might affect the signal sent by one sex and the receiver in the other (Alexander, 1962; Lofstedt, 1993). This hypothesis seems unrealistic: it is not immediately obvious that the diverse organs used in sending, receiving and processing signals would be controlled by the same genes. The other hypothesis is that the genetic correlations would result from close linkage on the chromosomes of different genes affecting sexual isolation in the two sexes. This could occur simply because the genes are fortuitously located close to each other on the chromosome (again, there is no obvious biological reason for this), or because

evolution favours such linkage. The latter scenario may occur if sexual isolation is a byproduct of sexual selection. In particular, runaway models of sexual selection based on female preference produce genetic correlations between male traits and female preferences (Lande, 1981). At present, however, there are no explicit models of the degree of linkage expected under runaway sexual selection or any other type of sexual selection).

The data from this study and the previous one on hybrid females (Coyne, 1989), as well as previous work on other species (see below), indicate that the genetic architecture of sexual isolation differs between the sexes. In the present study, the average effects in backcrosses of the X, second and third chromosome respectively on the probability of copulation with *D. mauritiana* females (all data from backcrosses), were 0·15, 0 (no detectable effect) and 0·17. In our previous study of hybrid females (Coyne, 1989), the effects of the chromosomes in hybrid females on their probability of copulating with *D. simulans* males were respectively 0·04, 0·26 and 0·20. The ordering of these effects differs quite strikingly between the studies. Because of dosage compensation, which doubles the amount of gene product produced by X-linked loci in males, the effect of a single X in the present study may be equivalent to that of two X chromosomes in females, so one might want to double the female X-effect when making this comparison. Even doing this, however, does not make the chromosome effects comparable between the sexes: the effects of each autosome in females would still be 2–3 times larger than that of the X, while in males the X and third chromosomes have comparable effects and the second none at all.

Our inability to detect a second-chromosome effect in females does not reflect a lack of statistical power. Given our sample sizes, we would have been able to detect a significant effect on copulation frequency as small as 8% – an effect only half that of each of the other major chromosomes.

This inequality of chromosome effects between the sexes is a common result in the few previous studies. In a genetic analysis of sexual isolation between *D. arizonae* (formerly *D. arizonensis*) and *D. mojavensis*, Zouros (1981) used species-specific allozyme variants to mark the chromosomes. Using pure *D. arizonae* females offered a choice between homospecific males and hybrid males, he found a significant effect of only the Y and fourth chromosomes on sexual isolation (the X was not examined). In hybrid females, on the other hand, only the second and fifth chromosomes had significant effects on sexual isolation; so there was no obvious correlation of chromosome effects between the sexes. Likewise, in hybridizations between subspecies of *D. paulistorum*, Ehrman (1961) observed no correlation of chromosome effects between the sexes, although in some crosses not all chromosomes were examined. In the two races of European corn borer *Ostrinia nubilalis*, sexual isolation is apparently caused by changes at only three genes, affecting the pheromone difference in females (a *cis v. trans* double bond), the perception of the pheromones by male antennal receptors, and the response of a male to a perceived stimulus (Roelofs *et al.*, 1987). Genetic analysis show that these genes are all distinct and assort independently (Roelofs *et al.*, 1987; Löfstedt *et al.*, 1989). The only work showing 'genetic coupling' between aspects of male and female sexual isolation is in the pair of butterfly species *Colias eurytheme* and *C. philodice*. Here, the male signals that attract females are the UV-reflecting wing patterns and the mixture of pheromones on the wings. Species differences in both pattern and one of the major pheromones (13-methyl heptacosane) reside largely on the X chromosome (Silberglied & Taylor, 1973; Grula & Taylor, 1979), as does the ability of females to discriminate among males (Grula & Taylor, 1980). This result is remarkable given the large number of chromosomes in these species (2N = 62), but it is not clear to what extent the similar X-effects reflect pleiotropy versus linkage.

Aside from these butterflies, then, there is little evidence for strong linkage – much less genetic identity – between alleles affecting sexual isolation in males and females. The meaning of this result for sexual-selection theory will depend on whether sexual isolation results from sexual selection, and whether various theories of sexual selection can be shown to predict close linkage of male- and female-specific genes.

What characteristics of *D. simulans* males might influence their acceptance by *D. mauritiana* females? They obviously do not include cuticular pheromones, as these are nearly identical among males of these two species (Jallon & David, 1987). There are at least two other possibilities: differences in male courtship 'song' (the pattern of wing vibration during courtship) or differences in other courtship behaviours (a combination of these characters is of course also possible). These species are known to differ in song patterns and frequencies of song (Cowling & Burnet, 1981; Robertson, 1983) as well as in the probabilities that different behaviours occur during courtship (Cowling & Burnet, 1981; Cobb *et al.*, 1985, 1986, 1988). Genetic analysis of these differences may give a clue to which traits are important in mate discrimination, as one may find a behavioural or song difference whose genetics correspond to the genetics of sexual isolation itself.

Finally, it should be noted again that reproductive isolation between these two species is enhanced by other factors, including sterility in male and female hybrids (True *et al.*, 1996*a*), and the short duration of interspecific copulation, which dramatically reduces sperm transfer (Coyne, 1993). As is often true in speciation, gene flow can be prevented by the joint action of several distinct characters.

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