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Genetics of morphological differences and hybrid sterility between *Drosophila sechellia* and its relatives

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Summary

We conducted classical genetic analysis of the difference in male genitalia and hybrid sterility between the island-dwelling sibling species *Drosophila sechellia* and *D. mauritiana*. At least two loci (one on each autosome) are responsible for the genital difference, with the X chromosome having no significant effect. In contrast, male hybrid sterility is caused by at least four gene loci distributed among all major chromosomes, with those on the X chromosome having the largest effect.

We also show that the large difference in ovariole number between *D. sechellia* and its mainland relative *D. simulans* is due to at least two gene substitutions, one on each major autosome. The X and the left arm of the second chromosome, however, have no significant effect on the character. This implies that the evolution of reduced ovariole number involved relatively few gene substitutions.

These results extend previous findings that morphological differences between *Drosophila* species are caused by genes distributed among all chromosomes, while hybrid sterility and inviability are due primarily to X-linked genes. Because strong X-effects on male sterility have been found in all three pairwise hybridizations among *D. simulans*, *D. sechellia* and *D. mauritiana*, these effects must have evolved at least twice independently.

1. Introduction

The only way to estimate the numbers and effects of genes causing speciation or morphological species differences is through genetic analysis requiring crossing of distinct species. Such analysis can, for example, answer the perennially-debated question of whether morphological evolution and speciation have a polygenic basis or are caused by a few 'macromutations'.

The *Drosophila melanogaster* subgroup contains some of the most promising material for such analysis. Four of its species can be intercrossed, all having mapped mutations that facilitate genetic analysis. These four, henceforth called the *D. melanogaster* complex, include two worldwide human commensals, *D. melanogaster* and *D. simulans*, and two species endemic to Indian Ocean islands, *D. mauritiana* and *D. sechellia*. Biochemical and chromosomal studies show that the island species, from Mauritius and the Seychelles respectively, are much more closely related to *D. simulans* than to *D. melanogaster* (Lemeunier & Ashburner, 1976, 1984; Coyne & Kreitman, 1986; Solignac *et al.* 1986; Cariou, 1987; Caccone *et al.* 1988).

Genetic studies of this complex have shown that species differences in morphology and mate discrimination are based on genetic changes at several loci distributed throughout the genome (Coyne, 1983, 1985*b*, 1989; Coyne & Kreitman, 1986). In contrast, genes causing postzygotic reproductive isolation (hybrid sterility and inviability) are invariably concentrated on the X chromosome (Coyne & Orr, 1989). This disparity between morphology and sexual behaviour on the one hand and postzygotic isolation on the other is also seen in other drosophilid and non-drosophilid species (Charlesworth *et al.* 1987; Coyne & Orr, 1989).

Two of the hybridizations producing these conclusions were *D. simulans*/*D. sechellia* and *D. simulans*/*D. mauritiana*. The two island species show remarkable parallels in the characters that distinguish them from *D. simulans* as well as in the genetic architecture of these differences (Coyne & Kreitman, 1986). Because these two species live on island groups separated by 1800 km, they were thought to result

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from two independent colonizations by *D. simulans* or its ancestor (Lachaise *et al.* 1988). If this were true, the two hybridizations would represent studies of two independent evolutionary events, and the genetic and morphological parallelisms (Coyne, 1985*b*) might imply recurring evolutionary forces.

The actual phylogeny of these three species is, however, unknown. Electrophoresis, mitochondrial-DNA restriction mapping, and nuclear-DNA sequencing show that the three pairwise genetic distances are nearly the same (Solignac *et al.* 1986; Coyne & Kreitman, 1986; Cariou, 1987), implying that the speciation events in the group were nearly simultaneous. A study of DNA hybridization, on the other hand, found that the two island species were slightly but significantly closer to each other than to *D. simulans* (Caccone *et al.* 1988). It is therefore possible that the two island species had a common ancestor more recently – although not much more recently – than either did with *D. simulans*.

This disparity between phylogenies based on DNA and biogeography would be resolved if the *D. sechellia* and *D. mauritiana* shared a common ancestor on a third island such as Madagascar (no such putative ancestor has been found), or if there were inter-island colonization between Mauritius and the Seychelles (this seems unlikely). Either of these two possibilities would mean that genetic analyses of the *D. mauritiana*/*D. simulans* and *D. sechellia*/*D. simulans* hybridizations are merely two studies of a single evolutionary event, and their similarities a phylogenetic artifact.

In the absence of an unambiguous phylogeny, one way to resolve the issue is through genetic analysis of all three pairwise hybridizations. Any patterns which hold in all three analyses would imply at least two evolutionarily independent events. We have previously described the genetic basis of differences in genital morphology and of hybrid male sterility in the *D. simulans*/*D. mauritiana* and *D. simulans*/*D. sechellia* hybridizations (Coyne, 1983, 1984; Coyne & Kreitman, 1986; Coyne & Charlesworth, 1986, 1989). Here we extend the pairwise comparisons via genetic analysis of morphological and reproductive differences between the two island species themselves.

In addition, we conduct a genetic analysis of a large difference between *D. sechellia* and *D. simulans* in a morphological character affecting life history: the number of ovarioles, the egg-producing chambers in the ovaries (Mahowald & Kambysellis, 1980). Females of *D. simulans* and the outgroup *D. melanogaster* have between 35 and 50 ovarioles (the character varies clinally), females of *D. mauritiana* about 30. In contrast, *D. sechellia* females have 20 or fewer ovarioles (David & Bocquet, 1975; Louis & David, 1986; unpublished data). The outgroup character implies that the low ovariole number of *D. sechellia* is a derived condition. These females also have much lower fecundity than those of the other three species, at least in the laboratory. Even under the best laboratory conditions, a *D. sechellia* female produces fewer than one egg per ovariole per day, roughly one-half the rate of the other three species. Taking into account their low ovariole number, *D. sechellia* females are roughly 25% as fecund as those of the other three species (Louis & David 1986; unpublished data).

The adaptive significance of the reduction in ovariole number (if any) is unknown; there seems to be no compensatory increase in other aspects of *D. sechellia*'s life history (Louis & David, 1986). The change may be connected with this species' unique ecology. While *D. melanogaster*, *D. simulans* and *D. mauritiana* are food generalists, *D. sechellia* apparently breeds only in fruits of the tree *Morinda citrifolia* (Rubiaceae), which are toxic to the other three species. The low fecundity of *D. sechellia* is not increased, however, when it is raised on *Morinda* (Louis & David, 1986, and unpublished data).

2. Materials and methods

(i) *Male fertility*

All of the crosses between *D. simulans*, *D. mauritiana* and *D. sechellia* give the same result: fertile hybrid females and sterile hybrid males. A few fertile males do appear in backcrosses (David *et al.* 1974; Coyne & Kreitman, 1986). The genetics of male sterility is best analyzed in these backcrosses, in which a male's chromosomes can be correlated with his fertility.

Our analysis used *D. mauritiana* strains marked with recessive mutations. For estimating the effects of the three major chromosomes segregating together, we used *pn*; *j*; *ir*, a strain containing the eye-colour mutation *prune* on the X chromosome (map position 1-2.9), the wing mutation *jaunty* on the second chromosome (2–48.7), and the eye-facet mutation *irregular* on the third chromosome (position unknown). Locations for these and other mutations are taken from *D. melanogaster* (Lindsley & Grell, 1968), whose X and second chromosomes are homosequential to those of these species (Lemeunier & Ashburner, 1984), from Sturtevant (1929), from J. S. F. Barker (personal communication) and from our own mapping experiments. The tiny fourth chromosome, less than 2% of the genome, was not studied.

D. mauritiana pn; *j*; *ir* males were crossed to *D. sechellia* females from an isofemale strain provided by Dr Hugh Robertson. The F_1 hybrid females were backcrossed to *D. mauritiana pn*; *j*; *ir* males, and the eight genotypes of male progeny scored for fertility. The presence of a mutant marker in these males indicates homozygosity (or hemizygosity) for a *D. mauritiana* chromosome segment linked to that marker, while a wild-type allele indicates heterozygosity for segments from both species (or hemizygosity for a *D. sechellia* X chromosome). Because of recombination in F_1 females, a marker does not necessarily identify the origin of an entire chromo-

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some; instead, the marker will be nonrandomly associated with 50 cM of genome on either side. The *prune* and *jaunty* markers will be associated with most of the X and second chromosome respectively, as the former marker is near the base of the X chromosome (about 65 map units long) and the latter near the middle of the second chromosome (about 110 map units long). *Irregular*, whose position is unknown, may mark as little as half of the third chromosome.

Testes of 4-day-old virgin males were crushed in Ringer's solution and examined under a compound microscope. As in our previous work (Coyne, 1984, 1985*a*; Coyne & Kreitman, 1986), fertility of a genotype was quantified as the percentage of its males having motile sperm. Males with as few as one motile sperm were scored as 'fertile'; those with no motile sperm or no sperm at all as 'sterile'. This is not a perfect measure of fertility because some males with only a few motile sperm are functionally sterile (Coyne & Kreitman, 1986; Orr, 1989). Our measure, is, however, correlated with offspring production, allows rapid scoring of many individuals, and does not confound fertility with mating ability.

In a further cross, we genetically subdivided the X chromosome using a *D. mauritiana* strain carrying the X-linked recessive mutations *prune* and *vermilion* (*pn*, 1–2.9; *v*, 1–35·0). The *pn*; *v* males were crossed to *D. sechellia* wild-type females, and the F_1 hybrid females backcrossed separately to *D. mauritiana pn*; *v* males and *D. sechellia* males. The four genotypes of males in each backcross were scored for fertility.

All crosses were reared at 24 °C on cornmeal-yeast-agar food.

(ii) *Genital morphology*

As in our previous work (Coyne, 1983; Coyne & Kreitman, 1986), we quantified genital size as the area of the posterior process of the genital arch. This area is about three times larger in *D. sechellia* than in *D. mauritiana* (Fig. 1). Using the *pn*; *j*; *ir* stock of *D. mauritiana* and the *D. sechellia* strain as described above, we produced eight classes of backcross males with different combinations of the species' chromosomes. Genitals were dissected from males of each genotype (as well as from the pure species and F_1 hybrids), mounted in Hoyer's solution (Coyne, 1983), and the outline of the genital process traced using an overhead projecting microscope. We determined the area of each tracing with a digitizing pad connected to a computer, and calculated the actual area using a micrometer scale projected beside the tracing. The length of the tibia on one foreleg was measured on each male to correct the genital area for any body size effects (see Coyne, 1983 for additional details).

All crosses were reared at 24 °C on cornmeal-yeast-agar food.

(iii) *Ovariole number*

Two sets of crosses were necessary for a genetic analysis of differences in this character, as our *D. simulans* stock having all of its chromosomes marked did not breed on the special rearing food. To investigate the X and second chromosomes, we used a *D. simulans* strain homozygous for the recessive markers *garnet*, *net*, and *cinnabar* [*g*, 1–42.3; *nt*, 2–0.0 (2L)], *cn*; 2–54.4 (2R)]. The *g*; *nt*, *cn* females were crossed to wild-type male *D. sechellia* from strain 85, a mixture of several isofemale lines collected on Cousin Island in 1985. The F_1 hybrid females were backcrossed to *D. simulans g*; *nt*, *cn* males, and ovariole number scored in the eight offspring classes from this backcross. The effect of the third chromosome was determined in a similar backcross using a *D. stimulans* stock homozygous for the recessive mutation *ebony* (*e*, 3–61).

Flies measured for ovariole number were reared at

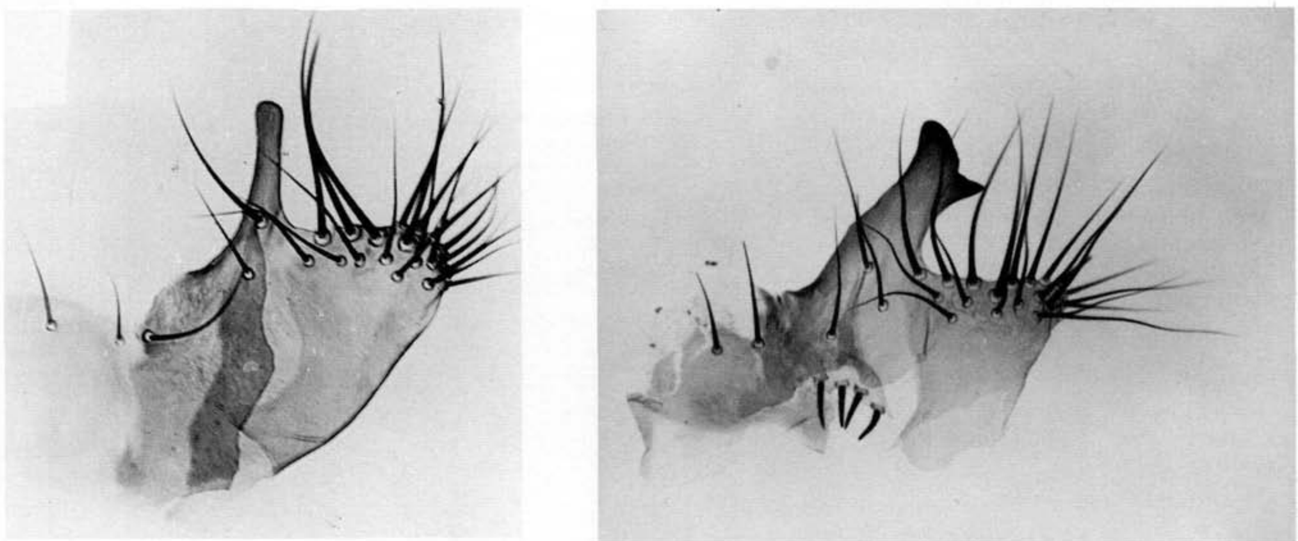

Fig. 1. Male genital arches (epandria) of *D. mauritiana* (left) and *D. sechellia* (right).

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constant density under optimal conditions at 25 °C. Parents of these flies were put into egg-laying chambers. After 24 h, groups of 50 eggs were picked, washed in 70% ethanol, and placed in vials containing a highly nutritious axenic yeast medium (David & Clavel, 1965). Females hatching from these eggs were stored in groups of 20 in yeasted cornmeal vials (genotypes were mixed within the vials), each vial also containing 10 young, wild-type *D. simulans* males. After 5 days, we dissected ovarioles from these mated females into a saturated solution of potassium bichromate. A single rear femur from each female was also measured to determine any effects of body size on ovariole number.

3. Results

(i) *Male fertility*

As observed previously (Coyne, 1984; Coyne & Kreitman, 1986), males from the pure species were highly fertile, while the F_1 males are completely sterile (we could not produce F_1 hybrids from the cross of *D. sechellia* males to *D. mauritiana* females). Male progeny from the backcross of F_1 females to *D. mauritiana* males were highly sterile (Table 1). Only 34% of the backcross males having all of their chromosome markers from *D. mauritiana* had motile sperm, indicating either cytoplasmic effects or, more likely, undetected recombination between the marker alleles and genes affecting fertility. The X chromosome had by far the largest effect on hybrid fertility; only one non-*prune* male of 976 examined had motile sperm. Using the log-likelihood CATMOD procedure of SAS (see Orr, 1987), the effect of the *prune* marker is highly significant ($\chi^2_1 = 34{\cdot}4$, $P < 0{\cdot}001$).

As in a previous analysis (Orr, 1987), the large effect of the X chromosome on fertility means that the effects of autosomes must be tested within the class of males having a *D. mauritiana* X chromosome [classes (1), (2), (3) and (4) in Table 1]: it is only in these classes that it is possible to detect autosomal effects. This nested analysis (Table 2) indicates that each of the two autosomes carries at least one gene with a large effect on hybrid fertility. The high probability associated with the interaction terms indicates that the autosomes act additively.

To determine whether the large X-effect was due to only one locus, we made backcrosses with the X-linked *prune* and *vermilion* alleles, the only markers available in these species. As in the first cross, parents are highly fertile and F_1 males sterile (Table 3). Analysis of the backcross data is difficult because one must use statistical models when more than one markers is segregating. This analysis, given in the Appendix, shows that it is highly unlikely $P < 0{\cdot}001$) that sterility in the backcross to *D. mauritiana* is caused by only a single gene, but that a satisfactory fit to a single-gene model is obtained in the backcross to *D. sechellia* (even in this case, however, the probability of a satisfactory fit is low). These results are not

Table 2. *Log-likelihood analysis of effects of autosomes on male sterility within the class carrying the 'prune' mutation [classes (1)–(4) in Table 1]. All sources of variance have one degree of freedom*

Source	χ^2	Probability
jaunty (chromosome 2)	20.72	< 0·001
irregular (chromosome 3)	37·72	< 0·001
jaunty × *irregular*	0·00	0·94
intercept	248·25	< 0·001

Table 1. *Male sterility in hybridization between* D. sechellia *and* D. mauritiana. *The table shows the proportion of males in each genotypic class having at least one motile sperm.* Prune (pn) *is on the X chromosome,* jaunty (j) *is on the second, and* irregular (ir) *is on the third. The female parent is designated first in all crosses.*

	No. with motile sperm	Total	Fraction with motile sperm
Pure species and F_1s			
D. mauritiana pn; j; ir	187	207	0·903
D. sechellia	174	200	0·870
F_1 (*D. sechellia* × *pn; j; ir*)	0	242	0·000
Backcross (F_1 × *pn; j; ir mauritiana*)			
(1) *pn; j; ir*	78	230	0·339
(2) *pn; j;* +	20	209	0·100
(3) *pn;* + ; *ir*	24	174	0·138
(4) *pn;* + ; +	7	227	0·031
(5) + ; *j; ir*	1	206	0·005
(6) + ; *j;* +	0	260	0·000
(7) + ; + ; *ir*	0	221	0·000
(8) + ; + ; +	0	289	0·000

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contradictory because the chromosome segments are tested in different genetic backgrounds that may interact differently with the X-linked 'sterility' genes (Wu & Beckenbach, 1983).

In sum, male sterility in this cross is caused by evolutionary divergence of at least four genes, with those on the X chromosome having the largest effect. These results are similar to those of our previous studies in the *D. melanogaster* complex (Coyne, 1984; Coyne & Kreitman, 1986).

(ii) *Genital morphology*

Table 4 gives the genital area and tibia lengths of both species, the reciprocal F_1 hybrids, and the backcross hybrids. The area in *D. sechellia* is about three times larger than that of *D. mauritiana*, and those of the F_1 hybrids are close to the midparental value. Considering the reciprocal F_1s together, there was no significant correlation between tibia length and genital area ($r_{72} = 0.123$, $P > 0.05$), so the areas of the two genotypes can be compared directly without correcting for body size. This difference is significant ($t_{72} = 2.29$, two-tailed $P < 0.025$), but it is in the direction opposite to that expected under either a genetic or a cytoplasmic hypothesis: F_1 males with *D. sechellia* cytoplasm and X chromosomes have smaller genitals than those of the reciprocal class. Not all genes differing among species must act in the same direction, of course, but because this difference was not seen in the backcrosses (see below), it may well be a random effect.

Tibia length and genital area were highly correlated in the combined data from the eight backcross classes ($r_{292} = 0.399$, $P < 0.001$). We corrected for this effect by using as each individual's genital area its residual deviation from the best-fit regression line between the two variables: genital area = 0.009242 (tibia length)–0.001861 (Table 4; higher-order regression coefficients did not improve the fit). The variances of these residuals were not heterogeneous among the backcross classes, nor was there a significant correlation between means and variances. We therefore analysed the chromosomal effects and interactions with a three-way fixed-factor analysis of variance.

According to this analysis (Table 5), both of the autosomes had large and significant effects on the character in the expected direction, although the second chromosome has a larger effect than the third. None of the interactions approaches significance, so there again is little evidence for epistasis. The effect of the X chromosome, although in the direction expected if it carries genes affecting genital morphology, is not significant. This lack of an X-effect does not substantiate the difference in genital area found between reciprocal F_1 males. The discrepancy could be caused by effects of the *D. mauritiana* Y chromosome or the *D. sechellia* cytoplasm on genital area (both of which we consider unlikely), or may simply be a random effect.

These species therefore differ by a minimum of two loci (one on each autosome) affecting genital morphology, while the X chromosome has little or no effect. This lack of effect is especially striking because the X chromosome is dosage-compensated, doubling its product in males.

(iii) *Ovariole number*

Table 6 gives the ovariole numbers of the pure species, the F_1 hybrid with a *D. simulans* mother (the reciprocal cross produced no offspring), and the female offspring of the two backcrosses. The ovariole number of hybrids was slightly lower than the mid-parent value,

Table 3. *Dissection of X-chromosome effects on male sterility in the* D. mauritiana/D. sechellia *hybridization.* Prune (pn) *is at 0.8 and* vermilion (v) *at 33.0 on the X chromosome. The female parent is given first in all crosses*

	No. with motile sperm	Total	Fraction with motile sperm
Pure species and F_1s			
D. mauritiana pn, v	183	204	0·897
D. sechellia	167	202	0·827
F_1 (*sechellia* × *pn, v*)	0	100	0·000
Backcross 1 (F_1 × *pn, v mauritiana*)			
pn, v	53	278	0·191
pn, +	1	205	0·005
+, *v*	13	201	0·065
+, +	2	284	0·007
Backcross 2 (F_1 × *sechellia*)			
pn, v	0	213	0·000
pn, +	17	197	0·086
+, *v*	2	189	0·011
+, +	31	215	0·144

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Table 4. *Genital area of genotypes in cross of* D. mauritiana × D. sechellia. Prune (pn) *is on the X chromosome*, jaunty (j) *on the second, and* irregular (ir) *on the third.* N = *37 for all genotypes except for the backcross classes* pn; j; ir *and* pn; +; ir *where* N = *36. In all crosses the female parent is given first*

Genotype (S.E.)	Genital area, $mm^2 \times 10^3$ (S.E.)	Tibia length, mm (S.E.)	Residual $\times 10^4$ (S.E.)
Pure species and F_1s			
D. mauritiana pn; j; ir	1·746 (0·035)	0·441 (0·002)	—
D. sechellia 85	5·311 (0·086)	0·469 (0·003)	—
F_1 (*sechellia* × *mauritiana*)	3·104 (0·061)	0·487 (0·003)	—
F_1 (*mauritiana* × *sechellia*)	3·323 (0·074)	0·474 (0·002)	—
Backcross (F_1 × *mauritiana*			
pn; j; ir)	1·944 (0·060)	0·443 (0·003)	−2·887 (0·589)
pn; j; +	2·341 (0·072)	0·461 (0·003)	−0·526 (0·688)
pn; +; *ir*	2·304 (0·058)	0·449 (0·003)	0·147 (0·606)
+; *j; ir*	2·066 (0·060)	0·438 (0·003)	−1·187 (0·634)
pn; +; +	2·659 (0·066)	0·467 (0·002)	2·064 (0·654)
+; *j;* +	2·334 (0·061)	0·457 (0·002)	−0·236 (0·592)
+; +; *ir*	2·360 (0·040)	0·449 (0·003)	0·770 (0·430)
+; +; +	2·635 (0·070)	0·465 (0·003)	2·004 (0·679)

Table 5. *Analysis of variance of corrected genital areas (residuals) of eight backcross progeny from Table 4. All factors have one degree of freedom except for the error term, which has 287*

Source	Mean square $\times 10^7$	F ratio	Probability (two-tailed)
X chromosome (*pn*)	2·86	2·07	0·15
Second chromosome (*j*)	43·81	31·70	4×10^{-8}
Third chromosome (*ir*)	18·86	13·65	3×10^{-4}
pn × *j*	1·01	0·73	0·39
pn × *ir*	2·12	1·54	0·22
j × *ir*	0·02	0·02	0·90
pn × *j* × *ir*	0·21	0·15	0·70
Error	1·38		

indicating slight dominance of the *D. sechellia* alleles.

There was no significant correlation between tibia length and ovariole number in the backcross with marked X and second chromosomes ($r_{846} = 0{\cdot}037$, $P < 0{\cdot}05$), so the data were not corrected for body size. Variances among backcross classes were not heterogeneous, nor was there a significant correlation between means and variances, so we applied a factorial analysis of variance to the untransformed data (Table 7).

Only one of the three chromosome segments studied – that marked with *cinnabar* – has a significant effect on ovariole number, although the effects of the X and 2L are in the direction expected if they carry 'ovariole' genes. Because *cinnabar* is on 2R near the centromere, it is likely that the gene or genes causing the effect are actually on 2R, although they could be closely linked to *cinnabar* but on the other arm. None of the interactions approach significance, indicating a lack of important epistasis. The ovariole number of the *g; nt, cn* backcross class is much lower than that of the pure *g; nt, cn D. simulans* stock, indicating either undetected recombination between the markers and the ovariole loci, loci on the third chromosome influencing the character, or both of these.

The second backcross (backcross B in Table 6) shows that the third chromosome does indeed carry genes affecting the character. In the lumped backcross classes there was again no correlation between ovariole number and tibia length ($r_{198} = 0{\cdot}13$, $P < 0{\cdot}05$). The untransformed ovariole numbers of the *ebony* and non-*ebony* classes were therefore compared with a simple *t*-test, which shows a highly significant differ-

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Table 6. *Ovariole numbers in* D. simulans, D. sechellia *and their hybrids.* Garnet *is on the X chromosome,* net *and* cinnabar *on the two arms of the second, and* ebony *in the middle of the third. In all crosses the female parent is given first. Standard errors are 0·002 for all tibia lengths*

Genotype	*N*	Ovariole number (S.E.)	Femur length (mm)
Pure species and F_1s			
simulans g; nt, cn	100	34·85 (0·33)	0·657
sechellia 85	100	16·40 (0·14)	0·677
F_1 (*g; nt, cn* × *sechellia 85*)	100	26·81 (0·17)	0·700
D. simulans e	100	35·15 (0·25)	0·693
Backcross A (F_1 × *g; nt, cn*)			
g; nt, cn	106	31·57 (0·36)	0·677
g; nt, +	106	29·00 (0·33)	0·677
g; +, *cn*	106	30·70 (0·35)	0·684
+, *nt, cn*	106	30·73 (0·42)	0·683
g; +, +	106	28·92 (0·33)	0·685
+, *nt,* +	106	29·00 (0·40)	0·690
+, +, *cn*	106	30·77 (0·37)	0·690
+, +, +	106	28·34 (0·38)	0·699
Backcross B (F_1 +/*e* × *simulans e/e*)			
e/e	100	32·48 (0·28)	0·693
+/*e*	100	28·81 (0·24)	0·698

Table 7. *Analysis of variance of ovariole numbers (residuals) of eight backcross progeny in* g; cn, e cross. *Data taken from Table 6. All factors have one degree of freedom except for the error term, which has 840*

Source	Mean square	*F* ratio	Probability (two-tailed)
X chromosome (*g*)	23·78	1·65	0·20
2L (*nt*)	32·50	2·25	0·13
2R (*cn*)	959·43	66·42	< 0·0001
g × *nt*	1·53	0·11	0·75
g × *cn*	0·47	0·03	0·86
nt × *cn*	0·08	0·005	0·94
g × *nt* × *cn*	29·44	2·04	0·15
Error	14·44		

ence in the expected direction ($t_{198} = 10{\cdot}14$, $P <$ 0·0001). We did not further subdivide this chromosome. Because *ebony* is near the middle of the chromosome, the gene or genes responsible could be on either or both arms.

Considering the two backcrosses, the average effect of the gene(s) linked to *ebony* is 3·7 ovarioles, and that of the gene(s) linked to *cinnabar* 2·2. The sum of these effects, 5·9, is still too small to account for the difference between the F_1 females and the pure *D. simulans g; nt, cn* stock (8·0 ovarioles). This disparity could again be due to either loose linkage between the markers and two ovariole loci, to additional genes undetected by this analysis, to maternal effects, or to epistasis between genes on the two autosomes.

The difference in ovariole number between the species is therefore caused by substitutions at at least two loci, one on each autosome. The X chromosome and left arm of the second chromosome have little or no effect on the character. Because two of the three arms examined do not significantly affect ovariole number, this species difference is probably not caused by many genes of small effect distributed throughout the genome.

4. Discussion

Our overall results parallel those of our earlier studies (Coyne, 1983, 1984, 1985; Coyne & Kreitman, 1986): morphological differences among species are based on genes distributed among several or many chromo-

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somes, with no particular concentration on the X, while postzygotic isolation (sterility and inviability of hybrids) is due largely to X-linked loci. Our conclusions from genital morphology and sterility, previously based on the *D. stimulans/D. mauritiana* and *D. simulans/D. sechellia* hybridizations, are therefore robust to the phylogeny of these three species.

Sterility of hybrids between *D. sechellia* and *D. mauritiana* is due to divergence of at least four genes, the maximum number detectable by the method of analysis. As in our previous studies and those of other workers (see Coyne & Orr, 1989), sterility is largely a polygenic phenomenon, but with an overwhelming X chromosome effect. It is not yet clear how many X-linked genes contribute to sterility, which could be caused by a few genes of large effect or many genes of small effect. Sorting this out will require more genetic markers (either morphological or molecular) and laborious recombination studies like that of Shrimpton & Robertson (1988).

This large effect of the X chromosome in male sterility has now been found in all three pairwise hybridizations among *D. simulans*, *D. sechellia*, and *D. mauritiana* (Coyne, 1985; Coyne & Kreitman, 1986). This genetic architecture must therefore have evolved at least twice independently, regardless of the true phylogeny of these species. This independent evolution supports the idea that X-linked heterogametic sterility and inviability is a recurring evolutionary phenomenon. Similar effects have been observed not only in other *Drosophila* groups, but also in other insects and at least one mammal (Coyne & Orr, 1989).

We have proposed that the X-linked sterility is a pleiotropic byproduct of the fixation of underdominant or advantageous recessive alleles (Coyne & Orr, 1989). Such alleles not only accumulate faster on the X chromosome than on autosomes (explaining the large X-effect for sterility), but also cause postzygotic isolation to evolve somewhat faster in heterogametic than in homogametic hybrids [explaining Haldane's (1922) Rule, the observation that if only one sex is sterile or inviable in species crosses, it is nearly always the heterogametic sex]. Although our explanation may be wrong, Haldane's Rule and the X-effect are widespread evolutionary phenomena that probably have the same cause.

Any study of the genetics of hybrid sterility may reveal allelic differences that have accumulated after reproductive isolation was complete and that therefore played no real role in speciation. The only way around this problem is to study taxa that are in an early stage of speciation. *D. mauritiana* and *D. sechellia* may fulfill this requirement. Although they are named as distinct species, they live in different places and have an incomplete degree of both pre- and postzygotic reproductive isolation. It is therefore impossible to judge if they are distinct biological species that could coexist in the same area without gene flow (Mayr, 1963). In addition, molecular analyses show that these taxa are very young (Coyne & Kreitman, 1986; Solignac *et al.* 1986; Cariou, 1987; Caccone *et al.* 1988). We are fairly confident, then, that our analyses reveal genetic changes occurring early in the process of speciation.

A minimum of two loci are responsible for the difference in genital morphology between *D. sechellia* and *D. mauritiana*, one fewer than the maximum of three detectable in our analysis. Our ability to reveal genes affecting this character is limited by the paucity of mutant markers in the two island species as well as by their rather small difference in genital area. There may be a slight effect of the X chromosome as well (the effect is nearly significant under a one-tailed test), but it would be very small and certainly not comparable in magnitude to the effect of either autosome. (Although half the size of each autosome, the X chromosome should have equivalent effects because it is dosage-compensated.) The two autosomes have roughly equal effects on the character, so that a single 'macromutation' cannot be responsible for most of the difference between the species. We also find no epistasis between chromosomes.

Finally, the genetic dissection of ovariole numbers does represent the analysis of a true evolutionary event. A minimum of two loci are also involved in the divergence of this character between *D. sechellia* and *D. simulans*. We again find a lack of epistasis, a general result in all of our genetic studies of morphology and behaviour (Coyne, 1983, 1985*a*, 1989; Coyne & Kreitman, 1986).

Two out of the three chromosome arms examined had no significant effect on the difference in ovariole number. Although we did not examine both arms of the third chromosome, the lack of effects of X and 2R suggest that relatively few genes are involved. There is no evidence that the character difference is based on many genes of small effect spread evenly throughout the genome.

There is surprisingly little evidence for the neo-Darwinian view that adaptations are often based on many loci of small effect. Our studies of the *D. melanogaster* complex show that differences in secondary sexual characters are polygenic (Coyne 1983, 1985; Coyne & Kreitman, 1986). These characters, however, probably evolved by sexual selection, possibly due to a gradual shift in female preference and the male character (Lande & Arnold, 1985). Such a slowly-moving optimum usually leads to polygenic evolutionary change (Fisher, 1958; Lande, 1983). A drastic shift in optimum, however, such as might occur when an island is colonized, may cause evolution based on a few alleles of large effect (Turner, 1975; Lande, 1983). Unfortunately, we do not know whether the low ovariole number of *D. sechellia* is a real adaptation or merely a byproduct of some other evolutionary change. It will be interesting to determine whether species differences on other characters not

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related to sexual selection also show a fairly simple genetic basis.

In sum, the studies reported here have confirmed the fundamental dichotomy in *Drosophila* between the genetics of morphological differences and reproductive isolation, and extended our conclusion that large X-effects on postzygotic isolation are recurring evolutionary phenomena.

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Appendix

Genetic analysis of the effect of the X chromosome on sterility

The hypothesis to be tested is that only a single locus on the X chromosome causes its sterility effect when present in the background of the other species. Our procedure is to calculate, using this assumption, the recombination and fertility parameters that best fit the observed data, and determine if there then remains a significant deviation from the single-locus model.

Consider first the backcross of F_1 females to *D. mauritiana* males. Let *s* be the 'sterility allele' from *D. sechellia* and s^+ the wild-type allele from *D. mauritiana* (Fig. 2). Let θ_s be the probability that a male carrying the *s* allele is sterile, and θ_f be the probability that a male carrying the s^+ allele is sterile.

θ_s and θ_f can be estimated from the frequency of non-motile males in the +, + and *pn*, *v* classes in the backcross:

$$\theta_s = 282/284 = 0{\cdot}993$$

$$\theta_f = 225/278 = 0{\cdot}809.$$

Let *r* be the probability that a crossover in the interval between *pn* and *v* falls to the left of the *s* locus, and $1-r$ be the probability that it falls to the right (Fig. 2). Then we have:

probability that *pn*, + male is sterile $= \pi_1 = r\theta_s + (1-r)\theta_f$,

probability that +, *v* male is sterile $= \pi_2 = r\theta_f + (1-r)\theta_s$.

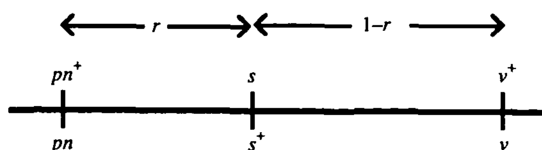

Fig. 2. Model for dissecting the effects of the X chromosome on hybrid male sterility. See text for details.

Denote the observed results as follows:

	Motile	Non-motile
pn, +	*a*	*b*
+, *v*	*c*	*d*

Then the log-likelihood of these data is given by:

$$\log L = c + a\log(1-\pi_1) + b\log\pi_1 + c\log(1-\pi_2) + d\log\pi_2.$$

The minimum value of $\log L$ ($-12{\cdot}387$) occurs at $r = 0{\cdot}93$. The expected values of *a*, *b*, *c* and *d* using the estimated parameters are $E(a) = 4{\cdot}08$, $E(b) = 200{\cdot}9$, $E(c) = 35{\cdot}8$, and $E(d) = 165{\cdot}2$; the observed values are 1, 204, 13, and 188 respectively. The χ^2 for the fit of calculated to observed data is 20·04 with one degree of freedom: this discrepancy between observed and expected under the single-locus model is highly significant ($P < 0{\cdot}001$). Examining the source of this discrepancy, we find that the expected number of motile individual in class *a* (*pn*, +) is very low, indicating that the sterility locus must be close to the *v* locus. On the single-locus model, one would then expect class *c* (+, *v*) to have rather high motility, as reflected in the expected motility of 0·17. The observed motility of this class is, however, only 0·06. This suggests that there is an additional sterility locus closer to *pn*.

The same type of analysis can be applied to the backcross of F_1 females to *D. sechellia*, exchanging wild-type for marker alleles. In this case,

$$\theta_s = 213/213 = 1{\cdot}000 \quad \text{and} \quad \theta_s = 184/215 = 0{\cdot}856.$$

Using the model:

	Motile	Non-motile
+, *v*	*a*	*b*
pn, +	*c*	*d*

the minimum of $\log L$ occurs at $r = 0{\cdot}88$, so that $E(a) = 3{\cdot}27$, $E(b) = 185{\cdot}77$, $E(c) = 25{\cdot}0$, and $E(d) = 172{\cdot}0$. The actual values are 2, 187, 17, and 180 respectively, giving a $\chi^2_1 = 3{\cdot}409$ ($P < 0{\cdot}065$). Although this value is nearly significant, we cannot statistically rule out only one gene on the X chromosome causing sterility in this backcross (the pattern is the same as before). Because the X chromosome is examined on different genetic backgrounds in the two backcrosses, we do not necessarily expect that the loci will work in the same way in these crosses.

It is therefore probable that at least two loci on the X chromosome have diverged to produce hybrid sterility, but the effect of both of them is only detectable in the backcross to *D. mauritiana*.

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