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Mapping and characterization of a 'speciation gene' in *Drosophila*

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Summary

Almost nothing is known about the identity of the genes causing reproductive isolation between species. As a first step towards molecular isolation of a 'speciation gene', I mapped and partly characterized a gene causing hybrid male sterility in *Drosophila*. This analysis shows that sterility of *D. melanogaster* males who carry the 'dot' fourth chromosome from *D. simulans* is due entirely to a very small region of the *D. simulans* chromosome (including only about 5 salivary gland bands or approximately 250 kb of DNA). Thus the hybrid sterility effect of the *D. simulans* fourth chromosome is almost surely due to a single gene of very large effect (here named *hms*, hybrid male sterile). *Hms* is zygotically acting, and the *D. simulans* allele of *hms* is completely recessive. Furthermore, complementation tests suggest that *hms* is not an allele of any known locus in *D. melanogaster*.

1. Introduction

There has been a great deal of speculation about the genetic changes causing speciation. While the founders of the modern synthesis argued that reproductive isolation usually results from the divergence of 'ordinary' genes by natural selection or genetic drift (Dobzhansky, 1937; Muller, 1940, 1942), a growing number of workers argue that speciation may often involve novel genetic processes. Kidwell (1983), Engels & Preston (1979) and Hurst & Pomiankowski (1991), for instance, suggest that hybrid sterility may result from the mobilization of transposable elements among hybrids. Rose & Doolittle (1983) suggest that reproductive isolation results from 'genomic resetting', i.e. the rapid and coordinated divergence of regulatory, not coding, regions. More recently, Frank (1991) and Hurst & Pomiankowski (1991) have suggested that genes causing meiotic drive – possibly satellite DNA sequences – are involved. Mayr (1988, p. 374) speculates that several of these kinds of changes may play some role in speciation.

In reality, we know almost nothing about the genetic changes that cause one animal species to become two: we still do not know if speciation usually involves 5 or 50 genes, what the normal functions – if any – of these genes are, and how alleles at these loci differ between two species (e.g. by point mutations, the insertion of transposable elements, etc.). We certainly cannot rule out the possibility that animal speciation often involves novel genetic processes.

Resolution of this question will require detailed genetic and molecular study of the genes causing reproductive isolation. Only one such 'speciation gene' has been molecularly isolated: Wittbrodt *et al.* (1989) cloned and characterized a gene that causes cancer among certain fish hybrids (see Discussion). Unfortunately, we remain completely ignorant of the molecular genetics of hybrid sterility. As a first step towards molecularly characterizing the genes causing hybrid sterility, several workers have attempted to map factors with large effects on hybrid male fertility (Coyne & Charlesworth, 1986, 1989; Pantazidis & Zouros, 1988; Orr, 1989*a*, *b*; Guenet *et al.* 1990). These studies have, however, suffered from a simple problem: they involve species that are rather poorly developed genetically. Mapping and characterization of these factors have proved difficult without abundant morphological markers, deletions, balancer chromosomes, restriction maps and other powerful genetic tools.

Here I analyse a factor that causes reproductive isolation in *Drosophila melanogaster*, where the necessary genetic tools are available. The study concerns the tiny 'dot' fourth chromosome of *D. simulans*, which was introgressed into *D. melanogaster* by Muller & Pontecorvo (1940). In an otherwise *D. melanogaster* genetic background, the '4-sim' chromosome causes

reproductive isolation: homozygous 4-sim males are completely sterile. All other genotypes of males and females are fertile. Here I map the region of the 4-sim chromosome causing hybrid male sterility. This mapping shows that 4-sim sterility is almost surely due to a single 4-linked gene of large effect. I also partly characterize this speciation gene.

2. Materials and methods

(i) *Stocks*

The 4-sim chromosome derives from Muller & Pontecorvo's (1940, 1942) experiment. (Throughout this paper I will refer to the *D. simulans* fourth chromosome introgressed into *D. melanogaster* as '4-sim'; similarly, I will refer to the *D. melanogaster* chromosome as '4-mel'.) Although *D. melanogaster* and *D. simulans* produce all sterile or inviable F_1 hybrids – precluding production of backcross or F_2 hybrids – Muller & Pontecorvo were able to simulate a backcross to *D. melanogaster* by crossing triploid *D. melanogaster* females to irradiated *D. simulans* males (irradiation causes the loss of paternal chromosomes from the embryo). Of the many 'partial hybrids' obtained, only one was fertile – a female who received only a Y and a fourth chromosome from *Drosophila simulans*. The 4-sim/ci^D stock (a balanced lethal-sterile), which I obtained from the Bowling Green Stock Center, is derived from this female.

There is, of course, the remote possibility that any of the 'hybrid sterility' or 'hybrid inviability' genes identified by Muller and Pontecorvo actually resulted from the X-irradiation of *D. simulans* males. In the case of 4-sim male sterility, however, this seems particularly unlikely: the 4-sim chromosome is cytologically normal (Slizynski, 1941, and below). Moreover, the chance that the only fourth chromosome introgressed alone into *D. melanogaster* happened to carry an induced mutation is extremely small. Ultimately, of course, this matter can only be resolved by molecular isolation of the gene(s) involved.

It should also be clear that one cannot tell whether any one of the factors identified by Muller and Pontecorvo was actually responsible for the initial appearance of reproductive isolation between *D. melanogaster* and *D. simulans* or simply diverged after the attainment of complete isolation. This difficulty besets the analysis of most speciation genes. Nonetheless, this factor displays the property best defining a speciation gene: it causes sterility only when present with genes from another species. I.e. it is a 'locus that may contribute to [reproductive] isolation' (Zouros, Lofdahl & Martin, 1988). Furthermore, it is clear that the factor(s) on the 4-sim chromosome do not cause sterility 'by themselves'. Instead, sterility obviously results from an incompatibility between the 4-sim chromosome and other gene(s) from *D. melanogaster*. Nothing is known about these other loci (except that they are not on the fourth chromosome). Here we simply hope to determine whether the effect of the 4-sim chromosome is due to one or several genes on the *D. simulans* fourth chromosome.

Because the 4-sim stock has been rarely used since its construction, it was necessary to verify the presence of the *D. simulans* chromosome within the stock. I did this in several ways: (1) because the *D. simulans* and *D. melanogaster* dot chromosomes are fixed for a large inversion difference that prevents their pairing (Horton, 1939; Slizynski, 1941), the 4-sim chromosome was easily seen in salivary gland preparations; (2) similarly, by crossing the *D. melanogaster* 4-sim stock to *D. simulans* and examining the salivary gland chromosomes of these hybrids, I confirmed that the 4-sim chromosome pairs normally with the *D. simulans* fourth; (3) I confirmed the sterility of homozygous 4-sim males (see below); (4) I confirmed that sterility maps within a large Minute deficiency at the proximal end of the fourth, as Muller & Pontecorvo (1942) claimed (see below); (5) I confirmed that the usually recessive mutation *cubitus interruptus* (4-0) becomes partly dominant when heterozygous with the 4-sim chromosome (data not shown), as Muller & Pontecorvo (1942) and Uphoff (1949) noted.

All the other stocks are described by Lindsley & Grell (1968).

(ii) *Fertility*

Fertility was scored in two ways. For some male genotypes, I counted the number of offspring produced by individual males. Single males were placed in a vial with three virgin females. Every 3 days (up to the ninth day), the flies were transferred to a fresh vial. All offspring emerging from a vial by day 18 were counted.

Because this procedure is tedious and time-consuming, fertility was usually scored by a much faster method: testes were dissected from 7-day-old males and examined under a compound microscope with dark-field optics (see Coyne, 1984). Males were classified into three sperm motility classes: (1) 'Many': abundant motile sperm were present (nearly filling the field of vision at 100× magnification). (2) 'Few': a few localized 'patches' of motile sperm were present. (3) 'None': no motile sperm were present. In most cases, there was little ambiguity in scoring a particular male into one of these three classes. However, to test the reliability of the sperm motility scoring, some male genotypes were scored by both the offspring-counting and the sperm motility methods. The results showed that sperm motility scores provide a very reliable indicator of male fertility (see below).

Unless otherwise indicated, all crosses were performed at 22 °C.

Table 1. *Fertility of 4-sim males. Fertility was assessed by counting number of progeny produced (mean number of offspring ± 1 S.D., N = number of fathers tested). Crosses were performed and progeny reared at 25 °C*

+/+	4-sim/+	4-sim/4-sim
358·9 ± 123·3 $N = 27$	355·6 ± 87·3 $N = 28$	0·0 $N = 15$

Table 2. *Fertility of homozygous and heterozygous* D. melanogaster *'4-sim' males. Fertility was assessed by observing sperm motility. Fertility of the top two genotypes was assessed at the same time; similarly, fertility of the bottom two genotypes was assessed simultaneously*

Genotype	Many	Few	None	χ^2
4-sim/ci^D	82	101	29	208·7***
4-sim/4-sim	0	69	196	
4-mel/ci^D	68	48	10	4·7
4-sim/ci^D	257	283	49	

3. Results

(i) *The phenomenon*

Homozygous 4-sim/4-sim males never produce any offspring (Table 1). However, males carrying a single copy of the 4-sim chromosome produce as many progeny as homozygous wild-type *D. melanogaster* males (Table 1). Thus the effect of the 4-sim chromosome on hybrid male sterility is completely recessive.

4-sim sterility is also recessive if fertility is assessed by sperm motility (Table 2): the distribution of males having 'Many' *vs.* 'Few' *vs.* 'No' motile sperm is identical among heterozygous 4-sim/4-mel males and control 4-mel/4-mel males. Most 4-sim/4-sim males, on the other hand, possess no motile sperm. However, as Table 2 shows, some 4-sim/4-sim males do produce a very few weakly motile sperm (typically, less than 25 or so sperm are motile). Because they produce so few motile sperm, 4-sim/4-sim males are easily distinguished from all other genotypes when microscopically assessing fertility.

(ii) *Mapping*

The fourth chromosome does not normally recombine (Hochman, 1976). Therefore, mapping of the 4-sim sterility gene(s) requires the use of chromosomal aberrations. Fortunately, because the 4-sim sterility effect is recessive, the gene(s) involved can be deletion mapped: 4-sim/Df(4) males will be sterile if the gene(s) involved are included within the deficiency.

I tested the fertility of 4-sim/Df(4) males using all available deficiencies. The results confirm Muller & Pontecorvo's (1942) claim that the gene(s) involved are located in the proximal end of the fourth, within the large Minute deletion, Df(4)M (Table 3). Moreover, sterility maps within the smaller Df(4)M^{62f} deletion, but *not* within the very small Df(4)M^{63a} deletion. The fertility of Df(4)M^{63a}/4-sim males proves that 4-sim sterility is not an artifact of deficiency males suffering from a Minute phenotype [Minute males are often partly sterile (Lindsley & Grell, 1968)]: Df(4) males are sterile only if the deletion includes material to the left or right of the Minute locus. Table 3 also shows that no additional sterility factors are located within Df(4)G, a large deletion at the tip of the fourth. These results show that the gene(s) causing hybrid male sterility are located in 101E-F and/or in 102A2-5–102B2-5 (Fig. 1). Thus the sterility gene(s) must be either within the centric heterochromatin to the left of Df(4)M^{63a} or to the right of this deletion. No genes are known in the former region.

I also constructed a synthetic deletion in 102B by combining the proximal and distal fragments of two translocations – T(2;4)DTD39 dpp$^{d\text{-}ho}$ and T(2;4)DTD40 dpp$^{d\text{-}ho}$ – that each have a breakpoint in 102B. Through a trivial series of crosses one can produce the genotypes, $2^P4^{D\,DTD40}/+;4^P2^{D\,DTD39}$/4-sim and $2^P4^{D\,DTD39}/+;4^P2^{D\,DTD40}$/4-sim. One of these must carry a deletion in 102B (we do not, however, know the size of this deletion). This deletion does not, however, harbour any hybrid sterility gene: both classes of males are perfectly fertile ($2^P4^{D\,DTD40}$/

Table 3. *Deletion mapping of 4-sim hybrid male sterility gene. Bal = ci^D or ey^D*

	Deficiency/4-sim			Bal/4-sim			
	Many	Few	None	Many	Few	None	χ^2
Df(4)M	0	12	45	67	56	5	117·5***
Df(4)M^{62f}	0	13	94	67	85	68	96·6***
Df(4)M^{63a}	84	71	17	45	57	11	2·5
Df(4)G	54	27	2	22	7	10	16·0***

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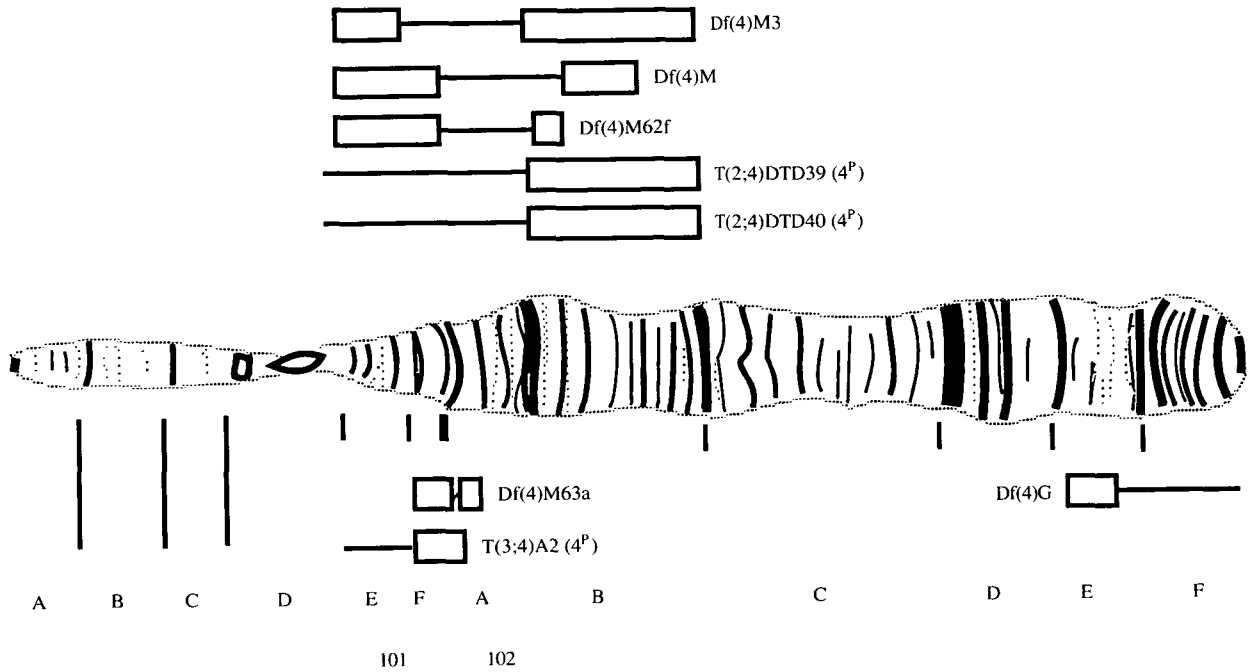

Fig. 1. Cytological location of the factor(s) on the *D. simulans* fourth chromosome causing hybrid male sterility. Chromosome rearrangements shown above the chromosome include the hybrid sterility gene(s); those shown below the chromosome do not. Open boxes represent uncertainty in the breakpoints. Results for Df(4)M^3, which is no longer available, are from the Muller papers (Lilly Library, Indiana University). The gene(s) causing hybrid sterility must be in 101F and/or 102A2-5–102B2-5. This figure is modified from that presented in Lindsley & Grell (1968), which is based upon Slizynski (1944).

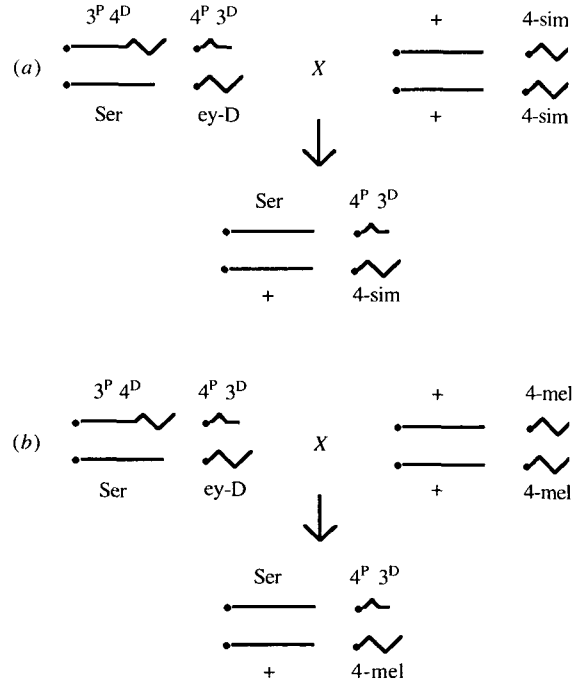

Fig. 2. Crosses used to map *hms* with respect to translocation breakpoint. The example shows use of T(3;4)A2. Cross A yields experimental males while cross B yields control males. Chromosome fragments (proximal vs. distal) are not drawn to scale.

+ ;$4^P2^{D\,DTD39}$/4-sim: 33 'Many': 7 'Few': 9 'None'; and $2^P4^{D\,DTD39}$/+ ;$4^P2^{D\,DTD40}$/4-sim: 39 'Many': 4 'Few': 3 'None').

I was, however, able to narrow down the location of this factor(s) further by using translocations with well-defined breakpoints in the Minute region. As Fig. 2 explains, one can produce males who carry a complete 4-sim chromosome but only the proximal fragment of a *D. melanogaster* chromosome. If the gene(s) causing sterility lie within this proximal segment, these males should be fertile (as the sterility effect is masked by the dominant *D. melanogaster* allele); if the sterility gene(s) is distal to this segment, these males will be sterile. To control for the effects of partial aneuploidy for the fourth on male fertility, control males were produced who differ from the above individuals only by carrying a *D. melanogaster* instead of a 4-sim chromosome.

Three translocations were used: T(3:4)A2 (breakpoint in 4 = 101F), T(2;4)DTD39 dpp$^{d\text{-}ho}$ (= 102B) and T(2;4)DTD40 dpp$^{d\text{-}ho}$ (= 102B). As Table 4 shows, 4-sim males carrying the proximal fourth material from T(3;4)A2 are sterile, although control males are fertile. Thus the hybrid sterility gene(s) is not in 101E or in 101F proximal to the T(3;4)A2 breakpoint; this result rules out most of the centric heterochromatin (Fig. 1). However, 4-sim males carrying the proximal fourth segment from either T(2;4)DTD39 dpp$^{d\text{-}ho}$ or T(2;4)DTD40 dpp$^{d\text{-}ho}$ are fertile, as are their controls (Table 4). Thus the gene(s) causing sterility is (are) within or proximal to 101B. More important, this result proves that *the D. simulans fourth chromosome harbours no additional hybrid sterility genes distal to 102B.*

In sum, 4-sim male sterility is caused by a gene or genes in 101F and/or in 102A2-5 – 102B2-5. Thus the entire sterility effect maps to a very small region

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Table 4. *Translocation mapping of 4-sim hybrid male sterility gene. Males carry one complete fourth chromosome (either 4-sim or 4-mel) and a proximal fragment of the* D. melanogaster *fourth from a translocation stock (see Fig. 2). The fourth-chromosome breakpoint of each translocation is given after the name of the translocation. The small sample sizes reflect the great difficulty in obtaining the desired genotypes. Notice that, although the T(2;4)DTD39 data approach statistical significance, the fertility difference is dubious: 4^P/4-sim males are slightly* more *fertile than 4^P/4-mel males*

	4^P/4-sim			4^P/4-mel			
	Many	Few	None	Many	Few	None	χ^2
T(3;4)A2 (101F)	1	3	26	17	22	4	43·8***
T(2;4)DTD39 (102B)	13	0	14	11	6	6	5·8
T(2;4)DTD40 (102B)	32	28	13	14	9	3	1·0

Table 5. *Complementation tests between* hms *and all known mutations in the Df(4)M region. All mutations above the dividing line are 'essential' (i.e. recessive lethal or sterile), while those below the line are visibles or P-element insertions. Bal = ci^D, ey^D or M^{57g}*

	Mutant/4-sim			Bal/4-sim			
	Many	Few	None	Many	Few	None	χ^2
1(4)1[a]	35	48	13	26	24	3	3·46
1(4)13	36	29	5	42	31	1	3·09
1(4)13[a]	48	10	0	35	12	7	9·09*
1(4)17	55	17	0	32	14	2	3·72
1(4)20	63	58	6	27	22	1	0·83
1(4)25	56	51	11	5	4	0	0·96
1(4)26	47	51	1	26	23	1	0·59
ar	60	28	2	34	22	4	2·69
Ce^2	35	13	1	46	9	0	2·89
ci^D	82	101	29	—	—	—	—
M^{57g}	67	82	20	22	38	2	5·16
Scn	73	4	7	—	—	—	—
P [R401.1]	71	57	1	—	—	—	—
ci	87	75	14	—	—	—	—
gvl	71	14	0	—	—	—	—

(including about 5 salivary bands), representing less than 15% of the cytological length of the tiny dot chromosome. This region should, therefore, include roughly 250 kb of DNA ($= 1{\cdot}7 \times 10^5$ kb [haploid genome size] $\times$ 0·01 [estimated percentage of genome that is 4-linked] $\times$ 0·15 [fraction of chromosome 4 involved]). It seems almost certain, therefore, that 4-sim hybrid sterility is caused by a single fourth-linked gene. Hereafter, this locus will be referred to as 'hybrid male sterile', *hms*.

(iii) *Characterization of* hms: *allelism tests*

Hms affords a unique opportunity to determine whether the genes causing hybrid sterility correspond to ordinary loci with normal functions within species: one can perform allelism tests between *hms* and all available mutations from the Minute region. Assuming that these mutations result from loss-of-function lesions (which seems likely), heterozygotes between *hms* and a mutation will be male-sterile if *hms* and the mutation are allelic. Unfortunately, it appears that no screens for 4-linked male steriles have ever been performed. (The one male 'sterile' known, *abdomen rotatum*, simply prevents males from successfully copulating; these males produce abundant motile sperm). Nonetheless a fairly large number of loci, including many lethals, have been identified in the Minute region of the fourth.

As Table 5 shows, all mutations in the Minute region complement *hms*. Thus *hms* does not appear to be an allele of any known locus in *D. melanogaster*. Because the Minute region has been saturated or nearly saturated for lethal mutations (Hochman, 1973), it seems unlikely that *hms* is an allele of a locus that is capable of mutating to lethality (unfortunately, a mutation at one lethal locus in this region, l(4)24 [Hochman, 1976], is apparently no longer available).

I also tested whether the *D. melanogaster* allele of *hms* has been inactivated by any translocation with a

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Table 6. hms *is zygotically acting. The 4-sim chromosome, when haploid, causes male sterility regardless of its parental origin. Control males carrying the fourth chromosome from* D. melanogaster *are fertile*

Genotype	Many	Few	None
4-sim (mat)/0	0	25	34
4-sim (pat)/0	0	83	48
4-mel (mat)/0	75	11	4
4-mel (pat)/0	36	9	6

breakpoint in the Minute region; if so, *hms*-translocation heterozygotes should be male-sterile. The following translocations were tested: T(3;4)A2, T(3;4)A28, T(3;4)86D, T(3;4)89E, T(3;4)ry^{ps1149}, T(2;4)CA36, T(2;4)DTD39 $dpp^{d\text{-}ho}$, T(2;4)DTD40 $dpp^{d\text{-}ho}$, T(3;4)A30. All *hms*-translocation heterozygotes are male-fertile (data not shown).

(iv) hms *is zygotically-acting*

Because only homozygous 4-sim/4-sim males are sterile, all sterile individuals have a mother who carries at least one copy of the 4-sim chromosome. Thus 4-sim sterility could require a maternal contribution; indeed, maternal effects on postzygotic reproductive isolation are well known (Orr, 1989*a*).

To determine whether 4-sim sterility involves any maternal product, males were produced who carry only one 4-sim chromosome and no *D. melanogaster* homologue; if maternal *hms* product is required for 4-sim sterility, haplo-4 males receiving the 4-sim chromosome from their mothers should be sterile, while those receiving it from their father should be fertile. These haplo-4 flies can be readily produced by using a C(4)RM stock (e.g. the cross of 4-sim/4-sim females × C(4)RM males yields many haplo-4 4-sim males, who are easily recognized by their Minute phenotype). To control for the effects of haploidy *per se* on male fertility, haplo-4 males carrying a *D. melanogaster* fourth chromosome were also produced. These control males are usually fertile regardless of the parental origin of their fourth chromosome (Table 6). More important, haplo-4 4-sim males are sterile regardless of whether the 4-sim chromosome is of paternal or maternal origin (Table 6). 4-sim male sterility must therefore result from the zygotic genotype at *hms*.

(v) hms *does not interact with testis-specific tubulin*

MAP (microtubule-associated protein) genes appear to represent a fairly large and important class of male-sterile loci in *D. melanogaster* (Fuller, 1986). The products of these MAP genes are associated with a testis-specific form of β2-tubulin, an important constituent of the sperm flagellar axoneme (Fuller, 1986). Although no 4-linked MAP genes are known (apparently no screen for them has been performed), the 4-sim sterility phenotype is similar to that of some MAP mutations: *hms* is recessive, zygotically expressed, and acts post-meiotically by disrupting sperm motility; a few weakly motile sperm are sometimes produced but are apparently not transferred through the vas deferens.

Interactions between B2t and certain MAP mutations are easily detected: although both the $B2t^n$ and MAP mutations are individually recessive, the $B2t^n/B2t^+$; MAP/MAP^+ double heterozygote is male-sterile (Fuller, 1986). I tested whether *hms* interacts with B2t by comparing the fertility of $B2t^n/B2t^+$ 4-sim/4-mel double heterozygotes with that of the single heterozygotes. *Hms* shows no interaction with B2t: double heterozygotes produce just as many progeny as single heterozygotes (Table 7). Thus, although we cannot entirely exclude the possibility the *hms* is structurally associated with testis-specific tubulin, we have no evidence that *hms* is a MAP gene.

4. Discussion

It has proved remarkably difficult to identify genes that have a large, easily scored effect on reproductive isolation. In fact, as noted above, only one gene reducing hybrid fitness has been molecularly isolated: Wittbrodt *et al.* (1989) characterized an X-linked gene which causes malignant melanoma among hybrids between the platyfish and the swordtail (genus *Xiphophorus*); the gene appears to code for a receptor tyrosine kinase. Unfortunately, it is not clear if this example is representative of the genetics of hybrid inviability: hybrid inviability probably does not usually result from malignancies. In any case, no gene causing hybrid sterility has been molecularly isolated.

Attempts to identify speciation genes in *Drosophila* have faced a simple problem – *D. melanogaster* does

Table 7. hms *shows no interaction with testis-specific β-tubulin. Two-way ANOVA on log-transformed data: 4-sim X* $B2t^n$ *interaction,* $F = 0{\cdot}329$, $P = 0{\cdot}57$. *Crosses performed and progeny reared at 25 °C*

+/+ +/+	+/TM3 4-sim/+	$B2t^n$/TM2 +/+	$B2t^n$/+ 4-sim/+
$358{\cdot}9 \pm 123{\cdot}3$ $N = 28$	$355{\cdot}6 \pm 87{\cdot}3$ $N = 27$	$180{\cdot}0 \pm 72{\cdot}6$ $N = 22$	$213{\cdot}9 \pm 116{\cdot}0$ $N = 29$

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not successfully cross with any other species. Although studies investigating other species of *Drosophila* have identified chromosome regions having a large effect on hybrid fertility (Coyne & Charlesworth, 1986, 1989; Orr, 1989; Pantazidis & Zouros, 1988), these regions are enormous by molecular genetic standards. It is thus unclear whether the effects of these regions reflect the action of one or many genes. Moreover, even if only one locus were involved, it is difficult to obtain an accurate map position for the locus. Last, because these species do not provide the powerful genetic tools available in *D. melanogaster*, it is doubtful whether these speciation genes could be readily cloned and characterized, even if they could be reliably mapped.

Because it escapes these difficulties, the 4-sim hybrid male sterility described by Muller & Pontecorvo (1940, 1942) provides an attractive system for analysis of speciation genes: one can study reproductive isolation within *D. melanogaster*, where an impressive array of genetic tools can be employed. The most important goal of the present study was to fine-map the sterility effect in order to determine whether it is probably due to a single gene: molecular analysis might prove prohibitively difficult if sterility is caused by more than one gene on the *D. simulans* fourth chromosome. The results show that sterility almost certainly involves a single 4-linked gene: sterility maps to a very small portion of the fourth (101F and/or 102A2-5–102B2-5), representing less than 15% of the cytological length of the dot chromosome and thus including roughly 250 kb of DNA. Indeed, this region only includes about 5 salivary bands. [It should be noted that the standard map of the fourth, produced by Slizynski (1944) and modified in Fig. 1, overestimates the number of salivary bands: Bridges (1935) and Hochman (1976) claim that the fourth harbours only about 50–75 bands, while Slizynski (1944) claimed that 137 bands are present. Recent electron microscope maps of the fourth (Sorsa, 1988) confirm the lower estimate. The map in Fig. 1 is based upon Slizynski's standard map only because it was traditionally used to define the breakpoints of the rearrangements shown.] This speciation gene is here named 'hybrid male-sterile', *hms*.

It was also possible to partly characterize *hms*. The results show that the *D. simulans* allele of *hms* is completely recessive. Obviously, then, the *D. simulans* fourth chromosome could only affect hybrid backcross, not F_1, male fertility. Although there are many possible explanations of this recessivity, it is possible that the *D. simulans* chromosome simply does not carry the *hms* locus, i.e. *hms* could be a neomorphic mutation in *D. melanogaster* or the ancestral *D. simulans* locus could have been transposed to some other location [interestingly, the gene inducing melanoma among *Xiphophorus* hybrids is physically absent from the X chromosomes of one of the species (Wittbrodt *et al.* 1989)]. At present, we have no way of distinguishing between these possibilities. The present results also show that hybrid male sterility depends on the zygotic, not maternal, expression of *hms*. This result is not surprising, as most male-sterile loci in *D. melanogaster* are zygotically acting (Lindsley & Lifschytz, 1972).

We know very little about the identity of *hms*. It is clear, however, that *hms* is not a non-complementing MAP gene: *hms* shows no interaction with testis-specific β-tubulin. More important, the results of the allelism tests suggest that *hms* does not correspond to any known locus in *D. melanogaster*. Because it appears that most of the essential loci in this region of the fourth chromosome have been identified by mutation (Hochman, 1976), *hms* is probably not an allele of a locus capable of mutating to lethality.

Had *hms* corresponded to some known locus in *D. melanogaster*, some longstanding questions about hybrid sterility would have been immediately answered. Most important, we would have known that hybrid sterility involves 'ordinary' genes having some function within the species. The actual result, however, does not allow us to resolve this issue. Although the view that speciation involves some novel genetic process might predict that *hms* would not correspond to any known gene, the view that speciation involves the divergence of normal genes can also easily explain this fact: while all available 4-linked mutations are lethals or visibles, most of the genes playing a role in spermatogenesis in Drosophila have little or no effect on viability (Lindsley & Lifschytz, 1972) and so would not have been detected in routine screens of the fourth chromosome.

Obviously, resolution of whether *hms* represents an 'ordinary' gene that has diverged between *D. melanogaster* and *D. simulans* by normal genetic processes will require molecular isolation of this gene. We are presently attempting to clone *hms*. Hopefully, such studies will finally allow us to elucidate the molecular genetic basis of speciation.

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