

Gene Transposition as a Cause of Hybrid Sterility in *Drosophila*

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We describe reproductive isolation caused by a gene transposition. In certain *Drosophila melanogaster*-*D. simulans* hybrids, hybrid male sterility is caused by the lack of a single-copy gene essential for male fertility, *JYAlpha*. This gene is located on the fourth chromosome of *D. melanogaster* but on the third chromosome of *D. simulans*. Genomic and molecular analyses show that *JYAlpha* transposed to the third chromosome during the evolutionary history of the *D. simulans* lineage. Because of this transposition, a fraction of hybrids completely lack *JYAlpha* and are sterile, representing reproductive isolation without sequence evolution.

Reproductive isolation can be a by-product of divergent evolution between populations and is a necessary step in speciation. Dobzhansky and Muller described a model for the evolution of reproductive isolation where functional divergence between interacting loci in different lineages yields incompatible interactions in their hybrids (1, 2). Although evidence for Dobzhansky-Muller interactions is well established, few genes involved in these incompatibilities have been identified and characterized (3). Furthermore, it remains unclear whether molecular evolutionary processes other than functional divergence cause postzygotic reproductive isolation (2).

The genetic basis of postzygotic isolation between *Drosophila melanogaster* and *D. simulans* has been studied previously by crossing triploid *D. melanogaster* females to heavily x-irradiated *D. simulans* males (4). This approach avoided the normal sterility and inviability of *D. melanogaster*-*D. simulans* F₁ hybrids, producing hybrids with backcross-like genotypes. One of these individuals, a fertile female, was used to establish a stock that carried the tiny "dot" fourth chromosome of *D. simulans* in an otherwise *D. melanogaster* genetic background. Hybrid males homozygous for the *D. simulans* fourth (*4-sim*) chromosome were completely sterile because mature sperm were immotile (5). Because the fourth chromosome does not recombine during meiosis (6), the location of the hybrid sterility gene(s) was mapped by using deletions and translocations to cytological regions 101E to 101F and/or 102A5 to 102B5 (5, 7).

It remained unclear, however, whether *4-sim* hybrid male sterility was genuine or an artifact of the x-irradiation used to construct the hybrid stock. By using recently characterized mutations that rescue the viability (8) and fertility (9)

of *D. melanogaster*-*D. simulans* F₁ hybrid females, we introgressed a new *D. simulans* fourth chromosome into an otherwise *D. melanogaster* background without use of radiation (Materials and Methods).

Hybrid male fertility in the new *4-sim* introgression line was scored by both sperm motility and number of offspring sired by individual males (Materials and Methods). Heterozygous *4-sim* males produce abundant motile sperm, whereas homozygous *4-sim* males typically produce immotile sperm (Table 1); sterile hybrid males thus show the same spermatogenic phenotype as those described previously (5). Although pure species *D. melanogaster* and heterozygous *4-sim* males (*ey^D/4-sim*) produce many offspring [232.1 ± 21.5 (SEM), $N = 13$, and 157.7 ± 13.9 , $N = 15$, respectively], homozygous *4-sim* males produce none (0.0 ± 0.0 , $N = 39$). In females, the *4-sim* chromosome has no effect on fertility. The *D. simulans* fourth chromosome thus causes true hybrid male sterility.

By using chromosomal deficiencies, we confirmed previous results (5, 7) showing that the gene(s) causing hybrid male sterility resides

within *Df(4)M101-62f*, which includes the proximal-most 21 genes on chromosome 4 (10). We dissected this region further by using deficiencies and genomic sequence data unavailable to earlier workers (Fig. 1). None of the new deficiencies uncovers hybrid sterility (Table 1 and Fig. 1). Assuming that *4-sim* hybrid sterility is caused by a single gene, we excluded all loci distal to *cubitus interruptus* as the cause of sterility. We also excluded *plexinB* on the basis of complementation tests. Our results thus show that one of the requisite loci for *4-sim* hybrid male sterility lies proximal to *plexinB* even if multiple genes within *Df(4)M101-62f* are involved (Fig. 1).

JYAlpha (*CG17923*), a 4.1-kb gene that encodes the alpha subunit of a Na⁺ and K⁺ adenosine triphosphatase (Na⁺/K⁺ ATPase), a transmembrane protein involved in ion exchange (11), was identified as a strong candidate from the four remaining loci in the region. Four mammalian isoforms of the Na⁺/K⁺ ATPase alpha subunit exist (12). One of these, $\alpha 4$, is expressed exclusively in testes (13) and is essential for sperm motility (14). *JYAlpha* from *D. melanogaster* (*JYAlpha^{mel}*) shows ~60% amino acid identity to mouse $\alpha 4$.

To test whether *JYAlpha* is the cause of *4-sim* hybrid male sterility, we performed complementation tests with the *4-sim* chromosome by using a *P*-element insertion in *JYAlpha^{mel}* (*P{y⁺,w⁺}JYAlpha*). Because *P{y⁺,w⁺}JYAlpha* does not appear to be a null mutation (Table 2), we remobilized the element. Two of the resulting excisions were chosen for analysis. *JYAlpha^{mel.8c}* truncated *JYAlpha^{mel}* after the first 268 amino acids, excluding the presumed active site. *JYAlpha^{mel.12a}* restored wild-type sequence at *JYAlpha^{mel}*. *JYAlpha^{mel.8c/4-sim}* males were sterile, and their sperm motility resembled that of *Df(4)M101-62f/4-sim* (Table 2). As expected,

Table 1. Hybrid male sterility and deficiency mapping. *ey^D* indicates *eyeless*-Dominant mutation.

Genotype	Sperm motility			χ^2
	Many	Few	None	
<i>ey^D/4-sim</i>	43	50	18	96.31***
<i>4-sim/4-sim</i>	0	32	92	
<i>Df(4)M101-62f/ey^D</i>	110	175	36	289.2***
<i>Df(4)M101-62f/4-sim</i>	0	59	211	
<i>Df(4)Gley^D</i>	87	71	11	3.22
<i>Df(4)G/4-sim</i>	146	118	8	
<i>Df(4)ED6369/ey^D</i>	29	8	0	1.10
<i>Df(4)ED6369/4-sim</i>	82	15	1	
<i>Df(4)ED6366/ey^D</i>	105	36	1	2.47
<i>Df(4)ED6366/4-sim</i>	132	66	2	
<i>Df(4)ED6364/ey^D</i>	205	34	1	9.09*
<i>Df(4)ED6364/4-sim</i>	125	43	2	
<i>Df(4)Δ3M/ey^D</i>	112	13	0	4.63
<i>Df(4)Δ3M/4-sim</i>	94	22	1	
<i>Df(4)Δ9M/ey^D</i>	131	11	0	1.98
<i>Df(4)Δ9M/4-sim</i>	99	12	1	

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*** $P \ll 0.0001$. *Although marginally significant ($P < 0.05$), this deficiency does not uncover the severe "none" sperm motility phenotype seen in *4-sim* hybrids.

JYAlpha^{mel.12a/4-sim} males were fully fertile (Table 2). *JYAlpha* is thus both necessary and sufficient for hybrid male sterility. Further analysis shows that *JYAlpha* is essential for sperm motility within *D. melanogaster* (Table 2).

Genes causing hybrid incompatibilities often evolve rapidly and show population genetic signs of divergence under positive natural selection (3, 15–17). However, preliminary analysis of *JYAlpha* revealed an apparent difference in its location between *D. simulans* and *D. melanogaster*. A BLAST search of the *D. simulans* whole genome assembly (18) suggests that *JYAlpha* is flanked proximally by *CG9766* and distally by *complexin* (*cpx*), two loci that reside on the right arm of the third chromosome (3R) in *D. melanogaster*, *D. simulans*, and their sister species (Fig. 2A). This 3R-linked locus represents the single best BLAST hit for *JYAlpha* (reciprocal e values = 0.0) and appears to be *JYAlpha*'s only location in *D. simulans*. We confirmed these results in several ways.

To determine *JYAlpha*'s chromosomal location in *D. simulans*, we performed crosses to track genetically the chromosome with which *JYAlpha* segregates. Strain-specific molecular markers in *JYAlpha*^{sim} confirmed that the locus segregates with chromosome 3 (Materials and Methods).

To confirm *JYAlpha*'s precise location within the third chromosome of *D. simulans*, we attempted to amplify polymerase chain reaction (PCR) product across the putative 3R-4 breakpoints from pure *D. simulans* C167.4, control pure *D. melanogaster*, and homozygous 4-sim hybrids. Amplification across both the proximal (*CG9766-JYAlpha*^{sim}) and the distal (*JYAlpha*^{sim}-*cpx*) breakpoints succeeded in pure *D. simulans* but failed in pure *D. melanogaster* and in homozygous 4-sim hybrids, as expected if *JYAlpha* resides on 3R in *D. simulans* but on 4 in *D. melanogaster* (Fig. 2B).

Next, we sequenced a large region of 3R from pure *D. simulans*. In particular, we sequenced ~9.8 kb from *D. simulans* C167.4; this region extends from *CG9766* proximally to *cpx* distally. In *D. melanogaster*, *CG9766* and *cpx* are adjacent genes on 3R (19). In *D. simulans*, however, the region between *CG9766* and *cpx* is interrupted by *JYAlpha* (Fig. 2A). *JYAlpha* is the only gene found on chromosome 4 of *D. melanogaster* that resides in this region of 3R in *D. simulans* (fig. S2).

We also attempted to PCR-amplify *JYAlpha* from homozygous 4-sim hybrids. We were unable to PCR-amplify *JYAlpha* product from homozygous 4-sim hybrids with use of any primer pairs, despite routine amplification from pure-species *D. melanogaster* and *D. simulans* individuals. This confirms that an intact *JYAlpha* locus does not exist on the *D. simulans* fourth chromosome.

Lastly, we asked whether *JYAlpha* is single-copy. We already possess strong genomic and genetic evidence that *D. melanogaster* carries a

single functional copy of *JYAlpha* (10) (Table 2). We also performed a Southern blot analysis of pure *D. melanogaster*, pure *D. simulans*, and

homozygous 4-sim flies. As expected from genome sequence data, *JYAlpha* appears to be single-copy in both species (fig. S3). Also as

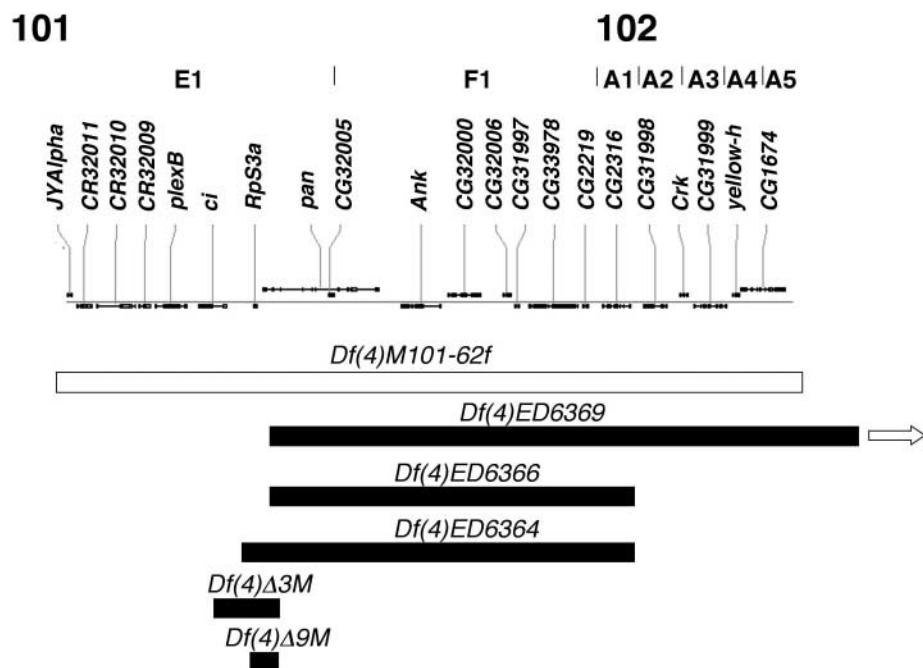


Fig. 1. Chromosome 4 region uncovered by *Df(4)M101-62f*. Horizontal bars represent deficiencies. White bars show deficiencies that uncover hybrid sterility when heterozygous with 4-sim; black bars show deficiencies that fail to uncover sterility when heterozygous with 4-sim. *Df(4)G* (not pictured) lies distal to *Df(4)M101-62f* and uncovers region 102E2 to 102F2. The distal breakpoint of *Df(4)ED6369* extends beyond *Df(4)M101-62f*. Map adapted from Entrez Genomes Build 4.3 (28).

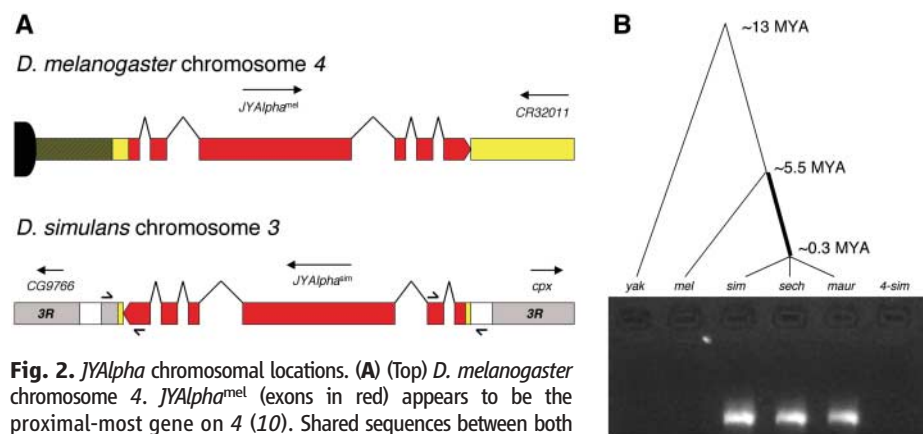


Fig. 2. *JYAlpha* chromosomal locations. (A) (Top) *D. melanogaster* chromosome 4. *JYAlpha*^{mel} (exons in red) appears to be the proximal-most gene on 4 (10). Shared sequences between both *D. melanogaster* chromosome 4 and *D. simulans* chromosome arm 3R are coded yellow. Upstream of *JYAlpha*^{mel}, chromosome 4 becomes highly repetitive (striped) and then “centromeric” (black). Arrows indicate the direction of transcription. (Bottom) *D. simulans* chromosome arm 3R. About 1.6 kb of sequence showing weak homology to sequence found on all major chromosomes (white) is present upstream and downstream of *JYAlpha*^{sim} (red). This sequence shows no significant homology to known DNA-mediated transposable elements (29). 3R material including *CG9766* and *cpx* is shown in gray. Half arrows give approximate primer locations for PCR across the 3R-4 breakpoints. Coding sequence between *JYAlpha*^{mel} and *JYAlpha*^{sim} shows no signature of divergence by positive selection, at least as crudely measured by the ratio of amino-acid changing to non-amino-acid changing substitutions: $K_a/K_s = 0.05$, consistent with purifying selection. (B) PCR-amplified region across the 3R-4 *CG9766-JYAlpha*^{sim} breakpoint. Analogous results were obtained from the *JYAlpha*^{sim}-*cpx* breakpoint. Divergence times are shown at speciation events on the phylogeny. The branch onto which the *JYAlpha* transposition event maps is bolded. *yak* indicates *D. yakuba*; *mel*, *D. melanogaster*; *sim*, *D. simulans*; *sech*, *D. sechellia*; *maur*, *D. mauritiana*; and 4-sim, 4-sim homozygotes.

expected, no hybridization was observed in our Southern blot for homozygous *4-sim* hybrids (fig. S3). This again demonstrates that no intact *JYAlpha* resides on chromosome 4 of *D. simulans*.

The cause of *4-sim* hybrid male sterility appears, therefore, to be surprisingly simple. A copy of *JYAlpha* exists on the fourth chromosome of *D. melanogaster* but not of *D. simulans*. Thus, a heterozygous *4-sim* hybrid male carries one *D. melanogaster* chromosome 4 and remains fertile, whereas a homozygous *4-sim* hybrid male lacks *JYAlpha* and is sterile. The sterility of this hybrid genotype reflects the complete absence of a locus essential for male fertility.

To determine the evolutionary direction of *JYAlpha*'s transposition, we performed PCR assays across the *3R-4* breakpoints in several species closely related to *D. melanogaster*. PCR amplification across both the proximal and distal break points succeeded in *D. sechellia* and *D. mauritiana*, showing that these species also carry *JYAlpha* on *3R* (Fig. 2B). The *D. sechellia* genome also confirmed that *JYAlpha* resides on *3R* in this species [Supporting Online Material (SOM) Text and fig. S2]. Amplification across the *3R-4* breakpoints did not succeed, however, in *D. yakuba*. Consistent with this, genome sequence data show that *JYAlpha* resides on the fourth chromosome in this species (SOM Text). Thus, *JYAlpha* appears to have resided ancestrally on chromosome 4 and was transposed to chromosome arm *3R* after the split of *D. melanogaster* from the *simulans* clade species but before the split of *D. simulans* from its sister species. This dates *JYAlpha*'s transposition to roughly 0.3 to 5.5 million years ago (20, 21).

The transposition of *JYAlpha* raises several evolutionary questions. Because the transposition

is evolutionarily old (at least $\sim 3 \times 10^6$ generations), it is unlikely that population genetic data would allow detection of a selective sweep associated with this event (22). Although the coding region of *JYAlpha* shows no obvious signs of divergence by positive natural selection between *D. melanogaster* and *D. simulans* (Fig. 2A legend), we cannot exclude a history of selection at this locus. Similarly, we cannot infer the exact mechanism of *JYAlpha*'s transposition. We can, however, rule out retroposition, because *JYAlpha^{sim}* possesses introns (Fig. 2A).

It has been hypothesized that movement of gene function between chromosomes might cause postzygotic isolation, either by simple transposition or translocation (*I*) or by gene duplication-transposition followed by divergent evolution (23). Although gene duplication-transposition events are fairly common in *Drosophila* (24–26), our results suggest that *JYAlpha* is currently single-copy in both *D. melanogaster* and *D. simulans*. But it seems likely that a *JYAlpha* duplication existed sometime during the evolutionary history of the *simulans* clade. In any case, *JYAlpha* represents a clear example of a gene transposition causing reproductive isolation.

These findings raise the possibility that gene transposition could be important in the evolution of reproductive isolation. Although the present example sterilizes only a fraction of F_2 hybrids and has no effect on the fertility of F_1 hybrids, an analogous gene transposition event between the sex chromosomes could sterilize or kill F_1 hybrids between allopatric populations. If, for example, a *Y*-linked gene essential for male fertility transposed to the *X* chromosome, crosses between transposed and nontransposed populations would yield sterile F_1 hybrid males in one direction of the hybridization, consistent with Haldane's rule (27). Similarly, transpositions between sex chromosomes and autosomes, or be-

tween autosomes, could affect a fraction of hybrid backcross or F_2 genotypes (23). Gene transposition events between chromosomes need not, therefore, be common to have a large effect on hybrid fitness, because any hybrid that lacks a single essential gene would be inviable or sterile. The transposition of essential genes could represent a largely overlooked cause of reproductive isolation.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5792/1448/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 to S2
References

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Table 2. *JYAlpha* complementation tests. *ci^D*, *cubitus interruptus*-Dominant mutation; *ey^D*, *eyeless*-Dominant mutation.

Genotype	Sperm motility			χ^2
	Many	Few	None	
<i>ci^D/4-sim</i>	91	45	10	173.1***
<i>P{y⁺,w⁺}JYAlpha/4-sim</i>	18	110	148	
<i>P{y⁺,w⁺}JYAlpha/ey^D</i>	71	61	27	
<i>P{y⁺,w⁺}JYAlpha/4-sim</i>	6	114	116	115.7***
<i>P{y⁺,w⁺}JYAlpha/ci^D</i>	186	20	2	
<i>P{y⁺,w⁺}JYAlpha/P{y⁺,w⁺}JYAlpha</i>	17	174	57	313.1***
<i>ci^D/4-sim</i>	94	25	3	
<i>JYAlpha^{mel.8c}/4-sim</i>	0	53	78	173.4***
<i>JYAlpha^{mel.12a}/ci^D</i>	67	13	0	
<i>JYAlpha^{mel.12a}/4-sim</i>	90	9	1	2.91
<i>JYAlpha^{mel.8c}/ci^D</i>	112	33	3	
<i>JYAlpha^{mel.8c}/JYAlpha^{mel.8c}</i>	0	37	53	151.7***
<i>JYAlpha^{mel.12a}/ci^D</i>	67	18	5	
<i>JYAlpha^{mel.12a}/JYAlpha^{mel.12a}</i>	92	8	2	8.35*

****P* < 0.0001. *Although marginally significant (*P* < 0.05), the effect is in the wrong direction and probably reflects a mild *ci^D* marker effect.