# Relative Paucity of Genes Causing Inviability in Hybrids Between *Drosophila melanogaster* and *D. simulans*

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ABSTRACT

Using deficiencies from *Drosophila melanogaster*, we looked for genomic regions in the sister species *D. simulans* that could cause lethality when hemizygous on a hybrid genetic background. Such genotypes allow hemizygous genes from one species to interact with heterozygous genes from other species and may correspond to the kinds of genotypes causing Haldane's rule, the observation that if only one gender is sterile or inviable in species hybrids, it is nearly always the heterogametic sex. A survey of roughly 50% of the *D. simulans* genome (114 chromosome regions) revealed only four regions causing hybrid lethality and five causing severe reductions in hybrid viability. However, the viability of all of these genotypes was at least partially restored by rearing hybrids at lower temperature or using different genetic backgrounds from *D. simulans*. We therefore detected no *D. simulans* chromosome regions causing unconditional hybrid lethality, although several regions were shown to be deleterious under most tested temperatures and genetic backgrounds. The relative paucity of "inviability genes" supports the idea, suggested by work on other species, that hybrid inviability between closely related species might be caused by interactions among relatively few genes, while hybrid sterility may involve many more loci.

MANY recent studies on the genetics of speciation have focused on regularities in reproductive isolation such as Haldane's rule (Haldane 1922), the generalization that if a species cross produces sterility or inviability in only one gender of hybrid offspring, it is almost invariably the heterogametic sex (see also Coyne and Orr 1989a,b; Wu and Davis 1993; Turelli and Orr 1995; Laurie 1997). This phenomenon appears in a wide variety of taxa, including birds and butterflies in which the heterogametic sex is female. Moreover, sterility or inviability of the heterogametic sex appears to be an important first step in speciation, as comparative studies show this condition to appear early in the evolution of complete postzygotic isolation (Coyne and Orr 1989a, 1997; Turelli and Begun 1997).

One genetic explanation of Haldane's rule, as well as the seemingly large effect of the *X* chromosome on postzygotic isolation (Coyne and Orr 1989b), is that the genes causing sterility and inviability of hybrids are often partly recessive (Orr 1993; Turelli and Orr 1995; Orr and Turelli 1996; Turelli and Begun 1997). In such cases, the heterogametic hybrids would be more debilitated than homogametic hybrids, as sterility or inviability caused by recessive genes would be exposed on hemizygous *X* chromosomes but masked in hybrids having two *X* chromosomes. Because genes causing hybrid inviability appear to do so in both males and females (Orr 1991; True *et al.* 1996), one would expect (as observed in the two aforementioned studies) that hybrid females would often be rendered inviable if homozygous for an *X* chromosome from one species, or that both sexes of hybrid might become inviable if a segment of autosome were made homozygous in a genetic background otherwise heterozygous for genes from both species.

Another question that has intrigued evolutionists is which *form* of postzygotic isolation evolves first: sterility or inviability? Early suggestions that both forms accumulate at the same rate (Coyne and Orr 1989a) may be erroneous (Wu 1992). Wu and Davis (1993) posited that genes causing sterility of hybrids evolve much more rapidly than those causing inviability. Studies of hybrid sterility genes have indeed shown that they are often quite numerous, even among closely related species (Naveira and Fontdevila 1986; Naveira 1992). Wu *et al.* (1996), for example, estimate that the sister species *Drosophila simulans* and *D. mauritiana*, whose hybridization produces fertile females and sterile males, differ by at least 120 loci causing male sterility, though this may be an overestimate because this figure was extrapolated from regions known in advance to have large effects on sterility (Coyne and Orr 1998). These observations have led to an alternative theory of Haldane's rule: male

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hybrids may be preferentially sterile or inviable because their postzygotic isolation is a pleiotropic byproduct of sexual selection, under which genes expressed in males evolve faster than those expressed in females (Wu and Davis 1993).

In contrast to the large number of genes typically involved in hybrid sterility, the few studies of hybrid *inviability* have implicated far fewer genes, although such work has often involved the introgression of large heterospecific chromosome segments and hence cannot accurately estimate gene number (Coyne and Orr 1998). Nevertheless, a higher number of sterility *vs.* inviability genes has been supported by three sets of genetic studies (Naveira and Fontdevila 1986, 1991; Naveira 1992; Carvajal *et al.* 1996; Hollocher and Wu 1996; True *et al.* 1996).

While these studies suggest that genes causing hybrid sterility accumulate faster than those causing hybrid inviability, there are a few uncertainties in the results. First, the aforementioned studies of hybrid inviability and sterility involved introgressing homozygous segments of genome from one species into a background homozygous for genes from another species. However, it is not clear whether these genes act like those posited to cause Haldane's rule. This rule derives from observations of $F_1$ hybrids, which not do not possess homozygous genes from one species interacting with homozygous genes from another, but rather hemizygous *X*-linked alleles interacting with *heterozygous* autosomal genes from another species. Thus, if sex-limited $F_1$ sterility and inviability involves partially recessive alleles, it must be based on *X*-linked genes of that type interacting with semidominant alleles from another species. Testing such genetic interactions [posited by Turelli and Orr (1995) to be a major cause of Haldane's rule] requires an experimental design different from those used previously. Second, previous studies (those of Naveira and his colleagues are an exception) examined rather large segments of introgressed foreign genome, so while there seem to be more segments causing hybrid sterility than inviability, we do not have a good idea of the relative number of these two classes of genes. Finally, all studies of hybrid sterility and inviability have tested single pairs of strains examined under a single set of environmental conditions, and we do not know whether "inviability and sterility genes" reflect fixed and unconditional interspecific differences or depend on specific environments and genetic background, as reported in some hybridizations (*e.g.*, Sturtevant 1929; Watanabe *et al.* 1977; Orr 1996).

Here we describe a study of the number of genes causing hybrid inviability when hemizygous in a heterozygous genetic background, *i.e.*, the types of genes that might be responsible for Haldane's rule. Our experimental design involves examining the effects of smaller chromosome segments than those used in previous work and also examines whether the expression of inviability genes depends on temperature and genetic background. This work involves the well-known species pair *D. melanogaster* and *D. simulans*; we summarize details of their hybridization in the next section.

## MATERIALS AND METHODS

**The species and their hybrids:** *D. melanogaster*, a cosmopolitan human commensal, is an outgroup to the three species *D. simulans* (also a cosmopolitan commensal), *D. sechellia*, and *D. mauritiana*. The last two species are endemic to islands in the Indian Ocean. The *D. simulans*/*sechellia*/*mauritiana* trio diverged about 0.6–0.9 mya, while the divergence between *D. melanogaster* and *D. simulans* is much older—roughly 2.5–3.4 mya (Hey and Kliman 1993).

The hybridization between *D. simulans* and *D. melanogaster* was first made fortuitously by Quackenbush (1910) and studied more extensively by Sturtevant (1919, 1920). When crossed, these species produce unisexual broods corresponding to the gender of the *D. melanogaster* parent. The unisexual hybrid offspring are sterile in both directions of the cross. When the hybridization involves *D. melanogaster* mothers, all male progeny die as late larvae, and the recovery of the female (but not male) offspring is enhanced by rearing larvae at lower temperatures. Some stocks of both species are much better than others at producing hybrids (Watanabe *et al.* 1977; Lee 1978). The reciprocal cross using *D. simulans* mothers usually yields only male offspring, with females dying as embryos; but with some stocks one can recover females by rearing the cross at low temperature (see Orr 1996). In general, *D. mauritiana* and *D. sechellia* behave similarly to *D. simulans* in these crosses.

The genetics of hybrid inviability in this group was elegantly reviewed by Hutter (1997), and relevant facts will be mentioned in the discussion. Recent work has revealed the presence of at least four rare "lethal rescue" mutants in some strains of both *D. melanogaster* and *D. simulans*. Such mutants can restore the normally inviable gender in the hybridizations (Hutter and Ashburner 1987; Hutter *et al.* 1990; Sawamura *et al.* 1993a,b; Sawamura and Yamamoto 1993, 1997). Lethality of offspring in the reciprocal hybridizations seems to be an independent phenomenon, because it not only occurs at different developmental stages (larval *vs.* embryonic) but also because a different set of genes rescues each type of lethality (Sawamura *et al.* 1993b). These rescue mutations might be either mutant alleles of genes that normally help cause the inviability of hybrids or perhaps only "switch" genes that override a more complicated polygenic system of inviability (Coyne 1992).

In contrast to inviability, little is known about the genetic basis of the sterility of these hybrids (see discussion). Davis *et al.* (1996) have recently described some strains that produce weakly fertile hybrids.

**Experimental design:** We looked for any segments of *D. simulans* chromosomes, that, when hemizygous in a hybrid background, caused inviability of the normally all-female offspring. Our purpose was not to study the causes of inviability (see Orr *et al.* 1997 for one hypothesis) but to assess the number of loci capable of causing it. Moreover, because inviability genes cause lethality in both male and female hybrids, our experimental design is able to detect them if they act recessively in the manner postulated by Orr (1993) and Turelli and Orr (1995) and also have a large effect on viability.

This design involved using stocks of *D. melanogaster* carrying known deficiencies that are kept heterozygous against balancer chromosomes containing dominant marker genes (Lindsley and Zimm 1992). Females from these balancer

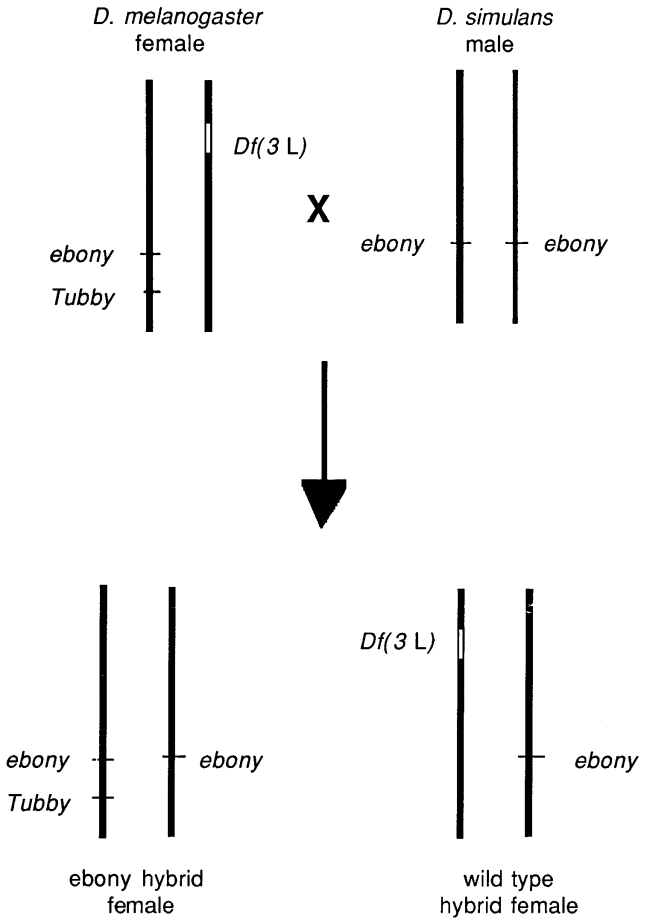


Figure 1.—Crossing scheme used to produce two classes of hybrid offspring, one of which is hemizygous for a small section of *D. simulans* genome uncovered by a deficiency from *D. melanogaster*. The chromosome containing the *Tubby* marker is the *TM6B* balancer. (This shows a cross investigating regions on the third chromosome.) See materials and methods for further information.

stocks were crossed to male *D. simulans* carrying a mutation that could be used in the female hybrid progeny to distinguish between hybrids carrying the *D. melanogaster* deficiency-carrying chromosome and hybrids carrying the alternative balancer chromosome.

Figure 1 gives an example of such a cross. Here, a *D. melanogaster* stock containing a third-chromosome deficiency balanced over the *TM6B* chromosome is crossed to a stock of *D. simulans* homozygous for *ebony*. Because *TM6B* balancers carry *ebony* (and usually *Tubby*, a dominant allele that is difficult to score in adults), hybrids carrying the *D. melanogaster* deficiency (genotypes henceforth called $Df/+_{sim}$) would be wild type in phenotype, while hybrids carrying the balancer (henceforth called $+_{mel}/+_{sim}$) would have the ebony phenotype. If the *D. melanogaster* deficiency in such a cross uncovered a region of the *D. simulans* genome causing hybrid lethality when hemizygous, this cross would produce ebony but no wild-type offspring.

Such *TM* balancers were used for most of the third-chromosome analysis. Similarly, most second-chromosome deficiencies in *D. melanogaster* are balanced against either *CyO* or *SM* balancers, which (with the exception of *SM5*) carry the recessive *cinnabar* mutation and the dominant wing mutation *Curly*. Deficiency-bearing and non-deficiency-bearing progeny could be distinguished by crossing these balancer stocks to a *D. simulans* strain homozygous for *cinnabar* (we preferred to use the eye-color mutation because *Curly* sometimes has variable expressivity, and so we could check progeny using two independent mutations). Most of our *D. melanogaster X*-chromosome deficiencies were balanced against *FM7*, which carries alleles of the *white* locus. Females from such strains were crossed to *D. simulans white* males. Females from *X*-chromosome deficiency stocks carrying *FM6* (which contains the dominant mutation *Bar*) were crossed to wild-type *D. simulans* males from the Florida City strain (see below), and Bar *vs.* non-Bar offspring were scored.

In a few cases, the nature of the balancer dictated that other *D. simulans* stocks be used to distinguish the two classes of hybrid offspring; we do not describe these crosses here but the information is available on request. In a few other cases, we made usable *D. melanogaster* deficiency stocks by combining deficiencies from some strains and balancers from others.

Over 150 suitable *D. melanogaster* deficiency stocks were obtained from a variety of sources, with the vast majority deriving from the "deficiency kits" assembled by K. Matthews at the Drosophila Stock Center at Indiana University. These kits contain 183 deficiency-carrying strains that, *in toto*, cover between 67 and 75% of the euchromatin. Of all stocks tested, 113 produced either at least one deficiency-carrying hybrid or more than five non-deficiency-carrying offspring in crosses to *D. simulans* (these were the criteria we used to determine whether deficiency-carrying hybrids were inviable or of low viability). The deficiencies we used, and their described breakpoints, are given in Table 1. The complete description of each *D. melanogaster* stock, including the balancers used, is available on request.

For each deficiency stock, we made five replicate crosses to *D. simulans*, each cross involving 15 males and 15 females. Crosses were made on banana/molasses/agar medium and reared at 24°. If no progeny were obtained from any of these crosses, no further hybridizations were made. If any progeny were obtained, we made at least five additional crosses under the same conditions. As is commonly observed, the *D. melanogaster*/*D. simulans* hybridization often does not go well because its success is highly dependent on the strains used.

**Conditionality of lethality:** Tests were made to determine whether any lethality seen in initial crosses was unconditional. When any of the crosses described above produced offspring showing either no or very few deficiency heterozygotes (the latter were arbitrarily defined as crosses producing fewer than one $Df/+_{sim}$ offspring for every eight $+_{mel}/+_{sim}$ offspring), we first examined whether the lethality was also seen when offspring were reared at lower temperatures. Crosses producing few or no $Df/+_{sim}$ offspring at 24° were hence remade (again in five replicate vials) and reared at 21°. If the hybrid offspring still showed an absence or a low ratio of deficiency-carrying heterozygotes, the crosses were remade and reared at 18°. We made initial crosses at higher temperatures because of evidence (cited above) that lower temperatures tend to rescue inviable hybrids in this cross.

In addition, crosses showing near or complete lethality of the $Df/+_{sim}$ genotype at 24° or at the two lower temperatures were crossed to independently derived stocks of the *D. simulans* "tester" mutation (see below) to determine the effect of genetic background in rescuing the initially lethal genotype.

In a few cases we also used *D. mauritiana* and *D. sechellia* (sister species of *D. simulans*) to determine whether any putative hybrid-lethal regions in *D. simulans* were also lethal in hybrids between its close relatives and *D. melanogaster*. This is not a test of the conditionality of lethality of *D. simulans* regions, but does show whether similar lethality can be seen in hybrids between *D. melanogaster* and the two species most closely related to *D. simulans*.

**TABLE 1**

**Numbers of offspring from crosses of balanced deficiency strains of *D. melanogaster* crossed to marker strains of *D. simulans***

| Deficiency | Breakpoints | No. of flies | |
|---|---|---|---|
| | | $Df/+_{sim}$ | $+_{mel}/+_{sim}$ |
| *Df(1)JC19* | 2F6; 3C5 | 17 | 8 |
| *Df(1)N-8* | 3C2–3; 3E3–4 | 15 | 27 |
| *Df(1)dm75e19* | 3C11; 3E4 | 10 | 14 |
| *Df(1)JC70* | 4C15-16;5A1-2 | 6 | 3 |
| *Df(1)C149* | 5A8-9;5C5-6 | 124 | 138 |
| *Df(1)N73* | 5C2;5D5-6 | 42 | 31 |
| *Df(1)JF5* | 5E3-5;5E8 | 32 | 25 |
| *Df(1)G4e[L]H24i[R]* | 5E3-8; 6B | 36 | 30 |
| *Df(1)Sxl-bt* | 6E2;7A6 | 101 | 56 |
| *Df(1)ct4bl* | 7B2-4;7C3-4 | 13 | 16 |
| *Df(1)C128* | 7D1;7D5-6 | 20 | 48 |
| *Df(1)RA2* | 7D10;8A4-5 | 4 | 32 |
| *Df(1)KA14* | 7F1-2;8C6 | 3 | 8 |
| *Df(1)v-L15* | 9B1-2;10A1-2 | 34 | 32 |
| *Df(1)N105* | 10F7;11D1 | 2 | 11 |
| *Df(1)JA26* | 11A1;11D-E | 2 | 9 |
| *Df(1)N12* | 11D1-2;11F1-2 | 41 | 74 |
| *Df(1)C246* | 11D-E;12A1-2 | 68 | 73 |
| *Df(1)g* | 12A;12E | 2 | 2 |
| *Df(1)RK4* | 12F5-6;13A9-B1 | 2 | 3 |
| *Df(1)sd72b* | 13F1;14B1 | 12 | 19 |
| *Df(1)4b18* | 14B8;14C1 | 16 | 0 |
| *Df(1)N19* | 17A1;18A2 | 112 | 114 |
| *Df(1)JA27* | 18A5;18D1-2 | 48 | 32 |
| *Df(1)JC4* | 20A1;20E-F | 0 | 27[a] |
| *DF(2L)net-PMF* | 21A1;21B7-8 | 34 | 54 |
| *Df(2L)ast2* | 21D1-2;22B2-3 | 64 | 113 |
| *Df(2L)JS32* | 23C3-5;23D1-2 | 119 | 263 |
| *Df(2L)23C;23E3-6* | 23C;23E3-6 | 233 | 250 |
| *Df(2L)ed1* | 24A3-4;24D3-4 | 15 | 28 |
| *Df(2L)ed-dp* | 24C3-5;25A2-3 | 15 | 16 |
| *Df(2L)cl-h3* | 25D2-4;26B2-5 | 6 | 15 |
| *Df(2L)J136-H52* | 27C2-9;28B3-4 | 32 | 79 |
| *Df(2L)spd* | 27D-E;28C | 96 | 100 |
| *Df(2L)N22* | 29C1-2;30C8-9 | 144 | 191 |
| *Df(2L)J39* | 31A;32C-E | 57 | 19 |
| *Df(2L)Prl* | 32F1-3;33F1-2 | 75 | 76 |
| *Df(2L)prd1.7* | 33B2-3;34A1-2 | 82 | 54 |
| *Df(2L)osp29* | 35B1-3;35E6 | 144 | 216 |
| *Df(2L)r10* | 35E1-2;36A6-7 | 327 | 418 |
| *Df(2L)H20* | 36A8-9;36E1-2 | 9 | 67 |
| *Df(2L)TW137* | 36C2-4;37B9-C1 | 198 | 227 |
| *Df(2L)TW84* | 37F5-38A1;39D3-E1 | 6 | 27 |
| *Df(2L)TW161* | 38A6-B1;40A4-B1 | 156 | 143 |
| *Df(2R)M41* | 41A | 14 | 23 |
| *Df(2R)pk78s* | 42C1-7;43F5-8 | 9 | 11 |
| *Df(2R)cn88b* | 42C;42E | 13 | 21 |
| *Df(2R)44CE* | 44C4-5;44E2-4 | 33 | 38 |
| *Df(2R)Np3* | 44D2-E1;45B8-C1 | 256 | 250 |
| *Df(2R)en-A* | 47D3;48A5-6 | 41 | 44 |
| *Df(2R)en30* | 48A3-4;48C6-8 | 20 | 21 |
| *Df(2R)vg135* | 49A-B;49D-E | 13 | 13 |
| *Df(2R)vg-C* | 49B2-3;49E7-F1 | 9 | 23 |
| *Df(2R)CX1* | 49C1-4;50C23-D2 | 38 | 672[b] |
| *Df(2R)vg-B* | 49D3-4;49F15-50A3 | 19 | 11 |
| *Df(2R)trix* | 51A1-2;51B6 | 70 | 91 |
| *Df(2R)Pcl 11B* | 54F6-55A1;55C1-3 | 32 | 64 |
| *Df(2R)PC4* | 55A;055F | 8 | 8 |

(*continued*)

**TABLE 1**

**(Continued)**

| Deficiency | Breakpoints | No. of flies | |
|---|---|---|---|
| | | $Df/+_{sim}$ | $+_{mel}/+_{sim}$ |
| *Df(2R)AA21* | 56F9-17;57D11-12 | 28 | 18 |
| *Df(2R)pu-D17* | 57B4;58B | 19 | 13 |
| *Df(2R) Px2* | 60C5-6;60D9-10 | 38 | 23 |
| *Df(2L)R-G5* | 62A10-B1;62C4-D1 | 14 | 17 |
| *Df(3L)R-G7* | 62B8-9;62F2-5 | 4 | 28 |
| *Df(3L)HR232* | 63C1;63D3 | 1 | 220[c] |
| *Df(3L)HR119* | 63C6;63E | 9 | 191[d] |
| *Df(3L)GN50* | 63E1-2;64B17 | 15 | 0 |
| *Df(3L)ZN47* | 64C;65C | 2 | 0 |
| *Df(3L)pbl-X1* | 65F3;66B10 | 13 | 9 |
| *Df(3L)66C-G28* | 66B8-9;66C9-10 | 22 | 2 |
| *Df(3L)h-i22* | 66D10-11;66E1-2 | 1 | 1 |
| *Df(3L)29A6* | 66F5;67B1 | 2 | 1 |
| *Df(3L)AC1* | 67A2;67D7-13 | 8 | 0 |
| *Df(3L)lxd6* | 67E1-2;68C1-2 | 12 | 7 |
| *Df(3L)vin5* | 68A2-3;69A1-3 | 7 | 0 |
| *Df(3L)Ly* | 70A2-3;70A5-6 | 4 | 7 |
| *Df(3L)fz-GF3b* | 70C1-2;70D4-5 | 32 | 26 |
| *Df(3L)fz-M21* | 70D2-3;71E4-5 | 9 | 2 |
| *Df(3L)th102* | 71F3-5;72D12 | 7 | 9 |
| *Df(3L)st-f13* | 72C1-D1;73A3-4 | 0 | 63[e] |
| *Df(3L)st4* | 72D10;73C1 | 8 | 7 |
| *Df(3L)st8P* | 72E4;73B4 | 14 | 17 |
| *Df(3L)81k19* | 73A3;74F | 0 | 94[f] |
| *Df(3L)st-e10* | 73A;73A | 420 | 333 |
| *Df(3L)st7P* | 73A1;73A7 | 157 | 116 |
| *Df(3L)W10* | 75A6-7;75C1-2 | 3 | 26[g] |
| *Df(3L)Cat* | 75B8;75F1 | 13 | 6 |
| *Df(3L)in61j* | 76F;77D | 2 | 3 |
| *Df(3L)rdgC-co2* | 77A1;77D1 | 8 | 1 |
| *Df(3L)ri-79C* | 77B-C;77F-78A | 10 | 1 |
| *Df(3L)1-16* | 80F1-h35L;80F1-35L | 22 | 10 |
| *Df(3R)Tpl10* | 83C1-2;84B1-2 | 32 | 33 |
| *Df(3R)Antp17* | 84B1-2;84D11-12 or 84A6; 84D14 | 12 | 6 |
| *Df(3R)dsx37* | 84D8;85B3-5 | 17 | 3 |
| *Df80(3R)by62* | 85D11-14;85F16 | 39 | 0 |
| *Df(3R)M-Kx1* | 86C1;87B1-5 | 86 | 5 |
| *Df(3R)T-32* | 86E2-4;87C6-7 | 101 | 29 |
| *Df(3R)ry615* | 87B11-13;87E8-11 | 83 | 6 |
| *Df(3R)ry506-85C* | 87D1-2;88E5-6 | 11 | 13 |
| *Df(3R)red1* | 88B1;88D3-4 | 18 | 18 |
| *Df(3R)ea* | 88E7-13;89A1 | 9 | 30 |
| *Df(3R)C4* | 89E;90A | 12 | 13 |
| *Df(3R)P14* | 90C2-D1;91A1-2 | 10 | 79 |
| *Df(2R)Cha7* | 91A;91F5 | 15 | 58 |
| *Df(3R)e-Ri* | 93B3;93D2-4 | 77 | 37 |
| *Df(3R)23D1* | 93F;94F | 105 | 27 |
| *Df(3R)mbc-R1* | 95A5-7;95D6-11 | 78 | 3 |
| *Df(3R)crbS87-4* | 95E8-F1;95F15 | 20 | 19 |
| *Df(3R)crbS87-5* | 95F7;96A17-18 | 35 | 19 |
| *Df(3R)XS* | 96A1-7;96A21-25 | 15 | 23 |
| *Df(3R)Tl-P* | 97A;98A1-2 | 0 | 146[h] |
| *Df(3R)D605* | 97E3;98A5 | 2 | 20[i] |
| *Df(3R)Dr-rvl* | 99A1-2;99B6-11 | 72 | 97 |
| *Df(3R)tll-g* | 99F1-2;100B4-5 | 3 | 6 |
| Chromosome *4* | 101A;102F | 32 | 64 |

For chromosome *4*, the figures under $Df/+_{sim}$ are offspring homozygous for the *D. simulans* fourth chromosome in a heterozygous *D. simulans/D. melanogaster* background, while those under $+_{mel}/+_{sim}$ are heterozygous for one fourth chromosome from each species in the same heterozygous background as the $Df/+_{sim}$ flies. $Df/+_{sim}$, hybrid offspring containing the deficiency; $+_{mel}/+_{sim}$, offspring lacking the deficiency and heterozygous for balancer.

[a–i] The indicated crosses are analyzed further in Table 2 (see results).

Although tests showed that the *white*, *ebony*, and *cinnabar* mutations scored in offspring do not markedly change their expression over the 18°–24° temperature range, some balancer chromosomes contained temperature-sensitive markers that might, if their expression disappeared at lower temperatures, give the false impression that the $Df/+_{sim}$ genotype was being rescued. [For example, when we crossed *D. melanogaster* *Df(3*R*)T1-P* $e^1ca^1$ */ TM3 Ser* females to *D. simulans ebony* males, we distinguished $Df/+_{sim}$ from $+_{mel}/+_{sim}$ progeny by scoring the Serrate wing phenotype because both the *TM3* balancer and the deficiency chromosome carried *ebony*. At 24° all progeny were Serrate, but wild-type progeny appeared when the cross was repeated at lower temperature (see below). This might reflect either an increased viability of the deficiency-containing chromosome, or perhaps only a much decreased expressivity of Serrate.] In the few such crosses showing such rescue when the mutant used to distinguish the two phenotypes was temperature sensitive (*Curly* is another example), we made control intraspecific crosses to test for reduced penetrance at lower temperature. In none of these cases did we find any effect of rearing temperature on penetrance of the marker (details of these crosses are available on request).

Finally, we produced $F_1$ interspecific hybrids homozygous for the entire *D. simulans* fourth chromosome (2% of the genome); the procedure is described below.

**Other fly stocks:** Besides the *D. melanogaster* deficiency stocks described above, we used the following *D. simulans* strains:

Florida City: An isofemale line collected in Florida City, Florida by J. Coyne and M. Kreitman in June 1985 (Coyne and Beecham 1987).

*white*$^{mky}$: The *white-milky* strain (an *X*-linked allele of the *white* locus) was obtained from A. Yamomoto. This stock crossed readily with stocks containing the *X*-chromosome balancer *FM7*, which itself carries the *white-apricot* ($w^a$) allele. If such a cross did not produce offspring, it was remade using the standard *white* mutation, obtained from J. S. F. Barker.

*cinnabar*: A second-chromosome mutation that spontaneously arose in the Florida City strain; it is identical to *D. melanogaster cinnabar*.

*ebony*$^d$: A third-chromosome mutation discovered by J. David segregating in an isofemale strain of *D. simulans* collected in Africa. If crosses using a *D. melanogaster TM* balancer did not produce progeny when crossed to this strain, we used, successively, two other *D. simulans* strains containing independently arising *ebony* mutations: one ($e^k$) provided by E. Khovanova, and the other ($e^b$) extracted from a *D. simulans scarlet, ebony* stock provided by J. S. F. Barker [this is likely to be the first *D. simulans ebony* mutation that was described by Sturtevant (1929)].

In a few cases we used *D. mauritiana* or *D. sechellia* mutant or wild stocks to determine whether regions in *D. simulans* behaving as hemizygous lethals in hybrids with *D. melanogaster* would also behave as hemizygous lethals when they came from the two sister species of *D. simulans*. To score offspring in such hybrids, we used *white* and *cinnabar* mutations present in both *D. mauritiana and D. simulans*, as well as the wild-type original isofemale line of *D. sechellia*, called "line 1" (see Coyne and Charlesworth 1997).

To produce a female *D. simulans*/*D. melanogaster* hybrid homozygous for *D. simulans* fourth chromosomes, we used a stock of *D. melanogaster* in which *D. simulans* fourth chromosome had been inserted by Muller and Pontecorvo (1940). Females homozygous for the *D. simulans* fourth chromosome are fertile, but males are sterile, and so this stock is kept balanced with males heterozygous for a *4-sim* chromosome and a *D. melanogaster* fourth chromosome carrying the dominant $ci^D$ mutation, which is homozygous lethal. This stock was provided by H. A. Orr, who recently determined using several methods that it still carried the *D. simulans* fourth chromosome (Orr 1992). We crossed *D. melanogaster* males heterozygous for the two species' fourth chromosomes (*4-sim*/$ci^D$) to *D. simulans* wild-type males from the Florida City stock. The wild-type hybrids are homozygous for the *D. simulans* fourth chromosome, while hybrids showing *cubitus-interruptus* are heterozygous for one fourth chromosome from each species; in both cases the remainder of the genome is heterozygous for genes from both species.

## RESULTS

Our main result (Table 1) is that none of the 113 regions of *D. simulans* genome made hemizygous against *D. melanogaster* deficiencies are unconditionally lethal in hybrid females. This was also the case for the entire *D. simulans* fourth chromosome, which appeared to have little effect on viability when homozygous in otherwise hybrid females. While several regions of *D. simulans* genome appeared to cause lethality when derived from a single *D. simulans* stock and tested at 24°, carriers of the heterozygous deficiency (*i.e.*, the hemizygous region from *D. simulans*) were always rescued when we repeated the cross at either a lower temperature or using another *D. simulans* tester stock. It is likely that the conditional lethality was due to the uncovered region from *D. simulans* and not to the *D. melanogaster* deficiency itself, because the balancer stocks of pure *D. melanogaster* are viable and the "lethal" deficiencies appeared in expected proportions when we made control crosses with *D. melanogaster* tester stocks (see below). In addition, a few *D. simulans* regions appeared to strongly (but not completely) reduce viability when hemizygous in hybrid females; again, nearly all of these appeared in higher proportions when tested at different temperatures or in different genetic backgrounds.

Table 1 shows the number of $Df/+_{sim}$ and $+_{mel}/+_{sim}$ progeny recovered for each cross producing at least one of the former offspring or five of the latter. (All of the progeny listed in this table are female, as is normal in crosses using a *D. melanogaster* mother. A few crosses occasionally produced a few males, but never in appreciable numbers.)

Calculating the cytological sizes of the deficiencies [we counted each of Bridges' (1935) 102 numbered regions as 1/102 or 0.98% of the genome], using the average length when only a range of breakpoints was given for a deficiency, and eliminating the overlap between deficiencies, we estimate that we tested ~49.8% of the *D. simulans* genetic map for hemizygous lethality. Because of the often weak correspondence between cytological and genetic length, this is of course only a crude estimate of the proportion of the genome tested. Nevertheless, the observation that a sample of nearly 50% of the *D. simulans* genome shows no hemizygous hybrid lethality implies that relatively few *D. simulans* genes or small chromosome regions can cause complete hybrid lethality.

Of 114 chromosome segments tested in the "initial cross" (*i.e.*, using a standard tester stock and rearing offspring at 24°), 4 showed a complete absence of deficiency heterozygotes, and another 5 a severe deficit of deficiency-carrying heterozygotes compared to controls (see materials and methods for definition of "severe deficit"). Of these 9 segments affecting viability, 1 was on the *X* chromosome, another on the second, and the remaining 7 on the third chromosome. For each of these 9 segments, we made control crosses within *D. melanogaster* and further interspecific crosses using other rearing temperatures and strains, as described in materials and methods. These additional crosses, the results of which are given in Table 2, nearly always led to substantial or full recovery of the offspring hemizygous for the *D. simulans* chromosome region.

Four hemizygous *D. simulans* segments appeared lethal in the standard tester cross, and all of the deficiencies uncovering them were recovered in expected proportions (0.47–0.52) in intraspecific control crosses. In further interspecific crosses, four of these *D. simulans* segments proved viable when the crosses were made at lower temperatures. In addition, each of these four balancer stocks produced offspring hemizygous for the *D. simulans* segment when crossed to a different *D. simulans* tester stock, indicating that lethality was also conditional on genetic background. Finally, some of the lethal *D. melanogaster* deficiencies were also rescued in crosses to *D. sechellia* and *D. mauritiana*, implying either genetic background effects or nonlethality of the corresponding genomic segments in these two island-dwelling species.

In the standard test cross, five *D. melanogaster* deficiencies appeared to cause severe but not complete inviability when heterozygous against *D. simulans* (Table 1). Again, each of these hemizygous *D. simulans* segments produced near-normal numbers of offspring in intraspecific controls (Table 2). As with the five hybrid-lethal deficiencies, all of these *D. simulans* "inviability deficiencies" were rescued when offspring were reared at lower temperatures, and some were also rescued by crossing to other heterospecific tester stocks.

Finally, five crosses (Table 1) produced hybrids containing only the *D. melanogaster* deficiency and not the balancer, though three of these crosses produced $<10$ offspring. Such a result is not surprising if the balancer-containing chromosome is lethal in hybrids (dominant mutations in *D. simulans*, for example, are often lethal when heterozygous in hybrids with *D. melanogaster*). We did not test these deficiencies further.

We therefore find no *D. simulans* regions either unconditionally lethal or nearly lethal when tested in a heterospecific genome. It is clear, however, that some deficiencies reduce hybrid female viability under many conditions. For example, the *D. simulans* segment corresponding to *Df*(2R)*CX1* was never seen in proportions $>0.34$ among hybrid offspring, regardless of rearing temperature or genetic background. In addition, the *D. simulans* segment corresponding to *Df*(3L)*81k19* was largely inviable regardless of genetic background, though it was recovered at lower temperatures. The most universally deleterious *D. simulans* segment was that uncovered by *Df*(3R)*Tl-P*, as a low proportion of deficiency-carrying heterozygotes appeared at all temperatures and genetic backgrounds, as well as when the deficiency was used to uncover corresponding segments of the sibling species *D. sechellia* and *D. mauritiana*. *Df*(3*R*)*Tl-P* occupies an entire cytological unit extending from 97A-98A1-2. A smaller deficiency uncovering the *D. simulans* region from 97B to 97D1-2 showed no lethality (Table 2), so if a single gene is responsible for the deleterious effects of *Df*(3*R*)*Tl-P*, it must lie in either 97A-97B or 97D-98A.

The absence of inviability genes is supported by the lack of large viability effects of the *D. simulans* fourth chromosome, which comprises two entire cytological units. This chromosome may lower viability somewhat when homozygous in an otherwise hybrid background, as it appeared in only 0.33 of offspring instead of the expected proportion of 0.5 ($\chi^2 = 10.7$, 1 d.f., $P < 0.001$). It should be noted that this chromosome does cause complete male *sterility* when homozygous in a *D. melanogaster* genetic background, and that the sterility maps to a small region of the chromosome (Pontecorvo 1943a,b; Orr 1992).

Considering all crosses simultaneously, there is no strong trend for lowered viability of deficiency-carrying females compared to their balancer-carrying sisters. Figure 2 shows the distribution of relative viabilities of all *Df*/+ offspring (measured as deficiency-containing offspring/total offspring) determined in the initial test cross. This distribution is centered around 0.5, with the mean proportion of deficiency offspring (excluding the fourth-chromosome test) being $0.487 \pm 0.024$ (SE). The distribution is not bell shaped because of the tails at 0 and 1 corresponding, respectively, to the lethal deficiencies and lethal balancers.

A sign test, however, does give evidence for some lowered viability of deficiency-carrying hybrids. Of the 114 sets of offspring described in Table 1, *Df*/+ offspring composed $<50\%$ of the progeny in 68 crosses and $>50\%$ of the progeny in only 41 crosses (in 5 crosses they occurred at exactly 50%). The comparison of 68 to 41 deviates significantly from a 50:50 ratio expected under the assumption of no reduced viability ($\chi^2 = 6.69$, 1 d.f., $0.005 < P < 0.01$). The same trend is seen among those crosses producing $>100$ progeny (20:9 ratio, $\chi^2 = 4.17$, 1 d.f., $P < 0.05$). Thus there is evidence for weak effects on hybrid viability. Moreover, it should be remembered that the uncovered *D. simulans* segments are being compared to genotypes carrying dominant-containing balancer chromosomes, which themselves almost certainly have lower viability than wild-type chromosomes. More complicated crosses would be needed

**TABLE 2**

**Additional crosses involving deficiencies that showed inviability or low viability in initial crosses**

| Strain from Table 1[a] | Strain crossed to *Df* strain | Temp. | Offspring | |
|---|---|---|---|---|
| | | | $Df/+_{sim}$ | $+_{mel}/+_{sim}$ |
| a. *Df(1)JC4* (first cross with sim $w^{mky}$) | Control (mel *w*) | 24° | 272 | 301 |
| | sim $w^{mky}$ | 21° | 170 | 216 |
| | | 18° | 112 | 163 |
| | mau *w* | 24° | 20 | 808 |
| | | 18° | 5 | 126 |
| | sec *w* | 24° | 1 | 2 |
| | | 18° | 100 | 108 |
| Overlapping deficiencies | | | | |
| *Df(1)A209* (20A; 20F) | sim $w^{mky}$ | 24° | 66 | 183 |
| *Df(1)GA90* (20C-20F) | sim $w^{mky}$ | 24° | 38 | 247 |
| b. *Df(2R)CX1* (first cross with sim *cn*) | Control (mel *cn*) | 24° | 550 | 914 |
| | sim *cn* | 21° | 6 | 298 |
| | | 18° | 79 | 155 |
| | sim $e^d$ | 24° | 0 | 99 |
| | sim $e^d$ | 21° | 28 | 291 |
| | sec *cn* | 24° | 3 | 47 |
| | mau *cn* | 24° | 50 | 268 |
| c. *Df(3L)HR 232* (first cross with $e^d$) | Control (mel *e*) | 24° | 250 | 189 |
| | sim $e^d$ | 21° | 321 | 231 |
| | sim $e^d$ | 18° | 137 | 114 |
| | sim $e^b$ | 24° | 163 | 176 |
| d. *Df(3L) HR 119* (first cross with $e^d$) | Control (mel *e*) | 24° | 392 | 281 |
| | sim $e^b$ | 24° | 320 | 279 |
| | sim $e^d$ | 21° | 86 | 124 |
| | | 18° | 94 | 75 |
| e. *Df(3L)st-f13* (first cross with $e^d$) | Control (mel *e*) | 24° | 335 | 304 |
| | sim $e^d$ | 18° | 46 | 32 |
| | sim $e^b$ | 24° | 10 | 16 |
| | sim $e^k$ | 24° | 57 | 51 |
| f. *Df(3L)81k19* (first cross with $e^d$) | Control (mel *e*) | 24° | 297 | 314 |
| | sim $e^d$ | 21° | 0 | 50 |
| | | 18° | 123 | 185 |
| | sim $e^b$ | 24° | 15 | 179 |
| | sim *e* | 18° | 30 | 62 |
| | sim $e^k$ | 24° | 0 | 3 |
| g. *Df(3L)W10* (first cross with $e^d$) | Control (mel *e*) | 24° | 187 | 213 |
| | sim $e^d$ | 21° | 8 | 2 |
| h. *Df(3R)T1-P* (first cross with $e^b$) | Control (mel *e*) | 24° | 185 | 185 |
| | sim $e^b$ | 21° | 11 | 101 |
| | | 18° | 30 | 121 |
| | sim $e^d$ | 21° | 10 | 157 |
| | | 18° | 121 | 166 |
| | sim $e^k$ | 21° | 0 | 193 |
| | sim FC | 24° | 0 | 79 |
| | | 21° | 56 | 180 |
| | | 18° | 119 | 298 |
| | sec 1 | 21° | 0 | 6 |
| | mau syn | 24° | 3 | 385 |
| | | 21° | 19 | 314 |
| | | 18° | 161 | 337 |
| Overlapping deficiency: *Df(3R)T1-X* (97B;97D1-2) | sim $e^d$ | 24° | 14 | 7 |
| | | 21° | 106 | 96 |
| i. *Df(3R)D605* (first cross with $e^d$) | Control (mel *e*) | 24° | 246 | 253 |
| | sim $e^d$ | 21° | 135 | 237 |
| | | 18° | 38 | 51 |

Nomenclature for offspring genotypes is identical to that of Table 1. For control crosses, which involve intraspecific crosses using *D. melanogaster,* figures given are the number of pure-species offspring containing the deficiency and lacking the deficiency, respectively. mel, *D. melanogaster*; mau, *D. mauritiana*; sec, *D. sechellia*; sim, *D. simulans.*

[a] Letters a–i correspond to the respective footnotes in Table 1.

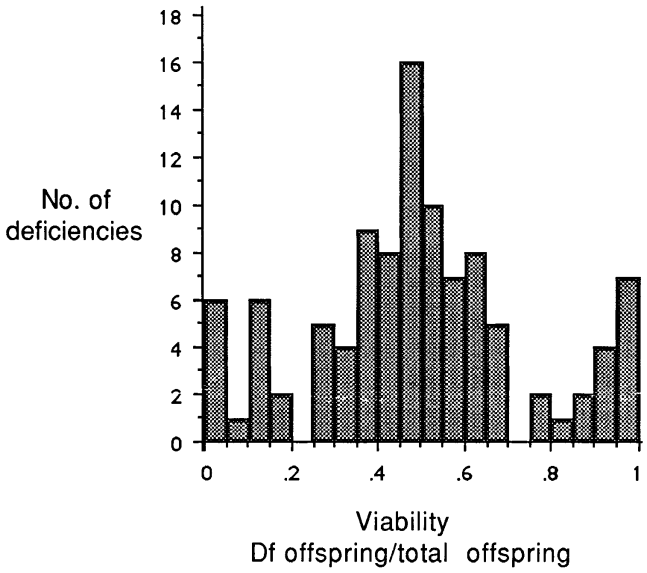


Figure 2.—Distribution of relative viabilities of the 114 *D. simulans* chromosome segments tested. Viabilities are defined for each cross as the ratio of "Df offspring" (*i.e.*, those offspring carrying the *D. melanogaster* deficiency and hence uncovering a corresponding region of *D. simulans* genome) divided by the total number of offspring.

to measure the viability of *Df*/+ progeny against wild-type hybrids having a similar genetic background.

The mean length of all deficiencies used (again excluding the fourth chromosome) is 0.573 ± 0.036 cytological units, or slightly more than half of a numbered section. To determine whether there was a correlation between the size of an uncovered *D. simulans* segment and its viability, we regressed the cytological length of the *D. melanogaster* deficiency against the proportion of deficiency-containing offspring (each segment was counted as one unit and we used fractional estimates based on Bridges' 1935 map). The slope of this regression line ($y = 0.47 + 0.031X$) did not differ significantly from zero ($F_{1,111} = 0.224$, $P = 0.64$). Moreover, the correlation between map length and Df-containing/total progeny (0.045) was also not significant. Longer segments of *D. simulans* genome therefore do not appear to cause greater inviability.

## DISCUSSION

In a survey of ~50% of the *D. simulans* genome, we found no regions that, when hemizygous, can cause unconditional lethality in hybrids with *D. melanogaster*. Although some regions caused either complete lethality or very low viability when tested initially, hybrids carrying the *D. simulans* regions were invariably rescued when reared at other temperatures or given a different genetic background from *D. simulans*. Although the total absence of unconditionally lethal regions was surprising, the conditional nature of inviability is consistent with previous work by Sturtevant (1929), Watanabe *et al.* (1977), and Lee (1978) showing that the viability of $F_1$ female hybrids from the cross between *D. melanogaster* females and *D. simulans* males depends on both genetic background and temperature, with more progeny appearing at lower temperatures. Male offspring from this cross, however, are not rescued by altered temperature or genetic background except when rare "rescue mutants" are used.

We conclude that in this pair of species there has been relatively little genetic divergence capable of causing hybrid inviability, at least for *D. simulans* segments hemizygous in a heterozygous *D. simulans*/*D. melanogaster* genetic background. The chance that we would not have detected one of these genes if there were five or more of them randomly distributed throughout the genome is $<3\%$ ($(1/2)^5$). As we discuss below, our results are consistent with previous work on this and other species pairs indicating that genes causing hybrid inviability are much rarer than those causing hybrid sterility.

We must emphasize that our experimental design assumes that hemizygosity for a *D. simulans* region, when uncovered by a deficiency, is equivalent to a homozygous *D. simulans* segment in causing hybrid lethality (that is, a single non-dosage-compensated dose of the *D. simulans* gene will have the same effect on viability as two doses). For three reasons, however, we feel that this is a fairly safe assumption. First, alleles *within* a species that are recessive lethal are almost always lethal when made hemizygous against a deficiency. If hybrid lethals act—as posited by Turelli and Orr (1995)—as partially recessive loss-of-function alleles in a hybrid background, we should easily see such alleles in our crosses. Even if there are semi-lethals that act as *semidominant* gain-of-function alleles (there are obviously no fully dominant lethals in this cross as normal $F_1$ hybrid females are viable), we should see a relative absence of those offspring carrying the *D. melanogaster* deficiency. Second, there is a known example in which a *D. simulans* allele causing postzygotic isolation in this hybridization behaves identically whether hemizygous or homozygous. The *D. simulans* fourth chromosome carries a small region that causes complete sterility when homozygous in a *D. melanogaster* background. This region was mapped by Orr (1992) using deficiencies, showing that a single dose of the *D. simulans* gene or genes produces, when hemizygous, the same phenotypic effect as a double dose. Finally, in the reciprocal hybridization using *D. simulans* mothers (see discussion below), double doses of entire *D. melanogaster* chromosomes or chromosome arms fail to cause lethality in an otherwise hybrid background. We are therefore confident that our experiment has the power to detect most hybrid lethals. Nevertheless, it should be noted that we may have missed some regions of *D. simulans* that cause lethality only when present in two doses in hybrids.

**Comparison to previous work on this hybridization:** Hutter (1997) reviews the genetics of inviability in

*D. simulans*/*D. melanogaster* hybrids. In the cross of *D. melanogaster* females to *D. simulans* males, death of the late-larval males apparently results from a recessive gene or genes on the *D. melanogaster X* chromosome interacting with semidominant, zygotically acting gene or genes from *D. simulans*. Larval inviability in this cross thus involves at least two genes. In the reciprocal cross (*D. simulans* mother) only male offspring appear, with females dying as embryos. As Hutter (1997) notes, the fact that male death in one direction of the hybridization occurs at a developmental stage different from that of females from the reciprocal hybridization implies the disruption of different developmental pathways, and hence different genes involved in the two types of inviability. One can conclude that there are probably a minimum of two genes causing inviability in each direction of the cross, or a minimum of four involved in total interspecific inviability. This conclusion is supported by the work of Hutter *et al.* (1990) and Sawamura *et al.* (1993c) who propose a model, based on the four known "rescue mutations," involving a total of four genes causing inviability in the two reciprocal crosses.

Other information on homozygous viability effects in this hybridization is scattered throughout the literature, and, while the authors do not discuss the implications about gene number, is consonant with our conclusion that there are few genes causing lethality in hybrids. For example, Orr (1991) crossed *D. simulans* attached-*X* females to *D. melanogaster* males and obtained viable $X_{sim}X_{sim}$ females on a background of *D. simulans* cytoplasm and heterospecific autosomal genome. The appearance of this genotype shows that entire homozygous *X* chromosomes from *D. simulans* do not cause complete inviability on a hybrid background. This is similar to our observation of a lack of *X*-linked *D. simulans* genes unconditionally lethal in a hybrid background, although the studies differ in that the cytoplasm of hybrids derived from *D. simulans* in Orr's experiment and from *D. melanogaster* in ours. Given the known effect of cytoplasmic incompatibility on female inviability, our observation of no lethality when *D. simulans X*-linked genes interact with *D. melanogaster* cytoplasm is further evidence against a multiplicity of inviability genes.

Yamamoto (1992) also showed that the lethality of male hybrids from a *D. melanogaster* mother does not involve the *D. simulans Y* chromosome. Finally, Ohnishi *et al.* (1982) and Coyne (1996), showed, using attached-autosome stocks of *D. melanogaster* crossed to *Y*-autosome translocation stocks of *D. simulans*, that each of the four autosomal arms of *D. melanogaster* produces viable female offspring when homozygous in a genetic background heterozygous for genes from *D. simulans* and *D. melanogaster*. Likewise, Coyne (1983) showed that at least some hybrid males having entire homozygous second or third chromosomes from *D. melanogaster* were viable in a heterozygous *D. simulans*/*D. melanogaster* genetic background, although these experiments had no power to detect less than complete inviability.

We conclude that in a hybridization between species separated for several million years, inviability is probably due to divergence at only a few loci. This is implied by the existence of rescue mutants that restore inviable hybrids. As Coyne and Orr (1998) note, the fact that inviability can be rescued by single mutants implies that lethality probably has a fairly simple developmental basis (how could single mutations restore viability if it were reduced by many different pathways?) and therefore also a simple genetic basis (hybrid inviability is likely to be a pleiotropic by-product of evolutionary change within lineages, and it is unlikely that such changes would all occur in the same developmental pathway).

A possible conflict with the above results was obtained by Pontecorvo (1943a,b) using mutant *D. melanogaster* triploid stocks crossed to irradiated *D. simulans* stocks, which yielded a variety of "artificial backcross hybrids" whose genetic constitution could be ascertained from their visible mutations. He observed that in both males and females, *D. melanogaster X* chromosomes acted as recessive lethals when both of the large autosomes were heterozygous, but hybrid viability was restored when either of two autosomes were homozygous for *D. melanogaster*, implying one recessive suppressor of lethality on each *D. melanogaster* autosome. He thus found a minimum of three genes involved in hybrid lethality, similar to the results described above. However, he also proposed that at least six additional loci *severely reduced* the viability of hybrids. The existence of these additional loci is somewhat uncertain, however, as sample sizes were extremely small and Pontecorvo also used mutant markers that may have reduced the viability of hybrids more than that of pure species.

Although we believe that few genes are responsible for the hybrid inviability in this hybridization, we unfortunately know little about the relative density of genes causing hybrid *sterility*. It is probably safe to say, however, that there are more sterility genes than inviability genes. In the studies of Pontecorvo (1943a,b), none of the viable offspring proved to be fertile, and even the tiny fourth chromosome of *D. simulans*, comprising only 2% of the genome, caused complete male sterility when homozygous in a *D. melanogaster* genetic background. Although one can occasionally produce a few fertile offspring in this hybridization using special mutants (Davis *et al.* 1996), these mutants are far less effective than those known to rescue hybrid viability.

**Comparison to other hybridizations:** Two other recent studies have supported not only the paucity of "hybrid inviability genes" in closely related species, but also a much higher relative density of "hybrid sterility genes." The most comprehensive work is that of True *et al.* (1996), who introgressed homozygous marked segments of *D. mauritiana* genome into a background homozygous for *D. simulans* genes. This pair of species is

much more closely related than are *D. simulans* and *D. melanogaster*, as the former pair diverged less than half as long ago (Hey and Kliman 1993). True *et al.*'s introgressed segments each averaged ~7% of the total *D. mauritiana* genome. A total of 185 introgressions were localized to 87 cytological positions, and ~50% of the position-introgressions caused male sterility when homozygous. The corresponding figure for female sterility, however, was only 5.4%. In addition, nine inserts at 5 positions caused homozygous *lethality* of both males and females, a figure similar to the proportion of inserts causing female sterility. (Curiously, all nine "inviability inserts" mapped to the third chromosome, as did most of our chromosome segments reducing viability.) Because of the large size of the inserts of True *et al.*, some of the nine different "inviability segments" may actually include identical loci, so one can estimate from their studies only that at least two or three *D. mauritiana* genes cause hybrid lethality when homozygous in a *D. simulans* background.

Hollocher and Wu (1996) introgressed homozygous second chromosomes from *D. mauritiana* and *D. sechellia* into *D. simulans*, with the average introgression also being rather long (57 cM). Two regions in *D. sechellia* and one in *D. mauritiana* produced complete inviability (other chromosomes were not examined). In these same introgressions, however, the level of male sterility was high and female sterility weak, again indicating a relative abundance of hybrid male sterility genes compared to female sterility genes or to inviability genes that affect both sexes.

The observation of some inviability genes in the crosses of True *et al.* (1996) and Hollocher and Wu (1996) does not necessarily contradict our inability to find such loci in a cross between an older pair of species. First, our experiment differed in design from these earlier studies: we examined the lethality effects of hemizygous (effectively homozygous) genes from one species in a heterozygous genetic background, while True *et al.* (1996) and Hollocher and Wu (1996) examined the effect of homozygosity for foreign genetic material on a background largely *homozygous* for genes from other species. One might expect to find more deleterious interactions from the latter design if, as posited by Turelli and Orr (1995; see also Orr and Turelli 1996), many genes causing hybrid inviability and sterility are partially recessive and so show their deleterious effects only when homozygous or hemizygous in hybrids. The experiments of True *et al.* (1996) and Hollocher and Wu (1996) allow for homozygous-homozygous incompatibilities between genes from the two species, while ours allow only hemizygous-heterozygous incompatibilities.

Second, the use of longer segments in the two earlier studies might increase the possibility for hybrid incompatibilities if, as posited by Muller (1942), Perez and Wu (1995), and Davis and Wu (1996), hybrid inviability requires the cooperation of several genes from the same species acting in a foreign genetic background. The introgression of longer chromosome segments would obviously make it easier to detect such incompatibilities.

Third, neither of the two studies cited above examined whether the hybrid lethality was conditional on temperature or genetic background, and so some of the "lethal" segments may have allowed viability under other conditions. Finally, of course, it is possible that the older species pair may simply differ by fewer inviability genes than the younger pairs.

One additional hybridization has recently provided evidence for a similar paucity of inviability genes. Carvajal *et al.* (1996) showed that male-limited inviability in hybrids between *D. buzzatii* and *D. koepferae* is apparently due to only three genes in two cytological regions. This is in strong contrast to the large number of loci, both *X*-linked and autosomal, causing male sterility in this hybridization (Naveira and Fontdevila 1986, 1991; Naveira 1992). Considering all published work, Carvajal *et al.* (1996, p. 15) conclude that "The total number of factors of hybrid inviability localized so far in Drosophila is surprisingly small, given the experimental effort devoted to this matter." The study of Carvajal *et al.* (1996) differed from ours in that it did not investigate conditional lethality, and in that it examined only homozygous chromosome segments from one species on a background largely homozygous for genes from the other species. (In this species pair, male inviability is not seen in $F_1$ hybrids but only in backcrosses, implying that recessive / recessive interactions play a large role in inviability.)

**Caveats and conclusions:** There must therefore be only a few small chromosome regions in *D. simulans* that, when hemizygous, cause unconditional inviability on an otherwise heterozygous *D. simulans* / *D. melanogaster* background. However, it is possible that we have underestimated the number of inviability genes for three reasons:

1. As noted above, genes causing inviability may do so only when homozygous on a genetic background homozygous and not heterozygous from that of the other species, as in the studies of Carvajal *et al.* (1996), Hollocher and Wu (1996), and True *et al.* (1996). We could not have detected such genes in our study. Such genes may cause inviability in backcrosses or $F_2$ crosses, but cannot play a role in the inviability of $F_1$ hybrids.
2. As posited by Perez and Wu (1995) and modeled by Orr (1995), hybrid incompatibilities may be complex, requiring interaction of *several* genes from *D. simulans* that could act together to cause inviability when co-introgressed onto a heterozygous hybrid background. We would not have seen such incompatibilities unless the interacting *D. simulans* inviability genes happened to lie within the span of single defi-

ciencies. Testing this hypothesis would require examining the effects of several *D. simulans* regions uncovered simultaneously by corresponding *D. melanogaster* deficiencies, an experiment not easily done.

3. Some of the *D. simulans* chromosome regions, though not unconditionally lethal in all genetic backgrounds or environments, might be effectively lethal in nature, particularly at higher temperatures. Moreover, all of our experiments were done in the benign environment of the laboratory, in which ample food and uncrowded vials may allow otherwise inviable genotypes to appear. There may thus be a substantial difference between what we counted as a hybrid lethal in our study and what would count as a hybrid lethal in evolution.

We suspect that there are indeed only a few genes capable of causing inviability in this hybridization, but that some of these may only act in a recessive/recessive way. The existence of rescue mutants somewhat militates against possibility 2, but only further genetic analysis will clarify this.

Finally, we do not know how many sterility genes are involved in this hybridization, but preliminary evidence (including the sterility gene or genes present on the tiny *D. simulans* fourth chromosome as well as the great difficulty in rescuing fertility as compared to inviability) indicates that, as in other Drosophila hybridizations, sterility genes are more numerous than inviability genes. This disparity is predicted by the hypothesis of Wu and Davis (1993) that the disproportionately large number of male hybrid-sterility genes is a pleiotropic byproduct of sexual selection, which may cause males within a species to accumulate more genetic change than females (but see Coyne and Orr 1998).

Regardless of the causes of sterility and inviability, however, our results agree with the conclusions of Hutter (1997, p. 180), who, after surveying the literature on the genetics of hybrid inviability, concluded that "no experimental study contradicts the view that a restricted number of major genes, along with a few minor genes, may be interacting within only a few related metabolic pathways." It is worth noting that if hybrid inviability involves only a few genes, these genes must therefore act as major factors, so that this aspect of speciation may not be highly polygenic. Moreover, the fact that there may be many genes causing hybrid *sterility* does not mean that sterility genes cannot act as major factors. As Orr (1995) notes, "sterility alleles" continue to accumulate after hybrid sterility is already complete, so that the initial attainment of full sterility might have involved only a few loci. Indeed, as shown by True *et al.* (1996) and Hollocher and Wu (1996), single chromosome regions can, when introgressed by themselves, cause complete sterility of male hybrids. Determining the number of genes causing speciation must involve counting only those genes causing incompatibilities up to the time that these incompatibilities completely prevent gene flow.

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