A Fine-Scale Genetic Analysis of Hybrid Incompatibilities in Drosophila

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ABSTRACT

The sterility and inviability of species hybrids is thought to evolve by the accumulation of genes that cause generally recessive, incompatible epistatic interactions between species. Most analyses of the loci involved in such hybrid incompatibilities have suffered from low genetic resolution. Here I present a fine-resolution genetic screen that allows systematic counting, mapping, and characterizing of a large number of hybrid incompatibility loci in a model genetic system. Using small autosomal deletions from *D. melanogaster* and a hybrid rescue mutation from *D. simulans*, I measured the viability of hybrid males that are simultaneously hemizygous for a small region of the *D. simulans* autosomal genome and hemizygous for the *D. melanogaster X* chromosome. These hybrid males are exposed to the full effects of any recessive-recessive epistatic incompatibilities present in these regions. A screen of ~70% of the *D. simulans* autosomal genome reveals 20 hybrid-lethal and 20 hybrid-semilethal regions that are incompatible with the *D. melanogaster X*. In further crosses, I confirm the epistatic nature of hybrid lethality by showing that all of the incompatibilities are rescued when the *D. melanogaster X* is replaced with a *D. simulans X*. Combined with information from previous studies, these results show that the number of recessive incompatibilities is approximately eightfold larger than the number of dominant ones. Finally, I estimate that a total of ~191 hybrid-lethal incompatibilities separate *D. melanogaster* and *D. simulans*, indicating extensive functional divergence between these species' genomes.

THE last decade has seen important progress in the genetics of speciation. In particular, there is now broad agreement on three aspects of intrinsic postzygotic isolation, the sterility and inviability of species hybrids:

i. Hybrid fitness problems evolve gradually as incompatible epistatic interactions accumulate between species (Coyne and Orr 1989, 1997; Sasa *et al.* 1998; Presgraves 2002; Price and Bouvier 2002). The so-called Dobzhansky-Muller model posits that incompatibilities evolve by the substitution of advantageous or neutral mutations in one species, which, having never been tested in combination with those in other species, have harmful effects when brought together in hybrids (Dobzhansky 1937; Muller 1940, 1942; Orr 1995, 1996).
ii. The alleles involved in these hybrid incompatibilities are thought to be, on average, partially recessive. This "dominance theory" neatly accounts for several phenomena, including Haldane's rule (the observation that *XY* hybrids typically suffer more severe hybrid problems than do *XX* hybrids; Haldane 1922) and F_2 hybrid breakdown (Muller 1942; Orr 1993; Turelli and Orr 1995, 2000).
iii. Incompatibilities causing hybrid male sterility accumulate faster than those causing other types of hybrid fitness problems (Wu and Davis 1993; Hollocher and Wu 1996; True *et al.* 1996; Wu *et al.* 1996; Naveira and Maside 1998; Presgraves and Orr 1998; Wu and Hollocher 1998; Singh 1999). The "faster-male" theory posits that the rapid evolution of hybrid male sterility is caused by the faster divergence of male-specific fertility genes, perhaps driven by sexual selection, or by the inherent sensitivity of spermatogenesis to the genetic perturbations experienced by hybrids (Wu and Davis 1993).

Nevertheless, progress in the fine-genetic and molecular characterization of hybrid incompatibility loci has been slow. Despite much effort, few studies have succeeded in precisely and systematically counting, fine mapping, and, ultimately, identifying a large number of "speciation genes." The reason is that the traits of interest, hybrid sterility and inviability, are by their very nature barriers to crossing and thus are refractory to standard genetic approaches. This problem is especially severe in one of our best model organisms, the fruit fly *Drosophila melanogaster*: All hybrids between *D. melanogaster* and its closest known relatives (*D. simulans*, *D. mauritiana*, and *D. sechellia*) are completely dead or sterile (Sturtevant 1920, 1929; Lachaise *et al.* 1986). Consequently, nearly a century's worth of accumulated genetic tools has not been brought to bear on the genetics of speciation, and genetic analyses of the *D. melanogaster*-*D. simulans* hybridization, despite an 80-year history, have suffered poor genetic resolution. The rough portrait that has emerged from this work is that a large

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number of hybrid male steriles but a modest number of hybrid lethals have accumulated between *D. melanogaster* and *D. simulans* (Muller and Pontecorvo 1940, 1942; Pontecorvo 1943a,b; Provine 1991; Sanchez *et al.* 1994; Coyne *et al.* 1998; Sawamura *et al.* 2000; Sawamura 2000).

Two methods have been used to circumvent the problematic sterility and inviability of *D. melanogaster* species hybrids. First, the discovery of hybrid rescue mutations—alleles that restore the viability and fertility of normally unfit hybrids—raised the possibility of introgressing foreign genes into a *D. melanogaster* background, where they might be further analyzed (Davis *et al.* 1996). Unfortunately, hybrid female fertility rescue has proved extremely weak, and so far only two small introgressions of the *D. simulans* second chromosome have been studied (Sawamura *et al.* 2000). Second, Coyne *et al.* (1998) used a battery of deficiencies (small chromosomal deletions) from *D. melanogaster* to uncover recessive *D. simulans* factors causing hybrid lethality in otherwise heterozygous F_1 females. Their screen of ~50% of the *D. simulans* genome revealed only a handful of hybrid lethals, none of which were unconditionally lethal. One reason why so few were detected may be that the screen was limited to incompatibilities involving a hemizygous (recessive) factor from *D. simulans* and a heterozygous (dominant) one from *D. melanogaster* (elsewhere in the genome). If most incompatibility alleles are recessive, as the dominance theory posits, then the most abundant class of hybrid incompatibility should be that in which the interacting loci are both homozygous or both hemizygous, *i.e.*, genotypes normally produced only in F_2 (or backcross) hybrids.

Here I present a new screen that modifies and combines these two approaches, taking advantage of both a hybrid rescue mutation from *D. simulans* and the genetic tools of *D. melanogaster* to test for the presence of these extreme recessive-recessive hybrid incompatibilities. In particular, I test the viability of F_1 hybrid males hemizygous for a small region of the *D. simulans* autosomal genome and simultaneously hemizygous for the *D. melanogaster X*. These males are exposed to the full effects of any recessive-recessive *X*-autosome hybrid incompatibilities in the regions tested. Using the many deficiencies available in *D. melanogaster*, I systematically screened most of the *D. simulans* autosomal genome for such incompatibilities.

Deficiency mapping in hybrids offers several advantages over recombination mapping. First, large numbers of the relevant genotypes can be assayed in the F_1 generation. Second, the existence and precise location of hybrid lethals in most cases can be easily confirmed using overlapping but independently derived deficiencies with defined cytological breakpoints on the polytene chromosome map (Bridges 1935). Third, the fitness effects of hybrid incompatibilities can be assessed without being confounded with recombination distance between the relevant locus and a genetic marker. Finally, and perhaps most important, the locations of hybrid lethals can be quickly narrowed to such small chromosome regions that the next step—identifying the particular genes causing hybrid lethality—becomes routine.

The present large-scale, fine-resolution screen reveals the existence of many new hybrid lethals between *D. melanogaster* and *D. simulans*. In later work, these hybrid lethals will be the subject of molecular study. Here, I use these data to address the following questions:

How many hybrid lethals separate *D. melanogaster* and *D. simulans*? This number is a proxy for functional divergence between the viability-essential components of the two species' genomes.

Where are the hybrid lethals? Are they randomly distributed throughout the genome or clustered in particular regions?

Is hybrid lethality caused by epistasis between incompatible loci, as predicted by the Dobzhansky-Muller model?

Are most hybrid incompatibilities recessive, as predicted by the dominance theory? There are few direct tests of the dominance of incompatible alleles. Furthermore, comparing the number of recessive hybrid lethals discovered here with the number of dominant ones from other studies provides a quantitative test of the dominance theory.

What is the probability that any two divergent substitutions, one from *D. melanogaster* and one from *D. simulans*, are incompatible, causing hybrid lethality?

What is the distribution of fitness effects of hybrid-lethal incompatibilities? Do most have weak or strong effects on viability? How does this distribution compare with that for hybrid male sterility, which often appears to have a polygenic basis (Naveira and Maside 1998; Wu and Hollocher 1998)?

At what stage of development do hybrid lethals act? If genes acting early in development are more evolutionarily constrained than later-acting ones, and thus less diverged, then embryonic hybrid lethals should be rarer than postembryonic ones.

MATERIALS AND METHODS

A screen for *X*-autosome incompatibilities: The screening method is diagrammed in Figure 1A and represents a modification of the crossing design of Coyne *et al.* (1998). Using many *D. melanogaster* (*mel*) stocks, each heterozygous for a deficiency chromosome with known cytological breakpoints and a dominantly marked balancer chromosome (Lindsley and Zimm 1992), I crossed *mel Deficiency/Balancer* (*Df/Bal*) females to *D. simulans* (*sim*) males carrying the hybrid rescue mutation, *Lethal hybrid rescue* (*Lhr*). *Lhr* rescues normally dead F_1 males from lethality that typically occurs at the larval-pupal transition (Watanabe 1979; Takamura and Watanabe 1980; Sawamura *et al.* 1993). Half of the F_1 hybrid progeny inherit

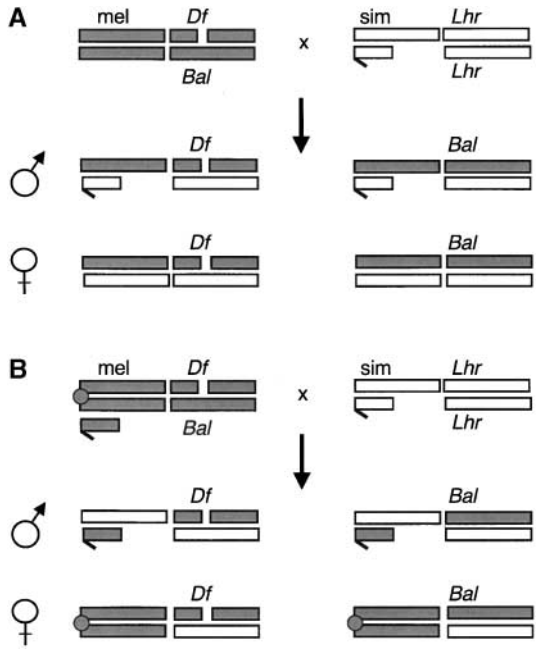

FIGURE 1.—Crosses used in F_1 deficiency screen for lethal hybrid incompatibilities. Sex chromosomes and one set of representative autosomes are shown. Gray, *D. melanogaster*; white, *D. simulans*. (A) *Deficiency/Balancer* females crossed to *Lhr* males produce (in the absence of hybrid lethality) four types of offspring in Mendelian ratios within each sex. (B) Attached-*X Deficiency/Balancer* female crossed to *Lhr* male. See text for further details.

the deficiency and half inherit the balancer. If hybrids of both sexes appear with both *Df* and *Bal* genotypes in roughly equal proportions, then no recessive hybrid lethal resides in the *sim* region uncovered by the deficiency. If, on the other hand, only *Df* hybrid males die, then a hybrid lethal resides in the *sim* autosomal region exposed by the deficiency. The fact that only *Df* hybrid males die, while their *Df* hybrid sisters do not, suggests either that the *sim* hybrid lethal is incompatible with a mostly recessive factor on the *mel X* (females are heterozygous for the *X*) or that the *sim* hybrid lethal is male specific. These possibilities are distinguished using follow-up crosses described below.

Table 1 shows the 193 deficiencies used. Together these deficiencies uncover ~69–77% of 2L (chromosome 2, left arm), 49–59% of 2R, 72–77% of 3L, and 65–79% of 3R. I did not test the small-dot fourth chromosome of *D. simulans* as it is known to carry no hybrid lethals (MULLER and PONTECORVO 1940, 1942; PONTECORVO 1943a; ORR 1992). All stocks are available through the Bloomington or Umeå Stock Centers (Bloomington Stock Center, http://flystocks.bio.edu/df-kit-infor.htm). Soichi Tanda kindly provided a stock with a newly extracted *Df(2L)al*. The most common dominant markers on chromosome 2 balancers were *CurlyO* and *Glazed*, and those on chromosome 3 balancers were *Stubble*, *Serrate*, *Tubby*, or a combination. When possible, deficiency stocks with a chromosome 3 balancer marked only with *Tubby* or *Ultrabithorax* were rebalanced over a different chromosome with a more easily scored marker (*e.g.*, *Stubble*). Complete descriptions of all stocks, including the particular balancers used, are available upon request.

Each species cross was made by mass mating 15–20 *mel Df/Bal* females with 15–25 *sim Lhr* males. All crosses were done at 24° and flies were reared on standard cornmeal-yeast-agar medium. For each deficiency, I repeated the cross at least four times and report the pooled number of progeny. Contamination was detectable in several ways: consistency among replicate crosses, the segregation of dominant markers among progeny, the presence of diagnostic hybrid male genitalia, and the absolute sterility of all progeny. Although the cross shown in Figure 1A is in principle straightforward, some stocks showed strong sexual isolation, requiring many replicate crosses to obtain sufficient progeny.

Most crosses deviated from the expected 1:1:1:1 segregation ratio of *Df* females, *Bal* females, *Df* males, and *Bal* males, because *Lhr* rescue of hybrid males is incomplete. Hybrid males were significantly rarer than females in most crosses, with an average of 45% rescue. In a few crosses, no hybrid males were rescued (see also GRANADINO *et al.* 1996); since these crosses are not informative, they are not reported here. For crosses that produced hybrid females and at least 10 *Bal* hybrid males, I estimated the relative viability of the deficiency by simply tabulating the ratio of *Df:Bal* progeny for each hybrid sex. I used χ^2 tests to detect deviations from the expected 1:1 ratio of *Df:Bal* progeny within each sex and Fisher's exact tests to detect differences in the ratio of *Df:Bal* progeny between sexes. I defined candidate *X*-autosome hybrid incompatibilities as those causing a significant deficit of *Df* hybrid males and a significantly smaller *Df:Bal* ratio in hybrid males than in hybrid females. I then classified hybrid incompatibilities by viability following the scheme of Crow and colleagues for deleterious mutations within species (reviewed in SIMMONS and CROW 1977; CROW and SIMMONS 1983): For regions causing significant reductions in *Df* hybrid male viability, those with viabilities $\leq$50% (*i.e.*, a *Df:Bal* ratio $<$0.50 at least for one of two overlapping deficiencies) were considered hybrid "semilethals," and those with viabilities of $\leq$10% were considered hybrid "lethals."

Further investigation of hybrid-lethal deficiencies: I performed three further tests for most of the regions that caused hybrid lethality. First, to ensure that the lethality of deficiencies is hybrid specific, I confirmed the viability of deficiencies in nonhybrids by crossing *Df/Bal mel* females to wild type, *Wolbachia*-free Canton-S *mel* males. (Note, however, that the very existence of the deficiency stocks shows that they are not lethal when heterozygous within species.)

Second, when possible, I confirmed the presence of each hybrid lethal by testing at least one other overlapping deficiency. Such confirmation with multiple, independently derived deficiencies rules out the possibility that a putative hybrid lethal is an artifact of the genetic background of any particular stock. Those regions shown to be lethal by a single deficiency, but which could not be confirmed because overlapping deficiencies were unavailable, should be viewed as candidate hybrid-lethal regions.

Third, I tested whether the exposed *sim* factors caused lethality by epistasis with the *mel X*. To do this, I constructed *mel* attached-*X* stocks, each carrying a hybrid-lethal deficiency over a balancer, and crossed females from these stocks to *sim Lhr* males. As Figure 1B shows, the attached-*X* chromosome forces mother-to-daughter inheritance of the *mel* attached-*X* and father-to-son inheritance of the *sim* free-*X*. Hybrid males from this cross thus inherit a *sim X* rather than a *mel X*, while the rest of their genotype, including their cytoplasm, remains identical in species origin to the hybrid males from the original cross (compare F_1 males in Figure 1, A and B). The important point is that if *Df* hybrid male lethality is caused by an incompatibility between a recessive *sim* autosomal factor and a recessive factor(s) on the *mel X*, then replacing the *mel X* with a *sim X* should rescue these males (as the *sim X* should be compatible with the *sim* autosomal factor). If, on the other hand, hybrid male lethality is caused by a male-specific incom-

patibility, replacing the *X* chromosome will not necessarily rescue hybrid males. The reason is that a male-specific incompatibility could involve an interaction between a recessive *sim* factor and a dominant *mel* factor anywhere in the *D. melanogaster* genome. Unfortunately, *mel* attached-*X* hybrid females from this cross, which would also have been informative, were not rescued in sufficient numbers (see RESULTS).

Lethal phase: For each incompatibility, I determined if hybrid lethality occurred at embryonic or postembryonic stages. If *Df* hybrid males suffer embryonic lethality, 25% of all embryos should die (*i.e.*, 50% of all male embryos). To test if embryonic lethality was sufficient to account for the absent *Df* hybrid males, I calculated the ratio of the number of dead (necrotized) embryos divided by half the number of hybrid females: dead embryos/[(*Df* females + *Bal* females)/2]. If this ratio was ≥ 1, the deficiency was scored as embryonic lethal. If the ratio was $\ll 1$, the number of dead embryos could not plausibly explain the deficit of *Df* hybrid males, and the incompatibility was scored as postembryonic lethal. Establishing the exact phase of lethality for postembryonic hybrid lethals is, unfortunately, complicated by background mortality and the imperfect degree of overall hybrid male rescue by *Lhr*.

RESULTS

Fine-mapping hybrid lethals: Table 1 gives the results for all tested deficiencies. The distributions of viability effects for the two sexes are shown in Figure 2. The most striking result is that while very few deficiencies uncover lethality in hybrid females, many uncover lethality in hybrid males (Figure 2): The distribution of viability for *Df* hybrid females is unimodal, with most crosses showing normal viability, *i.e.*, roughly equal representation of *Df* and *Bal* females. In contrast, the distribution of viability for *Df* hybrid males is bimodal, with one peak at normal viability and a second near complete lethality. Rare "escaper" males bearing hybrid-lethal deficiencies are typically weak and sometimes show developmental anomalies (*e.g.*, malformed abdominal tergites, bristles, wings, and eyes). In all, 40 nonoverlapping autosomal regions in *D. simulans* cause significant lethality when uncovered in *Df* hybrid males. Recessive hybrid lethals are thus common in the *sim* autosomal genome.

Of these regions, 20 are hybrid-lethal and 20 are hybrid-semilethal incompatibilities (Table 1). These regions appear to be distributed randomly among the two major autosomes ($\chi^2 = 3.333$, d.f. = 1, $P = 0.068$) and the four autosomal arms, with 14 on 2L, 14 on 2R, 6 on 3L, and 6 on 3R ($\chi^2 = 3.195$, d.f. = 3, $P = 0.363$; Figure 3). I performed further analyses on 20 hybrid lethals and 3 hybrid semilethals (these semilethals were nearly classifiable as lethal; *i.e.*, the ratio of *Df*:*Bal* hybrid males was ≤ 0.12 for all 3). The lethal effects of these deficiencies are hybrid specific because none caused strong haploinsufficiency when heterozygous in pure species individuals (Table 2). Of the 23 hybrid lethals and semilethals, I confirmed 18 (78%) with overlapping deficiencies that yielded consistent results (Table 1). Each of these regions thus contains at least one hybrid incompatibility factor.

It is important to note, however, that more hybrid lethals probably exist than the above numbers suggest because the available deficiencies from *D. melanogaster* cumulatively uncover only 64–73% of the autosomal genome. Correcting for this incomplete coverage yields an estimate closer to ~27 hybrid lethals and ~27 hybrid semilethals. These remain minimum estimates as each region could harbor more than one hybrid lethal. However, the fact that most deficiencies fail to uncover hybrid lethality suggests that hybrid lethals are sparsely distributed. Therefore, either most hybrid-lethal regions harbor a single hybrid-lethal locus or hybrid lethals are tightly clustered. If the former is true, as seems most plausible, it follows that many incompatibilities have major effects on hybrid viability.

The recessivity of the incompatible factors on the *mel X* can be illustrated in three ways. First, if factors on the *mel X* were completely dominant (and the incompatibilities are not sex specific), *Df* hybrid female viability would be correlated with *Df* hybrid male viability, with slope ≈ 1. (The slope of this relationship can be thought of as a proxy for the mean dominance coefficient of incompatible factors on the *mel X*.) Instead, while there is a weak positive correlation between *Df* hybrid female and male viability, the slope of the least-squares relationship is $b = 0.103$—a value $\ll 1.0$—consistent with the general recessivity of incompatible factors on the *mel X* (Figure 4; Spearman's $r = 0.326$, d.f. = 163, $P < 0.0001$; statistics use only crosses producing ≥ 10 *Bal* hybrid males). Second, of 16 regions that cause significant lethality in *Df* hybrid females, 9 cause significantly stronger lethality in *Df* hybrid males (*e.g.*, Table 1, line 4). Third, and most convincing, 30 of 40 deficiencies that caused significant lethality in *Df* hybrid males have *no* discernable effects in *Df* hybrid females. These findings strongly suggest that the incompatible factors on the *mel X* are also overwhelmingly close to completely recessive.

These results are consistent with those of YAGYU and YAMAMOTO (1996), who independently performed similar crosses using 15 deficiencies on chromosome 2.

Are hybrid lethals epistatic? The hybrid-lethal and semilethal *sim* autosomal factors detected above could be either incompatible with factors on the *mel X* or male-specific and incompatible with a dominant *mel* factor anywhere in the genome. To distinguish these possibilities, I used attached-*X* crosses shown in Figure 1B to test the viability of *Df* hybrid males that are genotypically and cytoplasmically identical to those above but possess a *sim X* rather than a *mel X*. These hybrid males are hemizygous for the *sim X* and hemizygous for a hybrid-lethal *sim* autosomal factor. If the lethal *sim* autosomal factors are incompatible with the *mel X*, changing the species origin of the *X* should rescue hybrid lethality. If, on the other hand, the *sim* autosomal factors cause male-specific hybrid lethality, changing the species origin of the *X* may not rescue hybrid lethality.

As Table 3 shows, 18 of the 18 hybrid-lethal deficien-

TABLE 1

Results from deficiency screen for hybrid-lethal *X*-autosome incompatibilities

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
Second chromosome									
1 *Df(2L)net-PMF*	021A01;021B07-08	49	54	0.907	16	33	0.485	152***	0.1140
2 *Df(2L)BSC4*	021B07-C01;021C02-03	126	112	1.125	10	4	2.500	252***	0.2699
3 *Df(2L)al*	021B08-C01;021C08-D01	114	107	1.065	1	76	0.013***	298***	<0.0001
4 *Df(2L)ast2*	021D01-02;022B02-03	162	223	0.726*	56	139	0.403***	580***	0.0020
5 *Df(2L)dp-79b*	022A02-03;022D05-E01	58	43	1.349	23	30	0.767	154***	0.1263
6 *Df(2L)D20*	022F04;023A01	20	18	1.111	5	8	0.625	51***	0.5230
7 *Df(2L)N6*	023A06;023B01	174	156	1.115	10	4	2.500	344***	0.2736
8 *Df(2L)JS17*	023C01-02;023E01-02	254	212	1.198	143	174	0.822	783***	0.0108
9 *Df(2L)drm-P1*	023F03-04;024A01	429	307	1.397**	61	63	0.968	860***	0.0628
10 *Df(2L)ed1*	024A03-04;024D03-04	47	33	1.424	21	7	3.000	108***	0.1728
11 *Df(2L)sc19-8*	024C02-08;025C08-09	150	122	1.230	17	3	5.667*	292***	0.0094
12 *Df(2L)sc19-4*	025A05;025E05	59	38	1.553	16	15	1.067	128***	0.4061
13 *Df(2L)cl-h3*	025D02-04;026B02-05	43	59	0.729	27	37	0.730	166**	>0.9999
14 *Df(2L)E110*	025F03-026A01;026D03-11	428	466	0.918	66	116	0.569*	1076***	0.0043
15 *Df(2L)BSC9*	026F01-07;027A02-B02	466	392	1.189	39	82	0.476**	979***	<0.0001
16 *Df(2L)Dwee-Δ5*	027A;028A	4	29	0.138*	0	22	0.000**	55***	0.1414
17 *Df(2L)J-H*	027C02-09;028B03-04	219	255	0.859	0	129	0.000***	603***	<0.0001
18 *Df(2L)spd*	027E;028C01-04	128	93	1.376	100	73	1.370	394**	>0.9999
19 *Df(2L)XE-3801*	027E02;028D01	50	64	0.781	35	50	0.700	199*	0.7725
20 *Df(2L)XE-2750*	028B02;028D03	172	166	1.036	114	115	0.991	567***	0.7981
21 *Df(2L)TE29Aa-11*	028E04-07;029B02-C01	115	91	1.264	66	20	3.300***	292***	0.0009
22 *Df(2L)N22-14*	029C01-02;030C08-09	144	134	1.075	95	119	0.798	492*	0.1219
23 *Df(2L)s1402*	030C01-02;030F, 030B09-10	146	169	0.864	102	127	0.803	544***	0.7274
24 *Df(2L)J2*	031B;032A	124	196	0.633**	1	38	0.026***	359***	<0.0001
25 *Df(2L)J1*	031B;031D	49	93	0.527*	12	11	1.091	165***	0.1103
26 *Df(2L)J3*	031D;031F	528	628	0.841*	39	143	0.273***	1338***	<0.0001
27 *Df(2L)J27*	031D01-11;031E01-E07	286	351	0.815	49	34	1.441	720***	0.0189
28 *Df(2L)FCK-20*	032D01;032F01-03	471	504	0.935	0	88	0.000***	1063***	<0.0001
29 *Df(2L)Prl*	032F01-03;033F01-02	87	86	1.012	4	47	0.085***	224***	<0.0001
30 *Df(2L)esc-P2-0*	033A01-02;033B01-02	95	90	1.056	61	50	1.220	296***	0.6307
31 *Df(2L)esc-P3-0*	033A01-02;033E	129	130	0.992	38	43	0.884	340***	0.7031
32 *Df(2L)esc10*	033A08-B01;033B02-03	312	274	1.139	191	175	1.091	952***	0.7896
33 *Df(2L)prd1.7*	033B02-03;034A01-02	136	144	0.944	74	127	0.583**	481***	0.0118
34 *Df(2L)b87e25*	034B12-C01;035B10-C01	34	44	0.773	33	33	1.000	144	0.5036
35 *Df(2L)osp29*	035B01-03;035E06	120	156	0.769	48	100	0.480**	424***	0.0289
36 *Df(2L)TE35BC-24*	035B04-06;035F01-07	19	7	2.714	12	9	1.333	47	0.3551
37 *Df(2L)r10*	035E01-02;036A06-07	247	216	1.144	46	120	0.383***	629***	<0.0001

(*continued*)

TABLE 1

(Continued)

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
38 *Df(2L)H20*	036A08-09;036E01-02	77	77	1.000	22	55	0.400**	231***	0.0020
39 *Df(2L)TW137*	036C02-04;037B09-C01	389	292	1.332*	0	8	0.000	689***	0.0012
40 *Df(2L)M36F-S5*	036D01-E01;036F01-37A01	374	319	1.172	0	13	0.000*	706***	<0.0001
41 *Df(2L)TW50*	036E04-F01;038A06-07	348	253	1.375*	0	10	0.000*	611***	0.0002
42 *Df(2L)M36F-S6*	036E06-F01;036F07-09	49	69	0.710	0	29	0.000***	147***	<0.0001
43 *Df(2L)TW3*	036F07-09;037B02-07	43	68	0.632	56	46	1.217	213	0.0200
44 *Df(2L)hk-UC2*	037B02-08;037C05	109	109	1.000	88	77	1.143	383*	0.5369
45 *Df(2L)pr-A16*	037B02-12;038D02-05	263	282	0.933	21	274	0.077***	840***	<0.0001
46 *Df(2L)VA23*	037B09-10;037D05	134	126	1.063	117	118	0.992	495	0.7193
47 *Df(2L)VA17*	037C01;037F05	342	279	1.226	7	256	0.027***	884***	<0.0001
48 *Df(2L)VA12*	037C02-05;038B02-C01	192	196	0.980	0	100	0.000***	488***	<0.0001
49 *Df(2L)TW2*	037D05-E01;038E06-09	182	226	0.805	2	85	0.024***	495***	<0.0001
50 *Df(2L)pr-A20*	038A03-04;038B06-C01	325	305	1.066	147	151	0.974	928***	0.5276
51 *Df(2L)TW161*	038A06-B01;040A04-B01	7	5	1.400	0	6	0.000	18	0.0377
52 *Df(2L)TW1*	038A07-B01;039C02-03	188	174	1.080	0	159	0.000***	521***	<0.0001
Centromere									
53 *Df(2R)M41A4*	041A	40	48	0.833	0	4	0.000	92***	0.1296
54 *M(2)41A*[d]	041A (h44-h46)	240	272	0.882	2	118	0.017***	632***	<0.0001
55 *Df(2R)M41A10*	041A	110	121	0.909	15	89	0.169***	335***	<0.0001
56 *Df(2R)M41A8*	041A	411	331	1.242*	47	182	0.258***	971***	<0.0001
57 *In(2R)bw[VDe2L]Cy[R]*	041A-B;042A02-03	506	331	1.529*	0	141	0.000***	978***	<0.0001
58 *Df(2R)nap14*	041BC/042A16-B01	113	152	0.743	0	7	0.000	272***	<0.0438
59 *Df(2R)nap19*	041E02-F01;043A02-B01	77	273	0.282*	0	77	0.000***	427***	<0.0001
60 *Df(2R)nap9*	042A01-02;042E06-F01	101	77	1.312	0	7	0.000	185***	0.0035
61 *Df(2R)ST1*	042B03-05;043E15-18	37	40	0.925	17	18	0.944	112**	>0.9999
62 *Df(2R)pk78s*	042F;043F08	120	146	0.822	23	87	0.264***	376***	<0.0001
63 *Df(2R)H3C1*	043F;044D03-08	118	129	0.915	84	60	1.400	391***	0.0468
64 *Df(2R)H3E1*	044D;044F	442	515	0.858	2	28	0.071*	987***	<0.0001
65 *Df(2R)Np3, bw1*	044D;044F	254	337	0.754*	0	66	0.000***	657***	<0.0001
66 *Df(2R)Np5*	044F10;045D09-E01	162	159	1.019	29	123	0.236***	473***	<0.0001
67 *Df(2R)B5*	046A;046C	136	132	1.030	62	60	1.033	390***	>0.9999
68 *Df(2R)X1*	046C;047A01	23	16	1.438	7	9	0.778	55**	0.3772
69 *Df(2R)stan1*	046D07-09;047F15-16	22	65	0.338**	6	26	0.231*	119***	0.6266
70 *Df(2R)stan2*	046F01-02;047D01-02	60	86	0.698	8	72	0.111***	226***	<0.0001
71 *Df(2R)E3363*	047A;047F	196	215	0.912	71	150	0.473***	632***	0.0002
72 *Df(2R)en-A*	047D03;048B02	148	158	0.937	14	150	0.093***	470***	<0.0001
73 *Df(wR)en-B*	047E03;048A04	138	178	0.775	0	101	0.000***	417***	<0.0001
74 *Df(2R)en30*	048A03-04;048C06-08	67	104	0.644	79	74	1.068	324*	0.0259

(*continued*)

TABLE 1

(Continued)

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
75 *Df(2R)en-SFX31*	048A-B	256	355	0.721*	120	341	0.352***	1072***	<0.0001
76 *Df(2R)CB21*	048E;049A	775	637	1.217*	44	33	1.333	1489***	0.7256
77 *Df(2R)vg-C*	049A04-13;049E07-F01	17	16	1.063	8	3	2.667	44**	0.3006
78 *Df(2R)vg135*	049A-B;049D-E	100	111	0.901	36	71	0.507*	318***	0.0226
79 *Df(2R)CX1*	049C01-04;050C23-D01	130	121	1.074	1	138	0.007***	390***	<0.0001
80 *Df(2R)trix*	051A01-02;051B06	54	54	1.000	0	33	0.000***	141***	<0.0001
81 *Df(2R)03072*	051A05;051C01	39	61	0.639	0	54	0.000***	154***	<0.0001
82 *Df(2R)Jp1*	051C03;052F05-09	348	401	0.868	53	45	1.178	847***	0.1632
83 *Df(2R)XTE-18*	051E03;052C09-D01	156	279	0.559***	36	50	0.720	521***	0.3280
84 *Df(2R)Jp4*	051F13;052F08-09	279	314	0.889	18	146	0.123***	757***	<0.0001
85 *Df(2R)WMG*	052A09-10;052D09-15	58	90	0.644	9	80	0.113***	237***	<0.0001
86 *Df(2R)Jp5*	052A13-B03;052F10-11	134	224	0.598***	54	133	0.406***	545***	0.0470
87 *Df(2R)Jp6*	052E03-05;052F	18	11	1.636	4	14	0.286	47*	0.0151
88 *Df(2R)Jp7*	052F05-09;052F10-11	248	281	0.883	96	135	0.711	760***	0.1791
89 *Df(2R)Jp8*	052F05-09;052F10-53A01	72	87	0.828	29	39	0.744	227***	0.7714
90 *Df(2R)P803-Δ15*	053E;053F11	666	536	1.243	21	19	1.105	1242***	0.7482
91 *Df(2R)rob1-c*	054B17-C04;054C01-04	249	324	0.769*	41	41	1.000	655***	0.2858
92 *Df(2R)Pcl7B*	054E08-F01;055B09-C01	195	244	0.799	154	152	1.013	745***	0.1175
93 *Df(2R)Pcl11B*	054F06-55A01;055C01-03	163	153	1.065	23	101	0.228***	440***	<0.0001
94 *Df(2R)PC4*	055A;055F	202	214	0.944	16	20	0.800	452***	0.7289
95 *Df(2R)PC29*	055C01-02;056B01-02	155	160	0.969	91	93	0.978	499***	>0.9999
96 *Df(2R)P34*	055E02-04;056C01-11	350	260	1.346*	12	13	0.923	635***	0.4117
97 *Df(2R)017*	056F05;056F015	273	303	0.901	202	212	0.953	990***	0.6989
98 *Df(2R)AA21*	056F09;057D11-12	83	82	1.012	23	65	0.354**	253***	0.0003
99 *Df(2R)Pu-D17*	057B04;058B	456	451	1.011	0	39	0.000***	946***	<0.0001
100 *Df(2R)PI13*	057B13-14;057D08-09	48	76	0.632	38	38	1.000	200***	0.1415
101 *Df(2R)PK1*	057C05;057F05-6	82	112	0.732	4	84	0.048***	282***	<0.0001
102 *Df(2R)Egfr5*	057D02-08;058D01	189	319	0.592***	3	25	0.120**	536***	0.0039
103 *Df(2R)Egfr18*	057E04-11;057F01	103	21	4.905***	99	0	—***	223***	<0.0001
104 *Df(2R)X58-7*	058B01-02;058E01-04	133	165	0.806	128	119	1.076	545*	0.1021
105 *Df(2R)59AD*	059A01-03;059D01-04	86	69	1.246	9	46	0.196***	210***	<0.0001
106 *Df(2R)or-BR6*	059D05-10;060B03-08	223	164	1.360*	53	29	1.828	469***	0.2674
107 *Df(2R)Px1*	060B08-10;060D01-02	77	93	0.828	57	39	1.462	266***	0.0304
108 *In(2LR)Px4*	060C05-06;060D01	56	59	0.949	9	34	0.265**	158***	0.0019
109 *Df(2R)Px2*	060C05-06;060D09-10	315	319	0.987	6	54	0.111***	694***	<0.0001
110 *Df(2R)Es1*	060E06-08;060F01-02	173	200	0.865	27	91	0.297***	491***	<0.0001

(*continued*)

TABLE 1

(Continued)

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
Third chromosome									
111 *Df(3L)emc-E12*	061A;061D03	312	348	0.897	107	214	0.500***	981***	<0.0001
112 *Df(3L)Ar14-8*	061C05-08;062A08	368	368	1.000	51	176	0.290***	963***	<0.0001
113 *Df(3L)Aprt-1*	062A10-B01;062D02-05	117	127	0.921	99	89	1.112	432*	0.3825
114 *Df(3L)R-G7*	062B08-09;062F02-05	86	102	0.843	75	97	0.773	360	0.7503
115 *Df(3L)HR119*	063C02;063F07	114	90	1.267	59	73	0.808	336***	0.0573
116 *Df(3L)GN19*	063F04-07;064B09-11	90	86	1.047	47	68	0.691	291**	0.0937
117 *Df(3L)ZN47*	064C;065C	170	225	0.756	2	28	0.071**	425***	<0.0001
118 *Df(3L)v65c*	064E01-13;065C01-D06	124	147	0.844	3	45	0.067***	319***	<0.0001
119 *Df(3L)XDI98*	065A02;065E01	73	58	1.259	71	37	1.919*	239**	0.1440
120 *Df(3L)RM5-2*	065E01-12;066B01-02	111	81	1.370	70	54	1.296	316***	0.8169
121 *Df(3L)ZP1*	066A17-20;066C01-05	285	294	0.969	155	31	5.000***	765***	<0.0001
122 *Df(3L)66C-G28*	066B08-09;066C09-10	213	161	1.323	57	15	3.800***	446***	0.0003
123 *Df(3L)h-i22*	066D10-11;066E01-02	34	41	0.829	30	43	0.698	148	0.6224
124 *Df(3L)Scf-R6*	066E01-06;066F01-06	73	72	1.014	57	71	0.803	273	0.3954
125 *Df(3L)29A6*	066F05;067B01	269	278	0.968	138	145	0.952	830***	0.9417
126 *Df(3L)AC1*	067A02;067D07-13	92	93	0.989	7	0	—	192***	0.0143
127 *Df(3L)lxd6*	067E01-02;068C01-02	457	461	0.991	2	13	0.154	933***	0.0070
128 *Df(3L)vin2*	067F02-03;068D06	164	191	0.859	19	58	0.328**	432***	0.0005
129 *Df(3L)vin5*	068A02-03;069A01-03	232	205	1.132	10	10	1.000	457***	0.8221
130 *Df(3L)vin6*	068C08-11;069A04-05	97	142	0.683*	25	2	12.500**	266***	<0.0001
131 *Df(3L)vin7*	068C08-11;069B04-05	57	49	1.163	17	0	—**	123***	<0.0001
132 *Df(3L)BK9*	068E;069A01	516	482	1.071	132	1	132.000***	1131***	<0.0001
133 *Df(3L)eygC1*	069A04-05;069D04-06	323	257	1.257	62	100	0.620*	742***	<0.0001
134 *Df(3L)iro-2*	069B01-05;069D01-06	113	85	1.329	6	0	—	204***	0.0420
135 *Df(3L)BSC10*	069D04-05;069F05-07	199	157	1.268	12	10	1.200	378***	>0.9999
136 *Df(3L)fz-GF3b*	070C01-02;070D04-05	138	145	0.952	65	54	1.204	402***	0.3255
137 *Df(3L)D-5rv12*	070C2;72A1	22	12	1.833	0	87	0.000	42***	<0.0001
138 *Df(3L)Brd12*	070E;071A01-02	71	52	1.365	22	1	22.000**	146***	0.0003
139 *Df(3L)Brd15*	071A01-02;071C01-02	142	117	1.214	34	0	—***	293***	<0.0001
140 *Df(3L)brm11*	071F01-04;072D01-10	56	48	1.167	11	14	0.786	129***	0.5043
141 *Df(3L)st-f13*	072C01-D01;073A03-04	265	361	0.734**	2	16	0.125*	644***	0.0071
142 *Df(3L)st-e4*	072D05-10;073A05-08	394	458	0.860	5	43	0.116	900***	<0.0001
143 *Df(3L)st-b11*	072D10-11;073D01-02	88	85	1.035	1	20	0.050**	194***	<0.0001
144 *Df(3L)81k19*	073A03;074F	109	116	0.940	13	36	0.361*	274***	<0.0066
145 *Df(3L)BSC8*	074D03-075A01;075B02-05	245	143	1.713***	41	49	0.837	478***	0.0028
146 *Df(3L)W10*	075A06-07;075C01-02	30	22	1.364	21	5	4.200*	78***	0.0484
147 *Df(3L)VW3*	076A03;076B02	23	14	1.643	17	14	1.214	68	0.6240
148 *Df(3L)XS543*	076B;077A	13	12	1.083	5	3	1.667	33*	0.6992

(*continued*)

TABLE 1

(Continued)

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
149 *Df(3L)kto2*	076B01-02;076D05	150	164	0.915	15	71	0.211***	400***	<0.0001
150 *Df(3L)XS-533*	076B04;077B	132	104	1.269	9	17	0.529	262***	0.0604
151 *Df(3L)rdgC-co2*	077A01;077D01	249	216	1.153	93	48	1.938**	606***	0.0115
152 *Df(3L)ri-79C*	077B-C;077F-78A	79	63	1.254	51	31	1.645	224***	0.3994
153 *Df(3L)Pc-2q*	078C05-06;078E03-079A01	447	167	2.677***	22	29	0.759	665***	<0.0001
154 *Df(3L)Ten-m-AL29*	079C01-03;079E03-08	63	75	0.840	28	19	1.474	185***	0.1283
155 *Df(3L)Δ1AK*	079F;080A	108	88	1.227	5	42	0.119***	243***	<0.0001
156 *Df(3L)2-30*	080Fj	168	146	1.151	84	5	16.800***	403***	<0.0001
Centromere									
157 *Df(3R)10-65*	081Fa	142	126	1.127	100	13	7.692***	381***	<0.0001
158 *Df(3R)6-7*	082D03-08;082F03-06	65	44	1.477	52	0	—***	161***	<0.0001
159 *Df(3R)Tpl10*	083C01-02;084B01-02	85	110	0.773	75	113	0.664	383*	0.4703
160 *Df(3R)dsx37*	084D08;085B03-05	594	504	1.179	271	145	1.869***	1514***	0.0001
161 *Df(3R)p13*	084F02;085B01	48	48	1.000	47	35	1.343	178	0.3674
162 *Df(3R)p-XT103*	085A02;085C01-02	99	85	1.165	9	16	0.563	209***	0.1345
163 *Df(3R)GB104*	085D12;085E10	146	156	0.936	8	2	4.000	312***	0.0585
164 *Df(3R)M-Kx1*	086C01;087B01-05	359	363	0.989	0	64	0.000***	786***	<0.0001
165 *Df(3R)T-32*	086E02-04;087C06-07	94	110	0.855	0	17	0.000**	221***	<0.0001
166 *Df(3R)E229*	086F6-7;87B1-2	48	30	1.600	7	0	—	85***	0.0481
167 *Df(3R)P-58*	087A4,5-6;87A9	62	100	0.620*	79	63	1.254	304**	0.0028
168 *Df(3R)ry615*	087B11-13;087E08-11	49	46	1.065	43	15	2.867*	153***	0.0066
169 *Df(3R)kar-Sz12*	087B1-3;87C8-9	101	100	1.010	100	12	8.333***	313***	<0.0001
170 *Df(3R)Po4*	088F07-089A02;089A11-13	101	229	0.441*	0	56	0.000***	386***	<0.0001
171 *Df(3R)P115*	089B07-08;089E07-08	75	78	0.962	15	5	3.000	173***	0.0333
172 *Df(3R)DG2*	089E01-F04;091B01-B02	160	214	0.748	3	94	0.032***	471***	<0.0001
173 *Df(3R)RD31*	089E02;090D	227	248	0.915	78	200	0.390***	753***	<0.0001
174 *Df(3R)C4*	089E03-04;090A01-07	75	72	1.042	54	65	0.831	266	0.3891
175 *Df(3R)Cha7*	090F01-F04;091F05	246	291	0.845	126	156	0.808	819***	0.7682
176 *Df(3R)Dl-BX12*	091F01-02;092D03-06	158	126	1.254	23	0	—**	307***	<0.0001
177 *Df(3R)H-B79*	092B03;092F13	153	117	1.308	50	26	1.923	346***	0.1873
178 *Df(3R)e-N19*	093B;094	354	331	1.069	33	109	0.303***	827***	<0.0001
179 *Df(3R)e-R1*	093B03-05;093D02-04	101	62	1.629*	61	49	1.245	273***	0.3157
180 *Df(3R)23D1*	094A03-04;094D01-04	59	91	0.648	24	48	0.500	222***	0.4592
181 *Df(3R)nau-11a4*	095A	92	70	1.314	57	30	1.900*	249***	0.2224
182 *Df(3R)mbc-30*	095A05;095C10-11	54	68	0.794	0	50	0.000***	172***	<0.0001
183 *Df(3R)mbc-R1*	095A05-07;095D06-11	214	169	1.266	1	82	0.012***	466***	<0.0001
184 *Df(3R)06624*	095C01;095C07	211	275	0.767*	132	83	1.590*	701***	<0.0001
185 *Df(3R)crb-F89-4*	095D07-D11;095F15	38	42	0.905	16	15	1.067	111***	0.8327

(*continued*)

TABLE 1

(Continued)

Deficiency	Cytological breakpoints	F_1 females			F_1 males			Total progeny[b]	Fisher's exact test[c]
		Df	*Bal*	Ratio[a]	*Df*	*Bal*	Ratio[a]		
186 *Df(3R)crb87-5*	095F07;096A17-18	9	9	1.000	10	18	0.556	46	0.3734
187 *Df(3R)96B*	096A21;096C02	66	53	1.245	37	14	2.643*	170***	0.0410
188 *Df(3R)Esp13*	096F01;097B01	503	315	1.597***	99	41	2.415***	958***	0.0377
189 *Df(3R)TI-P*	097A;098A01-02	119	108	1.102	15	3	5.000	245***	0.0129
190 *Df(3R)D605*	097E03;098A05	121	116	1.043	69	37	1.865*	343***	0.0186
191 *Df(3R)3450*	098E03;099A06-08	132	109	1.211	49	44	1.114	334***	0.8066
192 *Df(3R)Dr-rv1*	099A01-02;099B06-11	30	33	0.909	23	30	0.767	116	0.7101
193 *Df(3R)X3F*	099D01-02;099E01 100F	209	149	1.403*	29	21	1.381	408***	>0.9999
Total								85972	

[a] Superscripts indicate significant deviation from 1:1 ratio of *Df:Bal* progeny using χ^2 tests. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

[b] Superscripts indicate significant deviation from 1:1:1:1 ratio of *Df* female:*Bal* female:*Df* male:*Bal* male using χ^2 tests; *, **, and *** as in note *a*.

[c] Fisher's exact probabilities that ratio of *Df:Bal* hybrid females is equal to ratio of *Df:Bal* hybrid males.

[d] *M(2)41A*^{1}, an X-ray-induced mutation, is likely a deficiency affecting multiple loci (Lindsley and Zimm 1992).

Figure 2.—Distribution of relative viability of *Df* hybrids (deficiency bearing/total) for (A) hybrid females and (B) hybrid males.

cies tested become viable when hybrid males are given a *sim X* instead of a *mel X*. (For two hybrid-lethal deficiencies, the attached-*X* stocks proved too weak to maintain and could not be tested.) Thus, the hybrid lethality of 18 of 18 *sim* autosomal regions depends on the species origin of the *X*, confirming that these are true hybrid lethals caused by incompatible epistatic interactions.

These attached-*X* crosses were also expected to produce hybrid females homozygous for the *mel X* (and thus genotypically identical to hybrid males from the original screen; compare female hybrids in Figure 1B to male hybrids in Figure 1A). If produced in sufficient number, we could further test the sex specificity of each hybrid incompatibility: If hybrid lethals are not sex specific, *Df* hybrid females from attached-*X* crosses should also be inviable. This outcome appears to hold in three cases (Table 3, lines 2, 4, and 13), but in general I obtained too few hybrid females to draw meaningful conclusions.

Lethal phase: I determined the lethal phase of 18 hybrid incompatibilities. Only one causes embryonic lethality (see Table 4). I compared this distribution to

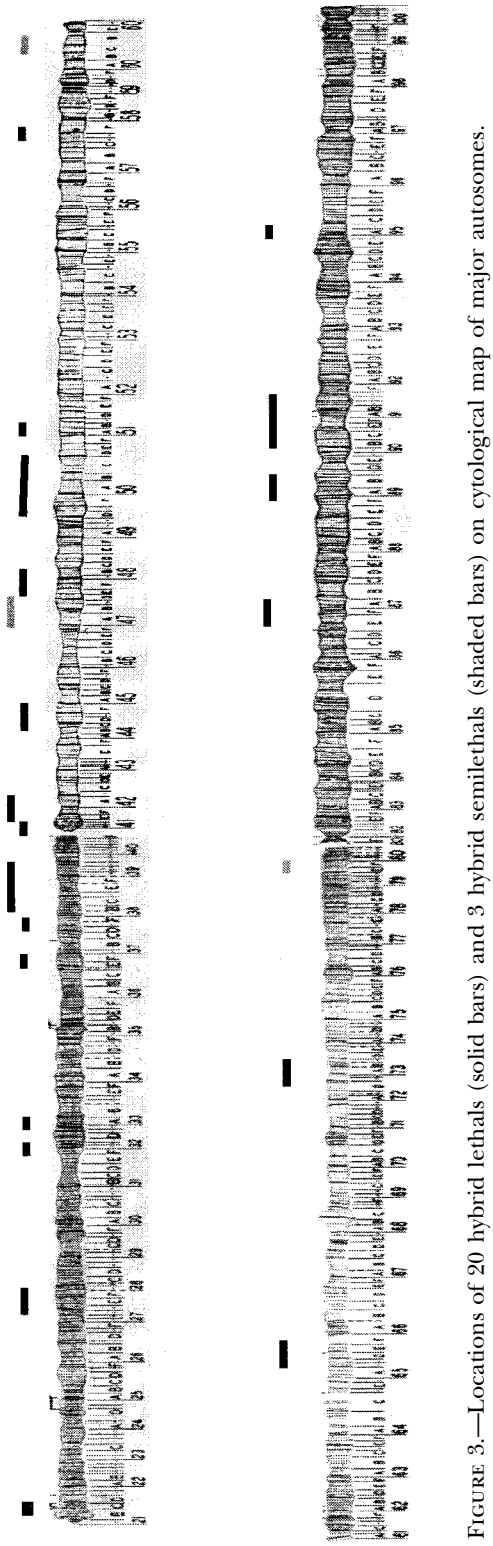

FIGURE 3.—Locations of 20 hybrid lethals (solid bars) and 3 hybrid semilethals (shaded bars) on cytological map of major autosomes.

that for lethal phases of mutations within species. In *D. melanogaster*, 60.5% of lethal *P* insertions cause embryonic lethality (TOROK *et al.* 1993; DEAK *et al.* 1997). There are thus significantly fewer embryonic lethal incompatibilities in hybrids than expected from the within-species data (1 embryonic *vs.* 17 postembryonic hybrid-lethal incompatibilities; 2515 embryonic *vs.* 1640 postembryonic within-species lethal mutations; Fisher's exact $P < 0.0001$). Thus hybrid-lethal incompatibilities more often afflict later, postembryonic stages of development.

DISCUSSION

This study yields three main results. First (and perhaps most surprising), many hybrid-lethal incompatibilities have evolved between *D. melanogaster* and *D. simulans*: The ~70% of the *D. simulans* autosomal genome examined harbors at least 20 factors that cause complete or near complete lethality in the presence of the *D. melanogaster X*. Taking into account certain corrections (see below), this value implies that the genome-wide number of recessive-recessive lethal incompatibilities is ~169. Second, the incompatible genes on both the autosomes and the *X* are nearly completely recessive and, as shown below, these recessive incompatibilities vastly outnumber dominant ones. These results provide strong support for the dominance theory. Third, all of the lethal hybrid incompatibilities tested involve an epistatic interaction with the *X* chromosome; *i.e.*, 18 of 18 are rescued when the *mel X* is replaced by a compatible *sim X*, as expected under the the Dobzhansky-Muller model.

While these data confirm the ubiquity of recessive, epistatic hybrid incompatibilities, they also allow us to go further. These data can be used to estimate four quantities: (1) the total number of hybrid lethals separating *D. melanogaster* and *D. simulans*; (2) the relative rates of evolution of dominant *vs.* recessive incompatibilities; (3) the probability that two randomly chosen divergent substitutions (one from each species) are incompatible, causing hybrid lethality; and (4) the fraction of viability-essential genes that have diverged to such an extent that they are no longer functionally compatible with alleles at interacting loci from the other species.

Dominance and the total number of hybrid lethals: By including data from previous genetic analyses, we can estimate the total number of hybrid lethals that have evolved between *D. melanogaster* and *D. simulans*. I make the simplifying assumption that hybrid lethality involves pairs of incompatible loci. Hybrid incompatibilities can then be classified into dominant-dominant, recessive-dominant, and recessive-recessive types (or, following TURELLI and ORR 2000, H_0, H_1, and H_2 incompatibilities, respectively, where the subscripts 0, 1, and 2 indicate the number of homozygous or hemizygous loci involved). More than two loci could be involved in a given incompatibility—so-called complex epistasis—but

TABLE 2

Deficiencies are not male lethal within *D. melanogaster* (*Df/Bal* females × Canton-S males)

Deficiency	Breakpoints	F_1 females			F_1 males			Total progeny
		Df	*Bal*	Ratio	*Df*	*Bal*	Ratio	
Df(2L)al	021C01;021C07	44	68	0.647	53	63	0.841	228
Df(2L)J-H	027C02-09;028B03-04	104	112	0.929	99	93	1.065	408
Df(2L)J2	031B;032A	111	118	0.941	119	111	1.072	459
Df(2L)Prl	032F01-03;033F01-02	76	95	0.800	58	81	0.716	310
Df(2L)TW50	036E04-F01;038A06-07	100	61	1.639	70	65	1.077	296
Df(2L)TW1	038A07-B01;039C02-03	51	64	0.797	54	53	1.019	222
M(2)41A1	041A	177	166	1.066	126	145	0.869	614
Df(2R)nap19	041E02-F01;043A02-B01	54	101	0.535	59	110	0.536	324
Df(2R)Np3	044D02-E01;045B08-C01	110	128	0.859	123	136	0.904	497
Df(2R)stan2	046F01-02;047D01-02	48	57	0.842	55	73	0.753	233
Df(2R)en-A	047D03;048B02	50	51	0.980	51	57	0.895	209
Df(2R)CX1	049C01-04;050C23-D02	68	68	1.000	69	64	1.078	269
Df(2R)trix	051A01-02;051B06	68	83	0.819	84	76	1.105	311
Df(2R)PK1	057C05;057F05-06	86	85	1.012	84	100	0.840	355
Df(2R)Px2	060C05-06;060D09-10	108	124	0.871	78	107	0.729	417
Df(3L)ZN47	064C;065C	109	127	0.858	125	138	0.906	499
Df(eL)st-f13	072C01-D01;073A03-04	96	88	1.091	68	64	1.063	316
Df(3L)Delta1AK	079E05-F01;079F02-06	128	98	1.306	109	93	1.172	428
Df(3R)M-Kx1	086C01;087B01-05	62	101	0.614	71	110	0.645	344
Df(3R)Po4	088F07-089A02;089A11-13	130	115	1.130	131	103	1.272	479
Df(3R)mbc-30	095A05-07;095C10-11	92	96	0.958	81	70	1.157	339
Total								7557

this should not seriously affect our estimates as long as the three incompatibility types (H_0, H_1, and H_2) are equally prone to such complex interactions.

H_0 incompatibilities: No H_0 incompatibilities separate *D. melanogaster* and *D. simulans*. The cross of *D. melanogaster* females with *D. simulans* males produces perfectly viable hybrid females that are heterozygous at every locus in the genome (Sturtevant 1920, 1929; but see Barbash *et al.* 2000 for temperature effects on viability of hybrid females).

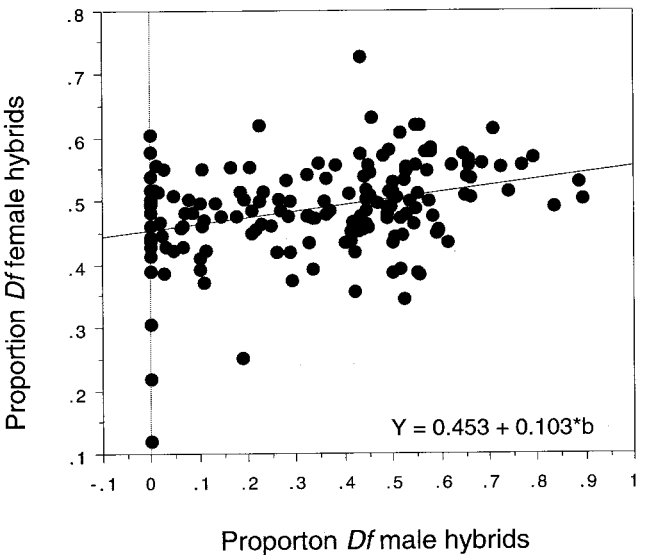

Figure 4.—Relative viability of *Df* hybrid females (*Df*-bearing females/total females) plotted against viability of *Df* hybrid males (*Df*-bearing males/total males).

H_1 incompatibilities: There are ~22 H_1 incompatibilities between *D. melanogaster* and *D. simulans*. This value comes from two kinds of data. The first involves studies of hybrid rescue mutations. There is now good evidence that such mutations are rare "compatible" alleles at normally incompatible loci (Hutter *et al.* 1990; Sawamura and Yamamoto 1997; Barbash *et al.* 2000; Orr and Irving 2000). We can therefore infer two H_1 incompatibilities from the existence of two known pairs of complementary hybrid rescue mutations (Hutter *et al.* 1990; Sawamura *et al.* 1993; Orr and Presgraves 2000). The second comes from Coyne *et al.*'s (1998) deficiency screen for H_1 incompatibilities. This screen uncovered five hybrid-lethal regions (this lethality was not unconditional, however, as some became viable at permissive temperatures or using different stocks). Coyne *et al.*'s number requires two corrections as only 50% of the *D. simulans* genome was screened, and the reciprocal experiment in which *D. melanogaster* regions are made hemizygous could not be done as no deficiencies are available in *D. simulans*. Correcting for these two considerations yields an estimate of ~20 hybrid-lethal regions. Thus ~22 H_1 incompatibilities separate *D. melanogaster* and *D. simulans*. This number is obviously rough, but as shown below, it differs qualitatively from that for H_2 incompatibilities.

TABLE 3

Hybrid lethality depends on the species origin of the *X*

Genotype of *D. melanogaster* female (× *Lhr D. simulans* males)[a]	Breakpoints	F_1 females			F_1 males			Total progeny
		Df	*Bal*	Ratio	*Df*	*Bal*	Ratio	
1 *C(1)M4, y2;Df(2L)al*	21C1;21C7	3	3	1.000	157	202	0.777	365
2 *C1(M4), y2;Df(2L)J-H*	027C02-09;028B03-04	12	45	0.267	808	799	1.011	1664
3 *C(1)M4, y2;Df(2L)Prl*	032F01-03;033F01-02	1	2	0.500	45	82	0.549	130
4 *C(1)M4, y2;Df(2L)pr-A16*	037B02-12;038D02-05	1	54	0.019	80	242	0.331	377
5 *C(1)M4, y2;Df(2L)TW2*	037D05-E01;038E06-09	0	1	0.000	61	75	0.813	137
6 *C(1)M4, y2;M(2)41A1*	041A	0	2	0.000	281	463	0.607	746
7 *C(1)M4, y2;Df(2R)nap14*	041BC;042A16-B01	0	0	—	26	18	1.444	44
8 *C(1)M4, y2;Df(2R)Np3*	044D02-E01;045B08-C01	0	1	0.000	63	205	0.307	269
9 *C(1)M4, y2;Df(2R)stan2*	046F01-02;047D01-02	0	0	—	87	125	0.696	212
10[b] *C(1)M4, y2; Df(2R)en-B*	047E03;048A04	0	0	—	6	92	0.065	98
C(1)M4, y2;Df(2R)en-A	047D03;048B02	0	0	—	131	140	0.936	271
11 *C(1)M4, y2;Df(2R)trix*	051A01-02;051B06	0	0	—	151	118	1.280	269
12 *C(1)M4, y2;Df(2R)PK1*	057C05;057F05-06	1	0	—	198	83	2.386	282
13 *C(1)M4, y2;Df(2R)Px2*	060C05-06;060D09-10	1	15	0.067	366	226	1.619	608
14 *C(1)M4, y2;Df(3L)ZN47*	064C;065C	0	0	—	3	7	0.429	10
15 *C(1)M4, y2;Df(3L)st-f13*	072C01-D01;073A03-04	0	0	—	183	132	1.386	315
16 *C(1)M4, y2;Df(3L)Δ1AK*	079E05-F01;079F02-06	0	0	—	119	70	1.700	189
17 *C(1)M4, y2;Df(3R)T-61*	086E03;087A09	0	0	—	221	191	1.157	412
18 *C(1)M4, y2;Df(3R)mbc-30*	095A05-07;095C10-11	0	2	0.000	106	41	2.585	149
Total								6547

[a] See Figure 1B for cross scheme.

[b] Two deficiencies were tested for this region. The initial cross, involving *Df(2R)en-B,* was anomalous as *Df* hybrid males are rare. Retesting with *Df(2R)en-A* clearly shows that hybrids hemizygous for this region become viable when given a *D. simulans X.*

H_2 incompatibilities: The total number of H_2 incompatibilities is large. We can estimate the genome-wide number by extrapolating from the number of *X*-autosome H_2 incompatibilities (see RESULTS). The *X* is roughly equivalent in size and gene content to one of the five major chromosome arms (*i.e.*, *X*, 2L, 2R, 3L, and 3R). Holding the *X* effectively homozygous (hemizygous) and screening the rest of the genome with deficiencies yielded ~27 lethal H_2 incompatibilities. If we could repeat this screen, successively holding the autosomal arms 2L, 2R, 3L, and 3R homozygous for *D. melanogaster* while scanning the rest of the *D. simulans* genome for lethal incompatibilities, we would expect to uncover another ~27 H_2 incompatibilities for each arm. We therefore expect ~135 hybrid lethals genome-wide. Using similar logic to correct for intra-arm H_2 incompatibilities brings the total number of H_2 incompatibilities to ~169. (Note that extrapolating from the number of *X*-autosome incompatibilities to the number genome-wide assumes that *X*-linked and autosomal loci diverge at similar rates; BETANCOURT *et al.*'s (2002) survey of divergence at >250 genes from *D. melanogaster* and *D. simulans* supports this assumption.)

Two conclusions follow from these calculations. First, summing across H_0, H_1, and H_2 incompatibility types gives an estimate of the total number of hybrid-lethal incompatibilities separating *D. melanogaster* and *D. simulans*: 191. (This does not include the hybrid semilethals found here; including them nearly doubles the estimate.) Given the conclusion from previous analyses that the number of hybrid lethals between these two species is small, this number comes as a surprise and reveals an unexpected degree of functional divergence.

These numbers also provide strong quantitative confirmation of the dominance theory: *D. melanogaster* and *D. simulans* are separated by no H_0 incompatibilities, by ~22 H_1 incompatibilities, and by ~169 H_2 incompatibilities. The number of hybrid lethals thus jumps nearly an order of magnitude as we increase the number of homozygous loci involved, leaving little doubt that most hybrid lethals are recessive. Why hybrid incompatibilities tend to be recessive remains a mystery. Although the similar effects of loss-of-function mutations within species and incompatibilities in hybrids have led to speculation that the latter mimic the former (STEBBINS 1958; ORR 1993; TURELLI and ORR 1995), several recent lines of evidence now appear inconsistent with this interpretation (BARBASH *et al.* 2000; ORR and IRVING 2000; ORR and PRESGRAVES 2000). But regardless of *why* most incompatibilities act as recessives, the present results leave little doubt that they do.

Epistasis and hybrid lethality: Using the above estimate of the total number of hybrid-lethal incompatibilities, along with a molecular estimate of the total number of divergent substitutions between *D. melanogaster* and *D. simulans*, we can estimate (to an order of magnitude)

TABLE 4

Summary of results for hybrid-lethal regions

Hybrid-lethal region[a]	Breakpoints[b]	*Df* hybrid male viability[c]	Confirmed?[d]	Lethal phase[e]	Lethality rescued by *D. simulans X*?[f]	*Df* lethal within *D. melanogaster*?[g]
1	021C02-03;021C08-D01	0.013	Y[h]	PE	Y	N
2	027C02-09;028A	0.000	Y	PE	Y	N
3	031F;032A	0.026	N	PE	—	N
4	032F01-03	0.043	Y	PE	Y	N
5	036E04-F01;036F07-09	0.000	Y	—	—	N
6	037D05;037F05	0.032	Y	PE	Y	N
7	038A07-B1;039C02-03	0.000	Y	E	Y	N
8	041A	0.009	Y	—	Y	N
9	041E02-F01;042A02-B01	0.000	Y	—	Y	N
10	044D03-08;044F10	0.042	Y	PE	Y	N
11*	047A01;047D01-02	0.272	Y	PE	Y	N
12	047E03;048A03-04	0.047	Y	PE	Y	N
13	049D-E;050C23-D02	0.007	N	PE	—	N
14	051A05;051B06	0.000	Y	PE	Y	N
15	057D08-09;057F05-06	0.056	Y	PE	Y	N
16*	060C05-06;060D01	0.188	Y	PE	Y	N
17	064E01-13;065C	0.069	Y	PE	Y	N
18	072D10-11;073A03-04	0.097	Y	PE	Y	N
19*	079E05-F01;079F02-06	0.119	N	PE	Y	N
20	086E02-04;086F06-07	0.000	Y	PE	Y	N
21	088F07-089A02;089A11-13	0.000	N	—	—	N
22	089E01-F04;091B01-B02	0.032	N	—	—	—
23	095A05-07;095C10-11	0.006	Y	PE	Y	N

[a] Twenty hybrid-lethal regions and 3 hybrid-semilethal regions (*).
[b] Breakpoints defining physical location of hybrid lethal are from combined information of multiple deficiencies (see Table 1).
[c] Viability, ratio of *Df:Bal* hybrid males for region (mean of multiple overlapping lethal deficiencies).
[d] Y, lethality confirmed by >1 overlapping deficiency (see Table 1); *N*, not confirmed.
[e] E, embryonic lethality; PE, postembryonic lethality.
[f] See Table 3 results.
[g] See Table 2 results.
[h] Hybrid lethality of region 021C02-03; 021C08-D1 was detected with *Df(2L)al* and then confirmed using a newly extracted *Df(2L)al* deficiency chromosome (see MATERIALS AND METHODS).

another important quantity from speciation genetics theory: the probability, p, that any two randomly chosen divergent sites (one from one species, one from the other) are incompatible in hybrids, causing (for our purposes) complete lethality. As Orr and Turelli (2001) show, the expected number of hybrid incompatibilities, I, between pairs of genes is

$$I = 2k^2t^2p,$$

where k is the genome-wide rate of substitution and t is the time since speciation, so that $2kt$ is the total number of substitutions separating the genomes of two species. By substituting estimates of I and kt and rearranging, we can solve for p. To ensure that our estimate of p for hybrid lethality is directly comparable to the estimate of p for hybrid male sterility calculated by Orr and Turelli (2001), I use the same data sources for k and t and follow their calculation exactly. (See appendix for fuller details and discussion of the calculation.) The total number of hybrid lethals is, from above, $I \approx 191$. *D. melanogaster* and *D. simulans* have accumulated $2kt \approx$ 156,000 nonsynonymous substitutions since they diverged $t = 2.5$ MYA (Hey and Kliman 1993; Li 1997). Solving the above equation thus gives $p \approx 1.6 \times 10^{-8}$. Two nonsynonymous substitutions chosen randomly (one from each species' genome) will therefore cause complete hybrid lethality $\sim 10^{-8}$ of the time. As discussed in the appendix, this value appears to be an order of magnitude smaller than the value for hybrid male sterility estimated by Orr and Turelli (2001).

Interestingly, p can be thought of as a crude index of the ruggedness of the molecular landscape. To see this, consider the extreme cases: When $p = 0$, negative epistasis is absent, all substitutions are compatible, and hybrid genotypes never fall into fitness valleys; when $p = 1$, however, negative epistasis is complete, all divergent substitutions after the first are incompatible, and all hybrid genotypes fall into fitness valleys. Thus, the exceedingly small value of p suggests that the molecular landscape is reasonably smooth: Substitutions that have never "seen" each other in their evolutionary histories are almost always compatible (or at least not lethal in combination).

Functional divergence: Finally, we can estimate the

fraction of viability-essential genes that have diverged to the extent that they are no longer functionally compatible. If hybrid-lethal incompatibilities involve *pairs* of loci, as assumed above, it follows from the Dobzhansky-Muller model that *both* loci must have diverged (Orr 1995). The above estimate of 191 hybrid-lethal incompatibilities thus implies that at least 382 loci have experienced significant functional divergence since the *D. melanogaster-D. simulans* split, *i.e.*, ~11% of all viability-essential genes (or 382/3600; see Spradling *et al.* 1999). This level of divergence at a class of genes that we might *a priori* expect to be relatively conserved (*i.e.*, those essential for viability) seems remarkable.

Two lines of evidence suggest that the genes regulating the earliest phases of development in the two species remain largely compatible. First, most hybrid genotypes survive embryonic phases of development only to die later (Carvajal *et al.* 1996). Second, my deficiency screen, as well as that of Coyne *et al.* (1998), could have detected any maternal-zygotic, embryonic lethal incompatibilities involving the *D. melanogaster* cytoplasm and hemizygous *D. simulans* autosomal factors. But very few of our incompatibilities can possibly fall into this category. Instead, most hybrid incompatibilities occur between zygotic genes acting at postembryonic stages. Since viability-essential genes are most often mutable to embryonic lethality within species, the paucity of embryonic *vs.* postembryonic hybrid lethals suggests that: (i) There are greater functional constraints (on gene sequence and/or expression) at early acting genes; (ii) most physiological and ecological adaptation in Drosophila occurs by divergence in postembryonic phases of development; or (iii) more genes are simultaneously active and thus prone to incompatible interactions during later stages of development. The latter possibility seems unlikely to account for the strong preponderance of postembryonic hybrid lethality as most genes exhibiting developmentally modulated expression during the Drosophila life cycle are expressed at some point during embryogenesis (>88%; Arbeitman *et al.* 2002).

Caveats: Using deficiencies and a hybrid rescue mutation to detect *X*-autosome incompatibilities makes two key assumptions. The first is that the effects of *D. simulans* autosomal regions when hemizygous are equivalent to when they are homozygous. If this assumption is incorrect, then instead of uncovering "hybrid lethals," the screen might simply uncover regions that are haploinsufficient. (Hemizygosity of the *D. melanogaster X* in males is equivalent to homozygosity because of dosage compensation.) Three facts, however, militate against this possibility (see also Coyne *et al.* 1998). First, although deficiencies can *uncover* recessive lethality within species, they are not inherently lethal as heterozygotes within species (Table 2).

Second, one might argue that deficiencies cause haploinsufficiency, but only in hybrids. Two observations rule out this possibility: deficiencies that cause lethality in hybrid males do not, in most cases, harm *Df* hybrid females; more important, even hybrid males that carry a hybrid-lethal deficiency become viable when carrying a *D. simulans*, rather than a *D. melanogaster*, *X*. Thus, the lethality of particular deficiencies depends on species genotype at background loci (*i.e.*, epistasis), as expected for hybrid incompatibilities.

Third, there are now two examples in which factors from *D. simulans* are known to behave identically in species hybrids, whether homozygous or hemizygous:

i. Muller and Pontecorvo (1940, 1942) discovered that the fourth chromosome of *D. simulans* causes hybrid male sterility when homozygous on an otherwise *D. melanogaster* genetic background. Orr (1992) fine mapped the region of the *D. simulans* fourth responsible using *D. melanogaster* deficiencies, thus confirming that the recessive, incompatible *D. simulans* factor causes sterility when homozygous or hemizygous.
ii. Sawamura *et al.* (2000) used a mutation that weakly rescues hybrid female fertility to introgress two small regions from *D. simulans* 2L into an otherwise *D. melanogaster* background. According to my results, these regions harbor three factors that each cause hybrid lethality when hemizygous in a genetic background containing a hemizygous *D. melanogaster X* and heterozygous autosomes (Table 4, lines 1, 3, 4). Sawamura (2000) found that, in an identical genetic background, these regions also cause hybrid lethality when homozygous.

Thus, both Orr's and Sawamura's results show that recessive hybrid incompatibilities behave identically when homozygous or hemizygous.

The second assumption of the deficiency analysis is that the 40 hybrid incompatibilities detected are independent of hybrid male rescue by *Lhr*. The primary cross (Figure 1A) was intended to rescue hybrid males from one incompatibility (involving the incompatible, wild-type allele Lhr^{sim}) and to then expose them to other potential incompatibilities. It is formally possible, however, that some of these hybrid lethals are actually suppressors of *Lhr* rescue. If true, these suppressors would still be of interest as interactors of a known hybrid incompatibility locus (*Lhr*). But this *Lhr*-suppressor scenario seems unlikely for several reasons. First, it seems unlikely that since the *D. melanogaster-D. simulans* split ~2.5 MYA, the only genetic pathways to evolve incompatibilities necessarily all involve *Lhr*. Second, such putative *Lhr* suppressors would necessarily be recessive *D. simulans* factors that suppress the rescue effects of a particular rare allele, *Lhr*. It is hard to believe that so many different recessive, conspecific loci are capable of such a trick. Third, *Lhr* rescues hybrid male lethality that normally occurs at the larval-pupal transition. But at least one hybrid lethal does not affect this phase of development, causing embryonic lethality instead (Table 4, line 7), and thus cannot be explained by *Lhr* suppression. Casual observations further suggest that

more detailed study of the postembryonic lethal phases will reveal other cases acting later than the larval-pupal transition.

Conclusions: This study shows that most hybrid incompatibilities are recessive and epistatic. The most surprising finding is the extraordinary degree of functional divergence that has occurred between *D. melanogaster* and *D. simulans* during the last 2.5 MY. Such extensive cryptic divergence would seem to confirm Muller's suggestion that "(t)wo groups of organisms which are not ordinarily allowed to cross with one another will thus automatically become increasingly immiscible, and their genic, chemical paths of evolution will diverge more and more . . . even in cases where their evolution is, from the phenotypic standpoint, strikingly parallel" (Muller 1939, p. 278).

An important aspect of the many hybrid incompatibilities identified here should not be overlooked: Their fine-scale resolution in a model genetic organism will greatly facilitate their routine molecular characterization. The 20 hybrid lethals and 20 hybrid semilethals discovered each reside in just a few cytological divisions. It is conceptually simple (although labor intensive) to move from identifying blocks of candidate genes using deficiency complementation tests to identifying the relevant genes using single-locus complementation tests. Establishing the molecular identity, function, and evolutionary history of a large collection of speciation genes is certain to reveal new patterns and to answer some long-standing questions in evolution: What are the normal functions of "speciation genes" within species? Are most functionally relevant substitutions concentrated in coding or regulatory regions? Is natural selection the primary force causing the fixation of divergent substitutions? Preliminary work in several of the hybrid-lethal regions identified here suggests that the molecular characterization of the genes responsible should be possible.

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APPENDIX: CALCULATION OF p

To obtain $2kt$, the total number of replacement changes separating *D. melanogaster* and *D. simulans*, I use Li's (1997) estimate of the rate of nonsynonymous substitution in Drosophila from 30 loci, 1.91×10^{-9}/site/year. To get the genome-wide rate of substitution, I take into account the number of relevant sites in the genome: 13,600 genes/genome $\times$ 1800 bp/gene $\times$ $\sim$2/3 are nonsynonymous sites $= 1.632 \times 10^7$. Multiplying gives the number of replacement changes per genome per year: $k = 0.0312$. Thus the total number of replacement changes that have accumulated between *D. melanogaster* and *D. simulans* during the last 2.5 MY is $2kt \approx 156{,}000$. Solving the equation gives $p \approx 1.6 \times 10^{-8}$.

We can check the plausibility of this estimate of p for hybrid lethality using data from another species pair. In particular, given p for hybrid lethality from above and the number of genome-wide substitutions separating a younger species pair, *D. mauritiana* and *D. simulans*, we can ask: How many hybrid lethals should we *expect* to find in a genetic analysis of *D. mauritiana-D. simulans* hybrids? (This assumes, of course, that p itself has not evolved substantially over the last few million years, as seems reasonable.) We already know the number of hybrid lethals between *D. mauritiana* and *D. simulans* to be $\sim$5–10 from the work of True *et al.* (1996) and so can compare it to the one predicted by the Orr-Turelli equation. To get the predicted number, we use $p = 1.6 \times 10^{-8}$ from above and the genome-wide number of nonsynonymous substitutions between *D. mauritiana* and *D. simulans*, $2kt = 48{,}000$ (see Orr and Turelli 2001). Solving for I, the predicted number of hybrid lethals is 18. Thus, given nothing more than p for hybrid lethality, as estimated from the *D. melanogaster-D. simulans* hybridization and a crude estimate of the number of substitutions, the equation comes remarkably close to predicting the true number of hybrid lethals.

The estimate of p for hybrid lethality is an order of

magnitude smaller than the one estimated for hybrid male sterility using genetic data from *D. simulans* and *D. mauritiana*, $p \approx 1.04 \times 10^{-7}$ (Orr and Turelli 2001). This discrepancy could be explained by either of the two putative causes of faster-male evolution (Wu and Davis 1993; Wu *et al.* 1996): (i) Spermatogenesis might be inherently more sensitive than viability to the incompatibilities experienced by hybrids, and thus a greater fraction of interspecific gene interactions cause hybrid male sterility; or (ii) our calculations have not taken into account that male-specific genes evolve faster, on average, than viability-essential genes (Begun *et al.* 2000; Swanson *et al.* 2001; Betancourt *et al.* 2002). As defined here and in Orr and Turelli (2001), p—the probability that any two divergent replacement changes (one from each species) from any gene in the genome are incompatible—ignores that not all genes interact with each other, that not all genes are mutable to sterility, that not all genes are mutable to lethality, that not all incompatibilities are protein-protein interactions, etc. In the absence of such perfect knowledge, it is convenient to estimate p as the genome-wide probability of incompatibility, averaging over all genes. But since male-specific genes evolve more rapidly than others (and, in particular, faster than the 30 loci used to estimate k from Li 1997 above), p for hybrid male sterility will be overestimated. Consequently, p for hybrid lethality and p for hybrid sterility are likely closer than they appear. The important point, however, is that both values are exceedingly small.