Dominance, Epistasis and the Genetics of Postzygotic Isolation

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ABSTRACT

The sterility and inviability of species hybrids can be explained by between-locus "Dobzhansky-Muller" incompatibilities: alleles that are fit on their "normal" genetic backgrounds sometimes lower fitness when brought together in hybrids. We present a model of two-locus incompatibilities that distinguishes among three types of hybrid interactions: those between heterozygous loci (H_0), those between a heterozygous and a homozygous (or hemizygous) locus (H_1), and those between homozygous loci (H_2). We predict the relative fitnesses of hybrid genotypes by calculating the expected numbers of each type of incompatibility. We use this model to study Haldane's rule and the large effect of *X* chromosomes on postzygotic isolation. We show that the severity of H_0 *vs.* H_1 incompatibilities is key to understanding Haldane's rule, while the severity of H_1 *vs.* H_2 incompatibilities must also be considered to explain large *X* effects. Large *X* effects are not inevitable in backcross analyses but rather—like Haldane's rule—may often reflect the recessivity of alleles causing postzygotic isolation. We also consider incompatibilities involving the *Y* (or *W*) chromosome and maternal effects. Such incompatibilities are common in Drosophila species crosses, and their consequences in male- *vs.* female-heterogametic taxa may explain the pattern of exceptions to Haldane's rule.

IN a landmark article, Dobzhansky (1936) reported the first thorough genetic analysis of any form of reproductive isolation: the sterility of F_1 male hybrids between *Drosophila pseudoobscura* and *D. persimilis*. Using stocks carrying visible mutations on each major chromosome arm, he backcrossed fertile F_1 females to both pure species and assessed the testis size (a proxy for fertility) of the resulting backcross males. He found that certain hybrid genotypes were consistently sterile, while others were consistently fertile. He posited that hybrid sterility and inviability often arise as a pleiotropic by-product of independent evolution in geographically separate lineages: alleles that enhance fitness on the "normal" genetic background may occasionally lower fitness when brought together in hybrids with alleles from another species. In this way, two taxa can become separated by an adaptive valley (corresponding to the unfit hybrids) without either lineage ever passing through such a valley. This resolves a paradox that plagued Darwin's (1859, Chap. 8) account of the origin of species: how can natural selection allow for the routine evolution of hybrid sterility and inviability, phenotypes that appear patently maladaptive?

This model of the evolution of postzygotic isolation was later elaborated by Dobzhansky (1937, p. 256) and Muller (1940, 1942), and it remains central to understanding speciation in allopatry. The model obviously focuses on developmentally mediated ("intrinsic") fitness loss in hybrids, not ecologically mediated selection against intermediate phenotypes (Rice and Hostert 1993). The so-called Dobzhansky-Muller model underlies many recent advances in our understanding of the evolution of postzygotic isolation (Orr 1995; Orr and Orr 1996; Gavrilets and Hastings 1996; Gavrilets 1997) and of the causes of Haldane's rule—the pattern that sex-specific hybrid problems typically afflict the heterogametic sex, a rule that holds across Drosophila, mammals, birds, and Lepidoptera, among other groups (Haldane 1922; reviewed by Laurie 1997; Orr 1997; Turelli 1998).

This article has two purposes. First, we hope to fill a vacuum in the theory of speciation. Although Dobzhansky's experimental approach has been followed in many studies of postzygotic isolation, there has been no attempt to predict the pattern of fitness differences seen across the hybrid genotypes produced. Are certain genotypes predictably less fit than others? Here, starting from the Dobzhansky-Muller model, we present a theoretical analysis of the expected fitness of different hybrid backcross and F_2 genotypes. This analysis differs from our previous ones (Orr 1993a; Turelli and Orr 1995) in several ways. For one, we consider types of incompatibilities that we previously ignored (*e.g.*, *X-X*, *X-Y*, and *Y*-autosomal, and maternal effects). For another, we consider classes of incompatibilities that appear only in backcross and F_2 hybrids, namely those between loci that

This article is dedicated to our pal King Coyne on the occasion of his fiftieth birthday.

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are *both* homozygous or hemizygous for incompatible alleles. As Muller (1940, pp. 204–205) recognized, such incompatibilities should have more severe effects than those acting in F_1 hybrids and will thus contribute to "hybrid breakdown," a form of postzygotic isolation that may often arise before F_1 problems.

This fuller analysis forces us to deal with several subtleties of the Dobzhansky-Muller model. Analysis of Dobzhansky-Muller interactions is more difficult than it first appears, as these interactions involve both dominance and epistasis: the severity of the interactions between two loci depends on the dominance of each incompatible allele. In addition, the dominance at one locus might well depend on the genotype at the other locus. In our previous work, we simplified our picture of Dobzhansky-Muller incompatibilities to render the analysis tractable and intuitive (*e.g.*, when studying Haldane's rule, we assigned all dominance effects to the *X*-linked locus involved in an *X*-autosomal incompatibility). Here we present a complete model of two-locus Dobzhansky-Muller incompatibilities.

We use this model to address the only two known patterns in the genetics of speciation: Haldane's rule and the "large *X* effect" (Coyne and Orr 1989a). Our previous conclusions about the central role of dominance in both patterns are supported but generalized. In particular, we show that a single dominance coefficient no longer suffices to characterize the hybrid incompatibilities experienced by backcross and F_2 genotypes.

Our second aim is to determine if the patterns of postzygotic isolation seen across hybrid genotypes in genetic studies of speciation are compatible with our model and to suggest new experimental tests.

GENETIC MODELS OF POSTZYGOTIC ISOLATION

We follow Dobzhansky (1937, p. 256) and Muller (1940, 1942) in assuming that the alleles causing inviability and sterility in hybrids have no such effects within lineages during their divergence from a common ancestor (see also Orr 1995; Orr and Orr 1996). For simplicity, we also assume that each species is fixed at all loci. Following Turelli and Orr (1995), we further assume that separate hybrid incompatibilities contribute additively to an underlying "breakdown score" that is translated into fitness by a monotone decreasing function, denoted *V*. (The additivity assumption is made for convenience and will be discussed below.) Hybrids become inviable/sterile when their breakdown score reaches a threshold value, *C*. Finally, we assume that loss of fitness in hybrids is caused by many incompatibilities between loci scattered throughout the genome. (Wu and Palopoli 1994 and Naveira and Maside 1998 review the evidence for this assumption.) This allows us to compare the expected breakdown scores of different genotypes.

Suppose that a hybrid genotype suffers n distinct incompatibilities and that the ith incompatibility contributes $e_i > 0$ to the breakdown score. (Note that specific loci may contribute to more than one incompatibility.) The fitness of this genotype is

$$W = V\left(\sum_{i=1}^{n} e_i\right), \tag{1}$$

where $W = 0$ if the breakdown score $S = \Sigma_{i=1}^{n} e_i \geq C$. Our analyses apply to taxa in which either males or females are the heterogametic (*XY* or *ZW*) sex. Because most relevant data come from Drosophila, however, we assume male heterogamety and note those occasions where female heterogamety alters our conclusions. Among individuals of identical genotype (*e.g.*, F_1 hybrid males), some are often fertile and others sterile (*e.g.*, Orr and Coyne 1989). Thus, whether any particular individual is fit or unfit must depend not only on its genotype but on environmental (including developmental) variation. It should be understood then, that given a hybrid breakdown score *S* and the mapping function *V*, we can predict only average fitness.

Formally, the assumption that many incompatibilities contribute to hybrid sterility/inviability is central to our analysis. Without loss of generality, we can assume that the critical threshold is $C = 1$. When a large number, n, of incompatibilities is required to reach this threshold, the effect of each must be roughly proportional to $1/n$. Hence, the variance of the breakdown scores must also be at most proportional to $1/n$ as the breakdown score is simply the sum of n random variables, each having mean on the order of $1/n$ and variance on the order of $1/n^2$. If we assume that n is large, we can approximate the fitness of a genotype by evaluating the function *V* in (1) at the expected breakdown score. This simplification is used throughout.

A model of two-locus incompatibilities: The central idea of our analysis is that hybrids can experience three different types of two-locus Dobzhansky-Muller incompatibilities, depending on whether the incompatible alleles at the interacting loci are homozygous or heterozygous. Imagine that one species' genotype is *AAbb* and the other *aaBB*, where *a* and *b* are incompatible in hybrids and *A* and *B* denote ancestral (and hence compatible) alleles. Further imagine that there are many such pairs of loci. We label the incompatibilities according to the number of loci that are homozygous for incompatible alleles. In an F_2 hybrid genotype, certain incompatibilities might be homozygous-homozygous (*e.g.*, *aabb*), which we denote H_2; each such incompatibility contributes, on average, h_2 to the hybrid breakdown score. Other incompatibilities might be homozygous-heterozygous (*aaBb* or *Aabb*), denoted H_1; each contributes, on average, h_1 to the breakdown score. All remaining incompatibilities are heterozygous-heterozygous (*AaBb*), denoted H_0; each contributes, on average, h_0 to the breakdown score. Note that any locus homozygous for

a compatible allele (*AA* or *BB*) cannot contribute to hybrid breakdown.

This last assumption merits comment. If the substitutions causing hybrid problems are driven by natural selection within species, one might expect that genotypes combining homozygous ancestral alleles at one locus with selectively favored alleles at another would enjoy enhanced fitness relative to the ancestral genotype, *AABB*. We assume, however, that any such effects are negligible compared with the deleterious Dobzhansky-Muller effects. Indeed these positive effects *must*, on average, be small compared to the Dobzhansky-Muller effects, or hybrids would not suffer large net fitness losses (relative to the parental means).

Initially, we consider autosomal-autosomal, *X*-autosomal, and *X*-*X* interactions, but ignore maternal effects and the *Y*. We also assume dosage compensation such that hemizygosity at *X*-linked loci is equivalent in fitness effect to homozygosity. (This assumption is not needed for our analysis of Haldane's rule, which centers on incompatibilities that either do or do not involve a hemizygous *X* chromosome; these correspond to H_1 *vs.* H_0 incompatibilities, irrespective of dosage compensation.) To compare the breakdown scores of different genotypes, we assume that there are *n* two-locus incompatibilities in a reference genotype that carries one set of autosomes and one *X* from each parental species, as in F_1 females. These incompatibilities are assumed to be randomly scattered throughout the genome. For normalization purposes, we use this reference genotype even when considering hybrid males.

To find the expected breakdown score for any particular hybrid genotype, we merely need to know the proportion of the genome that is homozygous (or hemizygous) from species 1 (p_1), the proportion that is homozygous (or hemizygous) from species 2 (p_2), and the proportion that is heterozygous for material from the two species (p_H, where $p_H = 1 - p_1 - p_2$). A simple combinatoric argument shows that, on average, a fraction $p_1p_2 + (p_1 + p_2)p_H + p_H^2$ of the *n* incompatibilities in the reference genotype appear in our hybrid genotype. In particular, our hybrid suffers an average of np_1p_2 H_2 incompatibilities, $n(p_1 + p_2)p_H$ H_1 incompatibilities, and np_H^2 H_0 incompatibilities. (These calculations take into account the fact that incompatibilities are generally "asymmetric"; *i.e.*, if introgression of allele A_1 from taxon 1 into taxon 2 lowers fitness, the reciprocal introgression of A_2 into taxon 1 does not, *e.g.*, Wu and Beckenbach 1983; Wu and Palopoli 1994, p. 292. Thus, although a fraction $2p_1p_2$ of all two-locus incompatibilities occur between loci in the portions of the genome included in p_1 and p_2, only *half* involve taxon 1 alleles in p_1 and taxon 2 alleles in p_2. We need not, however, discount the term p_H^2 by a factor of 2 as all potentially incompatible alleles from both species are present in heterozygous regions of the genome.) The expected breakdown score is thus

$$E(S) = n[p_1p_2h_2 + (p_1 + p_2)p_Hh_1 + p_H^2 h_0]. \quad (2)$$

This simple result forms the foundation of our analyses. We now use it to consider the two best known phenomena in the genetics of speciation: Haldane's rule and the large *X* effect.

Haldane's rule for inviability: The case of hybrid inviability is simpler than that of sterility as the same genes likely affect the viability of both hybrid males and females (Orr 1989a,b; Wu and Davis 1993). For F_1 females, all loci are heterozygous ($p_H = 1$), and (2) gives

$$E(S_f) = nh_0. \quad (3)$$

Let g_X be the fraction of all loci causing incompatibilities that are *X*-linked (*cf.* Turelli and Orr 1995; Turelli and Begun 1997). When *n* is large and the *X* and autosomes evolve at equal rates, we can estimate g_X as the fraction of the genome that is *X*-linked. Thus, in F_1 males, some proportion of the genome is *X*-linked (g_X) and the rest is autosomal. Because the *X* is the only material that is hemizygous, $p_1 + p_2 = g_X$, $p_1p_2 = 0$ (either p_1 or p_2 must be zero as the *X* in hybrid males derives from one species only), and $p_H = 1 - g_X$. Thus,

$$E(S_m) = n[g_X(1 - g_X)h_1 + (1 - g_X)^2h_0]. \quad (4)$$

In the limiting case in which none of the genome is *X*-linked ($g_X = 0$), (3) and (4) show that hybrid males and females have the same expected breakdown scores and Haldane's rule for inviability cannot arise (by this mechanism). As noted by Presgraves and Orr (1998), the case $g_X = 0$ arises in the genus Aedes in which the *X* and *Y* chromosomes are largely homologous and freely recombine. And, in fact, Haldane's rule for inviability does not occur in this genus.

More generally, (3) and (4) show that if $g_X > 0$, Haldane's rule is expected, *i.e.*, $E(S_m) > E(S_f)$, whenever

$$\frac{h_0}{h_1} < \frac{1 - g_X}{2 - g_X} = \frac{1}{2 + g_X/(1 - g_X)}. \quad (5)$$

Condition (5) allows for *X*-*X* incompatibilities that afflict only females and is, not surprisingly, somewhat more stringent than our previous condition, $h_0/h_1 < 1/2$ (or equivalently $d < 1/2$), which ignored *X*-*X* incompatibilities (Orr 1993a; Turelli and Orr 1995). For instance, for taxa such as *D. melanogaster* with $g_X \approx 0.2$, (5) requires $h_0/h_1 < 4/9$. As expected, (5) reduces to $h_0/h_1 < 1/2$ when *X*-*X* incompatibilities are ignored. Condition (5) also implies that whenever F_1 males show reduced viability, "unbalanced" F_1 females, who inherit both *X*'s from one species, should be less viable than normal F_1 females, a fact confirmed by experiment (Orr 1993b; Wu and Davis 1993; *cf.* Coyne 1985).

When there are unequal rates of evolution between the *X* and autosomes, g_X no longer equals the proportion of the genome that is *X*-linked. If, for instance, the *X* evolves faster than the autosomes, as suggested by Charlesworth *et al.* (1987), g_X will exceed the fraction

of the genome that is *X*-linked. The above results still hold, however.

Haldane's rule for sterility: There are good reasons for thinking that different loci will affect hybrid male *vs.* female fertility. First, mutagenesis experiments within *D. melanogaster* show that the overwhelming majority of steriles afflict one sex only, while most lethals affect both sexes (reviewed in Ashburner 1989). Second, backcross and introgression data show that chromosome regions that cause the sterility of one hybrid sex typically do not affect the other (Orr and Coyne 1989; Orr 1992; Hollocher and Wu 1996; True *et al.* 1996).

This opens the door to a possibility that does not arise with hybrid inviability: when different loci affect the two sexes, we have no guarantee that substitutions ultimately afflicting males will occur at the same rate as those afflicting females. Indeed there is evidence that alleles causing sterility in hybrid males accumulate faster than those causing sterility in females, at least in Drosophila and mosquitoes (Hollocher and Wu 1996; True *et al.* 1996; Presgraves and Orr 1998). Such "faster-male" evolution might well contribute to Haldane's rule for sterility (Orr 1989a; Wu and Davis 1993). We consider this effect below and show how dominance and faster-male effects can be taken into account simultaneously.

Incompatibilities affecting hybrid female *vs.* hybrid male sterility may differ in both number and average effects. Let n_f (n_m) denote the number of incompatibilities in our reference genotype that affect hybrid females (males). We assume that the average effects of H_0 and H_1 incompatibilities are h_0 and h_1 for females and $\tilde{h}_0$ and $\tilde{h}_1$ for males. Thus, the expected breakdown scores of F_1 females and males (from Equation 2) are

$$E(S_f) = n_f h_0 \tag{6a}$$

and

$$E(S_m) = n_m[g_X(1 - g_X)\tilde{h}_1 + (1 - g_X)^2\tilde{h}_0], \tag{6b}$$

where g_X denotes the fraction of all loci involved in male-sterilizing incompatibilities that are *X*-linked.

Under this model, Haldane's rule for sterility is expected [$E(S_m) > E(S_f)$] whenever

$$\frac{h_0}{h_1} < \left(\frac{n_m\tilde{h}_1}{n_f h_1}\right)\left((1 - g_X)^2\frac{\tilde{h}_0}{\tilde{h}_1} + g_X(1 - g_X)\right). \tag{7}$$

To simplify the notation, we let $\tau = n_m\tilde{h}_1/(n_f h_1)$ and assume that the ratio of effects for H_0 *vs.* H_1 incompatibilities is the same in females and males, *i.e.*, $h_0/h_1 = \tilde{h}_0/\tilde{h}_1 \equiv d_0$. Note that τ quantifies the relative cumulative effects of incompatibilities contributing to hybrid male *vs.* hybrid female sterility, taking into account both their numbers and average effects. Criterion (7) shows that both faster-male evolution, as measured by $\tau > 1$, and dominance, as measured by d_0, can contribute to Haldane's rule. We can express (7) either as a bound on τ or a bound on d_0, namely

$$\tau > \frac{d_0}{(1 - g_X)^2 d_0 + g_X(1 - g_X)} \tag{8a}$$

or

$$d_0 < \frac{\tau g_X(1 - g_X)}{1 - \tau(1 - g_X)^2}. \tag{8b}$$

As before, the constraint (8b) on the dominance parameter d_0 is somewhat more restrictive when *X-X* incompatibilities are considered than the analogous expression (B2) of Turelli and Orr (1995), which ignored *X-X* incompatibilities. If $\tau = 1$, condition (8b) reduces to (5), *i.e.*, the condition for inviability. When none of the genome is *X*-linked ($g_X = 0$) (8a) becomes $\tau > 1$. In this case, dominance cannot play a role in Haldane's rule and faster-male evolution is required.

In general, both faster-male evolution *and* dominance might contribute to Haldane's rule for sterility in male-heterogametic species. To assess their relative contributions, one can consider the ratio of male to female breakdown scores. This ratio, which must exceed 1 for Haldane's rule in male-heterogametic species, is

$$R = \tau(1 - g_X)\left(1 - g_X + \frac{g_X}{d_0}\right). \tag{9}$$

Ratio (9) reveals that Haldane's rule is promoted by faster-male evolution (large τ) *and* by greater recessivity of the factors causing hybrid sterility (small d_0). Both effects can be seen in Figure 1. If τ is sufficiently large, Haldane's rule will occur for any level of dominance. Conversely, in female-heterogametic species, *R* must be <1 to produce Haldane's rule; this is clearly made more

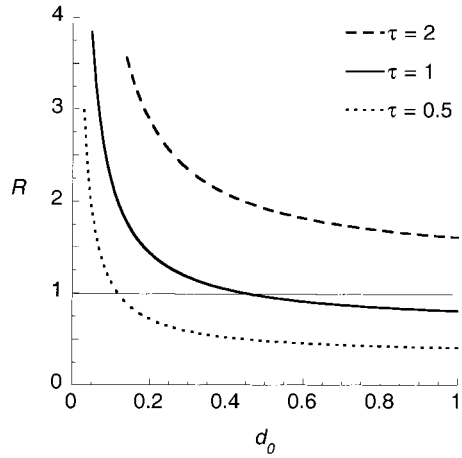

Figure 1.—Contributions to Haldane's rule from dominance and faster-male evolution. *R* is plotted from Equation 9. When $R > 1$, Haldane's rule is obeyed "on average." The calculations assume that 20% of the genome is *X*-linked ($g_X = 0.2$), as in *D. melanogaster*. The solid curve corresponds to the case of no faster-male evolution ($\tau = 1$), so that $d_0 < 4/9$ is required for Haldane's rule. The dashed curve corresponds to faster-male evolution in a male-heterogametic species ($\tau = 2$), and the dotted curve to faster-male evolution in a female-heterogametic species ($\tau = 0.5$).

difficult by faster-male evolution ($\tau > 1$). The resulting conditions correspond to $\tau < 1$ in a male-heterogametic species. As illustrated in Figure 1, extreme recessivity is required to produce Haldane's rule in this case.

Comparative analyses support the idea that faster-male evolution acts *against* Haldane's rule for sterility in female-heterogametic species: Haldane's rule is more common for sterility than for inviability in male-heterogametic species, while the reverse appears true in female-heterogametic species, as expected if faster-male evolution acts in both groups (Wu and Davis 1993; Turelli 1998). This pattern should not, however, obscure an even more striking one: Haldane's rule holds for 42 of 43 cases of unisexual hybrid sterility in birds and Lepidoptera (Laurie 1997); *i.e.*, *females* are overwhelmingly sterile in these taxa, despite presumed faster-male evolution. Put differently, when dominance and faster-male effects are pitted against one another, dominance predominates, yielding virtually no exceptions to Haldane's rule. This suggests two possibilities that are not mutually exclusive: H_0 incompatibilities have much smaller effects than H_1 incompatibilities; or, as discussed below, *Y*-associated incompatibilities play a large role in postzygotic isolation. We address another important aspect of faster-male evolution in the discussion.

Our analyses of Haldane's rule did not require us to consider homozygous-homozygous incompatibilities, which cannot appear in F_1 hybrids. Thus, our previous simple "dominance" analyses (Orr 1993a; Turelli and Orr 1995) sufficed to correctly identify the conditions for Haldane's rule. But as we now show, a more detailed model is required to understand the full consequences of Dobzhansky-Muller interactions.

The large *X* effect: As noted by Coyne and Orr (1989a), one of the most striking features of genetic studies of postzygotic isolation is that in backcrosses the *X* chromosome appears to have a disproportionately large effect on hybrid male fitness relative to comparably sized autosomes. There has, however, been a great deal of confusion about the biological significance, if any, of this so-called large *X* effect. Wu and Davis (1993) and Hollocher and Wu (1996) have argued that the effect is an artifact of backcross analysis: in any backcross, *X* substitutions in males replace a hemizygous *X* from one species with a hemizygous *X* from the other. Autosomal substitutions, on the other hand, replace a *single* autosome from one species with a single one from the other. Thus, they argue, hemizygous *X* substitutions should have larger effects on hybrid fitness than heterozygous autosomal ones. Orr (1997) and others, however, suggest that the large *X* effect may *not* be an inevitable artifact of backcross analysis but *evidence for* the partial recessivity of the genes causing postzygotic isolation. A quantitative treatment is clearly required to find the conditions under which large *X* effects are expected.

Consider a backcross analysis of male sterility in which fertile F_1 females are crossed to males from taxon 1. When assessing the effect of "foreign" loci, note that the introgressed segments of X_2 are necessarily hemizygous, while the introgressed autosomal segments are heterozygous. To quantify the *X* effect, we require an explicit comparison between *X*-linked and autosomal introgressions. Although discussions of the large *X* effect have been mostly qualitative, at least two quantitative criteria can be used. A large *X* effect might be declared if a hemizygous *X* introgression has (1) a greater effect than a heterozygous autosomal introgression that is *twice as large*; or (2) more than *twice the effect* of an equal-sized heterozygous autosomal introgression. The first criterion requires fewer assumptions, but the second (used by Coyne and Orr 1989a, among others) leads to similar results.

We first use criterion 1, comparing *X*-linked and autosomal introgressions from species 2 into a background homozygous for species 1. For the *X* introgression, let $p_2 = q$, where q is the fraction of the haploid genome that is introgressed, and $p_1 = 1 - q$. For the autosomal introgression, let $p_H = 2q$ (*i.e.*, it is twice as large as the *X* introgression) and $p_1 = 1 - 2q$. The expected breakdown scores are

$$E(S_X) \equiv E(S|p_2 = q, p_1 = 1 - q) = nq(1 - q)h_2 \quad (10a)$$

and

$$E(S_{auto}) \equiv E(S|p_H = 2q, p_1 = 1 - 2q) = n[(1 - 2q)2qh_1 + 4q^2h_0]. \quad (10b)$$

The *X* introgression has a greater effect when $E(S_X) > E(S_{auto})$, which requires $q[(1 - q)(h_2 - 2h_1) + 2q(h_1 - 2h_0)] > 0$ or

$$h_2 - 2h_1 + \frac{2q}{1 - q}(h_1 - 2h_0) > 0. \quad (11)$$

Our dominance-theory explanation of the large *X* effect (Turelli and Orr 1995) posited

$$\frac{h_0}{h_1} < \frac{1}{2}, \quad (12)$$

which constrains only the relative effects of H_0 and H_1 incompatibilities. But (11) shows that H_2 interactions are also important. The natural extension of our "dominance" constraint (12) is

$$\frac{h_1}{h_2} < \frac{1}{2}; \quad (13)$$

i.e., homozygous-homozygous incompatibilities have more than twice the deleterious effect of heterozygous-homozygous ones. The important point is that when dominance conditions (12) *and* (13) are met, so is (11).

The analysis above is idealized. Real backcross analyses involve comparing many genotypes, some of which

carry the "foreign" X (or portions of it) and some of which do not. We thus extend our analysis to more realistic situations in which the "background" for an introgression includes heterozygous material (*i.e.*, $p_H > 0$). In this case, we compare $E(S_X) = E(S|p_2 = q, p_H = r, p_1 = 1 - r - q)$ with $E(S_{auto}) = E(S|p_H = r + 2q, p_1 = 1 - r - 2q)$. Again, (12) and (13) suffice to give $E(S_X) > E(S_{auto})$. Thus, no matter what the rest of the hybrid genome looks like, introgressing part of the X will lower hybrid fitness more than introgressing twice as much autosomal material when (12) and (13) hold.

Criterion 2 is trickier to apply: while it is easy to compare the average breakdown scores of X *vs.* autosomal introgressions, we must actually compare the *fitness* effects of X *vs.* autosomal introgressions. But these are known only if we know the function V that maps breakdown score onto fitness. For simplicity, then, we assume that the fitness function is nearly linear over the relevant range of values, so that comparing breakdown scores themselves suffices. If fitness declines faster than linearly (*e.g.*, Kondrashov 1988; Charlesworth 1990), our conditions will be too restrictive.

We again first compare X-linked *vs.* autosomal introgressions from taxon 2 into a background homozygous for taxon 1. The breakdown score is initially zero in both cases, and we want to compare $E(S_X) = E(S|p_2 = q, p_1 = 1 - q)$ with $2E(S_{auto}) = 2E(S|p_H = q, p_1 = 1 - q)$. With (2), we see that $E(S_X) > 2E(S_{auto})$ if

$$h_2 - 2h_1 - \frac{2q}{1-q} h_0 > 0. \tag{14}$$

If we impose our standard "dominance" condition (12) on h_0/h_1, we see that (14) is satisfied when

$$\frac{h_1}{h_2} < \frac{1}{2 + q/(1-q)}. \tag{15}$$

For small introgressions ($q \approx 0$), this reduces to (13), $h_1/h_2 < 1/2$. For larger introgressions, it is more restrictive. But even if an X-linked introgression involves, say, 20% of the genome, (15) requires $h_1/h_2 < 4/9$ to produce a large X effect, which is only slightly more stringent than (13).

Next we consider introgressions into backgrounds in which some of the autosomal genome is already heterozygous. We want to compare $E(\Delta S_X) \equiv E(S|p_2 = q, p_H = r, p_1 = 1 - r - q) - E(S|p_H = r, p_1 = 1 - r)$ with $E(\Delta S_{auto}) \equiv E(S|p_H = r + q, p_1 = 1 - r - q) - E(S|p_H = r, p_1 = 1 - r)$. Using (2), we see that $E(\Delta S_X) > 2E(\Delta S_{auto})$ if

$$(h_2 - 2h_1)(1 - q - r) + 2r(h_1 - 2h_0) - 2h_0 q > 0. \tag{16}$$

For small introgressions ($q \approx 0$), conditions (12) and (13) are again sufficient. We assume that both (12) and (13) hold and ask what additional constraints on h_1/h_2 and h_0/h_1 are imposed by (16). In general, these depend both on the size of the introgressions, q, and on the extent to which the genetic background is heterozygous, r. Condition (13) implies that the left-hand side of (16) decreases as q increases. Thus, assuming $q \leq 0.2$, the most restrictive conditions on h_1/h_2 and h_0/h_1 occur with $q = 0.2$. By also considering the left-hand side of (16) as a function of r, one can show that (16) is always satisfied if

$$\frac{h_0}{h_1} < \frac{4}{9} \quad \text{and} \quad \frac{h_1}{h_2} < \frac{4}{9}. \tag{17}$$

Our key biological conclusion is that large X effects, by either criterion 1 or 2, are *not* automatic consequences of comparing hemizygous X with heterozygous autosomal substitutions. Instead, large X effects imply that the alleles causing hybrid sterility and inviability are fairly recessive. Indeed, large X effects are essentially guaranteed if condition (17) is met (in Drosophila). The more extreme the recessivity of the incompatible alleles, the more pronounced the large X effect. The "faster X" mechanism of Charlesworth *et al.* (1987) may also, of course, contribute to the large X effect.

Comparisons among other backcross genotypes: There are two different classes of backcross to consider, depending on whether one crosses F_1 hybrid males or females to a parental species. When F_1 males are backcrossed, the resulting progeny all inherit an X and a complete set of autosomes maternally. In this case, H_2 incompatibilities are impossible, and (2) reduces to

$$\begin{aligned} E(S) &= n[p_H(1 - p_H)h_1 + p_H^2 h_0] \\ &= nh_1[p_H(1 - p_H) + p_H^2 d_0], \end{aligned} \tag{18}$$

where p_H is the fraction of the genome that is heterozygous and $d_0 = h_0/h_1$. When these incompatibilities, rather than those involving the Y or maternal effects, dominate hybrid performance, the lowest fitness results when

$$p_H = \frac{1}{2(1 - d_0)}. \tag{19}$$

For $d_0 < \frac{1}{2}$, the right-hand side of (19) is between 0.5 and 1.0. Thus we predict that introgressions of "intermediate" size will have the most devastating effects on hybrid fitness.

When F_1 females are backcrossed, the resulting male progeny can inherit an X that is incompatible with the haploid set of autosomes inherited paternally. This can produce H_2 incompatibilities so that all three terms in (2) may be nonzero, precluding simple predictions analogous to (19).

Other incompatibilities affecting hybrids: Data from several well-known hybridizations have shown important effects of Y-X (*e.g.*, Orr 1987), Y-autosome (*e.g.*, Zouros *et al.* 1988; Heikkinen and Lumme 1991), and maternal-zygotic incompatibilities (Sawamura 1996; Hutter 1997). Indeed our review of the literature suggests that

TABLE 1

***Y* chromosome and/or maternal effects on postzygotic isolation in Drosophila**

Species pair	*Y* effect[a]	Maternal effect[b]	References[c]
melanogaster-simulans			
Inviability	—	Yes	1
Male sterility	Yes	No	2
simulans-mauritiana			
Male sterility	Yes	No	3
simulans-sechellia			
Male sterility	Yes	No	3
mojavensis-arizonae			
Male sterility	Yes	No	4
pseudoobscura-persimilis			
Male sterility	Yes	No	1
Female "sterility"	—	Yes	1
pseudoobscura Bogota-USA			
Male sterility	No	No[d]	1
buzzatii-koepferae			
Male sterility	Not known	No	1
Male inviability	—	No	1
subobscura-madierensis			
Male sterility	Yes	No	1
Female inviability	—	Yes	1
virilis-texana			
Male sterility	Yes	No	1
virilis-novamexicana			
Male sterility	Yes	No	5
virilis-lummei			
Male sterility	Yes	No	6
montana-texana			
Female inviability	—	Yes	1
hydei-neohydei			
Male sterility	Yes	No	1
Female sterility	—	No	1
Inviability	—	No	1

When tallying the results of species crosses (see text), hybridizations were counted as single events; *i.e.*, reciprocal crosses were not tallied separately. Different forms of isolation within a single species cross were, however, counted separately.

[a] *Y* effects were tallied only in cases of hybrid male sterility. "Yes" indicates that the *Y* chromosome is involved in at least one direction of the species cross, but not necessarily both. A dash indicates that the entry does not involve hybrid male sterility and so *Y* effects are not expected.

[b] "Yes" indicates that maternal effects are involved in at least one direction of the species cross. "No" indicates that no maternal effects are known given reasonably thorough genetic analysis; in many cases, however, no explicit test of maternal effects was performed.

[c] Most citations for species crosses are available in reference 1, Coyne and Orr's (1998) recent review (see their Table 1.1). Other references are as follows: 2, Muller and Pontecorvo (1942); 3, Johnson *et al.* (1993); 4, Zouros *et al.* (1988); 5, Heikkinen and Lumme (1998); and 6, Falileeva and Mitrofanov (1997).

[d] Contrary to Orr (1989b), this hybridization does not appear to involve maternally acting genes (H. A. Orr, unpublished results).

both *Y* and maternal effects on postzygotic isolation are extraordinarily common. We surveyed all known genetic analyses of both hybrid sterility and inviability in Drosophila; the results are shown in Table 1. We found that 10 out of 11 species crosses involving hybrid male sterility show *Y* chromosome effects in at least one direction of the cross. Similarly, 4 out of 18 hybridizations show maternal effects; excluding male sterility, this figure rises to 4 out of 7. Moreover, the observation of nonreciprocal hybrid female inviability—or even sterility—in Drosophila (Coyne and Orr 1989b) suggests *prima facie* that maternal effects on hybrid fitness are common, as reciprocal F_1 females have identical nuclear genotypes. [In the *D. melanogaster-D. simulans* and *D. montana-D. texana* hybridizations, for instance, females are lethal in one direction of the cross only. (These taxa belong to different subgenera, so their hybrid outcomes are phylogenetically independent.) Analysis of each has proven the role of maternal factors (reviewed in Wu and Davis 1993 and Sawamura *et al.* 1993).]

Given their frequent roles, it is important to incorporate *Y* and maternal effects into our analysis. This can be accomplished via Equation 1, by assuming that *Y* and maternal incompatibilities simply add to the total breakdown score, which we now denote S_T. Keeping with our earlier notation, we denote the cumulative breakdown score attributable to *X-X*, *X*-autosome, and autosome-autosome incompatibilities by *S*. Additional contributions from *Y*-associated and maternal-zygotic incompatibilities are denoted S_Y and S_{MZ}. We discuss each in turn.

Y-linked incompatibilities: We consider both male-heterogametic (*XY*) and female-heterogametic (*ZW*) taxa, but, for ease of discussion, refer to the sex-limited sex chromosome as the *Y* and its partner as the *X*. For simplicity, we focus on $E(S_Y)$. Our qualitative predictions do not require that the number of *Y*-associated incompatibilities is large. Indeed the "large *n*" assumption is clearly implausible for *Y*-associated incompatibilities, as (1) the *Y* carries few complementation groups, at least in *D. melanogaster* and *D. hydei* (Ashburner 1989, Chap. 20); and (2) in at least one hybridization (*D. mojavensis*-*D. arizonensis*), the *Y* interacts with a single autosome (Zouros *et al.* 1988). Because the *Y* has no essential somatic function, at least in *D. melanogaster* (Ashburner 1989), we might expect the *Y* to play a role in hybrid sterility but not inviability.

Because the *Y* is hemizygous, we treat the *Y*-linked partner in any incompatibility as effectively homozygous. We do not distinguish between *Y-X* and *Y*-autosome incompatibilities and assume that each occurs in proportion to the fraction of the genome that is *X*-linked *vs.* autosomal. Let n_Y denote the number of incompatibilities between *Y*-linked loci from taxon 1 and loci in a complete haploid set, including an *X*, from taxon 2. In F_1 and backcross genotypes, these incompatibilities can occur in two forms, depending on whether the non-*Y* partner is homozygous or heterozygous. Incompatibilities involving homozygous partners have average effect y_2, whereas those involving heterozygous partners have average effect y_1.

Because the *Y* is largely heterochromatic, we have no *a priori* basis for estimating the fraction of all incompatibilities that involve this chromosome and therefore no basis for drawing quantitative conclusions about S_Y *vs.* *S*. But, by considering $E(S_Y)$ alone, we can still draw interesting, albeit qualitative, conclusions. If the *Y* is inherited from taxon 1 and the source of the rest of the nuclear genome is described by the fractions p_1, p_2, and p_H, we get

$$E(S_Y) = E(S_Y|Y_1, p_1, p_2, p_H) = n_Y(p_2y_2 + p_Hy_1). \quad (20)$$

For F_1 males (or F_1 females in female-heterogametic species), we expect that $p_2 = g_X$ and $p_H = 1 - g_X$. Thus,

$$E(S_Y|\text{heterogametic } F_1) = n_Y[g_Xy_2 + (1 - g_X)y_1]. \quad (21)$$

Obviously, $S_Y = 0$ for the homogametic sex. Thus, *Y*-associated incompatibilities always afflict only the heterogametic sex and promote Haldane's rule (Muller 1942; White 1945, p. 225).

Y-associated incompatibilities also relax the constraints on the dominance, h_0/h_1, required for Haldane's rule for sterility, particularly in female-heterogametic species. For two reasons, this effect is likely to be especially important in taxa having small sex chromosomes. First, in the absence of *Y* effects, the upper bound on h_0/h_1 needed for Haldane's rule when faster-male evolution acts is proportional to g_X when g_X is small (see 8b). Thus, in female-heterogametic species with relatively small *X*'s (on the order of 10% of the genome or less), such as birds (Abbott and Yee 1975) and lepidoptera (Robinson 1971, Chap. VIII), extreme recessivity would ordinarily be required to obtain Haldane's rule. Second, even if rare, *Y*-linked incompatibilities involve the potentially stronger H_1 and H_2 incompatibilities, whereas interactions not associated with the *Y* involve H_0 and H_1 incompatibilities.

Given this expected disproportionate effect of the *Y* in small-*X* taxa, it is interesting to note that Haldane's rule for sterility shows only a *single* exception in Lepidoptera and birds (Laurie 1997)—despite the presumption of faster-male evolution, which opposes Haldane's rule, in these groups (Wu and Davis 1993; Turelli 1998). This suggests that the combination of dominance and *Y* chromosome effects much more than compensates for any faster-male evolution.

Next, we consider the role of the *Y* in the large *X* effect. Consider a study of F_1 male sterility in which F_1 females are backcrossed to taxon 1. As above, we can compare the values of S_Y produced by an *X*-linked introgression of size *q* *vs.* an autosomal introgression of size 2*q*. These are

$$E(S_Y|p_2 = q, p_1 = 1 - q) = n_Yqy_2 \quad (22a)$$

and

$$E(S_Y|p_H = 2q, p_1 = 1 - 2q) = n_Y2qy_1, \quad (22b)$$

respectively. Hence, an *X* introgression yields a larger *Y*-associated contribution to S_T if

$$\frac{y_1}{y_2} < \frac{1}{2}. \quad (23)$$

Note that if incompatibilities involving the *Y* are highly recessive (*i.e.*, $y_1/y_2 \ll \frac{1}{2}$), the relative effects of *Y*-autosomal interactions will be negligible unless the "foreign" autosomal segments are homozygous. Thus, our analysis suggests a bias toward finding *Y-X* incompatibilities *vs.* *Y*-autosomal. Indeed, in the best-known case of a *Y*-autosomal incompatibility—that between the *Y* of *D. arizonae* and a region of the fourth chromosome of *D. mojavensis*—flies that are *homozygous* for the incompatible autosomal segment are sterile whereas flies that are

heterozygous show normal fertility (Pantazidis *et al.* 1993). The *Y*-autosome incompatibility acting in *D. virilis* and *D. texana* hybrids is also detectable only when the autosomal partners are homozygous (Lamnissou *et al.* 1996).

Maternal-zygotic incompatibilities: Again we focus on $E(S_{MZ})$ but recognize that the data suggest that maternal incompatibilities may involve few factors. These incompatibilities arise from interactions between loci in two different diploid genomes—that of the mother and her offspring—and they may generally affect viability only as maternal control of development is surrendered fairly early in development (Sawamura 1996). Such interactions are not surprising given that, in Drosophila, many genes deposit maternal transcripts in the unfertilized egg, and the transfer from maternal to zygotic control of development is gradual (Lawrence 1992). Maternal effects are distinct from cytoplasmic effects involving interactions between nuclear genes and maternally inherited cytoplasmic factors, such as microbes and mitochondria. Although cytoplasmic effects, such as those associated with Wolbachia, may contribute to reproductive isolation between some taxa (Hoffmann and Turelli 1997; Werren 1997), they seem to be relatively unimportant in producing the sex-limited hybrid viability differences on which we focus (Hurst 1993).

Maternal-zygotic incompatibilities may occur in three forms depending on whether the incompatible alleles are homozygous or heterozygous. To distinguish these incompatibilities from those acting *within* the offspring genome, we denote them by M_0, M_1, and M_2. There may be a qualitative distinction between the two types of M_1 incompatibilities (depending on whether the mother or offspring is homozygous for an incompatible allele), but we assume they have equal average effects. We assume that the average effect of M_i incompatibilities is m_i for $i = 0, 1, 2$ and that there are n_{MZ} maternal-zygotic incompatibilities between a cytoplasm produced by a taxon 1 mother and her F_1 hybrid daughters. Obviously, these would all be M_1 incompatibilities involving homozygous maternal loci and heterozygous loci in the offspring. We assume that a fraction g_X of these incompatibilities involve loci on the zygotic *X*. With backcross analyses involving hybrid mothers, a wide range of incompatibilities can appear. We focus on only those that occur in hybrids with taxon 1 mothers.

If the offspring genome is characterized by the fractions p_1, p_2, and p_H, as before, the expected contribution to their breakdown score from maternal-zygotic interactions is

$$E(S_{MZ}|p_1, p_2, p_H) = n_{MZ}(m_2p_2 + m_1p_H). \quad (24)$$

This reveals a qualitative difference between the consequences of these incompatibilities in male-heterogametic *vs.* female-heterogametic taxa.

In male-heterogametic species, the only relevant difference between F_1 females and males is that females suffer from M_1 incompatibilities between the paternal *X* and the maternal cytoplasm, whereas males do not (Patterson and Stone 1952, pp. 435–436, 489; Wu and Davis 1993). This appears in the breakdown scores as

$$E(S_{MZ}|F_1 \text{ homogametic female}) = n_{MZ}m_1 \quad (25a)$$

and

$$E(S_{MZ}|F_1 \text{ heterogametic male}) = n_{MZ}(1 - g_X)m_1, \quad (25b)$$

since $p_H = 1$ in females, but $p_H = 1 - g_X$ and $p_1 = g_X$ in males. Thus females in male-heterogametic taxa suffer more from maternal incompatibilities than males; and incompatibilities involving the *X* can give rise to exceptions to Haldane's rule in these taxa. Indeed, Sawamura (1996) has argued that incompatibilities between maternally acting genes and the zygotic *X* can plausibly explain all known exceptions to Haldane's rule for viability in Drosophila. Our work suggests that these exceptions should be especially common in taxa with larger *X*'s.

In contrast, in female-heterogametic species, F_1 males have $p_H = 1$, but F_1 females have $p_H = 1 - g_X$ and $p_2 = g_X$. Thus,

$$E(S_{MZ}|F_1 \text{ heterogametic female}) = n_{MZ}[m_2g_X + m_1(1 - g_X)] \quad (26a)$$

and

$$E(S_{MZ}|F_1 \text{ homogametic male}) = n_{MZ}m_1. \quad (26b)$$

This shows that maternal-zygotic interactions *contribute to* Haldane's rule for inviability in female-heterogametic species—as the *heterogametic* sex gets its cytoplasm from one species and its *X* from another—whenever M_2 incompatibilities are more severe than M_1. It would be surprising if this very weak "dominance" constraint is not satisfied.

Thus, in female-heterogametic species, dominance and maternal effects act in concert to promote Haldane's rule, whereas they act in opposition in male-heterogametic species. Maternal effects may, therefore, explain both the prevalence of exceptions to Haldane's rule for viability in Drosophila (and many of these exceptions appear evolutionarily independent; Sawamura 1996) *and* the virtual absence of exceptions in birds and Lepidoptera (Laurie 1997).

Complex genetic interactions: We have focused on two-locus incompatibilities as they capture the essence of the Dobzhansky-Muller mechanism and are easily modeled. But hybrid inviability and sterility may be produced by more complex interactions involving three or more loci (*e.g.*, Carvajal *et al.* 1996). In the appendix, we present an alternative analysis based on three-locus interactions. Although the three-locus results are much more complex than those of our two-locus analysis, our central conclusion remains clear: dominance can ex-

plain both Haldane's rule and the large *X* effect if homozygosity for an incompatible allele has more than twice the effect of heterozygosity on the incompatibility score (see A5).

HYBRIDIZATION DATA

While the above theory makes several predictions, existing data do not yet allow critical tests. Our purpose here therefore is merely to show (1) what predictions can be made; and (2) how these predictions *can* be tested with hybrid backcross data.

Data from introgressions: A large body of introgression data supports our assumption that H_2 incompatibilities are more severe than H_1. True *et al.* (1996), for instance—in a study of 87 chromosome regions introgressed from *D. mauritiana* into *D. simulans*—found that many introgressions cause complete male or female sterility or inviability when homozygous. Although they made no quantitative measures of heterozygous fitness, the fact that introgressions proceeded through (obviously viable and fertile) heterozygous hybrids suggests that introgression heterozygotes were reasonably fit. (True *et al.* did not lose a large number of introgression lines, which would have indicated frequent severe heterozygous problems.) Similarly, Hollocher and Wu (1996), in a study of 18 second chromosome introgressions from *D. mauritiana* into *D. simulans*, report that none significantly reduces sterility or inviability when heterozygous, although ". . . individuals homozygous for these same regions show dramatic increases in both." More quantitative conclusions can be drawn from the *D. buzzatii-D. koepferae* (formerly *D. serido*) work of Naveira and collaborators.

D. buzzatii-D. koepferae: These species obey Haldane's rule for sterility. They have $N = 6$ chromosomes, with four autosomes and an *X*, all of roughly equal size, and a tiny sixth. Naveira and Fontdevila (1986) studied male fertility in a large set of *X* and heterozygous autosomal introgressions, using homology-dependent polytene pairing to assay the size of introgressions. Their chief result is that male fertility is a function of the size of heterozygous autosomal introgressions (Naveira and Maside 1998; see also Marín 1996). Heterozygous introgressions of up to 30% of a large autosome ($p_H \approx 0.3/5 = 0.06$) essentially never cause male sterility, whereas introgressions of >40% of a large autosome ($p_H \approx 0.4/5 = 0.08$) almost always do. These introgressions correspond to $p_1 = 1 - p_H$ in formula (2) and the expected breakdown scores are given by (18). These autosomal data imply that

$$n(0.056h_1 + 0.004h_0) < C \tag{27a}$$

and

$$n(0.073h_1 + 0.006h_0) > C. \tag{27b}$$

In contrast, Naveira and Fontdevila (1986) found that *X*-linked introgressions of as little as 4% of the *X* always produce male sterility (corresponding to $p_1 \approx 0.04/5 = 0.008$ and $p_2 = 1 - p_1 \approx 0.992$). Indeed, they argue that introgressions as small as 1% of the *X* cause sterility (*i.e.*, $p_1 \approx 0.002$). These hemizygous introgressions cause all H_2 incompatibilities, implying that

$$n(0.002)h_2 > C. \tag{28}$$

Combining (27a) and (28), we get $(0.056)h_1 < (0.002)h_2$ or

$$\frac{h_1}{h_2} < 0.036. \tag{29}$$

This suggests a level of recessivity for hybrid steriles comparable to that of within-species lethals in *D. melanogaster* (Crow 1993), although the biochemical bases of recessivity may well differ within and between species. The more conservative result that introgressing 4% of the *X* suffices to produce male sterility implies $h_1/h_2 < 0.14$. These quantitative results are obviously consistent with our qualitative interpretation of True *et al.*'s and Hollocher and Wu's introgression data. It should be noted, of course, that Naveira and Fontdevila's results might be complicated by faster-*X* evolution: we cannot rule out the possibility that a larger number of hybrid male steriles have evolved on the *X* than comparable-sized autosomal regions.

H_0 vs. H_1 incompatibilities: We have less direct evidence about the magnitudes of h_0 and h_1. However, Coyne *et al.* (1998) recently found several regions from *D. simulans* that—when made hemizygous with *D. melanogaster* deficiencies—cause temperature-dependent inviability of otherwise heterozygous *D. melanogaster-D. simulans* hybrid females. Though few such regions were found, their existence shows they individually satisfy $h_0 \ll h_1$.

Hybrid backcross analyses: We now turn to traditional backcross/F_2 analyses. Our approach is statistical: we pool *all* hybrid backcross or F_2 genotypes that have the same p_1, p_2, and p_H. Consider, for instance, a backcross between two Drosophila species possessing five roughly equal-sized chromosome arms, one of which is the *X*. We pool all data obtained when a single autosomal arm is introgressed, or two autosomal arms are introgressed, etc. We appreciate that experiments repeatedly show that particular chromosomes have large effects on backcross fitness while others do not, but our primary goal is not to explain the detailed outcomes of *particular* species crosses but to search for statistical regularities.

In a species cross, any of the incompatibilities discussed above might act. While we can make some predictions about the relative roles of *X*-autosomal *vs. X-X* incompatibilities, we have no theory allowing us to predict how often, for instance, *Y*-linked or maternally acting genes might contribute to—or even dominate—postzygotic isolation. Thus we concentrate on cases in which *Y* and maternal effects are absent or small.

We make one further simplification. The above theory requires that we know p_1, p_2, and p_H. Unfortunately,

TABLE 2

Data from Zouros *et al.* (1988) on the fraction of *D. mojavensis-D. arizonensis* hybrid backcross males with immotile sperm and theoretical expectations

	Data		Expected breakdown scores	
p_H	Fraction (N)	95% C.I.	Two-loci: $E(S)/nh_1$[a]	Three-loci: $E(S)/nh_2$[b]
0	0.10 (10)	(0.002, 0.45)	0	0
0.2	0.25 (24)	(0.10, 0.47)	$0.16 + 0.04d_0$	$0.064 + 0.048d_1 + 0.008d_0d_1$
0.4	0.93 (29)	(0.77, 0.99)	$0.24 + 0.16d_0$	$0.072 + 0.144d_1 + 0.064d_0d_1$
0.6	1.0 (22)	0.87[c]	$0.24 + 0.36d_0$	$0.048 + 0.216d_1 + 0.216d_0d_1$
0.8	1.0 (6)	0.61[c]	$0.16 + 0.64d_0$	$0.016 + 0.192d_1 + 0.512d_0d_1$

C.I., confidence interval.
[a] See (18), $d_0 = h_0/h_1$.
[b] These follow from (A1); $d_1 = h_1/h_2$ and $d_0 = h_0/h_1$.
[c] 95% lower bound.

most backcross studies in Drosophila involve taxa obeying Haldane's rule, so that backcrosses must proceed through F_1 females. Because females recombine, markers remain associated with only some (inexactly known) chromosome region. We do not, therefore, know p_1, p_2, and p_H. This difficulty does not arise when backcrosses are performed through F_1 males, as there is no recombination in Drosophila males. Thus we consider only backcrosses that proceed through F_1 males. We know of two relevant studies: *D. mojavensis-D. arizonae* (formerly *arizonensis*) and *D. hydei-D. neohydei*. We focus on the first for purposes of illustration.

D. mojavensis-D. arizonae: Backcross hybrid males are sterile if they have a *Y* from *D. arizonae* and are homozygous for the fourth chromosome of *D. mojavensis.* These species have five chromosomes of roughly equal size (including the *X*), and a dot sixth chromosome that we will ignore. To avoid the complications of *Y*-linked incompatibilities, we focus on the backcross of F_1 males (from *D. mojavensis* mothers) to *D. arizonae* females. Table 2 of Zouros *et al.* (1988) reports sperm motility for these males, all of whom carry an *X* and *Y* from *D. arizonae.* In their Figure 1, Zouros *et al.* (1988) pool their data into five classes, roughly corresponding to the fraction of the genome that is heterozygous (and ignoring the identity of the heterozygous chromosomes). Their categories correspond to $p_H = 0$, 0.2, 0.4, 0.6, and 0.8. For all of these males, $p_1 = 1 - p_H$, so the expected breakdown scores are given by (18). Using their pooled data, we constructed exact 95% confidence intervals, based on the binomial distribution, for the fraction of males with immotile sperm. For two of the five classes ($p_H = 0.6$, 0.8), all of the males have immotile sperm. In these cases, we constructed an exact 95% lower bound.

The data are given in Table 2 along with the predicted breakdown scores from our two-locus analysis, Equation 18, and the three-locus analysis from the appendix. Because of small samples, there is only one statistically significant jump between adjacent fractions in the table—that from $p_H = 0.2$ (6/24) to $p_H = 0.4$ (27/29) ($P < 10^{-5}$).

It is worth noting that our large *n* assumption likely does not hold here. This is suggested by several lines of evidence. First, $X_mY_a\,A_mA_a$ F_1 males (where *A* denotes a haploid set of autosomes) are fertile, whereas $X_aY_m\,A_aA_m$ males are all sterile. As both F_1 males have the same expected breakdown scores, this difference reveals heterogeneity in the numbers of incompatibilities between "replicate" *X* chromosomes. Second, Zouros and collaborators have shown that hybrid male sterility is caused by *X*-autosome and autosome-autosome incompatibilities, not *Y* effects. But the data in Table 2 of Zouros *et al.* (1988) show statistically significant heterogeneity among the individual chromosomal classes combined in the $p_H = 0.2$ class—a heterogeneity that is inconsistent with large *n*.

Despite this, it seems worth asking if the pooled data in the first column of Table 2 agree with our predictions (given in the last two columns), where we assume that higher expected breakdown scores will be associated with greater sperm immotility.

First consider the two-locus predictions. From (18), as p_H increases, the expected breakdown score, $E(S)$, rises to a maximum of $nh_1/[4(1 - d_0)]$ at $p_H = 1/[2(1 - d_0)]$ (see Equation 19), then falls to $nh_1(0.16 + 0.64d_0)$ at $p_H = 0.8$. Because we ignore *Y* effects, the expected breakdown score for males with $p_H = 0.8$ in Table 2 is the same as for the sterile $X_aY_m\,A_aA_m$ F_1 males. Indeed, both *are* sterile, as they suffer the same incompatibilities. Note that if $d_0 < 2/7$, $E(S)$ for $p_H = 0.6$ is larger than $E(S)$ for $p_H = 0.8$. As expected, the $p_H = 0.6$ males are also all sterile. The lack of statistical power precludes more detailed tests, but the data suggest the kind of inferences possible. For instance, if the difference between the fractions of males with motile sperm for $p_H = 0.4$ and $p_H = 0.8$ were statistically significant, we could conclude that the corresponding breakdown scores must satisfy $0.24 + 0.16d_0 < 0.16 + 0.64d_0$, so that $d_0 > \frac{1}{6}$.

Now consider the three-locus predictions. The most

interesting difference between the two- and three-locus predictions is that the latter implies that the largest breakdown score occurs for *smaller* p_H than in the two-locus case. This can be seen in the breakdown scores for $p_H = 0.2$ *vs.* $p_H = 0.8$. In the two-locus model, $E(S)$ is always larger for $p_H = 0.8$. In contrast, assuming that $d_0 = d_1 = d$, the three-locus model implies that $E(S|p_H = 0.8) < E(S|p_H = 0.2)$ if $d < 0.197$. Thus the observation that males from this cross with $p_H = 0.8$ are less fit than those with $p_H = 0.2$ suggests that most of the *D. mojavensis/arizonae* incompatibilities act more like two- than three-locus ones (or that these incompatibilities are less recessive than suggested by our inferences from other data).

DISCUSSION

Our understanding of speciation has been characterized by several steps in which large but nebulous problems have been reduced to smaller but sharper ones. During the modern synthesis, for instance, "the origin of species" was largely reduced to "the origin of reproductive isolation" (Dobzhansky 1937; Mayr 1942). Over the last decade, it has become clear that the origin of developmentally mediated (though not ecologically mediated; see Hatfield and Schluter 1999) postzygotic reproductive isolation can often be reduced to the origin of Dobzhansky-Muller incompatibilities (Hutter *et al.* 1990; Orr 1995, 1997; Hutter 1997). A clear understanding of speciation thus requires a clear understanding of Dobzhansky-Muller incompatibilities.

Fortunately, the Dobzhansky-Muller mechanism is simple enough that it can be captured in mathematical models. Here we have presented a complete model of two-locus Dobzhansky-Muller interactions. To simplify our analysis, we assumed that the number of incompatibilities is large and that individual incompatibilities contribute linearly to a "breakdown score," such that higher scores lead to lower fitness (see Equation 2). Either assumption may be incorrect and more general models could be constructed. Increased generality would, however, lead to more ambiguous predictions, dependent on a proliferation of parameters whose values are unknown. We have also assumed that dosage compensation renders the effects of hemizygotes equivalent to those of homozygotes. This assumption is irrelevant to our analysis of Haldane's rule, as F_1 individuals do not experience H_2 incompatibilities and all of their H_1 incompatibilities involve the hemizygous *X* (or *Z*) chromosome. Our assumption is, however, critical to our analyses of backcrosses. Fortunately, essentially all of the relevant data come from Drosophila in which dosage compensation occurs. For taxa like birds and lepidoptera, in which dosage compensation appears absent (Chandra 1994; Suzuki *et al.* 1999), one could add parameters that distinguish hemizygous from homozygous interactions. At present, however, this does not seem worthwhile as we have no data with which to estimate the required parameters.

We have used our model to address several questions in the genetics of speciation, including Haldane's rule and the large *X* effect.

Haldane's rule: Table 3 summarizes the forces hypothesized to contribute to Haldane's rule. We indicate whether each might act for hybrid sterility and/or inviability and in male and/or female heterogametic taxa. We first consider dominance alone.

Roughly speaking, our analysis shows that Haldane's rule arises if the factors causing postzygotic isolation act as partial recessives, *i.e.*, if H_1 incompatibilities are somewhat more than twice as severe as H_0 ones. This condition emerges if either the same genes affect males and females or if male and female incompatibilities evolve at the same rate.

Fortunately, we possess data allowing rough inferences about the magnitude of h_0/h_1. First, in two Drosophila hybridizations, "normal" F_1 females (who suffer H_0 incompatibilities only) are viable, while "unbalanced" females carrying an attached-*X* stock (and who thus suffer some H_1 incompatibilities) are inviable (Orr 1993b; Wu and Davis 1993). Similarly, Presgraves and Orr (1998) showed that mosquitoes that lack a hemizygous *X* (and so suffer H_0 incompatibilities only) do not show Haldane's rule for viability, while other mosquitoes

TABLE 3

Factors expected to contribute to (+), oppose (−), or have no effect on (0) Haldane's rule

	Male heterogametic		Female heterogametic	
Factors	Inviability	Sterility	Inviability	Sterility
Dominance	+	+	+	+
Faster-male evolution	0	+	0	−
Y-associated incompatibilities	0	+	0	+
Maternal-zygotic incompatibilities	−	0	+	0

The *Y* has no essential somatic function. It is not expected, therefore, to play a role in hybrid inviability (hence the "0" entries). Similarly, maternal gene effects cease early in development. They are not expected, therefore, to play a regular role in a "late" phenotype like adult fertility (hence the "0" entries).

that possess a hemizygous *X* (and thus suffer H_1 as well as H_0 incompatibilities) *do* show Haldane's rule for inviability. Together, these findings suggest that h_0/h_1 is small, at least for inviability. Additional indirect support comes from a comparative analysis of the time course of increasing postzygotic isolation between pairs of Drosophila with "small" *vs.* "large" *X* chromosomes (corresponding to roughly 20% *vs.* 40% of the genome). As expected if h_0/h_1 is small, Haldane's rule occurs at a smaller average genetic distance between large-*X* than small-*X* pairs (Turelli and Begun 1997).

With hybrid sterility, our analysis is more complex, as different loci appear to affect males *vs.* females, allowing for the possibility that male- and female-expressed genes evolve at different rates (Orr 1989a; Wu and Davis 1993). If so, the conditions under which Haldane's rule arises are set both by dominance and by any difference in the numbers/effects of incompatibilities affecting male *vs.* female fertility (see Figure 1). As expected, faster-male evolution (Wu and Davis 1993) always promotes Haldane's rule in male-heterogametic species but acts against it in female-heterogametic ones. Greater recessivity, on the other hand, *always* facilitates Haldane's rule.

There is now considerable evidence that faster-male evolution occurs for genes causing postzygotic isolation (reviewed in Wu *et al.* 1996; Laurie 1997; Orr 1997; Turelli 1998). Nonetheless, our analysis—together with several other lines of evidence—suggests that the *extent* of faster-male evolution may have been overestimated. There are four reasons for thinking this. First, in taxa in which faster-male evolution and dominance are opposed—the former working against Haldane's rule and the latter for it—comparative work shows nearly perfect conformity to Haldane's rule. In birds and Lepidoptera, Laurie (1997) showed that 42 of 43 species crosses obeyed Haldane's rule for sterility, while Wu and Davis's (1993) review found *no* exceptions; *i.e.*, *females* are nearly universally sterile despite phenotypic evidence for faster-male evolution based on sexual selection. This suggests that faster-male evolution is moderate enough to be overcome by dominance and *Y*-associated incompatibilities.

Second, the relative *rates* of accumulation of male-sterilizing *vs.* female-sterilizing substitutions can be easily overestimated from the observed excess of hybrid male over female steriles. Hollocher and Wu (1996), for instance, found that male steriles were 4-fold more common than female steriles in *D. sechellia-D. simulans* hybrids and 23-fold more common in *D. mauritiana-D. simulans*. While these ratios are subject to large error—True *et al.* (1996), for instance, found a 9-fold (not 23-fold) excess in *D. mauritiana-D. simulans*—it would seem safe to conclude that hybrid male steriles are, say, six times more numerous than female. It does *not* follow, however, that male-expressed genes evolve six times faster than female-expressed genes.

The reason is simple. If there have been K_f substitutions at female-expressed genes and K_m at male genes and all incompatibilities involve pairs of loci, the expected numbers of hybrid female *vs.* male incompatibilities are

$$n_f = \binom{K_f}{2} p \approx \frac{K_f^2}{2} p \quad \text{and} \quad n_m = \binom{K_m}{2} p \approx \frac{K_m^2}{2} p, \tag{30}$$

where p is the probability that two diverged genes are incompatible (Orr 1995). For the sake of simplicity, we assume this probability is the same in males and females. Thus, $n_m/n_f \approx (K_m/K_f)^2$ and the ratio of male-to-female substitution rates is

$$R_K = \frac{K_m}{K_f} \approx \sqrt{\frac{n_m}{n_f}}. \tag{31}$$

Thus, if genetic analysis suggests $n_m/n_f \approx 6$, the ratio of *substitution* rates is only $R_K \approx 2.4$. Moreover, this figure must be an overestimate as we have assumed that all incompatibilities result from two-locus interactions. If, instead, incompatibilities are due exclusively to three-locus interactions, the rate of male-to-female evolution would be roughly the cube root of n_m/n_f, which is yet smaller ($\approx$1.8). The point is that—if male incompatibilities are more common than female *and* complex incompatibilities are common—the ratio of male-to-female substitution rates must be much smaller than the observed ratio of male-to-female incompatibilities. This reflects the fact that Dobzhansky-Muller incompatibilities "snowball," accumulating at least as fast as the square of the substitution rate (Orr 1995).

Third, the fact that autosomal regions cause male sterility more often than female sterility when introgressed into a foreign species does not necessarily reflect faster evolution of autosomal male-expressed than female-expressed genes. The reason is that introgressions confront different genetic backgrounds in males and females. Autosomal introgressions confront a "foreign" *Y* chromosome in males but not females. To the extent that the *Y* plays an important role in hybrid male sterility—and our review of the literature strongly suggests it does—one would expect more male than female sterility in introgression experiments even if male and female autosomal genes evolve at the *same* rate.

Last, while proponents of faster-male evolution often cite the fact that male reproductive tract proteins evolve faster than nonreproductive ones, recent studies indicate that *both* male and female reproductive tissues evolve at high rates. Indeed, Civetta and Singh (1995) could not reject the null hypothesis of no difference between the rates of divergence of testis and ovary proteins between Drosophila species (male:female = 1.07), although both sets of proteins evolve significantly faster than those in nonreproductive tissues.

We are not suggesting that faster-male evolution for hybrid sterility does not occur. It almost certainly does

[it is hard to see how else one could explain the fact that taxa lacking a hemizygous *X* obey Haldane's rule for sterility (Presgraves and Orr 1998)]. We merely suggest that its extent may have been overestimated.

Our extensions to our basic model—incorporating *Y* and maternal effects—also have important bearings on understanding Haldane's rule. The consequences of *Y*-linked incompatibilities are simple: *Y* effects always promote Haldane's rule in both male- and female-heterogametic species. Moreover, *Y* effects may have disproportionately greater effects in taxa, like birds and Lepidoptera, that have relatively small *X* chromosomes. Our review of genetic analyses of postzygotic isolation in Drosophila leaves no doubt that *Y* effects are very common, at least for male sterility. The consequences of maternal effects are more subtle: maternal-zygotic incompatibilities contribute to Haldane's rule in female-heterogametic species (as the *XY* sex gets its cytoplasm from one species and its *X* from another) but work against Haldane's rule in male-heterogametic species (as the *XX* sex gets its cytoplasm from one species and one *X* from another). This asymmetry suggests that maternal effects might explain both the prevalence of exceptions (for viability) in Drosophila (Sawamura 1996) *and* the near absence of exceptions to Haldane's rule in birds and Lepidoptera (Laurie 1997).

The "faster *X*" hypothesis of Charlesworth *et al.* (1987) is not included as a separate factor in Table 3, as it represents, in effect, an increase in the size of the *X* relative to the autosomes and cannot by itself explain Haldane's rule (Orr 1997). An increase in the "effective size" of the *X* merely places slightly more stringent bounds on the dominance coefficient needed to obtain Haldane's rule for inviability (see Equations 5 and 8b). In contrast, a larger *X* promotes Haldane's rule for sterility via an increase in the prevalence of *X*-*Y* interactions (21). Finally, a larger *X* boosts the role of maternal-effect-*X* interactions in producing exceptions to Haldane's rule for viability in male heterogametic species (25), but facilitates Haldane's rule for viability in female-heterogametic species (26). Faster-*X* evolution may also, of course, supplement dominance as a force contributing to the large *X* effect.

Large *X* effect: Our analysis shows that large *X* effects are *not* an inevitable consequence of backcross analysis. Substitution of a hemizygous *X* does not invariably lower hybrid fitness more than twice as much as substitution of a similarly-sized heterozygous autosome. Instead, large *X* effects arise if the genes causing postzygotic isolation are fairly recessive. Here "recessive" refers to two comparisons. Because backcross hybrid males suffer from H_0, H_1, *and* H_2 incompatibilities, the ratios h_0/h_1 and h_1/h_2 are both relevant. This highlights the shortcomings of previous attempts, including ours, to understand the implications of dominance in Dobzhansky-Muller incompatibilities. Because such interactions involve both dominance and epistasis, no single dominance parameter fully captures the behavior of hybrid lethals/steriles. While this did not affect our earlier analyses of Haldane's rule—as F_1 hybrids cannot suffer H_2 incompatibilities—it plays a key role in backcross hybrids and thus in discussions of the large *X* effect.

As noted previously, we have qualitative information about h_1/h_2 from the introgression experiments of Hollocher and Wu (1996) and True *et al.* (1996). Similarly, Orr (1992) showed that flies that are otherwise pure *D. melanogaster* and that are heterozygous for the dot fourth chromosome from *D. simulans* are essentially perfectly fertile (an H_1 incompatibility), while those homozygous for the *D. simulans* fourth are completely male sterile (H_2). Similarly, work in the haplodiploid wasp *Nasonia vitripennis* shows that backcross females (who are diploid and suffer only H_0 and H_1 incompatibilities) are much more fit than backcross males (who are haploid and, hence, suffer *only* H_2 incompatibilities between their hemizygous loci; Breeuwer and Werren 1995). Our analysis also shows that some information about dominance can be extracted from traditional backcross analyses. In particular, our reexamination of backcross data from *D. mojavensis vs. D. arizonae* shows that they are at least qualitatively consistent with $h_1 < h_2$. The data from *D. buzzatii vs. D. koepferae* suggest that h_1/h_2 may be quite small.

Evidence bearing on the dominance theory may also emerge from quantitative trait loci (QTL) studies performed for quite different reasons. Interspecific QTL analyses often uncover distorted segregation ratios at marker loci (*e.g.*, Paterson *et al.* 1991; Bernacchi and Tanksley 1997). Though often referred to as "segregation distortion," these biases likely reflect the inviability of hybrid genotypes, not meiotic drive: certain combinations of chromosome regions from two species cause partial inviability, distorting marker ratios from Mendelian expectations. Obviously, such data can be used to detect and map hybrid lethals. Less obviously, QTL data can provide information on the dominance of such factors. Bernacchi and Tanksley (1997), for example, have noted a pattern characterizing QTL studies of plants: "segregation distortion" appears more often in F_2 analyses (where H_2 incompatibilities arise) than in backcross analyses (where H_2 incompatibilities do not arise in most plants as they typically lack sex chromosomes). Indeed Bernacchi and Tanksley conclude that "[t]his difference may result from increased manifestation of deleterious and subdeleterious allelic combinations in the F_2 populations, possibly associated with recessive epistatic factors." These QTL data also suggest that hybrid lethals are fairly recessive in *plants*, where we lack much direct data. (It is, however, already clear that plants often suffer Dobzhansky-Muller incompatibilities, *e.g.*, Christie and Macnair 1984.) Additional support comes from the common observation that F_2 hybrids often exhibit lower average fitness than F_1 hy-

brids, as expected from the formation of H_2 incompatibilities.

The weight of the evidence suggests, then, that both h_0/h_1 and h_1/h_2 are fairly small. If so, the dominance theory provides a powerful explanation of Haldane's rule and the large *X* effect for both inviability and sterility in all species having heteromorphic sex chromosomes.

In sum, we believe our analysis sheds considerable light on the genetics of speciation by postzygotic isolation. Our model's one obvious merit is that it is firmly grounded on the known genetic mechanism underlying postzygotic isolation—Dobzhansky-Muller incompatibilities. Such a model would seem, therefore, to provide a more solid basis for future work than the abstract theories and verbal speculations that all too often characterized discussions of speciation.

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APPENDIX: THREE-LOCUS INCOMPATIBILITIES

The text focuses on the simple case of two-locus epistatic incompatibilities; but hybrid dysfunction may be caused by more complex interactions involving three or more loci. To explore the robustness of our conclusions, we consider three-locus interactions here. We denote the interacting loci *A*, *B*, and *C* and use subscripts to indicate species identity.

Three interacting loci produce five distinct types of incompatible genotypes, all of which are assumed to consist of two alleles from one taxon that are incompatible with a third allele from the second taxon. We again assume incompatibility effects are asymmetric, in that if allele A_1 contributes to an incompatibility when introgressed into taxon 2, the reciprocal introgression of allele A_2 into taxon 1 has no deleterious effect. The five types of incompatibilities are labeled according to the number of loci that are homozygous for alleles involved in the incompatibility, with the exception that there are two distinct types of incompatibilities involving two homozygous loci; these are distinguished by using H_2 *vs.* $H_{1,1}$. The types of incompatibilities are

H_0, all three loci heterozygous: $A_1A_2B_1B_2C_1C_2$;
H_1, one locus homozygous: *e.g.*, $A_1A_2B_1B_2C_1C_1$;
H_2, two loci homozygous for alleles from one taxon: *e.g.*, $A_1A_2B_1B_1C_1C_1$;
$H_{1,1}$, two loci homozygous for alleles from different taxa: *e.g.*, $A_1A_1B_1B_2C_2C_2$; and
H_3, all three loci homozygous: *e.g.*, $A_2A_2B_1B_1C_1C_1$.

Let h_i denote the average effect of H_i incompatibilities for $i = 0, 1, 2, 3$; and let $h_{1,1}$ denote the average effect of $H_{1,1}$ incompatibilities.

Again let n denote the number of three-locus incompatibilities that occur in a reference genotype containing one *X* chromosome and one complete set of autosomes from each of the hybridizing taxa. When calculating the average number of these incompatibilities that will afflict any specific hybrid genotype, it is important to note that there are two types of three-locus incompatibilities: those in which two alleles from taxon 1 interact negatively with an allele from taxon 2 and those in which an allele from taxon 1 interacts negatively with two alleles from taxon 2. More complex interactions (involving reciprocal effects of both alleles at a locus) are expected to occur infrequently and will be ignored. Among the n incompatibilities, on average half should fall into each of the two types.

To find the expected breakdown score for any particu-

lar genotype of F_1, backcross, or F_2 hybrid, we need to know the proportion of the genome that is homozygous (or hemizygous) from species 1 (p_1), the proportion homozygous from species 2 (p_2), and the proportion heterozygous for material from the two species (p_H, where $p_H = 1 - p_1 - p_2$). The expected breakdown score is

$$E(S) = n\Big[p_H^3 h_0 + \frac{3}{2}p_H^2(p_1 + p_2)h_1 + \frac{1}{2}p_H(p_1^2 + p_2^2)h_2 + 2p_1p_2p_Hh_{1,1} + \frac{1}{2}(p_1^2p_2 + p_1p_2^2)h_3\Big]. \quad \text{(A1)}$$

The logic of the derivation will be illustrated by considering the term proportional to $p_1^2p_2$. Let I_1 denote the portion of the genome characterized by p_1 and let I_2 denote the portion characterized by p_2. Of the n three-locus incompatibilities in the reference genotype, a fraction $3p_1^2p_2$ will, on average, involve two loci in I_1 and one locus in I_2. Of these, on average, half will involve two alleles from taxon 1 and half will involve two alleles from taxon 2. By definition, two of the alleles in the incompatibilities under consideration must reside in I_1. Since I_1 is inherited from taxon 1, none of the second class of incompatibilities—involving two alleles from taxon 2—can contribute to the $p_1^2p_2$ term. Of the first class of incompatibilities—involving two alleles from taxon 1—only one-third will have both taxon 1 alleles in I_1. Thus, on average, only one-sixth of the $3np_1^2p_2$ three-locus incompatibilities involving two loci from I_1 and one from I_2 will occur in these hybrids, and all of them will be H_3 incompatibilities. Each term in (A1) can be derived similarly. We now use (A1) to determine conditions for Haldane's rule.

Haldane's rule for inviability: For F_1 females, all loci are heterozygous ($p_H = 1$), and thus

$$E(S_f) = nh_0. \quad \text{(A2)}$$

In F_1 males, $p_1 + p_2 = g_X$, the fraction of the genome that is *X*-linked, $p_1p_2 = 0$, and $p_H = 1 - g_X$; so

$$E(S_m) = n\Big[(1 - g_X)^3h_0 + \frac{3}{2}(1 - g_X)^2g_Xh_1 + \frac{1}{2}(1 - g_X)g_X^2h_2\Big]. \quad \text{(A3)}$$

Thus Haldane's rule occurs on average [*i.e.*, $E(S_m) > E(S_f)$] whenever

$$\frac{h_0}{h_1} < \left[\frac{1}{2} - \frac{g_X}{6}\left(3 - \frac{h_2}{h_1}\right)\right] \Big/ \left[1 + \frac{1}{3}\left(\frac{g_X^2}{1 - g_X}\right)\right]. \quad \text{(A4)}$$

When g_X is very small, this reduces to $h_0/h_1 < \frac{1}{2}$. In general, the bound on h_0/h_1 depends on h_2/h_1. When $h_1/h_2 \leq \frac{1}{2}$, it suffices to have

$$\frac{h_0}{h_1} < \left[\frac{1}{2} - \frac{g_X}{6}\right] \Big/ \left[1 + \frac{1}{3}\left(\frac{g_X^2}{1 - g_X}\right)\right]. \quad \text{(A5)}$$

For Drosophila species with ~20% of the genome *X*-linked, (A5) yields $h_0/h_1 < 0.46$, whereas our two-locus criterion (5) yields $h_0/h_1 < 0.44$.

We can obtain conditions for Haldane's rule for sterility and the large *X* effect by straightforward extensions of the arguments presented in the text.