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Sex chromosomes and speciation in *Drosophila*

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Abstract

Two empirical rules suggest that sex chromosomes play a special role in speciation. The first is Haldane's rule— the preferential sterility and inviability of species hybrids of the heterogametic (XY) sex. The second is the disproportionately large effect of the X chromosome in genetic analyses of hybrid sterility. Whereas the causes of Haldane's rule are well established, the causes of the 'large X-effect' have remained controversial. New genetic analyses in *Drosophila* confirm that the X is a hotspot for hybrid male sterility factors, providing a proximate explanation for the large X-effect. Several other new findings— on faster X evolution, X chromosome meiotic drive, and the regulation of the X chromosome in the male-germline— provide plausible evolutionary explanations for the large X-effect.

The two rules of speciation revisited

Speciation— the process by which new biological species arise— corresponds to the evolution of reproductive barriers that limit the potential for genetic exchange between populations [1, 2]. For geographically isolated populations, reproductive barriers evolve as incidental by-products of genetic divergence. Eventually, 'good species' come to be completely isolated by one or more reproductive barriers that take the form of, *e.g.*, incompatible courtship signals that prevent mating (prezygotic isolation) or incompatible gene interactions that cause the sterility or lethality of species hybrids (postzygotic isolation; Box 1). The past decade has seen good progress in the molecular characterization of the 'speciation genes' involved in reproductive barriers. In particular, the recent identification of genes causing intrinsic postzygotic isolation has begun to provide important information about the functions of these genes within species and on the population genetic forces that shape their evolutionary history [3,4]. I will not review the details of particular speciation genes here as they have been amply discussed elsewhere [3,4]. Instead, this review will focus on new developments that concern an older but still controversial problem— the special role of sex chromosomes in the evolution of postzygotic reproductive isolation between animal species.

The idea that sex chromosomes play a special role in speciation is based on two empirical rules that characterize speciation in animals: Haldane's rule and the so-called 'large X-effect' [5,6]. Haldane's rule refers to the preferential sterility or inviability of species hybrids of the heterogametic (XY) sex: in crosses between many recently diverged species, XY hybrids are often sterile or inviable whereas their XX siblings are not [7]. In cases of unisexual hybrid sterility or inviability, Haldane's rule holds in 95% (n = 131) and 100% (n = 26) of species crosses in *Drosophila* and mammals, respectively, in which males are the XY sex, and in 97% (n = 87) and 96% (n = 114) of species crosses in birds and butterflies, respectively, in which

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females are the XY (or ZW) sex [1,8,9]. Thus, Haldane's rule depends not on sex per se but on sex chromosomes. The second, and related, rule is the large X-effect— the disproportionately large effect of the X chromosome versus autosomes in backcross genetic analyses of hybrid sterility and inviability (also known as “Coyne's rule” [10]; Figure 1). Evidence for large X-effects comes from a wide range of taxa including mouse, *Drosophila*, birds and Lepidoptera (*i.e.*, moths and butterflies) [5,6].

It can safely be argued that modern speciation genetics was launched by attempts to explain these two rules (*e.g.*, [11]) and doing so remains an important task as the existence of these rules suggests that generalities underlie the evolutionary and genetic basis of speciation in all animals [5,6]. Although most speciation geneticists agree on the causes of Haldane's rule (Box 2), the causes and significance of the large X-effect have been questioned— so much so that the large X-effect is now rarely discussed, even by its original proponents [1]. However, several recent developments suggest that the time is right to reconsider the large X-effect. Here, I review new findings bearing on its existence, its significance, and its possible causes in *Drosophila*, the source of most of the new data. In particular, I review new genetic analyses that show that hybrid male sterility genes are conspicuously concentrated on the X chromosome, and I consider three models that might explain why.

Significance of the large X-effect

Coyne and colleagues first called attention to the importance of the X chromosome in speciation [5,6,11,12]. Although the evidence for large X-effects in backcross analyses has never been disputed, its meaning has. On the one hand, the large X-effect could signify something special about sex chromosomes during speciation, *e.g.*, that the X chromosome is a hotspot for speciation genes. On the other, the large X-effect could be a trivial consequence of the general recessivity of speciation genes, signifying nothing about the X per se [13,14]. As Wu and Davis [14] correctly note, backcross analyses provide an unfair contrast between hemizygous X effects and heterozygous autosomal efects (Figure 1): recessive speciation genes on the X are fully expressed in backcross hybrid males whereas those on autosomes are largely masked. Thus, because any X vs. autosome difference in the density of speciation genes is confounded by dominance, the large X-effect might tell us more about the limits of backcross analyses than about the X chromosome.

To separate the effects of density versus dominance, high-resolution chromosomal mapping data are necessary in which the effects of dominance are controlled. Over the last 15 years, high-resolution mapping data have accumulated steadily as backcross analyses have given way to fine-scale introgression studies (Figure 2). In these analyses, small chromosomal segments are moved from one species into the genetic background of another by repeated backcrossing, made homozygous and then tested for hybrid fertility and viability effects (Figure 2). Introgression analyses therefore contrast the fitness effects of hemizygous X-linked segments with homozygous autosomal segments [14]. In some studies, the sizes of chromosomal segments have been determined using either cytological [15] or molecular markers. The results from early analyses between *Drosophila* species were mixed. Whereas some studies found evidence consistent with a higher density of hybrid male sterility genes on the X chromosome [15-19], others did not [13]. Part of the difficulty in settling this seemingly straightforward question is that confounding technical artifacts could not always be excluded (*e.g.*, previous studies could not exclude possible X vs. autosome differences in introgression size or publication bias; reviewed in [16,20]). To date, only one study has phenotyped and estimated the sizes of a large collection of X-linked and autosomal introgressions in a single experiment. In a genetic analysis between two island endemic species that began diverging ~400,000 years ago [21], Masly and Presgraves [16] assayed the hybrid fitness effects of 142 introgressions from *D. mauritiana* in an otherwise *D. sechellia* genome. The data show that the X chromosome

harbors roughly four times as many hybrid male sterility factors as an average, comparably sized, autosomal arm (Figure 3). These results confirm previous analyses [15,18,19] involving other *Drosophila* species pairs and provide strong evidence that the X chromosome harbors a higher density of hybrid male sterility factors than the autosomes.

It is important to be clear about what these findings say about the large X-effect. First, while the new findings show that the large X-effect is not a trivial methodological consequence of dominance, they do not exclude a role for dominance. Both dominance and the higher density of hybrid male sterility factors might contribute to the large X-effect. Importantly, however, theory shows that as the rate at which hybrid male steriles accumulate on the X increases, dominance quickly becomes unimportant [22,23]. Second, the large X-effect was originally believed to apply to all forms of intrinsic postzygotic isolation— hybrid sterility in the XY sex, hybrid sterility in the XX sex, and hybrid inviability [5,6]. Despite earlier claims, evidence for large X-effects for hybrid inviability or for hybrid sterility in the XX sex is meager. Until more evidence accrues for other forms of isolation, the large X-effect— as a "rule of speciation"— is best limited to the sterility of the XY sex [22,23]. The newer analyses relate only to hybrid male sterility— and to the XY sex in *Drosophila*, in particular. Fine-scale genetic analyses in non-*Drosophila* species are needed to determine if the large X-effects seen in other taxa (*e.g.*, mammals, birds, Lepidoptera) also reflect a higher density of X-linked hybrid sterility factors.

Evolutionary causes of the large X-effect for hybrid male sterility

The genetics of speciation now faces the challenge of determining why the X chromosome accumulates hybrid male sterility factors so rapidly. The simplest explanation— that the large X-effect reflects a greater concentration of fertility genes on the X— can be ruled out immediately. Classical mutagenesis studies show that loci mutable to male sterility are randomly distributed throughout the genome [24], and whole-genome expression analyses show that genes with male-biased expression are, if anything, under-represented on the *Drosophila* X chromosome [25,26]. At least three other kinds of evolutionary explanations for the large X-effect remain, and important developments bearing on the plausibility of each have appeared recently.

Faster X evolution

There are several reasons why X-linked loci might evolve faster than autosomal loci [27]. X-linked and autosomal loci can differ in effective population size, mutation rate, and the efficacy of natural selection. In one of the original attempts to explain the large X-effect, Charlesworth et al. [12] showed that X-linked loci will evolve faster than autosomal loci so long as newly arising beneficial mutations are, on average, partially recessive (see also [6]). (Note here that the dominance of favorable mutations within species says nothing about the dominance of their incidentally deleterious side effects in hybrids.) Although the expected signature of 'faster X evolution' is clear, empirical tests in *Drosophila* have proven to be frustratingly inconclusive. As Table 1 shows, different analyses have arrived at different conclusions [28-38; J. Parsch, pers. comm.].

Genome-wide analyses of lineage-specific evolution— DNA sequence evolution in seven *Drosophila* lineages [28,32,34-36] and protein evolution across 12 *Drosophila* lineages [35] — together suggest weakly elevated substitution rates on the X chromosome in some lineages but not in others. It is not clear to what extent this lineage-specific faster X evolution reflects X vs. autosome differences in mutation rate or in the efficacy of natural selection [28,35]. Statistical tests that distinguish mutation and selection by combining data on DNA polymorphism and divergence do not detect an excess of genes with histories of recurrent adaptive evolution on the X chromosome relative to the autosomes in *D. melanogaster* or in

D. simulans [28,30]. However, a new analysis that accounts for sex-specific gene expression finds that genes with male-biased expression patterns experience moderately higher rates of adaptive protein evolution when X-linked (J. Parsch, pers. comm.). This is a potentially important finding as nearly every speciation gene identified to date shows individually significant evidence of recurrent adaptive evolution [39-42; but see 43]. However, given the paucity of genes with male-biased expression on the X [25,26], it seems unlikely that their moderately higher rate of adaptive evolution can, by itself, explain the 2.5- to 4-fold excess of hybrid male sterility genes on the X [16,18].

Sex ratio meiotic drive

A second class of explanations for the large X-effect involves recurrent bouts of evolutionary conflict [44,45]. Mendelian transmission through the male germline is often subverted by selfish meiotic drive (or segregation distorter) elements that kill or incapacitate sperm bearing allelic rivals so that drive element-bearing sperm are preferentially transmitted. Because drive elements impose direct fitness costs on their targets and indirect fertility costs on their bearers, they often elicit the evolution of unlinked suppressors. Coevolutionary arms races between drivers and their suppressors can, in principle, cause the evolution of hybrid male sterility in several ways. For instance, different species can become fixed for different driver-suppressor systems that, in hybrids, could be unleashed (if, *e.g.*, suppressors are incompletely dominant) causing mutual sperm destruction. In addition, drive elements could show aberrant expression in hybrids, triggering meiotic or spermatogenic checkpoint arrests that result in sterility. Last, evolutionary conflict between drivers and suppressors could cause the rapid, lineage-specific evolution of sperm-related genes, giving rise to hybrid incompatibilities that disrupt spermatogenesis.

Two recent studies provide evidence that genes involved in drive or suppression within species can contribute to hybrid male sterility. Tao and colleagues [46] showed that the *D. mauritiana* allele of the autosomal gene *too much yin (tmy)*, when introgressed into an otherwise *D. simulans* genome, unleashes a cryptic driver on the *D. simulans* X chromosome; in combination with other genes, *tmy* also contributes to hybrid male sterility. Similarly, Orr and Irving [47] discovered X-chromosome drive in the very weakly fertile F_1 hybrid males of two young subspecies, *D. pseudoobscura bogatana* and *D. p. pseudoobscura*. Subsequent genetic analyses show that the factors causing hybrid male sterility and hybrid drive co-localize and have the same patterns of complex epistasis, strongly suggesting that the same genes are involved [47,48]. It seems clear, then, that the genes involved in cryptic meiotic drive and suppression can contribute to hybrid male sterility.

For recurrent drive and suppression to explain the large X-effect, two things must be true. First, species must harbor multiple fixed, but otherwise cryptic, drive elements. The failure to unmask cryptic drive in species hybrids is one reason why the drive hypothesis was initially discarded [49-51]. However, it now appears that the choice of genotypes and species in these early surveys was simply unlucky as multiple *Drosophila* species are now known to carry cryptic drive systems, and some carry more than one. *D. simulans*, for instance, harbors at least three cryptic X-chromosome drive systems, each involving different suppressor loci [46,52-54]. Whether this situation is typical remains unclear, however, as some species subject to similarly intense genetic scrutiny have been shown to harbor cryptic drive (one in *D. pseudoobscura bogatana*; [47]) whereas others have not (*e.g., D. melanogaster* and *D. sechellia*; reviewed in [55]).

Second, to explain the large X-effect, drive elements must preferentially accumulate on the X chromosome. Population genetic theory shows that, under most conditions, X- and Y-linked drive elements can invade populations more readily than autosomal elements [45]. Consistent with the theory, X-linked drive has been identified in at least a dozen *Drosophila* species

[56], whereas autosomal drive has been observed just once. Unfortunately, this discrepancy could reflect a clear observational bias: sex chromosome drive distorts sex ratios whereas autosomal drive, unless associated by chance with a genetic marker, is usually undetectable [56,57]. Other complications exist. For drive elements to contribute to speciation they must not only invade; they also must become fixed. Although X-linked drivers enjoy better probabilities of invasion than autosomal drivers, there is good reason to believe that the reverse is true for probabilities of fixation [55-57]. In addition, unlinked suppressors of X-linked drive can accumulate on the Y or on the autosomes. If X-linked drivers are typically silenced by 3 or 4 autosomal suppressors then, assuming a *Drosophila*-like karyotype, there would be little or no preferential accumulation of divergence on the X chromosome: for every X-linked driver fixed, each autosomal arm, on average, would fix a suppressor.

The idea that meiotic drive contributes to *Drosophila* speciation seems increasingly plausible. However it remains to be seen if the fixation of cryptic drive elements on the X chromosome — as observed in *D. simulans* and in *D. pseudoobscura bogatana*— is sufficiently common to explain Haldane's rule and the large X-effect.

Regulation of the X chromosome in the male germline

The third class of explanation for the large X-effect involves the regulation of the X chromosome in the male germline. During the early stages of spermatogenesis in male heterogametic taxa, the X chromosome undergoes transcriptional inactivation and chromosomal condensation before the autosomes (reviewed in [58]). (The normally inactive W chromosome of female heterogametic species [*i.e.*, ZZ males and ZW females] becomes active during oogenesis [59].) In a classic paper, Lifschytz and Lindsley [60] argued that X chromosome inactivation is a critical stage of spermatogenesis that, if disrupted, causes male sterility. As evidence, they cited cytological observations and showed that 75% (85/110) of reciprocal X;autosome translocations in *D. melanogaster* cause dominant male sterility whereas autosome;autosome and Y;autosome translocations do not (females remain fertile regardless of translocation type). Although compelling, their genetic data provide only indirect evidence, leading some to question the existence of X inactivation in *Drosophila* [26,61-63]. (Spermatogenic X inactivation is well-established in marsupials, eutherian mammals, grasshoppers and nematode worms [64-67].) However, new evidence from *D. melanogaster* should settle the matter. Hense et al. [68] generated 47 transgenic lines, each harboring a construct comprising the *lacZ* reporter driven by the testis-specific promoter of an autosomal gene, *ocnus*. The 27 autosomal insertions behaved as expected, producing robust testis-specific expression; the 20 X-linked insertions, however, showed dramatically reduced expression. The uniformly low expression of X-linked insertions— regardless of their position on the X chromosome— supports the idea of chromosome-wide transcriptional inactivation of the X during spermatogenesis.

Given the ubiquity of X inactivation in male heterogametic species, Lifschytz and Lindsley [60] suggested that its possible disruption in species hybrids could contribute to Haldane's rule [69,70]. Their original argument can be extended to the high density of hybrid male sterility factors on the X chromosome: if ‘foreign’ X-linked introgressions are recognized as ‘non-X’ by the X inactivation machinery, they might mimic X;autosome translocations thereby causing sterility [16]. Moreover, if X inactivation is disrupted in hybrids, then X-linked genes that are normally silenced during spermatogenesis should show aberrant over-expression. Interestingly, this is the pattern that has emerged from recent surveys of gene misexpression in the testis, but not the whole bodies, of *Drosophila* species hybrids [71,72]. Only a small fraction, ~9%, of autosomal genes that are misexpressed in hybrid testis are overexpressed (the remaining 91% are underexpressed). Consistent with the idea of disrupted X chromosome

inactivation, the X chromosome is significantly enriched for genes that are overexpressed in hybrid testis (34-55%) [72].

The other difference in the regulation of the X and the autosomes in the *Drosophila* male germline involves dosage compensation. In somatic cells, the X chromosome is hypertranscribed so that the ratio of transcripts from X-linked and autosomal genes in males roughly equals that in females [73]. But, like X inactivation, the existence of dosage compensation in the *Drosophila* male germline has been uncertain [63]. Using whole-genome microarrays, Gupta et al. [61] have now shown that dosage compensation occurs in the male germline of *D. melanogaster*. However, somatic and germline dosage compensation appear to occur via distinct mechanisms in *Drosophila* as only one of the five dosage compensation complex (DCC) proteins, *maleless*, is present in male germline cells where, unlike in somatic cells, it shows no association with the X chromosome [63].

Spermatogenic X inactivation and dosage compensation provide two processes that distinguish the X chromosome from autosomes and male from female fertility. Both processes require that the X chromosome is recognized— probably via *cis*-acting X chromosome-specific sequence motifs— and appropriately regulated by RNA and/or protein complexes. If the molecular basis for recognition and regulation diverges between species, then incompatibilities disrupting either process could cause hybrid male sterility that maps disproportionately to the X chromosome. Interestingly, recent findings show that four of the five protein-coding genes of the somatic DCC have histories of recurrent adaptive evolution in the *D. melanogaster* lineage [74,75]. It will be important to determine if the genes involved in germline dosage compensation have a similar history. For the moment, the overexpression of X-linked genes in hybrid testis suggests that, if germline dosage compensation is disrupted, it does not cause the loss of X chromosome hypertranscription [72]. Clearly, further experimental work is needed to determine the molecular bases of spermatogenic X inactivation and male germline dosage compensation, to test if either is disrupted in hybrids and, if so, to determine why.

Concluding remarks

The findings reviewed here leave little doubt that the X chromosome plays a special role in speciation. In *Drosophila*, the generally recessive behavior of hybrid male sterility factors and their exceptionally high density on the X chromosome provide proximate genetic explanations for the large X-effect and, in turn, for Haldane's rule. Speciation genetics must now determine why hybrid male sterility evolves so rapidly on the X chromosome. The answer is important not just for explaining the large X-effect but also for what it says about evolution within species. For example, is the recurrent fixation of selfish sex ratio meiotic drive systems a regular feature of the evolutionary history of species with sex chromosomes? If X inactivation or germline dosage compensation is disrupted, then we must ask why the molecular bases of these processes should diverge between species so quickly? The answers could come from straightforward experiments that assay X inactivation and germline dosage compensation in species hybrids. Or the answers might have to wait for the considerably harder task of identifying a large sample of the DNA sequences that cause hybrid male sterility. Like Haldane's rule (Box 2), it is entirely possible that the large X-effect will be a composite phenomenon with multiple genetic and evolutionary causes.

Box 1. The evolution and genetics of intrinsic postzygotic isolation

The evolution of hybrid sterility and inviability corresponds to the gradual accumulation of incompatible genetic interactions between species. Dobzhansky [2] and Muller [76] first noted that geographically isolated— and hence independently evolving— populations inevitably fix new genetic variants that function well in one genetic background but not in the other. To understand why, consider two populations with the same two-locus genotype,

aabb. As the populations diverge, mutation *A* might arise and become fixed in the first population (yielding *AAbb*), and *B* might arise and become fixed in the second (yielding *aaBB*). Importantly, as these substitutions occur, neither population passes through a sterile or inviable intermediate genotype. However, during their evolutionary history, the *A* and *B* alleles never occur in the same genetic background and, consequently, their interaction is never exposed to natural selection. If the *A* and *B* alleles are by chance functionally incompatible, disrupting hybrid gametogenesis or development, then hybrids might well be sterile or inviable. The Dobzhansky-Muller model thus shows how divergence between populations can incidentally give rise to epistatic hybrid incompatibilities that cause reproductive isolation.

Box 2. The causes of Haldane's rule

Haldane's rule— the preferential sterility or inviability of hybrids of the XY sex— has multiple causes [14,20,77]. First, the dominance theory posits that the alleles causing hybrid sterility and inviability typically behave as recessives. Thus, XY hybrids suffer disproportionately because they experience the full effects of any X-linked recessive hybrid sterility or inviability factors whereas their XX siblings are mostly protected. Several genetic analyses provide evidence that recessive incompatibility alleles accumulate faster between species than dominant alleles [16,19]. Second, the faster-male theory posits that incompatibility factors causing hybrid male sterility accumulate much faster than those causing other hybrid fitness problems [14]. Faster-male evolution will therefore contribute to Haldane's rule in species with XY males. Genetic analyses confirm that hybrid male sterility genes accumulate much faster than other kinds of incompatibilities [13,16,18,19], but the reasons are not entirely clear. One possibility is that sexual selection drives the rapid divergence of male-specific fertility factors, giving rise to male-specific incompatibilities. Consistent with this idea, *Drosophila* genes with male-biased expression patterns show faster rates of DNA sequence evolution and gene expression divergence [78]. It is too soon, however, to exclude the second possibility that spermatogenesis is particularly sensitive to genetic perturbations and is thus easily disrupted in hybrids [14,60]. Third, growing evidence makes it increasingly plausible that repeated bouts of sex chromosome meiotic drive and suppression could contribute to Haldane's rule [44-48].

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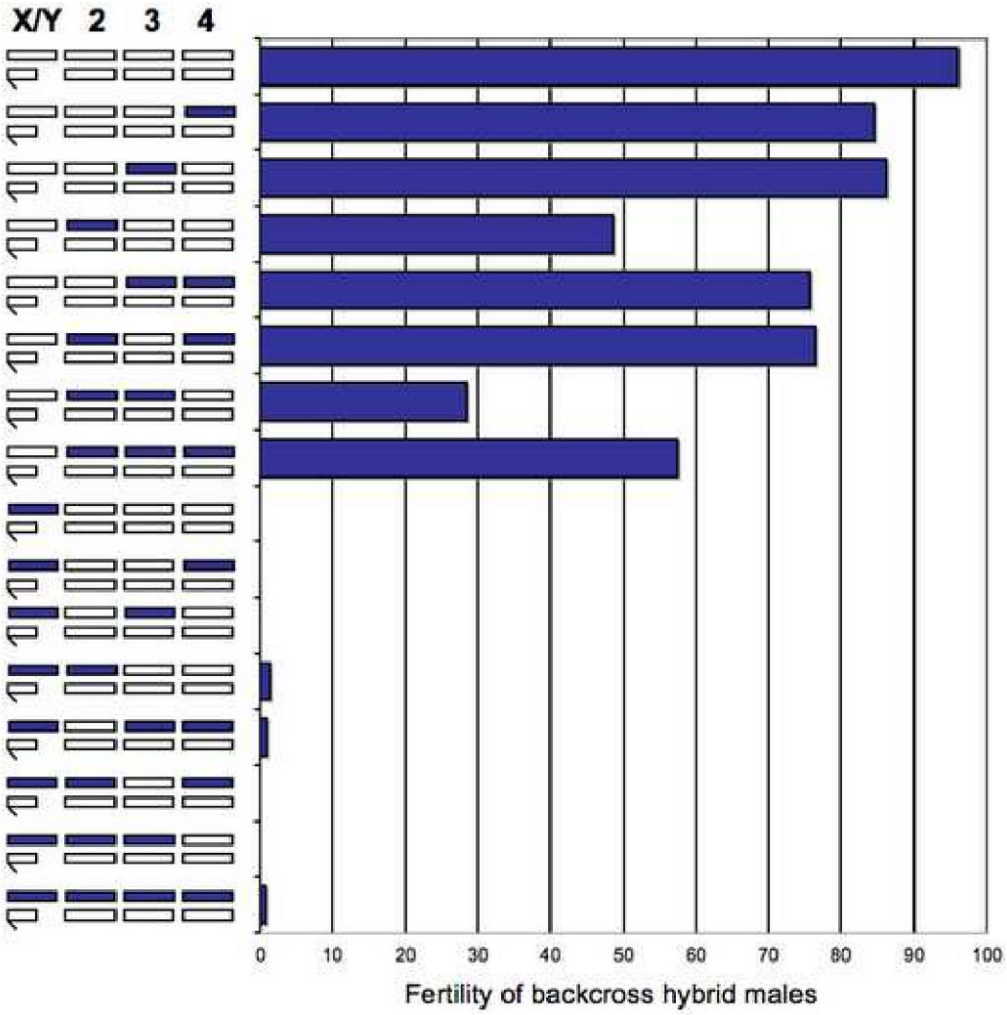

Figure 1. The large X-effect in *Drosophila* hybrids

The X chromosome has a conspicuously large effect on the sterility of backcross hybrid males between *D. pseudoobscura* (white) and *D. persimilis* (purple; modified from [79]). The x axis shows the percentage of fertile male flies obtained in the different chromosomal backcrosses that are depicted on the y axis. Similar results have been obtained in backcross analyses of hybrid sterility between many other species pairs, including *Drosophila*, mammals, Lepidoptera and birds (reviewed in [6]).

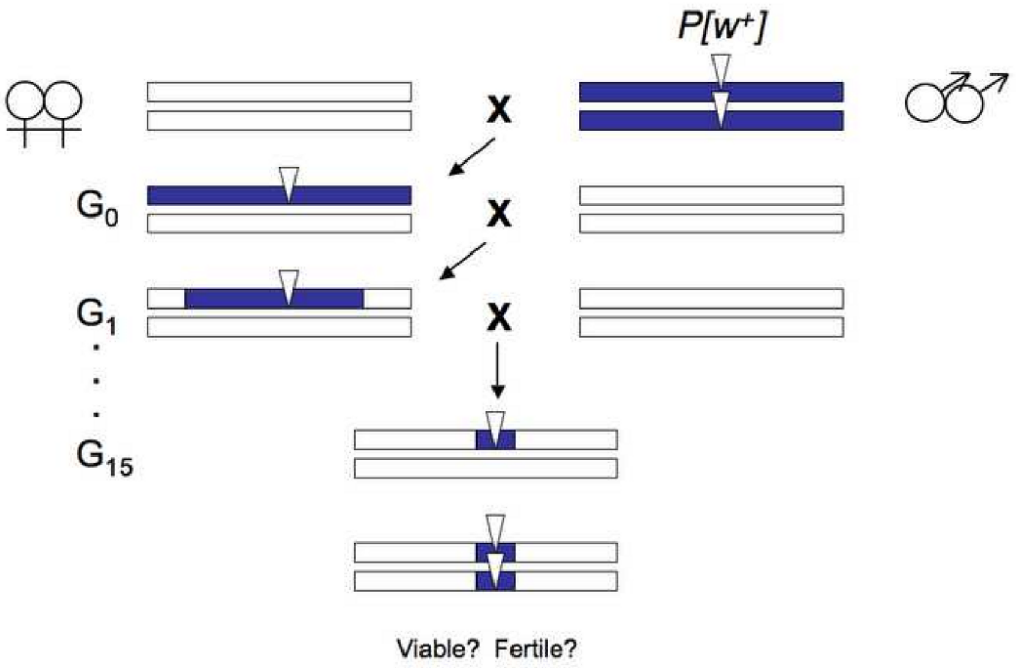

Figure 2. Introgression analyses of hybrid incompatibilities

Introgression studies move small chromosomal regions from one species (purple bars) into the genomic background of a closely related sister species (white bars) by repeated backcrossing through fertile hybrid females. Introgressed chromosomal segments typically bear a selectable dominant genetic marker (*e.g.*, a *P*-element bearing the w^+ eye color marker) and become progressively smaller with each generation of backcrossing. After several generations of backcrossing, the foreign introgression is made homozygous and tested for its effects on hybrid fitness.

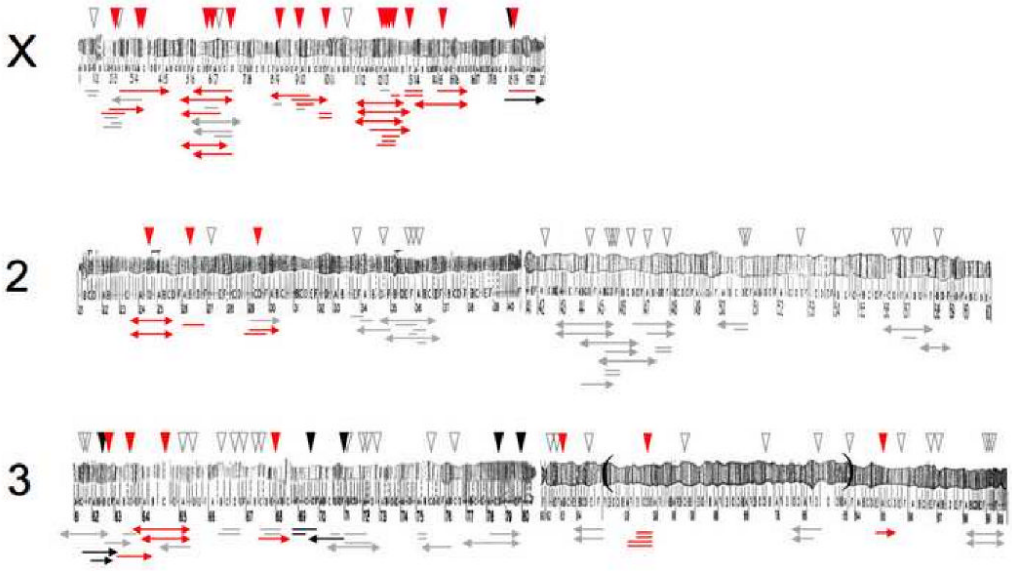

Figure 3. The X chromosome is a hotspot for hybrid male sterility factors

The density of introgressions from *D. mauritiana* that cause hybrid male sterility (red triangles) in an otherwise *D. sechellia* genomic background is four times higher on the X chromosome than on chromosomes 2 and 3. A chromosomal inversion is bracketed by parentheses on chromosome arm 3R. Gray and black triangles mark fertile and hybrid lethal introgressions, respectively. Introgression sizes are depicted beneath the chromosomes (modified from [16]).

Table 1

Tests for faster X evolution in *Drosophila*

Species	Number of Genes	Analysis	Result	References
D. melanogaster - *D. simulans*[a]	254 genes	d_N[b] and d_N/d_S[c]	X ≈ A	[30]
D. melanogaster	1,841 duplicate genes	d_N/d_S	X > A	[38]
D. melanogaster	13 duplicate genes	MK[d]	X > A	[39]
D. pseudoobscura - *D. melanogaster*	9,184 genes	d_N	X ≈ A	[35]
D. pseudoobscura - *D. melanogaster*	8,453 genes	d_N	X > A	[33]
D. pseudoobscura - *D. melanogaster*	110 genes	d_N/d_S	XR > 3L[e]	[32]
D. pseudoobscura - *D. melanogaster*	2,646 genes	d_N/d_S	XR ≈ 3L	[37]
D. melanogaster lineage	98 genes	MK	X ≈ A	[34]
D. melanogaster lineage	337 genes	MK	X ≈ A	[31]
D. melanogaster - *D. simulans*	50 genes with male-biased expression	MK	X > A	[28]
D. melanogaster - *D. simulans*	41 genes with female-biased expression	MK	X ≈ A	[28]
D. melanogaster - *D. simulans*	45 genes with no sex-biased expression	MK	X ≈ A	[28]
D. melanogaster - *D. simulans* and *D. melanogaster*-*D. yakuba*	597 genes with male-biased expression	d_N/d_S	X > A	[28]
D. melanogaster - *D. simulans* and *D. melanogaster*-*D. yakuba*	645 genes with female-biased expression	d_N/d_S	X ≈ A	[28]
D. melanogaster - *D. simulans* and *D. melanogaster*-*D. yakuba*	3,254 genes with no sex-biased expression	d_N/d_S	X > A	[28]
D. simulans, *D. melanogaster* and *D. yakuba* lineages	genome-wide, 50 kb windows	divergence at all sites[f]	X > A	[29]
D. melanogaster, *D. sechellia*, *D. erecta*, *D. ananassae*, *D. pseudoobscura*, *D. willistoni*, *D. mojavensis*, *D. virilis*, and *D. grimshawi* lineages	6,698 genes	amino acid divergence	X ≈ A	[36]
D. persimilis lineage	6,698 genes	amino acid divergence	X > A	[36]
D. yakuba and *D. simulans* lineages	6,698 genes	amino acid divergence	A > X	[36]
D. melanogaster, *D. sechellia*, *D. simulans*, *D. yakuba* and *D. erecta*	8,510 genes	d_N/d_S	X ≈ A	[36]
D. sechellia and *D. simulans* lineages	8,510 genes	d_N/d_S	X > A	[36]
D. melanogaster-*D. simulans* (3L) *vs. D. pseudoobscura*-*D. persimilis* (XR)	2,392	d_N/d_S	XR > 3L	[36]

[a] Molecular divergence can be estimated between two species (*e.g.*, *D. melanogaster* - *D. simulans*) or along a single lineage (*e.g.*, *D. melanogaster*).

[b] d_N = number of nonsynonymous differences per nonsynonymous site.

[c] d_N/d_S = nonsynonymous differences per nonsynonymous site standardized by synonymous (neutral) differences per synonymous sites.

[d] The McDonald-Kreitman (MK) test contrasts the numbers of replacement and synonymous fixed differences with the numbers of replacement and synonymous polymorphisms.

[e] Orthologous genes that are X-linked (XR) in *D. pseudoobscura* and close relatives but autosomal (3L) in *D. melanogaster*.

[f] For d_S, X ≈ A in the *D. simulans* lineage.