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THE CONTRIBUTION OF GENE MOVEMENT TO THE "TWO RULES OF SPECIATION"

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The two "rules of speciation"—the Large X-effect and Haldane's rule—hold throughout the animal kingdom, but the underlying genetic mechanisms that cause them are still unclear. Two predominant explanations—the "dominance theory" and faster male evolution—both have some empirical support, suggesting that the genetic basis of these rules is likely multifarious. We revisit one historical explanation for these rules, based on dysfunctional genetic interactions involving genes recently moved between chromosomes. We suggest that gene movement specifically off or onto the X chromosome is another mechanism that could contribute to the two rules, especially as X chromosome movements can be subject to unique sex-specific and sex chromosome specific consequences in hybrids. Our hypothesis is supported by patterns emerging from comparative genomic data, including a strong bias in interchromosomal gene movements involving the X and an overrepresentation of male reproductive functions among chromosomally relocated genes. In addition, our model indicates that the contribution of gene movement to the two rules in any specific group will depend upon key developmental and reproductive parameters that are taxon specific. We provide several testable predictions that can be used to assess the importance of gene movement as a contributor to these rules in the future.

KEY WORDS: **Gene relocation, genomics, hybrid incompatibility, sterility, X chromosome.**

Interest in the genetic basis of speciation has exploded in the last few decades, fueled in large part by the application of new molecular genetic techniques to classical genetic studies of reproductive barriers between species (Coyne and Orr 2004). Much of this work—done in animal species with heterogametic sex-determination—supports the existence of what have been called the "two rules of speciation" (Coyne and Orr 1989): the Large X-effect (the disproportionate influence of the X chromosome on the expression of interspecific hybrid incompatibility, particularly male sterility) and Haldane's rule (the observation that the heterogametic [XY or ZW] sex is disproportionately weaker or sterile in interspecific hybrids). Because of the prevalence of these patterns in a broad range of animal species (Coyne and Orr 2004), intense interest has focused on explaining their mechanistic basis, both with theoretical models (e.g., Orr and Turelli 2001) and emerging empirical data (e.g., Laurie 1997; Orr 1997; Masly and Presgraves 2007). These efforts have generated a set of plausible, often nonexclusive, explanations for the two patterns (Coyne and Orr 2004). However, disagreement remains over the predominant underlying genetic mechanism(s) responsible. The ubiquity of these patterns initially suggested that each might be due to a single common mechanism in heterogametic animal groups (Orr 1997). However, empirical support for several alternative hypotheses suggests that the genetic mechanisms contributing to these rules are likely multifarious (Orr 1993; Wu and Davis 1993; Coyne and Orr 2004). Importantly, all currently favored models are implicitly or explicitly based on the Dobzhansky–Muller model of hybrid incompatibility (Dobzhansky 1936; Muller 1939), which proposes that hybrid breakdown is due to interactions between alternative alleles at two or more loci that have arisen and been fixed in isolated lineages, and that fail to interact appropriately when brought together in hybrids.

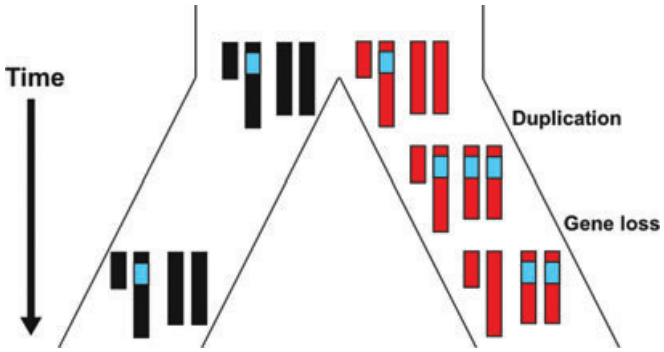

Figure 1. Gene relocation during lineage splitting. In one daughter lineage a gene is duplicated from its ancestral chromosomal location (in this case, the X chromosome) to a distant chromosomal location (in this case, an autosome). The gene in the ancestral location is subsequently lost.

Here, we revisit an alternative model of the evolution of hybrid incompatibilities that could also contribute to both rules of speciation: gene movement. Dobzhansky (1937, pp. 252–253) was one of the first (see also Haldane 1932, p. 75) to propose the physical movement of genes between distant chromosomal regions in alternative lineages as a potential mechanism for Haldane's rule (Fig. 1), and Muller (1942, p. 88) suggested that the same mechanism might explain the observed recessivity of many hybrid incompatibility loci:

> *By some types of transfers in the positions of genes, effects similar to those of complementary genes can be produced in hybrid recombinants that come to contain the given gene in neither position.*

More recently, a similar idea has been proposed in terms of the divergent resolution of gene duplicates in two daughter lineages that diverge after a duplication event (Werth and Windham 1991; Lynch and Force 2000). However, these proposals have collectively received minimal empirical attention from the speciation community (see Masly et al. 2006; Scannell et al. 2006; Bikard et al. 2009, for exceptions). This may be because rates of gene movement are thought to be insufficiently high to explain the prevalence of the rules of speciation, and/or because these patterns are also observed in hybrids between species that do not differ by evident large-scale chromosomal movements (Coyne and Orr 2004). In addition, unlike F2 incompatibility, F1 incompatibility cannot simply be due to duplications and deficiencies in the heterogametic sex (Coyne and Orr 1989) but must also involve either the effects of dosage compensation, postmeiotic gene expression, and/or expression or functional divergence to cause F1 hybrid problems (see below). Although these difficulties still pertain to gene movement as a universal and exclusive explanation of the "two rules," here we argue that this phenomenon should be more seriously considered as a contributing mechanism to these rules, alongside more common explanations.

We first lay out a model in which gene movement specifically between the X chromosome and autosomes can generate patterns of hybrid inviability and sterility consistent with the two "rules." We identify several specific mechanisms by which gene movement can produce F1 and later generation sterility and/or inviability, focusing on the sex-specific and chromosome-specific effects characteristic of the two rules. Using data emerging from large comparative genomic datasets, we show that gene movements are substantially more frequent than previously imagined based simply on large-scale karyotypic changes. In addition, these movements disproportionately involve the X and, in XY systems, involve genes that are strongly enriched for male reproductive expression. We identify the biological parameters under which gene movement is expected to make the most substantial contribution to the two rules, and evaluate current data on these biological parameters. Finally, we lay out several predictions of this hypothesis that are testable with further empirical data. Our goal is to present evidence for a plausible additional mechanism for these two rules, as well as explicit predictions that can be used to evaluate the importance of the model.

The "Two Rules of Speciation"

The "Large X-effect"—the disproportionately large effect of the X chromosome on the expression of hybrid incompatibility, especially male sterility—is supported by two general classes of empirical observations: genetic mapping experiments, in which the largest incompatibility effects map to the X chromosome in backcross or other later generation hybrids (reviewed in Coyne and Orr 2004); and genetic analyses of natural interspecific hybrid zones, in which the X chromosome is the least mobile chromosome across the hybrid zone (e.g., Tucker et al. 1992; Dod et al. 1993; Payseur et al. 2004; Macholan et al. 2007; Teeter et al. 2008). The most detailed data now available suggest that this effect is due to higher densities of male sterility loci on the X chromosome, rather than X-linked sterility loci having larger individual phenotypic effects (at least in *Drosophila*; Tao et al. 2003; Masly and Presgraves 2007). The precise mechanistic explanation for this numerical enrichment of sterility loci on the X chromosome remains controversial (Vicoso and Charlesworth 2006); proposed hypotheses include elevated rates of nonsynonymous evolution in X-linked genes ("Faster-X;" Charlesworth et al. 1987), pleiotropic effects of elevated rates of evolutionary change due to sex-chromosome meiotic drive (Frank 1991; Hurst and Pomiankowski 1991; Tao and Hartl 2003), disrupted dosage compensation on the X chromosome in heterogametic hybrids (Orr 1989), and/or disrupted X-inactivation in hybrid males (Masly and Presgraves 2007). There are difficulties with all of these proposed mechanisms (recently reviewed in Masly and Presgraves

2007; Presgraves 2008), and each remains contested as a complete explanation for the Large X-effect.

Named after its originator (Haldane 1922), Haldane's rule—the observation that the heterogametic sex is more often inviable or infertile among hybrids—has been observed in hundreds of heterogametic animal hybrids from groups as diverse as insects, mammals, birds, and reptiles (cf. Table 8.1 in Coyne and Orr 2004). Based on detailed empirical and theoretical work, it is now well accepted that Haldane's rule likely results from the combined effect of hemizygosity in the heterogametic sex and the frequent recessivity of hybrid incompatibility loci; that is, their greatest effect is observed when homozygous or hemizygous loci from one species are exposed in the background of the other species. Under these conditions, F1 males (when males are heterogametic) experience the full deleterious effect of any hemizygous X- (or Y-) linked incompatibility loci; these effects are masked in F1 females. Importantly, the Large X- (or Large Z-, in ZW systems) effect can act to amplify Haldane's rule—the larger the relative effect of the X (or Z) on hybrid incompatibility, the greater the differential effect observed in the two sexes. In this sense, the two rules of speciation are mechanistically linked: given partial or complete recessivity of hybrid incompatibility factors, mechanisms that enrich the density of incompatibility loci on the X chromosome (the Large X-effect) can contribute to explaining the observations underlying Haldane's rule. In our discussion later, we primarily focus on the Large X-effect, returning to Haldane's rule at the end. Throughout, it is important to note that the Large X-effect is experimentally observed in backcross or other later generation hybrids (where the effects of individual chromosomes can be clearly differentiated and quantified), whereas Haldane's rule applies to observations in F1 hybrids only.

Gene Movement and the Large X-Effect: A Hypothesis

We propose an additional mechanism for the Large X-effect: the greater apparent density of incompatibility loci on the X chromosome could also be due to the dysfunctional consequences of moving genes or genetic functions (defined below) between the X chromosome and autosomes (Fig. 1). These negative consequences come about because crosses between lineages that have the same gene on different chromosomes can produce gametes or offspring that do not contain either copy of the relocated gene, result in offspring that do not properly maintain dosage balance, or have mechanically dysfunctional X-inactivation and/or misexpression following X-inactivation (Table 1, Fig. 2). None of these mechanisms require a sequence change in the relocated gene. If relocated genes have also diverged in function, then additional mechanisms can also contribute to hybrid inviability or sterility (Table 1). The precise way in which gene movement can contribute to hybrid dysfunction and the Large X-effect depends upon the specific mechanism of gene movement and on details of developmental and reproductive biology of the X. We briefly introduce these mechanisms below, returning to the evidence for each in the next section.

There are three mechanisms by which genes can move between chromosomes. The first involves the simple movement of a gene from one chromosome to another with no duplicative intermediate. Movement of large chromosomal segments is observed frequently in nature (Dobzhansky 1937), although to produce inviability/sterility this mechanism requires that the population must initially be segregating null (and presumably inviable/sterile) genotypes before the moved gene becomes permanently established in its new location. Second, a gene duplication followed by loss of a gene can result in gene movement without intermediate null genotypes (relocation). This process can happen either in a single lineage, with the fixation of a new duplicate on an alternative chromosome followed by loss of the original locus (Fig. 1), or it can happen when a duplication precedes a population split, followed by the loss of alternative copies in each lineage (Werth and Windham 1991; Lynch and Force 2000). Importantly, for both of these processes it is unnecessary for the relocated gene to have diverged in function for it to contribute to hybrid incompatibility (Table 1, Fig. 2, Fig. S1); all that is required is a genomic "map change" (i.e., a change in the location of homologous genes between species; Dobzhansky 1937; Lynch and Force 2000). These map changes also include interarm gene movements within a chromosome, as these can be sufficiently physically distant to segregate independently of each other in recombinant populations. A third possibility is that a duplication event results in the change in location of a genetic function, rather than simply the gene itself. This can come about when two duplicates in a single lineage partition the original single-copy gene's multiple functions (i.e., "subfunctionalization;" Force et al. 1999). As a result, a subset of functions of the original gene can change genomic locations even though there is still a homologous gene in the original position (Lynch and Force 2000). Subsequent hybridization with a lineage that does not contain two copies, or that has partitioned the genetic functions between copies in an alternative manner, will still result in dysfunctional offspring (Table S1). In genomic mapping (including deletion) studies, null loci due to any of the above mechanisms will be indistinguishable from loci that cause negative interlocus epistasis ("Dobzhansky–Muller Interactions"; Dobzhansky 1936; Muller 1939). In addition, because many of the effects of gene movement are due to the formation of genotypes that lack sufficient functional gene copies, these loci are by definition recessive. This is exactly the property of gene movement that Muller (1942) invoked to explain the recessivity of hybrid incompatibilities.

Table 1. Mechanisms of sterility via gene relocation between X chromosomes and autosomes. Relocation is assumed to involve duplication followed by loss of the gene copy at the original ancestral location (Fig. 1); other movement mechanisms are addressed in Table S1. For brevity, we focus primarily on mechanisms that can explain F1 hybrid patterns; F1 effects apply also to F2 or other later generation hybrids. "X-to-A" denotes genes or gene functions that have moved from an ancestral position on the X chromosome to a new position on an autosome; "A-to-X" denotes the inverse direction of movement. Mechanisms that act when there is no difference between ancestral and moved gene can also apply in cases in which there is a sequence or functional change between moved genes (e.g., a movement of gene function). The principal cause of hybrid problems is in bold.

Difference between ancestral and moved gene	Basis of sterility	Challenges
None	• Creation of **null genotypes** in recombinant gametes from F1, or in F2 individuals	Only applies to F1 sterility in systems with postmeiotic gametic gene expression
	• Misexpression due to random X-inactivation **dosage** compensation in females plus upregulation of active X: half F1 males have underexpression (1x), half F1 males have overexpression (3x); F1 females are a mosaic of under- and overexpression (1 or 3x) (Fig. 2)	Only applies to mammal-like dosage compensation
	• Misexpression due to hypertranscriptional **dosage** compensation on X in males: all F1 males in one cross direction have underexpression (1x), all F1 males in the other cross direction have overexpression (3x); F1 females have normal expression (2x) (Fig. 2)	Only applies to *Drosophila*-like dosage compensation
	• Misexpression/**dosage** imbalance after meiotic sex-chromosome inactivation (MSCI): X-to-A genes continue to be expressed; A-to-X genes are no longer expressed	
Sequence/functional	• A-to-X or X-to-A genes interfere with normal **X-inactivation** in somatic or meiotic cells	Requires functional divergence in X-inactivation machinery following movement event; only observed in F1s if disruption occurs when X-inactivation machinery is heterozygous
	• **Derived function** in daughter gene produces incompatibility with other lineage (e.g., new male-biased autosomal genes interfere with spermatogenesis)	Lacking direct empirical evidence

Although gene movement that occurs exclusively between autosomes can contribute to F2 and later generation hybrid incompatibilities, it is specifically movement involving the sex chromosomes that is relevant to the two rules of speciation. Only these movements can contribute to the enrichment of incompatibilities on the X chromosome, and/or heterogametic-specific effects in F1 hybrids (Fig. 2, Figs. S2 and S3). This is because the deleterious effects of gene movements involving the X can be revealed in heterogametic hybrids, due to their hemizygosity. In addition, because unique mechanisms are used to balance sex chromosome dosage between males and females and to ensure correct meiotic segregation in the heterogametic sex (reviewed in Lucchesi et al. 2005; Payer and Lee 2008; Turner 2007), gene movements that involve the sex chromosomes can be subject to unique dysfunctional effects in hybrids. Under our hypothesis, these unique effects fall into three main classes: null genotype effects (in both F2s and F1s), dosage effects, and X-inactivation effects (Table 1, Table S1). These deleterious consequences do not require any functional divergence in moved genes or their interacting loci, with the exception of X-inactivation effects.

First, under a gene movement hypothesis, null genotypes specifically associated with the X chromosome will be more common in segregating hybrid populations because the sex chromosomes are hemizygous. For each X-autosome movement, 9.325% of all F2s will lack a copy of the locus; from autosome–autosome movements, only 6.25% of all F2 individuals are null. If the moved gene performs an essential function, any null F2 hybrid will experience a fitness deficit. Although F1 individuals will not themselves have doubly null genotypes, one-fourth of all female gametes and three-fifths of all male gametes from F1s will carry null genotypes. The consequences of these null genotypes can be observed in F1 generation, however, if the moved locus acts postmeiotically in the gametes of these individuals. Therefore, the importance of this mechanism for F1 hybrid problems will depend

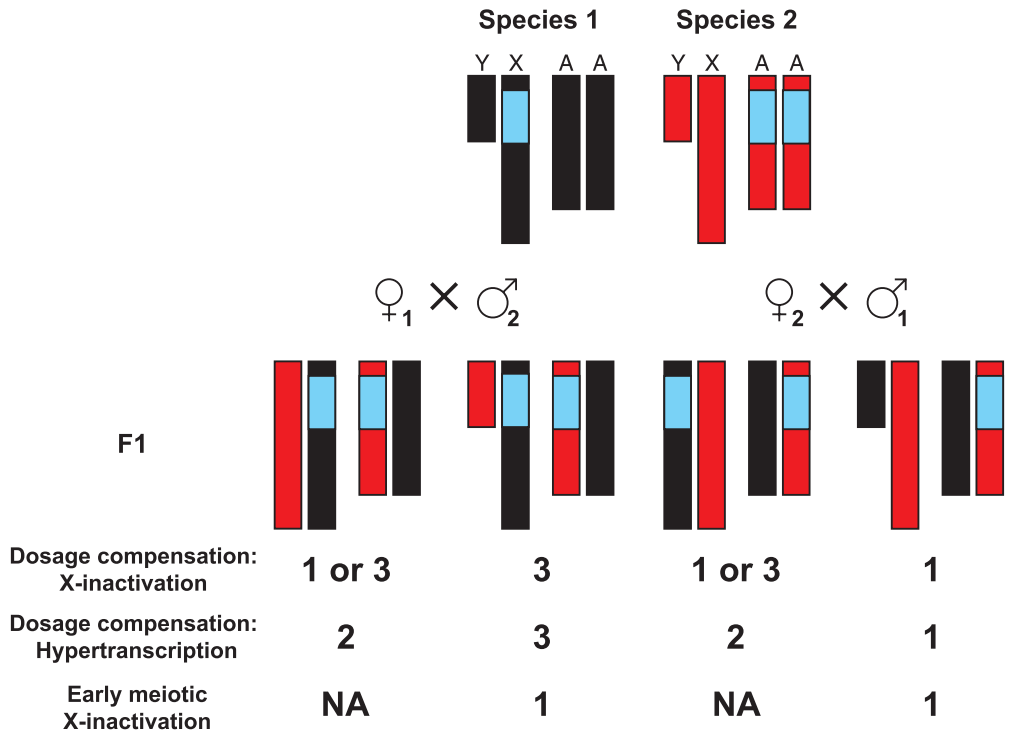

Figure 2. **Hybrid dysfunction from X-A gene relocation. Species 1 and 2 differ in the chromosomal location of a gene (shaded box) due to gene movement (only males of each parental species are shown). The four F1 hybrids from both possible directions of the species cross are illustrated. Numbers represent numerical values of gene product in each F1 genotype. F1 hybrids have a deficit or excess of gene copies or gene product in: somatic tissue due to dosage compensation via X-inactivation plus upregulation of the active X (mammals; expectation = 2,2); somatic tissue due to dosage compensation via hypertranscription in males (*Drosophila*; expectation = 2,2); in reproductive tissue following precocious meiotic X-inactivation (expectation = 0,2). Expectations are based on gene product in each parental species (species1, species2).**

upon the frequency or ubiquity of postmeiotic gene expression in any particular lineage.

Second, genes that have moved between the X and autosomes will experience changes in their dosage due to normal X chromosome dosage compensation mechanisms. This can contribute to hybrid problems that are specifically associated with the X chromosome if altering locus-specific dosage has large fitness consequences. For example, in *Drosophila* dosage compensation is achieved by hypertranscription of the X in males. In lineages differentiated by an X-A gene movement this compensatory mechanism leads to overexpression of the moved locus in some male hybrids, but underexpression in others (Table 1, Fig. 2); an equivalent gene movement among autosomes has no effect on sex-specific dosage compensation in hybrids (Fig. S3). Conversely, in mammals somatic dosage compensation occurs through random X-inactivation in the homogametic sex (i.e., one X in XX females) and upregulation of the active X in both sexes (Nguyen and Disteche 2006). Genes moved between X and autosomes can show transgressive expression phenotypes in both male and female F1s (Table 1, Fig. 2). In either case, the contribution of this mechanism to hybrid problems depends upon the degree of sensitivity to gene-dosage effects (haploinsufficiency or extra-diploid fitness effects) in any particular lineage. Note that dysfunctional dosage compensation has previously been proposed as an explanation for male-specific hybrid sterility when dosage compensation is achieved by X-hypertranscription in males, such as in *Drosophila* (reviewed in Masly and Presgraves 2007); however, an X-A gene movement hypothesis has explanatory power across different mechanisms of dosage compensation.

Finally, gene movements involving the X chromosome could interfere with normal X-inactivation processes, either during somatic X-inactivation in mammals (with dosage consequences already addressed above), or during meiotic sex-chromosome inactivation (MSCI: where the X is precociously inactivated before the autosomes during male gametogenesis in XY systems; Hense et al. 2007; Mueller et al. 2008) (Table 1). Improper MSCI during spermatogenesis has previously been proposed as an explanation of the Large X-effect (Lifschytz and Lindsley 1972; Masly and Presgraves 2007; Presgraves 2008). In particular, normal X chromosome condensation in hybrid spermatogenesis could be disrupted when the X-inactivation machinery fails to recognize heterospecific portions of the X chromosome as X-linked material, due to very high levels of sequence divergence between parental species at, for example, orthologous X-linked genes or

noncoding *cis*-regulatory elements that mediate MSCI (Masly and Presgraves 2007; Presgraves 2008). Rather than orthologous sequence divergence between species, under our hypothesis the disruption of X-inactivation by "non-X" sequence could be due to gene movement between X and autosomes; that is, X-inactivation machinery in lineages missing a gene on the X fails to properly condense heterospecific chromosomes that have that gene present on the X. This is a more rapid way to achieve sequence differences between X chromosomes in closely related lineages. Nonetheless, it does require additional functional divergence between lineages, specifically in the response of inactivation machinery to the presence/absence of a locus on the X chromosome; otherwise the presence (absence) mutation would cause sterility as soon as it arises within species. The plausibility of this process as a mechanism contributing to the Large X-effect relies on the sensitivity of X-inactivation to the presence/absence of individual genes on the X chromosome.

Under our hypothesis, all three general classes of sex and/or X-specific consequences of gene movement lead to sex-enriched or sex chromosome enriched deleterious effects in hybrids, as is required to explain the rules of speciation. Note that dysfunctional dosage compensation could have both viability and fertility effects in hybrids, whereas disruptions specifically of MSCI will exclusively affect fertility (Table 1). Therefore, the sterility consequences of X-A movement could be numerically greater than the viability consequences.

Gene Movement and the Large X-Effect: Empirical Support

Given the hypothesis laid out above, for gene movement to make a substantial contribution to the expression of hybrid problems and to the two rules several empirical conditions must be met. First, the relevant gene movements must be sufficiently frequent between recently differentiated taxa (i.e., the species pairs that show evidence for the two rules). Second, gene movements must have sufficiently deleterious effects on viability and fertility in hybrids. Under our model, lineages that have frequent postmeiotic gene expression, that are sensitive to altered gene dosage effects, and/or that experience dysfunctional X-inactivation due to segmental changes, will be particularly sensitive to the consequences of gene movement. Here, we discuss data relevant to whether and where these conditions are met in empirical systems. We focus on data from *Drosophila* and mammals, only because these are the taxa for which most data are available.

FREQUENCY AND NATURE OF GENE MOVEMENT

Several lines of evidence emerging from comparative genomic data indicate that (1) movement of homologous genes between chromosomes appears to occur much more frequently than initially inferred from large-scale karyotypic change; (2) gene traffic between chromosomes preferentially involves the X chromosome; and (3) moved genes involve an excess of loci with male reproductive functions. These observations indicate that gene movement could contribute specifically to the increased density of loci with male-sterility effects on the X chromosome.

Overall rates of gene movement

Genomic comparisons indicate that gene movement, including movement between chromosomes, is common across eukaryotes (Betran et al. 2002; Coghlan and Wolfe 2002; Drouin 2002; Emerson et al. 2004; Bai et al. 2007; Bhutkar et al. 2007; Jiang et al. 2007; Potrzebowski et al. 2008; Meisel et al. 2009). Most genomic studies of gene movement involve cases in which both duplicates are still present, largely because the presence of two paralogs within a single genome makes it easier to identify interchromosomal movement (see below). However, a recent study used whole-genome sequences from 12 *Drosophila* species to identify single-copy genes that have been positionally relocated (Bhutkar et al. 2007). These authors found 514 high-confidence relocated genes, which translates to a rate of 1.4–2.1 relocations per million years (depending on the divergence time used). This estimate is conservative, because many lower confidence movements were also identified. To obtain an estimate of the rate of gene relocation in mammals, we searched the Ensembl database (Hubbard et al. 2007) for high-confidence one-to-one orthologs between the human and Rhesus macaque genomes that were not located on homologous chromosomes. We were able to identify 178 pairs of orthologs that appear to have been relocated between chromosomes (Table S2). Assuming a human–macaque divergence time of 24 million years, this number implies a rate of 3.7 relocations per million years. Therefore, both *Drosophila* and mammal data provide good evidence for frequent gene relocation. As explained above, gene relocation can contribute to hybrid incompatibility simply because a genomic map change has occurred.

In cases of gene duplication where both paralogs are still present, however, expectations are more complex. Although no hybrids will have zero gene copies, all F1 hybrids and 50% of F2 hybrids will have gene dosage that differs from both parental species. Additional mechanisms—such as differential partitioning of function among duplicates (Lynch and Force 2000)—can contribute further to incompatibility (Table S1; Fig. S1 and see below). Although there are no good estimates of the frequency with which paralogs are subfunctionalized, there are data on the frequency with which paralogs reside on different chromosomes. If only a fraction of these paralogs has deleterious dosage effects or has partitioned functions differently among lineages, then this fraction will contribute to hybrid

incompatibility. Estimates of the rate of interchromosomal duplication via only retrotransposition—that is, the insertion of a new duplicate reverse-transcribed from the original locus' mRNA—suggest that between 0.5 and 2 new functional genes are fixed on new chromosomes every million year in *Drosophila* and mammals (Bai et al. 2007; Marques et al. 2005; Potrzebowski et al. 2008; Vinckenbosch et al. 2006). More sparse data on rates of interchromosomal movement via DNA-mediated duplication indicate that such events occur very frequently in mammals (Friedman and Hughes 2004; Jiang et al. 2007; McGrath et al. 2009), somewhat frequently in *C. elegans* (Semple and Wolfe 1999; Coghlan and Wolfe 2002), and less frequently in *Drosophila* (Bhutkar et al. 2007; Fiston-Lavier et al. 2007; Meisel et al. 2009). These data indicate that genome-wide rates of gene duplication between chromosomes are likely to fall between 2.7 (*Drosophila*) and 11.5 (mammals) events per million years. Considering both gene relocation and gene duplication, rates of gene movement are sufficiently high (at minimum 4.4 or 15.2 movements/million years, for *Drosophila* and mammals respectively) for gene movement to potentially contribute to the accumulation of reproductive isolation during speciation, although the importance of this contribution will depend both on the timing of movements with respect to the lineage divergence and on how many duplicative movements affect hybrid fitness. These data also suggest that gene movement might be a more important contributor to reproductive isolation between mammalian lineages than among *Drosophila* species, as further discussed below.

Frequency of X-autosomal movement

If gene movement contributes to hybrid incompatibility, differences in rates of movement among chromosomes will affect where incompatibility loci are preferentially found. A growing body of data supports the observation that traffic of gene duplicates between chromosomes preferentially involves the X chromosome, and we show here that gene relocation in both *Drosophila* and mammals also preferentially involves the X. Using the published data on relocated genes in *Drosophila* (Bhutkar et al. 2007), we were able to polarize gene movement among the six chromosome arms (i.e., Muller elements). Figure 3 shows that there is a statistically significant excess of genes relocating off Muller element A, which acts as the X chromosome in all *Drosophila* (with Neo-X: two-tailed binomial test, $P = 1.3 \times 10^{-4}$; without Neo-X: $P = 3.4 \times 10^{-5}$). A slight excess of movement off Muller element D can also be seen, likely to be due to the fact that there have been two independent fusions of element D to the X chromosome ("neo-X chromosomes") among the 12 *Drosophila* species considered. Using the data on orthologs between human and macaque introduced above, we can show that there are an excess number of gene relocations involving the X chromosome in mammals (FET, $P = 9.2 \times 10^{-8}$), although without a third (outgroup) genome we are unable to polarize gene movements as we can with *Drosophila*. Data from duplicated genes retaining both paralogs also demonstrate an excess of movement involving the X in both *Drosophila* (Betran et al. 2002; Dai et al. 2006; Meisel et al. 2009) and mammals (Emerson et al. 2004; Potrzebowski et al. 2008). Interestingly, there is a difference in patterns of gene duplicative movement between mammals and *Drosophila*: mammalian data indicate frequent movement both on and off the X (Emerson et al. 2004), whereas in *Drosophila* the movement is biased off the X chromosome and onto autosomes, but not vice versa (Betran et al. 2002). Nonetheless, the direction of gene movement does not influence whether X-autosome movement could contribute to the Large X-effect; all that is required is a chromosomal change that involves the X chromosome.

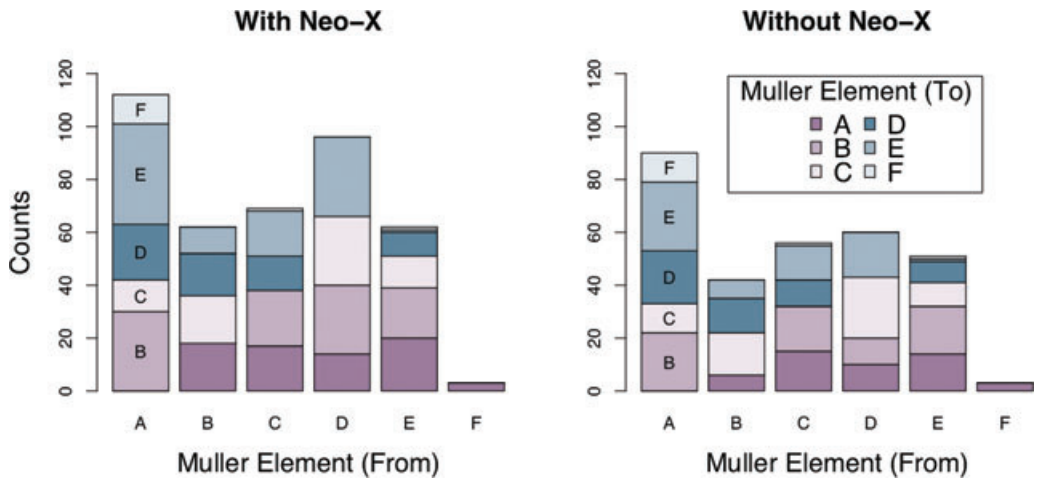

Figure 3. **Frequency of orthologous gene relocations between Muller elements among 12 *Drosophila* species. Data were reanalyzed from Bhutkar et al. (2007). Muller element A corresponds to the X chromosome in all species. With or without the neo-X, there is a statistically significant excess of genes relocating off the X chromosome and onto autosomal Muller elements (see the text).**

The average magnitude of this bias toward gene movement involving the X can also be estimated from data. From the relocation analyses above, we find that rates of X-A movement are 3.5X (mammals) and 1.46–2.27X (*Drosophila*) higher than expected, based on the relative size and gene content of the X chromosome. (For *Drosophila*, the lower and upper bounds of the estimate are based on both X-to-A and A-to-X movement together, or only on X-to-A movement, respectively, without including the Neo-X; estimates with the Neo-X are comparable (data not shown).) Similarly, rates from the movement of duplicates in *Drosophila* (Betran et al. 2002), mouse (Emerson et al. 2004), and human (Emerson et al. 2004) are estimated to be 1.3–2.1X, 3.76–4.09X, and 3.8–3.99X (respectively) higher than expected; estimates based on both X-to-A and A-to-X movement together, or only on X-to-A movement, provide lower and upper bounds. All estimates indicate that chromosomal movement is significantly X-A biased ($P < 0.001$). In comparison, although phenotypic patterns consistent with a qualitative Large X-effect are common (see Coyne and Orr 2004; Presgraves 2008, for recent reviews), there are few quantitative estimates of the relative density of X-linked versus autosomal hybrid incompatibility loci. The limited data currently available suggest that the density of hybrid sterility factors on the X chromosome is approximately two to three times that found on the autosomes (estimates of X to autosome ratio: 1.8 – 3 (Masly and Presgraves 2007); 2.5 (Tao et al. 2003); 2 (Moehring et al. 2006)). Note that the magnitude of the bias in X-A movement and X-enrichment for sterility need not be directly comparable in order for X-A gene movement to contribute to the Large X-effect. For example, because gene movements involving the X can be subject to unique functional consequences (see above), individual X-A movements might be more likely to result in hybrid problems than A-A movements.

Despite the clear bias toward movements involving the X chromosome, estimates of the absolute rates of moved genes appear to be insufficient to explain all of the X chromosome enrichment for sterility factors in some cases, especially as not all gene movements will necessarily contribute to hybrid dysfunction. In particular, data on gene movement among lineages within the *Drosophila melanogaster* subgroup give an estimated average of 2.12 movements/million years that specifically involve the X chromosome (Meisel et al. 2009). Although this is a conservative estimate (gene movement events are often systematically and substantially underestimated even from well-annotated genome sequences; M. V. Han and M. W. Hahn, and MWH, unpubl. data), at least nine X-linked male sterile loci have been detected between a species pair in this clade that diverged ~250,000 years ago (Masly and Presgraves 2007); this density cannot be explained solely by an average rate of several gene movements per million years. There are very few data on the relative numbers of X-linked versus autosomal sterility factors in other heterogametic systems (see Good et al. 2008; Kitano et al. 2009, for some recent studies). However, given the higher estimates of overall gene movement rates and higher X-bias in gene movements among mammalian lineages, it is possible that gene movement explains a greater proportion of the X-linked loci in mammalian (and perhaps other similar) groups, in comparison to *Drosophila*.

Preferential male sterility effects of moved genes

Male sterility is often the first postzygotic reproductive barrier to evolve between many animal species (Coyne and Orr 2004). This observation holds true for the Large X-effect (at least in *Drosophila* where it has most comprehensively been studied): the greater effect of the X chromosome is most frequently expressed as male sterility, although Large X-effect for inviability and female sterility have occasionally been observed (e.g., Coyne and Orr 1989; Orr 1993; although see Presgraves 2008). The gene movement hypothesis provides one potential explanation for why a Large X-effect is frequently observed for male sterility in XY-male systems. Substantial emerging data in *Drosophila*, mouse, human, and several other mammals indicate that moved genes preferentially involve loci associated with male reproductive function, as evidenced by male-specific or male-biased gene expression (Table S3). This is also the case specifically for gene relocation data in *Drosophila*. For example, Bhutkar et al. (2007) found that for 42% (39/94) of loci relocated between chromosomes and for which there was gene expression data, the relocated gene was expressed in the *D. melanogaster* testes. (Using these data, we found that 10 of these testes-expressed loci were associated with the X chromosome; nine involve X-to-A movements, and only one was A-to-X.) In a more comprehensive analysis of *Drosophila*, an estimated 87.5% of duplicated retrogenes were expressed in the testis, whereas 55% of the parents of these retrogenes were expressed in the testes (roughly equivalent to the genome-wide average); more specifically, 95.5% of X-to-A duplicated retrogenes have testis-expression, whereas 50% of the parents of the X-to-A retrogenes have testis-expression (Meisel et al. 2009), indicating a very strong bias in moved genes to male reproductive expression. Although expressed genes need not necessarily be functional, a recent paper (Kaessman et al. 2009) reviews evidence emerging from molecular evolution, comparative genomics, and direct functional studies, that a large number of retrogenes have evolved functional roles, especially in the male germline.

In addition to an apparent bias in male-reproductive expression in moved genes, the mechanisms responsible for gene movement might naturally bias gene movement toward reproductive (and specifically male) loci, rather than genes with viability effects. For example, the chance of movement via RNA-based gene duplication is likely to be closely related to the abundance of RNA transcripts in the germline (Kaessman et al. 2009), with

more highly transcribed genes presenting more available targets for movement. Testes undergo hypertranscription during early haploid stages of spermatogenesis (Schmidt 1996; Kleene 2001); therefore testes-expressed genes might be particularly prone to this mechanism of gene movement. (Interestingly, hypertranscription during spermatogenesis has also been implicated in the creation of de novo testes-specific genes in *Drosophila*; Levine et al. 2006; Begun et al. 2007.)

If gene movement preferentially involves genes with male-specific expression, then incompatibilities due to gene movements will also preferentially involve male functions. The disproportionate involvement of both the X chromosome and male-specific expression in gene movement therefore could contribute to the specific enrichment of male sterility factors associated with the X chromosome. Note that this is the case regardless of the fact that male-function genes (as determined by male-biased gene expression patterns) are generally underrepresented on the X chromosome in *Drosophila* (Parisi et al. 2003). The enrichment of male-specific expression in moved genes might also contribute to an explanation of why hybrid male sterility appears to accumulate much faster than other kinds of incompatibilities among species (Wu and Davis 1993; Coyne and Orr 2004; Presgraves 2008), at least among species in which the male is the heterogametic sex.

LIKELIHOOD OF DELETERIOUS CONSEQUENCES OF X-A GENE MOVEMENT

The likelihood that gene movement events involving the X chromosome will have uniquely deleterious consequences in hybrids depends on details of the underlying developmental and reproductive biology of lineages experiencing gene movements. As we identified above, important factors include (1) the frequency and ubiquity of postmeiotic gene expression; (2) sensitivity to gene copy number variation/dosage effects; and (3) sensitivity to the disruption of X-inactivation (Table 1).

Effects of null genotypes

Any gene relocation will result in F2 individuals that do not contain even a single copy of the moved gene. Therefore, the potential effects of gene movements could be felt at any developmental stage in F2s, and these effects will be greater for X-A movements than for A-A movements (see above). In contrast, genes that have undergone lineage-specific X-A movement can influence F1 sterility only when expressed postmeiotically, because some of these postmeiotic gametes will contain null genotypes. Therefore, the propensity for postmeiotic gene expression will also influence the likelihood that gene movements contribute to hybrid postmeiotic failure.

There is clear evidence for postmeiotic gene expression in mouse; for example, a recent study estimates that ~18% of mouse X-linked genes are expressed postmeiotically in spermatogenic cells (Mueller et al. 2008). In comparison, postmeiotic gene expression is conventionally thought to be rare or absent in *Drosophila* males (Schafer et al. 1995). Nonetheless, recent data show that specific *Drosophila* spermatogenic loci are postmeiotically expressed. In particular, Barreau et al. (2008) identify 24 loci with unambiguous male postmeiotic gene expression in *D. melanogaster* (also see Vibranovski et al. 2009). Using the *Drosophila* 12-genomes data we found that at least five different movement events have resulted in eight of these 24 loci being relocated on different chromosomes in at least one lineage (Table S4). All movements appear to be relocations (i.e., there is no "ancestral copy" in the lineage with the movement), and one of these relocations involves X-A movement. That is, among *Drosophila* species, gene relocation has occurred in postmeiotically expressed male genes and therefore could act as a basis for F1 sterility under a gene movement hypothesis, although for a small number of genes. Overall, however, the current data indicate that the frequency with which genes are expressed postmeiotically differs substantially between *Drosophila* and mammals (mice), suggesting that mammals might be more susceptible to negative fertility consequences of X-A gene movement in F1 hybrids.

Sensitivity to altered dosage

Altered gene-specific dosage in hybrids could result in sterility or inviability between lineages differing in X-A movements (Table 1, Fig. 2). The number and magnitude of potential hybrid problems depends upon the sensitivity of developmental and reproductive processes to increases and decreases in gene-specific dosage. In *D. melanogaster*, classical studies have uncovered a limited number of chromosomal regions that show evidence of haplo-insufficiency for viability or fertility, or for which duplications compromise viability or fertility. For example, in a survey of most of the second and third chromosomes, Lindsley et al. (1972) detected only 48 chromosomal regions with triploid or haploid viability or fertility effects. In a more recent systematic genome-wide analysis in *D. melanogaster*, Marygold et al. (2007) identified or confirmed 65 *Minute* loci that show haplo-insufficiency (with phenotypic consequences for development, viability, and fertility). Interestingly, 11 of these loci appear to have arisen within the Drosophilidae via gene duplication from a still-functional parental copy; of these 11 duplicate pairs, eight involved X-to-autosome duplications (five via retrotransposition), and in four of these cases there are data indicating that the autosomal copy has testis-enriched expression (Marygold et al. 2007). These data demonstrate that haplo-insufficient loci can undergo interchromosomal gene movement, appear to be enriched for X-A movement, and that there is male-specific gene expression in the moved copy. Other fine-scale deletion studies have uncovered haplo-insufficient loci with male-sterility effects (Ryder et al. 2007), but more detailed gene-specific duplicate data are

not yet available. Based on the data currently available, however, these observations suggest there might be limited numbers of loci (perhaps <100) for which *D. melanogaster* is sensitive to gene-specific dosage effects. No equivalent data are available in other *Drosophila* species.

The available data on dosage-sensitivity in mammals are more disparate, but suggest that mammals have much greater sensitivity to gene-specific dosage effects than *Drosophila*. For example, dosage effects of gene duplications or deletions underlie dozens of human disease disorders (Lupski 1998), with phenotypic effects ranging from mental retardation to infertility (Shaw and Lupski 2004; Lupski and Stankiewicz 2006; Conrad and Antonarakis 2007). A rapidly growing literature in human medical genetics is focused on gene copy number variants (CNVs) as causes of disease (Lupski and Stankiewicz 2006); similar effects are seen in mouse (Inoue and Lupski 2002; Walz et al. 2004). Because these data have emerged primarily from disease studies, it is unknown how pervasive gene-specific dosage effects are across the entire genome. However, extrapolation from these studies suggests that hundreds of loci might exhibit deleterious effects in haploid or extra-diploid dosage. Overall, if these data from *Drosophila* and mammals are indicative of the potential consequences of dosage changes in a hybrid background, they provide suggestive evidence that sensitivity to dosage effects might be more pervasive in mammals, and therefore might contribute to the importance of gene movement as a mechanism of hybrid problems in this group. There are no comparable data on heterogametic species in groups outside mammals and *Drosophila*.

Sensitivity of X-inactivation to disruption

Gene movement involving the X chromosome can interfere with normal X-inactivation, including MSCI in the male germline (Table 1). Whether this process contributes to the Large X-effect depends on the sensitivity of X-inactivation to the presence/absence of individual genes on the X chromosome. Unfortunately there are few data on the frequency or magnitude of this sensitivity. In *D. melanogaster*, improper male MSCI can result from reciprocal X-A translocations that produce dominant male sterility (Lifschytz and Lindsley 1972; see Presgraves 2008), although these observations are from large-scale chromosomal movements rather than the movement of individual genes. In contrast, simply carrying an X chromosome gene insertion is not sufficient to cause fertility problems, as indicated by transgenic studies in which small X-linked insertions do not necessarily cause sterility effects (e.g., Hense et al. 2007). Conversely, in humans, autosome-to-X movement of short DNA sequences (≤1 gene) have been associated with ovarian dysfunction; however, in these cases the underlying mechanism appears to be improper dosage of the moved gene (due to X vs. autosomal position effects), rather than disruption of X-inactivation per se (Rizzolio et al. 2007). More data are necessary to assess the plausibility of this specific mechanism, and whether it differs between taxonomic groups.

Gene Movement and the Large X-Effect: Predictions

If gene traffic on and off the X chromosome does contribute to the Large X-effect, we can make several predictions about the expected occurrence and size of this effect given rates of gene traffic in particular systems, and the proposed consequences of this gene movement for hybrid fitness.

First, the Large X-effect is expected to be weaker in organisms that show no X chromosomal bias in gene traffic. Given the increasing ease of collecting whole genome information on gene movement, it will likely be easier to use patterns of gene movement to predict bias in the location of sterility loci than vice versa. This being said, there are species with sequenced genomes and evidence for the two rules for which data on gene movement could be collected (e.g., *Anopheles gambiae*; M. Toups and MWH, unpubl. data). As more data are collected on both gene movement and sterility, this prediction can be evaluated.

Second, in organisms with an X chromosomal bias in gene movement, the proportion of the genome that is X-linked versus autosomal should affect the observed size of the Large X-effect. In particular, we predict that the more autosomes within a genome (i.e., the more "targets" for X-A movement), the proportionally larger the expected Large X-effect, assuming autosomes and the X chromosome are of roughly equal size. To explain this prediction, consider a hypothetical genome in which there is only one pair of autosomes in addition to the sex chromosomes. In this case, all gene traffic between X and autosomes will involve this autosome, so that there is no expectation of a Large X-effect (traffic to the Y chromosome makes this expectation more complex). In comparison, in a genome with many potential autosomes as traffic partners (i.e., many gene movement recipients and/or donors), the X chromosome will appear to have a much larger individual effect on sterility in comparison to the average autosome effect. Appendix 1 outlines a formal treatment of the influence of the genomic X-to-autosome ratio on the predicted size of the Large X-effect resulting from X-A movement, and demonstrates that a larger effect of the X chromosome is expected when the X chromosome occurs in a genome with numerically more autosomes.

Third, some numbers of hybrid inviability or sterility loci that have already been mapped in species crosses are expected to be due to gene movements rather than conventional Dobzhansky–Muller incompatibilities. Although there are no definitive cases of X-A movement causing incompatibilities, Masly et al. (2006) have shown that gene movement from chromosome 3R to 4 is responsible for the expression of hybrid male sterility in later

generation hybrids between *D. melanogaster* and *D. simulans* (see Bikard et al. 2009 for a similar finding in *Arabidopsis*). The likelihood that mapped incompatibilities are due to gene movement, and which specific loci might be responsible, can also be evaluated by assessing whether gene movement has occurred within regions known to harbor such loci. For example, using deficiency mapping in *D. melanogaster-simulans* hybrids, Presgraves (2003) identified 23 regions conferring recessive hybrid lethality or semilethality (see Table 4 in Presgraves 2003). Of the higher and lower confidence gene movement events identified in Bhutkar et al. (2007), we found that 1 and 4, respectively, occur in these 23 hybrid inviability regions when considering gene movements that followed the *melanogaster–simulans* split only (the high-confidence movement is X-A). These data show that moved loci clearly overlap with known hybrid inviability regions and identify individual moved genes as potential candidates underlying these inviability effects. Note that hybrid inviability, not sterility, was the phenotype examined in (Presgraves 2003); ideally, this prediction can be evaluated for hybrid sterility regions (e.g., Tao et al. 2003) once appropriate genomic data are available. The dosage-sensitive or postmeiotically expressed moved genes we have highlighted above might also be good candidates for known hybrid sterility and inviability QTL in *Drosophila*.

Fourth, for loci that are involved in gene movements we can make specific predictions about their phenotypic effects in hybrids, especially for hybrid problems that are due to dysfunctional dosage. For example, if dosage compensation is achieved by hypertranscription of the X chromosome in males, for every X-A gene movement half of the F1 males will experience a deficit in gene expression of the moved gene and half will experience an excess (females will exhibit normal gene expression; Fig. 1, Fig. S1). That is, F1 males will have more transgressive gene expression phenotypes than females. There are currently no data on the relative prevalence of gene misexpression in male versus female hybrids, but this prediction could be tested using comparative whole-genome expression profiling of hybrid males and females in *Drosophila* and similar systems. In contrast, when somatic dosage compensation occurs through random X-inactivation in the homogametic sex and upregulation of the active X in both sexes (as in mammals), a gene movement hypothesis predicts transgressive somatic gene expression in both hybrid males and females (Table 1, Fig. 2). Accordingly, dosage dysfunction due to X-A gene movement in mammals should produce a Large X-effect for both male and female inviability. Data on when and where the Large X-effect predominantly acts in mammals are rare, but this expectation could be assessed with more direct analyses of which sexes and which developmental stages exhibit a large X-effect in these groups.

Finally, our predictions explicitly relate to male heterogametic systems, but should in principle be predictive in female heterogametic systems in which female sterility is preferentially observed. For example, if all biological processes influencing gene movement in male-heterogametic systems are identical in female-heterogametic systems (but simply apply to the alternative sex), we predict that Z-A gene movements preferentially involve female-biased rather than male-biased genes. To our knowledge, there are currently no data on the relationship between sex-biased gene expression and gene movement in female heterogametic systems, although it has been shown that genes with female-biased expression are underrepresented (and male-biased are overrepresented) on the Z chromosome in the female ZW chicken (Kaiser and Ellegren 2006; Mank and Ellegren 2009). Moreover, new data indicate that meiotic sex chromosome inactivation (MSCI) occurs during female ZW gametogenesis in chicken (Schoenmakers et al. 2009), supporting similar predictions for some hybrid effects of gene movement in ZW systems.

Gene Movement and Haldane's Rule

Although we have primarily focused on the influence of X-A gene movement on the Large X-effect, our analysis is also relevant to the other "rule of speciation"—Haldane's rule. Because the X is hemizygous, enrichment of hybrid incompatibility factors on the X can contribute to Haldane's rule if these factors act recessively (Turelli and Orr 2000). We have already discussed several mechanisms for F1 inviability or sterility due to X-A movements that are relevant to observations of Haldane's rule. In addition, gene movements that involve the Y chromosome can also potentially contribute to the expression of Haldane's rule in F1s (Figs. S2 and S4), although they do not contribute to the Large X-effect unless they involve X-Y gene movements (Fig. S2). The Y chromosome is known to have effects on male hybrid fertility in at least 10 *Drosophila* species crosses (reviewed in Turelli and Orr 2000). Recent data from *Drosophila* indicate that Y-linked gene content turns over rapidly among lineages, including at least two Y-linked gene losses, and multiple gene movements between the Y chromosome and autosomes or the X among species within the *Drosophila* lineage (Koerich et al. 2008). All such events create the potential for male-specific problems in species hybrids.

Overall, however, gene movement is arguably a stronger explanatory hypothesis for the Large X-effect than for Haldane's rule. This is in part because recombination and segregation of genotypes in F2 generations can reveal dysfunctional interactions, including those involving X-A gene movements, not present in F1 hybrids. Accordingly, X-A gene movements provide more potential mechanisms to explain the Large X-effect than to explain Haldane's rule, consistent with the general observation that the expression of inviability and sterility is generally stronger in later generations of hybrids (Coyne and Orr 2004). In addition,

no assumptions about developmental or reproductive effects of gene movements (e.g., dosage dysfunction, failure of postmeiotic gene expression, etc.) must be made in considering their possible role in the Large X-effect, in contrast to mechanisms of F1 inviability and sterility that are required to explain Haldane's rule (see above). Whether the assumptions underpinning our proposed mechanisms for F1 sterility are sufficiently reasonable, and the consequences sufficiently numerous, to substantially contribute to Haldane's rule will ultimately require more empirical data. Regardless, it is clear that other mechanisms also contribute to this sex-specific pattern (Orr 1997; Coyne and Orr 2004).

Conclusions

Elevated rates of gene movement involving the X chromosome, the overrepresentation of male reproductive functions among moved genes, and the potential for gene movement to lead to dysfunctional genetic consequences in hybrids are all well-established phenomena. However, the combined significance of these processes for patterns of reproductive isolation is underappreciated. Our hypothesis is that elevated traffic between the X and autosomes can lead to sex-specific and sex chromosome specific effects on hybrid inviability and fertility, effects that are consistent with the expression of the two rules of speciation. Given this, we argue that interchromosomal gene movement (via both relocation and duplication) is a plausible contributing mechanism to the two rules of speciation, and a hypothesis worthy of more empirical attention. Better estimates of rates of interchromosomal gene movements, their timing with respect to speciation events, and their phenotypic consequences, from a wider diversity of relevant systems, are required to resolve the relative contribution that gene movement might make to the two rules of speciation. In particular, it remains to be seen whether gene movement is sufficiently frequent and its consequences in hybrids sufficiently deleterious to substantially contribute to the two rules.

We recognize that both rules of speciation are likely to be due to the composite or joint effects of several underlying genetic mechanisms, only one of which might be gene movement. Gene movement alone is very unlikely to be an exclusive explanation of hybrid incompatibility between species. For example, the simplest gene movement hypothesis is one that suggests pairwise interactions underlie hybrid incompatibility (i.e., the chromosomal locations of both the original and the relocated homologue in the two diverged lineages). However, in *Drosophila* a more complex genetic basis (i.e., the involvement of three or more interacting loci) is commonly observed for hybrid male sterility (reviewed in Wu and Palopoli 1994; Coyne and Orr 2004). Although this "complex conspecific epistasis" (Coyne and Orr 2004) does not exclude a contributing role for moved genes, hybrid incompatibilities that require multiple interacting loci in one or both species are hard to reconcile with a gene movement model involving a single-gene relocation. In addition, of the half dozen examples in which the molecular genetic basis of hybrid incompatibility has been identified, several are unambiguously due to epistatic interactions between divergent genes, and suggest no obvious role for gene movement (Orr et al. 2007).

Although it is unlikely to be a complete explanation of the two rules, there are still attractive aspects of a gene movement hypothesis that make it worth further empirical assessment. First, it can provide an unambiguous mechanism for obtaining F2 and later generation hybrid problems, without the need for functional differentiation between lineages. Even in F1s, functional divergence is not required for gene movement to produce hybrid problems under specific developmental and reproductive conditions. Second, it suggests several empirically testable predictions. Indeed, because of the heroic efforts required to identify the underlying genetic basis of hybrid incompatibility, evaluation of the predictions we lay out above will likely be the easiest approach to evaluating whether gene movement could be important for the two rules of speciation. Assessing these predictions with data on gene movement, sex-specific gene expression, and hybrid incompatibility loci, in a broader range of systems—especially female heterogametic groups—will be particularly helpful. Third, it provides an underlying mutational mechanism for two previous hypotheses for one or both rules of speciation (i.e., dysfunctional X-inactivation and dysfunctional dosage compensation in hybrids). Fourth, it suggests that there might be interesting mechanistic differences underlying the two rules in different groups of species, depending upon their developmental and reproductive biology. In particular, gene movement will be most influential in lineages that are subject to strong gene-specific dosage sensitivity, frequent postmeiotic gene expression, and deleterious effects of small-scale structural changes on X-inactivation. The current literature indicates that these effects might be more important in mammals than in *Drosophila*, a testable proposition given more data from these groups. Interestingly, rates of X-biased gene movement are also substantially higher in mammalian lineages. Assuming that gene relocations do contribute to the two rules of speciation, it will also be interesting to assess the ways in which a mechanism of gene movement might produce different expectations from the classical Dobzhansky–Muller model of genic incompatibilities, upon which almost all current speciation genetics theory is based (Coyne and Orr 2004).

Finally, our model also suggests an unanticipated consequence of whole-genome sequence analysis—providing insight into a biological process as fundamental as the formation of new species. Indeed, if our model is correct, a complete explanation of the two "rules of speciation" should ultimately include an explanation for why gene movement preferentially involves the

X chromosome. Although several hypotheses have been proposed to explain this enrichment (Wu and Yu 2003; Ellegren and Parsch 2007; Sturgill et al. 2007; Vibranovski et al. 2009; Vicoso and Charlesworth 2009), none is well supported at present. Accordingly, resolving this question might become an unexpected but important goal in future studies of the genetics of speciation.

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Appendix 1

INFLUENCE OF THE RATIO OF SEX CHROMOSOMES TO AUTOSOMES ON EXPRESSION OF A LARGE X-EFFECT FOR HYBRID INCOMPATIBILITY, UNDER A GENE MOVEMENT HYPOTHESIS

Here, we formally demonstrate that the X-effect on hybrid sterility caused by gene movement increases relative to the average autosome-effect as the number of chromosomes increases, assuming that autosomes and the X chromosome are of equal size on average. That is, the Large X-effect gets larger as the sex chromosome to autosome ratio in a genome becomes smaller. For illustration, we present two simple cases followed by a general solution.

Case I: Single autosome and an X chromosome

Let x be the monoploid number (i.e., the number of chromosomes in a single nonhomologous set) of equally sized chromosomes. In the present scenario, $x = 2$ (one autosome and the X; Fig. A1). We define C as the set of chromosomal arms in the genome, such that in this example $C = \{X^{(1)}, X^{(2)}, A^{(1)}, A^{(2)}\}$, because there is one autosome and an X chromosome, each partitioned into two arms as denoted by the superscript. Let α be the rate in n/t units of interchromosomal gene movement between the X and the autosome, where n is the number of movements and t is an arbitrary time unit. Let β be the rate in n/t units of intrachromosomal movement between chromosome arms. We make the simplifying assumption that in hybrid crosses, recombination occurs between but not within chromosomal arms, and thus treat the rate of gene movement within an arm to be 0 (see also Fig. S5). As with normal Dobzhansky–Muller incompatibilities, each gene movement

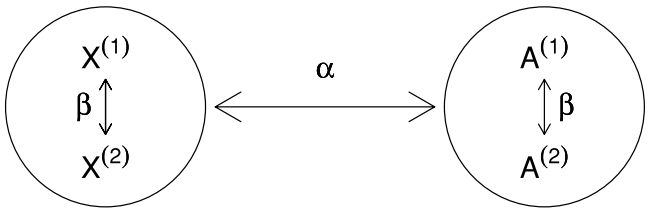

Figure A1. X-A gene movement when the number of unique homologous chromosomes (monoploid number) is 2. Chromosomes arms are assumed to be equally sized. α = rate of X-A gene movement; β = rate of both intrachromosomal and A-A gene movement. Although we have only drawn a single line connecting X and A, gene movement is taking place between all pairwise combinations of chromosome arms.

can give rise to a single incompatibility and two incompatibility loci, one on each chromosome arm. Assuming for simplicity that every gene movement gives rise to an incompatibility, the number of incompatibilities as function of time is $I(t) = 1/2n(t)$, where $n(t)$, the number of incompatibility loci as a function of time, is

$$n(t) = \sum_{i \in C} \sum_{j \in C} n_{ij} = 4(2\alpha + \beta)t$$

where n_{ij} is the number of movements from chromosomal arm i to chromosomal arm j. Note that $n_{ij} = n_{ji}$, because every gene movement must involve both chromosomal arms. The number of incompatibility loci on a given arm, a, of the X chromosome and autosome, respectively, is

$$\begin{aligned} n_{X^{(a)}} &= \sum_{j \in C} n_{X^{(a)}j} + \sum_{i \in C} n_{iX^{(a)}} \\ &= 2 \sum_{j \in C} n_{X^{(a)}j} \\ &= 2(2\alpha + \beta)t \\ n_{A^{(a)}} &= \sum_{j \in C} n_{A^{(a)}j} + \sum_{i \in C} n_{iA^{(a)}} \\ &= 2 \sum_{j \in C} n_{A^{(a)}j} \\ &= 2(2\alpha + \beta)t. \end{aligned}$$

The effect, defined as number of incompatibility loci, of the X chromosome relative to that of the autosome is thus

$$\frac{n_X}{n_A} = \frac{n_{X^{(1)}} + n_{X^{(2)}}}{n_{A^{(1)}} + n_{A^{(2)}}} = \frac{4(2\alpha + \beta)t}{4(2\alpha + \beta)t} = 1.$$

When the monoploid number $x = 2$, there can be no Large X-effect due to gene movement, because every movement involves both the X and the single autosome, and movement between arms of the same chromosome is, by assumption, the same on the X and autosome.

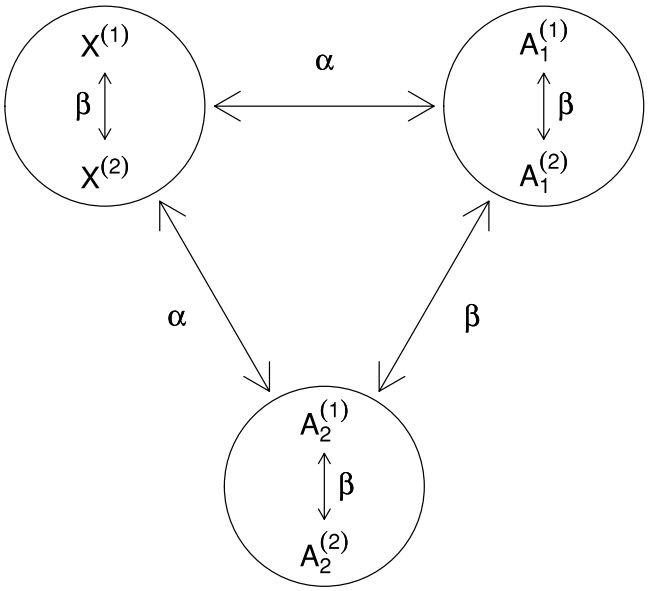

Figure A2. X-A gene movement when the number of unique homologous chromosomes (monoploid number) is 3. Chromosomes are assumed to be equally sized. α = rate of X-A gene movement; β = rate of both intrachromosomal A-A gene movement. Although we have only drawn a single line connecting chromosomes, gene movement is taking place between all pairwise combinations of chromosome arms.

Case II: Two autosomes and an X chromosome

In the next simplest case (monopoloid number $x = 3$) there is a single X chromosome and two unique autosomes (A_1 and A_2), all of equal size (Fig. A2). $C = \{X^{(1)}, X^{(2)}, A_1^{(1)}, A_1^{(2)}, A_2^{(1)}, A_2^{(2)}\}$, while α and β retain the same meaning as in Case 1. The number of incompatibility loci at time t is now

$$n(t) = \sum_{i\in C}\sum_{j\in C} n_{ij} = 4(8\alpha + 7\beta)t.$$

The number of incompatibilities on a given arm of the X and autosome is

$$n_{X^{(a)}} = 2(4\alpha + \beta)t$$
$$n_{A_i^{(a)}} = 2(2\alpha + 3\beta)t.$$

There will be a Large X-effect when

$$\frac{n_X}{n_{A_i}} = \frac{4(4\alpha + \beta)t}{4(2\alpha + 3\beta)t} > 1.$$

This condition is met when $\alpha > \beta$ (i.e., there is a higher rate of gene movement between X and autosomes than between autosomes).

General solution

We have shown that there can be no Large X-effect due to gene movement when $x = 2$, but that a Large X-effect can occur when $x = 3$. Here, we show that the trend toward increased Large X-effect with a smaller X to autosome ratio holds for all x so long as the rate of gene movement between the X and autosomes is higher than that between autosomes. In general, $C = \{X^{(1)}, X^{(2)}, A_1^{(1)}, A_1^{(2)}, \ldots, A_{x-1}^{(1)}, A_{x-1}^{(2)}\}$. The number of incompatibility loci as a function of the monoploid number and time is

$$n(x,t) = \sum_{i\in C}\sum_{j\in C} n_{ij} = 4\left[4(x-1)\alpha + \beta + \beta\sum_{i=1}^{2x-3} i\right]t.$$

Noting that the series $\sum_{i=1}^{n} i = \frac{n(n+1)}{2}$, this result simplifies somewhat to

$$n(x,t) = 4\left[4(x-1)\alpha + ([x-1][2x-3]+1)\beta\right]t.$$

The number of incompatibilities on a given arm of the X or an autosome is

$$n_{X^{(a)}}(x) = 2\left[2(x-1)\alpha + \beta\right]t$$
$$n_{A_i^{(a)}}(x) = 2\left[2\alpha + (2x-3)\beta\right]t.$$

There will be a Large X-effect when

$$\frac{n_X}{n_{A_i}} = \frac{4\left[2(x-1)\alpha + \beta\right]t}{4\left[2\alpha + (2x-3)\beta\right]t} > 1.$$

Again, this condition is met when $\alpha > \beta$. Next, let $\gamma = \alpha/\beta$, the relative rate of gene movement between the X and autosomes versus that between autosomes. After simplifying and substituting, the equation describing the Large X-effect as a function of monoploid number is

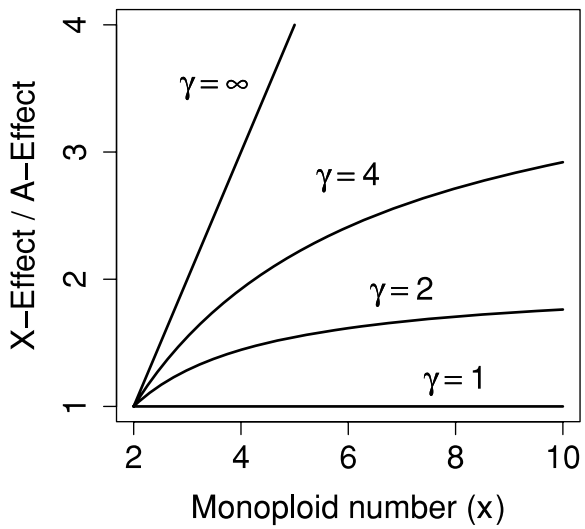

Figure A3. Relationship between the number of unique homologous chromosomes (monoploid number) in a genome and the relative magnitude of the Large X-effect (with respect to the average autosome effect) on hybrid incompatibility. Four values of γ (the relative rate of X-A vs. A-A gene movement) are considered. At γ = 1, there is no Large X-effect; at γ < 1, a "Small X-effect" is expected.

$$f(x) = \frac{2(x-1)\gamma + 1}{2(\gamma + x) - 3}.$$

Consequently, for $\gamma > 1$, the Large X-effect increases with increasing monoploid number (Fig. A3). Note that, in *Drosophila* species crosses (where the monoploid number is 4, although chromosomes are not equally sized) for observed values of the Large X-effect (i.e., X-Effect/A-Effect $\cong$ 2–3; see the main text), the expectation under this simple model is that $\gamma > 4$.

Supporting Information

The following supporting information is available for this article:

Figure S1. Hybrid dysfunction from X-A gene movement, where both ancestral and daughter gene copies are retained in one lineage.
Figure S2. Hybrid dysfunction from X-Y gene movement.
Figure S3. Hybrid dysfunction from A-A gene movement.
Figure S4. Hybrid dysfunction from Y-A gene movement.
Figure S5. Hybrid dysfunction from intrachromosomal gene movement.
Table S1. Mechanisms of sterility due to gene movement between X chromosomes and autosomes.
Table S2. Human–macaque orthologs differing in chromosomal location.
Table S3. Studies that associate male reproductive functions with gene movement.
Table S4. Postmeiotically expressed male genes in *D. melanogaster* and evidence for their chromosomal movement among *Drosophila* species.

Supporting Information may be found in the online version of this article.

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