PERSPECTIVES

The molecular basis of speciation: from patterns to processes, rules to mechanisms

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Abstract

The empirical study of speciation has brought us closer to unlocking the origins of life's vast diversity. By examining recently formed species, a number of general patterns, or rules, become apparent. Among fixed differences between species, sexual genes and traits are one of the most rapidly evolving and novel functional classes, and premating isolation often develops earlier than postmating isolation. Among interspecific hybrids, sterility evolves faster than inviability, the X-chromosome has a greater effect on incompatibilities than autosomes, and hybrid dysfunction affects the heterogametic sex more frequently than the homogametic sex (Haldane's rule). Haldane's rule, in particular, has played a major role in reviving interest in the genetics of speciation. However, the large genetic and reproductive differences between taxa and the multi-factorial nature of each rule have made it difficult to ascribe general mechanisms. Here, we review the extensive progress made since Darwin on understanding the origin of species. We revisit the rules of speciation, regarding them as landmarks as species evolve through time. We contrast these 'rules' of speciation to 'mechanisms' of speciation representing primary causal factors ranging across various levels of organization—from genic to chromosomal to organismal. To explain the rules, we propose a new 'hierarchical faster-sex' theory: the rapid evolution of sex and reproduction-related (SRR) genes (faster-SRR evolution), in combination with the preferential involvement of the X-chromosome (hemizygous X-effects) and sexually selected male traits (faster-male evolution). This unified theory explains a comprehensive set of speciation rules at both the prezyotic and postzygotic levels and also serves as a cohesive alternative to dominance, composite, and recent genomic conflict interpretations of Haldane's rule.

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Speciation genetics: a history in brief

The pace of scientific progress often follows a punctuated mode of change, and our understanding of how populations evolve into species is no exception. One hundred and fifty years ago, Charles Darwin and Alfred Wallace revolutionized biology by providing the much-needed mechanisms of natural selection and common ancestry to precursory evolutionary thought (Darwin *et al* 1858). Yet, for nearly eight decades after '*Origins*' was published, the very mechanisms that enabled species to evolve in isolation still remained unexplored. This general lack of attention on speciation mechanisms could be traced back to Darwin's treatment of species as nominal constructs. Darwin emphasized the transition from races (or populations) to varieties and species as a gradual continuum (Darwin 1859) and, for the most part, neglected the establishment of isolation factors that reduce gene flow among populations (Mayr 1942). Thus, while the species problem initiated the Darwinian revolution, the lack of an operational species definition constrained the investigation of the mechanisms involved in the formation of species.

It was only after the neo-Darwinian synthesis of the 1930's and 1940's that the species problem was seriously reexamined. Observations of such incompatibilities as lethality and sterility among hybrids of different species were finally reconciled with Darwin's gradualist view of population and species divergence. Population genetics played a central role by demonstrating that reproductive isolation evolves gradually as a byproduct of genetic divergence over time,

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and that problems associated with hybrid fitness are the result of an accumulation of deleterious epistatic interactions between species collectively known as 'Dobzhansky–Muller incompatibilities' (Dobzhansky 1937; Muller 1940). In other words, hybrid incompatibilities arise as a result of gradual accumulated genetic changes which may have been adaptive or neutral in their respective populations (Wallace 1890; Dobzhansky 1937; Muller 1940, 1942; Orr 1995; Johnson 2000; Gavrilets 2003). Not only did the Dobzhansky–Muller incompatibility model provide an elegant explanation to Darwin's difficulty in understanding the evolution of hybrid sterility (see chapter entitled '*Hybridism*' (Darwin 1859); also called 'Darwin's Dilemna' in Coyne and Orr 2004), but it also helped prompt the introduction of the biological species concept and catalyzed the investigation of isolation models (Dobzhansky 1937; Mayr 1942).

After the neo-Darwinian synthesis, another relatively long period of stasis in the field of speciation genetics ensued. The Dobzhansky–Muller model of reproductive isolation remained pretty much in the background, and speciation as a field of genetic investigation remained relatively inactive until Haldane's rule was resurrected 25 years ago (Bock 1984; Coyne 1985). Briefly, it was J. B. S. Haldane (a long-serving editor of this journal) who drew attention to the peculiar nature of species hybrid incompatibilities and pointed out that, "when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex" (Haldane 1922). Haldane's rule is followed remarkably well by a wide variety of animals including many insects, mammals (XY males), birds and lepidoptera (ZW females) (Wu and Davis 1993; Laurie 1997; Orr 1997), and is taken as a genetic footprint of speciation. The generality of Haldane's rule coupled with the appearance of a discrete phenotype (unidirectional hybrid sterility) provided geneticists with an experimental approach via backcross genetics to investigate the speciation problem. Dobzhansky first made use of backcross methods with *Drosophila pseudoobscura* and *D. persimilis* to study the genetic basis of speciation (Dobzhansky 1937) but due to the lack of sufficient phenotypic markers in his experimental system, did not pursue hybrids beyond the F_2 generation.

Since the late 1980's, studying the genetic basis of Haldane's rule using the backcross approach became the *tour de force* among speciation geneticists. Investigations of Haldane's rule have provided a great deal of information about the genetic basis of speciation by precipitating a diversity of explanations concerning its primary cause. Increased and more focussed attention on both empirical and theoretical grounds for understanding the genetic basis of Haldane's rule over the last two decades has identified new generalized patterns or rules. A second rule of speciation, the large effect of the X-chromosome on hybrid traits such as sterility, inviability and morphology, was identified by Coyne and Orr (1989a) in *Drosophila*. Further, through meta-analyses, primarily among species of the genus *Drosophila*, it was shown that sexual genes and traits tend to evolve faster and often are the only differentiating markers between closely related species (Civetta and Singh 1998; Singh and Kulathinal 2000), and premating isolation and hybrid sterility evolve faster than, respectively, postmating isolation and hybrid inviability (Coyne and Orr 1989b, 1997). Yet, as far as the investigation of speciation was concerned, Haldane's rule remained the predominant approach and provided a powerful framework for the study of postzygotic isolation.

Recent advances in genotyping and sequencing technologies have made the genetics of speciation accessible to a wider range of researchers studying a diverse cross-section of taxa. A new generation of genetic tools and resources are presently being deployed by speciation geneticists to track microevolutionary processes and phylogenetic relationships, in addition to understanding the genetics of species incompatibilities. This article focusses on the latter, i.e., elucidating the genetic and molecular basis of speciation (see figure 1 for a yearly growth comparison of publications in the speciation literature). We first provide an updated review of the various explanations of Haldane's rule—from genic to genomic to organismal. We then discuss other general patterns or 'rules' of speciation including those that have received less attention. We maintain that the observed faster evolution of premating isolation results from the faster evolution of sex and reproduction-related (SRR) genes and traits, and that this faster-SRR evolution also results in hybrid sterility generally evolving before hybrid inviability. We propose that a hierarchical faster-sex theory, unifying faster-SRR, hemizygous X-effects, and faster-male evolution, provides a general framework and sufficient explanatory power for not only Haldane's rule, but for other rules of speciation, thus connecting the rapid divergence of sex and reproductive-related traits to the evolution of reproductive isolation.

Explaining Haldane's rule

Ever since its rediscovery, Haldane's rule has facilitated the study of the early stages of speciation (Coyne and Orr 1989a). The Dobzhansky–Muller incompatibility model (Dobzhansky 1937; Muller 1940) provided a basic framework to understand why hybrid incompatibilities arise at different rates between males and females, and challenged researchers to explore all possible heterospecific interactions in the F_1 hybrid. For example, early explanations of Haldane's rule argued that differences between hybrid male and hybrid female incompatibilities were the result of imbalances between the X-chromosome and autosomes derived from divergent species (Muller 1942). Other early theories of Haldane's rule implicated heterospecific X–Y interactions (Haldane 1922; Coyne 1985). Also proposed were interactions between loci on the Y-chromosomes and autosomes, possibly driven by the fixation of deleterious genes on the Y-chromosome and the selection of modifiers on the autosomes

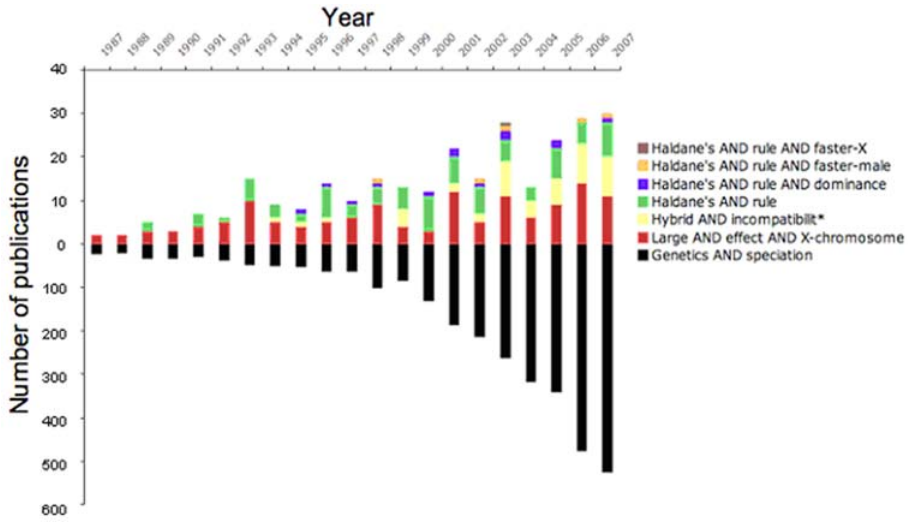

Figure 1. Number of publications per year for various terms in PubMed from 1987 to 2007. Publications that include the terms, 'hybrid incompatibilites' and 'Haldane rule', are in the top half, while publications that include, 'genetics' and 'speciation' are in the lower half. Note the difference in scale between halves. Terms and words were searched in the title and abstract. Searches were case insensitive and the boolean logic operator, 'AND', was applied.

(Zouros *et al*. 1988). Related to the X–Y interaction hypothesis were early incarnations of meiotic drive (Wu and Beckenbach 1983; Frank 1991; Hurst and Pomiankowski 1991; Dermitzakis *et al*. 2000). Findings of meiotic drive systems between species crosses (Mercot *et al*. 1995; Cazemajor *et al*. 1997; Montchamp-Moreau and Joly 1997; Orr and Presgraves 2000; Tao *et al*. 2001; Presgraves 2007) have revived interest in this mechanism based on the competing actions of autosomal and X-chromosomes (for more details, see section on genomic conflicts below). It was also argued that epigenetic conformational differences in the chromatin of sex chromosomes caused hybrid male sterility and the large effect of the X-chromosome on hybrid fitness (Jablonka and Lamb 1991). Additionally, incompatibilities involving non-nuclear genetic components were hypothesized to result in Haldane's rule. Heterospecific interactions, for example, between components of the nuclear genome and maternal effects (Sawamura 1996) as well as endosymbionts (Bordenstein *et al*. 2001) were previously shown to cause asymmetrical hybrid effects.

Past explanations of Haldane's rule have covered the bounds of chromosomal imbalance theories, as well as extended over a rather broad set of alternative hypotheses to incorporate other aspects of population biology, not just its chromosomal complement. Current explanations of Haldane's rule can be grouped into four categories: (i) those based on the hemizygosity of the incompatible sex, (ii) those based on the conflict between competing genomic elements, (iii) those based on the selective differences between male and females, and (iv) multifactorial causes. Below, we have highlight these four categories of Haldane's rule explanations (see table 1 for a detailed list).

Hemizygosity

That the sterile/inviable hybrid sex often contains a hemizygous chromosome (i.e., Haldane's rule) suggests that recessive factors may play an important role in the asymmetry of hybrid incompatibilities. A number of explanations for Haldane's rule specifically point to this difference in chromosomal endowment between the sexes. Currently, one of the leading hypothesis of Haldane's rule is dominance theory. This theory holds that most alleles causing hybrid incompatibility act as partial recessives in hybrids (Muller 1940, 1942; Orr 1993; Turelli and Orr 1995). Haldane's rule then follows since heterogametic hybrids show the full effect of any recessive X-linked incompatibility. Although containing twice the amount of incompatibilities, homogametic hybrids are heterozygous for the X-chromosome and are 'masked' from recessive incompatibilities. One simple prediction of dominance theory is a negative correlation between the size of the X-chromosome and the time required for heterogametic hybrid sterility to appear between a closely related species pair (Turelli and Orr 1995). This follows from the assumption that a larger X-chromosome would contain more hemizygous genes affecting hybrid incompatibility leading to the appearance of hybrid sterility sooner. In accordance to expectations, *Drosophila* species with large X-chromosomes are found to evolve hybrid sterility faster than species with relatively smaller X-chromosomes (Turelli and Begun 1997).

Higher rates of fixation on the X-chromosome versus autosomes can also explain Haldane's rule and may be due to the lower overall effective population size or, alternatively, more efficient selection on the X-chromosome in a male haploid environment (Charlesworth *et al*. 1987). This faster-X evolution also offers an explanation for the large effect of

Table. 1 Mechanisms of Haldane's rule.

Hemizygosity
X–Y interaction: Genetic changes and coadaptation between loci on the X and Y chromosomes within species leading to X–Y incompatibility between species. Most likely to affect fertility.
Y–autosome interaction: Evolutionary changes in the Y-chromosome and selection of autosomal suppressors within species leading to Y-autosome incompatibility between species. Most likely to affect fertility.
X–autosome interaction: Evolutionary changes in the X-chromosome and selection of autosomal suppressors within species can lead to X-autosome incompatibilities between species. Due to the large number of genes on the X-chromosome, it likely affects both fertility and viability.
Faster-X: X-linked loci evolve at a faster rate, and therefore have a greater chance of being part of a Dobzhansky–Muller incompatibility. This higher evolutionary rate may be due to greater selection on the hemizygous chromosome, or a lower effective population size relative to autosomes.
Conformational change: Evolution of X–Y conformational changes within species can lead to X–Y incompatibility and meiotic breakdown in hybrids.
Dominance theory: X–A incompatibilities are recessive or partially recessive on average and, therefore, are expressed first in the heterogametic sex.
Genomic conflicts
Endosymbionts / transposable elements: Incompatibilities between the X-chromosome and cytoplasmic or exogenous autosomal factors can lead to male (XY taxa) or female (ZW taxa) sterility.
Meiotic drive: Conflict between driver and suppressor elements on different chromosomes can lead to meiotic breakdown in hybrid males.
Faster-heterogametic sex: A revision of meiotic drive theory making it applicable to the conflict between the X-chromosome and autosomes in the heterogametic sex in general rather than to males *per se.*
Differential selection between sexes
Faster-male: Rapid evolution of male sterility due to stronger sexual selection in males or the higher sensitivity of spermatogenesis to genic imbalance in hybrid males. Only applies to XY male taxa.
Sexual antagonistic conflict: Rapid evolution of sterility due to pleiotropic effects of competing evolutionary strategies between the sexes.
Sex-biased gene flow: Sex-biased inferiority to gene flow varies according to the sex and chromosome involved. Heterogametic inferiority (in fitness) is a stronger barrier to reciprocal gene flow compared with homogametic inferiority. Such differential strength may affect divergence in speciation and result in Haldane's rule.
Multifactorial causes
Composite theory: This theory holds that Haldanes rule results from multiple causes and that no single explanation is sufficient for all taxa.
Hierarchical faster-sex: Faster evolution of sex and reproductive-related genes/traits in combination with sex-specific variable evolution of fitness modification by such factors as dominance and faster-male evolution. This theory is sufficient and applicable to all sexual taxa.

the X-chromosome relative to autosomes in hybrid sterility in *Drosophila* (Hollocher and Wu 1996; True *et al*. 1996; Tao *et al*. 2003). While a handful of studies found no evidence of a higher rate of X-chromosome evolution (Bauer and Aquadro 1997; Betancourt *et al*. 2002), other studies have found higher X-linked divergence (Thornton and Long 2002; Counterman *et al*. 2004) particularly those concerned with male-specific genes (Torgerson and Singh 2003; Stevison *et al*. 2004).

One problem with theories invoking the hemizygosity of the X (or Z) in many species outside the genus *Drosophila* is that the X-chromosome provides a relatively minute contribution to the total number of hybrid interactions. Many lepidopterans, for example, have much reduced X-chromosomes compared with *Drosophila* but have evolved hybrid sterility as fast, if not faster than *Drosophila* (Presgraves 2002). In addition, a significant underrepresentation of male-expressed genes harboured on the X-chromosome has been observed

in *Drosophila* (Parisi *et al*. 2003; Ranz *et al*. 2003) and *Caenorhabditis* (Reinke *et al*. 2000). A similar underrepresentation of late-acting X-linked spermatogenic genes in mammals has also been reported (Wu and Xu 2003), thus reducing the overall number of possible X-linked interactions. On the other hand, the hemizygosity of the X-chromosome may have made it more prone to evolve special genetic and epigenetic features involved in dosage compensation, gene inactivation, and achiasmy, making it more sensitive to hybrid incompatibilities (Jablonka and Lamb 1991).

Genomic conflicts

Alternatively, one may model the incompatible hybrid and its deleterious interactions as the consequence of an evolutionary arms race. Extracellular elements such as endosymbionts and transposable elements that have coevolved in a host may produce deleterious effects when placed in a hybrid background. *Wolbachia*-induced hybrid sterility was found to evolve at a much faster rate than hybrid sterility evolving via normal genic processes in the parasitic wasp *Nasonia* (Bordenstein *et al*. 2001). This example argues the case for incompatibilities resulting from newly formed interactions between the genome of the host and its invader.

Selfish interests also persist within the host's genome itself and the preferential involvement of the heterogametic sex in hybrid incompatibilities, i.e., Haldane's rule, may be a direct consequence of the conflict between linkage groups. Sexual dimorphism provides the potential for conflicting strategies between males and females to evolve at a very rapid rate (Rice 1984, 1996). One prediction is that, over time, sexually antagonistic genes should accumulate preferentially on the X-chromosomes (Rice 1984). Thus indirectly, sex chromosomes may become part of the raw material of tools for sexual selection to operate on.

In contrast to the 'battle of the sexes', a nonadaptive genomic-level explanation based on the conflict between the X-chromosome and autosomes has recently been proposed (Tao and Hartl 2003). The roots of this theory go back to early models of meiotic drive based on genetic systems involving driver and responder alleles that create sex-ratio distortion with distorters and suppressors on the X-chromosomes and Y-chromosomes (Frank 1991). With little or no recombination between them, X-chromosomes and Y-chromosomes are ideally suited for the evolution of meiotic drive and the genes involved are expected to affect spermatogenesis. Meiotic drive genes and their respective suppressors will behave as coadapted genetic systems within species, yet will be mismatched and noncoadaptive between species (i.e., in F_1 hybrids), with X-chromosomes and Y-chromosomes originating from different species. The evolution of X–Y meiotic drive genetic systems can explain Haldane's rule, as well as the large effect of the X-chromosome. One problem with X–Y meiotic drive theory is the small number of driver/target loci found on the Y-chromosome. In *Drosophila*, estimates are ~15 loci (Carvalho *et al*. 2001; Carvalho and Clark 2005). However, a recent study found that the gene-poor Y-chromosome may, in fact, control the expression of hundreds of autosomal and X-linked genes (Lemos *et al*. 2008). It is possible that distorters may target heterochromatin via RNAi-mediated silencing on other chromosomes, thus affecting chromosome condensation (Ferree and Barbash 2007; Tao *et al*. 2007b). In interspecific hybrids, disruption of normal condensation can result in the failure of sperm to develop to maturity.

In the recently proposed 'faster-heterogametic sex' theory (Laurie 1997; Tao *et al*. 2003), meiotic drive systems are assumed to affect gametogenesis in the heterogametic sex (instead of in males *per se*), and the drive generally involves the X-chromosome and autosomes instead of the X-chromosomes and Y-chromosomes. In *Drosophila*, earlier tests of the involvement of X–Y meiotic drive theory in Haldane's rule indeed produced negative results (Coyne *et al*. 1991; Coyne and Orr 1992; Johnson *et al*. 1992), but recent results based on X-autosomal interactions suggest otherwise (Dermitzakis *et al*. 2000; Tao *et al*. 2001). An X-autosome meiotic drive system provides a far greater number of interacting gene targets that can drive hybrid incompatibilities than earlier proposed X–Y meiotic drive systems.

Other recent studies have implicated the general mechanism of genomic conflict in speciation. Brideau *et al*. (2006) performed a rigorous genetic analysis of the Dobzhansky–Muller interaction pair, *Hmr* (from *D. melanogaster*) and *Lhr* (from *D. simulans*), which together had been reported to cause hybrid lethality. Their analysis, however, revealed the necessary presence of at least one more interacting locus. The authors suggest that genomic conflicts within species have generated an accumulation of diverged heterochromatin repeats and transposable elements that have become necessary components of this particular incompatibility. Presgraves (2007) also suggests that proteins involved in nuclear transport, including nuclear porins identified from Dobzhansky–Muller incompatibility screens of hybrid lethality in fruit flies (Presgraves *et al*. 2003), may also be rapidly evolving due to genomic conflicts.

Differential selection between the sexes

Haldane's rule exhibits a sex-biased phenomenon and as such warrants explanations based on sex-dependent selective differences between males and females. The faster-male hypothesis, the other leading hypothesis of Haldane's rule, attempts to explain the higher frequency of hybrid male sterility as compared with female sterility between taxa where the heterogametic sex is male (Wu 1992; Wu *et al*. 1996) and posits that sex-specific factors involved in hybrid sterility evolve faster in males than in females. The two main explanations offered by the faster-male hypothesis are stronger sexual selection on male fertility within species, and higher sensitivity of spermatogenesis to genetic and developmental perturbation (Haldane 1922; Wu and Davis 1993; Laurie

1997). Faster-male evolution has been demonstrated in a variety of taxa that follow Haldane's rule, including taxa with either male or female heterogamy (Wu and Davis 1993), taxa with no heterogamy such as certain mosquito species (Presgraves and Orr 1998), and even taxa that harbour patterns of incompatibility contrary to Haldane's rule (Malone and Michalak 2008).

Of course, the faster-male theory, in principle, should apply to both male-heterogametic and male-homogametic taxa, and comparative studies among the former show, as the theory would predict, relatively far more cases of hybrid-male sterility than female sterility or inviability (Wu 1992; Wu and Davis 1993; Wu *et al*. 1996). Molecular and morphological evidence supports the fact that both male and female sexual traits are evolving rapidly, although not equally (Civetta and Singh 1995, 1998). The reason behind more effective male evolution is not only male–male and sperm competition (Parker 1970) and female choice (Eberhard 1985), but also male sex-drive (Singh and Kulathinal 2005). Male sex-drive forces the male fitness to evolve faster as a result of their programmed desire to gain the upper hand in mating with females. Male sex-drive not only results in faster-male evolution, but also the evolution of new male-specific variants and traits. The origin of male-specific sexual traits and their effect on females was something that was also noticed by Darwin and he placed this under the broader banner of sexual selection (Darwin 1871).

Multifactorial causes

As an intriguing solution to the speciation problem, interest in explaining Haldane's rule intensified over the last two decades and understanding the genetics of speciation became synonymous with understanding the earliest stages of the genetics of postzygotic reproductive isolation. The first problem with this approach is that Haldane's rule attempts to summarize a general pattern based on taxon-specific factors. Different taxa possess varying degrees of selection, different sex chromosome complements, and differing sex-determination and dosage compensation mechanisms. Such parameters create clade-specific environments that may eventually generate the sex-biased asymmetries. The second problem with equating speciation to postzygotic reproductive isolation is that Haldane's rule itself is a composite rule that lumps together a number of factors (Wu and Davis 1993). The conviction that Haldane's rule represents the 'first stage of speciation' (Coyne and Orr 1989a) contrasts the premise that Haldane's rule is a composite phenomenon which includes both hybrid inviability and sterility.

Unfortunately, other general patterns of speciation have been neglected in order to work within the prescribed framework of Haldane's rule. Haldane's rule has certainly precipitated an impressive wealth of data and ideas, but in the process of cataloguing all the factors that cause it, less attention was paid to other early-acting patterns or rules. While representing an early stage of speciation, Haldane's rule certainly does not represent the first stage: the observation of sex-biased hybrid asymmetry (Haldane's rule) appears to be secondary to other primary rules of evolutionary change.

Rules of speciation

Below, we present an encompassing and progressive set of rules important for speciation to proceed, as well as briefly highlight some of the principal mechanisms that drive these rules. These rules represent general patterns that have been primarily observed in *Drosophila*, where much work on understanding the genetics of speciation has historically occurred. It remains to be seen if all these rules apply to other taxa and, if so, in what general capacity. We order these speciation rules as they are expected to appear during species divergence: from early to later stages of speciation.

Sexual genes and traits are rapidly evolving and generate novelty

The rapid evolution SRR genes and traits, especially during the early stages of speciation, has an immediate impact on fitness. Sex-related genes may evolve faster due to sexual selection (Lande 1981; Kirkpatrick 1982) or sexual conflict between the sexes (Rice 1996; Parker and Partridge 1998) and a large set of gene targets are often present in males. A variety of biological phenomena related to mating and reproduction provide a working foundation for the rapid evolution of sex and reproduction-related (SRR) genes. Some of these phenomena include sperm competition and female preference (Parker 1970; Civetta and Clark 2000), sexual antagonistic evolution (Rice 1984; Arnqvist *et al*. 2000; Knowles and Markow 2001; Rowe and Arnqvist 2002), coevolution between male and female traits at the molecular level (Swanson and Vacquier 2002), and male sex-drive (Singh and Kulathinal 2005). Together, these adaptive mechanisms constitute the faster-SRR hypothesis. The rapid evolution of sex genes addresses all levels of reproductive isolation and helps to reinforce other known speciation patterns (see below). Yet, the neutral alternative that sexual genes and traits may simply be under less functional constraint must also be entertained. We, however, note that these two hypotheses are not necessarily mutually exclusive. The presence of a larger genetic landscape potential may be instrumental in providing a larger cache of variants for selection to act upon and for novelty to take hold (Kulathinal *et al*. 2003).

Premating isolation evolves faster than postmating isolation

While postzygotic isolation provides a working model for most speciation geneticists, we must also not forget about the abundant examples of premating isolation barriers, such as mating behaviour, species-specific pheromones, and other barriers to reproduction between species. In a meta-analysis comparing hundreds of species hybridizations in *Drosophila*, Coyne and Orr (Coyne and Orr 1989b, 1997) demonstrated that, generally, premating isolation between species evolves well before postmating isolation. Much of this signal was

found among sympatric species pairs, but faster premating isolation has also been documented among natural isolated populations in a number of cases (Butlin and Ritchie 2001). For example, it was demonstrated that African populations of *D. melanogaster* display strong female mating preferences to males of the same population and discriminate against males from other populations (Hollocher *et al*. 1997b). This premating isolation is likely be driven by population-specific sexual selection. In plants, prezygotic isolation barriers are thought to contribute more to the total reproductive isolation than postzygotic barriers (Rieseberg and Willis 2007). In fact, many of these prezygotic barriers appear to be prepollination barriers that limit pollen transfer via mechanical, ecological, and temporal means. Postpollination, but still prezygotic, mechanisms including a greater difficulty in heterospecific fertilization as well as other gametic incompatibilities are common among plant species. However, while prezygotic isolation appears to be stronger in nature, it is more difficult to quantify than postzygotic isolation due to its more continuous and less binary nature (postzygotic isolation identifies sterile versus fertile hybrids and lethal versus viable hybrids).

Faster accumulation of hybrid sterility over hybrid inviability factors

It is becoming increasingly clear that hybrid sterility usually evolves faster than hybrid inviability among closely related species of *Drosophila* (Coyne and Orr 1989b), lepidoptera (Presgraves 2002), and birds (Price and Bouvier 2002). Moreover, the between-species ratio of hybrid sterile to hybrid lethal is much higher than the within-species ratio of sterile to lethal mutations, as measured in *D. melanogaster* (Lindsley and Lifschytz 1972). This enrichment of hybrid sterility is consistent with growing evidence that sex and reproduction-related genes and traits evolve faster than non-sex genes (Civetta and Singh 1995; Singh and Kulathinal 2000; Swanson and Vacquier 2002). Recent reports on the divergence of sex-biased transcripts in *D. melanogaster* (Parisi *et al*. 2003; Ranz *et al*. 2003) demonstrate that SRR genes comprise a substantive fraction of the transcriptome. These studies also suggest that sex-biased and sex-specific genes evolve faster than other genes (Rice and Chippindale 2002; Parisi *et al*. 2003; Ranz *et al*. 2003). Taken together, it is not surprising that fertility is more affected in the hybrid. Further, hybrid lethals may harbour hybrid sterility; hence, the relative contribution of hybrid sterility may be grossly underestimated (Barbash and Ashburner 2003).

Preferential involvement of the X-chromosome in hybrid incompatibilities (large X-effect)

The large X-effect has already been earmarked as the second of 'two rules of speciation', with Haldane's rule being 'the first rule' (Coyne and Orr 1989a). This observation originates from interspecific backcross hybrids: substituting an X-chromosome from one species has a disproportionate effect on hybrid fitness compared to an autosomal substitution of similar size. In other words, introgressing a X-chromosome into a hybrid background would generate a far worse sterility or inviability phenotype than would introgressing an autosome. This rule appears to also hold when using small and comparable X versus autosome introgressions that have been made homozygous in *Drosophila* (True *et al*. 1996; Tao *et al*. 2003; Masly and Presgraves 2007). A number of explanations have been put forward to explain this large X-effect including a higher density of hybrid male sterility factors, the faster evolution of X-linked genes, and epigenetic phenomena that affect the disruption of dosage compensation and germ-line X-inactivation.

Sexual asymmetry in Dobzhansky–Muller incompatibilities (Haldane's rule)

As a later-stage speciation rule, Haldane's rule is an amalgam of numerous mechanisms that are also the determinants of other speciation rules (table 2). The one position that most Haldane's rule enthusiasts have agreed upon is that any one single mechanism is not sufficient to explain its ubiquity. Currently, two major hypotheses are used to explain mechanistically why Haldane's rule is so well supported empirically in species with heterogametic males, such

Table 2. Explanatory capacity of various speciation mechanisms on rules of speciation.

Rules of speciation	Dominance	Faster-male	Composite	Faster-heterogametic	Faster-SRR	Hierarchical faster-sex
Preferential evolution of sexual traits	no	XY males	no	no	yes	yes
Faster premating than postmating isolation	no	yes	no	no	yes	yes
Faster hybrid sterility than hybrid inviability	no	yes	yes	yes	yes	yes
Large effect of the X-chromosome	yes	no	yes	yes	no	yes
Hybrid asymmetry (Haldane's rule)	yes	XY males	yes	yes	no	yes

as *Drosophila*. Faster-male evolution, the first leading hypothesis, provides a sufficient biological and evolutionary explanation for the evolution of hybrid male sterility in the early stages. Strong female mating discrimination (Hollocher *et al*. 1997a) and the presence of sperm competition (Clark *et al*. 1995) implicate selection on male traits in many species of *Drosophila*. Of course, faster-male evolution does not provide a common explanation across all taxa, particularly ones with female heterogamy.

Dominance theory, the other leading explanation of Haldane's rule, applies for both XY and ZW taxa, but fails to apply in a tested case of chromosomally nondimorphic taxa (mosquitoes) where males, despite being homomorphic, show higher reductions in hybrid male fitness (Presgraves and Orr 1998). The dominance theory makes the assumption that species-specific divergent alleles are incompatible in the heterospecific background but says nothing about the types of genes and networks diverging within species. As a general theory, dominance theory does not depend on the details as to why adaptive or neutral alleles should act as mostly recessive in the hybrids, why sexual traits appear to be affected more often than nonsexual traits in species hybrids (Civetta and Singh 1998), why premating isolation evolves faster than postmating isolation (Coyne and Orr 1989b, 1997), why hybrid sterility arises faster than hybrid inviability (Wu 1992), or why there are more male-sterility factors than female-sterility factors (Hollocher and Wu 1996; True *et al*. 1996; Sawamura *et al*. 2000; Tao *et al*. 2001). More recent versions of dominance theory (Orr and Turelli 1996; Turelli and Orr 2000) attempt to solve these problems by parameterizing such factors as the relative contribution of male versus female factors and the X-chromosome in order to produce a more comprehensive or 'composite' theory .

Hierarchical faster-sex theory of speciation

We still remain far from understanding the functional nature of genetic divergence during the early stages of speciation (Lewontin 1974). However, if we assume, as the evidence suggests, that sex-related genes and traits generally evolve faster than non-sex genes and traits, the evolution of hybrid incompatibilities can be treated as a two-level process (figure 2). We predict that (i) hybrid sterility would evolve faster than hybrid inviability as a direct result of the faster evolution of genes involved in sexual systems such as mating and gametogenesis, and (ii) the evolution of recessive factors on the hemizygous chromosome and faster-male evolution act simply as reinforcing agents of change.

We propose, by way of synthesis, a new hierarchical theory that is based on the large pool of sex and reproduction-related (SRR) genes and modified by hemizygous sex

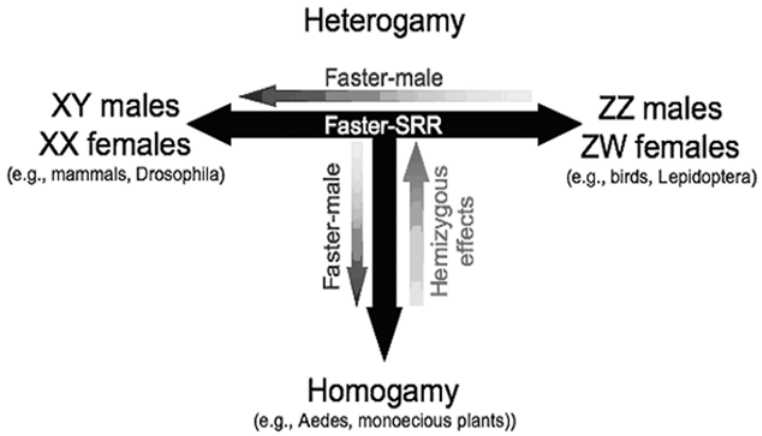

Figure 2. Hierarchical faster-sex theory and the relative effects of its components on generating Haldane's rule in various sexual taxa. The evolution of hybrid incompatibilities is a two-level process. At the primary level, faster-SRR evolution drives divergence in all taxa. Faster-SRR exemplifies the faster divergence, preferential novelty, and larger repertoire of genetic factors involved in sex and reproduction. At the secondary level, mechanisms involving hemizygous X-effects and faster-male evolution generate lineage-specific effects, depending on the relative contributions of, respectively, sex chromosomes and sexual selection in that taxa. Hemizygous X-effects embodies the effect of greater selection on hemizygous sex-specific genes, as well as the preferential role of recessive X-linked genes in generating asymmetrical hybrid incompatibilities (i.e., dominance theory). Preferential X-chromosome involvement may be also due to special chromatin effects from heterospecific sex chromosomal pairing or a greater density of incompatible factors on the X-chromosome. Faster-male evolution describes the more rapid evolution of male traits and genes.

chromosomes and sexual selection. This proposed unified theory, denoted as the 'hierarchical-faster sex' theory, provides a functional and complete explanation of the rules of speciation including Haldane's rule. The sequential order of evolutionary processes reflects the hierarchical role of sex-related genes, X-linked recessivity, and faster-male evolution. It is imperative to differentiate these evolutionary mechanisms from the so-called rules or empirical generalizations of speciation. Faster-SRR, hemizygous X-effects, and faster-male evolution are mechanisms that generate each of the five generalizations or rules of speciation. These rules are generally obeyed well (Coyne and Orr 1989b; Coyne and Orr 1997; Laurie 1997; Orr 1997) and can be categorized as a 'primary' rule (the rapid evolution of sexual traits) and 'secondary' rules (premating > postmating, hybrid sterility > hybrid inviability, large X-effect, and Haldane's rule) of speciation.

Although partially overlapping, a distinction must be made between the mechanisms of faster-SRR gene evolution and faster-male evolution. The former relates to the distribution of genes whose effects are primarily directed at fertility at the gene level, while the latter applies to sexually selected male genes affecting traits such as size and strength, acting primarily at the organismal level. Faster-male traits have a direct or indirect effect on male reproductive output and make up the subset of traits that are involved in female–male or male–male interactions (i.e., sexual selection).

The hierarchical faster-sex theory, as explained above, is basically an adaptive explanation as all its three sex-related components: faster-SRR evolution, faster-male evolution and, to a large extent, hemizygous X-effects, are based on genetic divergence between populations predominantly brought about by the action of selection. The hierarchical faster-sex theory, as presented here, presupposes that hybrid sterility is the result of the divergence of genes affecting predominantly fertility, and not simply a pleiotropic effect of general divergence at a large number of non-sex-biased loci. The faster-SRR, hemizygous X-effects, and faster-male components are distinct and, respectively, apply to three different levels of organization—genic, chromosomal and organismal. This makes the hierarchical faster-sex theory a unified, multilevel, and comprehensive explanation of Haldane's rule, as well as other rules of speciation (table 2).

Explaining the exceptions to dominance and faster-male theories

A cohesive theory should be able to explain the rules as well as their exceptions. Exceptions to the dominance and faster-male hypotheses of Haldane's rule can be explained by the hierarchical faster-sex theory (figure 2; table 2). First, the hierarchical faster-sex theory is applicable to both male-heterogametic and female-heterogametic taxa, as well as to non-heterogametic taxa (Presgraves and Orr 1998). Second, exceptions to dominance theory, such as the case of the mosquito (members of *Aedes* lack dimorphic sex chromosomes) can be explained by stronger sexual selection in males (Wu and Davis 1993). Third, the partial recessivity of genes causing sterility is only necessary in female heterogametic taxa and not male heterogametic taxa; faster-male evolution is sufficient to drive the evolution of patterns such as Haldane's rule in male heterogametic taxa. Fourth, the large effect of the X-chromosome observed in species crosses may be partly attributed to the faster divergence of sex-related genes only, and not all genes on the X chromosome (Torgerson and Singh 2003; Stevison et al. 2004). This is particularly noteworthy considering that there is an underrepresentation of SRR genes on the X-chromosome. Fifth, there need not be a correlation between the size of the X-chromosome and the evolution of hybrid sterility in lepidoptera (Presgraves 2002) as only a small number of sex-related genes may be involved for incompatibilities to arise. While, a correlation was found in *Drosophila* (Turelli and Begun 1997), the much reduced size of the X-chromosome in most other taxa that follow Haldane's rule points to a smaller contribution of hemizygosity. Sixth, the unified theory predicts a conflict between faster-male evolution and dominance theory in the case of female-heterogametic taxa such as birds and lepidoptera. Hemizygous X-effects will then favour the evolution of hybrid female sterility and the faster-male component would favour hybrid male sterility. Meta-analyses in birds reveal that both hybrid heterozygotes (females) and homozygotes (males) become sterile before they become inviable (Price and Bouvier 2002). The faster evolution of hybrid female inviability compared to hybrid male sterility in lepidoptera may be a pleiotropic effect of the divergence of sex-related genes.

Since the hierarchical faster-sex theory focuses primarily on the evolution of fertility and sterility—generally the initial stage of speciation—less attention can be afforded to the inviability aspect of Haldane's rule. That heterogametic inviability sometimes appears before homogametic sterility may best be explained by indirect arguments. We speculate that due to functional overlap between sex-related and inviability genes, hybrid lethality appears first in the same sex as hybrid sterility. Also, females are more likely to show correlated effects between hybrid sterility and inviability than males since, unlike males whose specialized stage of spermiogenesis makes them more prone to sterility; oogenesis exposes females to both sterility and inviability. For example, the only known case of viability rescue of F_1 females between *D. melanogaster* and *D. simulans* also restores female fertility (Barbash and Ashburner 2003). This may explain why, in many lepidoptera species, hybrid female inviability appears before sterility, and full female sterility and inviability before male sterility (Presgraves 2002). X-cytoplasm interactions may also be preferentially involved in the case of female inviability.

Hierarchical faster-sex versus meiotic drive theories

The relative importance of the hierarchical faster-sex versus the 'faster-heterogametic sex' (Laurie 1997; Tao and Hartl

2003) hypothesis is difficult to tease apart as both are similar in many respects. Both theories posit the initiation of the process of speciation by the evolution of genes whose main (drivers in the case of genomic conflict) or pleiotropic (hierarchical faster-sex theory) effects on hybrid fitness are negative, which in turn selects for fitness modifiers. Both theories can explain Haldane's rule in either XY and ZW taxa and both can explain the large effect of the X-chromosome. Further, these theories predict a relatively larger number of hybrid male sterility genes in XY taxa and hybrid female sterility genes in ZW taxa. A major difference, of course, is that the hierarchical faster-sex theory is based on selection affecting individual Darwinian fitness whereas the faster-heterogametic theory is based on selection affecting genomic (chromosomal) fitness.

Renewed interest in meiotic drive systems in natural populations, including the mapping of distorters and suppressors (Tao *et al*. 2007a,b), will provide a new dimension of data relevant to this ongoing debate. While many regions causing hybrid incompatibilities have been mapped, currently only a handful of genes have been identified. Mapping meiotic drive genes, hybrid sterility genes and hybrid inviability genes will reveal much about the mapped genes' functions and how they have evolved. For example, *Odysseus*, an X-linked gene involved in hybrid sterility between *D. mauritiana* and *D. simulans*, has been shown to be involved in spermatogenesis and has undergone accelerated evolutionary change in the *D. mauritiana* lineage (Ting *et al*. 1998)

An interesting approach to disentangle faster-heterogametic and hierarchical faster-sex theories is to explore the relative frequency of hybrid sterility and meiotic drive systems during the early stages of speciation. According to the faster-heterogametic theory, meiotic drive genes should accumulate at a relatively faster rate than other genes affecting normal sexual systems, premating or postmating. The difficulty in testing this prediction is that although the faster-heterogametic hypothesis posits that recurring bouts of meiotic drive will generate a genomic landscape of numerous coevolving factors, the result of this arms race is essentially hidden from view within species. A recent study tested for cryptic sex-ratio distortion among introgressed *D. sechellia/D. mauritiana* hybrids (Masly and Presgraves 2007). Such introgression studies serve to unmask hidden sex-ratio distortion. Among, over 50 fertile-introgression lines, they did not find a single line that displayed consistent sex-chromosome meiotic drive in hybrids. These results demonstrate the paucity of sex-ratio distortion processes in *Drosophila*. Further, since most non-*Drosophila* taxa harbour a relatively small X-chromosome, the evolution of X–A and X–Y meiotic drive systems will likely not be as extensive as observed in *Drosophila* and thus cannot provide a ubiquitous explanation for Haldane's rule. The presence/absence of sex-biased lines from fertile hybrid introgressions in female-heterogametic taxa such as lepidoptera would help elucidate which forces are of greater general importance.

Conclusions

Our understanding of how species form has profoundly evolved since Darwin and Wallace's revolutionary ideas were presented at the Linnean Society of London 150 years ago. Despite the long-term intractability of the genetic mechanisms of speciation, we have seen substantial recent progress in understanding how speciation advances. Haldane's rule has stimulated the genetics of speciation field by providing a firm investigative framework. In the process, more empirical generalizations or rules of speciation have been identified, including the rapid evolution of reproductive traits and genes, the faster progression of premating over postmating isolation, the early development of hybrid sterility over hybrid inviability, and the preferential involvement of the X-chromosome in hybrid sterility. The identification of such rules, even though they may prove more consistent in some taxa compared to others, allows us to evaluate the relative importance of different mechanisms of speciation. We believe that a renewed emphasis on studying the evolution of sex and reproduction-related traits and genes will significantly advance our current understanding of the speciation process, particularly among organisms that do not fall under the Haldane's rule banner (e.g., do not have heteromorphic sex chromosomes) or do not follow the rule itself. After all, speciation is primarily a problem among sexual organisms and it follows that its molecular bases should be based on mechanisms involving the role of sex and reproduction-related genes and chromosomes.

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