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REVIEW

Genetics of postzygotic isolation and Haldane's rule in haplodiploids

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The process of speciation has puzzled scientists for decades, but only recently they have they been able to reveal the genetic basis of reproductive isolation. Much emphasis has been on Haldane's rule, the observation that the heterogametic sex often suffers more from hybridization than the homogametic sex. Most research on Haldane's rule has focused on diploid organisms with chromosomal sex determination. We argue that species lacking chromosomal sex determination, such as haplodiploids, also follow Haldane's rule and thus should be included in the definition of this rule. We provide evidence for Haldane's rule in *Nasonia* wasps and describe how haplodiploids can be used to test the different theories that have been proposed to explain Haldane's rule. We discuss how the faster-male and faster-X theories can shape speciation differently in haplodiploids compared to diploids.
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Introduction

How new species arise has puzzled evolutionary biologists since the publication of Darwin's 'The Origin of Species'. Although much has been learned about speciation processes in one-and-a-half century of research, studies into the genetic basis of speciation are largely of recent time (Orr *et al*., 2004; Wu and Ting, 2004). Two main categories of reproductive isolation are traditionally distinguished: prezygotic isolation comprising all possible processes that prevent fertilization, and postzygotic isolation covering all phenomena of reduced fertility and viability of hybrid individuals (Coyne and Orr, 2004). The genetics of pre-zygotic isolation is mostly concerned with the genes that are subject to sexual selection, for example genes for courtship behaviour, chemical communication and colour patterns. The genetics of post-zygotic isolation is aimed at identifying the genetic causes for sterility and inviability of hybrids. As sterility and inviability may have many different causes, the genetics of postzygotic isolation appears to cover a larger array of genes.

Much effort is currently put into uncovering the (changes in) genes and molecules that lead to postzygotic hybrid incompatibilities. Such genes are sometimes referred to as 'speciation genes' and the field of study as 'genetics of speciation' (Orr *et al*., 2004). A number of genes causing hybrid sterility or inviability have been identified (Mallet, 2006; Noor and Feder, 2006), but details on their exact function and interactions with other genes are often still missing. Although it is too early to infer general patterns of gene interactions causing hybrid incompatibilities, these studies have already been instrumental in suggesting that hybrid dysgenesis may often be due to disrupted interactions between nuclear genes (nuclear–nuclear incompatibilities) or between nuclear and cytoplasmic genes (cytonuclear incompatibilities). A gene involved in causing nuclear–nuclear incompatibilities is the nuclear pore protein (nup96; Presgraves *et al*., 2003). The gene plays a role in cytonuclear trafficking of RNAs and proteins and causes inviability in hybrids between *Drosophila melanogaster* and *D. simulans*. Recent studies also point towards alterations in gene regulation as possible causes for hybrid incompatibilities (Barbash *et al*., 2003; Brideau *et al*., 2006; Landry *et al*., 2007). Research on cytonuclear incompatibilities has recently focused on the disruption of oxidative phosphorylation due to mismatches between nuclear and mitochondrial encoded subunits (Rawson and Burton, 2002; Ellison and Burton, 2006; Niehuis *et al*., 2008).

The role of reproductive mode in the evolution of reproductive isolation has received very little attention. Almost all animal species that have been studied in the context of the genetics of speciation are diploid and sexual, with a large focus on *Drosophila*. Interestingly, many genic incompatibilities in a variety of species involve the X or Z chromosome (True *et al*., 1996; Presgraves, 2003; Masly and Presgraves, 2007), and this lies at the basis of Haldane's rule. Haldane's rule states that when in the offspring of two different animal races, one sex is absent, rare or sterile, that sex is the heterozygous (heterogametic, that is, XY or ZW) sex (see below). In other words, the haploid sex chromosome in diploids appears to have a disproportionate effect on hybrid incompatibility. This opens the question to what extent our current knowledge of speciation genetics applies to other reproductive systems, such as

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haplodiploidy. Under haplodiploidy, males develop from unfertilized eggs and are haploid, whereas females arise from fertilized eggs and are diploid (Figure 1). Thus, in haplodiploids, the complete genome is always present in a single copy in males, similar to the sex chromosome in one of the sexes under heterogametic sex determination. Even though the system of haplodiploidy offers intriguing opportunities for testing existing theories on speciation, the number of studies focusing on postzygotic isolation in haplodiploids is still limited (Table 1).

In this paper, we discuss the role of haplodiploid reproduction in the genetics of postzygotic isolation. First, we consider whether and how Haldane's rule applies to organisms with haplodiploid reproduction in the absence of heteromorphic sex chromosomes. We argue that the explanations that have been proposed as the basis of Haldane's rule can also be applied to haplodiploids. We show how haplodiploids can be very instrumental in testing some of these hypotheses. Throughout we address a number of intriguing questions. If the haploid (heterozygous) sex chromosome is so important in the genetics of postzygotic isolation in diploids, what will be the effect if the whole genome is inherited in a haplodiploid fashion? Is the evolution of gene variants and gene regulation inherently different under haplodiploidy? Do genes that are male biased in their expression and always undergo selection in a haploid background diverge in a manner that is different from genes expressed in a diploid background? Our overall aim is to consider the effects of haplodiploidy on the evolution of postzygotic isolation and the possible implications for the rate of speciation in haplodiploid organisms.

Does Haldane's rule apply to haplodiploids?

Haldane's rule describes the phenomenon that in hybrids the heterozygous or heterogametic sex suffers more often from hybridization than the homogametic sex (Haldane, 1922). It has been established as one of the best-followed rules in evolutionary biology, and has been shown to apply to mammals, birds and insects (see Orr, 1997; Presgraves, 2002). As Haldane's rule describes only those cases where one sex suffers from hybridization, part of the research on Haldane's rule has focused on why some hybridizations result in differential sex-specific effects, whereas other hybridizations affect both sexes equally (Coyne and Orr, 1997). The results showed that for the more diverged taxa, both sexes tend to suffer

Table 1 Overview of studies on postmating and postzygotic isolation in haplodiploids

Species	*Type of reproductive isolation*	*Reference*
Leptopilina heterotoma	Cytoplasmic incompatibility	Vavre *et al*., 2000, 2001
Nasonia vitripennis	Cytoplasmic incompatibility	Breeuwer and Werren, 1990
longicornis		Bordenstein *et al*., 2003
*girault*i		Tram *et al*., 2006
*Nasonia longicornis girault*i	No hybrid incompatibilities	Bordenstein *et al*., 2000
*Nasonia vitripennis girault*i	F_2 male hybrid inviability, no sterility	Breeuwer and Werren, 1995 Gadau *et al*., 1999 Niehuis *et al*., 2008
Pachycrepoideus dubius	No incompatibility	Vavre *et al*., 2002
Tetranychus urticae	Cytoplasmic incompatibility	Vala *et al*., 2003 Gotoh *et al*., 2007
Tetranychus urticae turkestani	Cytoplasmic incompatibility	Breeuwer, 1997
Tetranychus urticae	F_1 female hybrid inviability and sterility	Perrot-Minnot *et al*., 2004
Trichogramma kaykai deion	F_1 female hybrid inviability and sterility	Jeong and Stouthamer, 2006
Trichopria drosophilae	Cytoplasmic incompatibility	Vavre *et al*., 2002

Most studies have shown that *Wolbachia* acts as an isolation mechanism by causing cytoplasmic incompatibility. In the strict sense, cytoplasmic incompatibility is prezygotic postmating isolation. Few studies have investigated the effect of hybridization at the genome level. They reveal that hybrid incompatibilities play a role in reproductive isolation.

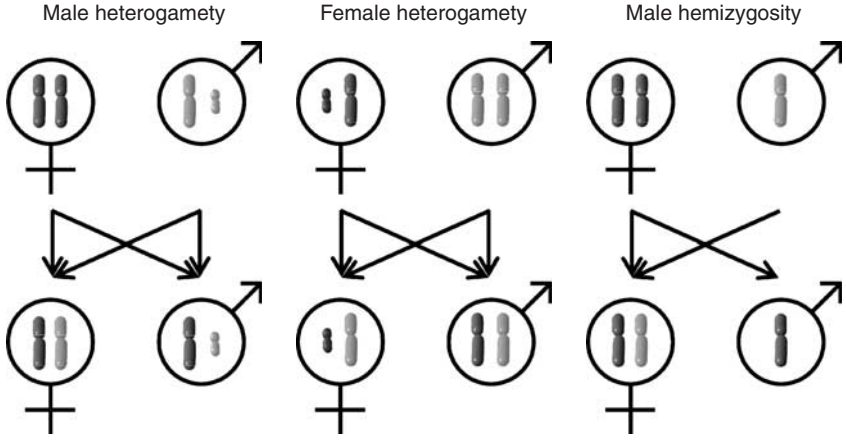

Figure 1 Inheritance under different sex determination mechanisms. Male hemizygosity includes XX-XO systems (only the sex chromosome is haploid) and haplodiploidy (the complete nuclear genome is haploid).

from the incompatibilities equally, whereas the less diverged taxa show more asymmetrical incompatibilities and thus tend to obey Haldane's rule. The conclusion from this study is that Haldane's rule applies to relatively early stages of the speciation process.

The simplest genetic model for explaining Haldane's rule is a two-locus two-allele system (reviewed by Orr, 1997). Both loci diverge independently in populations under isolation, through drift and/or selection. Upon reunion of both populations, the different alleles of the two genes are combined into a single individual. This results in negative epistatic interactions, the so-called Dobzhansky–Muller (DM) interactions (see Box 2 in Wu and Ting, 2004). Even though it is now widely accepted that DM interactions cause hybrid incompatibilities (Bierne *et al*., 2006; Payseur and Place, 2007), the reason why mostly the heterogametic sex suffers from these incompatibilities has found less consensus. The three main theories about the genetic basis of Haldane's rule (the dominance, faster-male and faster-X theories) will be discussed later.

Haldane's rule has now been shown to apply to 151 animal species-pairs with an XX-XY and 161 with a ZZ-ZW sex determining system (from Table 1 in Orr, 1997 and Table 1 in Presgraves, 2002). All these organisms are diploid and have chromosomal sex determination in which one sex is heterozygous and the other homozygous for a pair of heteromorphic sex chromosomes. A closer look at the three theories that seek to explain Haldane's rule shows that it can even be applied to species without chromosomal sex determination. Hence, Haldane's rule can be interpreted as an intermediate step in speciation that is applicable to all sexually reproducing organisms. Haplodiploids, which occur among Rotifera, Nematoda, Arachnida and a significant fraction of all insect species (Mable and Otto, 1998), form a large group of organisms that may obey Haldane's rule. Although the number of investigated haplodiploids is still limited, data indicate that haploid males suffer more from hybridization than diploid females when only one sex is affected by the hybridization (see below). However, to accommodate the inclusion of haplodiploids in the rule, its definition needs to be somewhat broadened into 'when in the offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterogametic or *hemizygous* sex'. Below we discuss how haplodiploids obey Haldane's rule.

The genetics of Haldane's rule and haplodiploidy

The dominance theory

Muller was the first to describe the mechanisms underlying Haldane's rule by introducing the dominance theory (reviewed by Orr, 1997). This theory assumes that there are two loci (a and b) interacting with each other: alleles a_1 and a_2 at locus a and b_1 and b_2 at locus b (a_2 and b_1 are fixed in species 1 and a_1 and b_2 are fixed in species 2), where a_2 and b_2 cause hybrid incompatibility. If these two loci are both autosomal, male and female hybrids have the same hybrid genotype ($a_1a_2b_1b_2$). Both alleles a_2 and b_2 have to be dominant in order for these hybrids to be inviable or sterile. If we assume that locus a is located on the X chromosome (under male heterogamety), there will be two effects: (1) males and females have different genotypes, and (2) the effect of hybridization on the males will depend on the direction of the cross. When a female of species 1 mates with a male from species 2, then male offspring will have $a_1b_1b_2$ genotype, which will not lead to hybrid incompatibilities. But if a female of species 2 hybridizes with a male of species 1, the male offspring will have $a_2b_1b_2$ genotype, whereas female offspring will have $a_1a_2b_1b_2$ genotype. For the female genotype to show hybrid incompatibilities still both a_2 and b_2 have to be dominant, whereas for the male genotype to show incompatibilities only the b_2 allele has to be dominant. Under such a scenario, incompatibilities have a higher chance of affecting males than females.

Under haplodiploidy, the dominance theory will lead to Haldane's rule only if the new mutations are (partially) recessive. Under haplodiploidy, females develop from fertilized eggs and are diploid, whereas males develop from unfertilized eggs and are haploid. This implies that the F_1 generation of a hybrid cross consists of hybrid females but pure-species males of maternal origin. The first generation of hybrid males is produced by the hybrid F_1 females. Although this is usually considered the F_2 generation, it is technically the F_1 hybrid male generation. The F_2 males will suffer more from the hybrid incompatibilities than the F_1 females because of their completely haploid genome. An interesting consequence of haplodiploidy is that DM interactions are not restricted to autosomes and the X chromosome as in diploids, but should occur between all autosomal pairs (Figure 2). The dominance theory is tested rather easily in the haplodiploids because all negative epistatic interactions are immediately expressed in haploid males.

The faster-male theory

Wu and Davis (1993) proposed that hybrid male sterility loci accumulate faster than hybrid female sterility loci, due to either the ease at which spermatogenesis is disrupted or sexual selection that drives a faster accumulation of mutations in males than in females. These simple assumptions lead to the second theory explaining Haldane's rule: the faster-male theory. This theory predicts that, because males evolve faster than females due to sexual selection, male hybrids have a higher chance of having disrupted gene interactions and thus show more incompatibilities than female hybrids. Evidence for faster evolution of males with regard to sterility was found by Hollocher and Wu (1996), Tao *et al*. (2003) and True *et al*. (1996), who performed introgression experiments with different *Drosophila* species. All these studies found more genomic regions causing sterility in males than in females. Meiklejohn *et al*. (2003) showed that male-biased genes have an accelerated rate of protein evolution when compared to genes that are female-biased in their expression and Parisi *et al*. (2003) found this increased rate of evolution only when comparing testes to ovaries but not when comparing somatic tissue. Metta *et al*. (2006), however, did not find faster evolution of the male genes in *D. pseudoobscura*, possibly due to a different action of sexual selection in this species.

Although many studies seem to support the faster-male theory, there are two problems with this theory.

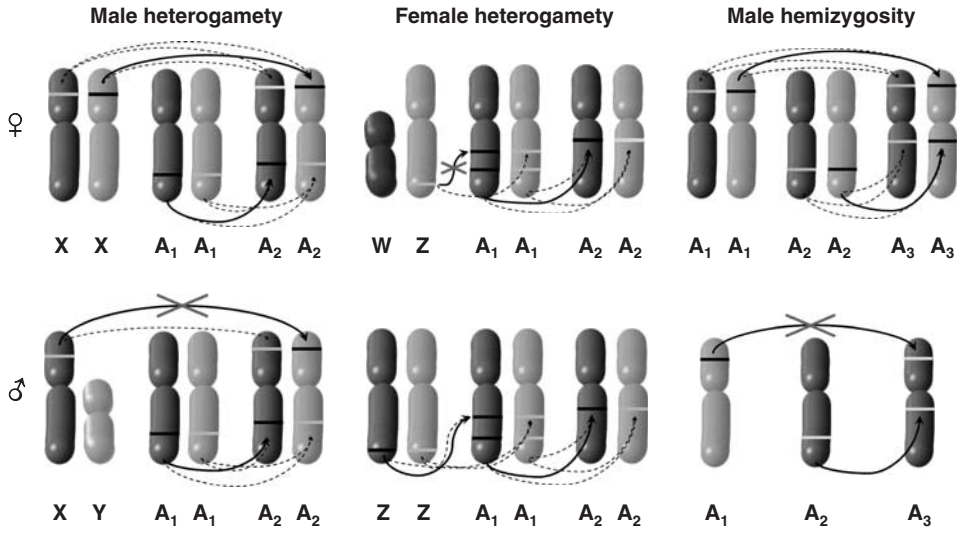

Figure 2 Simplified model of epistatic interactions in hybrids under different modes of sex determination. Differently coloured chromosomes are from different species. Dominant alleles are black and recessive alleles are grey. The epistatic interaction that is occurring is represented by the solid black arrow, whereas the epistatic interactions that do not occur due to recessiveness are represented by dotted arrow. Negative epistatic interactions are indicated by a cross through the arrow. A = autosome.

Table 2 Comparison of the different theories underlying Haldane's rule and their implications under different types of reproduction

	Diploidy		*Haplodiploidy*	
	Type of interaction	*Speed of gene divergence*	*Type of interaction*	*Speed of gene divergence*
Dominance theory	**Interaction between sex chromosome and any autosome**	Male-biased, female-biased and unbiased genes have same speed	**Interaction between any of the autosomes**	Male-biased, female-biased and unbiased genes have same speed
Faster-male theory	Interaction between any of the autosomes Interaction between genes involved in spermatogenesis	Male-biased genes have greater speed of divergence	Interaction between any of the autosomes Interaction between genes involved in spermatogenesis	Male-biased genes have greater speed of divergence
Faster-hemizygous-chromosome theory	**Interaction between sex chromosome and any autosome**	**Male-biased > unbiased > female-biased genes on X or Z chromosome**	**Interaction between any of the autosomes**	**Male-biased > unbiased > female-biased genes on whole genome**

Differences in predictions between haplodiploidy and diploidy are shown in bold.

First, it explains only sterility and not inviability. It is generally considered that male sterility loci do not affect female fecundity, whereas inviability loci affect both males and females (Lindsley and Tokuyasu, 1980; Johnson and Wu, 1993; Orr, 1993; Turelli and Orr, 1995; Hollocher and Wu, 1996; Coyne and Orr, 1998; Johnson, 2000). Moreover, male sterility appears to evolve faster than inviability, as shown by Hollocher and Wu (1996) and Tao and Hartl (2003), who found male sterility loci in a far greater frequency than loci causing lethality in *Drosophila* species. Scientists reconcile the faster-male theory with the 'inviability problem' by accepting that both the dominance and the faster-male theory are needed to explain Haldane's rule. The second problem with the faster-male theory is that it does not explain Haldane's rule under female heterogamety, where the female sex is more affected by hybridization. Again, scientists solve the 'female heterogamety problem' by accepting that Haldane's rule is a composite phenomenon that is not explained by only one theory (Wu and Davis, 1993). To explain Haldane's rule under female heterogamety, the forces of the dominance theory must be so strong that they overcome the faster-male effect.

If the faster-male theory is responsible for Haldane's rule in haplodiploids, then male-biased genes should diverge faster than the female-biased or neutral genes as in diploids. This increases the chance that the male sex suffers from hybridization. The difference between the dominance and the faster-male theory is that the faster-male theory predicts that genes with a male-biased expression diverge faster than female-biased or unbiased genes, and cause hybrid incompatibilities regardless of their dominance level. As under diploidy male sterility loci are expected to be distributed over the entire genome (see Table 2). This calls for studies of sex-specific gene expression and evolution in haplodiploids, which are not yet available to our knowledge.

The faster-X theory

The large X-effect refers to the observation that many of the regions involved in hybrid incompatibilities are

located on the X chromosome. Although many studies have provided evidence in favour of this effect (Masly and Presgraves, 2007 and references therein), results that contrast this have also been found (Hollocher and Wu, 1996). Charlesworth *et al*. (1987) had suggested that the X chromosome had a larger effect on incompatibilities than the autosomes and proposed the faster-X theory. It assumes that new beneficial mutations within a species' genome are recessive and would therefore accumulate more easily in a hemizygous state resulting in an increased rate of evolution of the X chromosome compared to the autosomes. A faster evolving X chromosome could lead to Haldane's rule if (1) the negative epistatic interactions are partially recessive and work between the sex chromosome and the autosomes (so in fact the dominance theory) or (2) the genes that cause hybrid incompatibilities affect only the heterogametic sex (if the faster evolving genes are expressed only in the heterogamous sex).

The faster-X theory is less supported by data than the dominance and the faster-male theories. True *et al*. (1996) found a higher density of male sterility loci on the X chromosome than on the autosomes, leading them to believe that this is due to faster accumulation of mutations. It leads to the prediction that mutations in genes on the X chromosome will have a male-biased or unbiased expression (the unbiased ones accumulate at a lower pace), whereas the female-biased genes on the X chromosome should have no increased mutation rate as compared to the autosomes.

At first sight, one might think that the faster-X theory does not apply to haplodiploids simply because they lack heteromorphic sex chromosomes. However, the underlying genetic mechanism of the faster-X theory could be very important for the evolution of haplodiploid species, because male hemizygosity of the complete genome might ease the fixation of beneficial mutations in male-biased and unbiased genes. Considering that the name of the faster-X theory is confusing for both female heterogamety and haplodiploidy, we will refer to this theory as the 'faster-hemizygous-chromosome theory' for the remainder of the article.

Under haplodiploidy, the faster-hemizygous-chromosome theory would give rise to Haldane's rule. As the whole genome goes through rounds of haploidy, *all* male-biased genes are expected to evolve faster, not just those located on the X chromosome as in diploids. Unbiased genes will diverge faster than female-biased genes because they are haploid in 1/3 of all cases under a 50:50 sex ratio (Table 2). Male-biased genes will have the largest divergence. This makes the distinction between the faster-hemizygous-chromosome theory and the faster-male theory less profound in haplodiploids when considering male-biased genes. In contrast, the difference between both theories is expected to be more clear when considering unbiased genes. Genes that are not sex-specifically expressed should be more diverged under the faster-hemizygous-chromosome theory than under the faster-male theory. A cautionary note is that under very low sex ratios (few males in the population), the unbiased genes will evolve mostly under diploidy because they exist for the larger part in females. Interestingly, if the faster-hemizygous-chromosome theory applies to hybrid incompatibilities in haplodiploids, it predicts that male-biased genes of haplodiploids diverge faster than those of diploids, due to the ease at which recessive mutations are selected for under haploidy.

Evidence for Haldane's rule in haplodiploids

Research on postmating and postzygotic isolation in haplodiploids has mostly revealed cytoplasmic incompatibilities due to *Wolbachia* infections (see Table 1). Studies focusing on genic incompatibilities in haplodiploids are scarce and usually do not proceed past the point of identifying the fitness effects of hybridization (see Box 1). In the mite *Tetranychus urticae* Perrot-Minnot *et al*. (2004) crossed different strains and in the parasitic wasp genus *Trichogramma* Jeong and Stouthamer (2006) crossed the two species *T. deion* and *T. kaykai*. Both studies found large hybrid incompatibilities in the F_1 females. This indicates that speciation has progressed past the point of Haldane's rule for these entities. However, there is a problem with identifying Haldane's rule in these cases. In diploids, a species can only undermine Haldane's rule when the homogametic sex suffers more from hybridization than the heterogametic sex. In haplodiploids, the effect of hybridization on males cannot be measured when the hybrid females are sterile or inviable, because hybrid males can only be produced in the F_2 generation by breeding F_1 hybrid females (Figure 1). Therefore, it is difficult to determine how many haplodiploid species follow Haldane's rule. But for those haplodiploid species for which hybrid breakdown of F_2 males has been found, pinpointing the genetics underlying Haldane's rule remains important.

Nasonia wasps have been used extensively in studies of the genetics of postzygotic isolation. Breeuwer and Werren (1995) looked at hybrid incompatibilities in crosses between *N. vitripennis* and *N. giraulti*. Slightly fewer F_1 females were found in one hybrid cross compared with the control crosses; however, most

Box 1 The biology of *Nasonia*

Haplodiploid wasps of the genus *Nasonia* have become a model system in speciation research. *Nasonia* are 2–3 mm large pupal parasitoids of several fly species and reproduce by arrhenotokous haplodiploidy; the males develop from unfertilized haploid eggs, whereas the females develop from fertilized diploid eggs. The *Nasonia* genus consists of three sister species: *Nasonia vitripennis*, *N. longicornis* and *N. giraulti*, with a putative fourth species *N. oneida* (J. Werren, personal communication). The cosmopolitan *N. vitripennis* was the first species to be described (Whiting, 1967), whereas *N. longicornis* and *N. giraulti*, occurring in western and eastern USA respectively, were discovered much later (Darling and Werren, 1990). The three species differ mainly in male wing size (Darling and Werren, 1990) and courtship display (van den Assem and Werren, 1994). All three species are reproductively isolated due to infection with different strains of *Wolbachia* (Bordenstein *et al*., 2001, 2003), but *Wolbachia* infections can be cured by antibiotics, which makes interspecies crosses possible. Various levels of prezygotic isolation due to differences in mating behaviour occur (van den Assem and Werren, 1994; Bordenstein *et al*., 2000; Beukeboom and van den Assem, 2001). However, as interspecific mate discrimination is not complete, curing and mating different *Nasonia* species opens up the possibility of studying the genetic basis of species differences and of reproductive isolation (Breeuwer and Werren, 1995; Bordenstein *et al*., 2001; Niehuis *et al*., 2008).

surviving females were viable and fertile. The fact that slightly fewer females were produced could not be linked to F_1 mortality because the egg production of the mothers was not measured. Data on crosses between *N. vitripennis* and *N. longicornis* show that the egg production in hybrid crosses is decreased without increasing the F_1 mortality (T Koevoets, unpublished results). The egg-to-adult survival probability of F_2 males was lower for the hybrids than the controls. In addition, the reciprocal hybrid crosses differed in the egg-to-adult survival probability, indicating both nuclear–nuclear and cyto-nuclear incompatibilities. To pinpoint the genic interactions that cause the hybrid breakdown between *N. vitripennis* and *N. giraulti*, Gadau *et al*. (1999) and Niehuis *et al*. (2008) mapped transmission ratio distortion loci in F_2 hybrid male adults. They identified multiple incompatibility loci, which were all dependent on the cytotype of the hybrid. However, the genes involved in the incompatibilities remain to be identified.

Sterility versus inviability

The studies by Breeuwer and Werren (1995), Gadau *et al*. (1999) and Niehuis *et al*. (2008) clearly show that F_2 hybrid males within the *Nasonia* genus suffer from inviability. However, Breeuwer and Werren (1995) and Bordenstein *et al*. (2001) found no evidence for sterility of hybrid males, although behavioural sterility in *Nasonia* hybrids has been reported (Beukeboom and van den Assem, 2001). This lack of hybrid sterility is remarkable because the study of *Drosophila* species has shown that sterility tends to evolve before inviability (Coyne and Orr, 1997). There are two possible explanations for lack of sterility in *Nasonia* hybrids. First, hybrid incompatibilities causing inviability in haplodiploids may be more frequent than those causing sterility. The reason is that incompatibility loci in haplodiploids can be spread over the whole genome and are not restricted to X or Z chromosome and autosome interactions. Second, as F_2 hybrid males are produced by F_1 hybrid females, these males can only be produced if the F_1 female is fertile. Therefore, there is selection for fertility in the first generation, which could also increase the fertility of the males of the next generation. There are two arguments that argue against this in *Nasonia* F_2 hybrid males: (1) there is no direct link between genotype and sterility because all F_1 females are similar (highly inbred lines were used and so all females are identical), and (2) female sterility loci are likely to be different from male sterility loci (Coyne, 1985).

Conclusion

Genetics of speciation research is gradually expanding towards a larger variety of organisms. We have argued that species with a haplodiploid mode of reproduction should receive more attention. They have traditionally not been included in research into the genetic basis of Haldane's rule. We have shown how the different theories that try to explain Haldane's rule also apply to haplodiploids and how haplodiploids can be used to test the genetic basis of Haldane's rule. We propose to slightly re-formulate Haldane's rule to include not only sexual species with a partial haploid genome (X or Z chromosome), but also those with a complete haploid genome (males under haplodiploidy). Although more haplodiploid taxa need to be studied, results thus far indicate that the haploid (male) sex is more often affected by hybrid dysgenesis than the diploid (female) sex. This is consistent with the dominance theory, but can also be explained with the faster-male and faster-X theory.

With the increased accessibility of microarray technology, gene expression studies are starting to make their entry into genetics of speciation research. This technology is crucial for distinguishing between the three main theories underlying Haldane's rule, by comparing the divergence of sex-specifically expressed genes between species. The dominance theory does not imply differences in evolutionary speed in genes with sex-biased expression. In *Drosophila*, more than 50% of the genes show a sex-specific expression (Ellegren and Parsch, 2007). The faster-male theory predicts that male-biased genes evolve faster than female-biased genes because sexual selection acts stronger on males. The faster X-theory makes the same prediction but the selective force in this case is the haploid background of the X-linked genes. Under haplodiploidy, all male-biased genes are selected in a haploid genetic background. Under the faster-X theory, we would therefore expect higher degrees of postzygotic isolation in haplodiploids as compared to diploids. If this is true, we may also expect stronger reinforcement to prevent maladaptive hybridization in haplodiploids. This, in turn, may lead to higher speciation rates in haplodiploid taxa. To our knowledge, comparative tests of speciation rates between haplodiploids and diploids have never been performed. Closely related taxa that differ in these reproductive modes may be particularly interesting to compare, such as haplodiploid and diploid beetles and mites. Stronger postzygotic isolation mechanisms in haplodiploids may also have contributed to the enormous diversity of the Hymenoptera with over 1 million estimated species.

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