The molecular evolutionary basis of species formation

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Abstract | All plant and animal species arise by speciation — the evolutionary splitting of one species into two reproductively incompatible species. But until recently our understanding of the molecular genetic details of speciation was slow in coming and largely limited to *Drosophila* species. Here, I review progress in determining the molecular identities and evolutionary histories of several new 'speciation genes' that cause hybrid dysfunction between species of yeast, flies, mice and plants. The new work suggests that, surprisingly, the first steps in the evolution of hybrid dysfunction are not necessarily adaptive.

Speciation occurs when populations, usually evolving in geographic isolation for extended periods, accumulate genetic differences that upon secondary contact cause reproductive incompatibilities[1]. New species may be isolated from one another by incompatible mating signals that prevent interbreeding, by incompatible ecological adaptations that when combined in hybrids render them unfit in either parental habitat, or by incompatible gene interactions that cause intrinsic hybrid dysfunction (for example, hybrid sterility or inviability)[2,3]. For most taxa, hybrid dysfunction is rarely the first form of reproductive incompatibility to evolve between species, but it is the only one that, once complete, is irreversible[4]. There are several genetic routes to the evolution of hybrid dysfunction. Polyploid formation in plants[5], the evolution of chromosomal rearrangement differences (for example, centric fusions and translocations)[6], and even infectious agents, such as the *Wolbachia* species of cytoplasmic bacteria[7], can contribute to hybrid dysfunction. But by far the most common route to the evolution of hybrid dysfunction is the incidental accumulation of incompatible gene interactions. As Dobzhansky[3] and Muller[4] showed, substitutions that are adaptive or nearly neutral in their own genomic background can be functionally incompatible with alleles that are present in foreign genomic backgrounds, causing hybrid sterility or inviability (BOX 1).

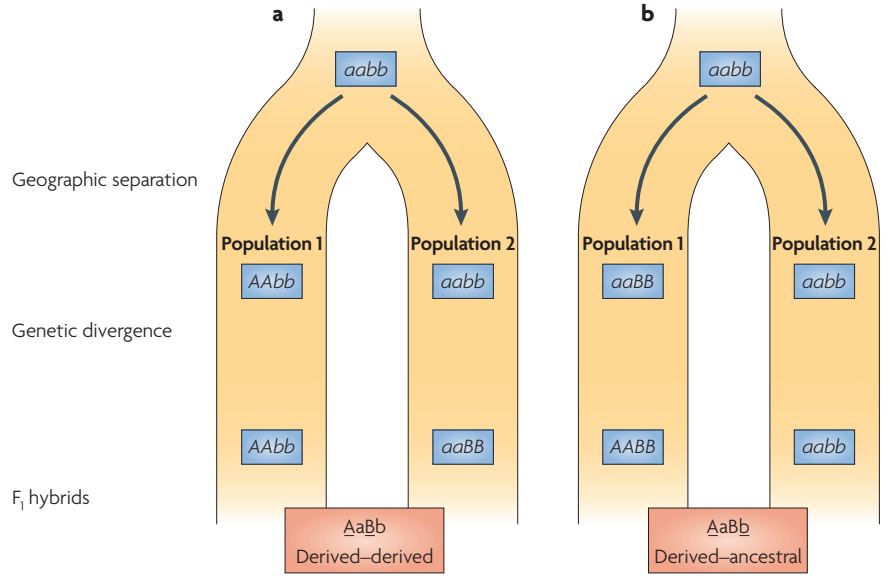
The Dobzhansky–Muller model has guided genetic analyses for more than 60 years, but only recently have some of the speciation genes that cause hybrid dysfunction been identified in yeast, mice, flies and *Arabidopsis* spp. (TABLE 1). Here, I review recent progress in our understanding of the normal functions of these genes within species, their hybrid phenotypes, and the population genetic forces that drive their interspecific divergence. Several genes that contribute to hybrid dysfunction between domesticated plant varieties have also been identified[8–11], but I will focus primarily on non-domesticated species. A surprising pattern has emerged from the still small but rapidly growing sample: contrary to the classic model of speciation — in which hybrid dysfunction evolves between species as an incidental by-product of their adaptation to different ecological niches[12] — the new findings suggest that the first steps in the evolution of hybrid dysfunction are not necessarily adaptive. Instead, as shown by the examples presented below, hybrid dysfunction often evolves as a by-product of the initial evolution of nearly neutral or, alternatively, selfish genetic changes that secondarily elicit adaptive compensatory changes at interacting loci.

Mutation pressure and hybrid dysfunction

Divergence by mutation-driven co-evolution. The feasibility of ecology-driven evolution of hybrid dysfunction has been demonstrated in experimental studies in fungi[13], but few good natural examples exist. One possible case of an ecologically based nuclear–mitochondrial hybrid incompatibility has been characterized between the yeast species *Saccharomyces cerevisiae* and *Saccharomyces bayanus*[14]. Hybrids homozygous for the *S. bayanus* allele of ATPase expression 2 (*Sb-AEP2*) in an otherwise *S. cerevisiae* genetic background suffer a respiratory defect and sporulation failure. The respiratory defect can be rescued by *S. bayanus* mitochondria, which implicates a gene encoded by *S. cerevisiae* mitochondria (but not any nuclear genes) as being incompatible with *Sb-AEP2*. Within species, the Aep2 protein normally binds the 5′ UTR region of the mitochondrially encoded oligomycin resistance 1 (*OLI1*) mRNA to facilitate translation. But, in hybrids, Sb-Aep2 fails to translate *Sc-OLI1* mRNA. The sequences of *AEP2* and the *OLI1* 5′ UTR have both evolved quickly since the *S. cerevisiae*–*S. bayanus* split. One possibility is that *AEP2* and *OLI1*, being involved in respiration, adapted to alternative carbon resources. Indeed, in competition experiments, *S. cerevisiae* reproduces faster in a glucose medium, whereas *S. bayanus* reproduces faster in a glycerol medium[14]. Hybrid dysfunction, in this case, might therefore have evolved as a by-product of ecological adaptation to different nutrient sources.

There is, however, a strong non-adaptive alternative[15]. To generate cellular energy, the *Saccharomyces sensu stricto* species have come to rely on fermentation more than respiration, even under aerobic conditions. This shift away from respiration seems to have entailed a relaxation of selective constraints on mitochondrial genes and hence an accelerated rate of substitution[16]. The mutation-driven rapid evolution seen at the 5′ UTRs of protein-coding genes of the *Saccharomyces* spp. mitochondria is mirrored by rapid evolution at their nuclear-encoded translation activator proteins[17]. Similar nuclear–mitochondrial hybrid incompatibilities have been reported

Box 1 | **The evolution of incompatible gene interactions between species**



Hybrid dysfunction does not, in general, result from an evolutionary change at a single locus. The reason is that if an ancestral population with genotype *aa* splits into two descendant species with genotypes *aa* and *AA*, the *Aa* genotype in which the *A* mutation first arose must at the very least be viable and fertile. Hybrids with the same single-locus *Aa* genotype cannot therefore be sterile due to an incompatibility between *A* and *a* alleles. Instead, hybrid dysfunction must usually be the result of evolution at two (or more) loci. Briefly, two substitutions that are individually innocuous or beneficial in their respective genetic backgrounds can be incompatible, causing, for example, sterility or inviability when they are brought together in hybrids (see the figure above). Incompatibilities can occur between two alleles that are functionally derived in the two separate species lineages (for example, alleles *A* and *B* in part **a**) or between an allele that is derived in one lineage but that retains the ancestral state in the other lineage (for example, alleles *B* and *a* in part **b**). In both cases, however, hybrid dysfunction results from incompatible epistatic interactions between genes that have diverged functionally between species. Determining what causes the evolution of incompatible substitutions is a major goal of speciation genetics.

between *Nasonia* wasp species[18] and between *Tigriopus* copepod populations[19], two taxa with exceedingly high mitochondrial mutation rates. Together, these cases suggest that some hybrid incompatibilities evolve as by-products of mutation-driven processes, perhaps accompanied by compensatory evolution at interacting nuclear genes, rather than ecological adaptation *per se*.

Hybrid dysfunction as a consequence of gene movement. Hybrid incompatibilities can evolve through another mutation-driven process: the duplication of genes, followed by the passive mutational silencing of alternative functionally redundant gene copies, can cause closely related species to have essential gene functions in different genomic locations[4,20]. As a result, some second filial generation (F_2) hybrids can have double-null genotypes, which bear only non-functional paralogous gene copies — one from each parent species — and no functional copies (FIG. 1). Genetic incompatibilities involving silenced duplicate genes have now been found segregating within species. In *Arabidopsis thaliana*, two recently duplicated copies of the histidinol-phosphate aminotransferase gene exist — *HPA1* on chromosome 5 and *HPA2* on chromosome 1 — but in 22 of 30 geographic isolates, one or the other gene copy has been incapacitated by degenerative mutations[21]. As a result, ~25% of pairwise crosses among geographic isolates suffer a two-locus genetic incompatibility that kills one-sixteenth of F_2 progeny — those that lack functional copies of either *HPA2* or *HPA1*.

Genetic incompatibilities involving silenced duplicate genes have also been found fixed between species. The male fertility-essential gene, *JYalpha*, is located on the fourth chromosome in *Drosophila melanogaster* but has moved to the third chromosome in *Drosophila simulans*. F_2-like hybrid males that are homozygous for the *D. melanogaster* third and the *D. simulans* fourth chromosomes are therefore sterile, as they lack any functional copies of *JYalpha*[22]. The frequency with which gene movement contributes to hybrid incompatibilities will undoubtedly vary among taxa with differences in the rate of gene duplication[23]. Along with the piecemeal duplication of individual loci, polyploidy events provide a sudden abundant substrate for the reciprocal loss of duplicated genes. A whole-genome duplication event occurred in the common ancestor of the *Saccharomyces sensu stricto* species complex, and the subsequent massive loss of redundant gene copies coincides with the emergence of several new yeast species[24].

Molecular arms races

Arms races with pathogens. Genetic incompatibilities can only occur between interacting genes that have functionally diverged from one another. It is perhaps unsurprising then that many of the hybrid incompatibility genes identified so far have evolved rapidly. Indeed, most show evidence of recurrent bouts of positive natural selection, as might be expected for genes caught up in open-ended molecular evolutionary arms races. Some of these arms races seem to involve ecological interactions with pathogens. In plants, for instance, genes involved in pathogen resistance have been implicated in a 'hybrid necrosis' phenotype that is characterized by tissue necrosis, dwarfism, wilting, cell death and often lethality[25,26]. In *A. thaliana*, progeny from ~2% of within-species crosses suffer similar phenotypes caused by five genetically independent epistatic interactions[25]. One of the incompatible epistatic interactions involves a disease resistance (*R*) gene, *DANGEROUS MIX 1* (*DM1*). Segregating *DM1* alleles, like other *R* genes[27], differ by many non-synonymous changes, which is consistent with a history of frequency-dependent selection. Gene expression profiles of F_1 hybrids between incompatible *A. thaliana* strains show that *DM1* triggers an immune response even in the absence of pathogens. Incompatible gene interactions involving divergent *R* genes can therefore cause autoimmune syndromes that result in necrosis. It will be important to establish whether divergent *R* genes generally contribute to the necrosis seen not just within species (or between domesticated varieties[10]) but in hybrids between species.

Arms races with selfish genes. Molecular evolutionary arms races can also involve non-ecological interactions. Like pathogens, selfish genetic elements that parasitize genomes — transposons, meiotic drive elements and gamete-killing segregation

distorters — have evolutionary interests that conflict with those of their hosts. Selfish genes manipulate host reproduction to facilitate their own non-Mendelian transmission, often at the expense of their hosts; and host genomes in turn evolve to suppress selfish genes or to compensate for their deleterious effects. The recurrent genetic conflict between hosts and their selfish genes can incidentally cause the evolution of hybrid dysfunction in two ways. First, hybrid dysfunction can result when otherwise suppressed selfish genes from one species are unleashed in the naive genomic background of another species. Second, hybrid dysfunction can result from incompatibilities between host genes that have evolved to silence or mitigate the effects of selfish genes.

There are several examples of selfish genes that have been unleashed in hybrids of *Drosophila* species. In crosses between *D. simulans* females and *D. melanogaster* males, F_1 hybrid males are viable but hybrid females typically die as embryos. Hybrid lethality is caused by an incompatibility between an unidentified maternal factor (or factors) from *D. simulans* and a dominant factor from *D. melanogaster*, *Zygotic hybrid rescue* (*Zhr*), which maps to the centric heterochromatin of the X chromosome[28]. *Zhr* is not a protein-coding gene but contains a block of 359-bp satellite repeats that are specific to *D. melanogaster*. Hybrid female embryos suffer an early mitotic defect in which the *Zhr* region of the *D. melanogaster* X chromosome fails to condense properly, resulting in lagging chromatids and mis-segregation[29]. The naive *D. simulans* maternal cytotype therefore lacks the appropriate proteins or RNAs necessary to regulate the *D. melanogaster*-specific satellite DNA. Rapid evolutionary change in species-specific satellite DNA quantity and composition can occur by neutral, nearly neutral[30] or selfish processes. The opportunity for genetic conflict arises when homologous chromosomes in the female germ line physically segregate into the four meiotic products: any centromere that is able to secure a position in the primary oocyte and avoid being shunted into one of the three polar bodies enjoys a transmission advantage. As the organization and composition of centric heterochromatin can influence the strength of centromeric meiotic drive in the female germ line[31], the rapid evolution of centromeric sequences and the proteins that bind them may reflect recurrent cycles of drive and suppression[32].

There are two other examples of selfish genes unleashed in hybrids. The first comes from crosses between two young subspecies, *Drosophila pseudoobscura bogotana* and *Drosophila pseudoobscura pseudoobscura*. Hybrid male offspring with a *D. p. bogotana* X chromosome and a *D. p. pseudoobscura* Y chromosome are

Table 1 | **Incompatibility genes within and between species**

Locus	Gene name	Species	Affected hybrids	Phenotype	Molecular function	Putative evolutionary basis	Refs
AEP2	ATPase expression 2	*Saccharomyces bayanus/ Saccharomyces cerevisiae*	F_2 hybrids	Sterility	Mitochondrial translational protein	Mutation pressure	14
OLI1	Oligomycin resistance 1	*S. bayanus/ S. cerevisiae*	F_2 hybrids	Sterility	F0-ATP synthase subunit	Mutation pressure	14
HPA2	*HISTIDINOL-PHOSPHATE AMINO-TRANSFERASE 2*	*Arabidopsis thaliana*	Intraspecies	Lethality	Histidine biosynthesis	Duplicate gene silencing	21
HPA1	*HISTIDINOL-PHOSPHATE AMINO-TRANSFERASE 1*	*A. thaliana*	Intraspecies	Lethality	Histidine biosynthesis	Duplicate gene silencing	21
JYalpha	*JYalpha*	*Drosophila simulans/ Drosophila melanogaster*	F_2-like hybrid males	Sterility	Na^+-K^+ ATPase	Duplicate gene silencing	22
DM1	*DANGEROUS MIX 1*	*A. thaliana*	Intraspecies	Lethality	Nucleotide-binding leucine-rich repeat disease resistance gene	Host–pathogen conflict	25
Zhr	*Zygotic hybrid rescue*	*D. melanogaster/ D. simulans*	F_1 hybrid females	Inviability	Repetitive DNA	Genetic conflict	28,29
Ovd	*Overdrive*	*Drosophila pseudoobscura bogatana/ Drosophila pseudoobscura pseudoobscura*	F_1 hybrid males	Sterility	DNA binding	Genetic conflict	34
Prdm9	PR domain-containing 9	*Mus musculus musculus/ Mus musculus domesticus*	F_1 hybrid males	Sterility	Histone 3 lysine 4 trimethyltransferase	Genetic conflict	37
Hmr	*Hybrid male rescue*	*D. melanogaster/ D. simulans*	F_1 hybrids	Inviability	DNA binding	Genetic conflict	40
Lhr	*Lethal hybrid rescue*	*D. simulans/ D. melanogaster*	F_1 hybrids	Inviability	DNA binding	Genetic conflict	41
Ods	*Odysseus*	*Drosophila mauritiana/ D. simulans*	F_2-like hybrid males	Sterility	Satellite DNA binding	Genetic conflict	42,43
Nup160	*Nucleoporin 160*	*D. simulans/ D. melanogaster*	F_2-like hybrids	Inviability	Nuclear pore protein	Host–pathogen/ genetic conflict	45
Nup96	*Nucleoporin 96*	*D. simulans/ D. melanogaster*	F_2-like hybrids	Inviability	Nuclear pore protein	Host–pathogen/ genetic conflict	46

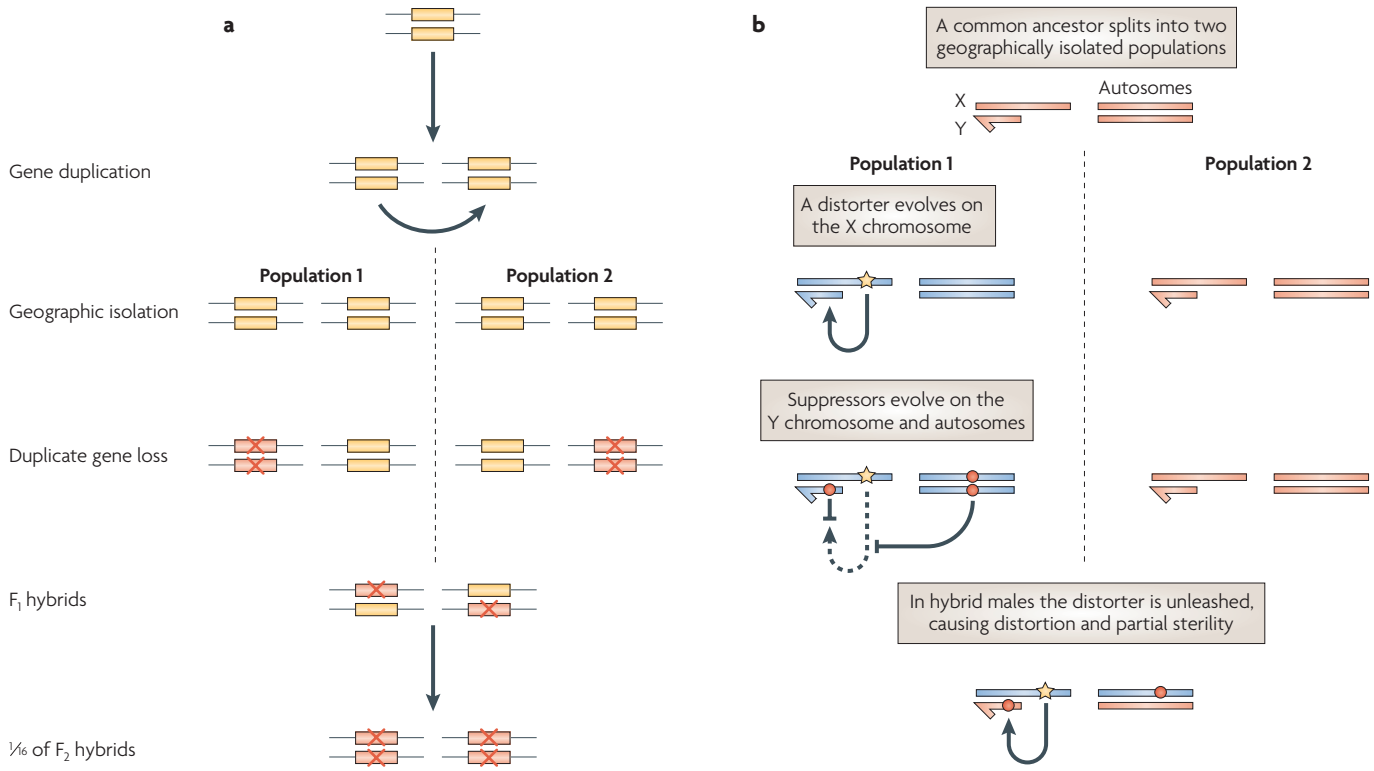


Figure 1 | **The molecular evolutionary basis of genetic incompatibilities that cause hybrid dysfunction. a** | Genetic incompatibilities can evolve through the reciprocal silencing of alternative duplicate gene copies[20]. When a functionally redundant gene duplication becomes established in a population, one or the other copy can be incapacitated by the neutral fixation of degenerative mutations. When degenerative mutations silence alternative gene copies in different populations or species, then (assuming independent assortment) one-sixteenth of F_2 hybrids will be doubly homozygous for non-functional paralogous genes. If the gene function is fertility- or viability-essential, these double-null F_2 hybrids will be sterile or inviable. **b** | Genetic incompatibilities can evolve as by-products of genetic conflict between selfish genes and host genes. When two populations evolve independently, different systems of selfish genes and host suppressors can accumulate. In one population, a segregation distorter might evolve on the X chromosome (yellow star) and obtain a transmission advantage by killing Y-bearing sperm during spermatogenesis (only the X chromosome, Y chromosome and one pair of autosomes are shown; the Y chromosome is hooked). The resulting fertility cost to the host and the distorted sex ratios among its progeny elicit the evolution of Y-linked and autosomal suppressors that silence the distorter. This genetic conflict of interest can trigger a molecular evolutionary arms race as the distorter evolves to escape suppression and suppressors evolve to silence the new distorter alleles. In F_1 hybrids, selfish distorters from one species occur in the naive genetic background of another species that is incapable of suppression. The selfish gene can therefore be unleashed in hybrids, causing distortion or, in some cases, sterility — as in F_1 hybrids between *Drosophila pseudoobscura bogotana* and *Drosophila pseudoobscura pseudoobscura*[33,34].

largely sterile but become very weakly fertile when aged and then, surprisingly, sire >90% daughters. These biased sex ratios are caused by a gamete-killing segregation distorter system: sperm bearing the *D. p. pseudoobscura* Y chromosome are destroyed by selfish distorter genes on the *D. p. bogotana* X chromosome during spermatogenesis. By killing Y-bearing sperm, X-linked distorters monopolize transmission at the expense of host fertility. The genetic causes of male sterility and segregation distortion in *D. p. bogotana–D. p. pseudoobscura* hybrids are identical[33]. Both require a multi-locus interaction among two X-linked *D. p. bogotana* factors, the *D. p. pseudoobscura* Y chromosome and the autosomes. One of the X-linked distorters, *Overdrive* (*Ovd*), was recently identified and shown to be necessary for both hybrid male sterility and segregation distortion[34]. *Ovd* encodes a DNA-binding protein with an evolutionary history that is consistent with the conflict scenario: a burst of non-synonymous substitutions occurred exclusively in the *D. p. bogotana* lineage, which gave rise to the allele responsible for sterility and segregation distortion in hybrids. It is important to note that the substitutions at *Ovd* spread in *D. p. bogotana* because of their inherent transmission advantage (that is, they cause segregation distortion) and not because they were beneficial to the host. Therefore, it seems that since the split of these two subspecies ~150,000 years ago, a selfish X-linked segregation distorter system invaded *D. p. bogotana* but was then silenced by the evolution of Y-linked and autosomal suppressors[33]. In F_1 hybrid males, which carry a naive and hence susceptible Y chromosome from *D. p. pseudoobscura* and a heterozygous set of mostly recessive autosomal suppressors from *D. p. bogotana*, *Ovd* (along with its co-distorter) is unleashed. For reasons that remain mechanistically unclear, *Ovd* overshoots the mark, causing nearly complete sterility rather than precisely eliminating Y-bearing sperm. A similar hybrid male sterility factor, *too much yin* (*tmy*), exists between *Drosophila mauritiana* and *D. simulans*: *tmy* from *D. mauritiana* unmasks one of three[35] usually suppressed X-linked distorters from *D. simulans* and, along with another *D. mauritiana* factor, causes hybrid male sterility[36].

Host genes that mediate genetic conflict. Hybrid incompatibilities also occur between host genes that mediate genetic conflict. The first speciation gene identified in mammals, PR domain-containing 9 (*Prdm9*), encodes a histone 3 lysine 4 trimethyltransferase that is involved in chromatin modification and causes sterility in hybrid males between *Mus musculus musculus* and *Mus musculus domesticus*[37]. Sterile hybrids experience spermatogenic arrest and abnormal sex chromosome body formation during the pachytene stage, which suggests that *Prdm9* disrupts meiotic sex chromosome inactivation (MSCI) in hybrids. This raises the questions of why MSCI — the early transcriptional silencing and heterochromatinization of sex chromosomes during spermatogenesis — exists and why its regulation might diverge between species. It is difficult to point to ecological reasons for divergence in the regulation of MSCI. But the so-called drive hypothesis posits that MSCI is one way that host genomes suppress the expression of segregation distorters on the X and Y chromosomes[38,39]. The molecular basis of MSCI might therefore diverge between species to suppress newly arising distorters or those that have evolved to escape suppression. As a result of genetic conflict over sex chromosome transmission, molecular incompatibilities can evolve between components of the MSCI machinery, causing sterility in hybrid males.

In addition to *Zhr*, three other host genes involved in hybrid incompatibilities in *Drosophila* spp. are likely to have evolved as by-products of interspecific divergence in heterochromatin and its regulation. In crosses between *D. melanogaster* females and *D. simulans* males, F_1 hybrid male offspring are killed by an incompatible interaction between the X-linked *Hybrid male rescue* (*Hmr*) gene from *D. melanogaster* and the autosomal *Lethal hybrid rescue* (*Lhr*) gene of *D. simulans*[40,41]. The HMR protein encodes a DNA-binding domain, whereas the LHR protein interacts with Heterochromatin protein 1 (HP1) and localizes to the centric heterochromatin, consistent with a role in the regulation of heterochromatic sequences. In hybrid males between the more closely related species *D. mauritiana* and *D. simulans*, the X-linked gene *Odysseus* (*Ods*) causes sterility[42], and new work shows that *Ods* from *D. mauritiana* aberrantly binds the *D. simulans* Y chromosome in hybrids[43]. The protein-coding sequences of *Hmr*, *Lhr* and *Ods* all have histories of recurrent positive selection[41,42,44]. Taken together, these findings show that the rapid co-evolution of heterochromatic sequences and their regulators has given rise to multiple incompatibilities that affect hybrids between species in the *D. melanogaster* subgroup.

Two other autosomal genes from *D. simulans*, *Nucleoporin 160* (*Nup160*) and *Nup96*, have been identified that are incompatible with (unidentified) factors on the *D. melanogaster* X chromosome, killing F_2-like hybrid genotypes[45,46]. Both encode protein components of the nuclear pore complex (NPC). NPCs are large macromolecular channels that perforate nuclear envelopes and mediate all cytonuclear transport in eukaryotes. Although the NPC comprises ~30 different proteins, the NUP160 and NUP96 proteins physically interact and, along with six other proteins, constitute the NUP107 subcomplex of the NPC. Despite the evolutionarily conserved function of NPCs, four genes that encode members of the NUP107 subcomplex have experienced recurrent adaptive evolution in *D. melanogaster*, and seven have experienced recurrent adaptive evolution in *D. simulans*[47]. Therefore, it seems that the proteins of the NUP107 subcomplex have co-evolved together within both species' lineages, incidentally giving rise to two lethal hybrid incompatibility genes. Why the NUP107 subcomplex has evolved rapidly remains unclear. But NPCs are known to interact with viruses and retrotransposons and may have evolved to suppress a segregation distortion system that manipulates nuclear transport[47,48].

Conclusions

The classic model for the evolution of hybrid dysfunction often assumes that incompatible gene interactions accumulate between species as they adapt to their differing external ecological circumstances. Surprisingly, however, there are few good examples of hybrid incompatibilities that support this model. Instead, it seems that most of the speciation genes that cause hybrid sterility or inviability evolved because genomes are intrinsically unstable, being susceptible to mutation pressure and invasion by pathogens and selfish genetic elements. Therefore, the first steps in the evolution of hybrid dysfunction may often be neutral or nearly neutral (mutation pressure) or even deleterious (pathogens and selfish genes) rather than adaptive. How then do we explain the strong signatures of adaptive evolution that are commonly found at hybrid incompatibility genes? These could occur for two reasons. Some signatures of positive selection undoubtedly reflect adaptation at host genes as they compensate for weakly deleterious mutations and the disruptions caused by pathogens and selfish genes. However, it is important to note that molecular population genetics alone cannot distinguish changes that are beneficial to the host from those that are selfish — beneficial substitutions and selfish substitutions leave the same signatures in the genome (consider, for example, the rapid evolution at *Ovd* in *D. p. bogotana*).

There are three big challenges going forward. First is the question of what forces are most important in the evolution of hybrid dysfunction — ecological adaptation, mutation pressure or molecular arms races with pathogens and selfish genes? The answer will almost certainly differ among taxa and therefore requires simply finding and characterizing more speciation genes from more systems. Even for the genes that have already been identified, further work is needed. Although their evolutionary histories provide the first hints of the importance of mutation pressure and evolutionary conflict, other possibilities, including ecology-based ones, have not been formally excluded.

Second, inferring the forces that drive the evolution of hybrid dysfunction may be harder for older species pairs. For instance, a speciation gene may have got the upper hand long ago by suppressing a selfish gene, thereby resolving the genetic conflict and leaving only sterility or inviability phenotypes to be observed in hybrids. Inferring the forces that drive the evolution of hybrid dysfunction may therefore require greater focus on younger species pairs in which conflicts are still unresolved and therefore still detectable in species hybrids.

Third, why do the genes involved in hybrid dysfunction tend to be those with high levels of sequence divergence? There are two extreme possibilities. One is that hybrid dysfunction might result from the cumulative effects of many sequence differences. And the other is that, as substitutions causing hybrid dysfunction might be exceedingly rare (that is, only a tiny fraction of fixed differences between species are incompatible[49]), genes with many fixed differences simply have more chances to experience an incompatible substitution. Distinguishing these alternatives might be achievable by moving beyond identifying incompatible genes to identifying incompatible substitutions.

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Competing interests statement

The author declares no competing financial interests.

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