Title: The molecular evolutionary basis of species formation revisited

Megan E. Frayer[1], Nemo V. Robles[2,3], María José Rodríguez-Barrera[2,4], Jenn M. Coughlan[1]*, and Molly Schumer[2,3,5]*

[1] Department of Ecology & Evolutionary Biology, Yale University, New Haven, CT, United States
[2] Department of Biology, Stanford University
[3] Centro de Investigaciones Científicas de las Huastecas "Aguazarca", A.C.
[4] Escuela Nacional de Estudios Superiores Juriquilla, Universidad Nacional Autónoma de México
[5] Freeman Hrabowski Fellow, Howard Hughes Medical Institute

*Co-supervised this work
*Correspondence: schumer@stanford.edu (M. Schumer)

Keywords

Hybrid; Reproductive Isolation; Genetic incompatibility; Intragenomic conflict; Introgression

Abstract

How do new species arise? This is among the most fundamental questions in evolutionary biology. The first genetic model for how reproductive barriers leads to the origin of new species was proposed nearly 90 years ago. However, empirical evidence for the genetic mechanisms that cause reproductive barriers took many decades to accumulate. In 2010, Presgraves presented a comprehensive review of the literature on known "speciation genes" and the possible evolutionary mechanisms through which they arose. Fifteen years later, with an explosion of studies that include both non-model and model organisms, the number of known incompatibility genes has increased ~7 fold. Here, we synthesize previous and new empirical examples to investigate the genetic mechanisms through which intrinsic incompatibilities arise and highlight current gaps in our understanding.

Main Text

Introduction

Evolutionary biologists have long been fascinated by the immense diversity of species and the mechanisms through which they form [1]. While many distinct mechanisms contribute to reproductive barriers between emerging species, including sexual and ecological selection on hybrids [2–5], there has been special interest in understanding **genetic barriers** (see Glossary) that prevent successful reproduction, perhaps because these barriers are viewed as "irreversible" when they are sufficiently strong [6]. Early work in evolutionary biology predicted that genetic barriers between species would arise via distinct genetic changes in each lineage [7–9]. The **"Dobzhansky-Müller" (DMI) model** of hybrid incompatibility predicts that neutral or adaptive substitutions that accumulate between diverging species may interact improperly in hybrids (Fig. 1), leading to reduced viability and fertility. While the general predictions of this model have been well supported by decades of genetic crosses in myriad species, only in recent years have the genes underlying these interactions and the mechanisms through which they evolve come into focus, aided by rapid technological advances. Work in the first decade of the 21st century focused on classical lab models with exceptional genetic tools including *Drosophila*, *Arabidopsis*, and *Saccharomyces* (reviewed in [6]), but advances in genomic tools for non-model species have enabled the discovery of hybrid incompatibilities in diverse taxa. Here, we review how the past 15 years of speciation research has led to a richer understanding of the potential mechanisms through which new species evolve, and deepened our knowledge of how incompatible alleles accumulate.

In addition, our review sheds light on how incompatibilities act in naturally hybridizing species and highlights key knowledge gaps.

What we knew about "Speciation Genes" and what we know today

In his seminal 2010 paper "The molecular evolutionary basis of species formation", Presgraves described all known genes involved in hybrid incompatibilities and outlined the first clues about how these incompatibilities arise based on empirical data (for theoretical predictions see [8–10]). Here, we revisit this work with a particular emphasis on incompatibility genes that have been identified since 2010. We focus our search on hybrid incompatibilities that act "intrinsically," meaning that these incompatibilities cause hybrid dysfunction regardless of the environment (but see [2]). Using both broad and targeted literature searches, we are able to identify 99 incompatibilities where at least one of the genes involved has been precisely identified (Table 1; see table legend for a description of our methodology). This large dataset allows us to begin to explore broad patterns in the data, while keeping in mind the many factors that impact DMI discovery and characterization.

One of the most striking differences when comparing our catalog of incompatibilities to the genes reported by Presgraves is a major expansion in the species in which hybrid incompatibilities have been identified (Fig. 2). Early work necessarily relied on species with exceptionally powerful genetic toolkits. While *Drosophila* and *Arabidopsis* continue to be overrepresented among organisms with precisely mapped hybrid incompatibilities, there has been substantial progress in mapping incompatibilities in less traditional models over the last decade. Table 1 includes 27 genera (13 of which include domesticated lineages; Fig. 2A), as opposed to

77 the 7 genera with mapped incompatibilities known in 2010 (3 of which included domesticated
78 lineages and were excluded from Presgraves's table). However, certain groups are notably
79 underrepresented, including vertebrates, where only seven incompatibilities have been mapped in
80 any species (Table 1).

81 Similarly, Table 1 covers a wider breadth of molecular mechanisms and phenotypes. Genes
82 involved in molecular processes from meiotic recombination to developmental patterning to adult
83 pigmentation have been shown to cause hybrid incompatibility (Table 1). These diverse molecular
84 functions are consistent with predictions of theoretical models that any interacting pair of genes
85 could become involved in hybrid incompatibilities [11]. Despite this diversity, Table 1 features
86 several instances where related genes have been implicated in incompatibility across different
87 species. For example, researchers have found repeated involvement of *RPP* genes in **hybrid**
88 **necrosis** in plants (Table 1). These observations raise the exciting possibility that the rate at which
89 hybrid incompatibilities evolve could differ across genes or pathways. However, it is also likely
90 that these observations are interconnected, with researchers more likely to prioritize
91 incompatibilities that are known in other systems. Moreover, biases may stem from the systems in
92 which incompatibility is most heavily studied– such as crop plants, which have undergone
93 domestication.

94

95 *A new understanding of genic drivers of incompatibility*

96 An expanded knowledge of the genes involved in hybrid incompatibilities allows us to
97 revisit hypotheses outlined by Presgraves [6] about the mechanisms that drive the evolution of
98 incompatibilities. In the majority of cases where hybrid incompatibilities have been precisely
99 mapped (Table 1), researchers have identified protein coding genes as the causal factors underlying

hybrid incompatibilities. Rapid evolution at the amino acid sequence level that disrupts protein-protein interactions appears to be the molecular cause of many of the known hybrid incompatibilities (e.g. [12–15]), and there are some documented cases of amino acid substitutions altering RNA-protein interactions [16]. In other cases, both evolved changes in expression and amino acid changes underlie hybrid incompatibility phenotypes. For example, in hybrids between swordtail fish species dysfunctional interactions between *Xmrk* and its repressors can cause melanoma [17]. Follow up work using cell culture experiments showed that both overexpression of the xmrk repressor *cd97* and amino acid changes in its sequence contribute to melanoma phenotypes in cell culture [18]. In several cases in Table 1, the causative variant is structural. This is the case for so-called "**presence-absence variants**", where duplication and reciprocal loss of a gene makes it possible for hybrids to inherit no functional copies [19–21,17,22].

Even with the massive progress reflected in Table 1, there are still relatively few studies that have successfully identified which mutations or regulatory changes lead to incompatibility. Interrogating these patterns is a high priority research area. An expanded knowledge of the mutations underlying incompatible interactions is not only important for our understanding of what types of evolutionary changes are more likely to lead to reproductive isolation, but can also greatly inform modeling efforts investigating the accumulation of incompatibility alleles (i.e. via the snowball effect or other processes; [23]), inferring the importance of evolutionary history in the emergence of hybrid incompatibilities, and determining how alleles interact at a molecular level to cause hybrid dysfunction.

Non-genic components of speciation

In addition to major progress in identifying new protein-coding genes involved in hybrid incompatibilities, research over the past decade has dramatically expanded our understanding of hybrid incompatibilities which are not driven by genes (Table 1; [24]). These mechanisms include structural changes in the genome that cause meiotic dysfunction, issues with inheritance of epigenetic modifications, or global perturbations to the gene regulatory landscape that cause hybrid dysfunction.

Our earliest understanding of the genetic basis of hybrid sterility came from broad scale differences in genome structure [25]. Karyotype differences contribute to reproductive isolation between many species, and are among the best understood incompatibilities in species that are not genetically tractable (e.g. muntjac deer; [26]). Karyotype differences generally lead to hybrid sterility when hybrids are unable to properly sort their chromosomes during meiosis. Similarly, extremely high levels of genetic divergence between chromosomes can impact success in crossing over during meiosis ([27,28]; Box 1). Structural changes, such as translocations, also play a crucial role in hybrid sterility due to failed pairing and meiosis in many plant lineages [29] and have been linked to hybrid incompatibility through a number of mechanisms. See [30–32] for several excellent reviews on this topic.

In addition to structural factors, other types of non-genic elements have been implicated in hybrid incompatibilities. Several families of transposable elements (TEs) have been linked to hybrid dysfunction in *Drosophila* [33–36]. For example, the copy number of P-elements significantly influences the frequency of **hybrid dysgenesis** [36,37]. Hybridization could also lead to genome-wide transposable element (TE) deregulation, called "genomic shock" [38]. Associations between general TE misregulation and hybrid dysfunction have been observed in

144 *Drosophila* [36,39] and *Caenorhabditis elegans* [40]. Hybrid-specific misregulation of TEs has
145 been reported in diverse taxa [41–45]. However, others have found limited evidence of TE
146 misregulation in hybrids [46,47] or misregulation with no clear impacts on hybrid **fitness** [48].

147 Other non-genic elements such as satellite DNA (long tandem repeats found in
148 heterochromatin regions) and non-coding RNAs play an important role in hybrid incompatibilities.
149 In hybrids between *Drosophila melanogaster and D. simulans*, the *mh* allele from *D. simulans*,
150 which typically regulates satellite DNA, interferes with the function of satellite DNA *359bp*
151 inherited from *D. melanogaster*, leading to disrupted genome integrity and female infertility [49].
152 Satellite DNA is often highly differentiated even between closely related species, although this
153 does not always result in an incompatibility [50]. Non-coding RNAs play diverse mechanistic
154 roles, including regulating gene expression, chromatin remodeling, and suppressing transposable
155 elements, among others [51], and have been implicated in several hybrid incompatibilities. For
156 example, seeds produced by crosses of multiple *Capsella* species are inviable due to a lack of
157 maternally deposited siRNAs in the endosperm, which leads to abnormal gene regulation and
158 ultimately developmental failure [52]. Some of the genes that are targeted by siRNAs have
159 previously been identified as incompatibility genes in other systems (such as *PHE1* in *Arabidopsis*;
160 [53]), and a similar process may also lead to hybrid seed failure in *Solanum* [54] and *Oryza* [55].
161 Since non-coding RNAs tend to evolve rapidly but retain their functional importance [56], they
162 may fall into a class of elements that are mechanistically likely to become involved in hybrid
163 incompatibilities.

164 Together, this work highlights the immense diversity of mechanisms through which hybrid
165 incompatibilities can evolve. While the importance of non-genic hybrid incompatibilities has been
166 appreciated since the inception of the field [57,58], and was discussed by Presgraves in 2010,

newly mapped non-genic incompatibilities are emerging as important mechanisms underlying incompatibility in the decade since, expanding the simple two-locus model originally proposed by both Dobzhansky and Müller.

Genetic architecture of speciation and its consequences for evolutionary outcomes

While understanding genetic interactions and their breakdown in hybrids is an interesting question in its own right, the increase in mapped incompatibilities allows us to begin to evaluate questions about both their mechanistic drivers and their evolutionary consequences. Here, we connect what we have learned from newly identified hybrid incompatibilities to classic evolutionary theory.

Symmetry and Complex Incompatibilities

Classic theoretical work made two major predictions about the architecture of incompatibilities. First, under a model of neutral evolution, researchers predicted that incompatibilities would be "asymmetrical," meaning that only one of the mismatched two-locus genotype combinations is expected to experience selection [59]. Although some incompatibilities fit this asymmetrical model (e.g. *Overdrive*; [60]), in many empirical cases, hybrid incompatibilities act "symmetrically," meaning that selection acts on both mismatched two-locus genotypes. **Symmetrical incompatibility** can arise through coevolution driving multiple substitutions in interacting genes [61,62]. While these differences in genetic architecture may seem subtle, they can have profound impacts on how genetic incompatibilities act after hybridization. With **asymmetrical incompatibilities**, hybridization tends to lead to a loss of genetic isolation

between species as a result of the compatible genotype combination spreading [21,63]. By contrast, symmetrical incompatibilities act as strong barriers to hybridization because all heterospecific two locus genotype combinations experience selection.

Similarly, theoretical models predicted that hybrid incompatibilities are likely to be "complex", meaning that they are expected to involve more than two interacting genes [11]. The intuition behind these theoretical models is that **complex incompatibilities** can evolve through more mutational paths that avoid low-fitness genotypic combinations. However, complex genetic interactions are notoriously difficult to detect and incompatibilities involving three or more genes are extremely rare in the empirical literature. Despite this, progress has been made in identifying [64] and mapping [14,65] complex incompatibilities, primarily in model organisms where large screens are possible. In some cases, complexity has been added to previously known incompatibilities. Bladen and colleagues [65] recently uncovered additional complexity in the Hmr-Lhr-gfzf incompatibility in Drosophila [66,67], mapping a novel locus in *D. sechellia* known as *Sechellia aversion to hybrid rescue* (*Satyr*). Similarly, work by Moran et al. [14] identified a novel example of a complex hybrid incompatibility in *Xiphophorus* (Fig. 4). F2 hybrids carrying *X. birchmanni* nuclear ancestry at *ndufa13* and *nufs5* and *X. malinche* mitochondrial ancestry are inviable. While this interaction initially appears to be the product of two simple incompatibilities with the mitochondrial genomes, Moran and colleagues found that harboring even one mismatched *ndufs5* allele sensitizes F_2 fish to the *ndufa13* incompatibility. This is a subtle three-way interaction that was only detectable because it was possible to generate nearly 1,000 hybrids in the laboratory. This highlights the difficulty of addressing this question in the current literature: while the fact that few complex incompatibilities have been identified in any species could hint that they are less common than theoretical models predict, it is equally likely that the technical issues impacting

their detection obscure their importance. Since it is challenging for even large experiments to have power to detect complex hybrid incompatibilities, progress in this area will likely require the development of new computational or experimental tools ([68]**;** See Box 2).

Snowball Theory

The rate at which two diverging lineages become fully reproductively isolated depends on how quickly they accumulate hybrid incompatibilities. While classic theoretical work predicts the presence of a "snowball" effect, where the number of genetic incompatibilities grows non-linearly with genome-wide genetic divergence between lineages [11], only a handful of studies have evaluated this empirically [81–83]. More recent work has suggested a snowball effect might not be expected under certain models of speciation [84,85] or certain models of gene interaction [23]. For example, a gene at the center of a highly connected gene network may be more prone to incompatible interactions, whereas modularity may reduce the opportunity for incompatibilities [23]. Moreover, to our knowledge, no similar theoretical work has been performed for the strength of selection on genetic interactions, which is arguably an equally important factor for understanding the emergence of new species. As a result, we are still very much in the dark about how the genetic architecture of incompatibilities scales with genetic divergence, with crucial implications for how quickly new species are expected to become isolated.

The evolutionary forces that drive speciation

Almost since the inception of the field, evolutionary biologists have searched for common mechanisms that drive the emergence of barriers to hybridization. Even in the decades when

empirical work on the genetic mechanisms of reproductive isolation was limited, theoretical and narrative predictions about the potential drivers of this process flourished and were heatedly debated [86–89]. With dozens more empirical cases in hand (Table 1), we can begin to evaluate some of these predictions.

"Classic" models for the evolution of incompatibility

Much of the classic speciation theory supposes that incompatibility loci fix as a result of genetic drift [90]**.** However, only a handful of incompatibility alleles to date clearly support this "neutral" model. Several incompatibilities caused by gene duplication are consistent with a model of neutral evolution [19,20,91,92]. In contrast, some of the best studied genes involved in hybrid incompatibilities exhibit elevated rates of molecular evolution, such as *Prdm9,* which is one of the most rapidly-evolving genes in many vertebrate genomes [93,94]. The importance of rapid evolution as a driver of hybrid incompatibilities has been apparent for decades [6,95], with verbal models for their evolution highlighting the importance of evolutionary **arms races** such as **intragenomic conflicts** and host-pathogen co-evolution. Evidence for the importance of these evolutionary forces has only strengthened with 15 additional years of research, with "selfish genetic elements" and "host-pathogen coevolution" being the two most common mechanisms proposed by authors as drivers of the evolution of the incompatibilities listed in Table 1 (Fig. 2B). Given its clear importance, there have been many excellent and in-depth reviews on the role of genetic conflict in driving hybrid incompatibility and other genomic processes [96–99]. However, despite empirical evidence of the importance of these processes, to our knowledge, they have yet to be integrated into theoretical models of hybrid incompatibilities, presenting an important (and addressable) knowledge gap for the field.

Another classic model for the evolution of hybrid incompatibilities is the evolution of incompatibility as a byproduct of substitutions driven by divergent ecological selection [90]. This model has a long and contentious history in speciation biology [100,101]. The most direct evidence that ecological divergence can lead to the accumulation of hybrid incompatibilities comes from experimental evolution in yeast and *Drosophila* [102–105]. However, empirical evidence of this process in nature is scarce and the degree to which adaptation drives the accumulation of intrinsic incompatibilities in nature is still poorly understood. Examples include strong hybrid incompatibilities between closely related populations adapted to different environments [106,107]**,** and hybrid breakdown associated with dysfunctional metabolism [108,109]. Nonetheless, since we lack knowledge of the precise genes or mechanisms involved in these cases, it remains challenging to distinguish whether ecological divergence is directly responsible for the accumulation of incompatibility alleles versus scenarios of **hitchhiking** or **linkage disequilibrium** [29,110,111].

Gene networks, complexity, and developmental mechanisms

While earlier studies reviewing genes involved in hybrid incompatibilities recognized the importance of compensatory evolution, insights from systems biology have led to new models for how incompatibilities might arise since Presgraves 2010. **Developmental systems drift** describes observations inspired by gene regulatory networks, where evolving biological systems can remain functionally 'equivalent' but have diverged in their underlying structure [68,112,113]. Theoretical work on this topic supports the inference that even biological systems under strong stabilizing selection can lead to the rapid evolution of incompatibility [114], and that this outcome is particularly likely in models of complex gene regulatory networks with functional redundancy.

Importantly, the developmental systems drift model does not require any form of adaptive divergence or genetic conflict within parental lineages for hybrids to experience strong selection. Studies over the past decade have highlighted the prevalence of gene expression misregulation in hybrids [113,115,116] and divergence in the genetic architecture of seemingly identical phenotypes among related species [117], both of which are predicted under a systems drift model (we note that gene misregulation does not necessarily derive directly from incompatibilities; [115]). More direct evidence has come from new empirical studies that have documented hybrid incompatibilities arising in conserved developmental pathways. In a pair of papers, Chang et al. [117,118] show that two highly conserved transcription factors that play the same developmental role *across Drosophila* species cause severe developmental incompatibilities in hybrids (Fig. 5) These results provide exciting empirical evidence for developmental systems drift in action, and its link to developmental dysfunction in hybrids.

Underappreciated evolutionary mechanisms: balancing selection and past introgression

Recent work has revealed evolutionary mechanisms that can lead to the emergence of hybrid incompatibilities that were not predicted by previous conceptual or theoretical models: **balancing selection** and **introgression**. Ancient balancing selection in yeasts has maintained a polymorphism in the ability to grow rapidly in galactose-rich environments (as opposed to glucose-rich environments), driven by three loci involved in galactose metabolism. When alleles from galactose- and glucose-adapted strains are introduced to each other in hybrids, certain combinations result in severe growth defects. In natural populations, the identity of the three loci matches the environmental condition (e.g. galactose alleles found in isolates from dairy-rich environments), and the two versions of the alleles themselves appear to be millions of years old

[119]. This suggests that ancient balancing selection has maintained functionally distinct sets of co-adapted alleles that result in incompatibility when combined in the same genetic background. Similar mechanisms may underlie some hybrid necrosis phenotypes in plants: NB-LRR proteins that are activated in immune responses to pathogens often harbor high levels of polymorphism, presumably driven by balancing selection that maintains alleles contributing to immunity [97,120]; Table 1). Beyond these specific examples, a large body of work has highlighted the importance of polymorphic hybrid incompatibilities [121]. These observations could be consistent with an underappreciated role of balancing selection in the maintenance of hybrid incompatibilities, or simply indicate that these variants are on their way to fixation or loss via natural selection or genetic drift.

Historically, researchers have predicted that hybridization between species should erode genetic incompatibilities. Although much theory and some empirical work support this hypothesis [122–124], a growing body of work suggests that hybridization can lead to complex patterns of reproductive isolation and potentially move alleles involved in incompatibilities between species. For example, recent work in *Xiphophorus* found that alleles involved in an incompatibility between *X. malinche* and *X. birchmanni* have introgressed from *X. malinche* into a third species, *X. cortezi*. Crosses between *X. birchmanni* and *X. cortezi* suggest that these introgressed alleles could be causing a phenotypically similar incompatibility in this species pair [125]. Similarly in *Mimulus*, patterns of organelle capture from the outcrossing *M. cardinalis* into selfing *M. parishii* may have facilitated cytoplasmic male sterility between *M. parishii* and a third species– *M. lewisii* [126]. Lastly, horizontal gene transfer of a **toxin-antidote system** among distantly related *Caenorhabditis* species has seemingly facilitated ongoing incompatibility within *C. briggsae* [40]. Together, this highlights the potential for past hybridization and gene transfer events to impact the

present-day distribution of hybrid incompatibilities between species and adds substantial complexity to our understanding of the evolution of hybrid incompatibilities. We speculate that in the case of both balancing selection and introgression, the increased genetic divergence between interacting genes (either driven by ancient balancing selection or movement of genes from a divergent lineage) may be contributing to incompatibility. We predict that these mechanisms may be common beyond the highlighted case studies, with major implications for our understanding of the evolution of reproductive isolation. Understanding when introgression leads to the maintenance, erasure, or transfer of incompatibility alleles will require significant strides in both theory and empirical works. This will also be aided by a greater understanding of how incompatibility genes behave in nature (see Box 3).

Evolutionary idiosyncrasies: patterns, processes and reading the phylogenetic tea leaves

With a rapid increase in the number of mapped hybrid incompatibilities, we have an opportunity to ask whether the mechanisms that drive the evolution of incompatibilities are shared across the branches of the tree of life. At first glance, it appears that the evolutionary mechanisms that underlie incompatibilities may vary across kingdoms, with coevolution with satellite DNA being especially common in *Drosophila* and host-pathogen coevolution remarkably common in plants, to name a few patterns that immediately emerge from our analysis (Fig. 2B). Furthermore, several cases where the same genes repeatedly become involved in hybrid incompatibilities may be driven by these common evolutionary pressures. For example, almost all incidences of hybrid necrosis across diverse plant species involve nucleotide-binding domain and leucine-rich repeat (NLR) genes [97].

It is important to note, however, that these discoveries do not occur in isolation. Each new mapped incompatibility spurs research into the consequences of particular genetic mechanisms, especially in closely related species. This makes unraveling phylogenetic patterns particularly challenging. However, we can look to examples where a particular mechanism has been investigated across diverse taxa. As one example, motivated by compelling evidence of the links between TE misregulation and hybrid dysgenesis in *Drosophila*, studies in several systems have found evidence for changes in TE regulation in hybrids, but few have found evidence that this is linked to lower viability or fertility in hybrids [36], suggesting that this mechanism may be somewhat lineage specific. By contrast, cytonuclear incompatibilities appear to be quite common across taxa and may represent a common evolutionary mechanism for the emergence of incompatibilities. Moreover, the convergent evolution of **genomic imprinting** in mammals and angiosperms could explain the seemingly parallel patterns of parent-of-origin growth defects underlying early onset hybrid inviability in these taxa [134]. Overall, the variance in mechanisms across systems highlights how little is known in general about the degree to which the evolutionary drivers of hybrid incompatibilities are shared versus lineage specific.

Concluding Remarks

Despite substantial progress in the past 15 years in identifying the genes underlying hybrid incompatibilities and the mechanisms through which they evolve, many outstanding questions remain (Box 4). With dozens of newly mapped hybrid incompatibilities, we find that several mechanisms previously synthesized by Presgraves [6] and others [135,136], such as intragenomic conflicts, remain an important force in the evolution of hybrid incompatibilities. However, we also

highlight new evolutionary scenarios that may play fundamental roles in the evolution of incompatibilities, including developmental systems drift, balancing selection, and introgression. Although we are now amassing some empirical examples of these processes, the relative importance of these evolutionary drivers remains unknown. In Box 2, we highlight new and promising approaches to begin to pursue these fundamental questions. Moreover, there is an urgent need to revisit classic theoretical models of how hybrid incompatibilities evolve in light of current empirical results and newer models for the evolution of hybrid incompatibility.

Box 1. Recombination, Sequence Divergence, and Isolation

Successful meiosis requires that a precursor cell accurately sorts one copy of each of its chromosomes into the future gametes. Pairing of homologous chromosomes is a crucial step in this process. If the paired chromosomes are too dissimilar, "anti-recombination" mechanisms can prevent crossing over and halt segregation, generally initiated by mismatch repair proteins ensuring homology [137]. While this mechanism typically prevents rare errors where non-homologous chromosomes pair during meiosis, a similar process may also come into play in hybrids. Specifically, if the two chromosomes that need to pair come from deeply diverged species, this may trigger anti-recombination pathways in nearly every meiosis, ultimately resulting in hybrid sterility.

This mechanism of reproductive isolation has been observed in yeast (Fig. 3). Early studies in yeast showed that the mismatch-repair system plays a key role in several instances of hybrid sterility between species [137] and between divergent lineages [138]. Hybrids of *Saccharomyces cerevisiae* and *S. paradoxus*, which exhibit ~12% sequence divergence, experience dysfunctional chromosomal segregation and high rates of aneuploidy [139]. Among *S. paradoxus* strains, even

relatively low levels of sequence divergence (1.4%) result in increased rates of spore inviability due to activation of anti-recombination mechanisms [139]. The suppression of mismatch-repair during meiosis rescues hybrid fertility, confirming the role of anti-recombination mechanisms in reproductive isolation between these species. While several mismatch repair genes have been implicated in this process (e.g. *MHS2* and *SGS1*), the underlying genetic divergence between the sequences plays a key role in meiotic failure and hybrid sterility [28].

Meiotic problems impacting chromosome pairing tend to be observed in species with extraordinary levels of genetic divergence at the nucleotide level, and the degree to which similar mechanisms may impact fertility in species with less extreme genetic divergence is unclear. That said, sequence divergence at the binding sites of *Prdm9*, which specifies the locations of meiotic double strand breaks in mammals and some other vertebrates, also drives hybrid sterility in mice through distinct mechanisms [140,141]. This suggests that there may be multiple ways in which recombination interacts with sequence divergence to impact successful meiosis, and future work may uncover further links between recombination and hybrid sterility.

Box 2: Promising new computational and experimental approaches

A major barrier to progress in research on hybrid incompatibilities is the high cost and labor of identifying causative genes. As one example, the interacting partner of *Xmrk* in hybrids between *X. maculatus* and *X. hellerii* took ~30 years to be identified [69]. Some recent experimental work has taken advantage of a combination of natural hybrids and admixture mapping approaches with lab-generated hybrids to combine the precision of mapping in the lab with the shorter ancestry tracts found in late generation hybrids [14,17]. However, few systems in which we can genetically map incompatibility in the lab also have active hybrid zones, precluding

this possibility for many research groups. We propose that an exciting possibility could come from adapting methods from other fields. For example, researchers focused on mapping the interactome have developed high-throughput and sensitive approaches to detect epistasis in cell lines [70]. Since it is increasingly possible to generate cell lines from non-model species [71,72], this approach could be accessible to many researchers, and could even be combined with a reciprocal hemizygosity test in F1 cell lines [73]. We note, however, that it would only allow researchers to assay a limited number of phenotypes. In cases where phenotypes associated with incompatibilities are known, other approaches such as targeted or single cell RNAseq, have allowed researchers to identify genes that are expressed or coexpressed in cell types of interest [74,75].

In addition to these experimental challenges, scans for hybrid incompatibilities notoriously suffer from low power because of the immense number of statistical tests required [76], but methodological advances have been slow. Most methods, including those developed by our groups, are underpowered and have high false positive rates (e.g. [77]). Researchers have used several effective approaches to improve power, such as first identifying segregation distortion in controlled crosses, and then performing scans for loci that interact with the distorter [14]. However, in a recent study we found that even with ~1800 hybrids, we only had power to detect segregation distorters that reduced survival by at least 30% [78], highlighting the likely presence of many biologically relevant incompatibilities that fall below the detection thresholds of most studies. Applications of new approaches from human genetics such as network-informed mapping [79] or machine learning approaches to identify signals in genetic data that have not been the focus of population genetic models could further improve power [80]. Progress in either experimental or computational tools could fuel major shifts in the field.

Box 3. Reproductive isolation in the wild

One major shortcoming of the current literature is a limited understanding of how hybrid incompatibilities are exposed in nature and the ways in which they act to impact reproductive isolation in the wild. Because the vast majority of hybrid incompatibilities have been identified in species that do not naturally hybridize (Table 1), it is impossible to evaluate their action in natural populations. The few exceptions - including mice, *Mimulus*, and swordtails - have yielded mixed results. Recently, Frayer and Payseur reported that most loci involved in reproductive incompatibility in mice do not prevent gene flow in natural hybrids [127]. By contrast, work in swordtails has indicated strong selection against mitonuclear incompatibilities and hybrid melanoma in wild populations, often resulting in changes in ancestry around these loci [128,129]. In hybrids between *Mimulus guttatus* and *M. nasutus*, some alleles show reductions in introgression in nature, while others do not [21,130,131].

An additional complexity is the growing realization that reproductive isolation is highly polymorphic in nature. While patterns of local ancestry in replicated hybrid zones are very consistent in some species [129], in other species pairs local ancestry patterns are highly variable [132]. While some of these patterns are likely driven by extrinsic factors, they could also reflect the outcomes of polymorphism in the underlying loci involved in hybrid incompatibilities. Indeed, many of the genes in Table 1 are polymorphic within their respective species. Just as asymmetric incompatibilities may be more likely to be removed by strong selection against hybrids (see section "Symmetry and Complexity"), an incompatible allele that is polymorphic within a population may also be easily removed by selection.

More broadly, we highlight that the way that incompatibility alleles act in naturally hybridizing species may be more complex than has long been anticipated [122,123,133].

Expanding our understanding of genetic incompatibilities in naturally hybridizing species and determining how frequently and under what conditions they play a role in preventing genetic exchange between species in nature should be a major priority for future work.

Glossary

- **Arms race:** The continuous co-evolution of genetic elements that experience antagonistic evolution, typically through intragenomic conflict or host-pathogen conflict.
- **Asymmetrical incompatibilities:** Genetic incompatibilities in which only one hybrid genotype exhibits reduced fitness (i.e. AAbb *or* aaBB).
- **Balancing selection:** When natural selection acts to maintain multiple alleles in a population.
- **Complex incompatibilities:** Hybrid incompatibilities involving more than two interacting genes or genomic regions.
- **Developmental systems drift:** A process by which the underlying genic structure of a phenotype evolves, while the phenotype itself remains relatively unchanged. Typically, this is conceptualized by a trait under stabilizing selection, with mutations shifting fitness away from the optima, with subsequent selection for compensatory mutations that move the population back towards the optima.
- **Dobzhansky-Müller model of hybrid incompatibilities (DMI):** A model to explain the evolution of intrinsic postzygotic reproductive isolation, by which populations diverge at two or more loci. While each new allele is neutral or increases fitness in the background in which it has evolved, combining these alleles in hybrids can result in dysfunction.
- **Fitness:** The ability of an organism to survive to maturity and reproduce.

- **Genetic barriers:** Genetic factors that prevent successful mating within or between populations.
- **Genomic Imprinting:** Gene expression that exhibits a parent-of-origin specific bias; typically caused by epigenetic modifications which preferentially silence expression of one parental copy of an allele.
- **Hitchhiking:** The process by which a neutral allele increases in frequency due to selection on a nearby allele.
- **Hybrid dysgenesis:** A phenotypic syndrome described in hybrids that includes reduced fertility and mal-formed reproductive organs. In described cases, this is typically caused by overactivity of transposable elements in hybrids.
- **Hybrid Necrosis:** A suite of stress-related phenotypes that are the outcome of immune system overactivation. This includes cell death (necrosis) and stunted growth (dwarfism).
- **Intragenomic conflict:** Co-evolution among genes within a genome caused by antagonistic natural selection, typically arising at different levels of selection. Examples include chromosomes that exhibit meiotic drive, or transposable elements and their respective repressors.
- **Introgression:** The incorporation of genetic material from one lineage into another through hybridization.
- **Linkage disequilibrium:** The statistical non-independence of alleles at different loci. This can be caused by physical proximity (i.e. linkage), non-random mating, or natural selection maintaining associations between two or more loci.
- **Presence-Absence variants:** A structural variant in which individuals vary in the presence or absence of a genomic region.

- **Speciation genes:** Genes associated with a hybrid incompatibility that play a role in reproductive barriers between species.
- **Symmetrical incompatibilities:** Genetic incompatibilities in which reciprocal hybrid genotypes both exhibit reduced fitness (i.e. both AAbb *and* aaBB genotypes).
- **Toxin-antidote systems:** A specific form of intragenomic conflict wherein "killer" gametes evolve an antidote and poison, the latter of which serves to incapacitate gametes that do not produce the antidote.

Acknowledgments

This work was supported by an NIH grant R35GM133774 to M.S. and NIH R35GM150907 to J.M.C. N.V.R was supported by an NSF Graduate Research Fellowship (grant no. DGE-2146755). M.E.F. was supported by an NSF Postdoctoral Research Fellowship in Biology (grant no. 2305853). Elements in Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5 were created with BioRender.com. We thank members of the Coughlan and Schumer labs for helpful feedback.

Declaration of interests

The authors declare no competing interests.

References

1. Darwin, C. (1859) *On the origin of species*, John Murray
2. Thompson, K.A. *et al.* (2024) The Ecology of Hybrid Incompatibilities. *Cold Spring Harb. Perspect. Biol.* 16, a041440
3. Garlovsky, M.D. *et al.* (2024) Synthesis and Scope of the Role of Postmating Prezygotic Isolation in Speciation. *Cold Spring Harb. Perspect. Biol.* 16, a041429
4. Mendelson, T.C. and Safran, R.J. (2021) Speciation by sexual selection: 20 years of progress. *Trends Ecol. Evol.* 36, 1153–1163
5. Shaw, K.L. *et al.* (2024) How Important Is Sexual Isolation to Speciation? *Cold Spring Harb. Perspect. Biol.* 16, a041427
6. Presgraves, D.C. (2010) The molecular evolutionary basis of species formation. *Nat. Rev. Genet.* 11, 175–180
7. Bateson, W. (1909) Heredity and variation in modern lights. In *Darwin and modern science*. (Seward, A. C., ed), Cambridge University Press
8. Dobzhansky, T. (1937) *Genetics and the Origin of Species*, Columbia University Press
9. Muller, H.J. (1942) Isolating mechanisms, evolution and temperature. *Biol. Symp.* 6, 71–125
10. Turelli, M. and Orr, H.A. (1995) The dominance theory of Haldane's rule. *Genetics* 140, 389–402
11. Orr, H.A. (1995) The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics* 139, 1805–1813
12. Chae, E. *et al.* (2014) Species-wide Genetic Incompatibility Analysis Identifies Immune Genes as Hotspots of Deleterious Epistasis. *Cell* 159, 1341–1351
13. Jhuang, H. *et al.* (2017) Mitochondrial–nuclear co-evolution leads to hybrid incompatibility through pentatricopeptide repeat proteins. *EMBO Rep.* 18, 87–101
14. Moran, B.M. *et al.* (2024) A lethal mitonuclear incompatibility in complex I of natural hybrids. *Nature* 626, 119–127
15. Kim, G. *et al.* (2024) Structure determinants of DANGEROUS MIX 3, an alpha/beta hydrolase, for triggering NLR-mediated genetic incompatibility in plantsbioRxiv, 2024.10.28.620575
16. Meiklejohn, C.D. *et al.* (2013) An Incompatibility between a Mitochondrial tRNA and Its Nuclear-Encoded tRNA Synthetase Compromises Development and Fitness in Drosophila. *PLOS Genet.* 9, e1003238
17. Powell, D.L. *et al.* (2020) Natural hybridization reveals incompatible alleles that cause melanoma in swordtail fish. *Science* 368, 731–736
18. Garcia-Olazabal, M. *et al.* (2025) Functional test of a naturally occurred tumor modifier gene provides insights to melanoma development. *G3 GenesGenomesGenetics* 15, jkae298
19. Masly, J.P. *et al.* (2006) Gene Transposition as a Cause of Hybrid Sterility in Drosophila. *Science* 313, 1448–1450
20. Bikard, D. *et al.* (2009) Divergent evolution of duplicate genes leads to genetic incompatibilities within A. thaliana. *Science* 323, 623–626
21. Zuellig, M.P. and Sweigart, A.L. (2018) A two-locus hybrid incompatibility is widespread, polymorphic, and active in natural populations of Mimulus*. *Evolution* 72, 2394–2405
22. Liao, B. *et al.* (2024) Dysfunction of duplicated pair rice histone acetyltransferases causes segregation distortion and an interspecific reproductive barrier. *Nat. Commun.* 15, 996
23. Guerrero, R.F. *et al.* (2017) Pervasive antagonistic interactions among hybrid

incompatibility loci. *PLOS Genet.* 13, e1006817
24. Dion-Côté, A.-M. and Barbash, D.A. (2017) Beyond speciation genes: an overview of genome stability in evolution and speciation. *Curr. Opin. Genet. Dev.* 47, 17–23
25. Wodsedalek, J.E. (1916) Causes of sterility in the mule. *Biol. Bull.* 30, 1-[56]-1
26. Yin, Y. *et al.* (2021) Molecular mechanisms and topological consequences of drastic chromosomal rearrangements of muntjac deer. *Nat. Commun.* 12, 6858
27. Ren, X. *et al.* (2018) Genomic basis of recombination suppression in the hybrid between Caenorhabditis briggsae and C. nigoni. *Nucleic Acids Res.* 46, 1295–1307
28. Bozdag, G.O. *et al.* (2021) Breaking a species barrier by enabling hybrid recombination. *Curr. Biol.* 31, R180–R181
29. Fishman, L. *et al.* (2013) Chromosomal Rearrangements and the Genetics of Reproductive Barriers in Mimulus (Monkeyflowers). *Evolution* 67, 2547–2560
30. Fuller, Z.L. *et al.* (2019) How chromosomal rearrangements shape adaptation and speciation: Case studies in Drosophila pseudoobscura and its sibling species Drosophila persimilis. *Mol. Ecol.* 28, 1283–1301
31. Zhang, L. *et al.* (2021) How Important Are Structural Variants for Speciation? *Genes* 12, 1084
32. Berdan, E.L. *et al.* (2024) Structural Variants and Speciation: Multiple Processes at Play. *Cold Spring Harb. Perspect. Biol.* 16, a041446
33. Orsi, G.A. *et al.* (2010) Drosophila I-R hybrid dysgenesis is associated with catastrophic meiosis and abnormal zygote formation. *J. Cell Sci.* 123, 3515–3524
34. Parhad, S.S. *et al.* (2017) Adaptive Evolution Leads to Cross-Species Incompatibility in the piRNA Transposon Silencing Machinery. *Dev. Cell* 43, 60-70.e5
35. Wang, L. *et al.* (2020) Adaptive evolution among cytoplasmic piRNA proteins leads to decreased genomic auto-immunity. *PLOS Genet.* 16, e1008861
36. Castillo, D.M. and Moyle, L.C. (2022) Hybrid incompatibility between Drosophila virilis and D. lummei is stronger in the presence of transposable elements. *J. Evol. Biol.* 35, 1319–1334
37. Serrato-Capuchina, A. *et al.* (2021) P-elements strengthen reproductive isolation within the Drosophila simulans species complex. *Evolution* 75, 2425–2440
38. McClintock, B. (1984) The Significance of Responses of the Genome to Challenge. *Science* 226, 792–801
39. Kelleher, E.S. *et al.* (2012) Drosophila Interspecific Hybrids Phenocopy piRNA-Pathway Mutants. *PLOS Biol.* 10, e1001428
40. Widen, S.A. *et al.* (2023) Virus-like transposons cross the species barrier and drive the evolution of genetic incompatibilities. *Science* 380, eade0705
41. Shan, X. *et al.* (2005) Mobilization of the Active MITE Transposons mPing and Pong in Rice by Introgression from Wild Rice (Zizania latifolia Griseb.). *Mol. Biol. Evol.* 22, 976–990
42. Kenan-Eichler, M. *et al.* (2011) Wheat Hybridization and Polyploidization Results in Deregulation of Small RNAs. *Genetics* 188, 263–272
43. Ungerer, M.C. *et al.* (2006) Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation. *Curr. Biol.* 16, R872–R873
44. Dion-Côté, A.-M. *et al.* (2014) RNA-seq Reveals Transcriptomic Shock Involving Transposable Elements Reactivation in Hybrids of Young Lake Whitefish Species. *Mol. Biol. Evol.* 31, 1188–1199

45. Metcalfe, C.J. *et al.* (2007) Genomic Instability Within Centromeres of Interspecific Marsupial Hybrids. *Genetics* 177, 2507–2517
46. Smukowski Heil, C. *et al.* (2021) Transposable Element Mobilization in Interspecific Yeast Hybrids. *Genome Biol. Evol.* 13, evab033
47. Göbel, U. *et al.* (2018) Robustness of Transposable Element Regulation but No Genomic Shock Observed in Interspecific Arabidopsis Hybrids. *Genome Biol. Evol.* 10, 1403–1415
48. Chen, M. *et al.* (2008) RNAi of met1 Reduces DNA Methylation and Induces Genome-Specific Changes in Gene Expression and Centromeric Small RNA Accumulation in Arabidopsis Allopolyploids. *Genetics* 178, 1845–1858
49. Brand, C.L. and Levine, M.T. (2022) Cross-species incompatibility between a DNA satellite and the Drosophila Spartan homolog poisons germline genome integrity. *Curr. Biol.* 32, 2962-2971.e4
50. Cabral-de-Mello, D.C. and Palacios-Gimenez, O.M. (2024) Repetitive DNAs: The "invisible" regulators of insect adaptation and speciation. *Curr. Opin. Insect Sci.* DOI: 10.1016/j.cois.2024.101295
51. Kaikkonen, M.U. *et al.* (2011) Non-coding RNAs as regulators of gene expression and epigenetics. *Cardiovasc. Res.* 90, 430–440
52. Dziasek, K. *et al.* (2024) Dosage-sensitive maternal siRNAs determine hybridization success in Capsella. *Nat. Plants* DOI: 10.1038/s41477-024-01844-3
53. Josefsson, C. *et al.* (2006) Parent-Dependent Loss of Gene Silencing during Interspecies Hybridization. *Curr. Biol.* 16, 1322–1328
54. Florez-Rueda, A.M. *et al.* (2021) Endosperm and Seed Transcriptomes Reveal Possible Roles for Small RNA Pathways in Wild Tomato Hybrid Seed Failure. *Genome Biol. Evol.* 13, evab107
55. Ishikawa, R. *et al.* (2011) Rice interspecies hybrids show precocious or delayed developmental transitions in the endosperm without change to the rate of syncytial nuclear division. *Plant J.* 65, 798–806
56. Pang, K.C. *et al.* (2006) Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. *Trends Genet.* 22, 1–5
57. Dobzhansky, T. (1937) Genetic Nature of Species Differences. *Am. Nat.* 71, 404–420
58. Kidwell, M.G. *et al.* (1977) Hybrid dysgenesis in Drosophila melanogaster: A syndrome of abberant traits including mutation, sterility and male recombination. *Genetics* 86, 813–833
59. Turelli, M. and Moyle, L.C. (2007) Asymmetric Postmating Isolation: Darwin's Corollary to Haldane's Rule. *Genetics* 176, 1059–1088
60. Phadnis, N. and Orr, H.A. (2009) A single gene causes both male sterility and segregation distortion in Drosophila hybrids. *Science* 323, 376–379
61. Lindtke, D. and Buerkle, C.A. (2015) The genetic architecture of hybrid incompatibilities and their effect on barriers to introgression in secondary contact. *Evolution* 69, 1987–2004
62. Schumer, M. *et al.* (2015) Reproductive Isolation of Hybrid Populations Driven by Genetic Incompatibilities. *PLOS Genet.* 11, e1005041
63. Bank, C. *et al.* (2012) The limits to parapatric speciation: Dobzhansky–Muller incompatibilities in a continent–island model. *Genetics* 191, 845–863
64. Lollar, M.J. *et al.* (2023) Hybrid breakdown in male reproduction between recently diverged Drosophila melanogaster populations has a complex and variable genetic architecture. *Evolution* 77, 1550–1563
65. Bladen, J. *et al.* (2024) A new hybrid incompatibility locus between D. melanogaster and

D. sechellia. *Genetics* DOI: 10.1093/genetics/iyae001
66. Barbash, D.A. *et al.* (2003) A rapidly evolving MYB-related protein causes species isolation in Drosophila. *Proc. Natl. Acad. Sci.* 100, 5302–5307
67. Brideau, N.J. *et al.* (2006) Two Dobzhansky-Muller Genes Interact to Cause Hybrid Lethality in Drosophila. *Science* 314, 1292–1295
68. Kuzmin, E. *et al.* (2018) Systematic analysis of complex genetic interactions. *Science* 360, eaao1729
69. Lu, Y. *et al.* (2020) Oncogenic allelic interaction in Xiphophorus highlights hybrid incompatibility. *Proc. Natl. Acad. Sci.* 117, 29786–29794
70. Simpson, D. *et al.* (2023) Mapping the Genetic Interaction Network of PARP inhibitor ResponsebioRxiv, 2023.08.19.553986
71. Allen, K.N. *et al.* (2024) Hypoxia exposure blunts angiogenic signaling and upregulates the antioxidant system in endothelial cells derived from elephant seals. *BMC Biol.* 22, 91
72. Alcock, D. *et al.* (2024) Generating bat primary and immortalised cell-lines from wing biopsies. *Sci. Rep.* 14, 27633
73. Stern, D.L. (2014) Identification of loci that cause phenotypic variation in diverse species with the reciprocal hemizygosity test. *Trends Genet.* 30, 547–554
74. Jung, M. *et al.* Unified single-cell analysis of testis gene regulation and pathology in five mouse strains. *eLife* 8, e43966
75. Hunnicutt, K.E. *et al.* (2025) Different complex regulatory phenotypes underlie hybrid male sterility in divergent rodent crosses. *Genetics* 229, iyae198
76. Wei, W.-H. *et al.* (2014) Detecting epistasis in human complex traits. *Nat. Rev. Genet.* 15, 722–733
77. Schumer, M. and Brandvain, Y. (2016) Determining epistatic selection in admixed populations. *Mol. Ecol.* 25, 2577–2591
78. Robles, N.V. *et al.* (2025) Admixture mapping reveals evidence for multiple mitonuclear incompatibilities in swordtail fish hybridsbioRxiv, 2025.01.30.635158
79. Muzio, G. *et al.* (2023) networkGWAS: a network-based approach to discover genetic associations. *Bioinformatics* 39, btad370
80. Adrion, J.R. *et al.* (2020) Predicting the Landscape of Recombination Using Deep Learning. *Mol. Biol. Evol.* 37, 1790–1808
81. Moyle, L.C. and Nakazato, T. (2010) Hybrid Incompatibility "Snowballs" Between Solanum Species. *Science* 329, 1521–1523
82. Matute, D.R. *et al.* (2010) A Test of the Snowball Theory for the Rate of Evolution of Hybrid Incompatibilities. *Science* 329, 1518–1521
83. Wang, R.J. *et al.* (2015) The Pace of Hybrid Incompatibility Evolution in House Mice. *Genetics* 201, 229–242
84. Maya-Lastra, C.A. and Eaton, D.A.R. (2021) Genetic incompatibilities do not snowball in a demographic model of speciationbioRxiv, 2021.02.23.432472
85. Unckless, R.L. (2023) Meiotic drive, postzygotic isolation, and the Snowball EffectbioRxiv, 2023.11.14.567107
86. Frank, S.A. (1991) Divergence of Meiotic Drive-Suppression Systems as an Explanation for Sex-Biased Hybrid Sterility and Inviability. *Evolution* 45, 262–267
87. Hurst, L.D. and Pomiankowski, A. (1991) Causes of Sex Ratio Bias May Account for Unisexual Sterility in Hybrids: A New Explanation of Haldane's Rule and Related Phenomena. *Genetics* 128, 841–858

88. Coyne, J.A. *et al.* (1991) Haldane's Rule Revisited. *Evolution* 45, 1710–1714
89. Charlesworth, B. *et al.* (1993) Meiotic drive and unisexual hybrid sterility: a comment. *Genetics* 133, 421–432
90. Gavrilets, S. (2014) Models of Speciation: Where Are We Now? *J. Hered.* 105, 743–755
91. Zuellig, M.P. and Sweigart, A.L. (2018) Gene duplicates cause hybrid lethality between sympatric species of Mimulus. *PLOS Genet.* 14, e1007130
92. Koide, Y. *et al.* (2018) Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. *Proc. Natl. Acad. Sci.* 115, E1955–E1962
93. Oliver, P.L. *et al.* (2009) Accelerated evolution of the Prdm9 speciation gene across diverse Metazoan taxa. *PLOS Genet.* 5, e1000753
94. Baker, Z. *et al.* (2017) Repeated losses of PRDM9-directed recombination despite the conservation of PRDM9 across vertebrates. *eLife* 6, e24133
95. Maheshwari, S. and Barbash, D.A. (2011) The Genetics of Hybrid Incompatibilities. *Annu. Rev. Genet.* 45, 331–355
96. Sweigart, A.L. *et al.* (2019) Making a murderer: The evolutionary framing of hybrid gamete-killers. *Trends Genet.* 35, 245–252
97. Li, L. and Weigel, D. (2021) One Hundred Years of Hybrid Necrosis: Hybrid Autoimmunity as a Window into the Mechanisms and Evolution of Plant–Pathogen Interactions. *Annu. Rev. Phytopathol.* 59, 213–237
98. Coughlan, J.M. (2023) The role of conflict in shaping plant biodiversity. *New Phytol.* 240, 2210–2217
99. Searle, J.B. and Villena, F.P.-M. de (2024) Meiotic Drive and Speciation. *Annu. Rev. Genet.* 58, 341–363
100. Agrawal, A.F. *et al.* (2011) Ecological Divergence and the Origins of Intrinsic Postmating Isolation with Gene Flow. *Int. J. Ecol.* 2011, 435357
101. Schluter, D. (2009) Evidence for Ecological Speciation and Its Alternative. *Science* 323, 737–741
102. de Oliveira, A.K. and Cordeiro, A.R. (1980) Adaptation of Drosophila willistoni experimental populations to extreme pH medium. *Heredity* 44, 123–130
103. Dettman, J.R. *et al.* (2007) Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature* 447, 585–588
104. Anderson, J.B. *et al.* (2010) Determinants of Divergent Adaptation and Dobzhansky-Muller Interaction in Experimental Yeast Populations. *Curr. Biol.* 20, 1383–1388
105. Ono, J. *et al.* (2017) Widespread Genetic Incompatibilities between First-Step Mutations during Parallel Adaptation of Saccharomyces cerevisiae to a Common Environment. *PLOS Biol.* 15, e1002591
106. Moyle, L.C. *et al.* (2012) Hybrid Sterility over Tens of Meters Between Ecotypes Adapted to Serpentine and Non-Serpentine Soils. *Evol. Biol.* 39, 207–218
107. Caisse, M. and Antonovics, J. (1978) Evolution in closely adjacent plant populations. *Heredity* 40, 371–384
108. Wang, S. *et al.* (2021) Signatures of mitonuclear coevolution in a warbler species complex. *Nat. Commun.* 12, 4279
109. Healy, T.M. and Burton, R.S. (2023) Genetic incompatibilities in reciprocal hybrids between populations of Tigriopus californicus with low to moderate mitochondrial sequence divergence. *Evolution* 77, 2100–2108
110. Wright, K.M. *et al.* (2013) Indirect Evolution of Hybrid Lethality Due to Linkage with

Selected Locus in Mimulus guttatus. *PLOS Biol.* 11, e1001497
111. Li, C. *et al.* (2023) Tight genetic linkage of genes causing hybrid necrosis and pollinator isolation between young species. *Nat. Plants* 9, 420–432
112. True, J.R. and Haag, E.S. (2001) Developmental system drift and flexibility in evolutionary trajectories. *Evol. Dev.* 3, 109–119
113. Mack, K.L. and Nachman, M.W. (2017) Gene Regulation and Speciation. *Trends Genet.* 33, 68–80
114. Schiffman, J.S. and Ralph, P.L. (2022) System drift and speciation. *Evolution* 76, 236–251
115. Kerwin, R.E. and Sweigart, A.L. (2020) Rampant Misexpression in a Mimulus (Monkeyflower) Introgression Line Caused by Hybrid Sterility, Not Regulatory Divergence. *Mol. Biol. Evol.* 37, 2084–2098
116. Runemark, A. *et al.* Hybridization and gene expression: Beyond differentially expressed genes. *Mol. Ecol.* n/a, e17303
117. Chang, W. *et al.* (2021) Gap genes are involved in inviability in hybrids between Drosophila melanogaster and D. santomea.bioRxiv, 2021.12.06.471493
118. Chang, W. *et al.* (2021) Rapid evolution of the functionally conserved gap gene giant in Drosophila.bioRxiv, 2021.07.08.451553
119. Boocock, J. *et al.* (2021) Ancient balancing selection maintains incompatible versions of the galactose pathway in yeast. *Science* 371, 415–419
120. Sicard, A. *et al.* (2015) Divergent sorting of a balanced ancestral polymorphism underlies the establishment of gene-flow barriers in Capsella. *Nat. Commun.* 6, 7960
121. Cutter, A.D. (2012) The polymorphic prelude to Bateson–Dobzhansky–Muller incompatibilities. *Trends Ecol. Evol.* 27, 209–218
122. Dobzhansky, T. (1964) Biology, Molecular and Organismic. *Am. Zool.* 4, 443–452
123. Owens, G.L. and Samuk, K. (2020) Adaptive introgression during environmental change can weaken reproductive isolation. *Nat. Clim. Change* 10, 58–62
124. Ostevik, K.L. *et al.* (2021) Morning glory species co-occurrence is associated with asymmetrically decreased and cascading reproductive isolation. *Evol. Lett.* 5, 75–85
125. Aguillon, S.M. *et al.* (2025) Pervasive gene flow despite strong and varied reproductive barriers in swordtails. *Nat. Ecol. Evol.* DOI: 10.1038/s41559-025-02669-9
126. Nelson, T.C. *et al.* (2021) Ancient and recent introgression shape the evolutionary history of pollinator adaptation and speciation in a model monkeyflower radiation (Mimulus section Erythranthe). *PLOS Genet.* 17, e1009095
127. Frayer, M.E. and Payseur, B.A. (2024) Do genetic loci that cause reproductive isolation in the lab inhibit gene flow in nature? *Evolution* 78, 1025–1038
128. Langdon, Q.K. *et al.* (2022) Predictability and parallelism in the contemporary evolution of hybrid genomes. *PLoS Genet.* 18, e1009914
129. Langdon, Q.K. *et al.* (2024) Swordtail fish hybrids reveal that genome evolution is surprisingly predictable after initial hybridization. *PLOS Biol.* 22, e3002742
130. Mantel, S.J. and Sweigart, A.L. (2024) Postzygotic barriers persist despite ongoing introgression in hybridizing Mimulus species. *Mol. Ecol.* 33, e17261
131. Farnitano, M.C. (2024) Causes and Implications of Variation in Reproductive Isolation Across Mimulus Monkeyflowers. Ph.D., University of Georgia
132. Mandeville, E.G. *et al.* (2017) Inconsistent reproductive isolation revealed by interactions between Catostomus fish species. *Evol. Lett.* 1, 255–268
133. Coughlan, J.M. and Matute, D.R. (2020) The importance of intrinsic postzygotic barriers

throughout the speciation process. *Philos. Trans. R. Soc. B Biol. Sci.* 375, 20190533
134. Soliman, H.K. and Coughlan, J.M. (2024) United by conflict: Convergent signatures of parental conflict in angiosperms and placental mammals. *J. Hered.* 115, 625–642
135. Johnson, N.A. (2010) Hybrid incompatibility genes: remnants of a genomic battlefield? *Trends Genet.* 26, 317–325
136. Nosil, P. and Schluter, D. (2011) The genes underlying the process of speciation. *Trends Ecol. Evol.* 26, 160–167
137. Hunter, N. *et al.* (1996) The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. *EMBO J.* 15, 1726–1733
138. Greig, D. *et al.* (2003) A role for the mismatch repair system during incipient speciation in Saccharomyces. *J. Evol. Biol.* 16, 429–437
139. Rogers, D.W. *et al.* (2018) Spore-autonomous fluorescent protein expression identifies meiotic chromosome mis-segregation as the principal cause of hybrid sterility in yeast. *PLOS Biol.* 16, e2005066
140. Bhattacharyya, T. *et al.* (2013) Mechanistic basis of infertility of mouse intersubspecific hybrids. *Proc. Natl. Acad. Sci.* 110, E468–E477
141. Davies, B. *et al.* (2021) Altering the Binding Properties of PRDM9 Partially Restores Fertility across the Species Boundary. *Mol. Biol. Evol.* 38, 5555–5562
142. Calvo-Baltanás, V. *et al.* (2021) Hybrid Incompatibility of the Plant Immune System: An Opposite Force to Heterosis Equilibrating Hybrid Performances. *Front. Plant Sci.* 11
143. Reifová, R. *et al.* (2023) Mechanisms of Intrinsic Postzygotic Isolation: From Traditional Genic and Chromosomal Views to Genomic and Epigenetic Perspectives. *Cold Spring Harb. Perspect. Biol.* 15, a041607
144. Kitano, J. and Okude, G. (2024) Causative genes of intrinsic hybrid incompatibility in animals and plants: what we have learned about speciation from the molecular perspective. *Evol. J. Linn. Soc.* 3, kzae022
145. Fernandez, A.A. and Morris, M.R. (2008) Mate choice for more melanin as a mechanism to maintain a functional oncogene. *Proc. Natl. Acad. Sci.* 105, 13503–13507
146. Krüger, J. *et al.* (2002) A Tomato Cysteine Protease Required for Cf-2-Dependent Disease Resistance and Suppression of Autonecrosis. *Science* 296, 744–747

A

Ancestral species

Gene duplication

Species divergence

Gene loss

Population A

Population B

F1 hybrids

Proportion of F2 hybrids inherit no functional genes

B

Species A nuclear genome

X

Species B mitochondrial genome

Nuclear genes from species A interact with mitochondrial genes from species B

Ancestry mismatch causes protein incompatibility

C

gap gene

Genetic divergence

tailless

?

Giant

Caudal, Bicoid

GAP

Population A

Population B

Same developmental output

Developmental drift results in hybrid inviability

D

Ancestral species

Male with spot

Female

Male without spot

Females prefer spotted males

Selection for spotted males

Species diverge

Species A without spot

Species B with spot

xmrk

regulator gene

Species B mates with species A

Proportion of F2 hybrids lacking a regulator develop melanoma

E

Fungi

Ancestral species

Immunity gene

Immunity regulator gene

Fungal resistance

Species diverge and adapt to fungal variants

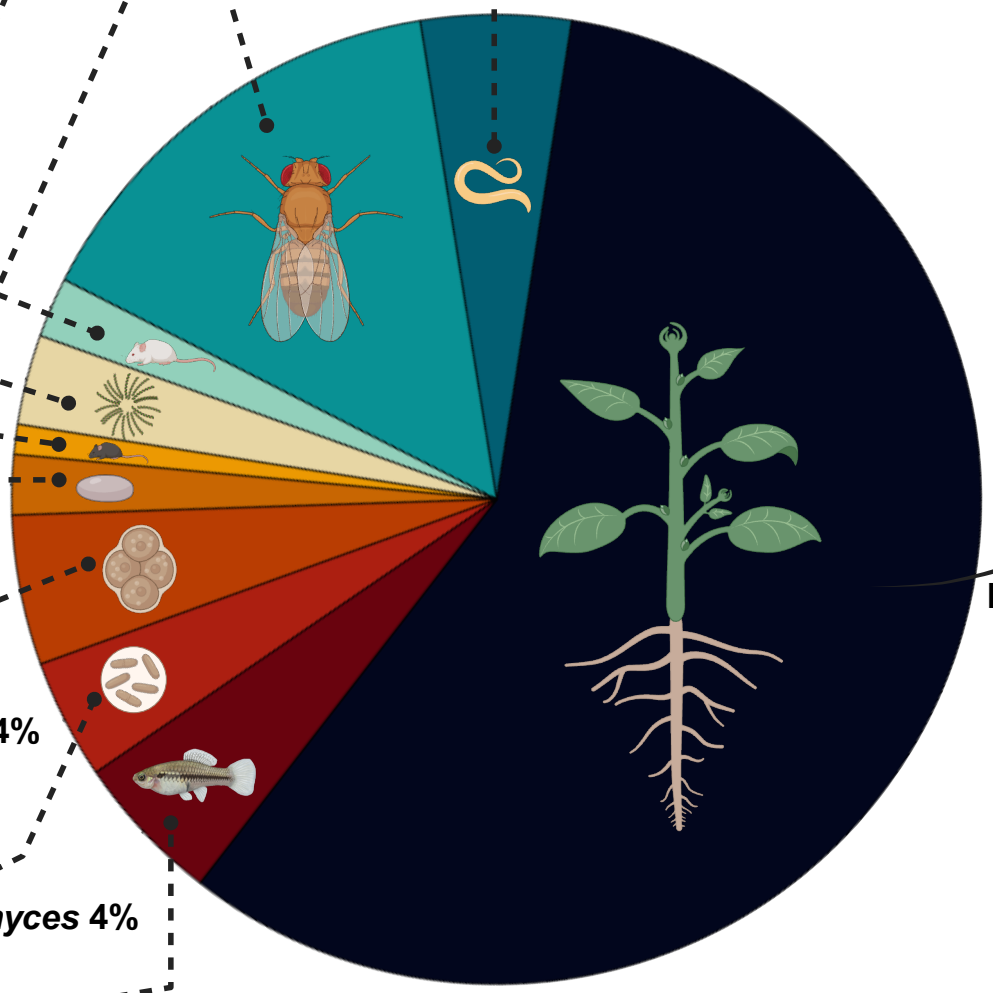
Population A

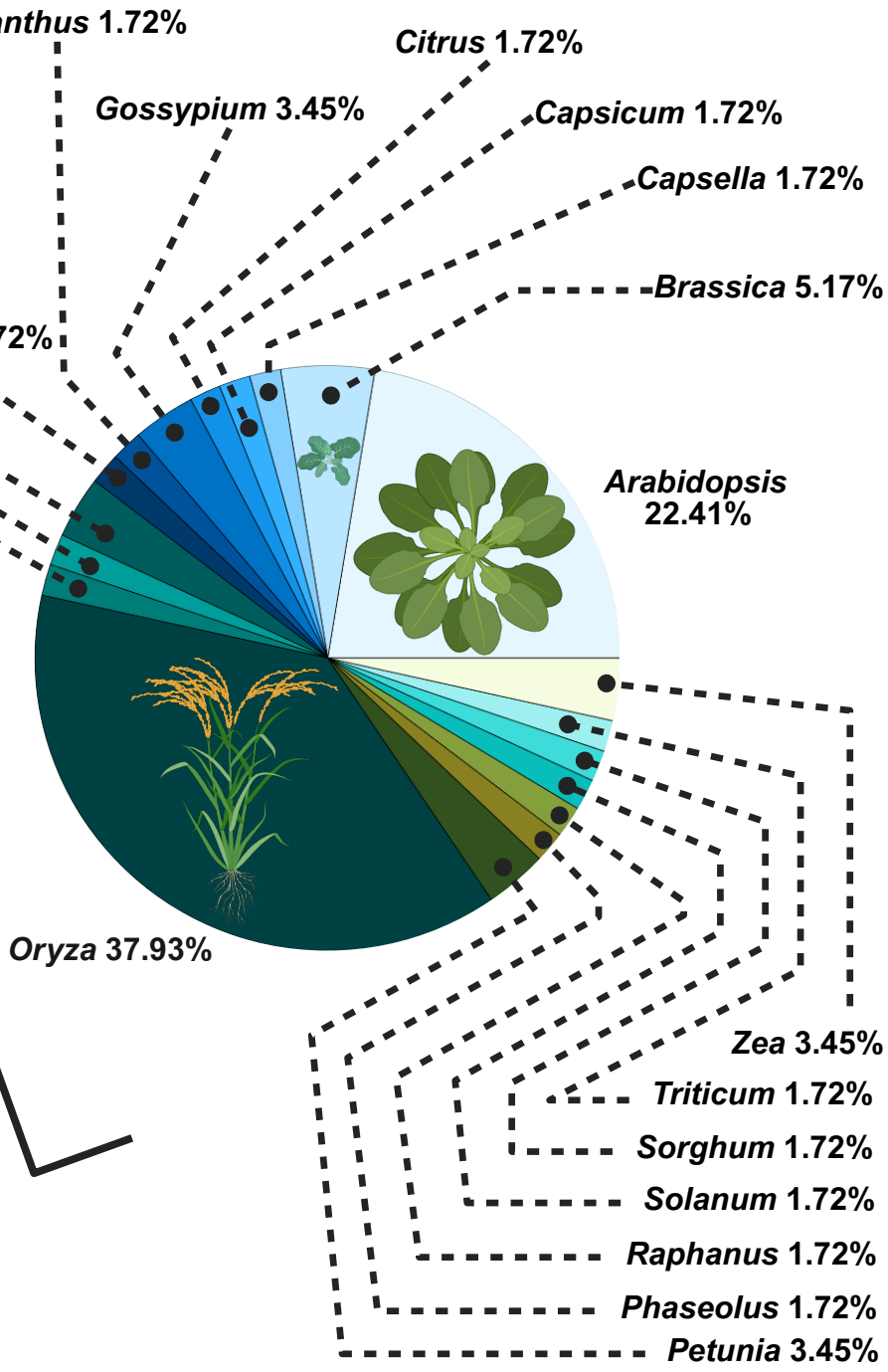
Population B

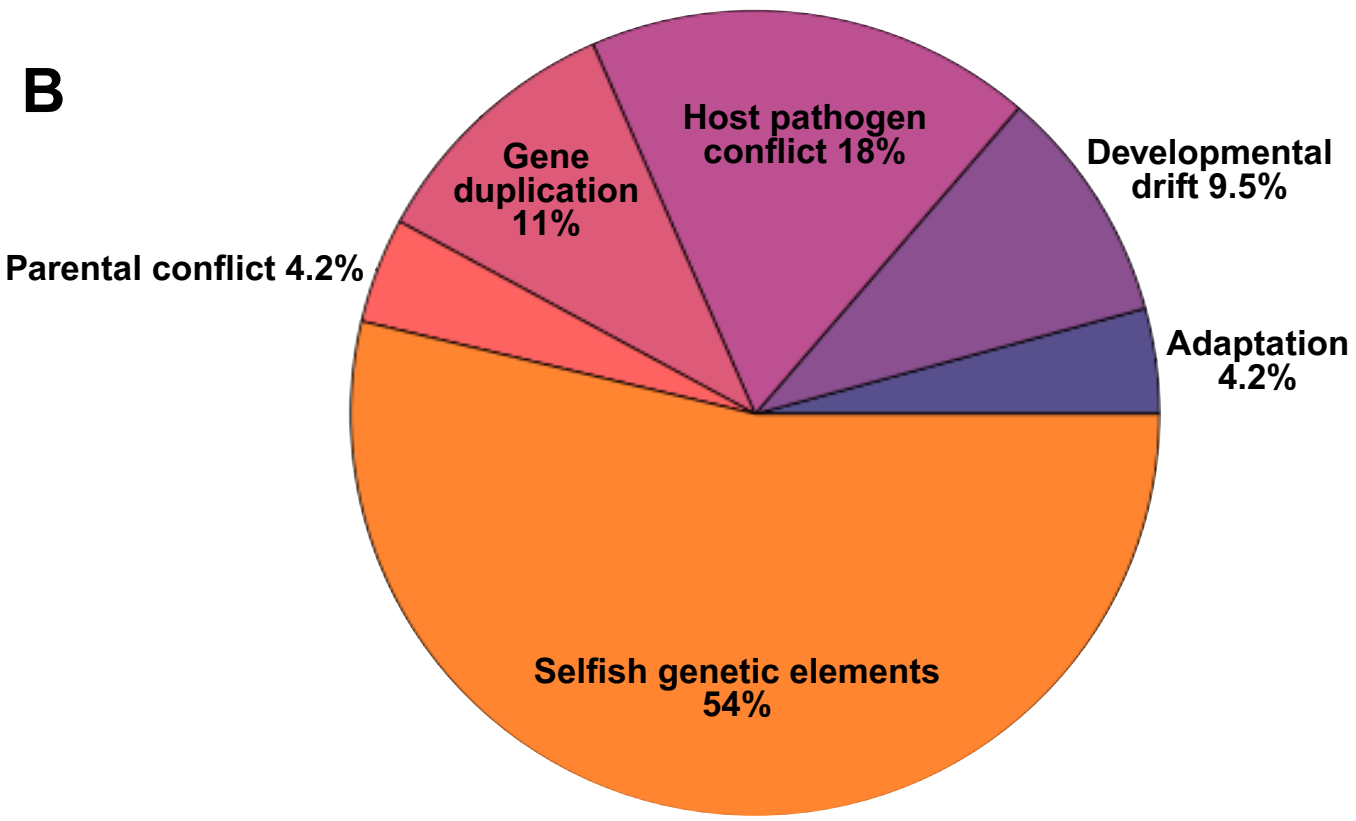
Misregulation of the immune response results in hybrid necrosis

Figure 1. Illustration of different evolutionary mechanisms that can contribute to the evolution of hybrid incompatibilities (Table 1). **(A)** Gene Duplication in the ancestral lineage followed by differential loss of duplicate copies in the daughter lineages can result in subset of hybrids inheriting no copies of a gene, which commonly results in inviability. **(B)** Coevolution between interacting proteins within lineages can result in dysfunctional interactions when mismatched proteins are introduced to each other in hybrids. Example shown here corresponds to a mitonuclear incompatibility from Moran et al. [14]. **(C)** Developmental systems drift describes the observation that genetic pathways underlying important biological processes can diverge over evolutionary timescales, but remain functionally conserved. The example shown here is drawn from Chang et al. [118,118], where the authors found that combining different versions of conserved developmental pathways in *Drosophila* hybrids can result in developmental defects. This example is further discussed in Fig. 5. **(D)** Adaptation and sexual selection can drive the fixation of variants that differ between species, and as a byproduct of this process, these variants can become involved in hybrid incompatibilities. Evidence for this particular process is sparse. Here we show an example from Powell et al. [17] where sexual selection may be important in the evolution of a melanoma incompatibility. Spotting patterns are sexually selected in some *Xiphophorus* species [145] but the gene underlying these spots often causes melanoma in hybrids. **(E)** Evolutionary arms races between pathogens and hosts can drive genetic changes between host lineages in genes involved in pathogen response. Misregulation of these genes in hybrids has been identified as a frequent cause of hybrid necrosis in plants. Example shown here corresponds to the case described by Kruger et. al. [146].

A


Peromyscus 1%
Neurospora 3%
Drosophila 5%
Caenorhabditis 5%
Mus 2%
Podospora 2%
Saccharomyces 4%
Schizosaccharomyces 4%
Xiphophorus 5%
Plants 58%


Nicotiana 1.72%
Helianthus 1.72%
Citrus 1.72%
Moricandia 1.72%
Gossypium 3.45%
Capsicum 1.72%
Mimulus 3.45%
Capsella 1.72%
Lactuca 1.72%
Brassica 5.17%
Arabidopsis 22.41%
Oryza 37.93%
Zea 3.45%
Triticum 1.72%
Sorghum 1.72%
Solanum 1.72%
Raphanus 1.72%
Phaseolus 1.72%
Petunia 3.45%


B
Host pathogen conflict 18%
Gene duplication 11%
Developmental drift 9.5%
Parental conflict 4.2%
Adaptation 4.2%
Selfish genetic elements 54%

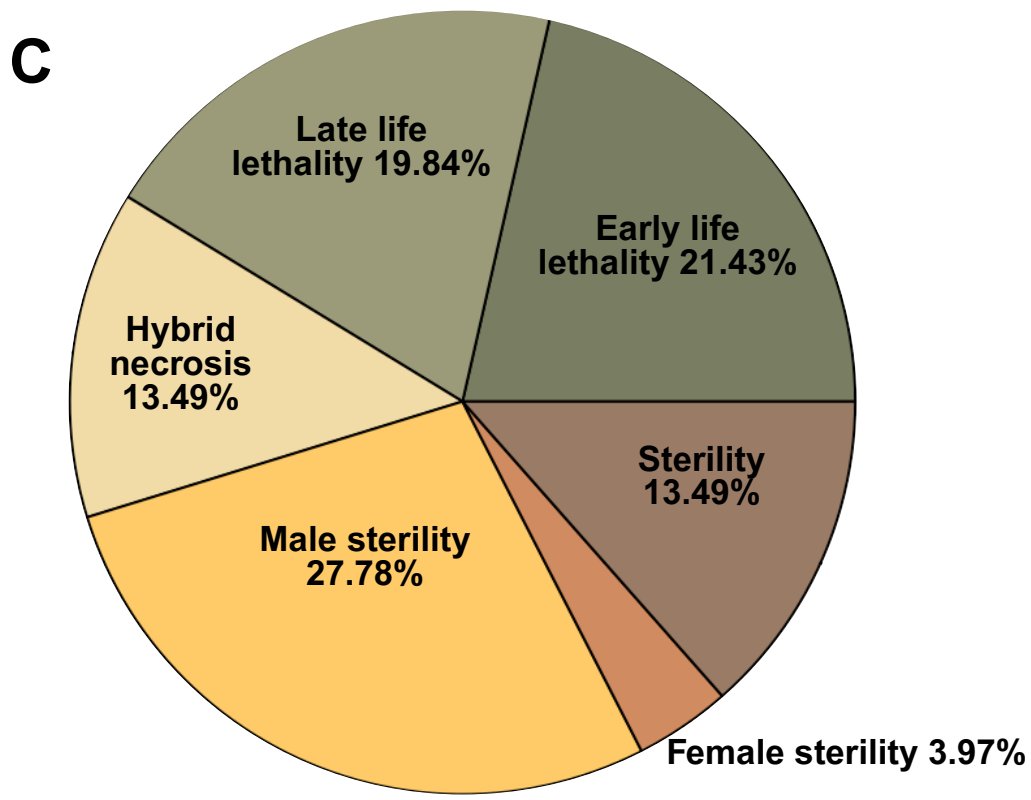

C
Late life lethality 19.84%
Early life lethality 21.43%
Hybrid necrosis 13.49%
Sterility 13.49%
Male sterility 27.78%
Female sterility 3.97%

Figure 2. Summary of results from our literature search and curation of hybrid incompatibility genes that have been precisely mapped. **A)** Despite recent progress, hybrid incompatibilities have been mapped in only a small subset of eukaryotic species. Shown here are results from Table 1 split by taxonomic group, with the inset summarizing plant genera where incompatibilities have been mapped. These results highlight several lineages that are absent from the existing literature, including amphibians and reptiles, among many others. **B)** Proportion of hybrid incompatibilities that have been identified categorized by the likely mechanism that drove their evolution (see Table 1). The "Adaptation" category includes cases involving both sexual selection and ecological adaptation. We note that although we plot only one mechanism per incompatibility – the one the authors of the original work viewed as most likely – many mechanisms are not mutually exclusive and a given hybrid incompatibility may span more than one category. **C)** Proportion of hybrid incompatibilities classified as a function of hybrid phenotype reported. Early Life and Late Life Lethality include cases of melanoma, abnormal development, biased sex ratio, and inviability. Female and Male Sterility refers to either sterility of an individual of a given sex or sterility of the male vs female gametes in hermaphroditic plants. If sterility is present in both sexes or not specified, it is listed under "Sterility".

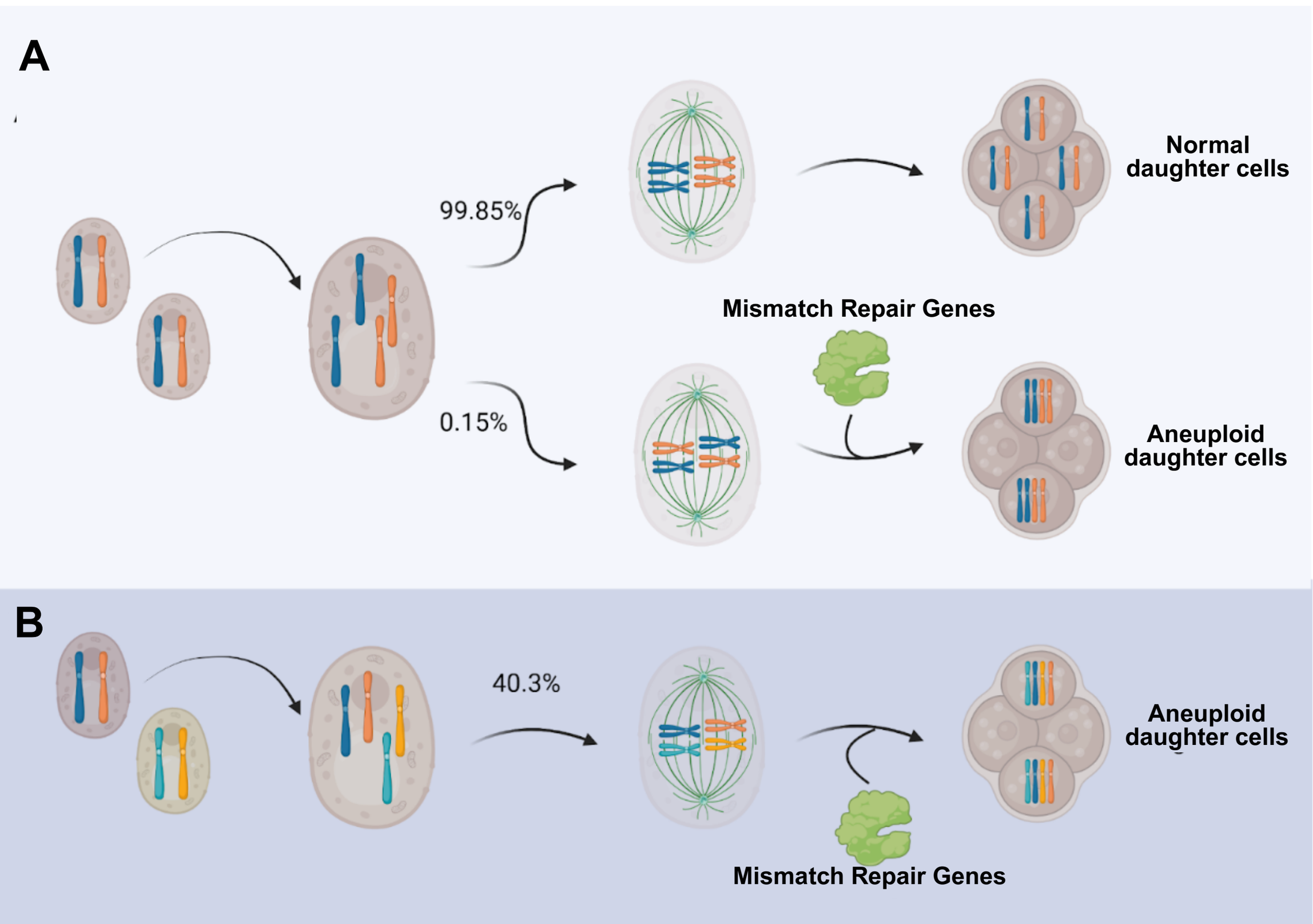
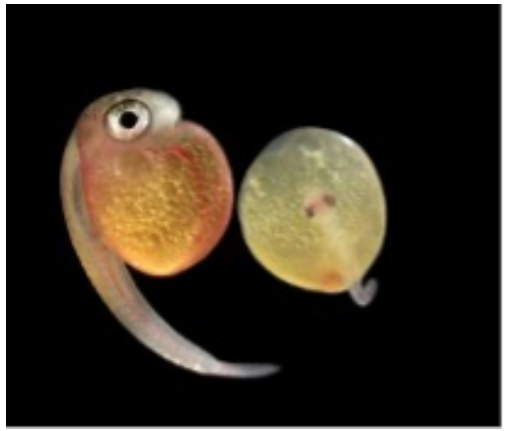

A
99.85%
Normal
daughter cells
Mismatch Repair Genes
0.15%
Aneuploid
daughter cells
B
40.3%
Aneuploid
daughter cells
Mismatch Repair Genes

Figure 3. During a typical *S. cerevisiae* meiosis **(A)**, chromosomes pair with their homologs, undergo recombination, and are then sorted into haploid gametes. In about 0.15% of meioses [139], the mismatch repair system detects a lack of similarity between pairs and halts segregation, producing tetrads with aneuploid cells. In hybrids between *S. cerevisiae* and *S. paradoxus* **(B)**, the mismatch repair system is often activated by the sequence divergence between homologous chromosomes derived from each species. This results in tetrads with aneuploid cells, and because it occurs at such a high rate, the hybrid yeast are rendered sterile.

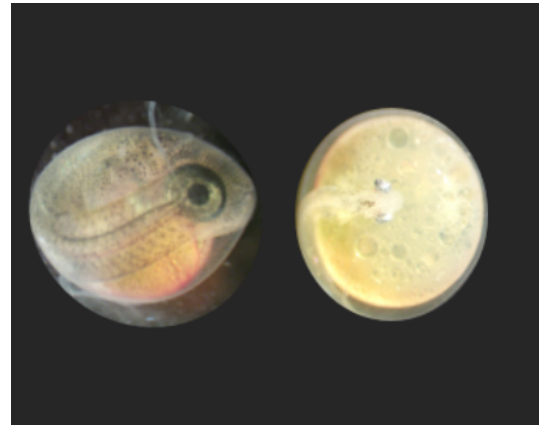
A

X. malinche x *X. birchmanni* F2



B

X. cortezi x *X. birchmanni* F2



C

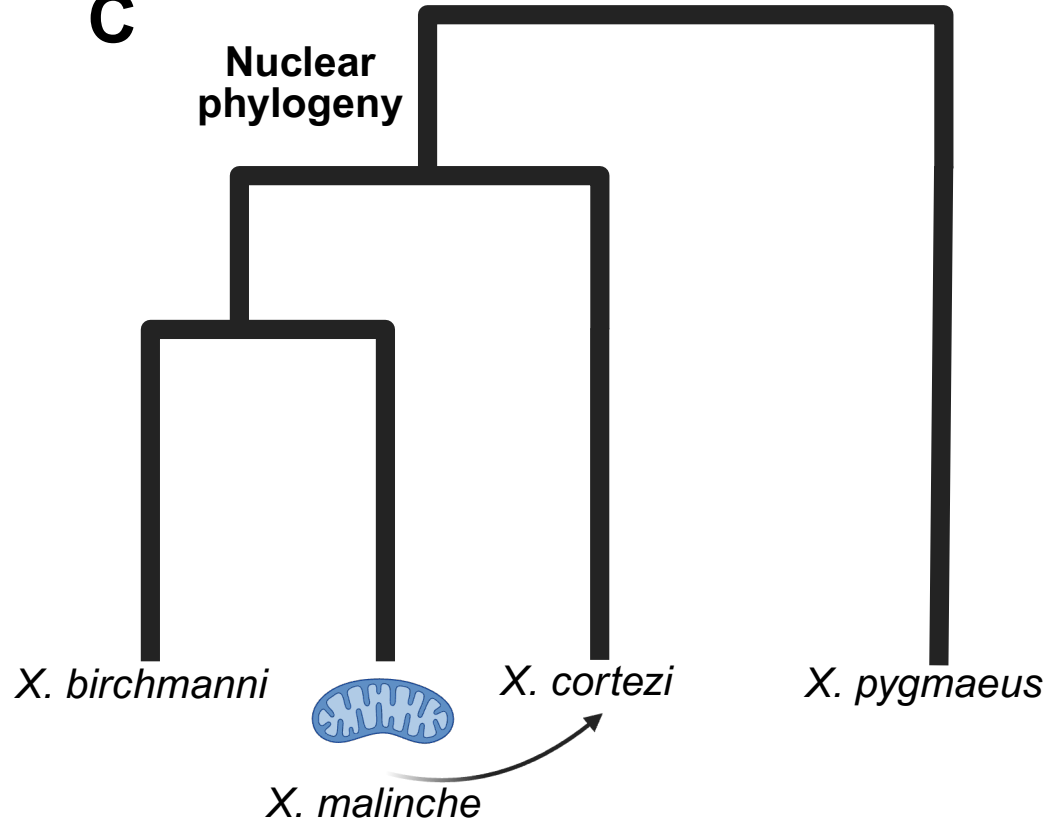
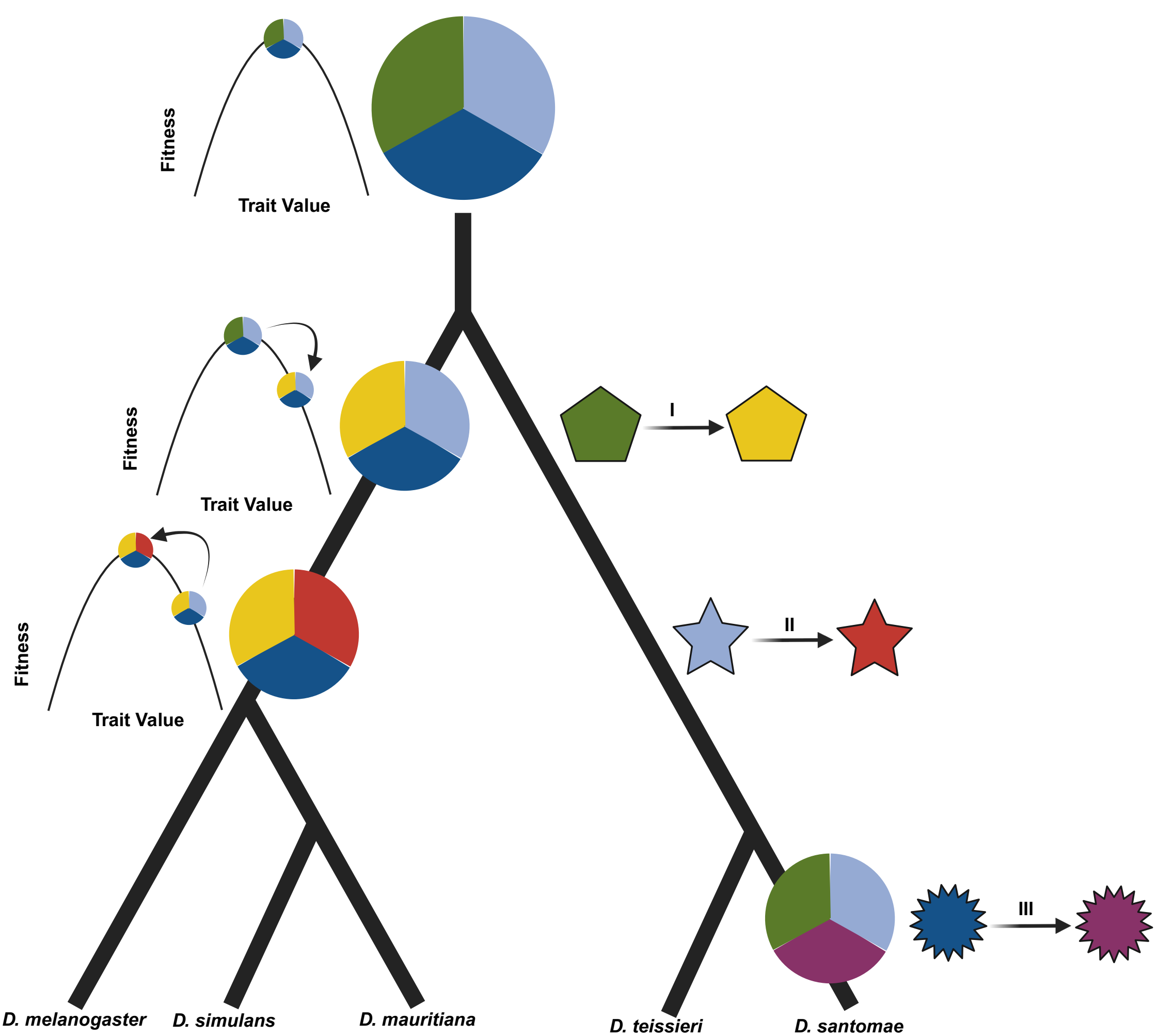
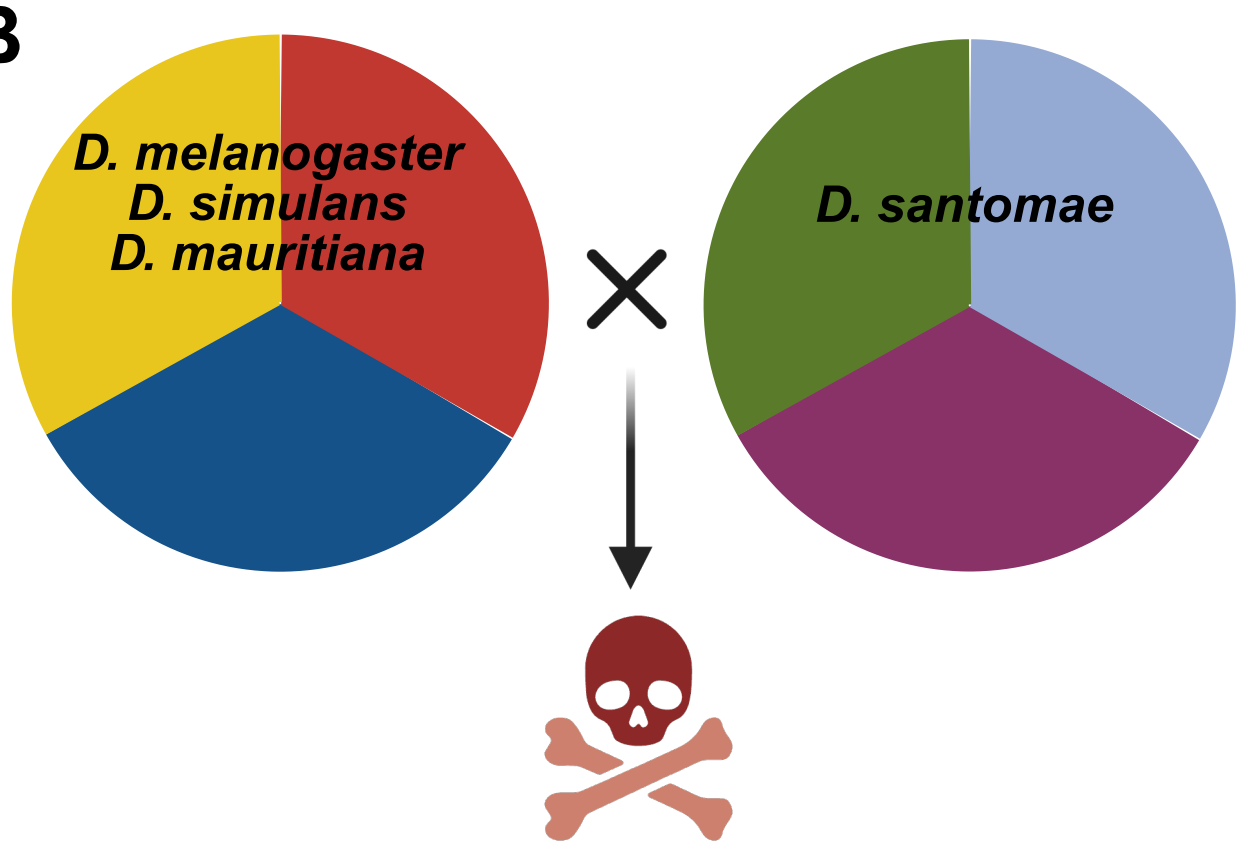


Figure 4. A) F2 hybrid with *X. malinche* mitochondrial ancestry and *X. birchmanni* heterozygous nuclear ancestry at *ndufs5* (left); F2 hybrid with *X. malinche* mitochondrial ancestry and *X. birchmanni* homozygous ancestry at *ndufs5* (right; [14]). **B)** F2 hybrid with *X. cortezi* mitochondrial ancestry and *X. birchmanni* heterozygous nuclear ancestry at *ndufs5* (left); F2 hybrid with *X. cortezi* mitochondrial ancestry and *X. birchmanni* homozygous ancestry at *ndufs5* (right;[125]). Ancestry mismatch at these loci has remarkably similar consequences for phenotypes and hybrid survival in *X. cortezi* x *X. birchmanni* hybrids as in *X. malinche* x *X. birchmanni* hybrids. Individuals with mismatched ancestry at *ndufs5* undergo arrested development *in utero* in both crosses and experience essentially 100% mortality. **C)** Ancient hybridization between *X. malinche* and *X. cortezi* has resulted in introgression of the mitochondria from *X. malinche* into *X. cortezi*.

A


Fitness
Trait Value
Fitness
Trait Value
Fitness
Trait Value
I
II
III
D. melanogaster
D. simulans
D. mauritiana
D. teissieri
D. santomae

B


D. melanogaster
D. simulans
D. mauritiana
D. santomae

C

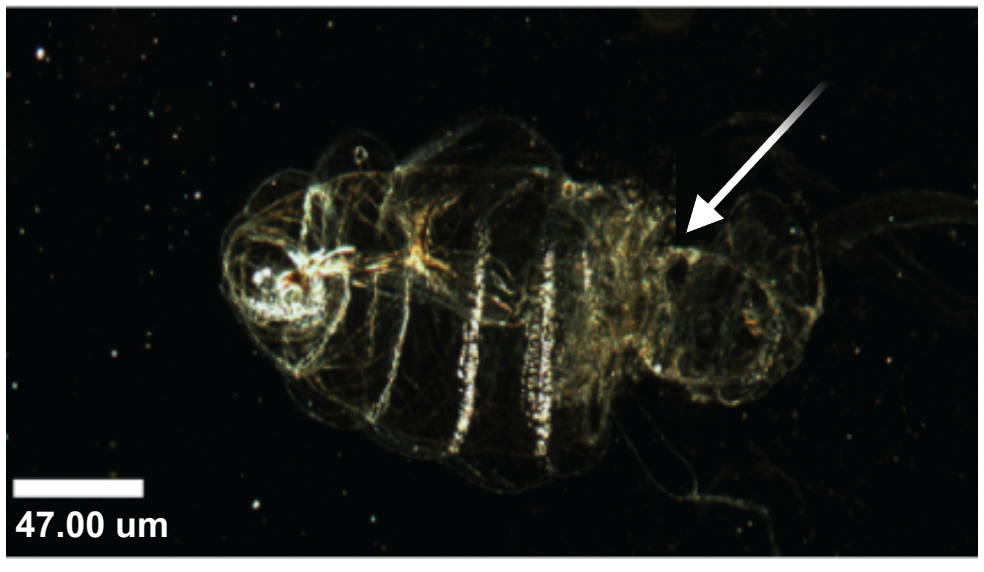

47.00 um

Figure 5. Hybrid inviability between species from the *D. melanogaster* subgroup and *D. santomea* is caused by developmental systems drift in pathways involving essential GAP genes. **A)** At least 3 loci control hybrid inviability between the *D. melanogaster* subgroup and *D. santomea*. The phylogeny shows a model of allelic evolution for two GAP genes that are essential for normal larval development, but cause hybrid inviability in crosses between the *D. melanogaster* subgroup and *D. santomea* (*Giant* and *Tailless*). On the left are fitness optima, illustrating that the ancestral combination of alleles existed at a fitness optimum. The developmental systems drift model predicts that changes from the fitness optima in a phenotype under stabilizing selection are restored by a compensatory mutation at another locus (we note at this time it is unknown which derived allele at *Giant* or *Tailless* were involved in compensatory mutations). Incompatibility is conferred by a three-way interaction involving a currently unidentified gene in *D. santomea*. **B)** These novel tri-locus genotypes interact negatively to cause hybrid death via abdominal ablation [117,118]. **C)** Image of abnormal development in *D. melanogaster* x *D. santomea* hybrids reveal a lethal abdominal ablation (photo credit to D.R. Matute).

Table 1. Compilation of known hybrid incompatibility genes, the predicted evolutionary mechanisms through which they evolved, organisms in which they occur, and associated phenotypes, if available. Note that data in this table includes genes curated from the primary literature as well as genes listed in previous review papers [6,98,142–144]. To identify empirical examples from the literature, we searched both Google Scholar and Web of Science, using forward and reverse searches to identify potential incompatibilities. We required that each incompatibility have at least one gene that is precisely mapped and a clear connection to a postzygotic barrier phenotype to be included in our table.

Gene	Interaction	Proposed Evolutionary Pressure	Species	Hybrid Phenotype	Hybrid Genotype	Molecular mechanism	Refs
Rf	*A. l. petraea* mitochondrial genome	selfish genetic elements	*Arabidopsis l. petraea x A. l. lyrata*	Male sterility	F2 hybrids carrying *A. l. petraea* mitochondria and lacking *A. l. petraea Rf.*		[1]
ACD6		host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Hybrid necrosis, Inviability, Late life lethality	F1 hybrids with *ACD6* from different populations.	*ACD6* encodes a transmembrane ankyrin repeat protein, which modifies pattern recognition receptors (PRRs) and triggers autoimmunity.	[2]
DM1 (SSI4)	DM2 (RPP1)	host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Hybrid necrosis, Inviability, Late life lethality	Hybrids with *DM2* from *A. thaliana* accession *Landsberg erecta* (*Ler*) interacts and *DM1*.		[3]
EDS1	DM2 (RPP1)	host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *DM2* from *A. thaliana* accession *Landsberg erecta* (*Ler*) and *EDS1*.		[4]
HPA1/HPA2		neutral (gene duplication)	*Arabidopsis thaliana (intraspecific)*	Early life lethality, Inviability	F2 hybrids homozygous for the non-functional allele at both loci.	Presence-absence variant	[5]
KPOK3A, KPOK3C	APOK3	selfish genetic elements	*Arabidopsis thaliana* (intraspecific)	Male sterility	Hybrids heterozygous for the antidote	Toxin (*KPOK3A, KPOK3C*) -antidote (*APOK3*) system	[6,7]
OAK		host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Inviability, Hybrid necrosis, Late life lethality	F1 hybrids with *OAK* alleles from different populations.	Novel promoter region	[8]

RFL24		selfish genetic elements	*Arabidopsis thaliana* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[9,10]
RPP4/5		host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Inviability, Hybrid necrosis, Late life lethality	F1 hybrids with *RPP4/5* alleles from different populations.		[11]
RPP7	RPW8/HR4	host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Inviability, Hybrid necrosis, Late life lethality	Multiple allelic combinations	Variation in the number of repeats in RPW8 modulates its ability to interact with RPP7.	[12]
SRF3	DM2 (RPP1)	host-pathogen conflict	*Arabidopsis thaliana* (intraspecific)	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *DM2* from *A. thaliana* accession *Landsberg erecta* (*Ler*) and *SF3*.		[13]
AGL62, AGL90		parental conflict	*Arabidopsis thaliana x A. arenosa*	Inviability, Early life lethality	F1 hybrids	Reduced expression of *AGL62* and *AGL90* leads to embryo arrest.	[14]
PHE1		parental conflict	*Arabidopsis thaliana x A. arenosa*	Inviability, Early life lethality	F1 hybrids	Maternal imprinting of PHE1 is disrupted.	[15,16]
ORF263, ORF193 (atp9)		selfish genetic elements	*Brassica juncea x B. tournefortii*	Male sterility	Hybrid males lacking restorer genes (unknown).		[17]
ORF224, ORF222		selfish genetic elements	*Brassica napus* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[18]
ORF14767 (msft-1)		selfish genetic elements	*Caenorhabditis briggsae* (intraspecific)	Inviability, Early life lethality	F2 hybrids	Toxin-antidote system	[19]
sup-35	*pha-1*	selfish genetic elements	*Caenorhabditis elegans (Hawaii strain x Bristol strain)*	Inviability, Early life lethality	F2 hybrids lacking *pha-1*.	Toxin (sup-35)-antidote (pha-1) system	[20]
zeel-1	*peel-1*	selfish genetic elements	*Caenorhabditis elegans (Hawaii strain x Bristol strain)*	Inviability, Early life lethality	F2 hybrids lacking *zeel-1*.	Toxin (*peel-1*)-antidote (*zeel-1*) system	[21,22]
Cni-neib-1 (F-box gene)	Cbr-shls-1 (phosphoglu-comutase)	host-pathogen conflict	*Caenorhabditis nigoni* x *C. briggsae*	Inviability, Early life lethality	F1 hybrids	The F-box protein degrades maternal and zygotic PGM	[23]

						from *C. briggsae* but not from *C. nigoni*.	
slow-1	grow-1	selfish genetic elements	*Caenorhabditis tropicalis* (intraspecific)	Inviability, Late life lethality	Hybrids lacking *grow-1*	Toxin (slow-1)-antidote (grow-1) system	[24]
NPR-1	*RPP5*	host-pathogen conflict	*Capsella grandiflora x C. rubella; C. rubella x C. orientalis*	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *RPP5* from *C. rubella* and *NPR1* from *C. grandiflora* or *C. orientalis*.		[25]
ORF456	CaPPR6	selfish genetic elements	*Capsicum annuum* (intraspecific)	Male sterility	Males lacking restorer genes		[26,27]
ORF374, ORF384	*Fh3g18750, Fh4g20550, Fh7g08550*	selfish genetic elements	*Citrus reticulata x C. maxima*	Male sterility	Males lacking restorer genes		[28]
hhl		selfish genetic elements	*Drosophila*	Inviability, Late life lethality	Hemizygous females with *D. melanogaster* X chromosome.		[29]
hlx	*su(hlx)*		*Drosophila mauritiana females x D. sechellia males; D. mauritiana females x D. simulans males*	Inviability, Early life lethality	Hybrids with *hlx* from *D. mauritiana* and recessive *D. sechellia* or *D. simulans* autosomal factors.		[30]
OdsH	Y chromosome heterochro-matin	selfish genetic elements	*Drosophila mauritiana x D. simulans*	Male sterility	Male hybrids	The *D. mauritiana OdsH* abnormally associates with the heterochromatic Y chromosome of *D. simulans*.	[31]
tmy		selfish genetic elements	*Drosophila mauritiana x D. simulans*	Biased sex-ratio, Sterility, Early life lethality	Hybrid males with *tmy* from *D. simulans* and lacking its respective suppressor (unknown).	The *D. simulans tmy* on the X chromosome destroys *D. mauritiana* Y chromosome sperm during spermatogenesis.	[32]
tmy	*broadie*	selfish genetic elements	*Drosophila mauritiana x D. simulans*	Biased sex-ratio, Early life lethality, Male sterility	Hybrid males with *tmy* and *broadie* from *D. simulans* that lack the *D. simulans tmy* suppressor (unknown).		[32]

HMR	*LHR, gfzf, Satyr*	selfish genetic elements	*Drosophila melanogaster females x D. simulans males*	Inviability, Early life lethality	F1 hybrids	Overexpression of *HMR/LHR* causes extensive mislocalization of *HMR* to *gfzf* sites in interspecies hybrids if *gfzf* from *D. simulans* is present.	[33,34]
giant		developmental systems drift or compensatory evolution	*Drosophila melanogaster x D. santomea*	Inviability, Abnormal development, Early life lethality	Hybrids with *D. melanogaster giant*.		[35,36]
giant	*tailless*	developmental systems drift or compensatory evolution	*Drosophila melanogaster x D. santomea*	Inviability, Abnormal development, Early life lethality	Hybrids with *D. melanogaster giant* and *tailless.*		[35,36]
mh	*zhr*	selfish genetic elements	*Drosophila melanogaster x D. simulans*	Inviability, Early life lethality	Hybrids with *mh* from *D. simulans* and *zhr* from *D. melanogaster*.	*mh* from *D. simulans* interferes with the function of satellite DNA in *D. melanogaster*.	[37,38]
tyr	*mt-TyrRS*	developmental systems drift or compensatory evolution	*Drosophila melanogaster x D. simulans*	Sterility, Abnormal development, Early life lethality	Hybrids with *tyr* from *D. simulans* and *mt-TyRS* from *D. melanogaster*.		[39]
ovd		selfish genetic elements	*Drosophila pseudoobscura bogotana x D. p. pseudoobscura*	Biased sex-ratio, Early life lethality, Male sterility	F1 hybrid males lacking *D. p. bogotana* Y-linked and autosomal suppressors.	The *Drosophila p. bogotana ovd* and unknown co-distorters on the X chromosome destroy *Drosophila p. pseudoobscura* Y chromosome sperm during spermatogenesis.	[40]
JYALPHA		neutral (gene duplication)	*Drosophila simulans x D. melanogaster*	Male sterility	F2 hybrids homozygous for the non-functional allele at both loci.	Presence-absence variant	[41]
nup96	*nup160*	host-pathogen conflict	*Drosophila simulans x D. melanogaster*	Inviability, Early life lethality,	Hemizygotes and homozygotes with *Nup96* and *Nup160* from *D.*		[42]

				Female sterility	*simulans* lacking a *D. simulans* X chromosome.		
shfr			*Drosophila simulans x D. melanogaster*	Biased sex-ratio, Inviability, Early life lethality	Hybrid females lacking the *shfr* gene.	The lethality of the *Shfr* locus is temperature-dependent.	[43]
dox	*nmy*	selfish genetic elements	*Drosophila simulans x D. sechellia; D. simulans x D. mauritiana*	Biased sex-ratio, Early life lethality, Male sterility	Hybrids with the *dox* distorter lacking an intact *nmy* gene.	*nmy* has undergone a recessive loss-of-function mutation due to a pair of inverted repeats which may allow *nmy* to create siRNAs from a repeat-induced stem loop structure.	[44]
Gh_D11G2949		host-pathogen conflict	*Gossypium hirsutum x G. barbadense*	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *Gh_D11G2949* from *G. barbadense* and an unknown *Le3* locus in *G. hirsutum.*		[45]
GoFLA19		neutral (gene duplication)	*Gossypium hirsutum x G. barbadense*	Male sterility	F2 hybrids	Presence-absence variant	[46]
ORF522		selfish genetic elements	*Helianthus annuus x H. petiolaris*	Male sterility	Hybrids lacking restorer genes (unknown).		[47]
RIN4		host-pathogen conflict	*Lactuca sativa x L. saligna*	Inviability, Hybrid necrosis, Late life lethality	F2 hybrids homozygous for RIN4 from *L. saligna* (partner locus unknown)		[48]
pTAC14		neutral (gene duplication)	*Mimulus guttattus x M. nasutus*	Inviability, Abnormal development, Late life lethality	F2 hybrids homoozygous for the non-functional allele of *pTAC14*.	Presence-absence variant	[49]
nad6	*RF1, RF2*	selfish genetic elements	*Mimulus guttatus x M. nasutus*	Male sterility	F2 males that lack *RF1* and *RF2*.		[50,51]

ORF108	*M. arvensis* mitochondrial genome	selfish genetic elements	*Moricandia arvensis x Brassica juncea*	Male sterility	Male hybrids carrying *M. arvensis* mitochondria.		[52]
Kcnq1 cluster, Phlda2, Ascl2		parental conflict	*Mus m. domesticus x M. spretus*	Inviability, Abnormal development, Late life lethality	F1 hybrids	Incorrect imprinting of paternal genes leads to the misexpression of growth regulators during development.	[53]
PRDM9	X-linked *Hstx2*	developmental systems drift or compensatory evolution	*Mus m. musculus x M. m. domesticus*	Male sterility	F1 hybrid males	*Prdm9*, *Hstx2*, and a minimum amount of heterogenic DNA lead to recombination failure and ultimately meiotic arrest.	[54]
Spk-2	*rsk*	selfish genetic elements	*Neurospora intermedia x N. metzenbergii*	Sterility	Hybrids with the *Spk-2* driver from *N. intermedia.*	Meiotic drive	[55]
Spk-3	*rsk*	selfish genetic elements	*Neurospora intermedia x N. metzenbergii*	Sterility	Hybrids with the *Spk-3* driver from *N. intermedia.*	Meiotic drive	[55]
Spk-1		selfish genetic elements	*Neurospora sitophila* (intraspecific)	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[56]
Nt6549g30		host-pathogen conflict	*Nicotiana tabacum x N. africana*	Inviability, Hybrid necrosis, Early life lethality	Hybrids with *Nt6459g30* from *N. tabacum* and an unknown partner from *N. africana*.		[57]
HSW1/HSW2/ EAF6		neutral (gene duplication)	*Oryza glaberrima x O. s. japonica*	Sterility	Hybrids lacking a functional copy of the *EAF6* protein.	Presence-absence variant	[58,59]
S1		selfish genetic elements	*Oryza glaberrima x O. sativa*	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[60,61]
S27/S28		neutral (gene duplication)	*Oryza glumaepatula x O. sativa*	Male sterility	Hybrids lacking a functional copy of *S27/S28*.	Presence-absence variant	[62]

Hwc3	Hwc1		*Oryza japonica* (interspecific)	Hybrid necrosis, Inviability	F1 hybrids	Hwc3 is an LRR protein, it appears to be upregulated in hybrids by Hwc1.	[63]
qHMS7		selfish genetic elements	*Oryza meridionalis x O. sativa*	Male sterility	Hybrids lacking the corresponding antidote.	Linked toxin (*ORF2*)-antidote (*ORF3*) system	[64]
ORF182, WA352, WA314	RF3, RF4 (unknown)	selfish genetic elements	*Oryza rufipogon* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[65,66]
ESA1			*Oryza rufipogon x O. sativa*	Female sterility	Backcross hybrids carrying *ESA1* from *O. rufipogon.*		[67]
Hwi1 (25L1/25L2)	*Hwi2*	host-pathogen conflict	*Oryza rufipogon x O. sativa*	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *Hwi1* from *O. rufipogon* and *Hwi2* from *O. sativa.*		[68]
DTE9 (OsMADS8)			*Oryza rufipogon x O. sativa japonica*	Inviability, Hybrid necrosis	Backcross hybrids to O. *sativa*.		[69]
Ckl1		host-pathogen conflict	*Oryza sativa japonica x O. s. indica*	Inviability, Hybrid necrosis, Late life lethality	Hybrids homozygous for *Ckl1* from *O. sativa japonica* and homozygous for *NBS-LLR* from *O. sativa indica.*		[70]
DPL1/DPL2		neutral (gene duplication)	*Oryza sativa japonica x O. s. indica*	Male sterility	F2 hybrids without a functional copy of *DPL*.	Presence-absence variant	[71]
HSA1a	*HSA1b*	selfish genetic elements	*Oryza sativa japonica x O. s. indica*	Female sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[72]
pf12A (ORF3, ORF4)		selfish genetic elements	*Oryza sativa japonica x O. s. indica*	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[73,74]

RHS13 (DUYAO/ JIEYAO)		selfish genetic elements	*Oryza sativa japonica x O. s. indica*	Male sterility	Hybrids lacking the corresponding antidote.	*DUYAO* targets mitchondrial protein *OxCOX11* and triggers cell death. *JIEYAO* reroutes *DUYAO* to autophagosomes.	[75]
S7 ORF3		selfish genetic elements	*Oryza sativa japonica x O. s. indica*	Female sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[76]
SaM	*SaF*	selfish genetic elements	*Oryza sativa japonica x O. s. indica*	Male sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[77]
S5		selfish genetic elements	*Oryza sativa japonica x O. s. indica (S5-i and S5-j)*	Female sterility	F1 hybrid females with *S5-i* and *S5-j* alleles (*ORF5+* and *ORF4+* genes).	The *ORF5+* protein possibly destroys the integrity of the cell wall. Signals are transmitted by the *ORF4+* protein, resulting in severe endoplasmic reticulum stress and female gamete abortion.	[78]
Sc		selfish genetic elements	*Oryza sativa japonica x Oryza s. indica*	Male sterility	Hybrids lacking the corresponding antidote.	Overexpression of *Sc-i* allele in the sporophyte selectively aborts pollen carrying *Sc-j* alleles.	[79]
ORF79, ORFH79	RF1A, RF1B	selfish genetic elements	*Oryza sativa* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[80,81]
S22A	S22B		*Oryza sativa x O. glumaepatula*	Male sterility	Backcross hybrids to O. *sativa*.		[82]
RPC4 (DGS1/DGS2)		neutral (gene duplication)	*Oryza sativa x O. nivara*	Male sterility	Hybrids lacking a functional copy of *RPC4*.	Presence-absence variant	[83]
qHMS1		selfish genetic elements	*Oryza satvia x O. meridionalis*	Male sterility	Hybrids with the toxin *qHMS1* from *O. sativa* and lacking the corresponding antidote (unknown).	Toxin-antidote system	[84]
Peg3		parental conflict	*Peromyscus maniculatus males x P. polionotus females*	Inviability, Abnormal development, Early life lethality	F1 hybrids	Incorrect imprinting leads to misexpression of growth factors.	[85,86]

ChiA1		host-pathogen conflict	*Petunia axillaris x P. exserta*	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *ChiA1* from *P. axillaris* and a chr7 region in *P. exserta*.		[87]
ORF402	Rf-PPR592	selfish genetic elements	*Petunia hybrida* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[88,89]
ORF239		selfish genetic elements	*Phaseolus vulgaris* (intraspecific)	Male sterility	Males lacking restorer genes (unknown).		[90,91]
Het-S		selfish genetic elements	*Podospora anserina* (intraspecific)	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[92]
Spok1, Spok2		selfish genetic elements	*Podospora anserina* (intraspecific)	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[93]
ORF138, ORS125/Rfo	RFK1	selfish genetic elements	*Raphanus sativus* (intraspecific)	Male sterility	Male hybrids lacking *RFK1*.		[94–97]
AEP2	*OLI*	ecological adaptation	*Saccharomyces cerevisae x S. bayanus*	Sterility, Abnormal development, Early life lethality	Hybrids homozygous for *AEP2* from *S. bayanus* and a primarily *S. cerevisae* background.	*AEP2* diverged as *S. bayanus* adapted to non-fermentable carbon sources. This has resulted in *Sb-AEP2* failing to translate *Sc-OLI1* mRNA.	[98]
Ccm1	15s rRNA	developmental systems drift or compensatory evolution	*Saccharomyces cerevisae x S. bayanus*	Inviability, Abnormal development, Late life lethality	Hybrids homozygous for a symmetrical mutation in *Ccm1*.	*Ccm1* has a lowered binding affinity to 15s rRNA resulting in reduced protein production.	[99]
PGM1	GAL2, GAL1/10/7	ecological adaptation	*Saccharomyces cerevisiae* (intraspecific)	Inviability, Late life lethality	Hybrids with the reference *PGM1* and alternative versions of *GAL2, GAL1/10/7*.	Alternative alleles allow yeast to utilize galactose while incompatible allele combinations result in yeast unable to grow on galactose.	[100]
MRS1, AIM22	*COX1*	developmental systems drift or compensatory evolution	*Saccharomyces cerevisiae x S. bayanus*	Inviability, Sterility, Early life lethality	Hybrids with *MRS1, AIM22* from *S. cerevisiae*, and the mitochondria from *S. bayanus*.		[101]

MRS1	*COX1*	developmental systems drift or compensatory evolution	*Saccharomyces cerevisiae x S. paradoxus*	Inviability, Sterility, Early life lethality	Hybrids with *MRS1* from *S. cerevisiae* and the mitochondria from *S. paradoxus.*	*MRS1* fails to remove intron from *COX1*.	[101]
wtf4		selfish genetic elements	*Schizosacchar-omyces kambucha x S. pombe*	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[102]
cw27		selfish genetic elements	*Schizosacchar-omyces pombe* (intraspecific)	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[103]
cw9		selfish genetic elements	*Schizosacchar-omyces pombe* (intraspecific)	Sterility	Hybrids lacking the corresponding antidote.	Toxin-antidote system	[103]
wtf13	wtf18-2	selfish genetic elements	*Schizosacchar-omyces pombe* (intraspecific)	Sterility	Hybrids spores lacking *wtf18-2*.		[104]
Rcr3	*Cf-2*	host-pathogen conflict	*Solanum lycopersicum x S. pimpinellifolium*	Inviability, Hybrid necrosis, Late life lethality	Hybrids with *Rcr3* from *S. pimpinellifolium* and *Cf-2* from *S. lycopersicum.*	*Rcr3* suppresses *Cf-2* which triggers autoimmunity.	[105]
ORF107	*RF1*	selfish genetic elements	*Sorghum bicolor (intraspecific)*	Male sterility	Male hybrids lacking *RF1*.		[106]
ORF256		selfish genetic elements	*Triticum aestivum x T. timopheevi*	Male sterility	Male hybrids lacking restorer genes (unknown).		[107]
Xmrk	*rab3d*	sexual selection	*Xiphophorus maculatus x X. hellerii*	Melanoma, Late life lethality	F2 hybrids lacking *rab3d* from *X. maculatus.*	*Xiphophorus* have independently evolved repressor(s) to control the activity of the proto-oncogene *xmrk* in some lineages. *xmrk* is not present in all *Xiphophorus* genomes.	[108,109]

atp5mg	mitochondrial genome	developmental systems drift or compensatory evolution	*Xiphophorus malinche x X. birchmanni*	Abnormal development, Early life lethality, Late life lethality	F2 hybrids with *X. malinche* mitochondria and *atp5mg* from *X. birchmanni.*		[110]
ndufs5	mitochondrial genome *(nd6/nd2)*	developmental systems drift or compensatory evolution	*Xiphophorus malinche x X. birchmanni*	Inviability, Early life lethality	F2 hybrids with *X. malinche* mitochondria and *ndufs5* from *X. birchmanni*.		[111]
Xmrk	*cd97*	sexual selection	*Xiphophorus malinche x X. birchmanni*	Melanoma, Late life lethality	F2 hybrids lacking *cd97* from *X. birchmanni*.	*Xiphophorus* have independently evolved repressor(s) to control the activity of the proto-oncogene *xmrk* in some lineages. *xmrk* is not present in all *Xiphophorus* genomes.	[112]
ndufa13	mitochondrial genome *(nd6/nd2)*	developmental systems drift or compensatory evolution	*Xiphophorus malinche x X. birchmanni; X. cortezi x X. birchmanni*	Inviability, Early life lethality, Late life lethality	F2 hybrids with *X. malinche* or *X. cortezi* mitochondria and *ndufa13* from *X. birchmanni*.		[111]
ORF355, ORF77, URF13	*RF2*	selfish genetic elements	*Zea mays mays (intraspecific)*	Male sterility	Male hybrids lacking *RF2.*		[113–115]
Dcl2	*Tdr1*, *Tpd2*, non-coding RNA hairpin	selfish genetic elements	*Zea mays mays x Z. m. mexicana*	Male sterility	Hybrids with *Tdr1* and *Tpd2* from *Zea m. mexicana* that lack the *Dcl2* variant from *Zea m. mays*.	*Tpd1* contains a non-coding RNA hairpin targeting *Tdr1* and *Dcl2*. *Tpd1* individuals possess a variant of *Dcl2*, which suppresses 22nt siRNA production and acts as an antidote. *Tpd2* is unlinked and required for full pollen fertility.	[116]

Table 1 References

1. Aalto, E.A. et al. (2013) Cytoplasmic male sterility contributes to hybrid incompatibility between subspecies of Arabidopsis lyrata. G3 Genes Genomes Genet. 3, 1727–1740
2. Świadek, M. et al. (2017) Novel allelic variants in ACD6 cause hybrid necrosis in local collection of Arabidopsis thaliana. New Phytol. 213, 900–915
3. Bomblies, K. et al. (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. PLOS Biol. 5, e236
4. Stuttmann, J. et al. (2016) Arabidopsis thaliana DM2h (R8) within the Landsberg RPP1-like Resistance Locus Underlies Three Different Cases of EDS1-Conditioned Autoimmunity. PLOS Genet. 12, e1005990
5. Bikard, D. et al. (2009) Divergent evolution of duplicate genes leads to genetic incompatibilities within A. thaliana. Science 323, 623–626
6. Simon, M. et al. (2022) APOK3, a pollen killer antidote in Arabidopsis thaliana. Genetics 221, iyac089
7. Ricou, A. et al. (2024) Identification of novel genes responsible for a pollen killer present in local natural populations of Arabidopsis thaliana.bioRxiv, 2024.10.07.616952
8. Smith, L.M. et al. (2011) Complex Evolutionary Events at a Tandem Cluster of Arabidopsis thaliana Genes Resulting in a Single-Locus Genetic Incompatibility. PLOS Genet. 7, e1002164
9. Gobron, N. et al. (2013) A Cryptic Cytoplasmic Male Sterility Unveils a Possible Gynodioecious Past for Arabidopsis thaliana. PLOS ONE 8, e62450
10. Durand, S. et al. (2021) A restorer-of-fertility-like pentatricopeptide repeat protein promotes cytoplasmic male sterility in Arabidopsis thaliana. Plant J. 105, 124–135
11. Chae, E. et al. (2014) Species-wide Genetic Incompatibility Analysis Identifies Immune Genes as Hotspots of Deleterious Epistasis. Cell 159, 1341–1351
12. Barragan, C.A. et al. (2019) RPW8/HR repeats control NLR activation in Arabidopsis thaliana. PLOS Genet. 15, e1008313
13. Alcázar, R. et al. (2010) Natural variation at Strubbelig Receptor Kinase 3 drives immune-triggered incompatibilities between Arabidopsis thaliana accessions. Nat. Genet. 42, 1135–1139
14. Walia, H. et al. (2009) Dosage-Dependent Deregulation of an AGAMOUS-LIKE Gene Cluster Contributes to Interspecific Incompatibility. Curr. Biol. 19, 1128–1132
15. Josefsson, C. et al. (2006) Parent-Dependent Loss of Gene Silencing during Interspecies Hybridization. Curr. Biol. 16, 1322–1328
16. Batista, R.A. et al. (2019) The MADS-box transcription factor PHERES1 controls imprinting in the endosperm by binding to domesticated transposons. eLife 8, e50541
17. Dieterich, J.-H. et al. (2003) Alloplasmic male sterility in Brassica napus (CMS 'Tournefortii-Stiewe') is associated with a special gene arrangement around a novel atp9 gene. Mol. Genet. Genomics 269, 723–731
18. Singh, M. and Brown, G.G. (1991) Suppression of cytoplasmic male sterility by nuclear genes alters expression of a novel mitochondrial gene region. Plant Cell 3, 1349–1362

19. Widen, S.A. et al. (2023) Virus-like transposons cross the species barrier and drive the evolution of genetic incompatibilities. Science 380, eade0705
20. Ben-David, E. et al. (2017) A maternal-effect selfish genetic element in Caenorhabditis elegans. Science 356, 1051–1055
21. Seidel, H.S. et al. (2008) Widespread Genetic Incompatibility in C. Elegans Maintained by Balancing Selection. Science 319, 589–594
22. Seidel, H.S. et al. (2011) A novel sperm-delivered toxin causes late-stage embryo lethality and transmission ratio distortion in C. elegans. PLoS Biol. 9, e1001115
23. Xie, D. et al. (2024) A newborn F-box gene blocks gene flow by selectively degrading phosphoglucomutase in species hybrids. Proc. Natl. Acad. Sci. 121, e2418037121
24. Ben-David, E. et al. (2021) Whole-organism eQTL mapping at cellular resolution with single-cell sequencing. eLife 10, e65857
25. Sicard, A. et al. (2015) Divergent sorting of a balanced ancestral polymorphism underlies the establishment of gene-flow barriers in Capsella. Nat. Commun. 6, 7960
26. Kim, D.H. et al. (2007) Isolation and characterization of the cytoplasmic male sterility-associated orf456 gene of chili pepper (Capsicum annuum L.). Plant Mol. Biol. 63, 519–532
27. Jo, Y.D. et al. (2016) Fine mapping of Restorer-of-fertility in pepper (Capsicum annuum L.) identified a candidate gene encoding a pentatricopeptide repeat (PPR)-containing protein. Theor. Appl. Genet. 129, 2003–2017
28. Wang, N. et al. (2022) Pan-mitogenomics reveals the genetic basis of cytonuclear conflicts in citrus hybridization, domestication, and diversification. Proc. Natl. Acad. Sci. 119, e2206076119
29. Cattani, M.V. and Presgraves, D.C. (2012) Incompatibility Between X Chromosome Factor and Pericentric Heterochromatic Region Causes Lethality in Hybrids Between Drosophila melanogaster and Its Sibling Species. Genetics 191, 549–559
30. Cattani, M.V. and Presgraves, D.C. (2009) Genetics and lineage-specific evolution of a lethal hybrid incompatibility between Drosophila mauritiana and its sibling species. Genetics 181, 1545–1555
31. Bayes, J.J. and Malik, H.S. (2009) Altered heterochromatin binding by a hybrid sterility protein in Drosophila sibling species. Science 326, 1538–1541
32. Tao, Y. et al. (2001) Sex-ratio segregation distortion associated with reproductive isolation in Drosophila. Proc. Natl. Acad. Sci. 98, 13183–13188
33. Cooper, J.C. et al. (2019) Altered Localization of Hybrid Incompatibility Proteins in Drosophila. Mol. Biol. Evol. 36, 1783–1792
34. Lukacs, A. et al. (2021) The Integrity of the HMR complex is necessary for centromeric binding and reproductive isolation in Drosophila. PLOS Genet. 17, e1009744
35. Chang, W. et al. (2021) Gap genes are involved in inviability in hybrids between Drosophila melanogaster and D. santomea.bioRxiv, 2021.12.06.471493
36. Chang, W. et al. (2021) Rapid evolution of the functionally conserved gap gene giant in Drosophila.bioRxiv, 2021.07.08.451553

37. Sawamura, K. and Yamamoto, M.-T. (1993) Cytogenetical localization of Zygotic hybrid rescue (Zhr), a Drosophila melanogaster gene that rescues interspecific hybrids from embryonic lethality. Mol. Gen. Genet. MGG 239, 441–449
38. Brand, C.L. and Levine, M.T. (2022) Cross-species incompatibility between a DNA satellite and the Drosophila Spartan homolog poisons germline genome integrity. Curr. Biol. 32, 2962-2971.e4
39. Meiklejohn, C.D. et al. (2013) An Incompatibility between a Mitochondrial tRNA and Its Nuclear-Encoded tRNA Synthetase Compromises Development and Fitness in Drosophila. PLOS Genet. 9, e1003238
40. Phadnis, N. and Orr, H.A. (2009) A single gene causes both male sterility and segregation distortion in Drosophila hybrids. Science 323, 376–379
41. Masly, J.P. et al. (2006) Gene Transposition as a Cause of Hybrid Sterility in Drosophila. Science 313, 1448–1450
42. Sawamura, K. et al. (2014) A Test of Double Interspecific Introgression of Nucleoporin Genes in Drosophila. G3 GenesGenomesGenetics 4, 2101–2106
43. Carracedo, M.C. et al. (2000) Location of Shfr, a new gene that rescues hybrid female viability in crosses between Drosophila simulans females and D. melanogaster males. Heredity 84, 630–638
44. Tao, Y. et al. (2007) A sex-ratio Meiotic Drive System in Drosophila simulans. II: An X-linked Distorter. PLOS Biol. 5, e293
45. Deng, J. et al. (2019) A CC-NBS-LRR gene induces hybrid lethality in cotton. J. Exp. Bot. 70, 5145–5156
46. Zhang, M. et al. (2021) Non-functional GoFLA19s are responsible for the male sterility caused by hybrid breakdown in cotton (Gossypium spp.). Plant J. 107, 1198–1212
47. Horn, R. et al. (1991) A mitochondrial 16 kDa protein is associated with cytoplasmic male sterility in sunflower. Plant Mol. Biol. 17, 29–36
48. Jeuken, M.J.W. et al. (2009) Rin4 Causes Hybrid Necrosis and Race-Specific Resistance in an Interspecific Lettuce Hybrid. Plant Cell 21, 3368–3378
49. Zuellig, M.P. and Sweigart, A.L. (2018) Gene duplicates cause hybrid lethality between sympatric species of Mimulus. PLOS Genet. 14, e1007130
50. Case, A.L. and Willis, J.H. (2008) Hybrid male sterility in Mimulus (Phrymaceae) is associated with a geographically restricted mitochondrial rearrangement. Evolution 62, 1026–1039
51. Barr, C.M. and Fishman, L. (2010) The nuclear component of a cytonuclear hybrid incompatibility in Mimulus maps to a cluster of pentatricopeptide repeat genes. Genetics 184, 455–465
52. Ashutosh et al. (2008) A novel orf108 co-transcribed with the atpA gene is associated with cytoplasmic male sterility in Brassica juncea carrying Moricandia arvensis cytoplasm. Plant Cell Physiol. 49, 284–289
53. Arévalo, L. et al. (2021) Haldane's rule in the placenta: Sex-biased misregulation of the Kcnq1 imprinting cluster in hybrid mice. Evolution 75, 86–100
54. Mihola, O. et al. (2009) A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science 323, 373–375

55. Vogan, A.A. et al. (2022) Meiotic drive is associated with sexual incompatibility in Neurospora. Evolution 76, 2687–2696
56. Svedberg, J. et al. (2021) An introgressed gene causes meiotic drive in Neurospora sitophila. Proc. Natl. Acad. Sci. 118, e2026605118
57. Ma, J. et al. (2020) Identification and editing of a hybrid lethality gene expands the range of interspecific hybridization potential in Nicotiana. Theor. Appl. Genet. 133, 2915–2925
58. Kubo, T. et al. (2022) Loss of OsEAF6, a Subunit of the Histone Acetyltransferase Complex, Causes Hybrid Breakdown in Intersubspecific Rice Crosses. Front. Plant Sci. 13
59. Liao, B. et al. (2024) Dysfunction of duplicated pair rice histone acetyltransferases causes segregation distortion and an interspecific reproductive barrier. Nat. Commun. 15, 996
60. Xie, Y. et al. (2017) Interspecific Hybrid Sterility in Rice Is Mediated by OgTPR1 at the S1 Locus Encoding a Peptidase-like Protein. Mol. Plant 10, 1137–1140
61. Koide, Y. et al. (2018) Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice. Proc. Natl. Acad. Sci. 115, E1955–E1962
62. Yamagata, Y. et al. (2010) Mitochondrial gene in the nuclear genome induces reproductive barrier in rice. Proc. Natl. Acad. Sci. 107, 1494–1499
63. Nadir, S. et al. (2019) A novel discovery of a long terminal repeat retrotransposon-induced hybrid weakness in rice. J. Exp. Bot. 70, 1197–1207
64. Yu, X. et al. (2018) A selfish genetic element confers non-Mendelian inheritance in rice. Science 360, 1130–1132
65. Luo, D. et al. (2013) A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. Nat. Genet. 45, 573–577
66. Tang, H. et al. (2017) Multi-step formation, evolution, and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. Cell Res. 27, 130–146
67. Hou, J. et al. (2019) ESA1 Is Involved in Embryo Sac Abortion in Interspecific Hybrid Progeny of Rice. Plant Physiol. 180, 356–366
68. Chen, C. et al. (2014) A two-locus interaction causes interspecific hybrid weakness in rice. Nat. Commun. 5, 3357
69. Kim, S.H. et al. (2022) A Novel Embryo Phenotype Associated With Interspecific Hybrid Weakness in Rice Is Controlled by the MADS-Domain Transcription Factor OsMADS8. Front. Plant Sci. 12
70. Yamamoto, E. et al. (2010) Gain of deleterious function causes an autoimmune response and Bateson–Dobzhansky–Muller incompatibility in rice. Mol. Genet. Genomics 283, 305–315
71. Mizuta, Y. et al. (2010) Rice pollen hybrid incompatibility caused by reciprocal gene loss of duplicated genes. Proc. Natl. Acad. Sci. 107, 20417–20422
72. Kubo, T. et al. (2016) Two Tightly Linked Genes at the hsa1 Locus Cause Both F1 and F2 Hybrid Sterility in Rice. Mol. Plant 9, 221–232
73. Zhou, P. et al. (2023) A minimal genome design to maximally guarantee fertile inter-subspecific hybrid rice. Mol. Plant 16, 726–738
74. Wang, D. et al. (2023) Two complementary genes in a presence-absence variation contribute to indica-japonica reproductive isolation in rice. Nat. Commun. 14, 4531

75. Wang, C. et al. (2023) A natural gene drive system confers reproductive isolation in rice. Cell 186, 3577-3592.e18
76. Yu, Y. et al. (2016) Hybrid Sterility in Rice (Oryza sativa L.) Involves the Tetratricopeptide Repeat Domain Containing Protein. Genetics 203, 1439–1451
77. Long, Y. et al. (2008) Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. Proc. Natl. Acad. Sci. 105, 18871–18876
78. Yang, J. et al. (2012) A Killer-Protector System Regulates Both Hybrid Sterility and Segregation Distortion in Rice. Science 337, 1336–1340
79. Shen, R. et al. (2017) Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nat. Commun. 8, 1310
80. Wang, Z. et al. (2006) Cytoplasmic Male Sterility of Rice with Boro II Cytoplasm Is Caused by a Cytotoxic Peptide and Is Restored by Two Related PPR Motif Genes via Distinct Modes of mRNA Silencing. Plant Cell 18, 676–687
81. Peng, X. et al. (2010) The mitochondrial gene orfH79 plays a critical role in impairing both male gametophyte development and root growth in CMS-Honglian rice. BMC Plant Biol. 10, 125
82. Sakata, M. et al. (2021) Domain Unknown Function DUF1668-Containing Genes in Multiple Lineages Are Responsible for F1 Pollen Sterility in Rice. Front. Plant Sci. 11
83. Nguyen, G.N. et al. (2017) Duplication and Loss of Function of Genes Encoding RNA Polymerase III Subunit C4 Causes Hybrid Incompatibility in Rice. G3 GenesGenomesGenetics 7, 2565–2575
84. You, S. et al. (2023) A toxin-antidote system contributes to interspecific reproductive isolation in rice. Nat. Commun. 14, 7528
85. Vrana, P.B. et al. (2000) Genetic and epigenetic incompatibilities underlie hybrid dysgenesis in Peromyscus. Nat. Genet. 25, 120–124
86. Loschiavo, M. et al. (2007) Mapping and identification of candidate loci responsible for Peromyscus hybrid overgrowth. Mamm. Genome 18, 75–85
87. Li, C. et al. (2023) Tight genetic linkage of genes causing hybrid necrosis and pollinator isolation between young species. Nat. Plants 9, 420–432
88. Young, E.G. and Hanson, M.R. (1987) A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. Cell 50, 41–49
89. Bentolila, S. et al. (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc. Natl. Acad. Sci. 99, 10887–10892
90. Abad, A.R. et al. (1995) Specific expression in reproductive tissues and fate of a mitochondrial sterility-associated protein in cytoplasmic male-sterile bean. Plant Cell 7, 271–285
91. Jia, M.H. et al. (1997) Nuclear fertility restorer genes map to the same linkage group in cytoplasmic male-sterile bean. Theor. Appl. Genet. 95, 205–210
92. Dalstra, H.J.P. et al. (2003) Sexual transmission of the [Het-s] prion leads to meiotic drive in Podospora anserina. Proc. Natl. Acad. Sci. 100, 6616–6621

93. Grognet, P. et al. (2014) Genes That Bias Mendelian Segregation. PLOS Genet. 10, e1004387
94. Bonhomme, S. et al. (1991) A 2.5 kb NcoI fragment of Ogura radish mitochondrial DNA is correlated with cytoplasmic male-sterility in Brassica cybrids. Curr. Genet. 19, 121–127
95. Iwabuchi, M. et al. (1999) Identification and expression of the kosena radish (Raphanus sativus cv. Kosena) homologue of the ogura radish CMS-associated gene, orf138. Plant Mol. Biol. 39, 183–188
96. Desloire, S. et al. (2003) Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family. EMBO Rep. 4, 588–594
97. Brown, G.G. et al. (2003) The radish Rfo restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. Plant J. 35, 262–272
98. Lee, H.-Y. et al. (2008) Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. Cell 135, 1065–1073
99. Jhuang, H. et al. (2017) Mitochondrial–nuclear co-evolution leads to hybrid incompatibility through pentatricopeptide repeat proteins. EMBO Rep. 18, 87–101
100. Boocock, J. et al. (2021) Ancient balancing selection maintains incompatible versions of the galactose pathway in yeast. Science 371, 415–419
101. Chou, J.-Y. et al. (2010) Multiple Molecular Mechanisms Cause Reproductive Isolation between Three Yeast Species. PLOS Biol. 8, e1000432
102. Nuckolls, N.L. et al. (2017) wtf genes are prolific dual poison-antidote meiotic drivers. eLife 6, e26033
103. Hu, W. et al. (2017) A large gene family in fission yeast encodes spore killers that subvert Mendel's law. eLife 6, e26057
104. Núñez, M.A.B. et al. (2018) A suppressor of a wtf poison-antidote meiotic driver acts via mimicry of the driver's antidote. PLOS Genet. 14, e1007836
105. Krüger, J. et al. (2002) A Tomato Cysteine Protease Required for Cf-2-Dependent Disease Resistance and Suppression of Autonecrosis. Science 296, 744–747
106. Tang, H.V. et al. (1996) Transcript processing internal to a mitochondrial open reading frame is correlated with fertility restoration in male-sterile sorghum. Plant J. 10, 123–133
107. Hedgcoth, C. et al. (2002) A chimeric open reading frame associated with cytoplasmic male sterility in alloplasmic wheat with Triticum timopheevi mitochondria is present in several Triticum and Aegilops species, barley, and rye. Curr. Genet. 41, 357–366
108. Adam, D. et al. (1993) Tumor suppression in Xiphophorus by an accidentally acquired promoter. Science 259, 816–819
109. Lu, Y. et al. (2020) Oncogenic allelic interaction in Xiphophorus highlights hybrid incompatibility. Proc. Natl. Acad. Sci. 117, 29786–29794
110. Robles, N.V. et al. (2025) Admixture mapping reveals evidence for multiple mitonuclear incompatibilities in swordtail fish hybridsbioRxiv, 2025.01.30.635158

111. Moran, B.M. et al. (2024) A lethal mitonuclear incompatibility in complex I of natural hybrids. Nature 626, 119–127
112. Powell, D.L. et al. (2020) Natural hybridization reveals incompatible alleles that cause melanoma in swordtail fish. Science 368, 731–736
113. Dewey, R.E. et al. (1987) A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize. Proc. Natl. Acad. Sci. 84, 5374–5378
114. Cui, X. et al. (1996) The rf2 Nuclear Restorer Gene of Male-Sterile T-Cytoplasm Maize. Science 272, 1334–1336
115. Zabala, G. et al. (1997) The Nuclear Gene Rf3 Affects the Expression of the Mitochondrial Chimeric Sequence R Implicated in S-Type Male Sterility in Maize. Genetics 147, 847–860
116. Berube, B. et al. (2024) Teosinte Pollen Drive guides maize diversification and domestication by RNAi. Nature 633, 380–388