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When Two Rights Make a Wrong: The Evolutionary Genetics of Plant Hybrid Incompatibilities

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Abstract

Hybrids between flowering plant species often exhibit reduced fitness, including sterility and inviability. Such hybrid incompatibilities create barriers to genetic exchange that can promote reproductive isolation between diverging populations and, ultimately, speciation. Additionally, hybrid breakdown opens a window into hidden molecular and evolutionary processes occurring within species. Here, we review recent work on the mechanisms and origins of hybrid incompatibility in flowering plants, including both diverse genic interactions and chromosomal incompatibilities. Conflict and coevolution among and within plant genomes contributes to the evolution of some well-characterized genic incompatibilities, but duplication and drift also play important roles. Inversions, while contributing to speciation by suppressing recombination, rarely cause underdominant sterility. Translocations cause severe F_1 sterility by disrupting meiosis in heterozygotes, making their fixation in outcrossing sister species a paradox. Evolutionary genomic analyses of both genic and chromosomal incompatibilities, in the context of population genetic theory, can explicitly test alternative scenarios for their origins.

Contents

1. INTRODUCTION

Hybrid incompatibilities, here defined as genic and structural interactions between divergent genomes resulting in the reduced fitness of interspecific or interpopulation hybrids, are a long-standing puzzle in evolutionary biology. How does bringing together two perfectly functional genetic programs in a hybrid somehow result in reproductive failure or death? Why, given natural selection as a major force in species divergence, do such deleterious incompatibilities evolve? When and where do hybrid incompatibilities act as barriers to gene flow during divergence and as direct contributors to speciation? While these questions transcend (eukaryotic) taxon, the answers depend in large part on the reproductive and developmental biology of a given organism. That is, how plant hybrids fall apart reflects how plants are put together. Thus, the study of plant hybrid incompatibilities touches many fields in plant biology, from molecular biology to the ecology of species interactions. Our goal here is to summarize recent work on the full breadth of plant hybrid incompatibilities from an evolutionary genetic perspective. This perspective necessarily includes both molecular mechanisms and potential speciation consequences (see the sidebar titled The Question of Consequences), but our primary focus is on recent progress toward understanding how and why incompatibilities evolve and on what they tell us about evolutionary processes within plant species.

2. GENIC INCOMPATIBILITIES

2.1. The Dobzhansky-Muller Model: An Epistatic Solution to the Puzzle of Unfit Hybrids

Darwin proposed that hybrid breakdown must arise incidentally when the "structures and constitutions" of two species are brought together into one individual (39, p. 266). However, because selection at the level of the individual cannot favor the production of unfit progeny, and he did not know of genes, Darwin could not explain exactly how hybrid dysfunction might evolve. Bateson (9), Dobzhansky (42), and Muller (110) eventually provided an elegant genetic solution to this evolutionary puzzle. In what is now commonly referred to as the Dobzhansky-Muller model (**Figure 1**), breakdown in hybrids is caused by two or more mutational differences between species that interact epistatically to cause low fitness. When these jointly deleterious mutations arise in independent lineages, they may both be neutral or adaptive in the ancestral genetic background

THE QUESTION OF CONSEQUENCES

Speciation is often (even always, eventually) accompanied by the evolution of genetic and genomic incompatibilities, but we do not assume here that the evolution of hybrid incompatibilities causes speciation. Some classes of incompatibility (e.g., seed lethality in F_1 hybrids) may generate strong barriers to gene flow between species, but some incompatibilities (e.g., cytonuclear male sterility) could actually accelerate interspecific introgression. Just as not every case of local adaptation results in speciation, not every gene or chromosome underlying an incompatibility is a step on an inevitable path to complete reproductive isolation. From an evolutionary perspective, a complementary approach to reconstructing the origins and locus-specific consequences of hybrid sterility and inviability is to measure them as postzygotic reproductive barriers (barriers to gene exchange that occur once a hybrid zygote is formed) within a framework that also quantifies premating barriers (those that reduce interspecific pollen transfer) and postmating prezygotic barriers (those that prevent fertilization after cross-pollination) in the wild. This "components of reproductive isolation" method of parsing barriers to interspecies gene flow is not new (145), but recently, it has been extensively applied to plants (106, 123; for a thorough summary of recent work on plant reproductive isolating barriers, also see 3).

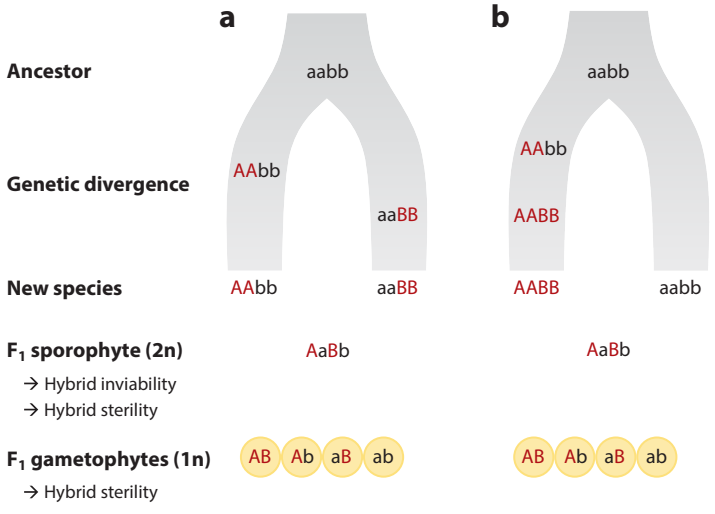

Figure 1

Evolution of incompatible gene interactions between plant species. Hybrid inviability arises from incompatibilities acting in the diploid (2n) sporophyte (or in the triploid endosperm) (see text and **Figure 2**), whereas hybrid sterility arises from incompatibilities either in the sporophyte or in the haploid (1n) gametophytes (*yellow circles*). For simplicity, only the F_1 generation is shown, but recessive sporophytic incompatibilities are also common in F_2 and backcross generations. (*a*) In the classic, two-locus model envisioned by Dobzhansky and Muller, an ancestral population splits into two geographically isolated populations that both accumulate genetic differences. In this scenario, hybrid dysfunction can evolve without any reduction in fitness because the incompatibility occurs between derived (*red*) alleles that fix independently in distinct lineages. (*b*) Hybrid incompatibilities might also evolve between derived (*red*) and ancestral (*black*) alleles if both substitutions occur within a single lineage. As with the classic model, these derived mutations may be adaptive or neutral. Alternatively, in a model involving intragenomic conflict, the two loci participate in a coevolutionary battle. In this scenario, if the A allele is a selfish genetic element that biases its own transmission at the expense of its host, the B allele could fix via selection to mitigate these negative fitness effects.

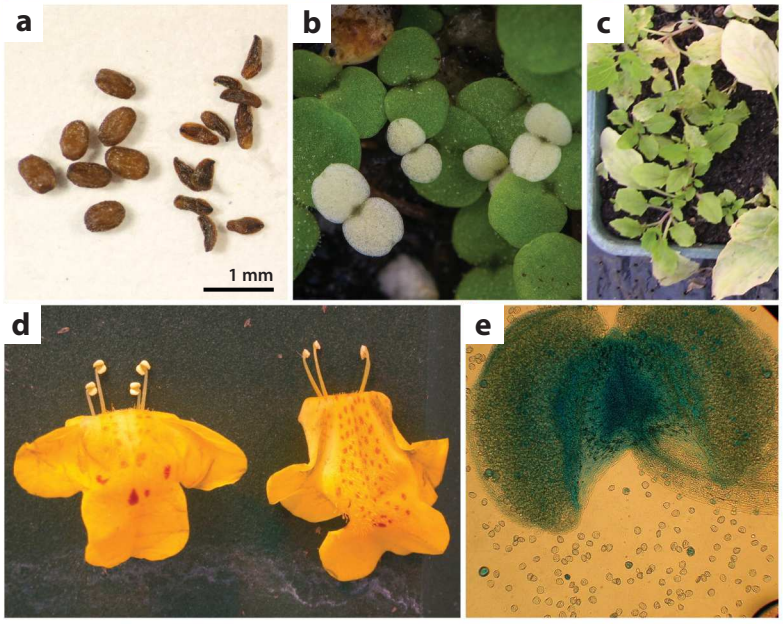

Figure 2

Intrinsic hybrid incompatibilities cause diverse phenotypes, as illustrated in the yellow monkeyflower *Mimulus guttatus* and its close relatives. (*a*) Hybrid seed lethality in F_1 hybrids of *M. guttatus* and *M. nudatus* (115). Self-fertilized *M. guttatus* seeds (*left*) and hybrid seeds (*right*) shown (photo courtesy of Elen Oneal). (*b*) Seedling chlorosis in hybrids between *M. guttatus* and *M. nasutus* (170) (photo courtesy of Adam Bewick). (*c*) Necrosis in hybrids between copper-tolerant and copper-intolerant *M. guttatus* populations. Adapted from 163 under the Creative Commons Attribution License (CC BY 4.0). (*d*) Cytoplasmic male sterility in *M. guttatus* × *M. nasutus* F_2 hybrids (7, 25, 26, 47). Anthers of fertile (*left*) and sterile (*right*) F_2 plants shown. Adapted with permission from 26. (*e*) Nuclear-nuclear male sterility in F_2 hybrids between *M. nasutus* and *M. guttatus*. Anthers of sterile hybrids produce abundant but inviable (unstained by lactophenol-aniline blue) pollen (photo courtesy of Camille Barr) (147, 148).

and are therefore not eliminated by natural selection. In flowering plants, many possible genetic interactions can cause hybrid breakdown and many evolutionary paths may lead to Dobzhansky-Muller incompatibility (**Figure 2**). Within the nuclear genome, incompatibilities often involve more complex interactions among multiple loci or might even occur at a single genetic locus between two or more alleles that evolve independently (111). They might also arise at duplicate genes when diverging populations lose function in different paralogs (104, 159). Incompatibilities can evolve between organellar (mitochondrial and chloroplast) and nuclear genomes and can involve haploid (gametophyte), diploid (sporophyte), and triploid (endosperm) components of the flowering plant life cycle. Regardless of the specific genetics, the key insight of this model is that hybrid incompatibility (and postzygotic reproductive isolation and speciation) can evolve as a by-product of neutral or adaptive divergence between geographically isolated populations.

Over the past decades, dissection of the genetic mechanisms underlying diverse incompatibilities has provided widespread empirical support for the Dobzhansky-Muller model. Nonetheless, we are only now beginning to resolve major questions about the molecular basis and evolutionary origins of hybrid incompatibility loci contributing to species divergence. For example, what types of molecular genetic changes and interactions cause hybrid dysfunction? Are there predictable genetic pathways to incompatibilities? How often do hybrid incompatibilities involve gene

Gametophyte: haploid, gamete-producing phase of the life cycle; pollen (male) and embryo sac (female) in flowering plants

Sporophyte: diploid phase of the life cycle; develops from the zygote

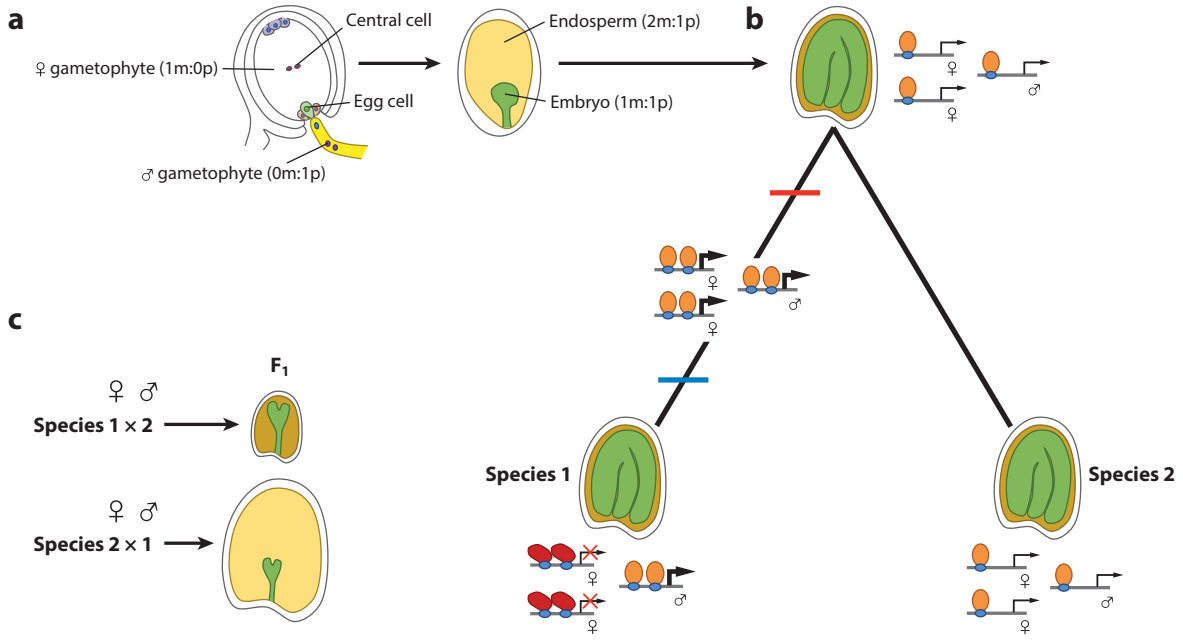

Figure 3

Parental dosage imbalance causes endosperm failure and hybrid seed lethality between species. (*a*) In double fertilization, the male gametophyte (pollen tube) releases two sperm into the ovule: One fuses with the egg nucleus to produce the diploid zygote, and the other fuses with the two nuclei of the central cell to form the triploid endosperm. In parentheses, the ratio of maternal (m) to paternal (p) genomes is given. (*b*) In this hypothetical scenario involving parental conflict, ancestral gene expression at a key regulator of endosperm development is biallelic. Over evolutionary time, Species 1 fixes a mutation (*red bar*) that increases expression of this gene and, consequently, endosperm proliferation. This mutation is advantageous to fathers but detrimental to mothers. A second mutation (*blue bar*) that reduces maternal expression of this target gene (e.g., via PRC2-mediated repression) then arises (58). This mutation fixes via selection to balance gene dosage. As a result of this conflict-driven coevolution, the endosperm developmental gene becomes imprinted (i.e., paternally expressed) in Species 1, whereas expression remains biallelic in Species 2. (*c*) Reciprocal crosses between the two species produce F_1 seeds with distinct developmental defects (e.g., precocious versus failed endosperm differentiation) (92). Note, although this figure depicts a simple, two-locus case, parental genome dosage imbalance might involve many genes (54, 125).

duplicates? Do the initial mutations increase in frequency by random genetic drift or natural selection? Do incompatibilities arise through the independent fixation of alleles that interact only in hybrids (as envisioned by Dobzhansky and Muller), or do they involve conflict and genomic coevolution within a single lineage? As the molecular variants that cause hybrid inviability and sterility are identified and their evolutionary histories within species revealed, plant systems are providing some of the first answers to these fundamental evolutionary questions.

2.2. Nuclear Genic Incompatibilities

Interactions among genes within the nuclear genome can lead to diverse forms of hybrid incompatibility, including F_1 seed lethality, later-acting F_1 and F_2 mortality via seedling and adult necrosis and chlorosis, and hybrid sterility via multiple gametophytic and sporophytic mechanisms.

2.2.1. Parent-of-origin effects on F_1 seed lethality implicate endosperm-embryo interactions and genomic imprinting. Hybrid seed lethality, a form of postzygotic incompatibility that acts early in the angiosperm life cycle, is usually associated with defects in the triploid endosperm, an important nutritive tissue for the developing embryo (**Figure 3*a***) (16, 73, 125, 154).

Deviations from the usual ratio of two-maternal to one-paternal genomes disrupts development of the endosperm (77), explaining the triploid block that arises from interploidy crosses, which double the genomic contribution of one or the other parent. A similar disturbance to endosperm balance also seems to cause hybrid seed lethality in many diploid crosses between species (see 92 for a recent review) and might represent a major source of hybrid incompatibilities in flowering plants.

Given the sensitivity of the endosperm to mismatches in parental genome dosage, improper genomic imprinting may be the molecular cause of both interploidy and interspecific seed lethality (see 58 for a recent review; 65, 66). Indeed, genomic imprinting provides a mechanistic explanation for the observation that reciprocal interploidy crosses often differ in seed phenotypes (16, 136, 151), as dosage changes in maternally versus paternally derived alleles likely have distinct consequences for endosperm and embryo development. Several studies in *Arabidopsis* have shown that imprinted genes, including both maternally and paternally expressed genes (MEGs and PEGs, respectively), are misexpressed in seeds derived from interploidy crosses (44, 79, 86, 162), and deregulation of specific PEGs have been directly implicated in F_1 seed failure associated with paternal excess (86, 162). Recent studies in *Arabidopsis*, *Mimulus*, and *Solanum* provide support for a mechanistic link between disrupted genomic imprinting and interspecific hybrid seed lethality. Reciprocal crosses between species have revealed parent-of-origin effects on endosperm developmental phenotypes (91, 115, 125) or genetic loci (54) associated with F_1 hybrid seed failure. Additionally, as in interploidy crosses, genomic imprinting is highly perturbed in the F_1 hybrid seeds of interspecific diploid crosses, with particularly severe deregulation of PEGs (18, 49, 82, 125). A loss-of-function mutation in the PEG *PHERES1* partially rescues seed inviability between *Arabidopsis thaliana* and *Arabidopsis arenosa* (78), providing strong evidence that this imprinted gene contributes to hybrid incompatibility and postzygotic isolation.

If, as early evidence suggests, genomic imprinting plays a central role in interspecific hybrid seed lethality in angiosperms, what does this imply about its evolutionary origins? Classic theory (65) posits that parental conflict over maternal investment has driven the evolution of genomic imprinting in the endosperm and its mammalian analog, the placenta (but see 85, 143 for alternative evolutionary scenarios). Under parental conflict, which should be restricted to outcrossing plant species (15), an evolutionary arms race between MEGs and PEGs over resource provisioning in the endosperm leads to predictable directionality in reciprocal hybrid lethality phenotypes: Maternal excess in F_1 seeds should attenuate endosperm growth (via increased dosage of MEGs), whereas paternal excess should promote it (via increased dosage of PEGs) (67). This expectation is largely borne out in crosses (16, 41). Of course, breakdown of a coevolved imprinting system in hybrids need not occur via a genetic arms race within one lineage, and addressing the evolutionary origins of embryo-endosperm lethality will require identification of the causal genes. Are imprinted genes the exclusive cause of hybrid seed lethality, or do other dosage-sensitive loci play a role (109, 138, 148)? When imprinted genes are involved, do they show molecular signatures of coevolution within species as predicted under the parental conflict scenario? Work within a few molecular model systems provides a strong functional framework for understanding hybrid seed lethality as the outcome of parental conflict. However, while imprinting status is sometimes conserved across species, there is also substantial turnover (69, 121, 158). Interspecific divergence in imprinting status might be due to intragenomic conflict, with new genes becoming imprinted to gain an advantage for one sex (e.g., **Figure 3*b***), but it could involve other selective mechanisms (161) or incidental proximity to transposable elements (57). Genetic mapping of loci underlying seed lethality in interspecific crosses (54, 125) offers a promising path toward identifying novel genes for hybrid seed lethality, testing their imprinting status, and revealing their evolutionary histories in natural populations.

Triploid block: F_1 seed inviability resulting from crosses between diploids and tetraploids

Genomic imprinting: parent-of-origin dependent gene expression due to differential epigenetic modifications established during male and female gametogenesis

Maternally expressed gene (MEG): gene carrying an imprinted paternal allele

Paternally expressed gene (PEG): gene carrying an imprinted maternal allele

2.2.2. Intraspecific balancing selection and gene duplication (plus drift) can lead to lethal incompatibilities. Incompatible interactions among nuclear genes can also act during later stages of plant development, causing hybrid inviability (from mild weakness to lethality) in cotyledons, seedlings, or even adult plants. Hybrid necrosis is a particularly common form of such inviability, which molecular analyses consistently show arises as an autoimmune response caused by incompatibilities between genes for plant defense against bacterial or fungal pathogens (reviewed in 14, 28, 150). Many of these disease resistance genes are arrayed in tandem clusters (27) and show exceptional variation in nucleotide sequence, copy number, and gene expression (75). Moreover, in natural plant populations, there are often signatures of diversifying and balancing selection at resistance genes, with some polymorphisms maintained for millions of years and across species (80). Hybrid necrosis alleles, too, are often found at intermediate frequencies within populations (1, 31, 137, 152). In one extreme case, two naturally co-occurring alleles of the *ACD6* immunity gene cause mild, temperature-dependent necrosis in lab-reared heterozygotes, but the same genotype shows no apparent defects in natural populations (152). Given the temperature sensitivity of *ACD6* and many other necrosis-causing genes, variable selection under fluctuating biotic and abiotic environments (i.e., if resistance alleles are costly in colder temperatures) may contribute to the maintenance of natural polymorphism.

Balancing selection on hybrid necrosis loci provides fascinating insight into the processes that maintain conditionally deleterious variation within species; however, how, and how often, these polymorphisms fix and become interspecific barriers remains an open question. To contribute to reproductive isolation, partners in Dobzhansky-Muller incompatibilities must come to differ among lineages, either through divergent selection or drift. In *Mimulus guttatus*, for example, an allele causing hybrid necrosis has risen to high frequency in a copper-mine population through linked selection on a locally adaptive copper-tolerance locus (163). If closely related species evolve specificities to different virulence factors, differences in hybrid necrosis allele frequencies might also arise, leading to interspecific incompatibility (e.g., such a scenario might explain the evolution of hybrid necrosis between species of lettuce) (76). Although the maintenance of shared polymorphism by balancing selection generally opposes divergence of incompatibilities, recent work in three species of *Capsella* show how such a scenario might be possible (137). Long-term balancing selection in outcrossing *Capsella grandiflora* has maintained two highly divergent alleles of the *NPR1* immune-response gene, which have differentially sorted into the recently derived selfing species *Capsella rubella* and *Capsella orientalis*. The allele shared by *C. grandiflora* and *C. orientalis* is incompatible with a *C. rubella*–specific mutation at the unlinked *RPP5* pathogen-response gene, leading to hybrid necrosis. This work argues that, by preserving genetic variation within an ancestral lineage, balancing selection might predispose descendant species to accumulate hybrid incompatibilities—particularly if those species evolve self-fertilization and fix distinct subsets of the variation. In support of this idea, an additional case of hybrid necrosis involving an outcrossing-selfing species pair in rice has followed a similar path: Incompatibility between an *Oryza rufipogon* allele at *Hwi1*, a locus under balancing selection, and an allele at *Hwi2* that is specific to the selfing *Oryza sativa* causes necrosis (31). Although more work is needed to determine how often disease resistance genes are a source of hybrid incompatibilities between species, these studies highlight a potential role for balancing selection (and drift in selfers) in the evolution of hybrid necrosis.

In contrast to incompatibilities involving disease resistance genes, two recently characterized hybrid lethality systems appear to involve divergent resolution of gene duplicates by degenerative mutations and (likely) genetic drift (104, 159). First, the progeny of crosses between certain accessions of *Arabidopsis thaliana* arrest as early embryos when they carry loss-of-function alleles at duplicate copies of an essential histidine biosynthesis gene (HISN6A and HISN6B, see 10; 11). Second, unlinked duplicate copies of the *pTAC14* homolog (170), a nuclear gene essential

for chloroplast development in *Arabidopsis* (53), cause chlorosis in hybrids between two species of *Mimulus* (monkeyflower). The *Mimulus nasutus* lineage carries only one (ancestral) copy of *pTAC14*; hybrid chlorosis arises in F_2 seedlings that are *M. nasutus* homozygotes (null/null) at the duplicate and carry only defective *M. guttatus* alleles at the ancestral locus. This genetic pattern suggests that this hybrid lethality in *Mimulus* derives from duplication and (likely neutral) nonfunctionalization within a single lineage. Such epistatic chlorophyll deficiency variants also segregate at low frequency within a single large population of *M. guttatus*, manifesting as epistatic inbreeding depression (as in 160). However, similar genetic patterns could result from ancestral duplication and then differential retention in descendent lineages; in either case, there is no need to invoke processes beyond mutation and drift (104). Thus, the hybrid lethality systems characterized to date provide no direct evidence of any form of divergent selection leading to the establishment of postzygotic incompatibility. More work in diverse systems is necessary to determine whether this is a robust pattern or an artifact of the few systems that have been investigated genetically so far.

2.2.3. Nuclear hybrid sterility: no smoking gun within parental species for gamete killers in hybrids? Hybrid sterility in seed plants can be caused by genetic incompatibilities that affect haploid gametophytes (i.e., pollen and embryo sacs in angiosperms) or the surrounding diploid sporophytic tissues (e.g., tapetum and ovule cells). Only a decade ago, researchers knew very little about the molecular or developmental basis of such incompatibilities, and not a single gene had been identified. Since that time, high-resolution genetic mapping of interspecific hybrid sterility has been performed in diverse systems (109, 138, 148), and there are now 10 cases (all in intersubspecific hybrids within the Asian cultivated rice *O. sativa* or in interspecific hybrids involving *O. sativa*) for which at least one of the causal genes of a nuclear Dobzhansky-Muller incompatibility has been identified (88–90, 101, 107, 112, 165–168). In addition, complex interactions causing gametophytic male sterility have been characterized at the genetic and molecular level in among-strain hybrids of *A. thaliana* (43, 138). Because other reviews have summarized the molecular details of many of these studies (32, 117, 150), we focus here on general patterns and their evolutionary implications.

One emerging theme is that plant hybrid sterility-causing incompatibilities seem to manifest more frequently in the haploid gametophytes than in the diploid sporophyte. This insight is not entirely new: Among the ~50 hybrid sterility loci classically identified between *O. sativa* ssp. *japonica* and *O. sativa* ssp. *indica*, gametophytic incompatibilities appear to be much more common (117). Recent work in other systems extends this pattern, with gametophytic incompatibilities now genetically mapped between populations of *Arabidopsis* (138), species of *Mimulus* (81), and species of *Oryza* (33, 112, 165). If these examples of hybrid sterility reflect a true bias toward incompatibilities that act in the gametophyte, what is its cause? One simple explanation could be a difference in the number of mutations that affect the two classes of hybrid sterility; perhaps the mutational target size is larger for the gametophyte than for the sporophyte. Additionally, just as with genes on heteromorphic sex chromosomes, recessive alleles are exposed in the haploid gametophyte. If genes causing hybrid sterility are often recessive, the gametophyte should express a larger number of incompatibilities than does the diploid sporophyte. A nonmutually exclusive possibility is that gametophytic genes are subject to unique evolutionary processes. For example, pollen competition and sexual selection might lead to faster rates of adaptive evolution in the male gametophyte (2, 59). Intragenomic conflict, too, might be particularly active in plant gametophytes: Any selfish genetic element that can disable gametes carrying the alternative allele will have a direct impact on its own transmission. Indeed, classic and recent crossing studies have often revealed so-called gamete killers that affect both transmission ratios and fertility (23, 100, 101, 126, 132, 167).

Despite the term gamete killer, however, genetically based pollen and embryo-sac mortality in hybrids (and the distortion of genetic transmission it causes) need not have its origins in genomic

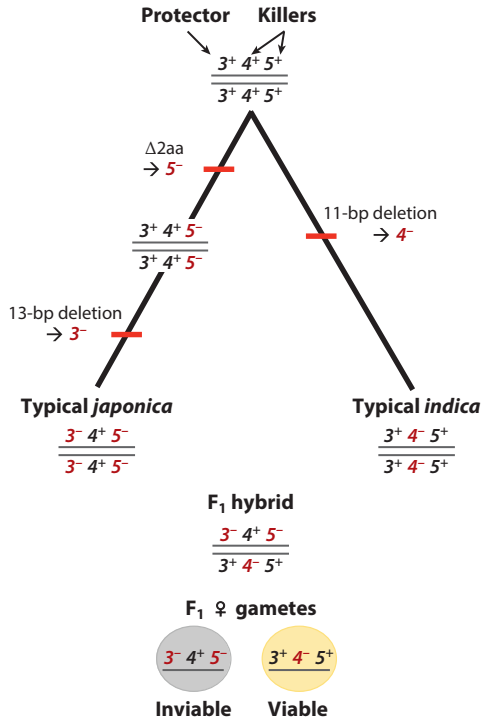

Figure 4

Three tightly linked genes, *ORF3*, *ORF4*, and *ORF5* (167) (indicated here by the numbers *3*, *4*, and *5*, respectively), regulate *S5* incompatibility, which causes embryo-sac abortion in *indica-japonica* heterozygotes. During female sporogenesis, killer alleles at *ORF4* and *ORF5* cause endoplasmic reticulum stress that results in premature programmed cell death and embryo-sac abortion, but these effects can be rescued by a protector allele at the adjacent *ORF3* gene. The presumed ancestral haplotype (at high frequency in wild species *Oryza rufipogon* and *Oryza nivara* and in the outgroup species *Oryza glumaepatula*) (116) carries functional copies of the protector ($ORF3^+$) and both killers ($ORF4^+$ and $ORF5^+$). In contrast, the typical *japonica S5* haplotype carries two amino acid–changing mutations (Δ2aa) that disable one of the killers ($ORF5^-$) and a 13-bp deletion that disables the protector ($ORF3^-$). The typical *indica* haplotype has an 11-bp deletion that incapacitates the other killer ($ORF4^-$). *S5* incompatibility causes an aberrant, gain-of-function phenotype in female gametes of *indica-japonica* hybrids that carry functional killer alleles ($ORF4^+$ and $ORF5^+$) in combination with a disabled protector ($ORF3^-$). Note that this mechanism of gamete killing implies a particular evolutionary history for this haplotype in *japonica*: Because the $ORF3^-$ allele is aborted in the presence of $ORF4^+$ and $ORF5^+$, the $ORF5^-$ allele must have arisen first to enable its spread. This evolutionary history with loss-of-function mutations occurring first in the killer and then in the protector is at odds with a classic scenario of genomic conflict.

conflict within species. In classic models of intragenomic conflict, selfish genomic elements spread because they manipulate host reproduction to bias their own transmission. Because these actions are often detrimental to host fitness, there is selective pressure for suppressors to neutralize their effects (21). This evolutionary sequence of events—gamete killers followed by suppressors—is at odds with the inferred histories of well-studied *Oryza* hybrid incompatibilities. One example is the *S5* locus, which causes female sterility in *japonica-indica* hybrids when gametes carry an incompatible combination of killer and protector alleles at three tightly linked genes (167) (**Figure 4**). These two subspecies typically carry haplotypes with loss-of-function alleles in distinct components of the killer-protector system. The *japonica S5* haplotype appears to have arisen through founder

Transmission ratio distortion: deviation from expected Mendelian genotypic ratios in progeny due to selection at meiotic, gametic/gametophytic, or zygotic stages

effects (drift), but the *indica* haplotype shows a signature of positive selection (116) suggesting it might have spread through adaptive or selfish substitutions. However, because transmission ratio distortion occurs exclusively in hybrid genetic backgrounds (the *indica* and ancestral haplotypes are perfectly compatible), selfish evolution seems unlikely. Similarly, the *Sa* locus, which is composed of two tightly linked genes that together cause *japonica-indica* hybrid male sterility, shows no evidence of recent conflict within subspecies or in their outcrossing progenitor, *O. rufipogon* (101). As with *S5* (see **Figure 4**), *Sa* gamete killing occurs only in hybrids, and the inferred sequence of mutations within *japonica* do not follow a classic conflict scenario. Although intragenomic conflict may have played a role in the initial formation of the *S5* and *Sa* haplotypes (i.e., the ancestral killer-protector combination might represent a resolved conflict), it does not seem to be the cause of current reproductive barriers between *japonica* and *indica*. Given the selfing mating system of both subspecies, this should not be a surprise; population genetic theory shows that selfish elements, which gain their advantage in heterozygotes, cannot spread in highly selfing populations (20).

Gametophytic-sporophytic incompatibility in hybrids between selfing (*M. nasutus*) and outcrossing (*M. guttatus*) yellow monkeyflower provides an evolutionary context in which a hybrid gamete killer could have evolved selfishly but no evidence that it did so. In this system, *M. guttatus* alleles at the *hms1* locus cause severe male sterility and partial female sterility in hybrids with *M. nasutus* alleles at a second unlinked locus (147). Additive effects and transmission ratio distortion in *hms1* heterozygotes point to gametophytic action. The *hms1* allele is at intermediate frequency in the *M. guttatus* population from which it was derived and is embedded in a genomic region that has undergone a recent selective sweep (148, 149). The recent rapid spread of *hms1* must have been driven by adaptation, selection on a linked locus (as in 163), or selfish gamete killing within *M. guttatus*. Intriguingly, crossing experiments suggest that *hms1* does not exhibit intraspecific gamete killing through either male or female function: The newly arisen incompatibility allele shows no transmission bias against other compatible variants segregating within the same population (81). Conversely, there is no evidence that female meiotic drive by a selfish centromeric variant found in the same population of *M. guttatus* directly contributes to hybrid sterility or any other species barrier (45). Thus, despite population conditions in *M. guttatus* ideal for selfish genetic elements (25, 45), there is as yet no functional link between within-species selfishness and the evolution of nuclear hybrid incompatibility. Other forms of linked and direct selection on *hms1* remain in play and are also amenable to testing with direct experimentation.

A key feature of many hybrid gamete killers is that they are often genetically complex, caused by two or more tightly linked epistatic genes (87, 88, 101, 167). Originally identified as single-locus incompatibilities (see 150), upon closer inspection, these gamete killers seem invariably to fracture into multiple, often paralogous genes. In one particularly complex example from *indica* and *japonica*, fine mapping revealed two tightly linked genes, each involved in an independent pollen killer system that includes interactions between sporophytic and gametophytic genes and additional modifier loci (89). The *hms1* locus in *Mimulus* exhibits similar complexity, with several independent effects on hybrid sterility and transmission ratio distortion revealed through fine mapping (81). In addition to these individual examples of hybrid incompatibilities, a recent genome-wide study of interactions among hybrid sterility loci between *Solanum lycopersicum* and *Solanum habrochaites* revealed a striking level of complexity with pervasive antagonism among incompatibility alleles (63). The picture emerging from such studies is one in which hybrid sterility is regulated by multiple, interconnected molecular networks potentially involving many genes. Although selection may certainly be involved, neutral evolution of gene regulation underlying large developmental networks might also lead to hybrid incompatibilities (118).

Molecularly characterized hybrid sterility systems also point to the importance of gene duplication in generating gametophytic incompatibilities. This possibility was first suggested by Oka

(114), who argued that loss-of-function alleles at duplicate genes might cause defects in pollen development. There is now clear empirical evidence for this scenario in three distinct *Oryza* crosses (one between subspecies of *O. sativa*, two between *O. sativa* and different wild species) (107, 112, 166). Gene duplicates also cause one additional case of sterility between accessions of *A. thaliana*, but the lack of functionality in one paralog is due to epigenetic silencing rather than sequence mutations (43). These examples, along with the hybrid lethality systems described in Section 2.2.2, suggest that divergent resolution of gene duplicates via mutation and genetic drift may be a common source of hybrid incompatibilities in plants.

Gynodioecious: both hermaphroditic and female (male-sterile) individuals within a population

2.3. Cytonuclear Incompatibilities

Organelles (mitochondria and plastids) are the essential powerhouses of eukaryotic cells. However, despite 2 billion years of co-option and coordination, cytonuclear coevolution is far from over, and this close relationship requires constant maintenance (22, 124). In flowering plants, dynamic cytonuclear interactions shape the reproductive biology of entire families (24), can contribute to individual fitness variation and local adaptation (61), and drive the repeated fine-tuning of key cellular processes within species (139) as well as the evolution of hybrid incompatibilities between species (reviewed in 22, 62, 98, 141). Here, we focus on two discrete classes of cytonuclear hybrid incompatibility, cytoplasmic male sterility (CMS) due to mitochondrial-nuclear mismatch and cytonuclear chlorosis (CNC) generally due to plastome-nuclear incompatibilities (PNIs). However, cytonuclear epistasis resulting in more subtle or environment-specific reductions in hybrid performance may also be common (131) and may interact with processes leading to these discrete incompatibilities (reviewed in 12).

2.3.1. Cytoplasmic male sterility: selfish organellar evolution in the flesh? Asymmetric hybrid sterility, in which reciprocal hybrids exhibit strikingly different patterns of fertility, is the rule across diverse organisms (153). In plants, interactions between maternally inherited CMS genes and nuclear restorer-of-fertility (*Rf*) alleles are the primary cause of reciprocal differences in hybrid male fertility. In addition to being ubiquitous in plant hybrids, CMS is expressed as segregating variation in the substantial fraction (up to 7%) of flowering plant species that are gynodioecious (24). Furthermore, theory argues that hybrid CMS, as well as gynodioecy, may be the outcome of selfish organellar evolution and nuclear coevolution (29, 50; reviewed in 133). Thus, understanding the mechanisms and origins of CMS in hybrids may reveal ubiquitous evolutionary processes affecting multiple aspects of plant diversification. CMS-*Rf* interactions are among the best-understood hybrid incompatibilities from molecular genetic perspectives as well (22; reviewed in 30, 34, 35, 68). Given recent reviews focusing on the molecular biology of crop CMS, we briefly review the molecular basis of CMS and restoration here and then focus on how evolutionary genomic studies of natural populations that can uniquely connect the functional loci underlying hybrid CMS to the processes predicted to drive their evolution (25).

The genetics of hybrid CMS are remarkably conserved across flowering plants. Phenotypically, CMS is generally characterized by anther sterility (failure to produce pollen or even anthers) in F_2 or backcross hybrids. Because it stably eliminates male function without negative effects on seed set (in outcrossing taxa), hybrid CMS is extensively used to enforce outcrossing during commercial F_1 hybrid seed production (reviewed in 34). This utility has led to the molecular characterization of CMS loci in at least 14 crop species (reviewed in 35). In almost all cases, CMS is caused by mitochondrial structural rearrangements that generate novel open reading frames. CMS open reading frames are generally chimeras consisting of coding sequences from an essential transmembrane respiratory protein (e.g., *atp6*, *cox1*) grafted to additional upstream or

downstream sequences (**Figure 5*a***). In a few cases, other mitochondrial variants may cause CMS; for example, nonchimeric mutations in *cox* genes are associated with hybrid CMS in gynodioecious sugar beets (38), and noncoding-RNA expression may explain segregating CMS in a population of the gynodioecious wildflower *Silene vulgaris* whose mitochondrial genome lacks chimeric open reading frames but exhibits elevated mutation rates (140, 142). Overall, however, mitochondrial

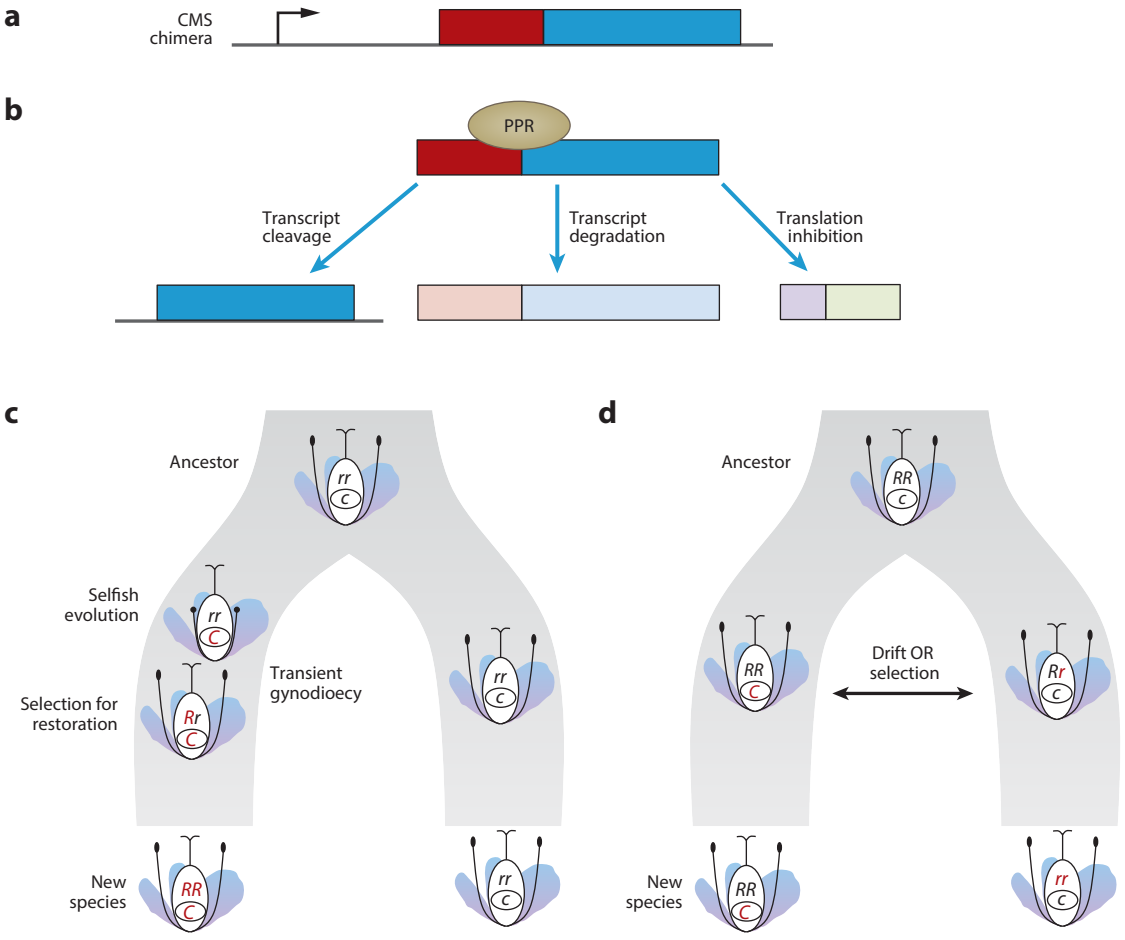

Figure 5

Cytoplasmic male sterility (CMS). (*a*) All confirmed CMS loci to date are chimeric mitochondrial open reading frames, often including sequence of an essential gene (*blue*) that has become physically associated with nonessential sequence (*red*) by a structural rearrangement and is coexpressed. (*b*) Processing by pentatricopeptide repeat (PPR) proteins is a common mechanism of restoration of male fertility in hybrids with CMS mitochondria. PPR proteins generally act by binding and processing, degrading, or blocking translation of CMS mRNAs. (*c*) In the selfish *CMS-Rf* (restorer-of-male-fertility) coevolution model, a selfish male-sterilizing mitochondrial variant (*C*) invades an ancestral hermaphroditic (*c rr*) population and increases in frequency owing to positive effects on seed number. Costs of sterility and frequency-dependent selection for maleness then favor the spread of nuclear restorer alleles (*R*), and *C* and *R* jointly go to fixation after a period of transient gynodioecy. Cryptic male sterility is revealed in F_2 hybrids of derived (*C RR*) populations with those still carrying (ancestral) *r* alleles. (*d*) In the agnostic CMS model, *R* is ancestral, and *C* can fix in one lineage (by drift or selection on other phenotypes) without any expression of male sterility. Meanwhile, *r* can fix in an independent lineage, again without necessary phenotypic effects. In hybrids, the same genetic combination is sterile as in the selfish scenario. These alternatives can be distinguished by examining the population genomic history of *R*, as only the selfish scenario predicts positive selection on both *C* and *R* within a single lineage.

rearrangements in a subset of essential respiratory genes are the primary mutational source for CMS genes. Because CMS transcripts are generally expressed in all plant tissues, the fact that they specifically disrupt pollen production without causing respiratory dysfunction elsewhere is intriguing from both evolutionary and functional perspectives (see 34, 35 for a detailed discussion of alternative functional explanations). Some CMS molecules appear to be intrinsically cytotoxic (e.g., *CMS-T* in maize), but there is broader evidence that mutations causing sporophytic CMS affect mitochondrial respiration just enough to starve the energetically demanding tapetal cells that support pollen development (34, 35). From an evolutionary perspective, the conserved basis for hybrid CMS may reflect constraints placed by extremely low nucleotide mutation rates in most plant mitochondria or selective filtering for CMS variants whose deleterious effects are restricted to anthers.

Pentatricopeptide repeat (PPR) proteins: large family of organelle-targeted proteins that bind and modify organelle RNA transcripts

The nuclear components of cytonuclear male sterility are also conserved, though less so. CMS often segregates as a recessive Mendelian trait (i.e., expressed in F_2 or backcross hybrids rather than in F_1), indicating that nuclear restorer (*Rf*) alleles are dominant (30). In most cases, a single *Rf* allele is sufficient to confer male fertility in hybrids, but redundant linked (7, 156, 157) or complementary unlinked (135) *Rf* alleles have been identified in a few systems. At least half the *Rf* genes that have been molecularly defined encode pentatricopeptide repeat (PPR) proteins (reviewed in 34, 52). The *Rf*-like (RFL) subfamily of PPR proteins, which often occur in species-specific tandemly duplicated chromosomal clusters (56), has been repeatedly implicated in the restoration of hybrid CMS (reviewed in 52). The RFL-PPR protein generally binds CMS transcripts and restores fertility via diverse mechanisms, including processing the CMS-associated transcript (**Figure 5*b***) and inhibiting translation of CMS proteins (reviewed in 37, 52). Non-PPR-protein *Rf* loci are even more diverse in function. For example, the glycine-rich protein *Rf2* in rice posttranslationally degrades the CMS-associated protein (74), and *Rf2* in CMS-T maize, a mitochondrial aldehyde dehydrogenase, may restore fertility by protecting anthers from CMS-caused oxidative stress rather than interacting directly with CMS transcripts or proteins (99). Expression and restoration of CMS can be dependent on the nuclear genetic background or environmental conditions, adding another layer of complexity (34, 128). Nonetheless, the repeated identification of PPR proteins as *Rf* loci in crop CMS systems (reviewed in 34, 35) and hybrids of wild species (7, 96) indicates that they play a special role in the evolution of cytonuclear incompatibility in plants and provides an empirical platform for exploring their origins in diverse systems.

Population genetic theory provides a selfish evolutionary scenario for the repeated evolution of hybrid CMS in plants (**Figure 5*c***). When organelles are maternally transmitted, organellar mutations that can even slightly increase female fitness by causing male sterility are predicted to spread rapidly within populations (29, 50). In the vast majority of angiosperms, this selfish genetic motive meets a developmental opportunity. Because male and female organs are in close proximity in hermaphroditic flowers, anther sterility may pleiotropically increase female organ size or the resources available for seed development (8, 40). In turn, selfish CMS spreading though a plant population exerts strong frequency-dependent selection for dominant nuclear *Rf* alleles (24; reviewed in 133). Under broad theoretical conditions, both CMS and *Rf* alleles should sweep to fixation, returning the population to its original, entirely hermaphroditic phenotypic state (29). Upon hybridization with lineages without matched *Rf* alleles, this cryptic CMS-*Rf* coevolution would be exposed as hybrid CMS. Alternatively, like other Dobzhansky-Muller incompatibilities, hybrid CMS can evolve via independent neutral or adaptive evolution in separate lineages (**Figure 5*d***). That is, a restorer of CMS in hybrids need not have evolved to restore it. The long history of selfish CMS evolution and coevolution of the RFL-PPR protein across flowering plants (51) sets the molecular stage for hybrid incompatibility to evolve by independent evolution in separate lineages as well as local conflict-coevolution dynamics. Furthermore, some CMS

mutations may be expressed in only a few sensitive nuclear backgrounds and thus may be hidden from selection even in the absence of matched restorers (128). Distinguishing between selfish and nonselfish scenarios speaks to the role of conflict in the evolution of hybrid incompatibility and to whether hybrid CMS should accelerate or impede interspecific gene flow.

Two indirect lines of evidence support the idea that selfish CMS-*Rf* coevolution is pervasive in flowering plants. First, the genetic basis and phenotypic expression of most hybrid CMS systems are consistent with the selfish model. Anther-limited effects of many CMS loci suggest that they represent a highly filtered subset of mitochondrial respiratory dysfunctions, reflecting a history of exposure and selection. Second, the RFL-PPR protein family specifically exhibits both high rates of tandem duplication (37, 52, 56) and classic signatures of diversifying selection on protein-coding sequence (51). Rapid evolution of RFL-PPR proteins is consistent with repeated *Rf* turnover driven by the spread of male-sterilizing mitochondrial mutations within species, which supports the selfish model of CMS-*Rf* coevolution. However, we emphasize that these patterns do not rule out the neutral alternative for any particular hybrid CMS system, and (as with many nuclear genic incompatibilities) other lines of evidence suggest more complex histories. For example, *rf* (sterility-causing) alleles in the majority of seven recently characterized *CMS-Rf* systems are loss-of-function mutations (as indicated in 128); this pattern is not consistent with a selfish evolutionary model in which the *rf* allele is ancestral, the CMS novel, and the *Rf* recently evolved for the purpose of restoration.

Hybrid CMS systems in wild taxa with genomic resources, such as *Mimulus* (7, 26) and *Arabidopsis* (96, 138), provide opportunities to test alternative evolutionary models, as interacting loci can be examined in the geographical and genomic contexts in which they evolved. Uniquely, a recent study in the outcrossing wildflower *M. guttatus* provides direct evidence of selfish CMS in action (25). In this system, interspecific hybrids with the Iron Mountain population *M. guttatus* cytoplasm and recessive *rf* alleles from closely related selfer *M. nasutus* produce no pollen (47). The molecular genetic basis of *Mimulus* hybrid CMS is similar to that in crop models: Iron Mountain CMS is a chimeric mitochondrial open reading frame containing the NAD6 sequence (26, 108), and processing of the CMS transcripts is associated with F_2 fertility (26). Iron Mountain *Rf* maps to a large cluster of tandemly repeated RFL-PPR proteins containing two tightly linked and redundant alleles, *Rf1* and *Rf2* (7). In addition, because CMS is fixed in the Iron Mountain population, but absent from the nearby Cone Peak *M. guttatus* population (26), explicit tests of selfish (**Figure 5*c***) and agnostic (**Figure 5*d***) models were possible: Only CMS-driven coevolution predicts divergent selection on the *Rf* region specifically in the Iron Mountain population. Whole-genome resequence data from both populations provided striking confirmation of the selfish CMS-*Rf* coevolution model: The *Rf* region is the largest Cone Peak–Iron Mountain F_{ST} outlier across the genome and also exhibits signatures of a recent, local selective sweep (25).

Confirmation of the selfish CMS model in *Mimulus* provides a template for further evolutionary investigation of hybrid CMS and cytonuclear coevolution. However, when considering both the origins of hybrid CMS and its consequences for speciation, context matters. Hybrid CMS is ubiquitous, agriculturally useful, and functionally interesting regardless of its origins, but it may make a very poor species barrier under a simple selfish scenario. Nuclear *Rf* alleles transmitted in pollen may spread far more rapidly than do CMS genes found only in seeds, thus potentially restricting selfish CMS mutations to the populations in which they originated. Furthermore, even a widespread selfish CMS might spread across species boundaries rather than act as a barrier upon natural hybridization (see 128 for review). In terms of origins, conflict models developed in specific reproductive and population genetic contexts should be transferred with caution to other plants. In particular, highly selfing taxa with reduced flowers (e.g., *A. thaliana*, *O. sativa*) should experience little evolutionary conflict between male and female fitness, which are tightly linked at the level

of both the individual flower and the population (20). Thus, although hybrid CMS is observed in interpopulation hybrids of *A. thaliana* (138) and rice (103), whether it evolved via a selfish process is very much an open question. Similarly, in dioecious (e.g., *Populus*) and monoecious (e.g., maize) species, CMS mutations should have relatively little opportunity to selfishly increase female fitness by eliminating male function, potentially enabling prediction of different incidences, genetic bases, and histories for hybrid CMS in such systems. As next-generation genomic approaches facilitate molecular evolution (51) and population genomic (25) studies of hybrid CMS loci in both wild and crop taxa, comparisons across this diversity provide an exciting opportunity for both mechanistic and evolutionary insights.

Cybrids: plants generated by protoplast fusion that carry nuclear and plastid genomes from genetically distinct individuals

2.3.2. Cytonuclear chlorosis: the breakdown of chloroplast-nuclear coordination. CNC or albinism in hybrids is not as common as CMS but occurs broadly across flowering plants (61). CNC is observed in *trans*-genus cybrids as well as in experimental hybrid progeny and is often (though not exclusively) caused by PNI (62). Mapping of either component of a cytonuclear interaction causing (usually) F_1 hybrid chlorosis is difficult because the phenotype is often lethal prior to reproduction and mitochondrial and plastome effects cannot be separated when both organelles are maternally inherited. Thus, most knowledge of the genetic mechanisms of CNC comes from cybrids and crosses in taxa with biparental inheritance of chloroplasts; the latter results in vegetative variegation (but viability) as compatible and incompatible chloroplasts sort during development (61). In *Atropa/Nicotiana* cybrids, albinism was traced to improper editing of the *Atropa* plastid ATPase A-subunit mRNA by the *Nicotiana* nuclear genome, and the evolution of species-specific editing sites was proposed as a general contributor to PNI (134). Rapid and coordinated evolution of the plastid (*rpo*) and nuclear (*sigma factor*) subunits of the plastid-encoded polymerase protein across the Geraniaceae may be a plausible source of hybrid PNI in *Pelargonium* (169). However, because the genetic loci underlying PNI in *Pelargonium* have not yet been mapped, it remains unclear whether both phenomena are consequences of highly elevated rates of plastid genome evolution in the Geraniaceae (64) or whether they are causally linked. In *Oenothera*, which exhibits PNI in numerous interspecific crosses (62), plastid genome rearrangements and disrupted expression of photosynthetic genes in chlorotic tissues also points to misregulation as the mechanistic basis of CNC. Mapping of the interacting nuclear loci, as has been done for a cytonuclear chlorophyll deficiency and sterility locus in *Pisum* (13), provides the opportunity to close this functional gap.

Two recent studies of intraspecific F_1 hybrid chlorosis in wildflowers suggest that CNC may also contribute to reproductive isolation in incipient plant species; thus, they provide new systems for understanding its origins. In *Silene nutans*, experimental F_1 hybrids between Eastern and Western lineages exhibit asymmetric chlorosis, which likely contributes to strong reproductive isolation (105). CNC in *S. nutans* hybrids may be due to PNI or to mitochondrial-nuclear mismatch, as mitochondrial dysfunction can also cause albinism (130). A gynodioecious species, *S. nutans* carries multiple CMS mitochondrial types within populations and exhibits high organellar divergence between isolated populations (55). Thus, the selfish CMS dynamics expected in gynodioecious taxa may have contributed directly or indirectly to elevated rates of organelle turnover and sequence evolution. In the hermaphroditic wildflower *Campanulastrum americanum*, similar patterns are clearly due to PNI: F_1 hybrids between distinct geographical lineages exhibit albinism and high mortality (6), and biparental inheritance of chloroplasts results in hybrid variegation and survival (4). In *C. americanum* as well as other members of the Campanulaceae (84), the plastid has unusually high rates of sequence evolution (5). Thus, as in *Oenothera*, high plastid mutation rates may be the primary drivers of rapid evolution of strong F_1 CNC. Whether this causes the fixation of deleterious (but nonlethal at each step) organellar mutations and compensatory nuclear

coevolution within one lineage or simply accelerates the fixation of plastid mutations that happen to be incompatible with nuclear genomes in other lineages remains an open question. Evolutionary genomic analyses of the nuclear partner(s) in these intraspecific incompatibilities may untangle these alternatives. Specifically, the one-lineage compensatory scenario predicts positive selection at the nuclear PNI gene(s) in the compatible lineage alone (as in 129), whereas the two-lineage scenario predicts either no differences in selection history or evolution within the incompatible lineage.

As with other forms of hybrid incompatibility, investigation of PNI in taxa with diverse mating systems and genomic architectures will enhance our understanding of its evolutionary origins. In selfers, apomicts, and permanent translocation heterozygotes such as *Oenothera*, linkage disequilibrium between organelle and nuclear genomes is also an important consideration. For example, a recent study in *A. thaliana* demonstrated hitchhiking of much of the nuclear genome during the selective sweep of a plastid herbicide-resistance mutation (48); essentially clonal evolution of all three genomes in such taxa should reduce intergenomic conflict but may accelerate drift and the need for local compensatory nuclear coevolution. Intriguingly, several groups with elevated organellar mutation rates and cases of CNC also contain gynodioecious taxa (e.g., Geraniaceae, Campanulaceae, *Silene*). Both CNC and gynodioecy may be conditioned on having highly error-prone organellar genomes; selection favoring novel CMS mutations in gynodioecious taxa (133) may further drive rapid organellar divergence. Untangling how mutation, drift, and selection interact to produce CNC in systems with diverse mutational spectra is an important evolutionary challenge that will also yield functional insights.

3. CHROMOSOMAL REARRANGEMENTS—THE ONCE AND FUTURE KINGS OF PLANT REPRODUCTIVE ISOLATION?

Most plant species differ in cytogenetic structure from their sister taxa, and chromosomal evolution has been recognized as an important correlate of plant species divergence since the invention of microscopy (146 and references therein). Whether rearrangements play a causal role in speciation has been more controversial. Chromosomal rearrangements, particularly inversions and translocations, can directly cause F_1 hybrid sterility and may also contribute to the evolution of species barriers by locally suppressing recombination in hybrids (reviewed in 70). Inversions (especially pericentric inversions) can cause sterility in a heterozygous individual when a crossover occurs within the inverted region, resulting in recombinant gametes with duplications or deletions of genetic material (70; reviewed in 127). Reciprocal translocations, if segregating randomly in heterozygotes, produce 50% unbalanced (duplication/deletion) gametes (145). In plants, such structural incompatibilities are particularly costly, as a large fraction of nuclear genes are expressed in haploid pollen (71). The number of inversions and translocations distinguishing species often correlates with the severity of F_1 hybrid sterility (60, 97). Furthermore, artificial chromosome doubling with colchicine can often restore sterile F_1 plant hybrids to fertility, indicating that structural heterozygosity during meiosis per se causes infertility (144 and references therein; 145). Nonetheless, rearrangements have fallen from favor as a direct cause of hybrid infertility and species barriers, on the basis of both theoretical and empirical difficulties (reviewed in 36). Theoretically, rearrangements that cause the most F_1 sterility are the most difficult to evolve: Any individual chromosomal variant that causes infertility in heterozygotes (underdominance) should be strongly selected against when it is at initial low frequency (155). This theoretical difficulty, combined with the ubiquity of both inversions and translocations in closely related plant species, creates an empirical paradox.

3.1. Inversions: Common Suppression of Recombination Without Direct Fertility Costs?

For inversions, this empirical paradox can be resolved in two ways. First, theory shows that inversions that suppress recombination in heterozygotes (either because recombinant gametes are lost or because crossing over is inhibited) are favored when recombination is disfavored (reviewed in 70). For example, when gene flow occurs across an ecological gradient, a novel inversion that locks together multiple, locally adaptive alleles will spread locally and come to define a distinct ecotype (83). Furthermore, once alternative chromosomal variants become associated with alleles under divergent selection despite gene flow, they are predicted to accumulate further locally adaptive or incompatible alleles (113, 127). Under the adaptation with gene flow scenario, even inversions with moderate underdominant effects on fertility may spread during parapatric speciation. Second, and as importantly, inversions do not cause sterility in the absence of crossing over, and inhibition of crossing over in rearranged regions may be common. Genetic mapping and genome scans in diverse plants implicate inversions (and associated suppression of recombination) in the maintenance of standing variation for fitness (95, 122), adaptive differentiation among populations (94, 102), and species differences (46). In most cases, however, inversions implicated in adaptation have no underdominant fertility costs, suggesting that suppression of recombination across large genomic regions can occur without gamete loss. In interspecific mapping populations, inversions are sometimes associated with underdominant hybrid sterility, but it is not yet clear where this is a direct effect or capture of genic incompatibilities (93). Notably, the one recent test of rearrangements as a direct cause of structural incompatibility (using artificial chromosome doubling) found no evidence that inversions suppress recombination via the loss of unbalanced gametes (144). Three inversions, two translocations, and underdominant pollen inviability were mapped in hybrids between *Mimulus lewisii* and *Mimulus cardinalis*, the classic models for plant ecological speciation (46 and references therein). Artificial chromosome doubling and comparative mapping demonstrated that the underdominant sterility was entirely structural and exclusively due to the two translocations. Thus, even under evolutionary conditions that allowed a strongly underdominant translocation to fix in each lineage (144), the species-diagnostic inversions have no direct effects on hybrid fertility. Similar work on other inversion-rich genera (e.g., in *Helianthus*) is necessary to turn an absence of evidence into a positive pattern, but inversions may generally contribute to speciation in the same way they contribute to polymorphism within populations and species—by suppressing recombination in heterozygotes without direct deleterious effects.

3.2. Crossing the Valley of Low Fitness to Speciation: The Enduring Mystery of Underdominant Translocations

Unlike inversions, heterozygous reciprocal translocations must cause severe underdominant effects (barring the strictly alternate segregation seen in a few taxa such as Onagraceae) (145). Nonetheless, they have been largely ignored in plant speciation and hybrid sterility research in the modern era of genetics and genomics. The few exceptions suggest that more attention is merited; in addition to being common in interspecific comparisons (60), translocations can contribute both directly and indirectly to the evolution of species barriers. In the sister monkeyflowers (*Mimulus*) described above, translocations directly cause severe hybrid sterility, suppress recombination in heterozygotes (though not as strongly as do inversions), and co-map with QTLs for putatively adaptive species differences (144). Rearrangements also accumulate rapidly in annual sunflowers (19). *Helianthus petiolaris* and *Helianthus annuus* (not sister taxa, but currently hybridizing) are separated by at least eight translocations that often co-map with underdominant sterility QTLs (likely due to

structural incompatibility) (93). Sunflowers are self-incompatible outcrossers with large effective population sizes, so drift cannot explain the prevalence of translocations in this system. Furthermore, *H. petiolaris* and *H. annuus* introgress freely, except very close to rearrangement breakpoints, suggesting little potential for spread by adaptive recombination suppression (83). Meiotic drive, particularly selfish chromosomal segregation during asymmetric female meiosis (female meiotic drive), may be a factor in *Mimulus*, *Helianthus*, and other outcrossing taxa in which translocations accompany speciation (e.g., Solanaceae) (164). Translocations can alter chromosomal structure (particularly centromere position and size) to cause overtransmission to the egg (reviewed in 120), and female meiotic drive by translocations has been posited as an explanation for large-scale patterns of chromosomal evolution in mammals (119). However they spread, the unavoidable effects of translocations on hybrid fitness are likely to be important in generating the strong postzygotic barriers required for speciation by reinforcement (72) and homoploid hybrid speciation (17). Determining what processes drive the evolution of species-diagnostic translocations and characterizing their roles in plant speciation remains a complex challenge, but their outsized impact on hybrid fertility and genome divergence suggests that translocations are a fruitful avenue for future research.

SUMMARY POINTS

1. Genomic tools bring tremendous opportunities to understand both the molecular mechanisms and evolutionary origins of hybrid incompatibilities.
2. Dobzhansky-Muller incompatibilities (genic interactions in hybrids) can arise via neutral or adaptive evolution in independent lineages and via conflict and coevolution within a single lineage.
3. Parental conflict and genomic imprinting are plausibly implicated in hybrid seed lethality, but it remains an open question whether conflict and coevolution within species is the primary pathway to embryo-endosperm breakdown in interspecific hybrids.
4. Long-term balancing selection on disease genes, or gene duplication and subfunctionalization (plus drift), may be important for the evolution of hybrid lethality.
5. Population genomic evidence supports selfish organellar evolution and nuclear coevolution as causes of CMS, but there is surprisingly little direct evidence (yet) that genomic conflict drives the evolution of most hybrid sterility and inviability in plants.
6. Population genetic theory, which predicts different dynamics in plants with different mating systems, is a necessary frame for understanding alternative evolutionary scenarios for the origin of hybrid incompatibilities.
7. Translocations, which almost always cause hybrid sterility when heterozygous, merit renewed interest (as inversions have received) as direct and indirect contributors to plant speciation.
8. Over the next decade, broadening our mechanistic understanding of incompatibility loci across the diversity of plants promises new insights into the processes that drive genomic divergence within species as well as the barriers between them.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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3. Provides a thorough review of current research on mechanisms of plant speciation.

15. Discusses the importance of mating systems in shaping the evolution of hybrid incompatibilities.

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Errata

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