Opinion

The polymorphic prelude to Bateson–Dobzhansky–Muller incompatibilities

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Speciation research has largely assumed that the genetic causes of reproductive isolation are the work of fixed, divergent alleles that interact to cause genetic problems in hybrids: Bateson–Dobzhansky–Muller incompatibilities. However, many recent studies demonstrate substantial heritable polymorphism within species for hybrid incompatibility, herein called variable reproductive isolation (VRI). In this review, I outline the causes and importance of this general phenomenon. I also identify the new challenges of quantifying the relative contributions to reproductive isolation of fixed alleles versus polymorphisms, and the change in these contributions over the course of speciation. Explicit integration of VRI into speciation theory will help to quantify the relative roles of genetic drift and selection in speciation, but this synthesis requires substantial new contributions from both theory and empirical studies.

Variable reproductive isolation between a pair of species

A challenge for evolutionary genetics is to understand how reproductive isolation (see Glossary) accumulates between species when it originates as variation within a species. How is it that alleles can combine to create dysfunctional hybrids when two nascent species mate, given that they recently shared a common ancestor in which individuals could freely interbreed? The Bateson–Dobzhansky–Muller (BDM) model of reproductive incompatibilities [1–3] provides a simple and powerful explanation for the genetic basis of reproductive isolation (Box 1). However, the theory surrounding BDM incompatibilities (BDMIs) chiefly considers genetic interactions between divergent alleles that have become fixed between species [4]. Although Bateson and Muller both mentioned polymorphism for testing of the BDM model [1,3,5] and the general recognition that it is important to understand the speciation process from the mutational origin of a BDMI allele through to fixation [6,7], the open secret of polymorphic incompatibility has remained an orphan topic in speciation research.

There is now explicit documentation for mammals, fish, arthropods, nematodes and plants that genetic variability within a species commonly contributes to variation in interspecific hybrid incompatibility (Table 1) [8,9]. In *Mimulus*, polymorphic loci contributing to reproductive isolation have been mapped and identified [10–12].

Glossary

Admixture mapping: population-based genetic mapping approach in which individuals have genetic backgrounds that are naturally heterogeneous owing to genetic mixing of different ancestral populations or incipient species.
Balancing selection: natural selection that acts to maintain polymorphism within a population, such as by rare-allele advantage (frequency-dependent selection) or heterozygote advantage (overdominance).
Bateson–Dobzhansky–Muller (BDM) model: dominant model for the evolution of intrinsic reproductive isolation, whereby divergent alleles at two or more loci interact negatively in hybrids to reduce fitness (Box 1).
BDM incompatibility (BDMI): combination of alleles at two or more loci that interact epistatically to detrimental effect in hybrid individuals (Box 1).
Epistatic interactions: contribution to a phenotypic effect that depends on the combined allelic state at two or more loci within an individual.
Fitness valley: in Sewall Wright's fitness landscape analogy for the distribution of fitness values as a function of genotype, as applied to the BDM model of speciation, distinct populations or species occupy peaks of high fitness and hybrids or genetic intermediates occur in lower-fitness valleys between fitness peaks (Box 1).
Genetic drift: selectively neutral changes in allele frequency within a population owing to random sampling of gametes in the formation of a finite number of zygotes.
Hybrid male sterility: infertility of males that have hybrid genetic backgrounds, a commonly observed aspect of incipient speciation and Haldane's rule.
Incipient species: populations sharing a recent common ancestor that are partially reproductively isolated; the species are capable of forming hybrid progeny, but hybrids suffer detrimental effects.
Inverse cline: ordinarily, genetic markers or phenotypes in a cline or hybrid zone increase monotonically from one end of the cline to the other. In the face of reinforcement, however, differences in allele frequency or phenotype might be greatest at the points closest to the contact zone, with intermediate values at points further from the cline centre, where individuals effectively experience allopatry, resulting in an inverse cline.
Neutral polymorphism: genetic variants at a locus that have no fitness differences relative to each other, often quantified at loci such as synonymous sites that are presumed to have negligible functional effects when altered.
Polygenic selection: selection on a phenotype whose trait value is influenced by variation at many genes.
Polyploidization: genome-wide chromosome duplication; common in plants, it can contribute to reproductive isolation, for example if triploid ($3n$) hybrids suffer reduced fitness relative to diploid ($2n$) and tetraploid ($4n$) parents.
Q_{st} and F_{st}: estimates of the fraction of genetic variation that is due to differences between populations, as inferred from phenotypic (Q_{st}) or genotypic (F_{st}) measurements (Box 2).
Reinforcement: process involving an increase in prezygotic reproductive isolation via natural selection when closely related species live in sympatry.
Reproductive isolation: collection of intrinsic (behavioral, physiological, developmental) and extrinsic (ecological) factors that prevent populations from interbreeding successfully.
Selective sweep: rapid increase in and fixation of a new beneficial allele, which eliminates variation at linked loci through genetic hitchhiking.
Snowball effect: theoretically predicted geometric increase over time between species in the number of BDMIs, which might or might not translate into a similar rate of change in overall reproductive isolation (Box 4).
Speciation gene: genetic locus that contributes to reproductive isolation.
Sympatric and allopatric populations: populations of different species that co-occur in the same place are said to be sympatric, whereas populations separated in space are allopatric.
Variable reproductive isolation (VRI): presence of heritable differences among individuals in the degree to which they are incompatible with a related species.

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Box 1. What is Bateson–Dobzhansky–Muller (BDM) incompatibility?

The standard description of the BDM model is as follows: mutations arise and fix independently at two (or more) loci in each of two lineages that derive from a recent common ancestor, giving, for example, the evolution of descendant genotypes *AAbb* and *aaBB* from ancestral genotype *aabb* (Figure Ia). The *Ab* and *aB* allele combinations might be beneficial or have no fitness difference relative to the ancestral *ab* combination. However, because the *A* and *B* alleles evolved independently of one another, they are said to have not been 'tested' in each others' company by natural selection. Subsequent hybridization between the daughter lineages will yield *AaBb* genotypes that might suffer diminished fertility or viability owing to negative epistatic interactions between the *A* and *B* alleles (Figure Ib). Such negative epistasis is the essence of BDM incompatibility (BDMI); I use the BDMI acronym to refer specifically to fixed incompatibilities, consistent with previous literature [26].

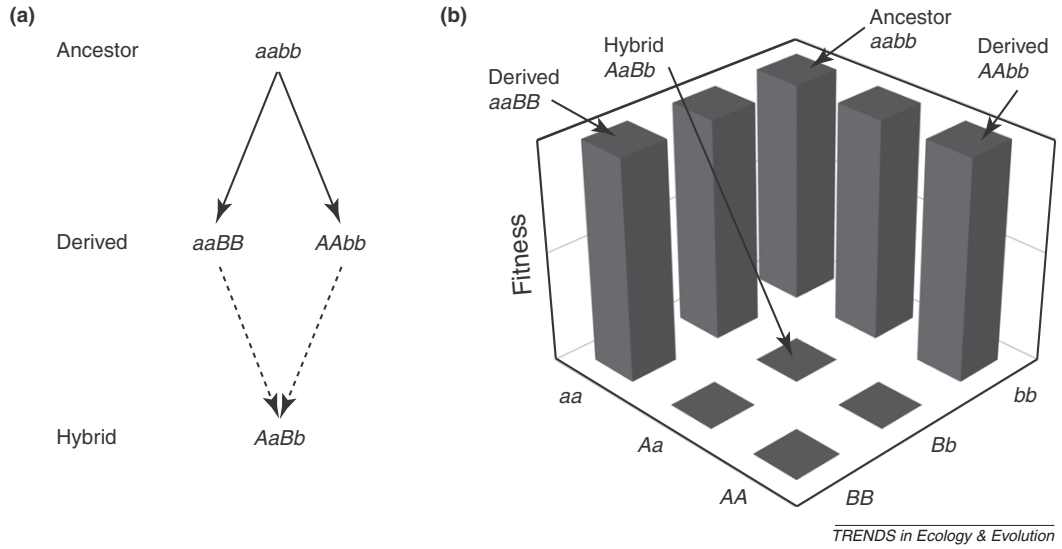

Figure I. Two graphical depictions of the BDM model. (**a**) Temporal evolution from an ancestral population fixed for alleles *a* and *b*, with two descendant populations that have fixed allele *A* in one and *B* in the other. Subsequent hybridization of the two descendant populations will yield hybrids that may experience a BDMI due to negative epistatic interactions between the *A* and *B* alleles. (**b**) Fitness landscape version of BDMI in which high fitness is experienced for the ancestral genotype (*aabb*), descendant genotypes (*aaBB, AAbb*), and intermediate genotypes in the single-locus polymorphic state (*aaBb, Aabb*). Hybrid genotypes (*AaBb*), however, would experience reduced fitness if *A* and *B* interact to create a BDMI.

Numerous other more anecdotal examples date back nearly a century [13–16] (see also references in [9,17,18]), and many well-known 'speciation genes' are, in fact, not fixed between species (e.g. *Hybrid male rescue* (*Hmr*), *Lethal hybrid rescue* (*Lhr*), *Odysseus* (*OdsH*), *Overdrive* (*Ovd*) and *Zygotic hybrid rescue* (*Zhr*) in *Drosophila* [19–23] and Meisetz (*Prdm9*) in *Mus* [24]). Furthermore, there is the potential for variation even in the degree to which polyploidization results in reproductive isolation between crosses of differing ploidy [25]. Despite the focus of theory

Table 1. Summary of study systems that document VRI

Organism		Type of VRI	Refs
Plants	*Arabidopsis* (thale cress)	Synthetic inviability or immune dysfunction[a]	[82,83]
		Male sterility	[84]
	Draba (Whitlow grass)	Synthetic sterility[a]	[85]
	Helianthus (sunflower)	Pollen and seed inviability	[86]
	Mimulus (monkey flower)	Male sterility and inviability	[10,11,45]
		Synthetic inviability[a]	[87,88]
	Oryza (rice)	F2 or later generation hybrid breakdown[a]	[89,90]
Nematodes	*Caenorhabditis* (roundworm)	Inviability	[91]
Arthropods	*Chorthippus* (grasshopper)	Male sterility	[27]
	Drosophila (fruit fly)	Male sterility	[15,18,28,54]
		Synthetic sterility and inviability[a]	[92]
	Nasonia (jewel wasp)	Mating frequency	[17]
	Tigriopus (copepod)	F2 or later-generation hybrid breakdown[a]	[93,94]
	Tribolium (flour beetle)	Inviability, developmental arrest and Haldane's rule	[60,95–97]
		Synthetic inviability, deformities and Haldane's rule[a]	[98,99]
Fishes	*Lepomis* (sunfish)	Inviability	[30]
Mammals	*Mus* (house mouse)	Male sterility	[14,100]

[a]Synthetic VRI phenotypes indicate that crosses were only performed intraspecifically to document negatively epistatic effects [47], with results discussed explicitly in a speciation context.

on a notion of instantaneous fixation of incompatibility alleles, often for mathematical convenience, polymorphism is inevitable during the interval between allele origin and fixation at incompatibility loci. The relevance of such polymorphism is particularly acute during the earliest stages of speciation [14,15], which is the timeframe of greatest biological significance and also of most interest to students of speciation.

And so, in this Opinion, I aim to bring renewed attention to variable reproductive isolation (VRI), the phenomenon of heritable variation in hybrid incompatibility, which I consider a fundamental, and yet neglected, aspect of speciation. First, I illustrate how VRI could work at the genetic level in the context of the BDM model (Box 1) and describe the range of potential evolutionary processes that could account for its presence. Next, I propose that explicit study of VRI will benefit our understanding of speciation, in part, by broadening the range of questions and approaches in the genetics of reproductive isolation (Table 2). I demonstrate how both standard and novel empirical approaches can be exploited in the context of VRI to provide powerful insights into the speciation process.

Derived and ancestral allele contributions to VRI

For biologists, the BDM model (Box 1) elegantly solves the dilemma of how each of two lineages from an ancestral population, once split from one another, could cross a fitness valley to generate reproductive isolation between them [26]. A major simplification, however, is that this model only considers interactions between alleles that have been fixed within each population of the incipient species. We know that alleles must have been polymorphic before they became fixed within their respective lineages. However, until now, many biologists have presumed that these polymorphisms are sufficiently transient that they may be neglected [4]. Here, I argue that this assumption should be revisited.

Let us consider a simple extension to the standard BDM explanation for the role of hybrid incompatibility in the process of speciation. In a population, extant variation for hybrid incompatibility at a given incompatibility locus could reflect either new derived mutations or ancestral polymorphisms. In either case, different alleles at the locus might differ in their degree of incompatibility as they interact with new derived mutations at another locus in the other lineage. Similar to the standard BDM model, we can presume that detrimental interactions between ancestral–ancestral pairings of alleles at different loci (so-called synthetically deleterious loci) will be negligible, because they would have been purged from the single ancestral population by selection. For example, let us imagine an ancestral population that is polymorphic at the a locus and monomorphic at the b and c loci, with alleles $\{a'/a'', b, c\}$ (Figure 1). This scenario allows us to simultaneously consider the nature of traditional (fixed) and polymorphic incompatibilities under two situations: when the incompatibilities involve ancestral and derived allele combinations. If new alleles arise in the separating lineages in order A–B–C and ultimately fix as in Figure 1, incipient species 1 will have alleles $\{A, b, C\}$ and incipient species 2 will have alleles $\{a', B, c\}$ or, potentially, $\{a'', B, c\}$. Now consider an intermediate stage in this process when

Table 2. Research questions to explore with VRI

Fundamental questions	Example approaches for study
How prevalent is VRI?	• Compare marker introgression among multiple transects for hybrid zones • Quantitative genetic analysis of hybrid crosses • Population genetic analysis of allele frequencies for genetically defined loci
What is the genetic architecture of VRI (few vs many loci, large vs small effects, dominance)?	• Genetic mapping • Quantitative genetic analysis of hybrid crosses
What portion of reproductive isolation is due to fixed and polymorphic factors?	• Theory on how VRI and fixed factors change over the course of speciation • Quantitative genetic analysis of hybrid crosses
What processes are responsible for VRI (e.g. genetic drift, local adaptation, balancing selection, mutation–selection balance)?	• Theory on neutral evolution within species for equilibrium variation for hybrid incompatibility • Clinal analysis of hybrid zones • Quantitative genetic analysis of hybrid crosses for families from same and different demes of each species • Molecular population genetic analysis of molecularly identified loci • Q_{st}–F_{st} analysis
Do evolutionary forces differ for polymorphic and fixed incompatibility loci?	• Meta-analysis of molecularly characterized loci
What is the relative importance of genetic drift and selection?	• Theory on neutral expectations for genetic variation for hybrid incompatibilities • Clinal analysis of hybrid zones for incompatibility vs neutral traits • Molecular evolution and population genetic analysis of molecularly identified loci • Q_{st}–F_{st} analysis
'Rules' of speciation questions	
Haldane's rule: do hybrid inviability and sterility differ in the incidence of VRI?	
Darwin's corollary: does VRI manifest more from one of the parents in hybrid crosses?	
Does VRI exhibit a large-X effect?	
Does VRI contribute to the 'missing snowball'?	
Reinforcement: do male and female traits differ in the incidence of VRI?	

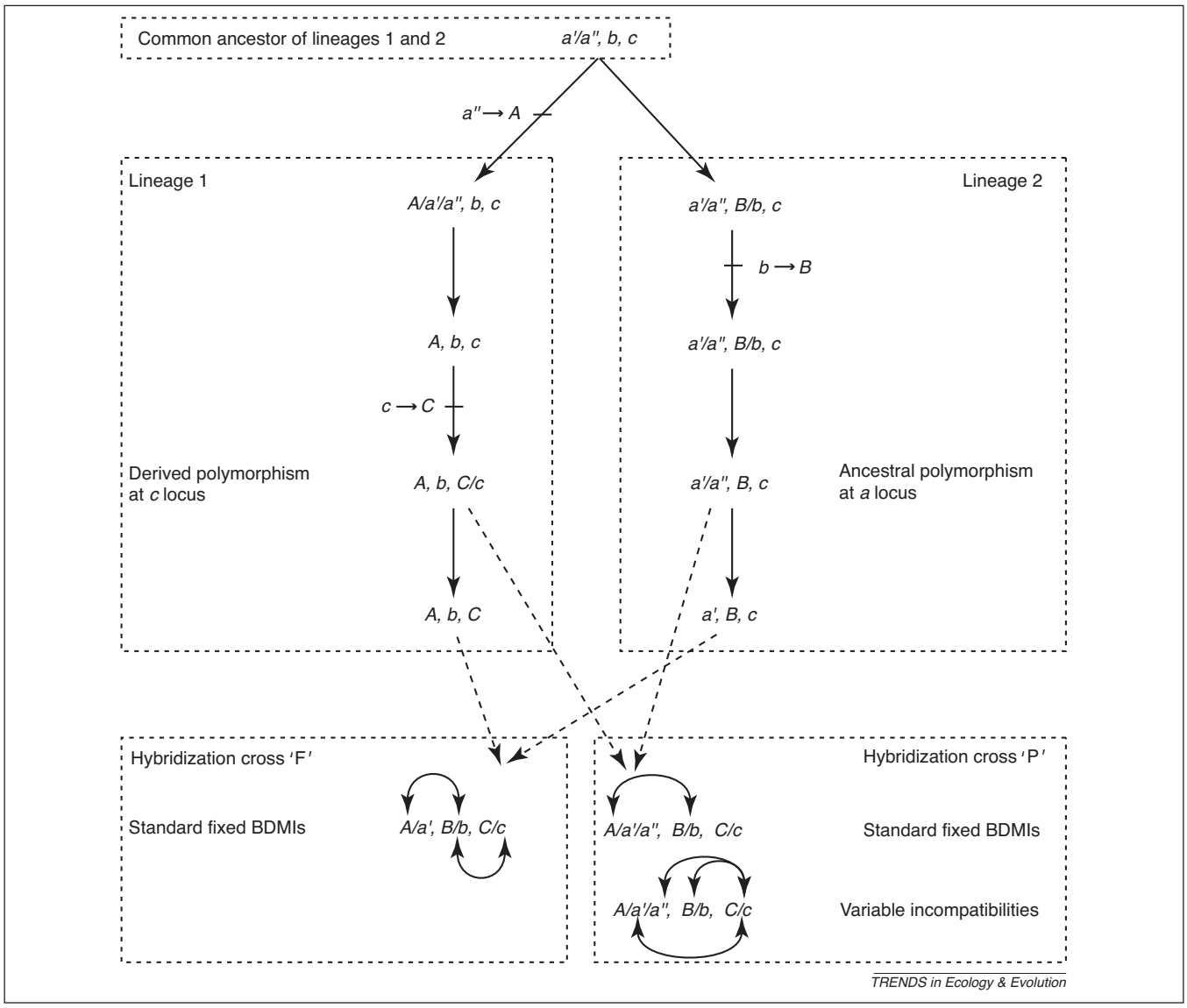

Figure 1. Depiction of the Bateson–Dobzhansky–Muller (BDM) model of reproductive incompatibility, explicitly documenting polymorphism within each lineage before fixation of potential incompatibility alleles. Broken lines represent hybridization between the separated lineages, either when both are fixed at all three loci (cross 'F' at bottom left, the standard depiction of the BDM model) or when some variation is still present (cross 'P' at bottom right, potentially causing VRI). When lineage 1 is crossed with lineage 2 before fixation of alleles *C* and *a*′, respectively, hybrids might experience incompatibilities that depend on the particular genetic backgrounds used in the cross. Such variable reproductive isolation might occur in combination with fixed incompatibilities that represent standard BDM incompatibilities (BDMIs). We can envision more elaborate combinations of alleles to create hybrid incompatibility, for example, if yet another allele arose at the *a* locus in lineage 2, if lineage 2 were polymorphic for alleles *b* and *B*, or if a new allele *A*′ arose in lineage 1.

incipient species 1 still segregates alleles *C* and *c*, and incipient species 2 harbors alleles *a*′ and *a*″ (Figure 1). During this time, crosses between these incipient species might form derived–ancestral incompatibilities between *B* and either *a*′ or *a*″, and yet these two interactions could yield different magnitudes of effect. Thus, the *a* locus can contribute to polymorphism within incipient species 2 for hybrid incompatibility. Similarly, *C* might form a derived–derived incompatibility with *B*, but *c* would not negatively interact with *B*; hence, the *c* locus can contribute to genetic variation within incipient species 1 for incompatibility. This scenario could also yield a standard BDMI: a fixed derived–derived incompatibility between *B* and *A* (Figure 1, Box 1). This slight modification of the standard depiction of the BDM model (Box 1), which simply permits allelic variation, provides a straightforward way of accommodating the empirical fact of VRI.

The BDM model predicts that derived BDMI alleles will contribute disproportionately to reproductive isolation [4]. Does VRI affect this prediction? During the early stages of speciation, VRI is probably the result of both ancestral–derived and derived–derived associations. However, ancestral–derived VRI will become relatively less common over time owing to the combined effects (i) of mutation constantly introducing new derived alleles and (ii) of ancestral polymorphisms becoming rarer over time from the inevitable fixation of alleles by genetic drift or selection. A larger current population size relative to the ancestral population would provide a larger mutational target for new incompatibilities, although any given derived allele would

consequently occur at a lower population frequency. Thus, in accord with standard BDM theory, VRI similarly predicts that derived alleles will be increasingly more important contributors to hybrid incompatibilities over the course of speciation.

The causes of VRI

Local adaptation, balancing selection and genetic drift of neutral polymorphisms all generate heritable intraspecific variation, and therefore could contribute to VRI. Additional potential factors causing VRI include the introgression of previously fixed BDMI alleles between incipient species [14,27], alleles that are detrimental within a lineage but are present owing to mutation–selection balance, or genomic conflicts such as those resulting from host–endosymbiont interactions [28,29]. Lopez-Fernandez and Bolnick further note that incomplete penetrance could cause non-heritable variability in reproductive isolation [30]. Here I consider how these population processes can cause VRI.

Local selection

Geographically restricted positive selection can fix different alleles in different subpopulations, leading to the maintenance of species-wide genetic variation. When individuals from such differentiated populations are crossed separately to another species, their different alleles might each have distinct epistatic effects on hybrid fitness, providing a source of VRI. Such selection need not be adaptive, but could reflect unique evolutionary responses by populations to endosymbionts or selfish genetic elements, especially for arms-race evolution [28,29]. Such genomic conflict is now thought to be of greater importance in speciation than previously recognized [31]. When this is considered together with the prevalence of local adaptation in nature, we should anticipate that VRI commonly results from population-specific selection.

By contrast, alleles driven by species-wide positive selection will not remain polymorphic for long, spreading quickly even for subdivided populations [32]. Consequently, such alleles will contribute primarily to fixed isolating factors rather than VRI. Indeed, it is often argued that selection, whether adaptive or not, has driven the fixation of most hybrid incompatibility loci [31,33]. A seeming paradox, then, is that VRI seems common, and yet molecularly characterized 'speciation genes' and traits often leave a signature of positive selection [31,34]. In addition to the possibilities of incomplete selective sweeps or special ancestral–derived allele relationships [33], three potential resolutions to this issue involve deme-specific positive selection (discussed above), polygenic selection and genetic conflicts. If adaptation commonly involves selection on many loci, then it can simply shift allele frequencies at trait loci, rather than fixing alleles via selective sweeps [35]. Consequently, polygenic species-wide positive selection could accentuate VRI by increasing the alleles involved in polymorphic incompatibilities to non-negligible frequencies, rather than fixing them outright. However, polygenic selection is unlikely to yield a strong molecular signature of positive selection at any given locus [35]. Alternatively, genetic conflicts could provide an explanation for the evidence of both polymorphism and positive selection on 'speciation genes' [31]. For example, the balance of selection on selfish elements between intrinsic advantageous and detrimental host effects, or arms-race evolution with suppressor alleles, might fail to yield allelic fixation species-wide, and yet could still leave the molecular imprint of positive selection.

Balancing selection

Balancing selection can maintain genetic variation within a single population. However, for balancing selection to provide a source of VRI, the timeframe of selection must be sufficiently recent that it is unique to the lineage of one species. Otherwise, the alleles would have been present in their common ancestor, precluding the potential for the alleles to have been epistatically 'untested' in the genetic background of the other species (Box 1). It remains an open question whether balancing selection might be common and recent, as could happen if frequency-dependent selection were to accelerate changes in the relative abundance of new alleles arising by mutation. Genes involved in disease susceptibility and self-incompatibility in plants might commonly fall into this category of polymorphic isolating factors [9]. Importantly, different crossing designs are needed to distinguish VRI caused by balancing selection or local adaptation, such as the use of replicate families derived from a single population or from several populations.

Neutral polymorphism

Variation that is selectively neutral within a species can also generate hybrid incompatibilities, although they will accumulate as fixed differences more slowly over time than when positive selection is involved because their evolution will be controlled by genetic drift [36]. Drift should therefore contribute fewer fixed hybrid-incompatibility factors than selection [36]. However, neutral evolution by drift might be responsible for VRI [8,27,37]. Such neutral evolution within species has a key practical implication: it enables researchers to build coherent null models of the levels of genetic variation and rates of accumulation of hybrid incompatibilities against which to test for an effect of selection. Although molecular population genetic approaches can help in determining whether the evolution at particular incompatibility loci was driven by positive selection [31], a restriction is that the loci must first be identified in exceptional detail, which limits the number and generality of such findings. Consequently, it would be valuable and complementary to build quantitative genetic null model frameworks that are amenable to comparisons of coarser-scale information about incompatibility regions and dysfunctional traits, as has been done for hybrid zone analysis [27,38].

Insights into speciation from VRI

Definition of the relative importance of genetic drift and selection presents a major challenge for ongoing study of speciation. VRI measurement should facilitate the estimation of how much reproductive isolation has accumulated owing to drift relative to positive selection within a lineage, by way of contrast between observations and neutral

patterns of genetic variance expected from drift alone. In addition, recognition of the role of VRI in the formation of new species lays bare the currently unexplored, but fundamental, questions of what proportion of hybrid incompatibility is caused by fixed versus polymorphic factors and how this proportion changes over the course of speciation. New theory is needed to help pin down the nature and number of polymorphic incompatibilities that are caused by neutral evolution within a lineage. Below I illustrate four examples of how empirical approaches can be used to address these issues.

Analysis of hybrid zone clines

Many incipient species form hybrid zones in which their natural ranges overlap. Analysis of genetic clines across such hybrid zones has provided an important source of insights into the speciation process [39], enlightened by theoretical work that predicts the shape of a cline for loci involved in BDMIs that are otherwise fixed on each side of the hybrid zone, and for which fixation is caused by either selection or drift [38,39]. Neutral loci that are unlinked to BDMI loci should have smooth and symmetric clines in contrast to the disjointed and asymmetric clines expected for genetic markers that are linked to loci involved in BDMIs [38,39]. Such clinal analysis also can detect VRI on each side of the hybrid zone and determine whether it results from selection or drift. For example, Shuker and colleagues exploited a natural *Chorthippus* grasshopper hybrid zone to test for selection and documented genetic variation for hybrid male sterility in locations far outside the hybrid zone [27]. By comparing the cline shape for male sterility to a putatively neutral trait, they concluded that within-lineage directional selection was not responsible.

Another promising avenue for clinal analysis is to look across multiple hybrid zone transects to reveal variability in the genetic basis of reproductive isolation [40]. Although comparison of introgression patterns of molecular markers for different transects can detect VRI [40,41], it is subject to a variety of complications [42,43]. Admixture mapping provides a complement to the analysis of clines in hybrid zones by using natural hybrids to identify incompatible genomic regions from measures of reproductive isolation [44]. VRI in the mapped regions could then be assessed by testing for discordant effects in different contact zones. In general, because clear null expectations have been established, the analysis of hybrid zones holds great potential for documenting VRI and for testing the relative roles of selection and drift in generating hybrid incompatibility.

Structured populations

Not all incipient species pairs form hybrid zones, but many do have ranges subdivided into local populations. Such population structure might facilitate VRI that manifests from interspecific crosses, as observed in the *Mimulus* species complex [10–12,45] and in cactophilic *Drosophila* [18]. If the genetic basis for such VRI can be determined, then standard methods of molecular population genetics can be applied to test for local adaptation [31]. Structured populations also facilitate the unique application of trait differentiation to test for selection on incompatibilities based on Q_{st} and F_{st} (Box 2) [46], although it would be valuable to develop more statistically powerful approaches. Given that population structure and local adaptation are common features of many species, this might be a frequent source of VRI in nature.

Differentiation among populations has been used to explore how negative genetic interactions, often termed synthetic lethality or negative epistasis, are somewhat comparable to interspecific hybrid incompatibilities. Several studies have explicitly considered their intraspecific findings in terms of the BDM model (Table 1). This approach, however, has been severely criticized [16] because it is not entirely clear how directly such intraspecific effects relate to interspecific hybrid incompatibility. In particular, synthetic lethal effects are only expected to manifest in F2 and backcross generations, not in F1 hybrids [47].

Standard laboratory crosses

In the laboratory, a quantitative genetic approach can be used to analyze hybrid crosses of multiple genetic backgrounds from each of two species. Analysis of variance, or more sophisticated statistical approaches [48], can be used to partition variation in hybrid incompatibility into between-species and within-species components. In other words, reciprocal crosses among distinct genetic backgrounds in a quantitative genetic framework can be used to answer the question: what fraction of overall hybrid incompatibility is explained by extant genetic variation and what fraction is due to fixed genetic factors? It is then possible to estimate the relative roles of drift and selection in creating fixed and polymorphic incompatibilities by comparison with the pattern of genetic variation that

Box 2. A Q_{st} test for selection on VRI

To quantify how VRI in a species is partitioned within and among populations, consider the phenotypic analog of F_{st}:Q_{st}, which describes heritable phenotypic differentiation [46]. Comparisons of Q_{st} and F_{st} within species generally reveal that $Q_{st}>F_{st}$, which implies that local adaptation for traits often overpowers the influences of migration and drift (but the QTL underlying the traits often do not reveal signatures of selection) [64–66]. What is expected when the trait is hybrid incompatibility and, instead of comparing subpopulations within a species, we compare different species with partial or nearly-complete reproductive isolation? This type of trait manifests only in hybrids and comprises inherently epistatic effects, and yet the phenotypic variation is undetectable and undefined in a given species by itself. If one of the species being tested is composed of structured populations, then we can compute Q_{st} for hybrid incompatibility and compare it to the distribution of F_{st} among the populations. For hybrid incompatibilities accumulating by drift, we should observe $Q_{st}=F_{st}$; for VRI resulting from local adaptation, we should observe $Q_{st}>F_{st}$; finally, $Q_{st}<F_{st}$ might result from VRI maintained at deleterious mutation–selection balance.

Some serious caveats to the Q_{st} approach are that estimates typically have very high variance, low precision with few populations, and sensitivity to underlying mutation rates; furthermore, the inherently epistatic nature of hybrid incompatibility as a trait may influence the form of the null relationship expected between Q_{st} and F_{st} [46,67–69]. Nevertheless, this example approach illustrates that it should be feasible to assess the importance of local adaptation and population structure generally as causes of VRI relative to VRI due to polymorphisms that are neutral within populations or at deleterious mutation–selection balance. I anticipate that more powerful methods can be developed.

might be expected from the evolution of BDMIs by drift alone. However, this requires appropriate definition of a neutral model of incompatibility evolution. In general, VRI provides an opportunity to further link population genetic and quantitative genetic models of incompatibility evolution in a modified BDM framework [4,38,48–52].

Given that incompatibility alleles can arise in either or both incipient species [4], what factors might cause them to differ in the relative incidence of VRI? An accelerated mutation rate in one lineage will cause a disproportionate occurrence of incompatibility loci in that lineage. Similarly, we should expect more variation for hybrid incompatibility within the species that has a higher mutation rate, presuming selective neutrality of incompatibility alleles within a species (additive genetic variance $V_g \approx 2NV_m$, where N is the effective population size and V_m is the mutational variance [49]). Furthermore, if the accelerated mutation rate is coupled to reduced effective population size, then drift will more quickly fix BDMI loci in one lineage. This could contribute to asymmetries in hybrid incompatibility related to Darwin's corollary to Haldane's rule [50]. Appropriately designed reciprocal crosses should help to reveal each of these features. Importantly, the cross designs needed to detect VRI are more complex than those typically used to quantify reproductive isolation, and will necessarily require more experimental effort to demonstrate family effects.

Genetic mapping

Determination of the molecular genetic basis of VRI is essential for dissecting in detail the evolutionary forces, trajectories, genetic interactions and molecular functions of loci involved in the origin and accumulation of reproductive isolation. Contrasting of phenotypic incompatibility effects in hybrids with genetic variation within species is essential to provide a baseline reference for evaluating the contribution of a locus to the difference between species [53]. Genes involved in hybrid male sterility in the *Mimulus* species complex have been identified by mapping and exhibit population specificity [10–12]. Explicit genetic mapping of VRI has been attempted in just one other system, cactophilic *Drosophila*, for which several quantitative trait loci (QTL) for hybrid male sterility were implicated [54]. Unfortunately, the chromosomal resolution was low. Future studies that exploit existing dense mapping resources or that combine genome-wide admixture mapping with next-generation sequencing and genotyping techniques [44] might prove valuable in determining the causal loci involved in VRI. In contrast to transient mapping populations, recombinant inbred lines (RILs; or near-isogenic introgression lines, NILs) based on interspecific crosses provide a long-term resource for investigating the genetic basis of reproductive isolation [55]. Construction of interspecific RIL libraries in a manner inspired by collaborative crosses and nested association mapping [56,57] that involve multiple genetic backgrounds would be a novel approach to identifying fixed and variable incompatibility loci simultaneously. Similarly, screens of deletion libraries against multiple heterospecific genetic backgrounds would be a powerful way of localizing VRI loci [58]. Such screens have been instrumental in testing the large-X effect in *Drosophila* [59] and could similarly be applied to determine whether sex chromosomes manifest a disproportional influence on reproductive isolation in terms of both VRI and fixed incompatibility loci (Table 2). Different forms of isolation might also vary in their propensity to reveal VRI. Specifically, traits more readily subject to positive selection might show VRI less often if the selected alleles are quickly fixed, as might occur for sexual selection on reproductive traits, although reports to date show no obvious bias against VRI for male sterility compared with inviability (Table 1). In the most amenable systems, mapping of genes involved in VRI and studies of their molecular evolution could be a faster and easier way to understand the relevant evolutionary forces compared to other, more laborious means, such as Q_{st}–F_{st} contrasts (Box 2).

Furthermore, trait mapping need not be limited to post-zygotic hybrid inviability and sterility, because it can also be applied to map intraspecific variation for interspecific pre-zygotic incompatibility [17] and Haldane's rule [60], including the genetic basis for extrinsic isolating barriers. In fact, the notion of VRI is implicit in many tests of reinforcement, such as inverse clines [61,62] and stronger sexual isolation in sympatric than in allopatric populations of closely related species [26]. Moreover, Coyne and Orr emphasized the potential of assessing sex-biased effects as a means of inferring the action of reinforcement [26]. In the context of VRI, the implication is that we might expect higher VRI in male-related traits than in female-related traits for species subject to reinforcement.

Uncovering the genetic architecture of VRI is another exciting avenue for research. Are there few polymorphic incompatibility loci that segregate alleles at intermediate frequency or partitioned among subpopulations, or do

Box 3. Partial reproductive isolation without fixed incompatibilities

Consider a concrete, simple example based on cross 'P' in Figure 1 (main text). Allele C occurs at frequency f_C in lineage 1 and forms incompatibilities with alleles B and a' in hybrids, but allele A does not form an incompatibility with B. Allele a' occurs at frequency $f_{a'}$ in lineage 2. The relative fitness is 1 for genotypes within either lineage 1 or 2. The mean relative fitness of hybrids, however, is:

$$\overline{w_{hyb}} = (1 - f_C) \cdot 1 + (f_C) \cdot [f_{a'} \cdot w_{CBa'} + (1 + f_{a'}) \cdot w_{CBa''}] \quad [I]$$

where $w_{CBa'}$ and $w_{CBa''}$ are the relative fitness of hybrids containing the subscript alleles. This example illustrates the simple fact that partial reproductive isolation $(\overline{w_{hyb}} < 1)$ can occur between the lineages despite the absence of any fixed BDMIs. Clearly, higher frequencies of f_C and $f_{a'}$ will induce lower mean hybrid fitness, as will stronger incompatibility effects (lower $w_{CBa'}$ and $w_{CBa''}$). Both local adaptation and balancing selection would contribute to high species-wide allele frequencies. However, the formation of reproductive incompatibilities depends on the product of the frequencies of incompatible alleles, which will be small if the alleles are rare. By contrast, should lineages have many such loci, their cumulative effects could still be substantial (akin to the snowball effect for fixed incompatibilities; Box 4). Theory for VRI should explore this quantitatively to consider expected neutral within-lineage allele frequency distributions, alternative fitness effect distributions, dominance in hybrids and the effects of the number of polymorphic loci involved (and the related issues of the mutation rate and the probability that a mutation will generate an incompatibility).

many loci have incompatibility alleles at low population frequencies? Does dominance in hybrids differ for polymorphic versus fixed incompatibility alleles? How prevalent is the divergent resolution of translocations [63]? Answers to these questions will unravel the roles of balancing selection, local adaptation, drift, and mutation–selection balance as contributors to VRI.

Does VRI contribute directly to speciation?

Despite emerging evidence that VRI is common, a gnawing question remains: how relevant are polymorphic incompatibilities to speciation *per se* if they might never become fixed and form BDMIs? One answer is that polymorphic incompatibilities are inherently relevant simply because polymorphism is an inevitable state between the emergence of a mutation and its fixation. A more productive view holds that polymorphic incompatibilities themselves can contribute substantively to overall reproductive isolation (Box 3), as well as to predicted patterns of isolation, such as the snowball effect (Box 4). The degree to which this is true depends on the genetic architecture of reproductive isolation. To understand the full extent of VRI in the speciation process, there is a need to better document its presence and genetic basis in nature, and to develop appropriate formal theory.

Concluding remarks

It is now clear that heritable intraspecific variation for interspecific hybrid incompatibility, VRI, occurs in a broad range of organisms (Table 1). This underexploited facet of speciation genetics needs rigorous theory to guide further empirical study. Even in the absence of theory, thoughtful investigation of VRI can shed light on a variety of fundamental topics in speciation (Table 2), including quantification of the relative contributions of within-lineage selection and genetic drift to reproductive isolation. To the extent that neutral evolution contributes to VRI and that VRI contributes substantially to overall reproductive isolation, genetic drift might play a more prominent role in speciation than is currently appreciated. With the recognition

Box 4. Implications of genetic variation for the snowball effect

Genetic variation is pertinent to tests of the snowball effect, the faster-than-linear accumulation of incompatibilities [4]. Recent tests for the snowball effect regressed the number of BDMIs against the time separating species pairs as measured by genetic distance [70,71]. Unfortunately, analyses to date have not incorporated a key feature of population genetic divergence. The total genetic distance between a pair of individuals from two species is:

$$K = 2\mu T + \pi \quad [I]$$

but the genetic distance relevant for BDMI accumulation and population splitting is just $2\mu T$ (where K is neutral-site divergence, μ is the neutral mutation rate, T is divergence time and π is neutral polymorphism) [72,73]. The average genetic distance between a pair of individuals at the instant of population separation is $\pi=4N\mu$ (where N is the effective population size) [74], the contribution of ancestral polymorphism. This distance is not negligible for closely related species. Consequently, the expected x-axis intercept for incompatibility–genetic distance regression is not zero, but is equal to π (Figure I). Regressions of BDMI counts on raw genetic distance forced through π rather than through the origin will effectively reduce the strength of empirical support for the snowball effect (Figure I), as demonstrated recently for data from tomatoes [75].

Importantly, a correction for ancestral polymorphism should also be applied to analyses of overall isolation as a function of genetic distance, especially for closely related species [76–81]. However, such analyses are distinct from tests of the snowball effect of incompatibilities: overall isolation is not necessarily expected to exhibit a faster-than-linear change with time [4].

The snowball effect presumes that observed incompatibilities are fixed [4], as do current empirical tests [70,71]. However, if VRI is present and unaccounted, then the fixed BDMI count might be overestimated more greatly for closely- than for distantly-related species pairs. This could mask a true geometric increase in the number of fixed BDMIs. Thus, VRI could provide an explanation for the ''missing snowball'' [76]. Consequently, it is crucial to understand how many incompatibilities involve segregating variants. Because each new derived allele can potentially interact to detrimental effect with an increasing number of loci in the other incipient species, VRI might exacerbate the standard snowball effect and contribute to strong overall reproductive isolation even at short genetic distances.

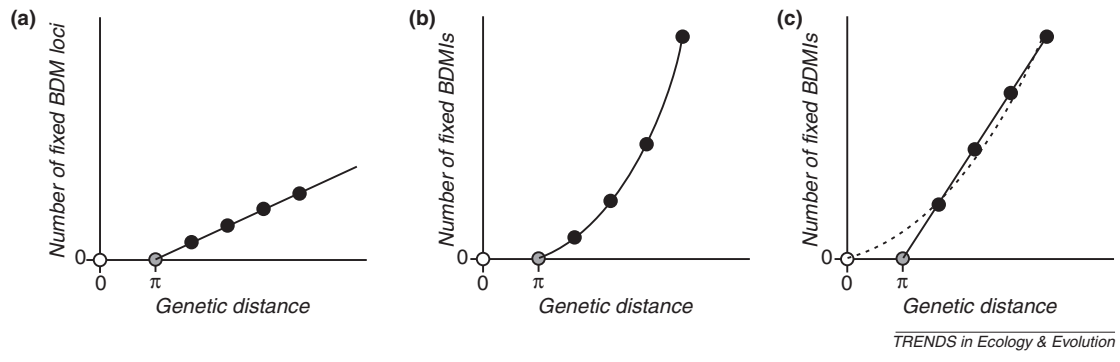

Figure I. The snowball effect represented in terms of the number of Bateson–Dobzhansky–Muller incompatibilities (BDMIs) as a function of genetic distance. **(a)** Theory predicts that the rate of fixation of alleles at BDM loci might be constant, with the number of loci accumulating linearly with a molecular clock (genetic distance between species), where fixation could result from either neutral evolution or from a constant rate of substitution of alleles by positive selection [4]. **(b)** Theory also predicts that even with such linear accumulation of fixed alleles involved in incompatibilities from the time at which separation of incipient species occurred, the number of pairwise or higher-order incompatibilities will increase geometrically [4]. Hypothetical observed numbers of fixed alleles causing BDMIs **(a)** and numbers of BDMIs **(b,c)** are indicated with black-filled circles. However, testing for a non-linear increase by fixing the curve through the origin (unfilled circle) could lead to a spurious inference if the pattern is actually linear; **(c)** shows a spurious dashed curve compared to a solid linear curve. Within a population with zero BDMIs, we expect individuals to differ genetically by distance π (gray circle) [74].

that VRI is an inherent part of how species become reproductively isolated, a new major question emerges: what fraction of the incompatibilities that cause reproductive isolation comprise fixed versus polymorphic factors over the time course of speciation? A quantitative solution to this problem will require substantial new contributions from both theoretical and empirical studies.

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