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Review

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The importance of intrinsic postzygotic barriers throughout the speciation process

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Intrinsic postzygotic barriers can play an important and multifaceted role in speciation, but their contribution is often thought to be reserved to the final stages of the speciation process. Here, we review how intrinsic postzygotic barriers can contribute to speciation, and how this role may change through time. We outline three major contributions of intrinsic postzygotic barriers to speciation. (i) *reduction of gene flow*: intrinsic postzygotic barriers can effectively reduce gene exchange between sympatric species pairs. We discuss the factors that influence how effective incompatibilities are in limiting gene flow. (ii) *early onset of species boundaries via rapid evolution*: intrinsic postzygotic barriers can evolve between recently diverged populations or incipient species, thereby influencing speciation relatively early in the process. We discuss why the early origination of incompatibilities is expected under some biological models, and detail how other (and often less obvious) incompatibilities may also serve as important barriers early on in speciation. (iii) *reinforcement*: intrinsic postzygotic barriers can promote the evolution of subsequent reproductive isolation through processes such as reinforcement, even between relatively recently diverged species pairs. We incorporate classic and recent empirical and theoretical work to explore these three facets of intrinsic postzygotic barriers, and provide our thoughts on recent challenges and areas in the field in which progress can be made.

This article is part of the theme issue 'Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers'.

1. Introduction

Reproductive barriers are the currency of speciation. These barriers can occur before or after a hybrid zygote is formed (i.e. pre- and postzygotic), and selection against hybrids may or may not be mediated by the environment (i.e. extrinsic versus intrinsic). While intrinsic postzygotic barriers were an initial focus of speciation research [1–4], recent work has highlighted the importance of ecology in reproductive isolation, namely prezygotic and extrinsic postzygotic barriers [5–8]. This wave of ecology-focused speciation work, paired with the observation that prezygotic barriers tend to reach completion before intrinsic postzygotic barriers (e.g. [9,10]), has led to the prevalent opinion that prezygotic barriers play a more important role in speciation than intrinsic postzygotic barriers, particularly early on [11–15]. Yet, the fact that intrinsic postzygotic barriers are common across all kingdoms of life, and are a hallmark of most 'good' species [16] suggests that their role in speciation may also be essential.

Here, we highlight the contribution of intrinsic postzygotic barriers to speciation and explore how this contribution may change as speciation proceeds. While speciation is a continuous process, we refer to early and late stages based on the degree of reproductive isolation between species, while acknowledging that reproductive isolation is not unidirectional and can be accumulated or lost (figure 1). While these stages roughly correspond to divergence times; wherein recently diverged populations or incipient species may represent earlier stages, and more divergent species pairs may represent later stages, it is not a perfect proxy for reproductive isolation. For example, speciation can be instantaneous (e.g. polyploid speciation [16,32] or 'single-gene speciation'

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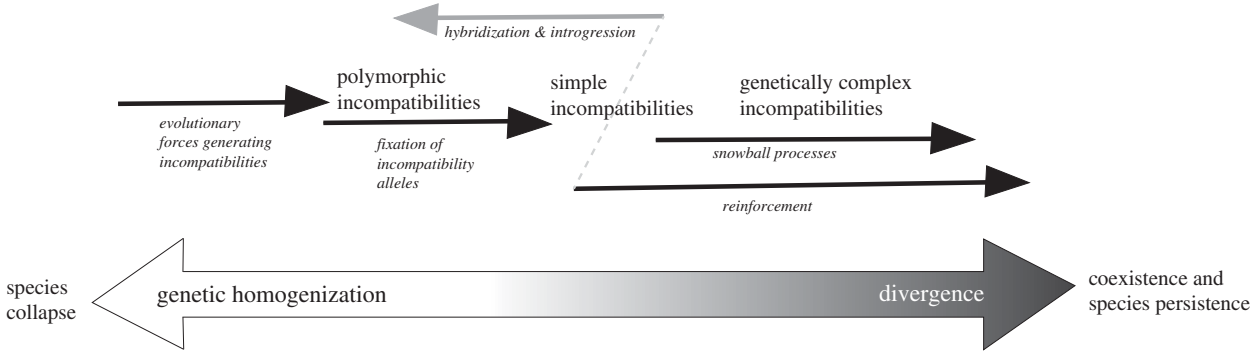

Figure 1. Intrinsic postzygotic barriers play a dynamic role throughout speciation. Incompatibilities can allow for the build up of divergence, while introgression can reverse incompatibilities by replacing incompatible allelic combinations with compatible ones (bottom arrow; lighter colour indicates increasing genetic homogenization, while darker colouring indicates increasing divergence). Three stages of incompatibilities are outlined: polymorphic (involving segregating alleles within species or between incipient species), simple (involving few interacting alleles), and genetically complex (involving many interacting incompatibility alleles with potential for genetic redundancy). The evolutionary processes that connect these stages are listed below the arrows in italics. Early in the speciation process, various evolutionary forces may generate polymorphic incompatibilities (outlined in [17–20]). These in turn may become species-wide, genetically simple incompatibilities if incompatibility alleles fix. Simple incompatibilities can become genetically complex if incompatibility alleles continue to accumulate in a snowball like fashion, in turn creating genetic redundancy [18,21–30]. Although these are drawn as discrete stages, we note that the path of speciation can be substantially more complex (for example, polymorphic incompatibility may be genetically complex). If there is sufficient selection against hybrids, and sufficient production of unfit hybrids, processes such as reinforcement can complete speciation ([31]; note the dotted line between 'hybridization & introgression' and 'reinforcement'). We highlight that divergence is reversible at almost any point along this continuum (although it becomes increasingly more difficult as incompatibilities become more complex). Also, all of these processes can occur relatively rapidly, and may not reflect divergence times between incipient species (e.g. incompatibilities may remain polymorphic for long periods of time if they are maintained by local selection, or reinforcement may happen relatively rapidly if there is strong selection against the production of unfit hybrids).

[33], but see [34–36]). Alternatively, relatively divergent species pairs may collapse owing to hybridization [37–39].

2. What do we mean by intrinsic postzygotic barriers?

Intrinsic postzygotic barriers manifest when hybrids exhibit lower fitness than either parent, regardless of the environment. Although many examples of intrinsic postzygotic barriers can easily be classified as such, this delimitation is not always clear. For example, barriers that originate prezygotically can also manifest again in hybrids (e.g. [40]). In addition, extrinsic factors can influence the severity of expression of intrinsic postzygotic barriers (e.g. [41,42]). While most research of intrinsic postzygotic reproductive barriers focuses on its most severe forms—hybrid sterility or inviability—these barriers may also manifest as reduced cognitive [43–45] or physiological ability [46,47], or subtler declines in fertility or viability (e.g. [48,49]).

3. The role of intrinsic postzygotic barriers may change throughout the speciation process

Intrinsic postzygotic barriers may be crucial for speciation, but their role is probably dynamic through time. Whether intrinsic postzygotic barriers contribute to the onset of speciation is unresolved. Incompatibilities commonly segregate within species [50–52] and between recently diverged incipient species [53–64], which may serve as an initial barrier. However, polymorphic incompatibility alleles can also be transient, in which case, their contribution to speciation is unclear. For example, incompatibility alleles may be lost by drift or selection before reaching fixation, or polymorphic incompatibility alleles may be polymorphic, because introgression has replaced incompatible allelic combinations with compatibles ones (e.g. [62,64–68]; figure 1). Determining whether polymorphic incompatibility alleles are transient is challenging (and in some cases impossible), and efforts to assess the fate of polymorphic incompatibility alleles requires knowledge of the specific alleles that contribute to reproductive isolation, large population genomic datasets to infer patterns of introgression and selection, and/or the ability to perform crosses. Still, some polymorphic incompatibilities will ultimately reach fixation (figure 1; box 1) and the observation that some barrier alleles predate speciation (e.g. [60,110]) suggests that intrinsic postzygotic barriers may be present at the onset of speciation.

Strong evidence that intrinsic postzygotic barriers play a role at low to intermediate levels of divergence stems from the commonality of these barriers between incipient and recently diverged species pairs. We amassed data from eight previously published comparative studies, comprising nine taxonomic groups, to determine whether significant intrinsic postzygotic isolation can appear early in divergence and whether this is common across taxonomic groups [9,111–117]. In each study, the degree of reproductive isolation was assessed as a function of genetic distance between species. However, these studies differ in how reproductive isolation and genetic distance were measured. Some used categorical indices of intrinsic postzygotic isolation [9,111–113], while others measured individual components of intrinsic postzygotic isolation [114–117]. Studies also varied in both the statistic calculated to infer divergence (e.g. Nei's D [9,111], phylogenetic distance [116], pairwise sequence distance [112,113,115], divergence in millions of years [117] or K_s [114]), as well as the genetic markers used (a small number of nuclear or mitochondrial genes [112–117] or allozymes [9,111]). While these methodological differences prevent us from quantitatively comparing studies, their amalgamation can qualitatively inform us of general patterns in speciation.

Box 1. The genetic basis of intrinsic postzygotic barriers.

The evolution of intrinsic postzygotic barriers baffled early biologists, as natural selection should never favour the production of unfit hybrids [69]. We now know that these barriers need not evolve in the face of selection, but often evolve as a byproducts of divergence between populations at two or more loci (i.e. DMIs; the Dobzhansky-Muller model of genetic incompatibilities [70–72]). DMIs are common across biological kingdoms [19,20,73], and can underlie both hybrid inviability and sterility [15,17,74], though there are other causes of intrinsic postzygotic barriers (i.e. changes in ploidy, structural genomic changes, differences in endosymbionts [16], meiosis defects owing to substantial sequence divergence [73], or global patterns of inappropriate gene expression as a result of gene regulatory divergence [75]). DMIs can involve interactions between nuclear, or nuclear and organellar genes (reviewed in [16]). They may involve substitutions in two diverging lineages or multiple derived substitutions in one lineage and a preserved ancestral allele in another (derived–derived versus derived–ancestral incompatibilities; [76,77]). Many studies have mapped the genetic location of incompatibility alleles, but few incompatibilities have been resolved to the level of genes [74]. Still, some general patterns have emerged from these mapping efforts while many questions remain.

First, the number of loci involved in DMIs varies greatly (from *simple incompatibilities* involving a single pair of interacting alleles [78]; to *complex incompatibilities* involving many interacting alleles [79]). The number of loci contributing to DMIs should increase through time, although the rate of increase is debated [18,21–28]. The number of loci involved in DMIs does not reflect the severity of the barrier; many severe inviability or sterility phenotypes are controlled by simple incompatibilities [29]. However, as the complexity of intrinsic postzygotic barriers increase, so does the potential for *genetic redundancy* (e.g. an increase in the number of incompatibility loci with no substantial increase in reproductive isolation). For example, hybrid inviability between *Drosophila melanogaster* and each of *Drosophila simulans* and *Drosophila santomea* is equally strong, but is controlled by roughly six times more loci in the latter cross [24].

Second, a knowledge of the genes responsible for intrinsic postzygotic barriers has provided insight into the underlying causes for their evolution. Of the incompatibility genes discovered to date, many exhibit genomic signatures of strong positive selection ([53,80–86], reviewed in [19,87,88]), although others do not [89–92]. Yet, the evolutionary drivers of most incompatibilities are unresolved. Several evolutionary explanations have been put forth as the underlying cause of incompatibilities, including local adaptation [93–96], hitchhiking [97,98], systems drift (i.e. stochastic evolution of the genetic basis of a trait without change to the phenotype [99–101]), gene duplication [102–104], intra-genomic conflicts [19,87,88], and host-pathogen conflict [20,50,105]. Although the original proposals [106,107] were met with skepticism [108,109], of the handful of incompatibilities for which the underlying genes have been identified, many seem to have evolved via conflicts. Yet, the relative importance of conflict, other types of selection and other processes, in the evolution of incompatibility alleles remains unknown.

Third, many species exhibit genetic variation for reproductive isolation. This variation can exist as segregating incompatibilities within a species [50–52], or as a genetic polymorphism for the ability of a species to cross to a close relative (e.g. [53–64] reviewed in [18]). The relative importance of polymorphic incompatibilities in speciation has been debated, and their contribution will depend on the allele frequencies of each incompatibility allele [18].

We find that substantial intrinsic reproductive isolation appears relatively early in divergence for most groups (figure 2). In fact, in eight of nine comparative studies, the first species pair to reach complete intrinsic postzygotic reproductive isolation is within the youngest half of species tested, and the distribution of genetic distances for species pairs with greater than 50% intrinsic postzygotic isolation is largely overlapping with the distribution of genetic distances for species pairs with less than 50% reproductive isolation (figure 2; we note that centrarchid fishes is the exception in both of these observations [117]). In addition, there are many examples of rapid evolution of intrinsic barriers. For example, hybrid sterility evolves rapidly in stalk-eyed flies [118], mountain pine beetles [63,119], and several plant lineages [120–122], sometimes in the absence of significant prezygotic barriers (e.g. [123]). Hybrid inviability has been shown to evolve rapidly in several plant species [60,124–127], as well as mammals [128]. In line with the rapid evolution of reproductive barriers is the observation that diverging populations tend to show a very sharp transition between freely sharing migrants to exhibiting no signs of migration [129–131] (although these patterns do not uniquely support a role of intrinsic postzygotic barriers). Understanding at what level of reproductive isolation this transition will happen is a key aim in speciation biology.

Not all species pairs evolve intrinsic postzygotic isolation rapidly. Variation exists within and between taxonomic groups for the rate of evolution of intrinsic postzygotic barriers. For example, complete intrinsic postzygotic isolation is not achieved in centrarchid fishes until approximately 28 Myr [117], while similar levels of isolation can be reached within approximately 3–5 Myr for birds, *Drosophila*, and *Streptanthus* (figure 2, [9,112,116]). Similarly, intrinsic barriers tend to evolve faster in mammals than birds or frogs [4,132,133]. Studies comparing species pairs with and without strong reproductive isolation at comparable levels of divergence can shed light on the underlying evolutionary drivers of intrinsic postzygotic barriers. For example, hybrid male sterility accumulates faster in the tropics in *Drosophila* [134]. In plants, mating system [135] and life history [115] affect the rate of evolution of hybrid inviability and sterility, respectively.

Lastly, intrinsic barriers probably play an important role late in the speciation process. Most divergent species show

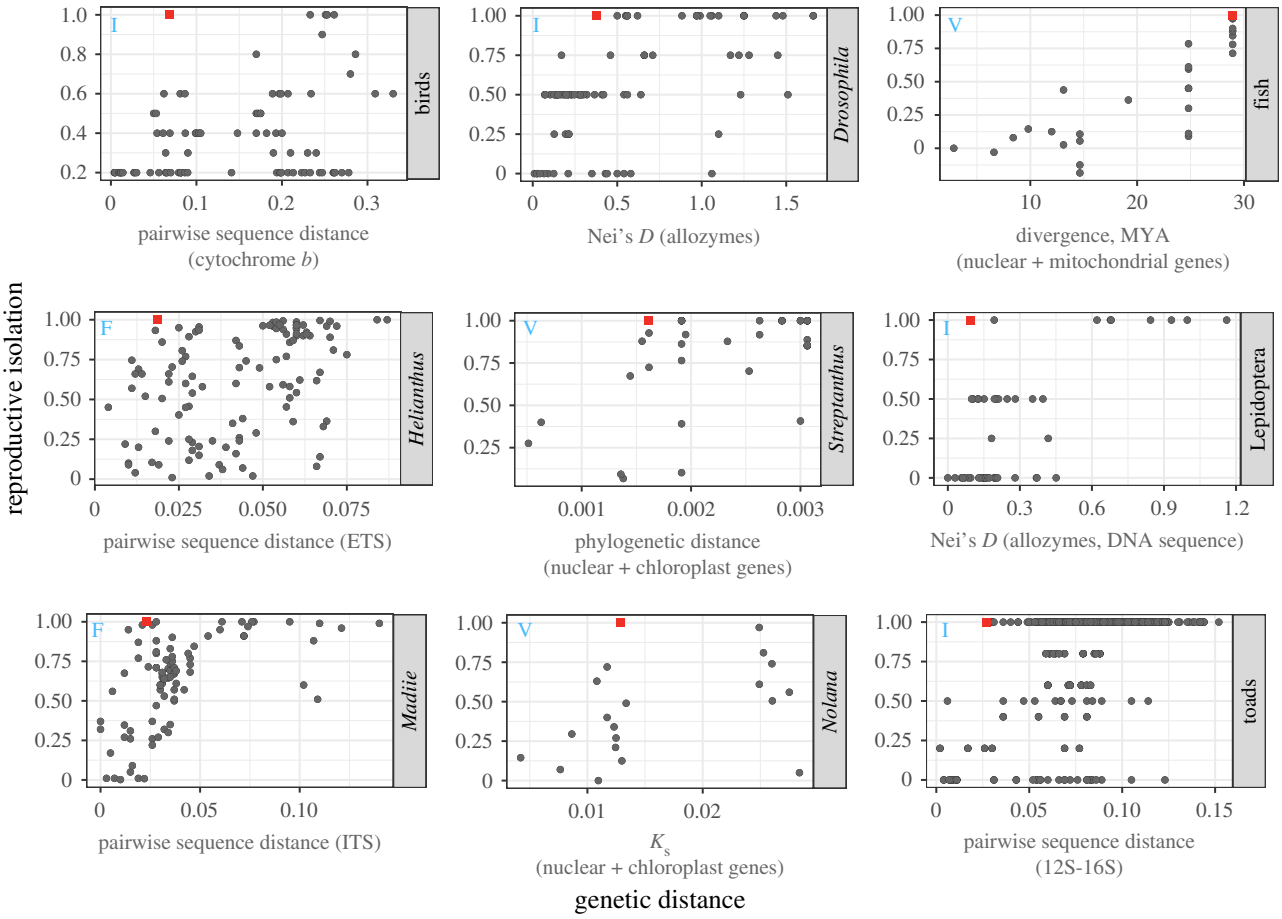

Figure 2. Intrinsic postzygotic reproductive isolation can evolve rapidly across many types of taxonomic groups. The rate of evolution of different types of intrinsic postzygotic reproductive isolation across nine different taxonomic groups. Scatterplots display the rate of accumulation of either hybrid inviability (V), sterility (F) or an index of both (I) for species pairs of differing divergence times, with the first species pair to achieve complete reproductive isolation (greater than 99.5% reproductive isolation) highlighted in red boxes. Note that the units of the *X*-axis differ among panels, as genetic distance was measured using different methods in each study (the statistic calculated as well as the markers used are listed as individual panel *X*-axis labels). References for the data are as follows: Birds [112], *Drosophila* [9], centrarchid fishes [117], *Helianthus* and *Madiie* [115], *Streptanthus* [116], Lepidoptera [111], *Nolana* [114], toads [113]. For both *Nolana* and *Streptanthus*, multiple components of intrinsic postzygotic reproductive isolation were measured. Here, we display reproductive isolation conferred by the number of viable seeds produced, as it is one of the earliest acting barriers. (Online version in colour.)

strong intrinsic reproductive isolation (figure 2). The accumulation of reproductive isolation may simply be a consequence of time (i.e. a deterministic outcome of genomic divergence). By contrast, it is also possible that the reason most 'good' species have strong intrinsic reproductive isolation is because strong intrinsic postzygotic isolation is essential to the speciation process. For example, species pairs with strong intrinsic postzygotic isolation may be less likely to collapse owing to hybridization upon secondary contact than weakly isolated species (e.g. Templeton effect [136]; reviewed in [16,37,137]). As species accumulate divergence, genetic incompatibilities are expected to increase in complexity and redundancy (box 1; [21,22]). This increased redundancy can greatly reduce the probability of introgression, and therefore the probability of species collapse (figure 1). Alternatively, intrinsic postzygotic barriers may play a generative role via reinforcement or positive feedbacks between pre- and postzygotic barriers [e.g. 138]. Determining whether the presence of strong intrinsic postzygotic barriers in divergent species is a deterministic outcome of time, or whether intrinsic postzygotic barriers play an essential role in species persistence are challenging, but essential goals in speciation biology.

We posit that intrinsic postzygotic barriers may play a dynamic role throughout the speciation process. Through time the underlying genetic architecture of intrinsic postzygotic barriers is expected to change, and this in itself can influence how these barriers contribute to speciation (figure 1; box 1). Early on, the evolutionary forces that give rise to intrinsic postzygotic barriers will generate polymorphic incompatibilities, some of which will fix between species (figure 1; box 1). Through time, these simple incompatibilities are expected to increase in genetic complexity and redundancy (figure 1; box 1). As these barriers increase in complexity, so too does the ability of intrinsic postzygotic barriers to prevent introgression at genome-wide scales. The presence of strong intrinsic barriers—whether simple or complex—can also generate reinforcing selection, which can ultimately complete speciation.

4. Intrinsic postzygotic barriers can contribute to speciation in multiple ways

The contribution of intrinsic postzygotic isolation to speciation is a contentious issue. While there is substantial theoretical work on this topic, empirical tests are challenging,

and may require a knowledge of barrier alleles, the ability to detect genome-wide rates of introgression, large comparative datasets and/or the ability to perform many controlled crosses. Here, we outline three important ways that intrinsic postzygotic barriers can contribute to speciation.

(a) Limiting introgression

The first and most obvious way in which intrinsic postzygotic barriers contribute to speciation is by dampening gene flow between diverging populations. By definition, all reproductive barriers should restrict introgression between species, but barriers may vary in the rate at which they prevent introgression and the extent to which particular barriers prevent introgression can be influenced by many factors. Secondary contact zones—whether natural, synthetic or simulated—present a unique opportunity to test how effective different types of barriers are, as well as the factors that affect the ability of particular barriers to restrict introgression [16,139]. Here, we discuss the efficacy of intrinsic postzygotic reproductive isolation as a barrier to gene flow by comparing intrinsic postzygotic barriers to other barriers, and discussing the factors that can influence the ability of intrinsic postzygotic reproductive barriers to prevent introgression.

(i) How well do intrinsic postzygotic barriers prevent introgression relative to prezygotic barriers?

Researchers have argued that prezygotic barriers are sufficient to maintain species boundaries [140], intrinsic postzygotic barriers are more effective than prezygotic barriers [40,141,142], both are equally efficient at preventing introgression [65,143], or that both are necessary to prevent species collapse [37,144]. Different conclusions mainly stem from differences in underlying assumptions (e.g. the genetic architecture of reproductive isolation) or what is considered evidence for the maintenance of species boundaries (e.g. whether hybrid zones remain 'bimodal' [140], or the diffusion of alleles relative to dispersal [40,141,145]). Empirical studies describe hybrid zones maintained predominantly by prezygotic barriers [146–151], postzygotic barriers [152–156] or a combination [157–163]. Thus, either prezygotic or postzygotic reproductive barriers may be sufficient to maintain a stable hybrid zone if sufficiently strong. Conversely, both prezygotic and postzygotic barriers are susceptible to gene flow, and either may dissolve if sufficiently weak [66,67,145,164].

One approach to estimate the efficacy of different barriers in preventing introgression is to assess how easily specific barrier alleles cross species boundaries relative to the rest of the genome. Alleles that play no role in reproductive isolation should diffuse across a secondary contact zone in a manner that is proportional to the migration rate, and as such should exhibit generally low differentiation [145,165]. By contrast, alleles that maintain species boundaries should show sharper clines [145,166], and should represent differentiated regions of the genome (although we note that this is not a valid assumption for single allele mate preference [167]). By comparing the rate of diffusion of alleles that contribute to pre- versus intrinsic postzygotic isolation, we may begin to understand how effective each of these types of barriers are. While seemingly straightforward, only a handful of studies have assessed the outcome of known barrier loci in hybrid zones (e.g. [62,64,168,169]), and studies comparing clines of known pre- versus intrinsic postzygotic barriers are lacking. Genomic scans for highly differentiated loci have been used to identify regions of the genome that appear resistant to gene flow and thus may contribute to reproductive isolation (e.g. [170–173]), but these analyses are also likely to contain regions of high differentiation that are unrelated to speciation [165,174,175]. In addition, genomic scans can only assess what alleles resist introgression. Barrier alleles that introgress between species cannot be identified using this approach. In order to holistically assess what alleles maintain species boundaries and which ones succumb to introgression, we suggest that researchers use both sympatric and allopatric populations to understand the distribution of both reproductive barriers (e.g. [68,176]), as well as the underlying alleles. While certainly a challenging endeavour, integrating quantitative genetic mapping methods to identify barrier loci in allopatric populations with population genomic tools to assess their fate in sympatry is a powerful approach to determine how different types of barrier loci fair in secondary contact.

Ultimately, speciation biologists are interested in understanding how reproductive barriers can allow for divergence to accumulate, and for gene flow to be prevented not just at specific barrier loci, but genome-wide. Theory predicts that even moderate intrinsic postzygotic isolation can substantially decrease the rate of introgression of both barrier and linked neutral loci [40,65,166]. One valuable test of the relative efficacy of pre- versus intrinsic postzygotic barriers in nature would be to characterize the extent of introgression and the distribution of introgressed alleles in secondary contact zones when the involved species are reproductively isolated via prezygotic, postzygotic or both types of barriers. With the advent of inexpensive sequencing technologies in combination with advanced computational methods for ancestry assignment (e.g. [158,177–179]), detecting and quantifying introgression across a wide variety of secondary contact zones is an achievable task, and in fact has been done to assess the genomic outcomes of hybridization, as well as map incompatibility loci in hybrid zones (e.g. [180,181]).

(ii) What factors affect the ability of intrinsic postzygotic barriers to impede introgression?

A rich theoretical literature suggests that the ability of intrinsic postzygotic barriers to prevent introgression depends on three aspects of barrier loci: (i) the genetic architecture, (ii) the genetic context, and (iii) the mechanism of evolution. We discuss each of these in turn.

Genetic architecture- or simply, the number and location of incompatibility loci, and their mode of action (i.e. dominance, additivity) can effect rates of introgression. Polygenic intrinsic postzygotic barriers should be more efficient than simple incompatibilities at hampering introgression across the genome (although this will also depend on the dominance, additivity and effect size of the loci involved; [67,145,166,182,183]). The rate of introgression for a neutral locus will depend on both the degree of linkage with and the amount of selection against linked incompatibility alleles. Therefore, if incompatibilities are randomly distributed throughout the genome, then as the number of incompatibility loci increases, so does the proportion of the genome that is linked to an incompatibility allele, and is thus protected against introgression (although, selection against any particular incompatibility locus will be lower for polygenic

incompatibilities than simple ones if selection against hybrids is equal). Curiously, the proportion of the genome that is protected against introgression may increase nonlinearly with time, as the number of loci involved in incompatibilities is expected to increase nonlinearly (assuming that an increase in the number of loci involved in an incompatibility also increases reproductive isolation or the genetic redundancy of strong intrinsic postzygotic barriers; [22,24,25,30]).

In addition, the dominance of incompatibility alleles, as well as whether they are sex-linked or autosomal will influence the generation (i.e. F_1, F_2 or later) that an incompatibility is revealed and the proportion of hybrids that are afflicted by the incompatibility. In theory, incompatibilities that manifest in the first generation of hybrids, or involve more dominant loci should be more effective at preventing introgression [30,67]. While there are few empirical tests of this prediction, recent work has shown that hybrid lethality involving two recessive alleles is relatively ineffective at preventing gene flow in sympatric populations of *Mimulus* [62]. Dominance and linkage can interact to influence the manifestation and maintenance of barriers. For dominant incompatibility loci, linkage between interacting alleles can maintain reproductive isolation in the face of substantial gene flow [164,184]. However, for incompatibilities involving two recessive loci, tight linkage among them will ensure a low probability that hybrids are homozygous for both incompatibility loci, and therefore the incompatibility will rarely manifest.

The genomic context of barrier loci can also impact patterns of introgression. Gene density and recombination can interact to influence rates of introgression such that regions of low recombination are more protected against introgression than regions of high recombination. This is because regions of high recombination have an increased probability that neutral alleles will recombine away from incompatibility alleles, particularly in gene-rich regions which are more likely to house incompatibility loci [180]. This has been shown theoretically [145] and empirically across scales: including variation in local recombination rate along the genome [180,181], within versus outside of structural changes that reduce recombination (e.g. inversions [185–187]), and between chromosomes that vary in the extent of recombination they experience (e.g. sex chromosomes versus autosomes: [168,188], although reduced introgression on sex chromosomes may also be a function of the higher density of incompatibility loci on sex chromosomes than autosomes; reviewed in [189]). The recombination landscape can vary within and between populations [190,191], and this may have implications for population or individual differences in the rates and landscape of introgression.

Finally, the degree to which intrinsic postzygotic barriers can prevent gene flow is also a product of the evolutionary drivers responsible for incompatibilities. Most theory suggests that incompatibility alleles that evolved neutrally are unlikely to be maintained in secondary contact [67,145]. This is because derived incompatibility alleles will experience a fitness cost when in the wrong genomic background, while ancestral alleles should be equally fit on all genomic backgrounds. By contrast, if incompatibilities have evolved via selection, the selective benefit of each incompatibility allele may outweigh the fitness cost when those alleles are found in the wrong genetic background. Yet, little work has been done to explore how different evolutionary drivers of incompatibilities can influence the stability of incompatibility alleles under scenarios of gene flow. It has been argued that incompatibilities which have evolved as a byproduct of adaptation to the same or similar selective pressures (e.g. mutation-order speciation) are unlikely to be maintained in the face of gene flow, as allelic combinations that have the highest global fitness will eventually spread to both species [15,192,193]. While this is intuitive for situations in which incompatibilities arise when populations are adapting to the same optima (e.g. [194]), it may not accurately describe incompatibilities that result from other selective processes (such as genomic conflicts and other co-evolutionary processes). Yet, to our knowledge, this has not been explicitly modelled. Therefore, new theory and simulations assessing how different evolutionary drivers affect the stability of different barriers in secondary contact are needed.

(b) Early onset of species boundaries via rapid evolution

The second major contribution of intrinsic postzygotic barriers to speciation is that these barriers can play a prominent role when they evolve early in the speciation process. Above, we point out that hybrid sterility and inviability often evolve rapidly and many incompatibility alleles exhibit signatures of rapid evolution (box 1). The rapid evolution of intrinsic barriers is perhaps unsurprising in light of the potential evolutionary drivers of incompatibilities. While only a handful of studies have determined the evolutionary drivers of incompatibility alleles, of the few examples amassed, co-evolutionary, conflict-driven processes appear to dominate (reviewed in [19,87,88]). Theory predicts that conflict-driven evolution should promote arms-race dynamics [195,196], which in turn would result in rapid evolution that is detectable at the molecular level. Several intrinsic reproductive barriers that are thought to evolve via conflict-driven processes also exhibit rapid evolution—either by appearing in recently diverged species [118,126] or by showing signals of positive molecular evolution [50,53,83,85].

While this review has focused on more severe hybrid defects, there is a host of less obvious intrinsic hybrid deficiencies that may appear even earlier in the speciation process. For example, in animals, hybrids have been shown to display transgressive metabolic phenotypes [46,47,197–199], and neurological defects [43–45,200]. In plants, many closely related crop varieties exhibit reduced vegetative growth, malformed roots and/or reduced fertility (sometimes referred to as 'hybrid weakness'; e.g. [201–204]; synthesized in [20]). The fitness consequences of these defects have rarely been studied (though see [197,200]), but may impose substantial selection against hybrids between recently diverged taxa (e.g. in crop plants, hybrid weakness has evolved within thousands of years). Determining the timing of when these types of barriers evolve, their commonality in natural populations, the degree of selection against them, and the evolutionary drivers responsible for them are all essential aims for speciation biologists.

(c) Reinforcement

Lastly, the third major role intrinsic postzygotic reproductive isolation can play in the speciation process is the generation of subsequent reproductive isolation. Specifically, the presence of strong intrinsic postzygotic barriers between sympatric taxa can lead to selection favouring increased prezygotic reproductive isolation owing to low fitness hybrids (reinforcement; [138,205]). While we focus on examples and theory in which reinforcing selection results from intrinsic

Box 2. Outstanding questions in speciation biology.

	question	potential approach(es)
origin, commonality, and importance of intrinsic postzygotic barriers	**1. what are the major evolutionary drivers of intrinsic barriers and how frequent are they in nature?**	a. genetically dissect reproductive barriers to determine if genes involved provide information of the evolutionary drivers (such as [50,85,91])
		b. compare the extent of reproductive isolation or diversification rates between groups that are known to differ in proxies for particular evolutionary drivers (e.g. mating system as a proxy for parental conflict and the evolution of hybrid seed inviability, *circa* [135]
	2. how common are less obvious intrinsic barriers (e.g. deficits in hybrid metabolism, neurology, or general 'hybrid weakness')? At what stage are these barriers important?	a. case studies quantifying hybrid defects, and when possible, the amount of selection against hybrids who carry them [e.g. 200]
		b. comparative studies that analyse the relative age of species pairs that produce hybrids with 'hybrid weakness' or other transgressive phenotypes
	3. is there interplay between intrinsic barriers and ecology? How important is this for speciation?	a. generate hybrids under multiple biologically realistic environmental conditions and measure viability or sterility [e.g. 41]
		b. assess hybrid sterility of viability in natural populations across an environmental gradient
intrinsic postzygotic barriers and introgression	**4. how effective are intrinsic barriers at preventing introgression in nature?**	a. map the genetic basis of incompatibility loci in allopatric populations, assess the fate of incompatibility alleles in contact zones (e.g. whether or not they are still present, or assess the steepness of clines across a hybrid zone for incompatibility loci versus neutral loci). Genetic mapping will of course be easiest in model systems in which laboratory crosses are possible. Although not as robust, if researchers are using non-model systems in which laboratory crosses are unattainable, using field collected hybrids for admixture mapping or RNAseq of barrier tissues (e.g. gametes) may be informative. We caution, however, that these natural hybrids may represent a non-random collection of incompatibility alleles
		b. experimentally evolve hybrid swarms between parental species with different types of reproductive barriers. Assess the prevalence of reproductive isolation through time and determine how quickly different types of barriers are lost from hybrid populations (or whether some persist)
		c. simulate genome-wide patterns of introgression for species pairs with differing types of reproductive barriers
	5. what is the relationship between the amount of introgression (or the composition of introgressed alleles) and divergence time?	a. assess rates and timing of introgression in natural or synthetic contact zones from species pairs of differing ages

(*Continued*.)

Box 2. (*Continued.*)

	question	potential approach(es)
		b. the same as above, but can use genomes of broadly sympatric taxa rather than explicit contact zones
		c. simulate genome-wide patterns of introgression between pairs of populations with differing divergence
	6. how do different evolutionary drivers that are responsible for the evolution of intrinsic barriers influence the stability of these barriers in the face of gene flow?	a. simulate secondary contact when incompatibilities are driven by different evolutionary mechanisms (e.g. neutrality, local adaptation, systems drift, conflict or other co-evolutionary dynamics), and assess the stability of simulated incompatibility alleles
intrinsic postzygotic barriers and species persistence and diversification	**7. how important are intrinsic postzygotic barriers for species coexistence and diversification?**	a. compare rates of accumulation of postzygotic intrinsic barriers in allopatry versus sympatry to determine if sympatric taxa are more likely to be strongly isolated (consistent with Templeton effect [136])
		b. simulate secondary contact between populations that are weakly or strongly reproductively isolated with pre- and/or postzygotic reproductive barriers and determine the probability of extinction
		c. determine whether levels of reproductive isolation are correlated with diversification rates [such as 244]

barriers, reinforcement may also be a consequence of extrinsic postzygotic reproductive isolation (reviewed in [31]). Reinforcement has primarily been studied in the context of increased mate preference in sympatry ([206–209]; or similarly, pollinator shifts in plants; [210–213]), but can manifest as any reproductive barrier that prevents parental investment in unfit hybrids, including increased gametic incompatibilities [214,215], ecological divergence that decreases the probability of heterospecific matings [216], or early embryo abortion in systems with substantial parental care [217]. Reinforcement can also have more direct consequences of the generation on biodiversity through processes such as cascading reinforcement (the increase in reproductive isolation between parapatric conspecific populations as a result of reinforcing selection between sympatric heterospecific interactions; e.g. [209,218–220]).

Reinforcement may be a common phenomenon across taxonomic groups, and is considered by some to be an essential step in speciation [205,221]. Patterns consistent with reinforcement have been described in a number of natural systems (e.g. [9,206–208,210–213,218,219,222–227]), and reinforcement has been experimentally evolved many times (e.g. [228–232]; reviewed in [233]). Reinforcement is often considered a final step in the speciation process; reinforcing selection can cause an increase in prezygotic reproductive isolation that finalizes speciation and allows coexistence between close relatives. Because of its association with the completion of reproductive isolation, there is a connotation that reinforcement may take a long time to evolve (e.g. [144,234]). However, three classes of empirical results show that reinforcement need not be a process that occurs only between highly divergent species. Firstly, in many experimental evolution studies of *Drosophila*, reinforcement can evolve in fewer than 10 generations [228–231]. Secondly, in large surveys of the accumulation of reproductive isolation through time (such as [9,222,235]), evidence for reinforcement rests on the observation that prezygotic barriers evolves more rapidly in sympatry than allopatry. In *Drosophila*, allopatric species pairs often exhibit strong prezygotic isolation at low to moderate levels of divergence [9,222]. Therefore, evidence of reinforcement in *Drosophila* stems from the observation that strong prezygotic reproductive isolation evolves between very recently diverged sympatric species pairs [9,222]. Of course, studies which have scored both pre- and postzygotic reproductive isolation for a large number of sympatric and allopatric species pairs are scarce, and so broad conclusions about the rate of evolution of reinforcement from large-scale comparative work are limited. Thirdly, reinforcement has been documented between recently diverged species pairs, such as in the *Drosophila subquinaria* species complex [208,236]. Thus, while reinforcement may signify later stages of speciation, the presence of reinforcing selection is not restricted to highly divergent species pairs.

Reinforcement may also manifest as ecological divergence that decreases the probability of heterospecific matings. This phenomenon is well described in the context of polyploids and their diploid progenitors ('minority cytotype principle'; [216]), but can also extend to diploid species. In essence, when two species co-occur and one is substantially less common than the other, the less common species can experience a potential cost to reproduction as it is more likely to engage in heterospecific matings than the more common species. Selection to reduce interspecific mating can result in ecological divergence [216]. In the context of polyploids

and their diploid progenitors, ecological divergence is common [237–241] and perhaps immediate [242]. Patterns of ecological divergence as a consequence of strong intrinsic postzygotic barriers among diploids are less established, but have been described in *Mimulus* [243].

5. Moving forward as a field

We outline three contributions of intrinsic postzygotic barriers to speciation and highlight how their role may change through time. Still, there are many remaining questions which can further our understanding of the role of intrinsic postzygotic barriers in speciation (box 2). Gaps in our knowledge can be categorized as questions pertaining to the origin, commonality, and strength of different types of intrinsic postzygotic barriers, the relationship between intrinsic postyzogtic barriers and introgression, and the role of intrinsic postzygotic barriers in species persistence and diversification. Answering these questions will require the integration of new theory and simulations, as well as the collection of more datasets that address both broad scale patterns of reproductive isolation and detailed dissections of specific barriers. Below we highlight how integrative studies can further our understanding of intrinsic postzygotic barriers.

Several questions remain on the origin, commonality and strength of different types of intrinsic barriers (box 2, questions 1–3). A potential major contributor to intrinsic postzygotic isolation are subtle intrinsic postzygotic barriers that affect hybrid physiological or cognitive ability, or general 'hybrid weakness' (box 2, question 2). Case studies that detail the diversity of hybrid defects with measurements of selection against these transgressive phenotypes, as well as comparative studies that assess the rate of accumulation of these types of barriers (such as [9,235]) can inform us of the commonality, strength, and timing of these types of barriers in nature.

While there is substantial theory highlighting the efficacy of intrinsic postzygotic barriers against introgression, explicit empirical work is needed (box 2, question 4). One approach is to combine quantitative genetics to map the genetic basis of reproductive isolation and population genomics to infer patterns of introgression of these alleles. Another approach is to pair large-scale quantifications the accumulation of intrinsic postzygotic isolation through time (as in [9,111–117,245–247]), with comparative population genomic studies to assess how the extent of introgression tracks with divergence times (as in [130]) and the accumulation of reproductive isolation. The later approach can also be used to test whether the extent of introgression is also nonlinear through time (box 2, question 5; [21,22,30]).

Lastly, intrinsic postzygotic barriers may be essential for species persistence [37,234], but empirical tests are needed (box 2, question 7). For example, the Templeton effect posits that only strongly isolated species pairs can persist in sympatry, as weakly isolated species pairs are more likely to go extinct or collapse via introgression [37,136,137,234]. One approach to test this would be to use large-scale comparative datasets to assess the rate of accumulation of intrinsic postzygotic isolation in sympatric and allopatric species pairs, with the prediction that sympatric species pairs should show higher intrinsic postzygotic isolation than similarly divergent allopatric species pairs. The role of reproductive barriers in speciation at macro-evolutionary time scales is also not well understood. For example, reproductive isolation is often abundant, but may not predict patterns of diversification [244,248]. Understanding if intrinsic postzygotic barriers contribute to macroevolutionary patterns of diversity, and what factors affect this process, is an important, but rarely studied aspect of speciation.

Data accessibility. All data presented in this paper were accessed from previously published datasets. These datasets have been referenced throughout the manuscript.

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