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NEWS AND VIEWS

Opinion

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Intrinsic incompatibilities evolving as a by-product of divergent ecological selection: Considering them in empirical studies on divergence with gene flow

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Abstract

The possibility of intrinsic barriers to gene flow is often neglected in empirical research on local adaptation and speciation with gene flow, for example when interpreting patterns observed in genome scans. However, we draw attention to the fact that, even with gene flow, divergent ecological selection may generate intrinsic barriers involving both ecologically selected and other interacting loci. Mechanistically, the link between the two types of barriers may be generated by genes that have multiple functions (i.e., pleiotropy), and/or by gene interaction networks. Because most genes function in complex networks, and their evolution is not independent of other genes, changes evolving in response to ecological selection can generate intrinsic barriers as a by-product. A crucial question is to what extent such by-product barriers contribute to divergence and speciation—that is whether they stably reduce gene flow. We discuss under which conditions by-product barriers may increase isolation. However, we also highlight that, depending on the conditions (e.g., the amount of gene flow and the strength of selection acting on the intrinsic vs. the ecological barrier component), the intrinsic incompatibility may actually destabilize barriers to gene flow. In practice, intrinsic barriers generated as a by-product of divergent ecological selection may generate peaks in genome scans that cannot easily be interpreted. We argue that empirical studies on divergence with gene flow should consider the possibility of both ecological and intrinsic barriers. Future progress will likely come from work combining population genomic studies, experiments quantifying fitness and molecular studies on protein function and interactions.

KEYWORDS
divergent ecological selection, gene interaction, intrinsic incompatibility, outlier scan, pleiotropy, speciation

1 | INTRODUCTION

The framework and terminology we use structures scientific inquiry and influences the way we set up research questions and evaluate data to understand the principles of evolution (Harrison, 2012).

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Binary distinctions between concepts can be helpful to aid communication, but they may lead to oversimplification—as has been the case for the distinction between "sympatric" and "allopatric" speciation (Butlin, Galindo, & Grahame, 2008). Here, we discuss the overlap between "intrinsic" and "ecological" barriers to gene flow (see definitions in Box 1), aiming our article mainly at empiricists working on adaptive divergence and speciation with gene flow. We argue that, while ecological barriers are considered crucial in driving these processes, empirical studies in these fields often neglect the possibility of intrinsic barriers. The main reason for this is that purely intrinsic barriers are unlikely to evolve under continuous gene flow: they are selected against under many scenarios, and incompatible alleles cannot spread in areas where diverging populations frequently interbreed (Bank, Bürger, & Hermisson, 2012).

However, there is a situation in which intrinsic barriers may evolve and be stable even under gene flow. That is the case when the very same loci that are under divergent ecological selection are also involved in intrinsic barriers (Baack, Melo, Rieseberg, & Ortiz-Barrientos, 2015; Bank et al., 2012; Dobzhansky, 1951; Gavrilets, 2000; Schluter & Conte, 2009). Early work, for example, by Dobzhansky (1951) already recognized that divergent selection on a locus can cause the evolution of intrinsic barriers as a by-product; this idea was further developed in later theoretical work (e.g., Bank et al., 2012; Barton, 2001; Chevin, Decorzent, & Lenormand, 2014). While a large part of this work has focused on divergence in allopatry, there are clear indications that the same mechanism can generate barriers even *under continuous gene flow* (Baack et al., 2015; Gavrilets, 1999; Schluter & Conte, 2009; Slatkin, 1982)—the point we emphasize in this article.

First, divergent ecological selection on a locus may favour alleles that are incompatible with alleles at other loci in the other population, producing intrinsic isolation as a by-product (i.e., derived-derived incompatibility, Box 1). Second, adaptive changes at ecologically selected loci can generate new "genetic environments," enabling further changes at interacting loci in the same population. These cascading changes can themselves lead to intrinsic incompatibilities with the ancestral allele (i.e., ancestral-derived incompatibility, Box 1). If such patterns are common, intrinsic barriers may well contribute to primary ecological divergence and act as a pathway to "ecological speciation" (Rundle & Nosil, 2005). Then, the distinction between "ecological" and "intrinsic" barriers (Box 1) becomes blurred, as does the distinction between adaptive divergence and speciation.

The connection between divergent ecological selection and intrinsic barriers also has practical consequences for studies investigating the genomic basis of adaptive divergence and ecological speciation. Genomic scans for loci showing high differentiation between populations have become very popular and are commonly interpreted to reveal loci under divergent ecological selection (Beaumont & Balding, 2004; Nosil, Funk, & Ortiz-Barrientos, 2009). The logic behind this approach is that loci underlying local adaptation are able to resist gene flow and should therefore be the most differentiated genomic regions detected as high F_{ST} peaks. However, there are various caveats, and high F_{ST} values may not always indicate divergent selection (Cruickshank & Hahn, 2014; Wolf & Ellegren, 2017). The field of evolutionary biology is now moving towards increasing the reliability of outlier scans by controlling for confounding factors (e.g., Burri et al., 2015), using experiments to test whether outlier loci indeed respond to selection (Soria-Carrasco et al., 2014), and using genetic manipulation to establish the organismal role of outlier loci (Colosimo et al., 2005). Still, as pointed out by Bierne, Welch, Loire, Bonhomme, and David (2011), loci showing high differentiation between ecologically divergent populations may actually reflect intrinsic barriers trapped at the transition between two environments (i.e., ecotone) after secondary contact, rather than loci under ecological selection. Here, we emphasize a different aspect: even during primary divergence, intrinsic barriers may evolve and colocate with the ecotone because they are caused by, or interact with, the loci under divergent ecological selection.

To help bridge the gap between theoretical and empirical studies, we discuss how gene interaction networks and pleiotropy may facilitate the evolution of intrinsic barriers driven by ecological selection, ask under which conditions these barriers contribute to a reduction in gene flow and discuss consequences for genome scans and similar analyses. By outlining explicit mechanistic scenarios, we aim to facilitate the search of loci contributing to both ecological and intrinsic barriers from empirical data.

In much of this short article, we restrict ourselves to cases of two-locus scenarios for the sake of simplicity, but we discuss implications of more complex incompatibilities below. Single-locus barriers are not considered for reasons of space, but in principle they can evolve by the same mechanisms as multilocus ones (although they may be restricted to certain types of genes as they require repeated evolution at a single locus).

In all of our scenarios, at least one locus—denoted as locus **A** in population 1—is under divergent ecological selection. In addition, alleles at locus **A** are incompatible with alleles at locus **B** (Figure 1). Locus **A** is therefore influenced by both ecological and intrinsic selection pressures. In contrast, locus **B** is involved in an incompatibility with locus **A**, but not necessarily under divergent ecological selection (Figure 1). There are two simple ways in which divergent selection can lead to intrinsic barriers in two-locus systems (Muller, 1942) (derived-derived and ancestral-derived); these are outlined in Box 1.

We emphasize that throughout this article, when we refer to "changes" at a locus, on the population level we mean a change in allele frequency at the locus in question. This does not necessarily imply fixation, but rather the emergence of a difference in allele frequencies between populations.

2 | MECHANISTIC REASONS FOR AN ASSOCIATION BETWEEN ECOLOGICAL AND INTRINSIC BARRIERS

Why should ecological barrier loci also be involved in intrinsic incompatibilities? One reason is that ecologically selected loci may change

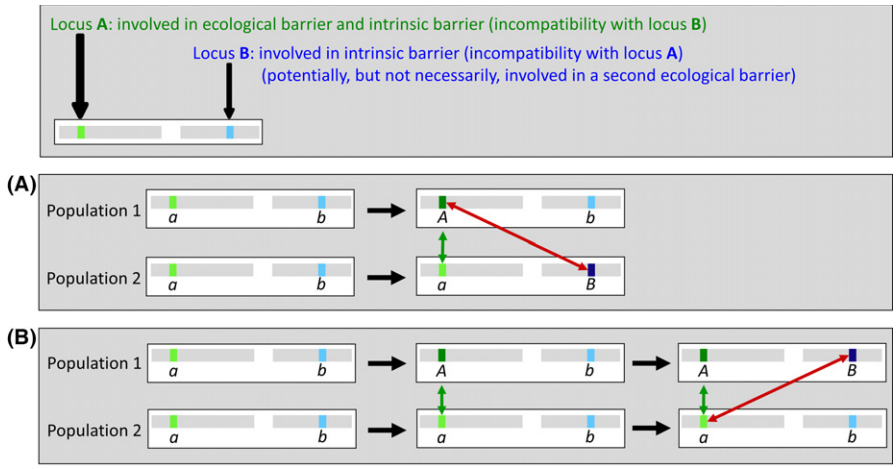

FIGURE 1 Evolution of incompatibilities as a by-product of divergent ecological selection. Shown are two hypothetical loci, **A** and **B**, situated on two different chromosomes and each with two alleles. A mutation from allele *a* to *A* indicates a locus under divergent ecological selection. Mutation from *b* to *B* can arise by divergent ecological selection or is selected for other reasons (e.g., compensating pleiotropic side effects of other mutations). Green arrow indicates divergent ecological selection between alleles of the same locus, and red arrow indicates intrinsic incompatibility between loci. Panel A: derived-derived incompatibility; panel B: derived-ancestral incompatibility

more rapidly than neutrally evolving ones. The more interdependencies between genes there are, the more likely it is that ecologically driven selection generates incompatibilities as a by-product. Indeed, no gene is an island; instead, genes are dependent on other genes and regulatory sequences through networks and feedback cascades (Phillips, 2008; Wright, 1968). They are also frequently pleiotropic and are likely to serve multiple functions depending on when and where they are expressed. Below we detail how, due to these interdependencies, intrinsic barriers may be caused by divergent ecological selection. We do not focus on their stability with gene flow yet (this topic is dealt with in the later sections).

We define a pleiotropic gene as a locus for which there is a high probability that an allelic substitution will have effects on more than one trait. A "trait" in this sense can be either morphological, behavioural or biochemical. For example, nucleotide substitution in a pleiotropic gene could lead to changes in both colour and size. At a molecular level, the product of a gene could be involved in several different molecular functions or could perform different tasks when expressed in different tissues (Paaby & Rockman, 2013).

Gene interactions occur whenever the products of different genes are part of the same functional network. This can happen via direct interaction, where two gene products, for example, form a protein complex, or one protein catalyses a conformational change in another. Because genes usually interact with several partners and are part of interaction cascades, the number of indirect interactions is arguably much larger.

Pleiotropy and gene interactions are related: on average, the more functions a gene has, the more traits it affects (pleiotropy) and the more other loci it interacts with (gene interaction). However, they can also act independently, that is, one can occur without the other for a specific gene.

Pleiotropy and gene interactions can lead to derived-derived incompatibilities. If a pleiotropic locus experiences nucleotide changes due to ecological selection on one trait, this can have effects on other traits or the molecular functions this locus underlies (Fig. S1A). Any such change in a trait or function in population 1 might be incompatible with another change in population 2. The more pleiotropic the locus is, the more traits or functions can potentially be altered, and the higher the risk of an adaptive mutation producing an incompatibility between populations as a side effect. Similarly, the more interactions a gene product is involved in, the higher the chance for an incompatibility with a gene product in another population (Fig. S1B).

Pleiotropy and gene interactions are also likely to cause ancestral-derived incompatibilities. Any change in population 1 might lead to follow-up changes at other loci within the same population (Pavlicev & Wagner, 2012), resulting in co-adaptation of genes (Fig. S1C). For example, adaptive change in the gene product of locus **A** can enable an adaptive conformational change in the physically interacting gene product of locus **B** (Fig. S1D). As another example, adaptive changes in a pleiotropic gene might cause negative pleiotropic side effects, and follow-up changes at other genes in the same population might compensate for these, again leading to co-adaptation (Lehner, 2011; Pavlicev & Wagner, 2012). The more pleiotropic effects or gene interactions there are, the more possibilities for changes at other loci emerge that would not have been possible in the previous background.

These considerations demonstrate that with pleiotropy and gene interactions, ecological selection may often cause intrinsic barriers. So, how common are pleiotropy and gene interactions according to empirical studies? Few studies (reviewed in Paaby & Rockman, 2013) have systematically tested for genomewide pleiotropy by reverse genetics, that is, by mutating single genes one by one and measuring the effects. Alternatively, pleiotropy can be measured by QTL studies. These approaches employed in yeast (Dudley, Janse, Tanay, Shamir, & Church, 2005), nematodes (Wang, Liao, & Zhang, 2010), mice (Wagner et al., 2008) and sticklebacks (Albert et al., 2008)

BOX 1

Definitions

Ecological barrier loci

An ecological (and extrinsic) barrier occurs when a locus is under divergent ecological selection and reduces gene flow between populations. This means ecological selection favours one allele in population 1 and another allele in population 2, leading to selection against unfit immigrants and/or the formation of hybrids that are maladapted in both environments (Nosil, 2012; Schluter, 2000). A purely ecological barrier is always environment-dependent and would not function as a barrier in a homogeneous environment (e.g., under standardized laboratory conditions).

Intrinsic barrier loci

We define intrinsic barriers (i.e., incompatibilities) as those where interactions between alleles result in lowered fitness of individuals carrying their combination. Such barriers may either involve alleles at the same or at different loci; we here focus on the latter and will not discuss the former for simplicity. Purely intrinsic barriers are environment independent, meaning they result in a lowered fitness of hybrids or recombinants in any relevant environment and under standardized laboratory conditions. Purely intrinsic barriers may evolve by drift, usually in allopatry (Turelli, Barton, & Coyne, 2001 and references therein). Alternatively, the incompatible alleles may each be favoured by selection that is uniform across environments in population 1 and 2 (e.g., global temperature increase), but if the alleles are combined within the same genotype they are incompatible. For example, population 1 may adapt by evolving allele *A* at locus **A** and population 2 adapts by evolving allele *B* at locus **B**, but when brought together allele *A* and *B* are incompatible with each other (i.e., mutation order speciation; Mani & Clarke, 1990).

Loci involved in both intrinsic and ecological barriers

Our focus is on loci that are involved in both intrinsic and ecological barriers. The ecological barrier occurs because the locus is under divergent ecological selection. The intrinsic effect results from the interaction of one or more alleles at the locus with one or more alleles at other interacting loci that cause reduced fitness of hybrids (i.e., intrinsic incompatibilities) (Figure 1). Loci involved in both intrinsic and ecological barriers will show evidence of divergent selection in the field, as well as reproductive isolation in a standardized laboratory environment.

Two ways of evolving intrinsic barriers

Derived-derived incompatibility

Derived-derived incompatibility can evolve when two interacting loci change in each of two diverging populations, that is, an ancestral genotype *aabb* evolves into *AAbb* in population 1 and *aaBB* in population 2 (Figure 1A). When the derived alleles (*A* and *B*) at the two loci are combined within the same individual (e.g., *AaBb*), they may be incompatible with each other (Dobzhansky 1936, 1951; Muller, 1942) leading to derived-derived incompatibility and intrinsic barrier (Orr, 1995).

Ancestral-derived incompatibility

Ancestral-derived incompatibility may emerge if divergent selection drives change in population 1 (e.g., the replacement of the ancestral genotype *aabb* by *AAbb*), and this enables a change in locus **B** in the same population, leading to *AABB* genotypes. Because the *B* allele only works when the *A* allele is present, combining the *B* allele with the ancestral allele *a* in hybrids (e.g., *aaBB*) generates ancestral-derived incompatibility leading into intrinsic barrier (Orr, 1995) (Figure 1B).

suggest that an average gene affects three to seven traits (Paaby & Rockman, 2013). Comprehensive screens and functional information are likely to be available for only a handful of genes, but an indication of pleiotropy is the number of splice variants a gene has. Generally speaking, the *Drosophila* genome contains three times more proteins than there are genes (Nei, 2013, p. 115), suggesting that on average a single gene produces three functional variants. As an extreme example, *Dscam*, a gene encoding a membrane protein and involved in development of *Drosophila*, has 24 exons and theoretically would be able to produce 38 016 different types of proteins (Nei, 2013, pp. 127–128). Of course, the crucial question is whether all these splice variants are functional, and whether they serve the same or different functions. In any case, pleiotropy is probably widespread in the genome.

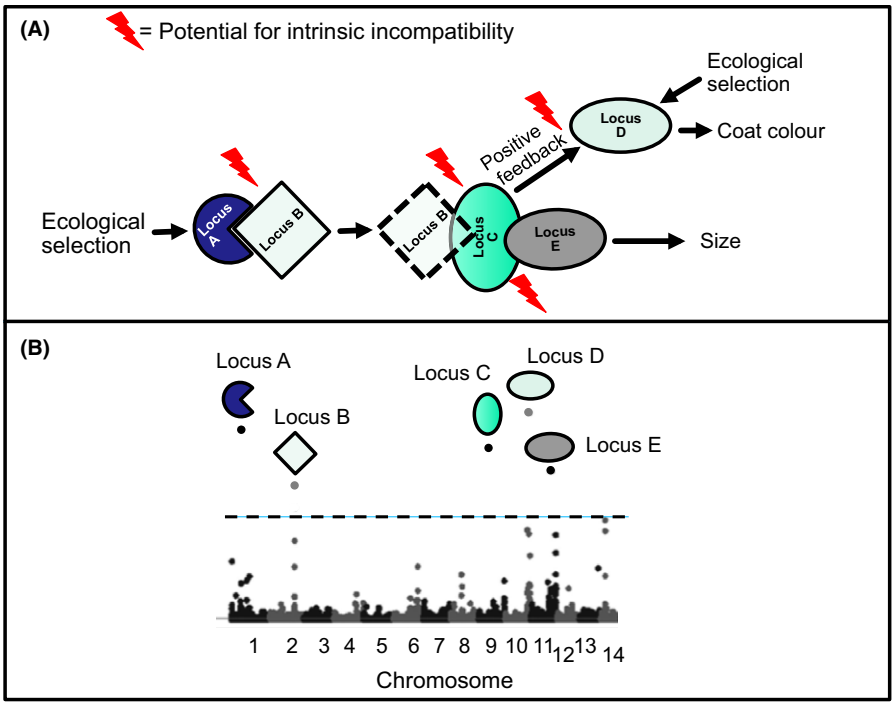

FIGURE 2 Gene interaction networks, pleiotropy and genome scans: Outliers may not be the direct target of divergent ecological selection, but only indirectly involved through gene interaction networks. Panel A shows the interaction network of the protein products of five loci. These loci reside on different chromosomes (panel B), but still do not evolve independently of each other, because they are involved in the same gene interaction pathway that underlies coat colour and size. In such an interaction network, there are multiple opportunities for incompatibilities to arise. For example, it is easy to imagine that a change in locus A, favoured by divergent ecological selection, enables the increase in frequency of a new allele at locus B in population 1. Similarly, selection on locus D could enable a change at locus C in population 2. The derived alleles at loci B and C could then be incompatible (while neither of them is directly under divergent selection); this would result in genome scan patterns as depicted in panel B (outliers shown above the dashed line)

A possible example of ecological selection driving evolution of intrinsic barriers because of pleiotropy comes from studies of hybrid necrosis in plants (Bomblies, 2010; Chae et al., 2014). One particular locus, *DM2*, shows signatures of diversifying selection at the sequence level. Selection is likely driven by pathogen pressure as the locus is involved in pathogen recognition (Chae et al., 2014). Interestingly, *DM2* interacts with at least five different loci causing necrosis and problems in hybrids, suggesting natural selection from parasites generates incompatibilities between *DM2* and loci interacting with it (Bomblies, 2010; Chae et al., 2014).

Molecular biology has also shown that interactions of gene products are ubiquitous. A study testing 1000 genes in yeast found that the number of confirmed interactions per gene varied from 1 to 146, with an average of 34 interactions per gene (Tong et al., 2004). Another study on gene essentiality identified 44 genes that are needed for the viability of the Sigma1278b isolate of *S. cerevisiae* but not for the standard S288c strain. Remarkably, genetic analysis revealed that in the majority of tested cases the differences in essentiality were influenced by at least four different loci in the genome suggesting complex multigenic interactions (Dowell et al., 2010). Even the classical case of DMI in *Drosophila* is genetically complex, where hybrid lethality of *Nup160* depends on one or more unknown additional factors in the autosomal background (Tang & Presgraves, 2015).

Interactions between different loci in the genome do not only arise because of gene–gene interactions; they arise also between regulatory sequences like transcription factors, microRNAs, siRNAs and their target regions. Loci that regulate gene expression, called eQTL, generally appear to affect a small number of gene expression traits, but typically a handful of eQTL hotspots affect abundances of hundreds to thousands of transcripts (Paaby & Rockman, 2013). Taking also regulatory variation into consideration, the number of interactions per locus is further increased and indeed, these types of complex interactions have been suggested to contribute to sterility of hybrids in the house mouse (Turner, White, Tautz, & Payseur, 2014).

2.1 | Moving towards more complex and realistic scenarios

In summary, we predict that the potential for ecological selection causing intrinsic barriers as a by-product is enormous because both pleiotropy and gene interaction are common. In fact, the simple two-locus scenarios described above are probably often an oversimplification, and intrinsic and ecological barriers can also be *indirectly* associated via gene interaction cascades (Figure 2). Imagine a linear cascade with four loci where there is interaction between loci **A** and **B**, loci **B** and **C,** and loci **C** and **D** (Figure 2). Divergent ecological

selection in population 1 favours a change in locus **A**, which enables the evolution of a new allele at locus **B**. In population 2, divergent selection favours a change in locus **D**, which enables the evolution of a new allele in locus **C**. The new alleles at loci **B** and **C** are incompatible. Importantly however, neither locus **B** nor locus **C** was the direct target of divergent selection (Figure 2). This example shows how divergent selection can lead to cascading genetic changes that indirectly produce intrinsic barriers.

Although two-locus scenarios are more commonly considered, intrinsic incompatibilities evolving in multilocus gene pathways have been studied in theoretical literature (Johnson & Porter, 2000; Lindtke & Buerkle, 2015; Porter & Johnson, 2002). Lindtke and Buerkle (2015) compared the classical Dobzhansky–Muller incompatibilities to incompatibilities evolving in gene pathways by simulating whole genomes in individuals of hybridizing species. They found that incompatible interactions that arise from genetic pathways (but not from classical DMIs) maintain species-specific differences even with high gene flow and at the same time allow introgression at large parts of the genome, a pattern consistent with empirical observations.

2.2 | Pleiotropic and connected genes can evolve under positive selection

Several authors have suggested that mutations in highly pleiotropic or interconnected genes are likely to have deleterious consequences (Fisher, 1930; Orr, 2000; Stern & Orgogozo, 2009). For this reason, they are less likely to respond to positive selection, being rather highly conserved. If this is true, they are less likely to evolve differences between closely related species and to serve as intrinsic incompatibilities, contradicting our above hypothesis.

However, ecological adaptation frequently requires changes in several traits; especially under gene flow, these changes may evolve more easily by mutations in a single pleiotropic locus compared to mutations in several independently segregating loci. This is because in the latter case the favourable allele combination is broken down every generation by recombination (Smadja & Butlin, 2011). Therefore, highly pleiotropic loci might be more effective in generating adaptive divergence, while at the same time being especially likely to generate intrinsic barriers as a by-product. Another argument for the involvement of highly connected or multifunctional loci in adaptive divergence is simply that there might be no other option. Adaptive changes may occur as long as their positive effect outweighs these negative side effects.

Empirical evidence for positive selection and fast evolution in highly connected genes is mixed. In humans, long-term positive selection is less likely in highly connected genes (i.e., genes that have multiple interaction partners) compared to genes with fewer connections (Luisi et al., 2015). In contrast, recent positive selection was more likely to target genes with higher centralities (i.e., highly connected) during human evolution (Luisi et al., 2015). In *Drosophila*, genes under long-term positive selection were significantly more connected than genes with no signatures of positive selection (Chakraborty & Alvarez-Ponce, 2016). There are also specific examples of highly pleiotropic or connected genes involved in divergence. For example, in contrast to the expectations of strong purifying selection and conservation, the highly pleiotropic gene Vitellogenin has been shown to experience bouts of recent selection between different honey bee races (Kent, Issa, Bunting, & Zayed, 2011), and chemosensory genes, which bind a wide range of chemicals, have been suggested to play a role in local adaptation to different host plants in pea aphids (Eyres et al., 2016; Smadja et al., 2012).

3 | THE ROLE OF DIFFERENT TYPES OF BARRIER LOCI IN DIVERGENCE AND SPECIATION

In the previous sections, we have discussed mechanisms why the emergence of intrinsic barriers as a by-product of divergent ecological selection may be common. Both the ecologically selected loci themselves and other interacting loci may be involved. However, we have so far only shown that adaptive mutations should often generate incompatibilities. An important question is whether these mutations, first usually present only in a single individual, will rise in frequency, specifically when there is gene flow between the diverging populations. Under which conditions does the additional intrinsic barrier drive the system closer to speciation, compared to a purely ecological barrier?

To understand the role of barrier loci influenced by both intrinsic incompatibility and ecological selection in reducing gene flow, we will first briefly look at the fate and stability of purely ecological and purely intrinsic barrier loci separately. Intrinsic barriers evolving as a by-product of divergent ecological selection will be affected by the dynamics of both of these.

3.1 | Purely ecological barrier loci

In contrast to many purely intrinsic barriers, ecological barriers may be stable even with gene flow and recombination because environmental heterogeneity continuously favours divergence (Fitzpatrick, Gerberich, Kronenberger, Angeloni, & Funk, 2015; Garant, Forde, & Hendry, 2006; Kawecki & Ebert, 2004). Stable barriers may evolve and be maintained as long as selection is strong enough to overcome the counteracting effect of gene flow (Haldane, 1930). The strength of the barrier, and its contribution to divergence and speciation, depends on the strength of ecological selection (and on the genetic architecture of the divergent trait). Strong selection against intermediate hybrids and/or against immigrants will lead to a marked reduction in gene flow and an abrupt change in allele frequencies where the environment changes, at least locally in the genome (Richardson, Urban, Bolnick, & Skelly, 2014; Vines et al., 2016).

3.2 | Purely intrinsic barrier loci

Purely intrinsic barriers can evolve either by genetic drift or under uniform selection (see Box 1); this will affect their dynamics, but in both cases, they are unlikely to increase in frequency where gene flow is high.

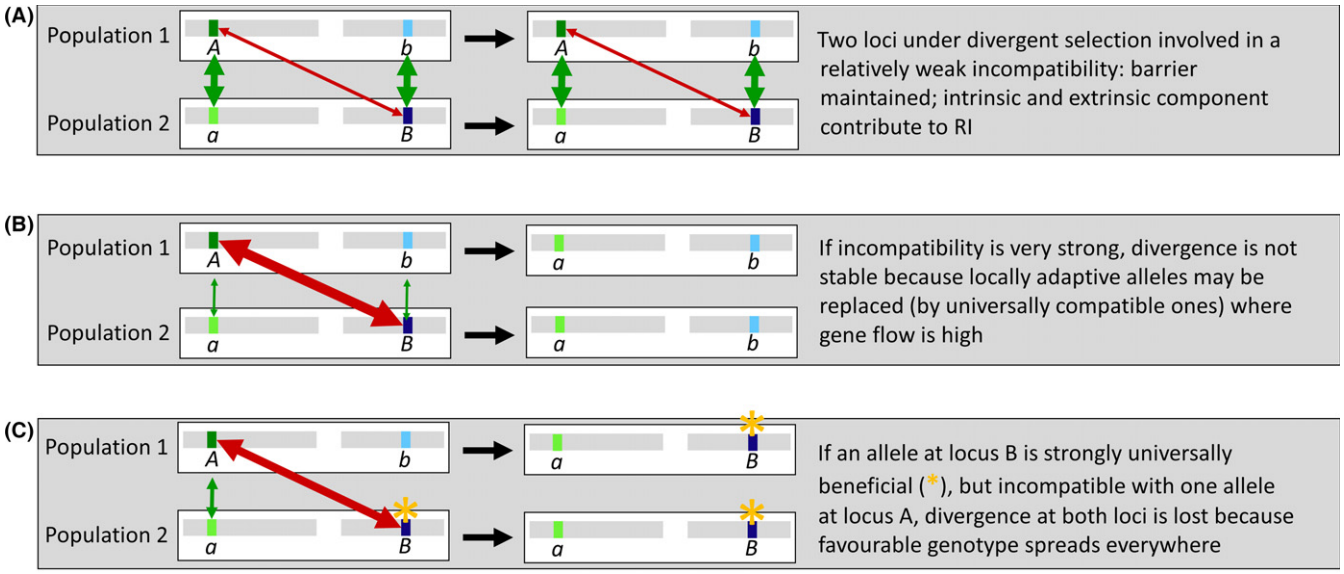

FIGURE 3 Examples of possible evolutionary dynamics of barriers between populations 1 and 2 under gene flow. Shown are two hypothetical loci, **A** and **B**. Green arrow indicates divergent ecological selection between alleles of the same locus, and red arrow indicates intrinsic incompatibility between loci. Arrow thickness indicates strength of selection. On the left, we show allele combinations that may have evolved by mutation (Figure 1); we do not make any statement about their frequency in the population. On the right, we show whether these combinations will be stable over time and potentially increase in frequency (Panel A) or be replaced by other combinations (panels B and C)

Intrinsic barriers arising under drift alone are likely to be maintained only in allopatric phases. This is because incompatible alleles without any selective benefits tend to be removed by selection under gene flow (Bank et al., 2012). Such barriers are therefore less likely to contribute to divergence and speciation with gene flow compared to barriers maintained by selection (but see the effects of additional factors below).

Intrinsic barriers may be more stable if different, but generally beneficial, mutations (e.g., alleles that confer adaptation to increasing global temperatures) become fixed in diverging populations ("mutation order speciation"; Mani & Clarke, 1990; Schluter, 2009). However, this process is also counteracted by gene flow because in this case, adaptive alleles will often spread between populations before incompatible alleles can establish (Nosil & Flaxman, 2011).

3.3 | Intrinsic barriers as a by-product of divergent ecological selection

Intrinsic barrier loci evolved as a by-product of divergent ecological selection should be affected by both the forces working on purely ecological, and purely intrinsic barrier loci. This results in antagonistic selection pressures under gene flow; ecological selection should favour divergence and the maintenance of a barrier despite gene flow; at the same time, intrinsic incompatibility at the locus should favour replacement with compatible alleles, decreasing the strength of the barrier.

The evolutionary fate of intrinsic barriers that evolved as a by-product of ecological selection has partly been explored in the theoretical literature (e.g., Agrawal, Feder, & Nosil, 2011; Bank et al., 2012; Gavrilets, 2000; Slatkin, 1982) and depends on various parameters of the system: the strength of divergent ecological selection on locus **A**, the strength of divergent ecological selection on loci interacting with locus **A** and the epistatic interactions between loci. In the following, we discuss a two-locus system for simplicity and restrict ourselves to a discussion of general principles. We note that more complex incompatibilities may show different dynamics, which we cannot explore here. In addition, physical linkage between ecological barrier loci and interacting loci could work towards maintaining ecologically driven incompatibilities. We will assume unlinked loci here, but emphasize that linkage needs to be considered in empirical work and in a deeper exploration of the topic in general. Another potentially important factor that we ignore here are dominance effects.

In general, if both of the interacting loci are affected by divergent ecological selection, as well as being involved in the intrinsic barrier, this increases the barrier stability as divergence at both loci is favoured by environmental selection (Agrawal et al., 2011). Figure 3A shows an example where both loci are under divergent ecological selection, and involved in an intrinsic incompatibility that is not extremely strong. In this case, the barrier will be maintained under gene flow, and both the intrinsic and the extrinsic components will contribute to the overall reproductive isolation (Slatkin, 1982).

However, even if both loci involved in intrinsic barriers are also under divergent ecological selection, the barrier is not necessarily stable under gene flow. For example, if the intrinsic barrier is extremely strong (e.g., all *AB* individuals die as juveniles), incompatible alleles are strongly selected against even if they are locally adaptive, so that universally compatible alleles are favoured in areas where diverging populations are in contact (Figure 3B). Therefore, in this case the intrinsic barrier component locally counteracts local

adaptation. However, in areas far away from the contact, local adaptation via incompatible alleles is possible (Gavrilets, 1997).

The involvement of locus **A** in an intrinsic incompatibility can even lead to the loss of both the ecological and the intrinsic barriers (Agrawal et al., 2011), potentially across the species' range. Consider the example in Figure 3C. Here, locus **B** has evolved an allele that is universally adaptive (e.g., that confers adaptation to generally rising temperatures—that is, a trait that would be adaptive in both populations), but this universally adaptive allele (*B*) is strongly incompatible with allele *A*. In this case, allele *B* will spread across both populations and "drag" the compatible allele *a* with it. Consequently, both the ecologically driven and the intrinsic barriers are lost (Figure 3C).

Of course, the examples presented here include only a small range of possible parameters, but they do show that, depending on the conditions, the additional intrinsic barrier that evolved as a by-product of ecological selection can have all kinds of effects on the overall barrier to gene flow. Both intrinsic and extrinsic barriers might contribute to reproductive isolation (Figure 3A); barriers might be lost locally (Figure 3B); or both barriers may be lost completely if a universally beneficial alleles take over (Figure 3C).

4 | EMPIRICAL EVIDENCE FOR INTRINSIC BARRIERS AS A BY-PRODUCT OF ECOLOGICAL SELECTION, AND FUTURE RESEARCH DIRECTIONS

The above discussions suggest that intrinsic barriers may frequently emerge as a by-product of divergent ecological selection even with gene flow. However, it is less clear whether they increase in frequency and contribute to divergence in the long term. To evaluate this better, evidence needs to come from two complementary directions. First, it is important to test how commonly the requirements for the evolution for intrinsic barriers as a by-product of divergent ecological selection are fulfilled. How common are pleiotropy and gene interactions in divergently selected loci? How often do empirically measured selection coefficients and epistatic interactions fall into the parameter ranges that enable stable intrinsic barriers? Second, it is possible to more directly test whether intrinsically selected loci are identical with or interact with, ecologically selected ones, and if these are common in natural systems.

There is already much evidence for the ubiquity of pleiotropy and gene interaction networks (see above). However, we need specific estimates for loci likely to become involved in ecological divergence. Estimating selection coefficients of new mutations and mapping interactions between loci becomes more and more feasible (e.g., Gerke, Lorenz, & Cohen, 2009; Wang et al., 2010), but is still an endeavour when done on nonmodel organisms.

Empirical work does point towards association between ecological and intrinsic barriers (Schluter & Conte, 2009). For example, multiple studies exposing *Drosophila* populations to different selective conditions produced intrinsic postzygotic isolation in addition to local adaptation (reviewed in Rice & Hostert, 1993). Such observations are potentially explained by ecologically selected loci generating intrinsic isolation as a by-product (Dobzhansky, 1951; Rundle & Nosil, 2005). Further evidence comes from yeast, where populations grown in two distinct environments for 500 generations evolved intrinsic postzygotic isolation affecting growth rate and meiosis (Dettman, Sirjusingh, Kohn, & Anderson, 2007), and where it has been shown that environmental selection can generate strong incompatibilities at the same loci as a by-product (Anderson et al., 2010). Fascinating work on plants demonstrates that loci putatively adapting to local parasite pressures are also involved in incompatibilities, generating hybrid necrosis (Bomblies, 2010; Chae et al., 2014). In sticklebacks, a functional mismatch in traits involved in niche use reduces the performance of F2 hybrids beyond that of additive genetic effects, suggesting epistatic interactions between genes underlying niche differentiation (Arnegard et al., 2014). This functional mismatch might lead to hybrid incompatibilities that are analogous to those underlying intrinsic reproductive isolation but depend on the ecological context (Arnegard et al., 2014).

However, the majority of the work demonstrating links between ecological and intrinsic barriers comes from systems where most of the divergence happened in allopatry. It shows that ecological selection can drive intrinsic barriers, but it does not prove that this happens with gene flow, where parameter combinations that allow for the evolution of intrinsic barriers are more restricted. More work is needed in systems of local adaptation with gene flow. To test whether loci involved in local adaptation also show negative epistatic interactions with other loci, outlier scans and hybrid zone studies need to be complemented with further experimental or functional evidence. Crosses under standardized conditions are important means of testing whether any intrinsic barriers exist (e.g., Hatfield & Schluter, 1999); the question is then whether intrinsic barriers map to the same loci as ecological barriers.

Another approach relies on detailed annotation of candidate genes, followed by computational approaches to explore whether they are likely to be pleiotropic or part of a common interaction network (e.g., genemania.org). If interdependence between candidate loci can be established, this raises the possibility of involvement of intrinsic incompatibilities. These could be tested by functional studies, for example CRISPR/Cas9 gene introduction or other transformation tests (potentially in more easily tractable model species), where replacement of a gene involved in an intrinsic barrier should result in reduced viability and/or sterility in all possible environments.

5 | PRACTICAL IMPLICATIONS

If ecological selection frequently causes intrinsic barriers as a by-product, we need to keep this in mind when analysing genome scans and similar data. As already mentioned, there are other challenges with outlier scans that need to be taken into account. A correct inference of selected loci is a prerequisite for the considerations below.

First, a locus may be under divergent ecological selection and act as an intrinsic incompatibility as well (e.g., locus **A** in Figure 3A).

Such a locus will show evidence of selection in outlier scans and may be associated with a divergently selected adaptive trait—but it is not clear what proportion of the reduction in gene flow is due to the ecological barrier effect. Ignoring this could lead to an overestimation of the strength of ecological selection acting on the locus.

Second, outlier loci may only *indirectly* be associated with divergent selection (see Section "2.1"). Imagine a locus under divergent ecological selection that is part of a gene interaction network (Figure 2). Then, ecological selection on one locus within the network can drive further changes at other interacting loci. Then, these secondary changes can cause intrinsic incompatibilities with derived alleles of the network from another population. Because alleles within the network are dependent on each other, linkage disequilibrium emerges among interacting loci and they will appear as independent outlier peaks in a genome scan (Figure 2). Importantly, some of these peaks are created by intrinsic barriers and are not the direct target of ecological selection. This idea has been developed in the "Selection Pleiotropy Compensation" model of adaptive evolution, which suggests that most adaptive signatures detected in genome scans could be the result of selection on a pleiotropic loci followed by compensatory changes, rather than of progressive character adaptations (Pavlicev & Wagner, 2012).

Interestingly, outlier loci might also be produced by changes that are universally adaptive, but which are confined to one genetic background: A universally adaptive allele will spread in the population where it is compatible with the genetic background, but cannot enter the second population due to incompatibilities, thereby producing an outlier signal.

6 | CONCLUSIONS

We conclude that the emergence of intrinsic barriers as a by-product of divergent ecological selection may be common, as suggested by the ubiquity of molecular interactions and pleiotropy. However, their role in speciation with gene flow is unclear. Depending on the conditions, the intrinsic barrier effect that evolved as a side effect of ecological selection may either strengthen or weaken the overall barrier to gene flow. More research is needed to estimate the relative importance of these effects; they may well differ between study systems and traits, depending, for example, on the nature and genetic architecture of ecologically selected traits. We need to consider the interdependencies between ecological selection and intrinsic incompatibilities in empirical studies of local adaptation and ecological speciation in order to correctly interpret the results of outlier scans and similar approaches.

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AUTHOR CONTRIBUTION

J.K. and A.M.W. discussed the idea and wrote the paper together.

DATA ACCESSIBILITY

Manuscript does not include primary data.

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