



HAL
open science

Coupling of barriers to gene exchange: causes and consequences

Erik B. Dopman, Kerry L. Shaw, Maria Servedio, Roger K. Butlin, Carole Smadja

► **To cite this version:**

Erik B. Dopman, Kerry L. Shaw, Maria Servedio, Roger K. Butlin, Carole Smadja. Coupling of barriers to gene exchange: causes and consequences. *Cold Spring Harbor Perspectives in Biology*, 2024, *Speciation*, 10.1101/cshperspect.a041432 . hal-04283248

HAL Id: hal-04283248

<https://hal.science/hal-04283248>

Submitted on 15 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Coupling of barriers to gene exchange: Causes and consequences

Erik B. Dopman¹, Kerry L. Shaw², Maria R. Servedio³, Roger Butlin^{4,5}, Carole M. Smadja⁶

¹*Department of Biology, Tufts University, Medford, Massachusetts, 02155, USA*

²*Department of Neurobiology and Behavior, Cornell University, Ithaca, New York, 14853, USA*

³*Department of Biology, University of North Carolina, Chapel Hill, North Carolina, 27599, USA*

⁴*Ecology and Evolutionary Biology, School of Biosciences, The University of Sheffield, Sheffield S10 2TN, UK* and ⁵*Department of Marine Sciences, University of Gothenburg, 40530 Gothenburg, Sweden*

⁶*Institut des Sciences de l'Evolution de Montpellier ISEM, Univ. Montpellier, CNRS, IRD, Montpellier, France*

Erik B. Dopman, ORCID: 0000-0002-8633-5527, erik.dopman@tufts.edu

Kerry L. Shaw, ORCID: 0000-0001-8300-0425, kls4@cornell.edu

Maria R. Servedio, ORCID: 0000-0002-3965-4445, servedio@email.unc.edu

Roger K. Butlin, ORCID: 0000-0003-4736-0954, r.k.butlin@sheffield.ac.uk

Carole M. Smadja, ORCID: 0000-0002-7285-1408, carole.smadja@umontpellier.fr

Corresponding author:

Erik Dopman, erik.dopman@tufts.edu

Abstract

Coupling has emerged as a concept to describe the transition from differentiated populations to newly evolved species through the strengthening of reproductive isolation. However, the term has been used in multiple ways, and relevant processes have sometimes not been clearly distinguished. Here, we synthesize existing uses of the concept of coupling and find three main perspectives: 1) coupling as the build-up of linkage disequilibrium among loci underlying barriers to gene exchange, 2) coupling as the build-up of genome-wide linkage disequilibrium, and 3) coupling as the process generating a coincidence of distinct barrier effects. We compare and contrast these views, illustrate the diverse processes involved and the complexity of the relationships among recombination, linkage disequilibrium, and reproductive isolation, and finally, we emphasize how each perspective can guide new directions in speciation research. Although the importance of coupling for evolutionary divergence and speciation is well-established, many theoretical and empirical questions remain unanswered.

Introduction

For many biologists, the evolution of **barriers to gene exchange** (see [Table 1](#) for definitions of terms in **bold**) is the major process by which boundaries form between newly emerging species (Coyne and Orr 1998; Harrison 1998). An analysis of such barriers—their causes, order of appearance, and relative strength both individually and collectively—should therefore provide fundamental insights into the process of speciation. Barriers arise first within species, for example due to local adaptation, and are likely to influence gene flow only in specific regions of the genome (Wu et al. 2001). At the completion of speciation, barriers prevent gene exchange throughout the genome, potentially becoming irreversible. In between, there may be an extended period during which the extent of gene flow varies across the genome, even when reproductive isolation is strong enough to allow populations to coexist. Modern genomic data have given unprecedented insights into these patterns of gene exchange.

Newly evolved species in nature can rarely, if ever, be characterized by the divergence of a single **barrier trait** or the existence of a single barrier to gene exchange. It seems logical that an accumulation of multiple barriers should make for stronger **reproductive isolation** and thus movement towards the completion of speciation, provided different barriers somehow work together. Completion of speciation is hard to define but here we take it to mean the complete (or nearly complete) cessation of gene flow, throughout the genome, without reference to whether 1) isolation is reversible (Stankowski and Ravinet 2021) or 2) the new species persist and coexist (Germain et al. 2021). From a genetic perspective, many regard genome-wide divergence to signify that the speciation process has passed a ‘tipping point’ towards strong reproductive isolation (Flaxman et al. 2014; Nosil et al. 2017). Thus, it is tempting to hypothesize that the evolution of multiple barriers to gene exchange is often required to complete speciation, and is necessary to prevent species collapse when nascent boundaries around divergent populations are challenged, for example by secondary contact.

Coupling, described generally as any process causing different barrier traits, **barrier loci**, or **barrier effects** to operate together in ways that generate a stronger overall barrier to gene exchange, has emerged as a concept to describe the transition from differentiated populations to newly evolved species through the build-up of reproductive isolation ([Fig. 1](#)). What influences whether coupling will occur? To answer this question, we must keep in mind that barriers arise within species under the influence of well-known evolutionary forces. For example, selection may drive a change in phenotype to different local optima in different populations, due to varying fitness across ecological, sexual, or genomic environments (Endler 1986, Schneemann et al., this volume). The physical organization of genomes creates evolutionary constraints on the origin and fate of genetic variation, rates of recombination, and the genetic architectures underlying phenotypic variation, all of which have consequences for multilocus structure (Lynch 2007; Mérot et al. 2020; Seehausen et al. 2014). Furthermore, demographic history injects chance associations of variation across the genome (Slatkin 2008). All of these features of organisms and populations influence both the strengthening (or weakening) of individual barriers to gene exchange and the tendency of different barriers to operate together. Tackling these complexities, both theoretically and empirically, is necessary to understand how strong reproductive isolation evolves and speciation is completed (Kulmuni et al. 2021).

Perhaps due to the intriguing centrality of ‘coupling’ in understanding evolutionary divergence and speciation, multiple uses of the term have arisen in the literature, and at the same time relevant processes have sometimes been discussed but not specifically called coupling. Here, we aim to consolidate and explain the historical and contemporary uses of the concept of coupling, discuss the range of processes that fall under this umbrella, highlight their specific and complementary aspects, and emphasize how these alternative views can help to guide future directions in speciation research.

Main text

Three views of coupling

We identified three main perspectives on the concept of ‘coupling’ represented in the literature (Fig. 2). Perspective 1 derives from Felsenstein’s 3-locus model (1981) that considers coupling as a process that builds **linkage disequilibrium** (LD) among loci underlying specific barrier traits. Perspective 2, influenced by Barton’s work on multilocus cline analysis (Barton 1983) and increasing access to genome-wide differentiation data, extends coupling to the build-up of genome-wide LD across both barrier and non-barrier loci. Finally, Perspective 3 considers coupling as any process generating a coincidence of barrier effects, whereby coincidence can be considered at either the phenotypic/organismal or genetic level, with or without build-up of LD (Butlin and Smadja 2018).

As these brief descriptions make clear, there are diverse ways to think about the concept of coupling. Although coupling is predominantly studied at the genetic level (all perspectives), barrier effects reduce the production or fitness of hybrids at the organismal level of organization (Perspective 3). The genomic scale at which coupling is addressed also varies, from a restricted set of individual genes and traits (Perspective 1) to multiple loci, entire genomes and manifold phenotypic differences (Perspectives 2 and 3). Finally, although discussions of coupling primarily focus on the association of different barrier effects and their underlying loci, the concept can be fruitfully extended to include coupling among barrier loci underlying a single barrier trait or effect (e.g., local adaptation) (Perspective 1, implicit in Perspective 2; see also Ritchie and Butlin, this volume), or coupling among barrier and non-barrier loci (Perspective 2).

Despite their differences, the three perspectives are not mutually exclusive and have many commonalities, as all aim to characterize the nature of barrier accumulation during speciation. One uniting aspect is that coupling has been regarded as both a pattern and a process across all perspectives. Coupling can describe the association between barriers present at a given phase in their accumulation, for example, the extent to which barrier loci act non-independently and influence non-barrier loci at a point in time, a particular pattern of physical linkage among barrier loci, or the magnitude of correlation between distinct barrier traits. Alternatively, coupling can be used to describe the process by which LD builds up and/or is maintained or how barrier effects become coincident. As is commonly the case when investigating long-term evolutionary phenomena, the empirical studies we surveyed tended to document patterns of coupling and infer process indirectly, whereas the theoretical studies tended to deal specifically with the combination of factors potentially responsible for the process of coupling (e.g., number of barrier loci, selection strength, recombination rate).

We do not wish to argue that one of the three perspectives on coupling discussed here is generally preferable over the others. In common with other terms in the language of speciation (Harrison 2012), ‘coupling’ can have subtly variable technical uses. What is important is that this flexibility is recognised and that authors make their specific meaning clear when they use the term.

Perspective 1. Coupling as the build-up of linkage disequilibrium among barrier loci

The view of coupling as the build-up of LD among barrier loci underlying specific barrier traits has been the basis for some in-depth theoretical investigations into the importance of various factors in the evolution of reproductive isolation, and for the interpretation of empirical patterns (Fig. 2). Early conceptual development came from a highly influential theoretical paper by Felsenstein (1981),

although coupling as a term was not used or discussed in this context until several decades after its publication.

Influence of Felsenstein's model and its extensions

The non-random association of alleles at different loci (LD) is an integral part of most speciation models because it captures the degree to which gene pools separate into different groups, a necessary component for the evolution of new species. LD can build-up in many ways (Charlesworth and Charlesworth 2010, Chapter 8). Allopatry creates LD among loci that diverge in frequency between populations, selection for local adaptation can generate LD among locally-favored alleles, and assortative mating generates LD between signal and preference loci. However, Felsenstein (1981) was the first to emphasize, explicitly, how progress towards speciation could be illustrated by the build-up of LD between barrier loci: two reducing fitness in hybrids (resulting from extrinsic incompatibilities due to local adaptation, loci B and C) and one for assortative mating (locus A). By doing so, he highlighted key factors influencing this process. First, a combination of migration and direct selection on the two local adaptation loci generates some LD between them. Second, indirect selection on the assortative mating locus due to initial LD with the local adaptation loci reinforces assortative mating. In turn, this enhances LD between local adaptation and assortative mating loci.

As further evolution of prezygotic isolation and LD among barrier loci depends on the balance between the selection coefficient at the ecological loci and recombination rates in the system, this work also clarified the role of favorable genetic architectures of barrier traits to minimize the influence of recombination on LD between post- and prezygotic barrier loci. Over the subsequent decades, Felsenstein's model had widespread influence on theoretical and empirical developments, including theoretical models and empirical studies of **reinforcement**, studies addressing the genetic basis and architecture of barrier traits, and the role of reduced recombination (reviewed in Butlin et al. 2021).

Although neither Felsenstein nor most later developers adopted the term 'coupling', Barton and de Cara (2009) and later Butlin and Smadja (2018) made explicit links between the build-up of LD among post- and prezygotic barrier loci and what they referred to as coupling processes. Barton and de Cara (2009) first expanded this view by showing that coupling of existing **two-allele barrier effects** enhances mean fitness in a very general way in unstructured populations, for any form of barrier effect, in any combination. Butlin and Smadja (2018) classified this and other types of reinforcement (classical or extended views, e.g., Felsenstein 1981, Kirkpatrick and Servedio 1999, Servedio 2009) as 'adaptive coupling' where the coincidence of barrier effects (and most of the time LD among barrier loci) is favored by selection (Fig. 3), but they extended the coupling framework further by arguing that coincidence and the build-up of LD can evolve as a by-product of other processes (Figs. 3, 4). Coupling viewed as the build-up of LD among barrier loci can therefore include non-adaptive scenarios and any possible associations among barrier loci underlying the same or different barrier effects, and among barrier loci underlying the same or different barrier traits of any type.

Deterministic and incidental factors favoring the build-up of LD among barrier loci

Support for certain components of adaptive coupling has been found in natural systems. Coincidence of post- and prezygotic barriers at the phenotypic level has been documented, specifically in areas where selection against hybridization is acting (i.e. reinforcement cases, Fig. 3C and reviewed by Servedio and Noor 2003; Hopkins 2013; Butlin and Smadja 2018). However, studies investigating LD between post- and prezygotic barrier loci and the effect of indirect reinforcing selection on prezygotic barrier loci in a reinforcement scenario remain rare (but see in *Phlox*, Hopkins and Rausher 2012 or house mice, Smadja et al. 2022). Interesting parallels occur in the context of spatial clines, where

enhancement of LD among barrier loci may evolve in response to indirect selection. In hybrid zones, theory predicts that overlapping clines of two barrier loci will tend to attract one another (Slatkin 1975; Barton 1983; Bierne et al. 2011). Attraction occurs because dispersal produces LD where clines overlap, which in turn generates indirect selection on the two loci and the further build-up of their LD. The effect is spatially asymmetric, making the clines move toward one another until their centers are coincident (e.g., Fig. 2 of Butlin and Smadja 2018). Cline attraction, therefore, reflects a process of coupling through the build-up of LD among barrier loci due to indirect selection. In line with Barton and de Cara's predictions, any combination of barrier loci (prezygotic, postzygotic, intrinsic, extrinsic) can become spatially 'coupled' (Bierne et al. 2011). We do not see 'spatial coupling' as distinct from other forms of coupling but we do encourage authors to distinguish clearly between LD within demes and LD among demes.

LD among barrier loci can also build-up as a by-product of other, non-adaptive processes ('by-product coupling,' Butlin and Smadja 2018). In reinforcement, for example, the need in some scenarios for initial LD between post- and prezygotic loci to drive further LD highlights the importance of generally understanding diverse processes generating LD, beyond adaptive responses (discussed further in Perspective 3). Allopatric divergence followed by the mixing of the divergent populations is one such process (Barton and Hewitt 1985) (Fig. 3A). More generally, long-term discontinuities in the distribution range (allopatry but also temporal disjunctions, partial extrinsic barriers, areas of low densities or any source of population structure) delay the spread of new mutations and therefore contribute to the creation of distinct allelic pools (Mallet et al. 2009). The mixing of these populations, through dispersal or erosion of the discontinuity, will then create LD between divergent loci, including potential loci involved in reproductive isolation. More transient changes in the distribution range can also bring together alleles that originate in different parts of a distribution (e.g., by human-mediated transport or habitat alteration, see Ålund et al., this volume), hence generating LD (Hewitt 1989; Lucek and Willi 2021) (Fig. 3B). In all geographic contexts, a variety of processes within populations such as the stochastic effects of mutation and genetic drift, the effects of selection, mating system and non-random mating can also create some degree of LD that can affect barrier loci incidentally (Ohta 1982; Kirkpatrick 1982; Barton 1995). Different regimes of such within-population processes operating independently in different populations may therefore create LD among barrier loci if populations mix. This includes divergent selection regimes acting independently on different barrier traits or loci but at coincident environmental boundaries, generating LD among barrier loci and resulting in a stronger overall barrier than would be caused by selection on any single trait or locus in isolation (Rice and Hostert 1993; Nosil et al. 2009; Nosil 2012, i.e., 'multifarious selection,' but see White and Butlin 2021). However, the full range of these processes generating LD has not been investigated systematically in the context of reproductive isolation.

Empirically, LD among barrier loci can only be confirmed once barrier loci have been identified. Approaches such as QTL, GWAS, genetic mapping, and genome scans of differentiation have been used to assess the genetic basis of specific barrier traits, although the identification of the causative genes and variants remains challenging (e.g., Wu and Ting 2004; Wolf et al. 2010; Kitano et al. 2022; Merrill, this volume). Still, to document LD among barrier loci and understand the factors favoring (or allowing) it, an effort to characterize them is essential. For at least one barrier effect, positive assortative mating, gene identification can potentially be facilitated by the coupling process itself because LD is expected to build at the loci underlying assortment (Kirkpatrick 1982), allowing for a 'genome scan of assortative mating' (Unbehend et al. 2021). A nice example of such resolution is provided by pheromone blend and pheromone response loci in the European corn borer moth, *Ostrinia nubilalis*, which are in strong LD between E- and Z-race populations despite being on different chromosomes, probably due to non-random mating (Unbehend et al. 2021). Similar patterns of long-range LD are found in reef fish for chromosomal intervals associated with visual-based non-random mating (Hench et al. 2019). In the corn borer moth system, Künther et al. (2022) also found elevated LD between unlinked loci underlying two different barrier effects, temporal and sexual

isolation, when the two barriers coincide in space compared to when they do not. More studies estimating LD among barrier loci across varying geographic conditions within species ranges are needed to gain insight into the factors promoting the build-up of LD in natural systems.

The role of recombination and genetic architectures: enhancement of LD is not universal

Although LD among barrier loci does not require physical linkage (Barton and de Cara 2009), the rate at which it builds up or is maintained in the presence of gene flow depends on recombination, as is discussed above. Many studies have thus explored how specific genetic architectures and recombination patterns—from pleiotropy through close physical linkage to large regions of reduced recombination—can facilitate coupling and whether selection favors modifiers of these patterns. Cases of **pleiotropy**, in the context of speciation, can correspond to situations where one allele affects more than one barrier trait, at least partly removing the necessity of building up LD among different barrier loci (Barton et al. 2007; Smadja and Butlin 2011; Ritchie and Butlin, this volume). **Magic traits** (Gavrilets 2004, Servedio et al. 2011; Maan and Seehausen 2012), **multiple-effect traits** (Smadja and Butlin 2011), and **one-allele mechanisms** (Felsenstein 1981) can also reduce or remove the role of LD in promoting reproductive isolation at equilibrium; they are discussed below (Perspective 3) (Fig. 4). Here, we focus on cases of two-allele barrier traits that have distinct genetic bases, even if empirically it can be difficult to distinguish strict cases of pleiotropy from very tight physical linkage (e.g., Ritchie and Butlin, this volume).

Tight physical linkage and other mechanisms, such as inversions, that reduce recombination between loci contributing to components of reproductive isolation have been found to promote speciation in certain contexts (Kirkpatrick and Barton 2006; Yeaman and Whitlock 2011; Ortíz-Barrientos et al. 2016; Schuldiner-Harpaz et al. 2022). In line with these theoretical predictions, some evidence has been found, via QTL or genomic approaches, for tight physical linkage among loci underlying different types of barrier effects, for example, between loci underlying local adaptation and mate preference (e.g., *Heliconius*: Merrill et al. 2011, 2019; *Gasterosteus aculeatus*: Bay et al. 2017) or habitat choice and local adaptation (e.g., pea aphids: Hawthorne and Via 2001). While some level of LD between mating trait and mate preference loci will arise as a consequence of non-random mating itself (Kirkpatrick 1982) and does not require physical linkage (e.g., Unbehend et al. 2021), tight linkage is sometimes found between trait and preference loci (e.g., *Laupala* crickets Xu and Shaw 2019). Finally, tight linkage is also found among loci underlying a single barrier trait (local adaptation: e.g., Roda et al. 2017; mating signal: e.g. *Heliconius*: Byers et al. 2021; *Laupala* crickets Xu and Shaw 2021; mate preferences: house mice: Smadja et al. 2022; pollinator syndrome: *Petunia*: Hermann et al. 2013), suggesting that coupling at this level might also be important to consider. QTL for multiple barrier traits map to inversions in some taxa, such as *Mimulus* (Lowry and Willis 2010), *Helianthus* (Huang et al. 2020) and *Littorina* (Koch et al. 2022) (see also Berdan et al., this volume on chromosomal speciation).

Although Felsenstein stressed the role of recombination in hindering speciation in his 1981 model, a closer look reveals that the effects of recombination on LD depend on the loci being compared (Fig. 5). Recombination between assortative mating and local adaptation loci does hinder the build-up of LD between them (D_{AB}), thus opposing ‘progress’ towards speciation. However, recombination between the two local adaptation loci, while eroding their LD (D_{BC}), increases the production of low fitness genotypes, the necessary source of selection for reinforcement and therefore the build-up of even greater LD between assortative mating and local adaptation loci (D_{AB} , Fig. 5). Therefore the build-up of LD between barrier loci does not always equate with more reproductive isolation, and recombination does not always work against speciation because the consequences of coupling (if defined as LD among barrier loci) depends on the function of the barrier locus. An erosion of LD

between certain barrier loci (reduced coupling) may be required for greater LD (enhanced coupling) between others.

Other models exploring the effects of recombination between loci involved in reproductive isolation have come to similarly inconsistent conclusions, both regarding the evolution of divergent allele frequencies between populations and the amount of LD maintained between them. A curve describing the equilibrium values of LD between genes controlling mating preferences and magic traits, for example, does not change with a decreasing recombination rate in a two-island model (Servedio and Bürger 2018). In contrast, under the same geographic assumptions, tighter physical linkage between ecological and mating trait loci directly increases LD between them at equilibrium, causing tightly linked sets of such loci to mimic magic traits in their effects on population divergence. Furthermore, Aubier et al. (2023) found that intermediate rates of recombination between such ecological and mating traits promoted the evolution of the strongest choosiness in a female choice model, provided that the allele for stronger choosiness is also physically linked to the mating trait locus. Finally, Aubier et al. (this volume) find that tighter physical linkage between components of different sets of preference and trait loci that each, independently, lead to premating isolation, has mixed effects on equilibrium levels of LD. In fact, when physical linkage between functionally different preferences and between functionally different traits is very tight, LD between the preferences and LD between the traits can evolve to be negative, due to complicated interactions that lead to recombinant males having the highest mating success. Because Shuldiner-Harpaz et al. (2022) find, in a separate study, that reduced recombination between mating preferences and ecological traits is expected to evolve under some conditions, we might expect these counterintuitive effects on coupling to occur not uncommonly in natural systems. Reduced recombination can therefore both promote and inhibit the buildup of LD depending on the particulars, making this area open for further research.

Inversions, which reduce recombination among loci that they capture, have been argued to promote the maintenance of LD among barrier loci (e.g., Noor et al. 2001; Rieseberg 2001; Butlin 2005; Kirkpatrick and Barton 2006; Feder and Nosil 2009). Trickett and Butlin (1994) examined the spread of an inversion capturing ecological and mating loci using a modification of Felsenstein's (1981) model, and found that physical linkage in an inversion will lead to a lower strength of assortative mating necessary in order to establish stable LD between the loci in the system (in this case 3-way LD). Feder and Nosil's (2009) simulation study found that only inversions that allow for exceedingly low recombination rates are particularly effective in promoting divergence in the frequency of alleles between populations, although Rafajlović et al. (2021) found that introducing genetic drift allowed substantial divergence to persist.

Aside from these static influences of recombination, several studies have found that the process of divergence or speciation with gene flow actually selects for modifiers of recombination through co-localization, either by pleiotropy or tight linkage, of loci underlying barrier traits. An evolutionary lowering of the recombination rate is expected between locally-adapted pairs of loci under broad conditions (Lenormand and Otto 2000; in the context of speciation this would follow the "adaptive-extrinsic" model of recombination reduction *sensu* Butlin 2005). The evolution of reduced recombination rates can occur in different ways, including the spread of linked or unlinked modifiers (e.g., Nei 1967) or the spread of inversions that capture the genes of interest. Models of local adaptation also show that clustering of locally favorable alleles may arise due to the physical transposition of loci from one chromosomal position to another (Ortíz-Barrientos et al. 2016; Yeaman 2013) or by changes in gene order following chromosomal rearrangements (Ortíz-Barrientos et al. 2016). The spread of an inversion that captures the genes of interest has been examined extensively in a speciation context when such inversions capture pairs of genes causing hybrid inviability or sterility (Noor et al. 2001; Feder and Nosil 2009) and when they capture sets of loci that lead to local adaptation (Trickett and Butlin 1994; Rieseberg 2001; Kirkpatrick and Barton 2006; Feder and Nosil

2009; Bürger and Akerman 2011; Charlesworth and Barton 2018; Mackintosh et al. 2022; for general review see Butlin 2005).

Perspective 1 concluding remarks

Perspective 1 specifically introduces a dichotomy between adaptive coupling, where non-random associations of barrier loci enhance fitness, and by-product coupling where LD increases as a consequence of any non-adaptive process (divergence in allopatry, migration and hybridization, population range shifts, recombination suppression, etc.). The main goal of recent studies has been empirically characterizing LD among barrier loci, and elucidating, often by mathematical modeling, the numerous factors initiating, maintaining, and enhancing LD, but the focus, thus far, has been on selective processes. As our review makes clear, neither coupling nor the effects of low recombination rates have been examined systematically, in terms of the types of loci involved and the geography of speciation. Low recombination does not always lead to high LD and low LD between one pair of barrier loci can correlate with stronger LD between other pairs of barrier loci. Evolutionary change in the overall barrier to gene exchange is then hard to predict. The simple view that coupling and LD are equivalent and that greater coupling leads to stronger reproductive isolation may need to be modified. As more than one coupling process may generally be at play, a strength of this perspective on coupling is that it allows the dissection of the myriad processes potentially responsible for the build-up of LD among specific barrier loci, traits, or effects. Such in-depth investigation can come at a cost, however, as it may not be possible to investigate all levels of LD (Smadja and Butlin 2011) or the full complement of barrier effects in a given system or theoretical model. A careful analysis of organismal life history can help guide study of the strongest and/or most important barrier effects (Perspective 3), but a genomic analysis of barrier coupling is also necessary to understand how barrier effects influence the build-up of LD at non-barrier loci (Perspective 2) (e.g., see Figs. 7, 8 in Künnerth et al. 2022).

Perspective 2. Coupling as the build-up of genome-wide linkage disequilibrium

The second perspective from which coupling has been considered is as a build-up of genome-wide LD, which affects both barrier and non-barrier loci (Fig. 2). We see this view as an extension of Perspective 1. Specifically, the effects of selection and recombination described above are envisioned to apply across many barrier loci, leading to patterns of coupling at genome-wide scales.

Factors driving the build-up of genome-wide LD

In 1983, Barton argued that multilocus behavior in hybrid zones depended on a parameter called the ‘coupling coefficient’, which describes the conditions under which all barrier loci act independently or tend to act together and affect gene flow at loci throughout the genome (Barton 1983). Either in a two-population model or in continuous space, a single barrier locus only impedes gene exchange at very closely-linked neutral loci ($r < s$, where r is the recombination rate between the neutral and the selected locus and s is the fitness reduction in a foreign environment or a foreign genetic background). When there are many barrier loci, the effect on gene flow at neutral loci depends on the ratio of total selection to recombination (the **coupling coefficient** $\theta = S/R$, where $S = ns$ and $R = nr$ for n barrier loci and r is now the recombination rate between adjacent barrier loci; Barton and Bengtsson 1986). There is a critical value of θ above which all barrier loci are in strong LD and so tend to act together and below which they act independently. In continuous space, the transition from independent to ‘coupled’ behavior results in the formation of stepped clines in allele frequency in which the steep central portion occurs because each locus experiences the total selection, S , rather than the locus-specific selection, s . When coupling is strong, neutral loci also experience substantial indirect

selection. However, the barrier experienced by neutral loci increases more continuously as θ increases (Barton 1983; Barton and Bengtsson 1986).

Multilocus cline theory was developed in the context of secondary contact where all of the barrier loci considered have alleles in population 1 that reduce fitness in population 2 for some reason (maladaptation, incompatibility with the local genetic background, difficulty in finding mates). In effect, initial strong LD among barrier loci was assumed as a result of divergence in allopatry followed by admixture, and coupling describes the process by which LD induces indirect selection on each barrier locus in addition to direct selection, leading to stronger overall selection. Critically, since initial LD is assumed, all processes that result in the original formation of these associations at barrier and at non-barrier loci (see Perspective 1, 3) could also be considered as part of the coupling process.

The development of ‘genome scan’ approaches to document the genomic distribution of barrier effects (reviewed by Ravinet et al. 2017), combined with Wu’s (2001) ‘genic view’ of speciation and a shift in focus towards divergence with gene flow, led to the creation of a related but distinct terminology and to a series of simulation studies that also address how the number of barrier loci in LD can increase and so influence the overall barrier to gene flow throughout the genome (Feder et al. 2012). ‘Divergence hitchhiking’ describes the local barrier effect around one, or a few closely-linked barrier loci that results in a peak of differentiation relative to the genetic background (an ‘island of differentiation’; Turner et al. 2005; Dopman et al. 2005; Via and West 2008). Theoretical expectations for a single locus (Charlesworth et al. 1997) were extended using simulations to situations with many loci (Feder and Nosil 2010). With enough loci and sufficiently strong selection, Feder and Nosil observed genome-wide barrier effects, which they dubbed ‘genomic hitchhiking’. Further theoretical developments investigated the mechanisms favoring the formation of islands of differentiation and the spread of barrier loci (Yeaman 2013, Yeaman et al. 2016). The term ‘coupling’ was not used in any of this work but it could be applied to either the process of genomic hitchhiking or the process of recruitment of new barrier loci from divergence hitchhiking mechanisms up to genomic hitchhiking mechanisms.

Flaxman et al. (2014) also investigated the dynamics of genomic differentiation over the course of a speciation process. They modeled divergence in a two-deme system with divergent selection and many loci that could mutate to locally-beneficial alleles, thereby allowing for an increasing number of barrier loci. Divergence was initially gradual but could undergo a transition to much more rapid divergence across the genome implying that a critical threshold value of Barton and Bengtsson’s (1986) θ was reached. This coupling required LD among sets of divergently selected alleles but it could occur without physical linkage of barrier loci on chromosomes, and it was marked by a rapid increase in genome-wide LD. Nosil et al. (2017) found that ‘bi-stability’ might occur in some parts of parameter space, which would allow external factors such as brief interruptions to migration to precipitate rapid switches from independent to coupled behavior. Flaxman et al. (2014) called the transition ‘genome-wide congealing’ but it has also been called ‘coupling’ (Flaxman et al. 2013; Schilling et al. 2018). Schilling et al. (2018) emphasize a distinction in behavior between selected loci, for which they observed a sharp transition, and neutral loci, which experienced a relatively smooth decrease in effective migration, again reflecting the earlier predictions of Barton and Bengtsson (Barton 1983; Barton and Bengtsson 1986).

In all of these scenarios, coupling can be used to describe the build-up (or maintenance) of LD among multiple barrier loci that enhances both differentiation at those loci and the barrier to gene flow in the rest of the genome (Fig. 1C). This is the general sense in which ‘coupling’ is used by Nosil et al. (2021). Like some other authors, they use ‘genomic coupling’ or ‘genome-wide coupling’ when the effect encompasses loci throughout the genome. In some cases, a sharp transition in divergence behavior of barrier loci is expected as the coupling coefficient, θ , passes a critical value. Before this transition, coupling may be evident as associations of barrier loci in LD, and associated genomic regions with

elevated neutral differentiation. Beyond this transition, the barrier to gene flow at neutral loci is expected to be much more genomically-widespread but this may not be immediately apparent in the genome-wide level of differentiation at neutral loci.

Evidence for the build-up of genome-wide LD

LD among multiple barrier loci is undoubtedly common in nature. It is expected, and observed, following secondary contact (Barton and Hewitt 1985). Hybrid zones formed in this way provide an opportunity to ask whether LD is maintained or whether it breaks down when the opportunity for gene flow and recombination occurs (evidence from hybrid zones is reviewed by Firneno et al., this volume). Similarly, studies of local adaptation frequently imply roles for multiple traits, and multiple loci per trait, that are differentiated between populations living in different environments, and so are in LD species-wide, as well as within populations that are connected by migration (see Table 1 in Bierne et al. 2011). This LD may have been generated by divergence in allopatry (as in many hybrid zones) or may have been built up by selection *in situ* (e.g., *Gasterosteus* lake-stream sticklebacks, Marques et al. 2016; *Senecio*, James et al. 2021), perhaps aided by gene flow (e.g., marine-freshwater sticklebacks, Jones et al. 2012) or introgression (e.g., *Heliconius*, The *Heliconius* Genome Consortium 2012). Rather few of these patterns have been resolved to causal loci and this is likely to be difficult where there are many barrier loci.

Some empirical evidence for sharp transitions from largely-independent behavior of barrier loci to genome-wide coupling is also available. Hybrid zones typically have broadly coincident allele frequency clines at many divergent loci as a result of secondary contact, physical barriers or steep environmental transitions (Barton and Hewitt 1985). However, these clines may be sigmoid and vary in width and precise geographical location, suggesting that they are responding independently to direct selection or selection on nearby loci. Alternatively, clines may be stepped (i.e. steeper nearer the center) and concordant (i.e. centered at the same place and of the same width), suggesting genome-wide coupling. Classic examples are the hybrid zones in mouse (*Mus musculus* and *M. domesticus*, Janoušek et al. 2012) and in toads (*Bombina bombina* and *B. variegata*, Szymura and Barton 1986), respectively, but the distinction is not always clear-cut (e.g., Vines et al 2016). In principle, coupling may apply only to a subset of loci, but this pattern has rarely been reported (*Hippocampus* seahorses, Riquet et al. 2019; *Anopheles* mosquitoes, The *Anopheles gambiae* 1000 Genomes Consortium 2017; and see above for effects of chromosomal rearrangements). Bimodality of phenotypes in the central parts of hybrid zones implies strong LD and also indicates strong isolation, perhaps where behavioral isolation is coupled with selection against hybrids (Jiggins and Mallet 2000).

Nosil et al. (2017) argued that the threshold transitions seen in multilocus models (Flaxman et al. 2014; Barton 1983; Barton and Bengtsson 1986) should be reflected in observed patterns of genome-wide divergence: either populations should show generally low divergence, perhaps with some islands of differentiation, or they should show high levels of divergence genome-wide, but intermediate patterns will be rare or absent. Firneno et al. (this volume) test this expectation in a hybrid zone context. A survey of >100 populations from 11 species of walking stick uncovered patterns consistent with this prediction (Riesch et al. 2017): genome-wide average F_{ST} fell either below 0.3 or above 0.6 but not in between, and strong differentiation was associated with coupling of polygenically controlled prezygotic isolation, rather than simply the build-up of local adaptation.

Perspective 2 concluding remarks

Coupling viewed as the build-up of genome-wide LD introduces the importance of understanding how LD among multiple barrier loci impacts the total barrier to gene flow and the parts of the genome not directly involved in reproductive isolation. Importantly, this view hypothesizes that indirect selection, as an emergent property of non-random associations among selected barrier loci,

creates a threshold or tipping point leading to two alternative stable states, motivating new research directions on the dynamics of coupling processes and conditions favorable to either gradual or sudden transitions to genome-wide independence. Pursuing the effort of estimating genome-wide LD/differentiation, the coupling coefficient, and cline shapes in empirical cases reflecting various stages in the speciation process will certainly help to clarify these conditions. However, a full understanding of coupling at large genomic scales also requires connecting genome-wide patterns of LD with the number and nature of the barrier traits involved, which is still rarely achieved (Perspective 1, 3). Where a strong overall barrier influences the whole genome, it may be hard to distinguish loci directly involved in reproductive isolation from loci experiencing genome-wide barrier effects, implying that the capacity to distinguish coupling processes diminishes as speciation progresses. In contrast, intermediate cases, where some loci show strong differentiation or stepped clines whereas others do not, open the opportunity to identify individual barrier loci, the extent of coupling between them, and potentially the associated phenotypes. Combining, in the same biological system, phenotypic and genetic characterisation of barrier traits with genome-wide analyses will further help in bridging this gap (Perspective 1, 3).

Perspective 3. Coupling as any process generating coincidence of barrier effects

The first two views consider coupling at the genetic level only, with a focus on LD among barrier loci. We identified a third view that encompasses these aspects while extending the concept of coupling beyond LD and the genic level to the organismal and phenotypic level (Fig. 2). Butlin and Smadja (2018) defined coupling as any process generating coincidence of distinct barrier effects. If any given barrier effect can be considered to define two populations that experience a reduction in gene flow, then ‘coincidence’ means that two or more barrier effects define the same pairs of populations (in geographic or niche space or in time). The focus on distinct barrier effects includes LD across loci contributing to different barriers but excludes LD among loci that are together responsible for any one barrier effect. Thus, LD between a mating signal locus and a mating preference locus would be excluded from this definition of coupling. This specific type of association is explored by Ritchie and Butlin (this volume) (‘genetic coupling’ of Alexander 1962; Butlin and Ritchie 1989).

A phenotypic focus

With its unique emphasis on the phenotypes (‘barrier effects’) that help keep populations distinct, this view is able to make meaningful inferences about speciation by documenting patterns of barrier coincidence (e.g., Table 1 in Bierne et al. 2011; Dopman et al. 2010; Sánchez-Guillén et al. 2014; Karrenberg et al. 2018), without knowledge of the underlying genotypes. A phenotypic focus is valuable because there are at least three forms of barrier coincidence that strengthen the overall barrier without leaving a detectable change in LD at causal barrier loci, the main signature of coupling ‘progress’ adopted by the first two views. Multiple-effect traits (Smadja and Butlin 2011) are cases where a single phenotypic trait influences multiple components of isolation (Fig. 4B). Thus, coincidence of barrier effects is a simple consequence of divergence in a single trait contributing to any combination of barrier effects. Convincing examples include temporal isolation arising from divergent selection on diapause phenology in insects (Kozak et al. 2019; Inskeep et al. 2022), ecological and mating isolation arising from mimetic selection and mate signaling divergence in wing color pattern in *Heliconius* butterflies (Kronforst et al. 2006; Chamberlain et al. 2009), and ecological and mating isolation arising from body size divergence in an experimental selection study of body size evolution in feather lice (Villa et al. 2019).

Coincidence can also occur without requiring LD if an allele has pleiotropic effects on two or more barrier traits, each contributing to a different barrier effect (Fig. 4A). Possible examples of pleiotropy

include loci that influence both male and female body size in fish or both daily and seasonal mating time in insects, but discriminating pleiotropy from tight linkage is an empirical challenge that may require confirmation by genetic modification of candidate genes (see Ritchie and Butlin, this volume). Differentiating multiple-effect traits from pleiotropic effects is useful because the two may differ in their propensity for coupling under spontaneous mutation. The mutation of any gene underlying a multiple-effect trait will alter all barrier components influenced by the trait and therefore each genetic mutation will contribute to coupling. In contrast, coupling of barrier effects will only increase due to pleiotropy for the subset of substitutions in genes underlying the traits that have pleiotropic effects. This potential for a larger mutational target size for direct coupling could make multiple-effect traits common contributors to speciation.

The idea of magic traits encompasses both multiple traits that are connected by pleiotropy (partially or completely) and multiple barrier effects that are connected via underlying traits (Servedio et al. 2011; Maan and Seehausen 2012). Although originally applied to the combination of pre- and post-zygotic isolation, it can be applied more generally. For many theoretical purposes, pleiotropy can be considered as equivalent to zero recombination between loci involved in different barriers, and it may not be necessary to specify the phenotypic traits that link genes to barrier effects. Therefore, the distinction between pleiotropy and multiple-effect traits assumes less importance.

Finally, enhanced coincidence of barrier effects can occur without increased LD, at least at the end of the process, through a one-allele mechanism (Felsenstein 1981 and see discussion in Butlin et al. 2021). In this case a barrier effect results from evolution of the same phenotype in both diverging populations (Fig. 4C). For example, evolution of greater philopatry in each of two populations will decrease gene exchange between them. However, since there is no divergence in this trait (i.e., greater philopatry evolves in both populations), no LD accrues between this trait and others. In many cases, one-allele effects can be seen as modifiers of existing barrier effects, which falls outside a strict interpretation of coupling as the coincidence of distinct barrier effects. Nevertheless, these modifier effects can be critical for the development of strong reproductive isolation, as in, for example, increased choosiness in mate preference or evolved imprinting mechanisms (Kopp et al. 2018), or where habitat choice arises in response to local adaptation (Berner and Thibert-Plante 2015). The plausibility of one-allele effects is strongly supported by theory (e.g., Kirkpatrick 2000; Servedio 2000, Kelly and Noor 1996) and while numerous possible examples have been described (Butlin and Smadja 2018; Kopp et al. 2018), few candidate one-allele genes have been identified (e.g., Ortíz-Barrientos and Noor 2005, a case where the one-allele effect might be considered a modifier of an existing component of behavioral isolation).

Population processes and initial LD

The theoretical models guiding research under the previous two perspectives often assume initial LD among barrier loci (Felsenstein 1981; Barton 1983). Perspective 3 specifically emphasizes how such associations may originally form as a by-product of population processes. In 1989, Hewitt convincingly argued that adaptive coupling, even within the context of hybrid zones, will be subsequent and secondary to any distribution range shifts that bring together spatially scattered barriers (Hewitt 1989). For example, extended periods of allopatry (e.g., on islands) allow modes of evolution rarely important in hybrid zones or in sympatry but which may help explain the initial origins of LD when populations meet. Foremost are situations of uniform selection (mutation order, Mani and Clarke 1990; Schluter 2009) or drift within isolated populations. Drift has limited empirical support for the evolution of barrier effects (Rice and Hostert 1993), but a wide range of conditions appear to facilitate the evolution of barriers to gene flow under uniform selection (Nosil and Flaxman 2011) and at least some ecologically-similar species pairs are thought to evolve by mutation-order processes (birds, Price 2007; *Senecio* wildflowers, Melo et al. 2019; vertebrates, Anderson and Weir 2022).

Information on the history of divergence and gene flow (e.g., Sousa and Hey 2013; Green et al. 2010) can indirectly inform us about the likelihood of coupling as a consequence of long-term allopatry or as an adaptive response, since adaptive coupling requires hybridization and recombination to expose alternative allele combinations to selection. Accumulating examples of divergence with gene flow in empirical systems suggest that conditions for adaptive coupling might be somewhat common (e.g., Roux et al. 2016). However, the possibility of periodic phases of allopatry probably cannot easily be discounted. Such transient changes to the distribution range might be one of the most probable forms of by-product coupling, considering theory suggests different barriers will likely have arisen in different places (Coyne and Orr 2004; Barton and Hewitt 1981, 1985; Hewitt 1989) and both palaeoclimatic evidence and contemporary observation show that changes in distribution range are commonplace over time (Hewitt 2000; Sardain et al. 2019; Capinha et al. 2015). Global cycles of climate fluctuation over the last 2.4 million years almost certainly resulted in population contractions and expansions that helped establish contemporary patterns of genetic structure and likely, initial coincidence of barriers that originally evolved in separate populations (Hewitt 2000, 1996; Searle 1993). A recent theoretical study of speciation with periodic gene flow (Linck and Battey 2019; 'MIM' model of He et al. 2019) emulated cycles of population expansion and contraction under Pleistocene glacial cycles and showed that speciation via DMIs occurred at rates that approached strictly allopatric speciation. More simulations of this type are needed, as it is unclear how other barrier effects (local adaptation, assortment, one- vs two-allele mechanisms) or coupling processes (reinforcement, introgression) might alter coupling dynamics.

If barriers do often arise in different places, for example from local adaptation and/or spatial discontinuities that slow the spread of mutations, populations separated by greater geographic or ecological distance are predicted to show stronger divergence for individual barrier traits/effects as well as greater correlations across distinct barrier traits/effects. Patterns of isolation-by-distance are common for neutral loci, and the same ought to be true for locally advantageous mutations and mutations whose spread is limited (Barton 2013). However, rather few studies have investigated these *a priori* expectations for barriers, such as a general increase in reproductive isolation with distance between populations (Edmands 2002). One rare case found this pattern for ethological isolation in a classic study of salamanders, *Desmognathus ochrophaeus* (Tilley et al. 1990). Developing the capacity to detect this and other coupling processes is an area in need of further research. Overall, broadening our understanding of the geographic distribution of barrier traits, effects, or loci should help explain the initial source of LD among barrier loci, a key assumption of many speciation models.

Perspective 3 concluding remarks

Coupling as the coincidence of distinct barrier effects between the same populations emphasizes important ways that coupling can occur without the build-up of LD and as a by-product of population processes. As our review indicates, distinguishing why coupling occurs in nature will usually require information on both barrier traits and effects (distinguishing multiple-effect traits, pleiotropy, one- vs two-allele mechanisms) and their geographic distribution as well as population history. This information is still quite rare in hybrid zone studies, where genomic patterns across geography, not barrier effects, are emphasized, but it is also rare in 'components of isolation' studies that document barrier effects and coincidence but rarely (geographic) variation in barrier interaction. Nevertheless, both of these empirical frameworks represent a strong base upon which to document variation in levels of barrier coincidence across the distribution of interacting taxa. By combining genomic analyses in these expanded systems, along with environmental and biogeographic data, adaptive (e.g., reinforcement, attraction of overlapping clines) and by-product (e.g., range shift, simultaneous evolution) hypotheses of coupling processes responsible for strengthening the overall barrier can be evaluated.

General conclusions and outlook

General agreement exists between theoretical predictions and empirical evidence for coupling. However, few studies have managed to identify and establish the relative roles of the various factors generating LD in an empirical system, and gaps remain for empirical support of certain aspects of theory. For example, more empirical tests are needed for by-product coupling, and for forms of adaptive coupling other than classic reinforcement. Examples of barrier effect coincidence or lack of coincidence ([Fig. 1B](#)) need to be combined with tests of predictions at the genetic level ([Fig. 1C](#)) (as in the mouse and *Phlox* examples, and in Kunerth et al. 2022). An alternative approach is to test the prediction that recombination rate variation around the genome will influence the pattern of introgression for polygenic barriers because the coupling of multiple barrier loci in regions of low recombination will impede introgression. There is evidence for this pattern in *Heliconius* butterflies (Martin et al. 2019), in *Xiphophorus* swordtail fish and in humans (Schumer et al. 2018).

Gaps also remain for theoretical conditions favoring coupling. New opportunities exist to establish the likelihood and existence of additional forms of coupling under the ‘extended view’ of reinforcement (e.g., a postzygotic barrier evolving in response to an existing premating barrier) (see Fig. 3 in Butlin and Smadja 2018), and the complex relationships between recombination, LD and barriers to gene flow need to be explored systematically. Finally, in addition to establishing its mechanisms, uncertainties persist about coupling’s pace, particularly the possibility of rapid transitions between states.

We provide further ideas for future work in [Table 2](#). These and other studies of coupling promise to bring us closer to an understanding of the origin of new species when strong reproductive isolation requires the evolution of multiple barriers to gene exchange. Although the importance of coupling for the speciation process seems well-established today, the speciation community’s view has only recently changed in the last decades as it has become clear that no single barrier is likely to lead to complete isolation and the coincidence of barriers is no longer seen as inevitable (as it is under strictly allopatric speciation). A greater emphasis on the dynamics of coupling during later-stage speciation therefore represents an exciting new area of growth for the field of speciation biology.

Acknowledgments

Tufts University provided support for this project to EBD. The CNRS provided support for this project to CMS. This is publication n° ISEM 2023-145. RKB acknowledges support from the Leverhulme Trust (RPG-2021-141) and the Swedish Research Council (2018-03695). MRS was supported by the National Science Foundation Grant DEB 1939290. KLS acknowledges support from National Science Foundation Grant IOS 2128521.

Author contributions

All authors conceived of, designed, and executed the overarching research goals. MRS re-analyzed Felsenstein’s (1981) mathematical model. EBD, CMS, and MRS created data visualizations. All authors contributed substantially to initial drafting, editing, and revising the final paper.

References

- Alexander RD. 1962. The evolution of mating behavior in arthropods. *Am Zool* **2**: 501-502.
- Anderson SAS, Weir JT. 2022. The role of divergent ecological adaptation during allopatric speciation in vertebrates. *Science* **378**: 1214-1218.
- Aubier TG, Bürger R, Servedio MR. 2023. The effectiveness of pseudomagic traits in promoting premating isolation. *Proc Royal Soc B*.
- Barton NH. 1983. Multilocus clines. *Evolution* **37**: 454-471.
- . 1995. Linkage and the limits to natural selection. *Genetics* **140**: 821-841.
- . 2013. Does hybridization influence speciation? *J Evolution Biol* **26**: 267-269.
- Barton NH, Bengtsson BO. 1986. The barrier to genetic exchange between hybridizing populations. *Heredity* **57**: 357-376.
- Barton NH, Briggs DEG, Eisen JA, Goldstein DB, Patel NH. 2007. *Evolution*. Cold Spring Harbor Laboratory Press, New York, NY, USA.
- Barton NH, de Cara MAR. 2009. The evolution of strong reproductive isolation. *Evolution* **63**: 1171-1190.
- Barton NH, Hewitt GM. 1981. Hybrid zones and speciation. in *Evolution and speciation* (eds. W Atchley, D Woodruff), pp. 109-145. Cambridge University Press, Cambridge, UK.
- . 1985. Analysis of hybrid zones. *Annu Rev Ecol Syst* **16**: 113-148.
- Bay RA, Arnegard ME, Conte GL, Best J, Bedford NL, McCann SR, Dubin ME, Chan YF, Jones FC, Kingsley DM et al. 2017. Genetic coupling of female mate choice with polygenic ecological divergence facilitates stickleback speciation. *Curr Biol* **27**: 3344-3349.
- Berner D, Thibert-Plante X. 2015. How mechanisms of habitat preference evolve and promote divergence with gene flow. *J Evolution Biol* **28**: 1641-1655.
- Bierne N, Welch J, Loire E, Bonhomme F, David P. 2011. The coupling hypothesis: Why genome scans may fail to map local adaptation genes. *Mol Ecol* **20**: 2044-2072.
- Bürger R, Akerman A. 2011. The effects of linkage and gene flow on local adaptation: A two-locus continent-island model. *Theor Popul Biol* **80**: 272-288.
- Butlin RK. 2005. Recombination and speciation. *Mol Ecol* **14**: 2621-2635.
- Butlin RK, Ritchie MG. 1989. Genetic coupling in mate recognition systems: What is the evidence? *Biol J Linn Soc* **37**: 237-246.
- Butlin RK, Servedio MR, Smadja CM, Bank C, Barton NH, Flaxman SM, Giraud T, Hopkins R, Larson EL, Maan ME et al. 2021. Homage to Felsenstein 1981, or why are there so few/many species? *Evolution* **75**: 978-988.
- Butlin RK, Smadja CM. 2018. Coupling, reinforcement, and speciation. *Am Nat* **191**: 155-172.
- Byers KJRP, Darragh K, Garza SF, Almeida DA, Warren IA, Rastas PMA, Merrill RM, Schulz S, McMillan WO, Jiggins CD. 2021. Clustering of loci controlling species differences in male chemical bouquets of sympatric *Heliconius* butterflies. *Ecol Evol* **11**: 89-107.

- Capinha C, Essl F, Seebens H, Moser D, Pereira HM. 2015. The dispersal of alien species redefines biogeography in the Anthropocene. *Science* **348**: 1248-1251.
- Chamberlain NL, Hill RI, Kapan DD, Gilbert LE, Kronforst MR. 2009. Polymorphic butterfly reveals the missing link in ecological speciation. *Science* **326**: 847-850.
- Charlesworth B, Barton NH. 2018. The spread of an inversion with migration and selection. *Genetics* **208**: 377-382.
- Charlesworth B, Charlesworth D. 2010. *Elements of Evolutionary Genetics*. Roberts and Company, Greenwood Village, CO, USA.
- Charlesworth B, Nordborg M, Charlesworth D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genet Res* **70**: 155-174.
- Coates BS, Kozak GM, Kim KS, Sun J, Wang YZ, Fleischer SJ, Dopman EB, Sappington TW. 2019. Influence of host plant, geography and pheromone strain on genomic differentiation in sympatric populations of *Ostrinia nubilalis*. *Mol Ecol* **28**: 4439-4452.
- Coyne JA, Orr HA. 2004. *Speciation*. Sinauer, Sunderland, MA, USA.
- Dopman EB, Perez L, Bogdanowicz SM, Harrison RG. 2005. Consequences of reproductive barriers for genealogical discordance in the European corn borer. *P Natl Acad Sci USA* **102**: 14706-14711.
- Dopman EB, Robbins PS, Seaman A. 2010. Components of reproductive isolation between North American pheromone strains of the European corn borer. *Evolution* **64**: 881-902.
- Edmunds S. 2002. Does parental divergence predict reproductive compatibility? *Trends Ecol Evol* **17**: 520-527.
- Endler JA. 1986. *Natural selection in the wild*. Princeton University Press, Princeton, NJ, USA.
- Feder JL, Gejji R, Yeaman S, Nosil P. 2012. Establishment of new mutations under divergence and genome hitchhiking. *Philos T R Soc B* **367**: 461-474.
- Feder JL, Nosil P. 2009. Chromosomal inversions and species differences: When are genes affecting adaptive divergence and reproductive isolation expected to reside within inversions? *Evolution* **63**: 3061-3075.
- . 2010. The efficacy of divergence hitchhiking in generating genomic islands during ecological speciation. *Evolution* **64**: 1729-1747.
- Felsenstein J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals. *Evolution* **35**: 124-138.
- Flaxman SM, Feder JL, Nosil P. 2013. Genetic hitchhiking and the dynamic buildup of genomic divergence during speciation with gene flow. *Evolution* **67**: 2577-2591.
- Flaxman SM, Wacholder AC, Feder JL, Nosil P. 2014. Theoretical models of the influence of genomic architecture on the dynamics of speciation. *Mol Ecol* **23**: 4074-4088.
- Gavrilets S. 2004. *Fitness Landscapes and the Origin of Species*. Princeton University Press, Princeton, NJ, USA.
- Germain RM, Hart SP, Turcotte MM, Otto SP, Sakarchi J, Rolland J, Usui T, Angert AL, Schluter D, Bassar RD, Waters MT. 2021. On the origin of coexisting species. *Trends Ecol Evol* **36**: 284-93

- Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Patterson N, Li H, Zhai WW, Fritz MHY et al. 2010. A Draft Sequence of the Neandertal Genome. *Science* **328**: 710-722.
- Harrison RG. 1998. Linking evolutionary pattern and process. in *Endless Forms* (eds. DJ Howard, SH Berlocher), pp. 19-31. Oxford University Press, New York, NY, USA.
- Harrison RG. 2012. The language of speciation. *Evolution* **66**: 3643-57.
- Hawthorne DJ, Via S. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* **412**: 904-907.
- He ZW, Li XN, Yang M, Wang XF, Zhong CR, Duke NC, Wu CI, Shi SH. 2019. Speciation with gene flow via cycles of isolation and migration: Insights from multiple mangrove taxa. *Natl Sci Rev* **6**: 275-288.
- Hench K, Vargas M, Hoppner MP, McMillan WO, Puebla O. 2019. Inter-chromosomal coupling between vision and pigmentation genes during genomic divergence. *Nat Ecol Evol* **3**: 657-667.
- Hermann K, Klahre U, Moser M, Sheehan H, Mandel T, Kuhlemeier C. 2013. Tight genetic linkage of prezygotic barrier loci creates a multifunctional speciation island in *Petunia*. *Curr Biol* **23**: 873-877.
- Hewitt GM. 1989. The subdivision of species by hybrid zones. in *Speciation and its consequences* (eds. D Otte, JA Endler), pp. 85-110. Sinauer Associates, Sunderland, MA, USA.
- . 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol J Linn Soc* **58**: 247-276.
- . 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907-913.
- Hopkins R. 2013. Reinforcement in plants. *New Phytol* **197**: 1095-1103.
- Hopkins R, Rausher MD. 2012. Pollinator-mediated selection on flower color allele drives reinforcement. *Science* **335**: 1090-1092.
- Huang K, Andrew RL, Owens GL, Ostevik KL, Rieseberg LH. 2020. Multiple chromosomal inversions contribute to adaptive divergence of a dune sunflower ecotype. *Mol Ecol* **29**: 2535-2549.
- Inskeep KA, Doellman MM, Powell TH, Berlocher SH, Seifert NR, Hood GR, Ragland GJ, Meyers PJ, Feder JL. 2022. Divergent diapause life history timing drives both allochronic speciation and reticulate hybridization in an adaptive radiation of *Rhagoletis* flies. *Mol Ecol* **31**: 4031-4049.
- James ME, Arenas-Castro H, Groh JS, Allen SL, Engelstadter J, Ortíz-Barrientos D. 2021. Highly replicated evolution of parapatric ecotypes. *Mol Biol Evol* **38**: 4805-4821.
- Janoušek V, Wang LY, Luzynski K, Dufkova P, Vyskočilová MM, Nachman MW, Munclinger P, Macholan M, Pialek J, Tucker PK. 2012. Genome-wide architecture of reproductive isolation in a naturally occurring hybrid zone between *Mus musculus musculus* and *M. m. domesticus*. *Mol Ecol* **21**: 3032-3047.
- Jiggins CD, Mallet J. 2000. Bimodal hybrid zones and speciation. *Trends Ecol Evol* **15**: 250-255.
- Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, Johnson J, Swofford R, Pirun M, Zody MC, White S et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**: 55-61.
- Karrenberg S, Liu X, Hallander E, Favre A, Herforth-Rahme J, Widmer A. 2019. Ecological divergence plays an important role in strong but complex reproductive isolation in champions (*Silene*). *Evolution* **73**: 245-261.

- Kautt AF, Kratochwil CF, Nater A, Machado-Schiaffino G, Olave M, Henning F, Torres-Dowdall J, Harer A, Hulseley CD, Franchini P et al. 2020. Contrasting signatures of genomic divergence during sympatric speciation. *Nature* **588**: 106-111.
- Kelly JK, Noor MAF. 1996. Speciation by reinforcement: A model derived from studies of *Drosophila*. *Genetics* **143**: 1485-1497.
- Kirkpatrick M. 1982. Sexual selection and the evolution of female choice. *Evolution* **36**: 1-12.
- . 2000. Reinforcement and divergence under assortative mating. *P Roy Soc B-Biol Sci* **267**: 1649-1655.
- Kirkpatrick M, Barton N. 2006. Chromosome inversions, local adaptation and speciation. *Genetics* **173**: 419-434.
- Kirkpatrick M, Servedio MR. 1999. The reinforcement of mating preferences on an island. *Genetics* **151**: 865-884.
- Kitano J, Ishikawa A, Ravinet M, Courtier-Orgogozo V. 2022. Genetic basis of speciation and adaptation: From loci to causative mutations. *Philos Trans R Soc Lond B Biol Sci* **377**: 20200503.
- Koch EL, Ravinet M, Westram AM, Johannesson K, Butlin RK. 2022. Genetic architecture of repeated phenotypic divergence in *Littorina saxatilis* ecotype evolution. *Evolution* **76**: 2332-2346.
- Kopp M, Servedio MR, Mendelson TC, Safran RJ, Rodriguez RL, Hauber ME, Scordato EC, Symes LB, Balakrishnan CN, Zonana DM et al. 2018. Mechanisms of assortative mating in speciation with gene flow: Connecting theory and empirical research. *Am Nat* **191**: 1-20.
- Kozak GM, Wadsworth CB, Kahne SC, Bogdanowicz SM, Harrison RG, Coates BS, Dopman EB. 2019. Genomic basis of circannual rhythm in the European corn borer moth. *Curr Biol* **29**: 3501-3509.
- Kronforst MR, Young LG, Kapan DD, McNeely C, O'Neill RJ, Gilbert LE. 2006. Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *P Natl Acad Sci USA* **103**: 6575-6580.
- Kulmuni J, Butlin RK, Lucek K, Savolainen V, Westram AM. 2020. Towards the completion of speciation: The evolution of reproductive isolation beyond the first barriers. *Philos Trans R Soc Lond B Biol Sci* **375**: 20190528.
- Kunther HD, Bogdanowicz SM, Searle JB, Harrison RG, Coates BS, Kozak GM, Dopman EB. 2022. Consequences of coupled barriers to gene flow for the build-up of genomic differentiation. *Evolution* **76**: 985-1002.
- Lenormand T, Otto SP. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* **156**: 423-438.
- Lewontin RC. 1964. Interaction of selection and linkage. I. General considerations; heterotic models. *Genetics* **49**: 49-67.
- Linck E, Battey CJ. 2019. On the relative ease of speciation with periodic gene flow. *bioRxiv doi: 101101/758664*.
- Lowry DB, Willis JH. 2010. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. *Plos Biol* **8**.
- Lucek K, Willi Y. 2021. Drivers of linkage disequilibrium across a species' geographic range. *PLoS Genet* **17**: e1009477.
- Lynch M, Walsh B. 2007. *The origins of genome architecture*. Sinauer, Sunderland, MA, USA.

- Maan ME, Seehausen O. 2012. Magic cues versus magic preferences in speciation. *Evol Ecol Res* **14**: 779-785.
- Mackintosh C, Scott MF, Reuter M, Pomiankowski A. 2022. The establishment of locally adaptive inversions in structured populations. *bioRxiv* doi: 101101/20221205519181.
- Mallet J, Meyer A, Nosil P, Feder JL. 2009. Space, sympatry and speciation. *J Evol Biol* **22**: 2332-2341.
- Mani GS, Clarke BC. 1990. Mutational order: A major stochastic-process in evolution. *Proc R Soc Ser B-Bio* **240**: 29-37.
- Marques DA, Lucek K, Meier JL, Mwaiko S, Wagner CE, Excoffier L, Seehausen O. 2016. Genomics of rapid incipient speciation in sympatric threespine stickleback. *Plos Genetics* **12**.
- Martin SH, Davey JW, Salazar C, Jiggins CD. 2019. Recombination rate variation shapes barriers to introgression across butterfly genomes. *Plos Biol* **17**.
- Melo MC, James ME, Roda F, Bernal-Franco D, Wilkinson MJ, Liu H, Walter GM, Ortíz-Barrientos D. 2019. Evidence for mutation-order speciation in Australian wildflower. *bioRxiv* doi: 101101/692673.
- Mérot C, Oomen RA, Tigano A, Wellenreuther M. 2020. A roadmap for understanding the evolutionary significance of structural genomic variation. *Trends Ecol Evol* **35**: 561-72.
- Merrill RM, Gompert Z, Dembeck LM, Kronforst MR, McMillan WO, Jiggins CD. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. *Evolution* **65**: 1489-1500.
- Merrill RM, Rastas P, Martin SH, Melo MC, Barker S, Davey J, McMillan WO, Jiggins CD. 2019. Genetic dissection of assortative mating behavior. *Plos Biol* **17**.
- Nei M. 1967. Modification of linkage intensity by natural selection. *Genetics* **57**: 625-641.
- Noor MAF, Grams KL, Bertucci LA, Reiland J. 2001. Chromosomal inversions and the reproductive isolation of species. *P Natl Acad Sci USA* **98**: 12084-12088.
- Nosil P. 2012. *Ecological speciation*. Oxford University Press, New York, NY, USA.
- Nosil P, Feder JL, Flaxman SM, Gompert Z. 2017. Tipping points in the dynamics of speciation. *Nat Ecol Evol* **1**: 0001.
- Nosil P, Feder JL, Gompert Z. 2021. How many genetic changes create new species? *Science* **371**: 777-779.
- Nosil P, Flaxman SM. 2011. Conditions for mutation-order speciation. *P Roy Soc B-Biol Sci* **278**: 399-407.
- Nosil P, Harmon LJ, Seehausen O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol Evol* **24**: 145-156.
- Ohta T. 1982. Linkage disequilibrium due to random genetic drift in finite subdivided populations. *P Natl Acad Sci USA* **79**: 1940-1944.
- Ortíz-Barrientos D, Engelstadter J, Rieseberg LH. 2016. Recombination rate evolution and the origin of species. *Trends Ecol Evol* **31**: 226-236.
- Ortíz-Barrientos D, Noor MAF. 2005. Evidence for a one-allele assortative mating locus. *Science* **310**: 1467-1467.
- Perini S, Rafajlović M, Westram AM, Johannesson K, Butlin RK. 2020. Assortative mating, sexual selection and their consequences for gene flow in *Littorina*. *Evolution* **74**: 1482-1497.
- Price T. 2007. *Speciation in birds*. Roberts and Company, Woodbury, NY, USA.

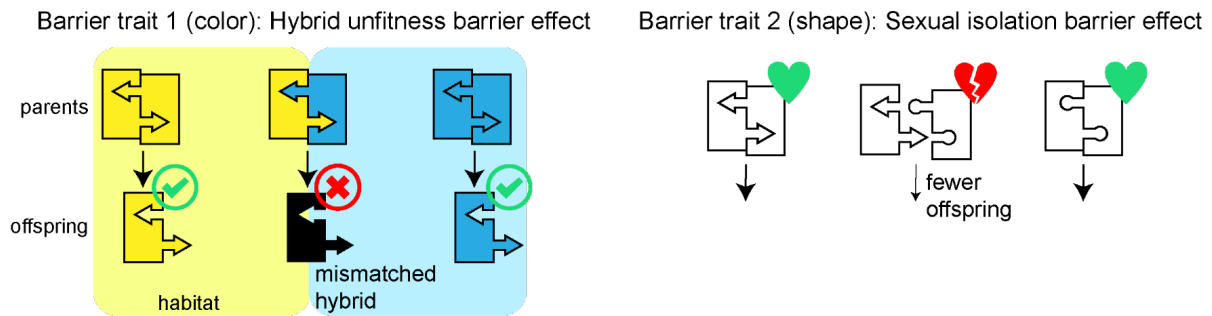
- Rafajlović M, Rambla J, Feder JL, Navarro A, Faria R. 2021. Inversions and genomic differentiation after secondary contact: When drift contributes to maintenance, not loss, of differentiation. *Evolution* **75**: 1288-1303.
- Ravinet M, Faria R, Butlin RK, Galindo J, Bierne N, Rafajlović M, Noor MAF, Mehlig B, Westram AM. 2017. Interpreting the genomic landscape of speciation: A road map for finding barriers to gene flow. *J Evolution Biol* **30**: 1450-1477.
- Rice WR, Hostert EE. 1993. Laboratory experiments on speciation: What have we learned in 40 years? *Evolution* **47**: 1637-1653.
- Riesch R, Muschick M, Lindtke D, Villoutreix R, Comeault AA, Farkas TE, Lucek K, Hellen E, Soria-Carrasco V, Dennis SR et al. 2017. Transitions between phases of genomic differentiation during stick-insect speciation. *Nat Ecol Evol* **1**: 0082.
- Rieseberg LH. 2001. Chromosomal rearrangements and speciation. *Trends Ecol Evol* **16**: 351-358.
- Riquet F, Liautard-Haag C, Woodall L, Bouza C, Louisy P, Hamer B, Otero-Ferrer F, Aublanc P, Beduneau V, Briard O et al. 2019. Parallel pattern of differentiation at a genomic island shared between clinal and mosaic hybrid zones in a complex of cryptic seahorse lineages. *Evolution* **73**: 817-835.
- Roda F, Walter GM, Nipper R, Ortíz-Barrientos D. 2017. Genomic clustering of adaptive loci during parallel evolution of an Australian wildflower. *Mol Ecol* **26**: 3687-3699.
- Roux C, Fraisse C, Romiguier J, Anciaux Y, Galtier N, Bierne N. 2016. Shedding light on the grey zone of speciation along a continuum of genomic divergence. *Plos Biol* **14**.
- Sachdeva H. 2022. Reproductive isolation via polygenic local adaptation in sub-divided populations: Effect of linkage disequilibria and drift. *PLoS Genetics* **18**: e1010297.
- Safran RJ, Scordato ESC, Wilkins MR, Hubbard JK, Jenkins BR, Albrecht T, Flaxman SM, Karaardic H, Vortman Y, Lotem A et al. 2016. Genome-wide differentiation in closely related populations: the roles of selection and geographic isolation. *Mol Ecol* **25**: 3865-3883.
- Sánchez-Guillén RA, Cordoba-Aguilar A, Cordero-Rivera A, Wellenreuther M. 2014. Rapid evolution of prezygotic barriers in non-territorial damselflies. *Biol J Linn Soc* **113**: 485-496.
- Sardain A, Sardain E, Leung B. 2019. Global forecasts of shipping traffic and biological invasions to 2050. *Nat Sustain* **2**: 274-282.
- Schilling MP, Mullen SP, Kronforst M, Safran RJ, Nosil P, Feder JL, Gompert Z, Flaxman SM. 2018. Transitions from single- to multi-locus processes during speciation with gene flow. *Genes-Basel* **9**: 274.
- Schluter D. 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737-741.
- Schuldiner-Harpaz T, Merrill RM, Jiggins CD. 2022. Evolution of physical linkage between loci controlling ecological traits and mating preferences. *J Evolution Biol* **35**: 1537-1547.
- Schumer M, Xu CL, Powell DL, Durvasula A, Skov L, Holland C, Blazier JC, Sankararaman S, Andolfatto P, Rosenthal GG et al. 2018. Natural selection interacts with recombination to shape the evolution of hybrid genomes. *Science* **360**: 656-659.
- Searle JB. 1993. Chromosomal hybrid zones in Eutherian mammals. in *Hybrid Zones and the Evolutionary Process* (ed. RG Harrison), pp. 309-353. Oxford University Press, New York, NY, USA.

- Seehausen O, Butlin RK, Keller I, Wagner CE, Boughman JW, Hohenlohe PA, Peichel CL, Saetre GP, Bank C, Brännström Å, Brelsford A. 2014. Genomics and the origin of species. *Nat Rev Genet* **15**: 176-92.
- Servedio MR. 2000. Reinforcement and the genetics of nonrandom mating. *Evolution* **54**: 21-29.
- Servedio MR. 2009. The role of linkage disequilibrium in the evolution of premating isolation. *Heredity* **102**: 51-56.
- Servedio MR, Bürger R. 2018. The effects on parapatric divergence of linkage between preference and trait loci versus pleiotropy. *Genes-Basel* **9**.
- Servedio MR, Noor MAF. 2003. The role of reinforcement in speciation: Theory and data. *Annu Rev Ecol Evol S* **34**: 339-364.
- Servedio MR, Van Doorn GS, Kopp M, Frame AM, Nosil P. 2011. Magic traits in speciation: 'Magic' but not rare? *Trends Ecol Evol* **26**: 389-397.
- Slatkin M. 1975. Gene flow and selection in a two-locus system. *Genetics* **81**: 787-802.
- Smadja CM, Butlin RK. 2011. A framework for comparing processes of speciation in the presence of gene flow. *Mol Ecol* **20**: 5123-5140.
- Smadja CM, Loire E, Caminade P, Severac D, Gautier M, Ganem G. 2022. Divergence of olfactory receptors associated with the evolution of assortative mating and reproductive isolation in mice. *Peer Community Journal* **2**.
- Sousa V, Hey J. 2013. Understanding the origin of species with genome-scale data: Modelling gene flow. *Nat Rev Genet* **14**: 404-414.
- Stankowski S, Ravinet M. 2021. Defining the speciation continuum. *Evolution* **75**: 1256-1273.
- Szymura JM, Barton NH. 1986. Genetic-analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata*, near Cracow in southern Poland. *Evolution* **40**: 1141-1159.
- The *Anopheles gambiae* 1000 Genomes Consortium. Genetic diversity of the African malaria vector *Anopheles gambiae*. *Nature* **552**: 96-100.
- The *Heliconius* Genome Consortium. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature* **487**: 94-98.
- Thompson KA, Peichel CL, Rennison DJ, McGee MD, Albert AYK, Vines TH, et al. 2022. Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. *PLoS Biol* **20**: e3001469.
- Tilley SG, Verrell PA, Arnold SJ. 1990. Correspondence between sexual isolation and allozyme differentiation: A test in the salamander *Desmognathus ochrophaeus*. *P Natl Acad Sci USA* **87**: 2715-2719.
- Trickett AJ, Butlin RK. 1994. Recombination suppressors and the evolution of new species. *Heredity* **73**: 339-345.
- Turner TL, Hahn MW, Nuzhdin SV. 2005. Genomic islands of speciation in *Anopheles gambiae*. *Plos Biol* **3**: 1572-1578.
- Tusso S, Nieuwenhuis BPS, Weissensteiner B, Immler S, Wolf JBW. 2021. Experimental evolution of adaptive divergence under varying degrees of gene flow. *Nat Ecol Evol* **5**: 338-349.

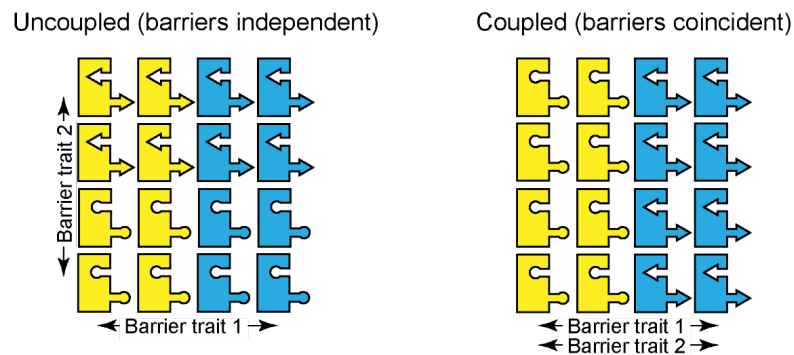
- Unbehend M, Kozak GM, Koutroumpa F, Coates BS, Dekker T, Groot AT, Heckel DG, Dopman EB. 2021. *bric* à *brac* controls sex pheromone choice by male European corn borer moths. *Nat Commun* **12**: 2818.
- Via S, West J. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Mol Ecol* **17**: 4334-4345.
- Villa SM, Altuna JC, Ruff JS, Beach AB, Mulvey LI, Poole EJ, Campbell HE, Johnson KP, Shapiro MD, Bush SE et al. 2019. Rapid experimental evolution of reproductive isolation from a single natural population. *P Natl Acad Sci USA* **116**: 13440-13445.
- Vines TH, Dalziel AC, Albert AYC, Veen T, Schulte PM, Schluter D. 2016. Cline coupling and uncoupling in a stickleback hybrid zone. *Evolution* **70**: 1023-1038.
- Westram AM, Stankowski S, Surendranadh P, Barton N. 2022. What is reproductive isolation? *J Evolution Biol* **35**: 1143-1164.
- White NJ, Butlin RK. 2021. Multidimensional divergent selection, local adaptation, and speciation. *Evolution* **75**: 2167-2178.
- White NJ, Snook RR, Eyres I. 2020. The past and future of experimental speciation. *Trends Ecol Evol* **35**: 10-21.
- Wolf JB, Lindell J, Backstrom N. 2010. Speciation genetics: Current status and evolving approaches. *Philos Trans R Soc Lond B Biol Sci* **365**: 1717-1733.
- Wu CI. 2001. The genic view of the process of speciation. *J Evolution Biol* **14**: 851-865.
- Wu CI, Ting CT. 2004. Genes and speciation. *Nat Rev Genet* **5**: 114-122.
- Xu MZ, Shaw KL. 2019. The genetics of mating song evolution underlying rapid speciation: Linking quantitative variation to candidate genes for behavioral isolation. *Genetics* **211**: 1089-1104.
- Xu MZ, Shaw KL. 2021. Extensive linkage and genetic coupling of song and preference loci underlying rapid speciation in *Laupala* crickets. *J Hered* **112**: 204-213.
- Yeaman S. 2013. Hybridization and the porous genome: Patterns of isolation and introgression in manakins. *Mol Ecol* **22**: 3195-3197.
- Yeaman S, Aeschbacher S, Bürger R. 2016. The evolution of genomic islands by increased establishment probability of linked alleles. *Mol Ecol* **25**: 2542-2558.
- Yeaman S, Whitlock MC. 2011. The genetic architecture of adaptation under migration-selection balance. *Evolution* **65**: 1897-1911.

Figures

(A) Barrier traits and barrier effects



(B) Degrees of barrier trait coupling



(C) Effect of coupling on genome-wide divergence

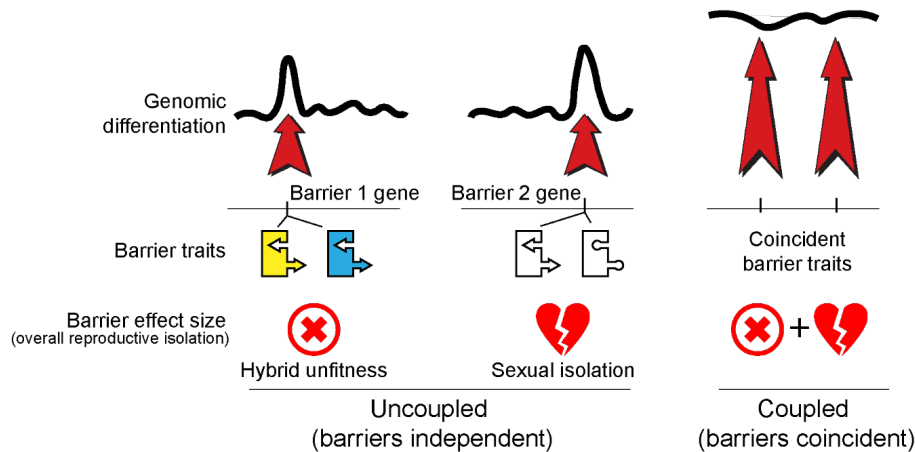


Fig. 1. Barrier traits, barrier effects, degrees of coupling and genomic consequences. (A) Phenotypic divergence of barrier trait 1 (color) from local adaptation leads to reduced fitness in hybrid offspring. Phenotypic divergence of barrier trait 2 (shape) leads to positive assortative mating and partial sexual isolation between individuals differing in mating traits. (B) Traits and barrier effects may be uncoupled (left) or coupled (right) to various degrees with each barrier defining a pair of populations. Coupled barriers (right) have coincident boundaries and define the same two populations (yellow vs blue), whereas uncoupled barriers (left) have non-coincident boundaries and define two different population pairs (barrier trait 1: yellow vs blue populations; barrier trait 2: arrow vs circle populations). Because they form barriers to gene flow between the same population pair, coincident

barriers are predicted to lead to stronger overall reproductive isolation. (C) Coupling can be used to describe the build-up (or maintenance) of LD among multiple barrier loci that enhances both differentiation at those loci and the barrier to gene flow in the rest of the genome. However, the consequences of increased LD among barrier loci on gene flow can be counterintuitive and may depend on the specific effect of the barrier loci (e.g., assortative mating vs local adaptation) (see Fig. 5 and the section, '*The role of recombination and genetic architectures*'). Whether coupling of different barrier effects increases overall levels of reproductive isolation and genetic differentiation compared to uncoupled situations is therefore an hypothesis that should be tested (Table 2).

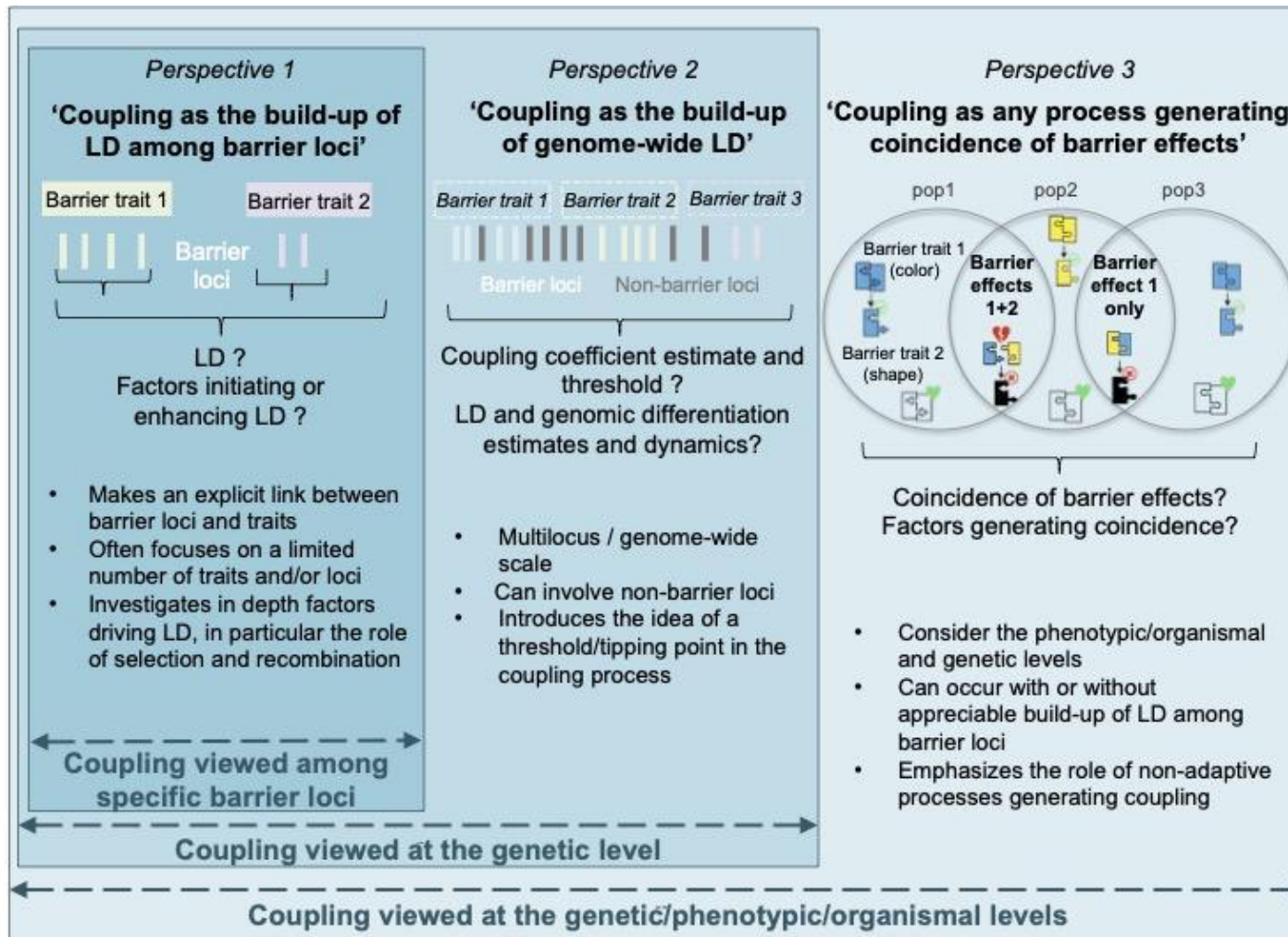


Fig. 2. **The three main perspectives on the concept of 'coupling' identified in the literature.** Perspective 1 considers coupling as the build-up of linkage disequilibrium (LD) among loci underlying specific barrier traits. Perspective 2 extends coupling to the build-up of genome-wide LD across both barrier and non-barrier loci. While the first two perspectives consider coupling at the genetic level only, with a focus on LD among barrier loci, perspective 3 encompasses these aspects while extending the concept of coupling further, to any process generating a coincidence of barrier effects, whereby coincidence can be considered at either the genetic or phenotypic/organismal level, with or without build-up of LD. Symbols used for perspective 3 are explained in Fig. 1.

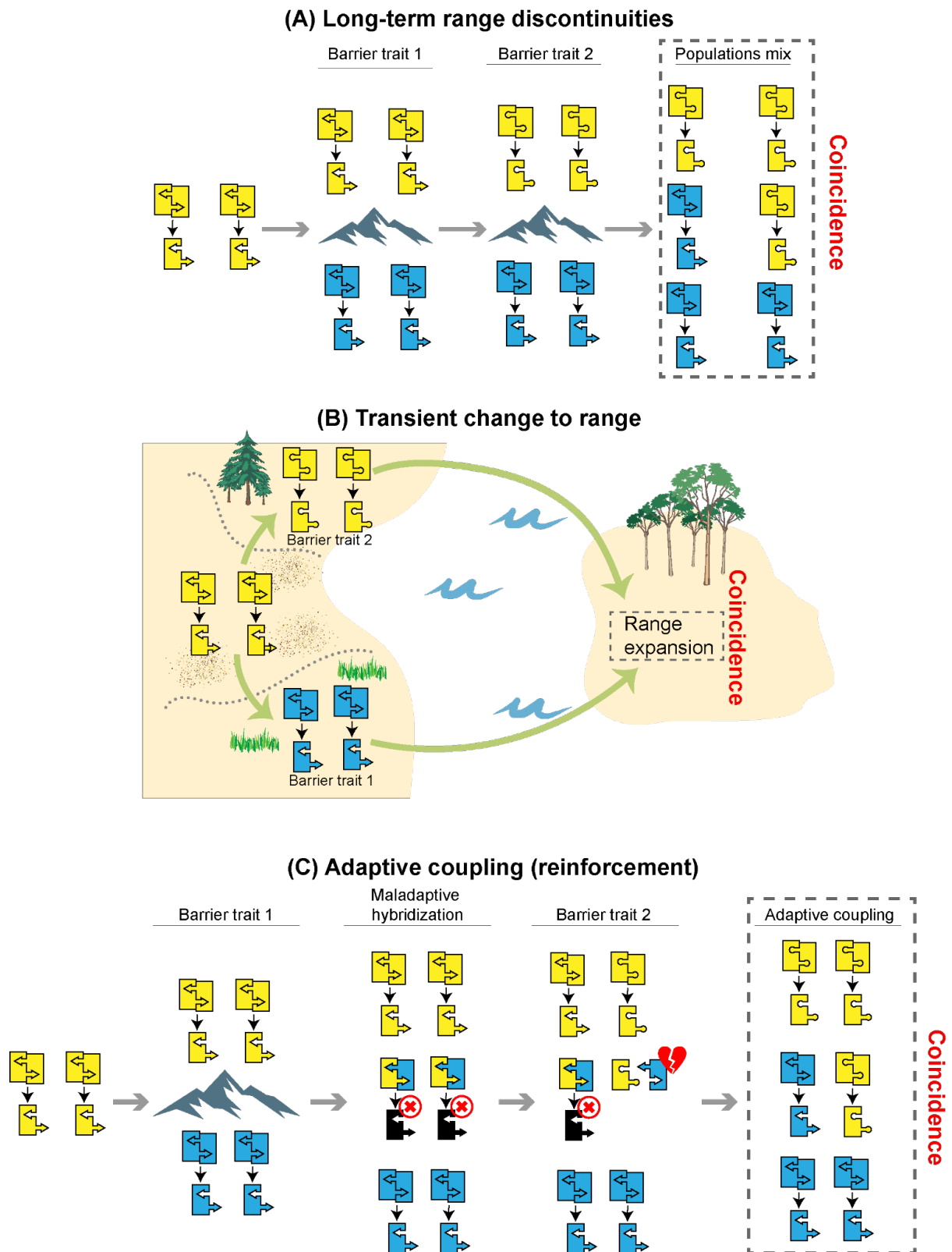


Fig. 3. **Coupling mechanisms enhancing LD.** (A) Long-term discontinuities in the distribution range such as allopatry contribute to the creation of distinct gene pools that, when mixed through erosion of the discontinuity, creates LD between loci involved in reproductive isolation. Symbols are explained in Fig. 1. (B) Transient changes in the distribution range can bring together genetic traits originating in different parts of a species distribution, for example through extinction and recolonization (not shown) or range expansion, thereby creating LD between loci including those underlying distinct

barrier effects. (C) Selection can favor the coincidence of distinct barrier effects by reinforcement, in which indirect selection on assortative mating (shape) due to initial LD with hybrid unfitness (color) strengthens LD between hybrid unfitness and assortative mating loci. More generally, LD will be favored among any combination of distinct barrier loci that show positive fitness epistasis.

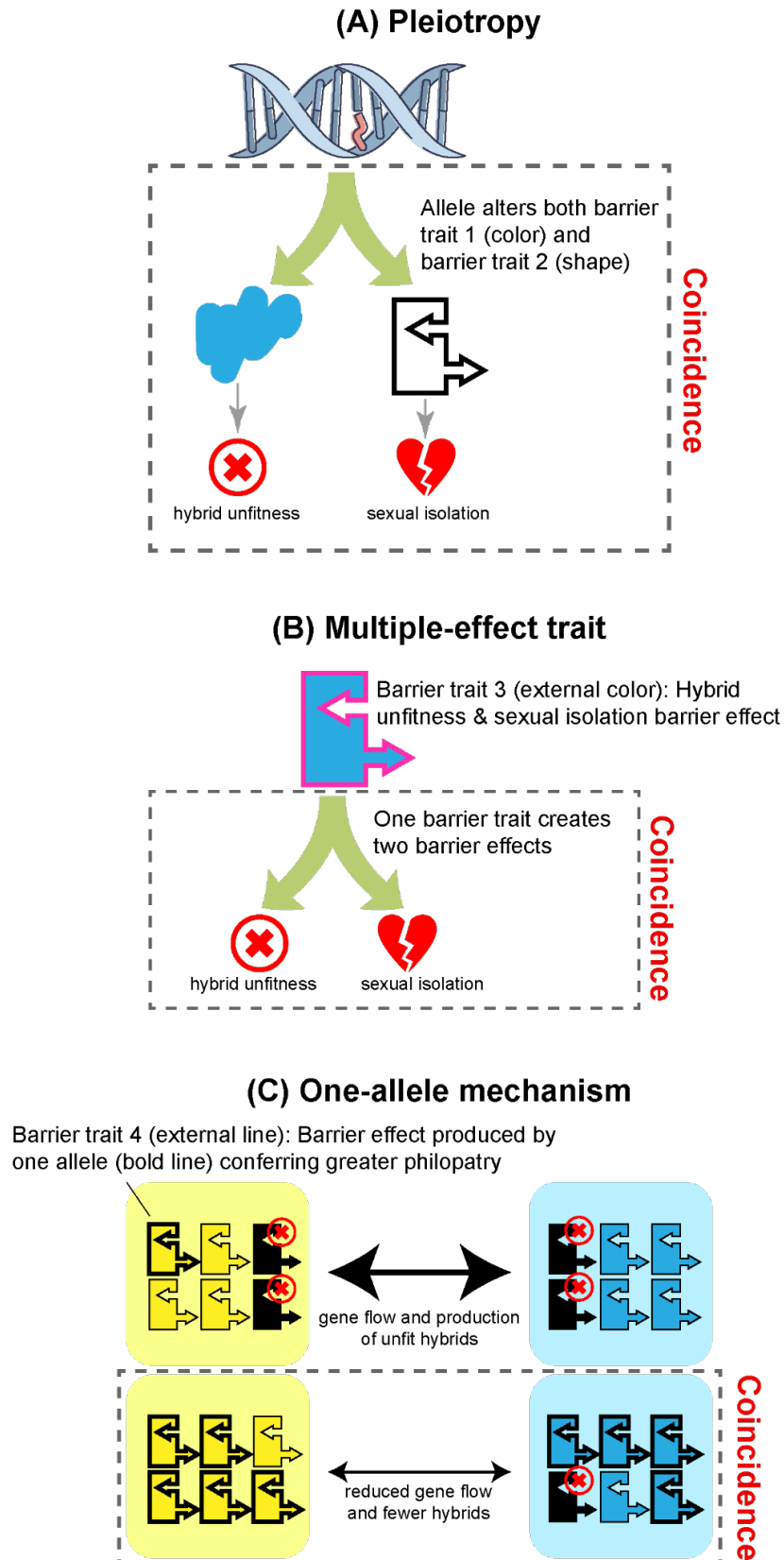


Fig. 4. **Non-LD mechanisms of coupling.** (A) Coincidence due to pleiotropy occurs when an allele alters multiple barrier traits (color, shape), thereby influencing multiple barrier effects (hybrid unfitnes, sexual isolation). Symbols are explained in Fig. 1. (B) Coincidence occurs as a simple consequence of divergence in a single trait (barrier trait 3, external color) influencing multiple barrier effects. Both (A) and (B) can be considered instances of ‘magic trait’ evolution. (C) For one-allele

mechanisms, a barrier effect results from the evolution of the same phenotype across diverging populations. For example, evolution of greater philopatry (barrier trait 4, bold external color) in each of two populations will decrease gene exchange between them, strengthening existing barrier effects (barrier trait 1) by reinforcement.

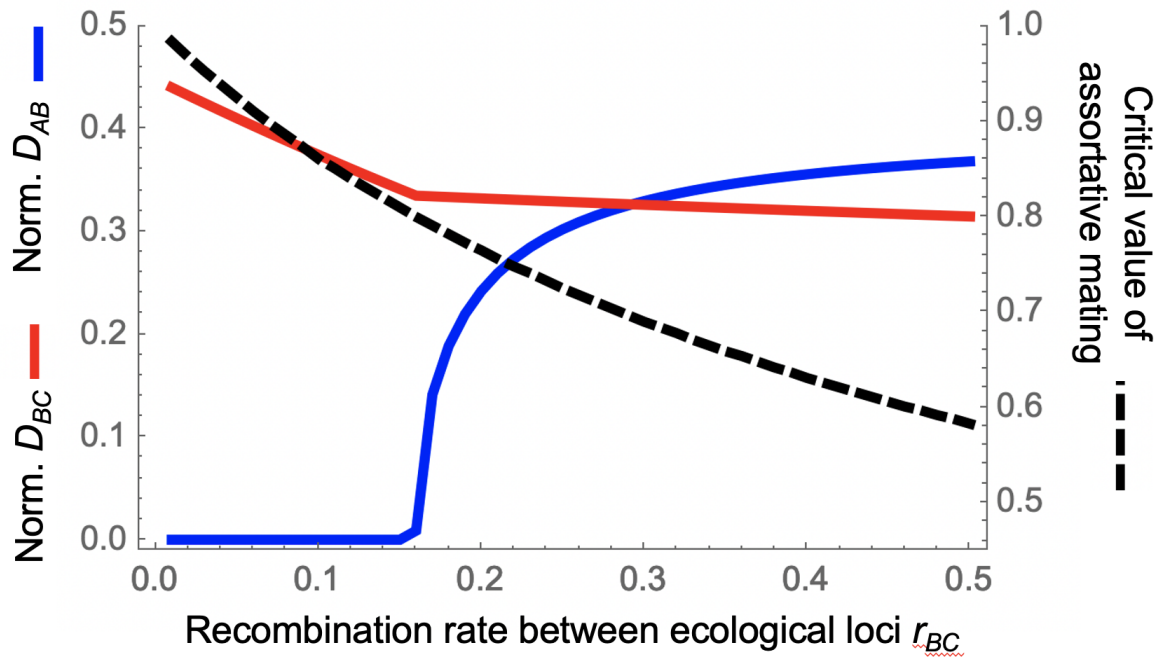


Fig. 5. **Contrasting effects of recombination rate.** Numerical iterations of the model of Felsenstein (1981) show the contrasting effects of increasing the recombination rate (r_{BC}) between the local adaptation loci that lead to extrinsic hybrid incompatibility (B and C). More recombination produces more hybrids, the source of selection for reinforcement and the build-up of LD between the assortative mating and local adaptation loci, which is Felsenstein's criterion for progress towards speciation. Specifically, greater r_{BC} lowers the threshold level of assortative mating necessary for the build-up of LD between the assortative mating and local adaptation loci (dashed line), in contrast to the effect of greater r_{AB} , which increases this threshold (not shown). Greater recombination between loci B and C has contrasting effects on the LD in the system, lowering the LD between B and C themselves (red line) but increasing the LD between the assortative mating locus A and the local adaptation loci (blue line). In other words the LD between A and B, considered by Felsenstein as progress towards speciation, increases under the same conditions where the LD between the locally adaptation loci, which some would also consider progress towards speciation through coupling, decreases. It is therefore clear that some care must be taken in the interpretation of coupling between these various sets of loci, the role of recombination, and whether speciation is occurring. Norm. D_{BC} and Norm. D_{AB} are normalized measures of LD (between the B and C, and A and B loci, respectively), calculated by dividing the LD by the maximum LD possible at the current allele frequencies (e.g., Norm. LD = LD/LD_{max}, where, for example, LD_{max} between B and C is $\min\{p_B(1-p_C), (1-p_B)p_C\}$, where p_B (p_C) is the frequency of allele B (C), from Lewontin (1964)). Qualitatively similar results are obtained when LD is not normalized. Details of the model can be found in Felsenstein (1981). Parameters are $r_{AB} = 0.5$, $m = 0.01$, $s = 1$ and for the red and blue lines $d = 0.8$.

Tables

Table 1. Definition of terms

Term	Definition	Further reading
Barrier effect	the contribution that a barrier locus or trait, or some combination of barrier loci or traits, makes to the barrier to gene exchange between two populations, also known as a 'component of isolation'	Butlin and Smadja 2018
Barrier locus	any locus that contributes, alone or in combination with other loci, to the barrier to gene exchange between two populations	
Barrier to gene exchange	a reduction in gene flow between two diverging populations relative to the gene flow expected between two populations of the same species that have a similar spatial arrangement. The barrier is expected to be locus-specific but the barrier experienced by neutral loci that are not closely-linked to barrier loci is sometimes used as a proxy for the overall barrier effect.	Westram et al. 2022 and associated commentaries
Barrier trait	any trait that contributes, alone or in combination with other traits, to the barrier to gene exchange between two populations	
Coupling coefficient	the ratio of total selection to total recombination. A critical value of this ratio is associated with a transition from locus-specific barriers to gene exchange to a genome-wide barrier	Barton 1983
Linkage disequilibrium	non-random association of alleles at different loci, regardless of whether those loci are physically linked or not	
'Magic trait'	a trait, or a set of traits with at least a partly-shared genetic basis, that contributes to more than one barrier effect (or component of RI); encompasses both barrier traits that are connected by pleiotropy (partially or completely) and multiple barrier effects that are connected via a single underlying trait	Servedio et al. 2011; Maan and Seehausen 2012
Multiple-effect trait	a phenotypic trait that contributes to more than one barrier effect	Smadja and Butlin 2011

One-allele mechanism	a contribution to reproductive isolation that results from evolution of a trait in the same direction, or substitution of the same allele(s), in two populations	Felsenstein 1981; Butlin et al. 2021
Pleiotropy	the case where a single allelic substitution causes changes in more than one phenotypic trait	Smadja and Butlin 2011
Reinforcement	origin or strengthening of a barrier effect in response to costs associated with existing barrier effects; the classic example is the strengthening of behavioral, prezygotic isolation in response to reduced fitness of hybrids	Servedio and Noor 2003; Butlin and Smadja 2018
Reproductive isolation (RI)	either the reduction in successful interbreeding between diverging populations (organismal view) or the reduction in gene flow caused by genetic differences between diverging populations (genetic view)	Westram et al. 2022 and associated commentaries
Two-allele mechanism	a contribution to reproductive isolation that results from evolution of a trait in different directions, or substitution of different allele(s), in two populations	Felsenstein 1981; Butlin et al. 2021

Table 2. Approaches to advance understanding of coupling

Approach	Description	Perspective	Example studies
Theory and simulation	Expectations and potential unique predictions of coupling via selection, population contraction/expansion, and range expansion; conditions favoring reinforcement under the expanded view; thorough analyses of the role of recombination rate in coupling, including interactions with initial conditions (e.g. geographic history) during coupling; systematic analysis of the effects on LD between different functional types of loci (pre mating isolation, local adaptation, postzygotic isolation, etc.) on build-up of RI; assessment of conditions that lead to positive vs. negative LD between different sets of loci during the speciation process, and how these relate to the overall build-up of LD and RI.	All	Aubier et al., this volume, Felsenstein 1981; Ortíz-Barrientos et al. 2016; Schuldiner-Harpaz et al. 2022

<p>Documenting barriers and levels of coincidence</p>	<p>Documenting barriers and levels of coincidence over distributions of interacting taxa (e.g., 'components of RI' and hybrid zone studies, taxa experiencing recent/on-going altered habitats or human-mediated movement), across a wide range of taxa, geographic regions and relationships among populations, population histories and levels of divergence. Ideally, combined analysis of barrier traits, barrier loci and barrier effects and how levels of barrier coincidence (uncoupled to fully coupled) and types of barrier effects (pre mating, local adaptation, postzygotic isolation, etc.) influences overall RI (measured under organismal or genetic views). Future empirical and theoretical studies could also more explicitly address and compare different 'levels' of coupling and their interplay (coupling among barrier loci underlying a single barrier trait; coupling among loci underlying different traits generating a single barrier effect; coupling among loci underlying different barrier effects).</p>	<p>Primarily 1 and 3 but hybrid zone studies also relate to 2</p>	<p>Firreno et al., this volume ; Kunerth et al. 2022; Karrenberg et al. 2019; Guevara Andino et al., this volume ; Ålund et al., this volume ; Perini et al. 2020</p>
<p>Landscape genetics</p>	<p>Analysis of whole genome data and fine-scale documentation of barrier traits or barrier effects across distributions to study the role of geographic separation and degree of barrier coincidence on genomic structure of populations. Some studies are doing this using phenotypic measures; more direct estimates of coupling by LD of identified barrier loci are needed.</p>	<p>Primarily 2 but adding barrier traits implicates 1 and 3</p>	<p>Edmands 2002; Safran et al. 2016; Coates et al. 2019</p>

Barrier gene identification	Analysis of functional and linked genetic variation at experimentally verified genes to identify and establish the relative roles of coupling mechanisms, including population processes, genomic architecture (rearrangement, recombination rate, pleiotropy, multiple-effect, one-allele, etc.), and adaptive coupling (see 'Experimental manipulation' below). Of particular importance is the identification of one-allele mechanisms at the genetic level (contrasting populations where the allele is under selection versus not under selection, e.g., sympatric versus allopatric populations in reinforcement scenarios after secondary contact). Identifying genes is critical for discriminating between barrier loci and non-barrier loci in hybrid zone or genome scan contexts, as otherwise cannot distinguish cause/effect and loci under direct selection versus indirect selection. Combined with environmental information (see 'Landscape Genetics' above) to test possible drivers, e.g., coupling from common drivers or from cline attraction.	Primarily 1, but also 3 especially where loci underlying multiple-effect traits and one-allele barrier effects can be included	distinct barriers: Kozak et al. 2019; Unbehend et al. 2021; Kautt et al. 2020; Merrill et al. 2011; local adaptation: Roda et al. 2017; mating signal: Byers et al. 2021; Ritchie and Butlin, this volume ; mate preferences: Smadja et al. 2022, Hench et al. 2019; pollinator syndrome: Hermann et al. 2013
Genomic patterns	Particularly where large-effect barrier loci cannot be identified, predictions about genomic patterns of divergence and gene flow need to be made and tested. Quantitative genetics approaches can be used to document the genomic distribution of loci underlying barrier traits or effects. Statistics like ancestry heterozygosity can be used to make inferences about barriers. Relationships between recombination and divergence can be used to test predictions about genomic architecture.	Primarily 2	Thompson et al., this volume ; Firreno et al., this volume ; Kautt et al. 2020; Riesch et al. 2017; Martin et al. 2019; Schumer et al 2018; Thompson et al. 2022
Experimental manipulation	Experimental manipulations to test which trait/gene combinations are favored (individual fitnesses), and how selection might strengthen combined barrier effects through coupling, as suggested by theory and indirectly by empirical patterns (e.g., reinforcement). Allows determination of whether coupled barrier effects are directly selected or whether they might be coupled by indirect selection, and of how LD with	Primarily 1	Villa et al. 2019; Rice and Hostert 1993; White et al. 2020; Tusso et al. 2021

	directed selected loci arose.		
--	-------------------------------	--	--