

# Does selection favour the maintenance of porous species boundaries?

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## Abstract

The endpoint of speciation has been viewed as complete isolation and the absence of gene flow between species. If the influx of genes from another species is maladaptive because species have different adaptations and genetic backgrounds, selection should favour the closing of species boundaries and zero gene flow, a process known as reinforcement. Recently, numerous cases of gene flow between species have been identified, many of which involved adaptive introgression of beneficial alleles. These cases could reflect transient states on the way to closed species boundaries or the result of declining strength or efficacy of selection for reinforcement as the level of gene flow approaches zero. An alternative hypothesis, however, is that selection favours porous species boundaries that allow beneficial alleles to cross, especially in changing environments. This perspective evaluates the conditions that would be needed for selection to favour porous species boundaries and the evidence for them. A contrast is made between hybridization in sexual eukaryotes and gene transfer via homologous recombination in bacteria. Current evidence is inconclusive on whether non-zero gene flow is favoured by selection. Studies are needed that quantify selection gradients on rates of gene flow and test for evolution towards intermediate values, especially experiments that manipulate conditions and track evolution for multiple generations. Estimation of gene flow networks for more clades and regional assemblages using emerging genome data will also allow the evolutionary determinants of interspecific gene flow to be better understood.

**Keywords:** porous, permeable, partial, incomplete, species, reinforcement, introgression, eco-evolutionary interactions, speciation

## Introduction

A central tenet for theories of speciation is that gene flow and local adaptation act in opposition (Felsenstein, 1981; Gavrilets, 2004; Tigano & Friesen, 2016). If different sets of alleles are favoured in one location or niche than in another, migration brings an influx of maladapted genes and counteracts divergence between populations. In simple models, divergence is a function of the strength of selection divided by the rate of gene flow (Haldane, 1932; Lenormand, 2002). Maximum polygenic divergence requires complete isolation, but appreciable divergence still occurs if selection is strong relative to gene flow (Feder et al., 2012; Schmutzer & Barraclough, 2019). In such models, gene flow is maladaptive because it prevents populations from diverging fully to the optima favoured by environmental conditions in each location or niche. As such, with genetic variation for the degree of migration or interbreeding between diverging populations, selection should favour a reduction in gene flow and closure of the species boundary (with closure defined as zero gene flow), known as reinforcement (Dobzhansky, 1937; Kulmuni et al., 2020a). In addition to local adaptation, genetic incompatibilities between populations generate selection against hybrids that further promote the closure of species boundaries (Dobzhansky, 1937).

Nonetheless, maintaining some level of gene flow between differentially adapted species might still be beneficial. For example, both species might face ongoing changes to their environments, not just a static local optimum (Blanquart et al., 2013). If so, maintaining a wider interconnected network

might provide a large source pool of beneficial mutations for adapting to those changes (Abbott et al., 2013; Barton, 2013, 2020). Perhaps some traits share a single global optimum for all populations, which could be reached via a shared gene pool, whereas others have local optima that diverge between niche-adapted populations (Barraclough, 2019). In this case, selection might favour porous boundaries that maintain low rates of gene flow (Barton, 2020; Harrison & Larson, 2014; Mallet, 2008; Servedio & Hermisson, 2020) or mechanisms that restrict gene flow at niche- or location-specific genes but not the rest of the genome (Martin et al., 2019). For instance, could an allele that conveys 98% preference to mate with a conspecific replace an allele that conveys 100% preference for conspecifics over successive generations by associating in highly beneficial new combinations with alleles from the other species? In an extreme model, whole clades or communities could retain non-zero levels of gene flow that permit globally beneficial alleles to spread, rather than each species adapting strictly in isolation.

Widespread evidence for gene flow between species would not necessarily mean, however, that porous species boundaries are adaptive. One alternative is that selection indeed favours closure of the species boundary, as in the classic reinforcement model, but either cases of gene flow are transient steps along this path (Stankowski & Ravinet, 2021) or the strength of selection declines as gene flow approaches zero, meaning that it is too weak for complete closure to occur (Bank et al., 2012). Alternatively, selection might not act directly on gene flow. Instead, the pattern of gene flow, whether a porous or

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closed species boundary, might be an emergent property and by-product of selection and other evolutionary forces operating within species (Nosil et al., 2009). Selection on species boundaries might usually be weak because the benefits of any mutation affecting interspecific transfer only accrue in linkage disequilibrium with transferred beneficial genes akin to indirect selection on the mutation and recombination rate (Roze, 2021), and only then after repeated backcrossing in sexual organisms. Nonetheless, a re-evaluation of the evolutionary forces acting on species boundaries is timely.

This perspective discusses theory and evidence for whether selection could act to maintain a non-zero rate of gene flow between species. My focus is on the eco-evolutionary aspects of environmental regimes, species interactions, and coexistence. I first outline conditions that would be required for introgressed alleles to benefit recipient individuals, not just for a single gene or as a transient feature of initial contact but with a sufficient net benefit over longer timescales to sustain selection for traits that promote a porous boundary. Next, I consider how ecological interactions between species affect whether species that exchange beneficial genes would coexist and persist, a further criterion for the high prevalence of porous boundaries. A comparison is made between sexual eukaryotes and bacterial divergence and gene transfer. By describing current evidence for porous species boundaries and adaptive rates of gene flow, I outline predictions and some future ways to test these ideas, especially experimental evolution in bacteria.

### When do alleles imported from another species bring fitness benefits?

Gene flow between species provides an additional source of genetic variation to supplement mutation. The difference between mutation and gene flow is that mutation is random, whereas variants arriving via gene flow have already been tested by selection in other species and can encompass haplotypes with multiple co-adapted substitutions. The fitness effect of incoming variants depends on the selective regime in the two species. When species are adapted to different local environments or niches, imported alleles for niche-specific genes are likely to be deleterious. Also, if genes are co-adapted to function within gene networks that also diverge between species, then incoming variants at other loci may also be incompatible with the alternative genetic background and generally reduce fitness (as in Dobzhansky–Muller incompatibilities, Satokangas et al., 2020; Turelli & Orr, 2000).

A beneficial effect for an imported allele can arise if alleles of high fitness in the donor species but currently absent in the recipient species are nonetheless beneficial in the recipient species (Edelman & Mallet, 2021). A sustained probability that this condition arises repeatedly, rather than as a one-off event, implies (a) directional or fluctuating selection on the recipient species (Blanquart et al., 2013) and (b) that there is useful variation in the donor species that is currently absent in the recipient species. One scenario is that the donor species is pre-adapted to an environmental condition that the recipient species is now facing. A potential example is the influx of warm-adapted genes from a species adapted to lower latitudes into a recipient species in high latitudes faced with a warming environment (Satokangas et al., 2023). Bidirectional gene flow would also bring an influx of cold-adapted genes to the low-latitude species, which would be selected against. Specific

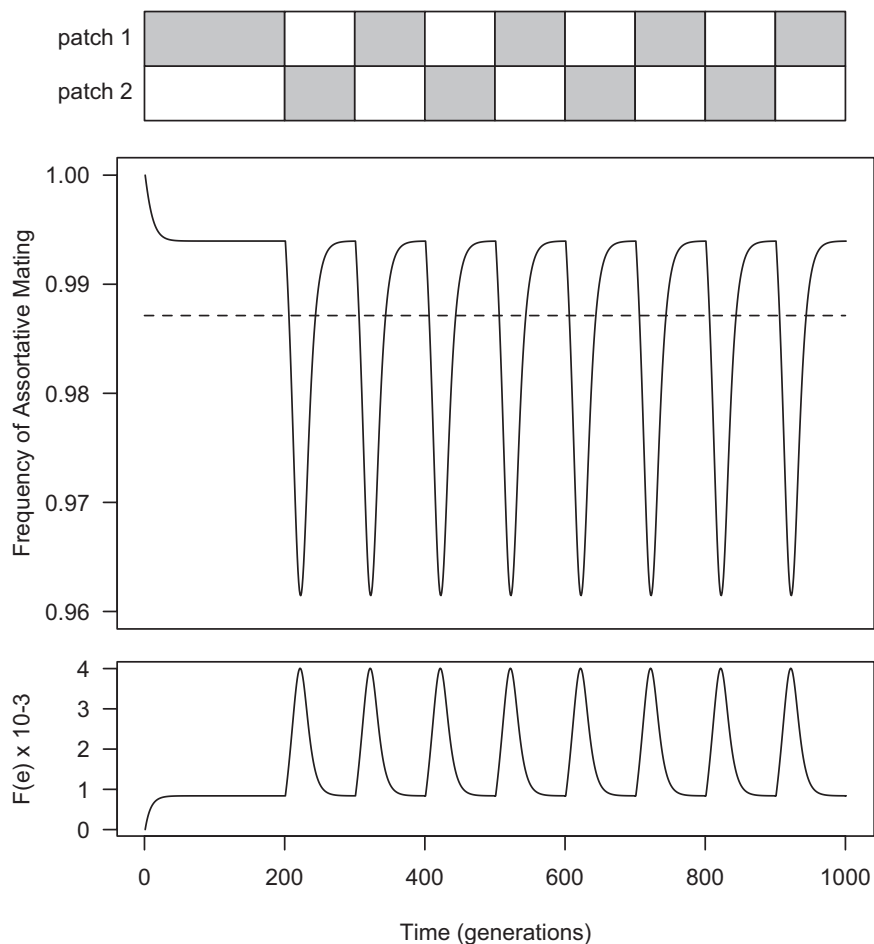
patterns of selection can favour bidirectional gene flow at low average rates when an environmental variable fluctuates in an opposing or uncorrelated way between two populations that have adapted to different resource availability in two niches (Figures 1 and 2 in later section, Barraclough, 2019).

There could also be scenarios where imported alleles are positively selected but do not involve the swapping of the environment. For instance, both species might face the same directional or fluctuating changes in a global environment, but the donor species has already fixed beneficial mutations that have yet to appear in the recipient. In a stochastic model, different globally beneficial mutations might arise first in each population, and bidirectional gene flow allows them to cross the species boundary and spread into both species. In this scenario, an allele that increases the chance of hybridization could be associated with beneficial gene combinations formed by hybridization between the two populations (especially if linked to one of the positively selected alleles). A low but non-zero rate of gene flow brings a large gene pool for adapting to shared optima between the two species while maintaining optimal divergence at niche-specific loci. The wording so far assumes that imported alleles have the same phenotypic and fitness effects in the recipient as in the donor species and are compatible with the alternative genetic background. Positive selection might, therefore, be more likely for genes that are less well connected and operate in simpler or more conserved gene networks, reducing the risk of incompatibility.

Even without the environmental contexts outlined above, it is possible that gene flow might facilitate the spread of beneficial variants despite a general background of deleterious effects, more akin to the effects of mutation (Edelman & Mallet, 2021). For example, an imported variant might, by chance, trigger a phenotypic effect in the recipient's genetic background that happens to be beneficial in the recipient species, irrespective of any match in environmental conditions. Although this would be random in the same way as mutation, such effects could be larger if imported variants accrued more differences from the recipient copy than would result from single mutations (and globally, deleterious mutations have already been weeded out by selection). Large phenotypic changes might even be triggered by a mismatch with the recipient's genetic background (Bui et al., 2015). For polygenic traits, each population will typically contain segregating variation that is potentially beneficial in the other, even when adapted to different environmental optima (Chevin et al., 2014; Yeaman & Whitlock, 2011). There would be no tendency towards positive fitness effects, but gene flow could still facilitate the spread of positively selected variants as a supplement to mutation. Finally, another category of genes that might favour gene flow is those under balancing selection, such as immunity genes that display negative frequency-dependence, where a supply of rare novel genes from another species could bring inherent benefits (Fijarczyk et al., 2018).

### When would selection for porous species boundaries occur?

Any of the scenarios in the previous section could favour residual levels of gene flow between niche-adapted species (see Servedio & Hermisson, 2020 for additional mechanisms based on sexual selection). If migration or mate choice preferences are under genetic control and tuneable to specific levels, then a value retaining low but non-zero rates could be



**Figure 1.** Selection for porous species boundaries in a simple haploid model based on [Diehl and Bush \(1989\)](#) of adaptive divergence between two patches with consistently different resource availability (optimum genotype  $AB$  in one patch, and  $ab$  in the other) but fluctuating physical environment (match determined by two different loci, with alleles  $C/c$  and  $D/d$ ). A fifth locus  $E/e$  determines whether  $AB$  and  $ab$  genotypes enter different mating pools ( $E$ ) or enter the two mating pools at random ( $e$ ). Selection coefficients for optimal niche axis genotype = 0.1, and for physical environment genotype = 0.2, relative to sub-optimal genotypes (see [Table 1](#)). Mutation rates = 0.0001 per generation. In this example, the physical environment fluctuates inversely in the two patches every 50 generations after an initial 200 generations (top panel). Immediately after the first switch, a more porous species boundary is favoured to generate the new optimal genotypes  $ABcd$  and  $abCD$  from the initial optimum genotypes of  $ABCD$  and  $abcd$ , in patches 1 and 2, respectively. The frequency of  $e$  alleles that cause hybrid matings increases (bottom panel) through their linkage disequilibrium with new optimal genotypes. This leads to a decrease in the frequency of assortative mating (middle panel), defined as the proportion of matings across the population that are either  $ab \times ab$  or  $AB \times AB$  (mean value shown by dashed line). The code is available at [github.com/tim-barra/PorousBoundaries](https://github.com/tim-barra/PorousBoundaries).

selected. Such theory mirrors models of selection acting on the mutation or recombination rate. Positive selection of an allele modifying the probability of interspecific mating derives from an association with new hybrid gene combinations that are beneficial in one or both populations. One difficulty for sexual organisms is that unless F1 hybrids have higher fitness than parental genotypes, the benefits of a genotype primarily derived from one population but with some variation from the other only arise after repeated backcrossing. In both models and experiments, the spread of mutator alleles tends to be transient under periods of extremely strong selection (i.e., stress), and similar criteria would likely apply to the spread of alleles that promote gene flow ([Raynes & Sniegowski, 2014](#)).

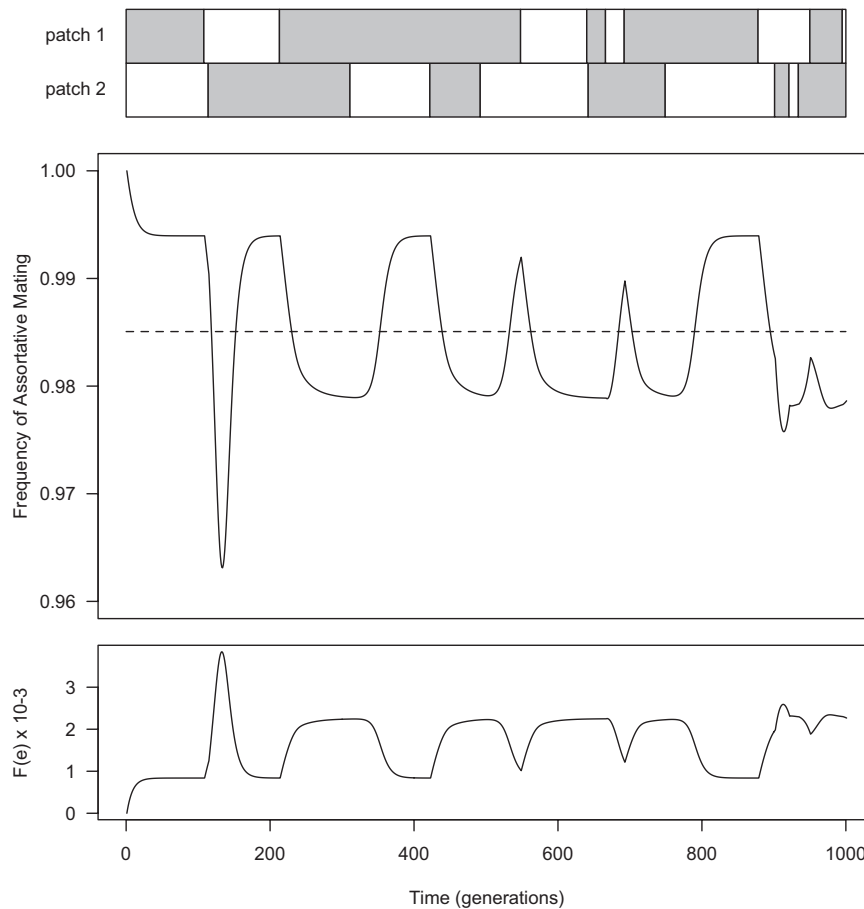
Another category of mechanisms could restrict gene flow at niche-specific loci but permit higher rates of gene exchange across the rest of the genome. For example, chromosomal inversions can protect niche-adapted loci from recombination while allowing high rates of gene flow at other loci that are under uniform selection between populations ([Yeaman, 2013](#)). Similarly, sex chromosomes or other genome regions

of low recombination rate can accumulate divergence ([Martin et al., 2019](#)).

Ongoing selection for non-zero gene flow between species requires that the conditions favouring gene flow are maintained over extended periods, which brings further assumptions than those simply for explaining a single episode of adaptive introgression. For example, many studies of gene flow in sexual organisms consider secondary contact between previously isolated populations. These populations accumulate genetic differences before contact, and adaptive introgression of alleles that are favourable in the alternative population can occur. Once those alleles have spread, however, selection might then act to reduce gene flow unless the selection regime favours ongoing gene flow or selection becomes too weak to completely close the boundary.

### A simple model of selection for porous species boundaries

A simple haploid model that encapsulates the conditions discussed above can be used to illustrate how selection might



**Figure 2.** Selection for porous species boundaries in a physical environment that fluctuates randomly over time. All details are the same as in Figure 1, except now the timing of shifts in the physical environment in each habitat type occur at random (top panel). A transient porous boundary (low frequency of assortative mating—middle panel, higher frequency of  $e$  alleles—bottom panel) is especially strongly selected when physical environmental conditions swap between patches, as occurs just after generation 100 in this random trial. Otherwise, the model fluctuates between two alternative states of mutation-selection balance depending on whether physical environments are the same in the two patches (e.g., around generation 250) or different (e.g., around generation 400). The latter conditions increase the strength of divergent selection between the patches and hence the strength of indirect selection against hybrid mating and  $e$  alleles. The code is available at [github.com/tim-barra/PorousBoundaries](https://github.com/tim-barra/PorousBoundaries).

act to maintain porous species boundaries (Figure 1 and Barraclough, 2019). The model considers two habitat patches of equal size but with different resources available (Diehl & Bush, 1989; Felsenstein, 1981). Resource use is determined by two unlinked loci, with optima  $AB$  in patch 1 and  $ab$  in patch 2 that are constant over time. Fitness effects are multiplicative (Table 1).

The physical environment fluctuates over time in both patches between conditions that favour unlinked two-locus genotype  $CD$  (white shading in bars at the top of plots in Figure 1) and those that favour genotype  $cd$  (grey shading in Figure 1). Fitness effects are multiplicative (and across all four loci combined) but with a different selection coefficient than the habitat resource loci, i.e., fitness of  $CD$  genotype in environment 1 is  $(1+s)^2$  whereas genotype  $cd$  or  $Cd$  is  $1+s'$ .

Assortative mating occurs as a pleiotropic effect of the same two loci that determine habitat resource use (Diehl & Bush, 1989). Specifically, it assumes two mating pools, early and late, where  $ab$  genotypes enter the early pool with probability  $m$ ,  $AB$  genotypes enter the late pool with probability  $m$ , and  $aB$  and  $Ab$  genotypes enter mating pools at random (Table 1). Random mating occurs within each mating pool. Another unlinked biallelic locus determines  $m$ : allele  $E$  gives  $m = 1$  causing  $ab$  genotypes to enter the early mating pool and

**Table 1.** Parameters for the habitat resource use loci.

Genotype	Patch 1 fitness	Patch 2 fitness	Probability of entering early mating pool	Probability of entering late mating pool
$ab$	$(1+s)^2$	1	$m$	$1-m$
$Ab, aB$	$1+s$	$1+s$	0.5	0.5
$AB$	1	$(1+s)^2$	$1-m$	$m$

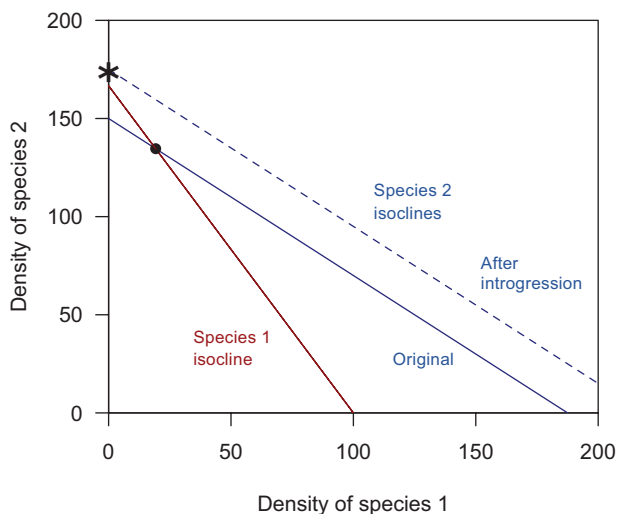
$AB$  genotypes to enter the late mating pool, whereas allele  $e$  gives  $m = 0.5$  whereby both  $ab$  and  $AB$  genotypes enter the mating pools at random. The frequency of assortative mating is recorded as the proportion of matings that are either  $ab \times ab$  or  $AB \times AB$ , which depends both on the frequency of  $ab$  and  $AB$  in the population and the frequency of  $e$  alleles and hence the mean value of  $m$ . The model starts with  $E$  alleles fixed and only  $ab$  and  $AB$  genotypes present, i.e., strict assortative mating. Partial gene flow evolves if the frequency of  $e$  alleles increases from zero. There is no direct selection on alleles  $e$  or  $E$ , any fitness effects derive from their association with alleles at the other loci. The mutation is bidirectional and occurs at rate  $\mu$  for all loci. An alternative model that treats  $m$  as a trait

with continuous values determined by a multiallelic modifier locus gives similar results (Barraclough, 2019). The code to run the model is at [github.com/tim-barra/PorousBoundaries](https://github.com/tim-barra/PorousBoundaries).

When the physical environment is constant over time, the frequency of assortative mating maintains a value close to 1.0 because hybrid individuals (and  $e$  alleles) are selected against as in the classical model of reinforcement (the first 200 generations in Figure 1). It remains fractionally below 1.0 in mutation-selection balance, highlighting the hypothesis that porosity could simply arise because selection is too weak to close the species boundary. Immediately after environmental change, however, if hybrid individuals have higher fitness than parental genotypes (which occurs when the physical environment imposes stronger selection than the resource use axis), there is transient selection for hybrid mating and an increase in the frequency of  $e$  alleles (resulting in a nearly eightfold increase in porosity in Figure 1). This occurs because each population is pre-adapted to environmental conditions that the other population suddenly faces, i.e., the physical environment swaps between the two habitat patches and optimum genotypes are produced by disassortative mating. While opposing environmental fluctuations between habitat patches seem unlikely, random fluctuations also lead to recurrent selection for a porous species boundary, as illustrated in Figure 2.

### Eco-evolutionary consequences of porous species boundaries

As well as being a potential target for selection, non-zero gene flow could influence the population densities of both species by altering ecological interactions and mechanisms



**Figure 3.** An illustration of how gene flow could lead to a change in competitive interaction between two co-occurring species, assuming a Lotka-Volterra model of competition. Change in the density of species  $i$  is modelled as  $\frac{dN_i}{dx} = r_i N_i \left( \frac{K_i - N_i - a_{ij} N_j}{K_i} \right)$ , with terms as defined in the main text. Blue solid line = the isocline for species 2 before introgression, showing that the change in density of that population is zero. Red solid line = initial isocline for species 1, showing where its density is zero. An initial stable equilibrium of coexistence occurs at the point indicated where the lines cross. Parameter values:  $K_1 = 100$ ,  $K_2 = 150$ ,  $a_{12} = 0.6$ ,  $a_{21} = 0.8$ . Blue dashed line = change in the isocline for species 2 after adaptive introgression from species 1, causing an increase in carrying capacity  $K_2 = 175$ . The new equilibrium is the extinction of species 1 (indicated by the asterisk).

of coexistence. While these effects might not generate direct selection on species boundaries, they could influence the long-term stability and persistence of species with porous boundaries. Assume that introgression occurs in one direction, from a donor to a recipient species. Transfer of beneficial alleles will increase mean fitness and hence either the population growth rate or carrying capacity of the recipient species (Lande et al., 2009). If the two species have no ecological interaction, there would be no impact on the donor species population. For instance, asymmetric introgression via rare hybridization in a narrow contact zone might have no direct impact on the donor species outside the contact zone because the major parts of each species range are geographically separated and do not interact.

In contrast, if the two species co-occur and compete and imported alleles improve the competitive ability of the recipient species, then transfer could have a negative effect on the donor species. For example, locally adapted alleles from an endemic plant species could introgress into an invasive species, which then allows the invasive to outcompete the local endemic (Viard et al., 2020). Or, in a broader case of gene transfer (see The same questions can be asked about gene transfer in bacteria), a bacterial species with a plasmid carrying antibiotic-resistance genes might be outcompeted by a competitor if it shares the plasmid with it (Coyte et al., 2022).

This can be visualized with a classical Lotka–Volterra model of competition between two species (Dorschner et al., 1987). Stable coexistence is obtained when interspecific competition is weak relative to intraspecific competition, which occurs when  $K_1 > a_{12} K_2$  and  $K_2 > a_{21} K_1$ , where  $K_i$  indicates the carrying capacity of species  $i$  in the absence of competitors and  $a_{ij}$  equals the competitive effect per individual of species  $j$  on species  $i$ . Assuming the transfer of beneficial alleles occurs from species 1 to species 2, then this might not influence the competitive equilibrium; for example, fixation of the alleles might change the intrinsic rate of population increase at low densities,  $r$ , which in this model does not affect stable coexistence. But if transferred alleles alter the carrying capacity or competition coefficient—presumably an increase if the alleles are beneficial in the recipient species—then under certain conditions, this could lead to the loss of stable equilibrium and out-competition of species 1 by species 2. Specifically, an increase in  $K_2$  greater than  $K_1/a_{12} - K_2$  or in  $a_{12}$  greater than  $K_1/K_2 - a_{12}$  would change the sign of the equalities and cause the extinction of species 1. In the example in Figure 3, extinction results from a ~16% change in the carrying capacity  $K_2$ . More generally, for any mechanism of coexistence between species (Chesson, 2000), it is possible that adaptive introgression could shift the balance in favour of the recipient and lead to a decline or even extinction of the donor species.

Different ecological interactions would lead to different ecological effects of gene flow. If the two species were mutualists, both populations would experience positive effects whenever gene flow led to an increase in the mean fitness of the recipient population. Population effects might also coincide for transfer from a predator to a prey (or parasite to a host), as both populations could increase if the prey growth rate increased, whereas the transfer of genes from a prey that improves the population growth rate of a predator would clearly harm the prey. In sexual organisms, where gene flow is usually considered between closely related species, it is more likely that those species would have neutral (if adapted to divergent niches) or competitive interactions than mutualistic

or predator–prey (because such large ecological shifts are rare).

The separation of donor and recipient populations is a useful abstraction for thinking about ecological effects. In sexual organisms, the formation of a hybrid individual involves symmetrical mixing, but asymmetric mate preferences and subsequent rounds of backcrossing and selection can generate asymmetry (Pickup et al., 2019). So, even in sexual organisms, donor and recipient status can often be ascribed. With symmetrical gene flow, the ecological effect would depend on the net effects of receiving new beneficial alleles versus donating beneficial alleles between both species. This could still entail a shift that disrupts coexistence.

How might these ecological effects on the population densities of two species connected by gene flow manifest in terms of selection on the species boundary? It is tempting to think that negative feedback of “sharing” beneficial alleles with a competitor might lead to selection for or against donation. The complication is that the negative effects of improved competitive ability would apply to all of the donor population, not just to genotypes that increase or decrease the probability of gene flow. In the notation used in the model above, individuals carrying alleles that increase or decrease their value of  $m$  would suffer the same negative effects of competition irrespective of their values of  $m$ . So, indirect selection is unlikely to act on alleles that modulate gene flow. Instead, it might provide feedback on the coevolution of traits that affect the strength of the ecological interaction. For example, a donor population whose competitor received a competitive boost from imported alleles could respond through divergence in its resource use or increased competitive ability (Yamamichi et al., 2022).

Even without causing selection on levels of gene flow, changes in ecological interaction strengths could still influence the frequency of different patterns of gene flow across taxa through differential persistence and extinction. Species pairs with porous boundaries might be more likely to persist as two species in cases with neutral or positive interactions rather than between species with strong competitive interactions. This mechanism is additional to the theoretical risk of extinction or collapse of a single species due to genetic swamping (Ma et al., 2019), where extinction is a direct effect of gene flow rather than an indirect effect via a shift in ecological interactions caused by gene flow and adaptive introgression.

### Evaluating evidence that selection maintains porous species boundaries

The hypothesis that selection favours non-zero gene flow between species requires several criteria to be met. First, there must be non-zero gene flow. Second, imported genes must bring net benefits. Third, there must be genetic variation in mechanisms that affect the rate of gene flow between species. Fourth, these conditions should be maintained or recurring over extended timescales rather than a transient feature of recent contact, with species able to persist. Finally, there should be evidence that selection indeed affects the level of gene flow.

To perform a semi-quantitative review of the evidence for these components in the recent literature, I surveyed papers recovered from Web of Science in July 2023 that were published in the years 2019–2023 (chosen to entail a manageable number) with search criteria targeting the topic of porous

species boundaries, refined by Web of Science Categories: Evolutionary Biology or Ecology or Genetics Heredity or Plant Sciences or Biochemistry Molecular Biology or Zoology or Biology. The terms “(species or speciation) and (boundar\* or barrier or isolation)” returned 16,010 papers, of which 886 (5.5%) contained the additional terms “(porous or permeable or introgression or reinforcement or ‘partial reproductive isolation’ or ‘incomplete reproductive isolation’),” which are the focal set here. I counted the incidences of key terms in titles, keywords and abstracts, and scanned titles and abstracts to identify papers that addressed key components of interest.

### Evidence of non-zero gene flow

There is now abundant evidence of cases of inter-species gene flow and transfer in all kinds of organisms (Edelman & Mallet, 2021). The proportion of species that have a porous boundary with at least one other species (whether as taxonomically named or recognized by an evolutionary species concept) is hard to estimate, however, because of the potential bias towards studies of interesting systems with hybrids that were tractable for classical genetics. A few studies have surveyed a wider clade or a set of species pairs, and the probability of introgression generally declines with increasing divergence (Gourbière & Mallet, 2010). For example, within the neotropical bird genus *Dendrocincla*, introgression events are reconstructed to occur up to around 2.5 million years of divergence between interacting species but not between older divergences (Pulido-Santacruz et al., 2020). Extrapolating crudely based on interspecific divergences estimated from DNA barcoding data for neotropical and North American birds as a whole, this would imply that perhaps 11%–33% of species have close enough living relatives for introgression to be feasible (Chaves et al., 2015; Lavinia et al., 2016). Increasing the availability of whole genome data across the Tree of Life will allow unbiased estimates to be made for more clades and whole regional or local assemblages, and correlates of high and low rates of gene flow will be tested.

### Evidence of net benefits to gene flow

There is abundant evidence of adaptive introgression between species (66/886 search papers 2019–2023 refer to it; see also the review of earlier literature by Edelman and Mallet (2021)). Among 115 studies of mammal hybrid zones that reported the consequences of hybridization, 49% reported negative consequences, 38% neutral, and 13% positive (Adavoudi & Pilot, 2022), referring to the transfer of adaptive variants through hybridization. Asymmetry is also a regular finding (for example, in 43/79 plant species hybrid zones surveyed by Pickup et al. (2019) and referred to in 95/886 of search papers 2019–2023). This can be associated with population size differences, such as when a widespread species hybridizes with local rare species (Schley et al., 2020), including invasive species acquiring locally adapted genes (Viard et al., 2020). The outcome can be genetic swamping (Ma et al., 2019) or evolutionary rescue and persistence of the rare species thanks to beneficial alleles acquired from the common one (DeVos et al., 2023; Vedder et al., 2022). Further conditions are needed, however, for selection to favour leaky boundaries. Specifically, it is not enough to observe some cases of adaptive introgression. Rather, there would need to be net or recurrent conditions during which the introgression of beneficial alleles outweighs any negative effects of hybrid formation (e.g., as in the scenario in Figure 1). A survey of 982 comparisons across

animal studies reached similar conclusions as older surveys (Arnold & Hodges, 1995), that hybrids are not always less fit than parental taxa (Arnold & Hodges, 1995; Muraro et al., 2022), which could provide conditions for selection of alleles promoting hybrid formation. Studies generally do not summarize the distribution of selection coefficients, but potentially, this could be estimated from the same data (Cruzan et al., 2021). The selection gradient on the rate of gene flow as a trait could then be calculated to see whether there is an intermediate optimum level or whether zero is favoured.

### Genetic mechanisms for controlling gene flow

Much literature on speciation concerns identifying the genetic basis of isolating mechanisms, providing ample evidence of heritable traits that affect the level of gene flow between species (Westram et al., 2022). These can be asymmetric as well, as might be favoured with asymmetry of costs and benefits between donors and recipients (Van Huynh & Rice, 2021). Of specific relevance is whether it is feasible to encode prezygotic barriers that deliver a particular level of gene flow, say restricting 1% of matings to interspecific. For example, mate choice might be efficient at 95% accuracy. Could genetic changes in the underlying traits reduce this to 90% or increase it to 99% accuracy (Bank et al., 2012)? Evidence for mechanisms enabling patchy isolation across the genome is more common, with sex chromosomes (27/886 search papers, all but one—(Hartmann et al., 2020)—reporting higher differentiation of sex chromosomes), inversions (16/886 papers) and variation in recombination rate across the genome (Martin et al., 2019; Wang et al., 2022) all featuring in the surveyed papers. Patchy separation of high and low gene flow regions across the genome might be the most feasible mechanism to maintain porous species boundaries, although observing variation is not evidence that selection acts on it.

### Non-zero gene flow is maintained rather than transient

Many studies of interspecific gene flow consider close relatives that re-establish contact in geographically restricted hybrid zones (242 of the search papers refer to hybrid or contact zones). Whether introgression is a transient feature or whether it recurs to maintain non-zero gene flow over longer periods in such cases is not generally clear. Some studies report evidence of swamping and predicted extinction of one species as a result of gene flow (Ma et al., 2019) or movement of zones over time (Van Huynh & Rice, 2021), which imply transience of gene flow or species persistence over the longer term. Others report evidence for stable maintenance of the hybrid zone (Schreiber & Pfenninger, 2021; Wang et al., 2020) but do not explicitly test for recurrent adaptive introgression events occurring over an extended period.

Fewer cases of gene flow between congeneric species that co-occur in ecological communities in broad sympatry seemed to be reported among the search list, i.e., outside the context of a geographical contact zone. It was harder, however, to identify search terms that clearly defined such cases. For example, 110 of the papers refer to “sympatry or sympatric NOT hybrid or contact zones,” of which 84 do not mention allopatry/allopatric either, but many of these still refer to geographical contact or reported no evidence of gene flow. Nonetheless, nine papers seemed to fit the category of broadly sympatric species with evidence of coexistence with non-zero gene flow: three on trees (Dang et al., 2019; Li et al., 2021;

Wei et al., 2021, of which two were on the same *Quercus* species), one on bromeliads (Mota et al., 2019), one seagrass (Sinclair et al., 2019), one on bryophytes (Pereira et al., 2019), one on abalones (Hirase et al., 2021), one on crossbill finches (Benkman et al., 2022), plus a further one on the syngameon concept in plants (Buck & Flores-Renteria, 2022), namely of multispecies networks connected by interbreeding. Notable further examples in the wider literature include *Rhagoletis* fruit flies (Feder et al., 1994), *Heliconius* butterflies (Edelman et al., 2019), Darwin's finches (Grant, 1993), ducks (Kraus et al., 2012), the *Anopheles* mosquitoes described above, and other examples reviewed in Edelman and Mallet (2021). Even famous examples such as the Darwin's finches on Isla Daphne Major might not be durable over longer timescales; however, genetic and morphological divergence between a species pair with a permeable boundary has declined over 30 years of observation (Enbody et al. 2023). Furthermore, some authors question how representative they are of bird species more broadly (Zink & Vázquez-Miranda, 2019).

The apparent high prevalence of geographical contact zones in the literature survey could be an artefact of historical interests and methods: comparisons of isolation within and outside contact zones allow tests for reinforcement, and geographical clines provide a useful framework for estimating selection (Barton & Hewitt, 1989). Broad-scale genome sequencing might reveal more cases of gene flow in other settings. Or perhaps introgression is indeed more frequent in cases of geographical contact zones than between well-established, co-occurring species. One hypothesis could be that co-occurring species generally compete for resources in their local area, and so gene flow of beneficial genes would disrupt the coexistence of the donor and recipient. Long-term coexistence and persistence of species with porous boundaries might be more likely when the bulk of the populations are separated in different regions.

### Selection affects the level of gene flow

Evidence for reinforcement, in terms of metrics of increased isolation in secondary contact zones compared to between allopatric populations, is common but not ubiquitous across studies reporting gene flow between diverging species (110/886 papers). This indicates selection on interbreeding and, thereby, potentially on gene flow. Most cases, however, did not distinguish whether selection would eventually close the boundary to zero or whether it favours a low but non-zero value. Only two of the search papers explicitly mentioned selection acting to keep the species boundary open. One was a theory paper exploring mechanisms favouring partial reproductive isolation (Servedio & Hermisson, 2020). The other demonstrated that loci that acted to reduce introgression at an earlier time point had shifted to favour introgression 10 years later in a wood ant hybrid zone (Kulmuni et al., 2020b). An earlier classic case is the spread of insecticide resistance genes between *Anopheles* mosquito species upon deployment of insecticide (Clarkson et al., 2014). *Anopheles colluzzi* and *A. gambiae* sensu stricto are adapted to different larval habitats, and prezygotic isolation is maintained through swarming behaviour (Diabate et al., 2009). Uniform selection caused by insecticide spraying in Ghana led to mutations in the voltage-gated sodium channel gene in *A. gambiae* sensu stricto, which then introgressed into the other species. There was a small increase in the number of observed hybrids during the early stages of the spread of resistance, but the

proportion of hybrid individuals remained less than 1%, consistent with weakening and then re-strengthening of the species boundary during a period of intense uniform selection. This example resembles the scenario modelled in Figure 1, where strong selection on a uniformly beneficial trait favours pulses of increased gene flow. Hybridization is observed in all populations across the range, with the periodical breakdown in assortative mating. Hybrids generally suffer a fitness disadvantage, but some are viable and stable introgression is observed (Lee et al., 2013).

### The same questions can be asked about gene transfer in bacteria

The above discussion focussed on the effects of gene flow in sexual eukaryotes, but interesting comparisons can be made with gene transfer in bacteria (Barraclough, 2019; Diop et al., 2022). Bacterial recombination and gene transfer occur through a wide range of mechanisms that differ from interbreeding in sexual organisms. The closest analogy to sexual recombination in evolutionary effects is homologous recombination via natural competence. Here, DNA is taken up from the environment, and if it is sufficiently homologous to part of the cell's genome, it can be recombined to replace that region. Theory and data show how high genome-wide rates of homologous recombination within populations can limit adaptive divergence in the same way that interbreeding limits sexual divergence (Diop et al., 2022; Fraser et al., 2007; Schmutzer & Barraclough, 2019). This phenomenon could form the basis of a bacterial species concept analogous to the biological species concept in sexuals (Ochman et al., 2005). For example, gene transfer could be restricted to occur within populations adapted to distinct niches but not between them, sometimes called niche-specific gene pools and analogous to a model of reproductive isolation in the sexual case (Polz et al., 2013). Or it could occur more widely within a community or clade (Diop et al., 2022).

One key difference from sexual gene flow is that genotypes with a beneficial heterospecific allele pasted into a parental genome can arise in a single event rather than via F1 and repeated backcrossing. Conditions for adaptive introgression might, therefore, be less restrictive than in sexual eukaryotes (where F1 and early backcross generations must have non-zero fitness if the benefits of adaptive introgression only emerge in later generations). However, the same question can be asked about bacterial homologous recombination as of sexual gene flow. Does selection maintain porous boundaries between species (which might be most unambiguously recognized when they co-occur as genetic clusters with distinct adaptive traits), or are rates of gene transfer by this mechanism an incidental outcome of other forces? The same conditions and kinds of evidence outlined for sexual organisms can be evaluated.

### Evidence of non-zero gene flow

Although homologous recombination occurs primarily within species (Ochman et al., 2005), introgression between taxa identified as separate species also occurs. A recent genomic survey of more than 2,600 bacterial species found that gene flow between bacterial lineages via homologous recombination steeply declines above between 90% and 98% sequence similarity, consistent with gene flow delineating species in a similar way as in sexual eukaryotes (Diop et al., 2022). Only

one putative case of introgression was detected for species with <73% sequence similarity. The amount of introgression varied among taxa: in more than half of cases <1% of the genome but sometimes over 20%. In the same way as was roughly estimated for birds, these results could be extrapolated to estimate the potential number of bacterial lineages connected by gene flow via this mechanism.

### Evidence of net benefits to gene flow

Genomic surveys have found genes showing high rates of transfer are enriched for functional roles consistent with benefits, such as defence-associated genes (González-Torres et al., 2019). More directly, bacteria offer enormous potential for experimental approaches that are difficult to implement in most eukaryotes (Power et al., 2021). For example, Rathmann et al. (2023) generated a library of hybrid genomes by transforming *Bacillus subtilis* and two congeneric donor species and measured selection coefficients of the hybrid genotypes in competition with the untransformed "parent." Between 2% and 5% of the hybrids were identified as having large positive effects, and in contrast to the distribution of fitness effects of mutations, there was no net shift to negative fitness in most growth conditions that were investigated (interpreted as evidence that gene flow differs from mutation because the variants have already been tested as functional in the donor). This study provides evidence not just for the benefits of a particular transfer but also for the net benefit of non-zero rates of transfer events between these species.

### Genetic mechanisms for controlling gene flow

Bacteria possess numerous mechanisms that control the secretion, uptake, processing, and recombination of DNA that could be suitable targets for selection (reviewed in Mell & Redfield, 2014). For example, natural competence is regulated by a range of genetic mechanisms and environmental cues, which include systems for detecting conspecific cells in the environment (as by quorum-sensing pheromone called competence-stimulating peptide in *Streptococcus pneumoniae* (Carrolo et al., 2009)). Other mechanisms, such as restriction enzymes and clustered regularly interspaced short palindromic repeats that defend against genetic parasites, can also affect the specificity of DNA available for incorporation (Oliveira et al., 2016). Modifications to such pathways could increase or decrease the specificity of homologous transfer. One difference from sexual eukaryotes is that the initial formation of a recombinant is intrinsically directional, with a recipient cell receiving DNA released from a donor. This could facilitate the spread of beneficial genes into a new genetic background, as no equivalent to backcrossing is required. It also means that separate mechanisms might change the probability of being a donor versus recipient of heterospecific DNA, which could respond to selection for asymmetric gene flow.

### Selection affects the level of gene flow

If conditions that favour gene flow between species are maintained or recur, then selection could act on the genetic mechanisms controlling the specificity of homologous recombination to modulate the rate of transfer. Tight regulation of competence and its dependence on environmental conditions referenced in the previous section are clearly shaped by natural selection. But, these mechanisms could have evolved to optimize intraspecific recombination and not necessarily to fine-tune non-zero rates of interspecific transfer. Evolution

experiments could be used to test many of the ideas outlined above by manipulating culture conditions of populations with and without transfer of cells or DNA between them and for different divergent and fluctuating environments over 1,000s of generations (e.g., [Tusso et al., 2021](#)). Perhaps uncertainty over species concepts in bacteria explains the scarcity of “speciation experiments” with bacteria. Classic work addressed adaptive divergence within a tube in *Pseudomonas fluorescens* ([Rainey & Travisano, 1998](#)) and the limiting effects of recombination within the host for ecological divergence of bacteriophage lambda ([Meyer et al., 2016](#)). Evidence that homologous recombination delineates bacterial species, but with porous boundaries and interesting pathways to control rate and specificity, provides a new stimulus to test when selection would increase or decrease the rate of gene flow. Genetic manipulation could also be used to generate mutants with higher or lower propensity for transfer ([Perron et al., 2011](#)), whose fitness can be compared in competition experiments.

My goal here is to illustrate similarities and differences between bacterial divergence and speciation in eukaryotes, as well as the potential benefits of bacterial experiments, to address the central question of this article. Of course, there are many more mechanisms of gene transfer in bacteria than highlighted here, which could raise similar questions (for example, does selection act to control the host range of conjugative plasmids, or is interspecific transfer an accidental by-product of selection for transfer within species?), as well as different ones (for example, what selection pressures determine the range of gene transfer when mediated by a vector such as transfer via viruses, i.e., transduction, where selection can act differently on the donor, vector, and recipient ([Sheppard et al., 2021](#))?). The host range for transfer might be broader in other mechanisms and more akin to horizontal gene transfer between distant relatives (also observed in sexual eukaryotes). Homologous recombination applies particularly to the core genome, whereas bacteria can have large accessory genomes that vary in presence, absence, and degree of sharing within and between species due to alternative mechanisms of transfer. Full consideration is beyond the scope of this article, but a similar evaluation of these other mechanisms would be fruitful.

## Conclusions

While there is strong evidence for gene flow and adaptive introgression between species, evidence for the more specific hypothesis that selection favours non-zero levels of gene flow is currently inconclusive. Several requirements seem to be often met. Conditions in which hybrid genotypes have greater fitness than parental genotypes can arise in fluctuating environments (e.g., [Figures 1 and 2](#)), even in the context of adaptive divergence. Empirical work on both eukaryotes ([Muraro et al., 2022](#)) and bacteria ([Rathmann et al., 2023](#)) shows that net fitness benefits in hybrid genotypes are not uncommon. Heritable traits that affect the probability of gene flow exist that could be targeted by selection. The alternative hypotheses that selection acts to reduce gene flow (but is too weak to close the species boundary) or that species boundaries are accidental by-products of selection within each species are equally credible, however. Yet, in many cases, these alternatives have not been directly tested.

Studies are needed that estimate the selection gradient on the rate of gene flow (either overall or as it varies across

genome regions) and then test for evolution towards an optimal, intermediate level of gene flow. In genetically tractable organisms such as yeast or *Drosophila* it might be possible to measure the distribution of fitness effects directly across panels of hybrid individuals as [Rathmann et al. \(2023\)](#) did for bacteria. Alternatively, the distribution of fitness effects of heterospecific alleles could be modelled from polymorphism patterns between two species connected by a porous boundary ([Barton, 1983](#)).

Testing for optimal non-zero rates of gene flow is hard for wild populations, and there is a need for more experimental studies of speciation in organisms with short generation times. Experiments could investigate the evolution of rates of gene flow and underlying mechanisms that control them under different patterns of environmental change. *Drosophila*, yeast and other fast-reproducing eukaryotes have been used for speciation experiments (reviewed in [White et al., 2020](#)), but bacteria offer a particularly useful but neglected opportunity. Under different combinations of divergent and uniform selection pressures on populations with manipulated rates of contact and potential for gene flow, how do mechanisms determining rates of gene transfer evolve? Real divergence and species barriers are messy, but theory and experiments provide a way to navigate the complexity.

For observational studies, more systematic surveys of how the probability of gene flow and introgression scales with evolutionary divergence are required to estimate the frequency and scope of interspecific gene flow as a contributor to adaptation. In addition, whole genome sequencing projects for whole communities and ecosystems will allow more unbiased estimates of the frequency of gene flow among co-occurring species.

Finally, levels of gene flow observed among co-occurring species in nature will depend on the impacts of gene flow on coexistence and long-term persistence, as well as on selection pressures acting on individuals within populations. Some types of species boundary might be more likely to permit long-term persistence of multiple species than others, depending on the ecological interactions and mechanisms of coexistence between the species. Specifically, it would be fruitful to investigate when the transfer of beneficial alleles among species is expected to increase or decrease the stability of co-occurrence among sets of species.

## Data availability

The code used to generate Figures 1 and 2 is available at [github.com/tim-barra/PorousBoundaries](https://github.com/tim-barra/PorousBoundaries).

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## Conflicts of interest

None declared.

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