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TARGET REVIEW

Hybridization and speciation*

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Abstract

Hybridization has many and varied impacts on the process of speciation. Hybridization may slow or reverse differentiation by allowing gene flow and recombination. It may accelerate speciation via adaptive introgression or cause near-instantaneous speciation by allopolyploidization. It may have multiple effects at different stages and in different spatial contexts within a single speciation event. We offer a perspective on the context and evolutionary significance of hybridization during speciation, highlighting issues of current interest and debate. In secondary contact zones, it is uncertain if barriers to gene flow will be strengthened or broken down due to recombination and gene flow. Theory and empirical evidence suggest the latter is more likely, except within and around strongly selected genomic regions. Hybridization may contribute to speciation through the formation of new hybrid taxa, whereas introgression of a few loci may promote adaptive divergence and so facilitate speciation. Gene regulatory networks, epigenetic effects and the evolution of selfish genetic material in the genome suggest that the Dobzhansky–Muller model of hybrid incompatibilities requires a broader interpretation. Finally, although the incidence of reinforcement remains uncertain, this and other interactions in areas of sympatry may have knock-on effects on speciation both within and outside regions of hybridization.

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Introduction

If hybridization is defined as reproduction between members of genetically distinct populations (Barton & Hewitt, 1985), producing offspring of mixed ancestry, then it occurs in almost all proposed processes of speciation. The only exceptions would be cases of completely allopatric or instantaneous speciation. Hybridization may cause interactions involving a wide range of types and levels of genetic divergence between the parental forms. This divergence may have accumulated in different ways including neutral divergence, local adaptation and coevolution. Any of these may generate novel phenotypes through interactions in hybrids, including both advantages of transgressive segregation and disadvantages mediated by intrinsic or environmentally mediated incompatibilities. Therefore, the consequences of hybridization and the role it might play in promoting or retarding speciation can be expected to vary widely both between different hybridizing taxa and at different stages of divergence.

Hybridization may occur in many different spatial contexts (Fig. 1). Some of these have been studied intensively, most notably the formation of hybrid zones at abrupt parapatric boundaries (Harrison, 1993) and the exchange of genes between locally adapted populations, such as host races in phytophagous insects (Dres & Mallet, 2002), where there may be no spatial separation at scales above typical dispersal distances. Hybridization may also differ in temporal context, for example, secondary contact after a period of independent evolution vs. continuous contact with divergent selection. Hybridization may follow habitat disturbance, range expansion or both (as in *Senecio,* Abbott *et al.*, 2003; or baboons, Zinner *et al.*, 2009; for example) and may occur in complex habitat mosaics combining some of the features of hybrid zones with those of local adaptation (as in Louisiana *Iris*, Arnold *et al.*, 2012; *Allonemobius* crickets, Ross & Harrison, 2002; or *Mytilus* bivalves, Bierne *et al.*, 2003).

Hybridization may be common and widespread, spatially or temporally localized or globally rare. It may influence a rare interacting population much more strongly than an abundant population, and its consequences may depend on whether populations are growing or contracting, local or invasive (Currat *et al.*, 2008). In all cases, the pattern of contemporary hybridization is potentially only a single snapshot of a complex and continuously changing interaction. The evolution of complete reproductive isolation may take hundreds to millions of generations. During this time, populations change in size and spatial distribution, perhaps cyclically due to periodical climate changes (Hewitt, 1996, 2011), and the processes that enhance or erode barriers to gene exchange, including hybridization, may occur at different stages or locations during this extended history (Fig. 1). Although many of the debates concerning outcomes of hybridization refer to specific scenarios, it is important to keep this spatial and temporal context in mind when considering the broad significance of hybridization.

In the context of speciation, hybridization may have several distinct outcomes, which have attracted very different levels of research interest. First, there may be a stable, or at least persistent, balance between selection and hybridization, with only some parts of the genome introgressing between hybridizing populations. This may be true both in tension zones (hybrid zones involving a balance between selection against hybrids and dispersal; Barton & Hewitt, 1985) and in populations adapted to distinct habitats (Nosil *et al.*, 2009). In either case, there may be no progress towards speciation but existing differentiation may be maintained, with the potential for future divergence when circumstances change. Alternatively, barriers to gene exchange may breakdown in such a situation, leading to a reduction or loss of differentiation (e.g. Taylor *et al.*, 2006). The opposite type of outcome involves an increase in the strength of any barriers to gene exchange and a progression towards larger areas of the genome being protected from introgression (Wu, 2001; Via, 2009). This outcome, where hybridization initiates speciation, is that which has probably attracted greatest controversy and therefore is given more attention here. Reinforcement (Servedio & Noor, 2003) is an example of one process that might be involved, where a premating barrier evolves in response to reduced hybrid fitness. Finally, and distinctly, hybridization might contribute to adaptive divergence between populations, and it might also result in the generation of new populations of mixed ancestry that remain distinct from both parental populations (hybrid speciation: Mallet, 2007; Abbott *et al.*, 2010). These new populations may be sexual or asexual, homoploid or polyploid. We do not consider asexual hybrid lineages here, but see Bullini (1994) for a review. Reinforcement and hybrid speciation, in particular, may have subsequent knock-on effects, facilitating or catalysing further speciation through the differences they generate between populations that are exposed to hybridization and those that are not.

Recent reviews of aspects of speciation (e.g. Fitzpatrick *et al.*, 2009; Nosil *et al.*, 2009; Sobel *et al.*, 2010; Wolf *et al.*, 2010a; Nei & Nozawa, 2011; Smadja & Butlin, 2011) have touched on the role of hybridization in speciation, but none has explicitly dealt with a discussion of the central role of hybridization in species divergence. Here, we recognize that hybridization is widespread, diverse in form and in its potential to contribute to individual speciation events. We focus on identifying key areas of current uncertainty, especially about the circumstances in which the different outcomes introduced above might be more or less likely. We aim to clarify the nature and importance of open questions in these areas and, wherever possible, suggest

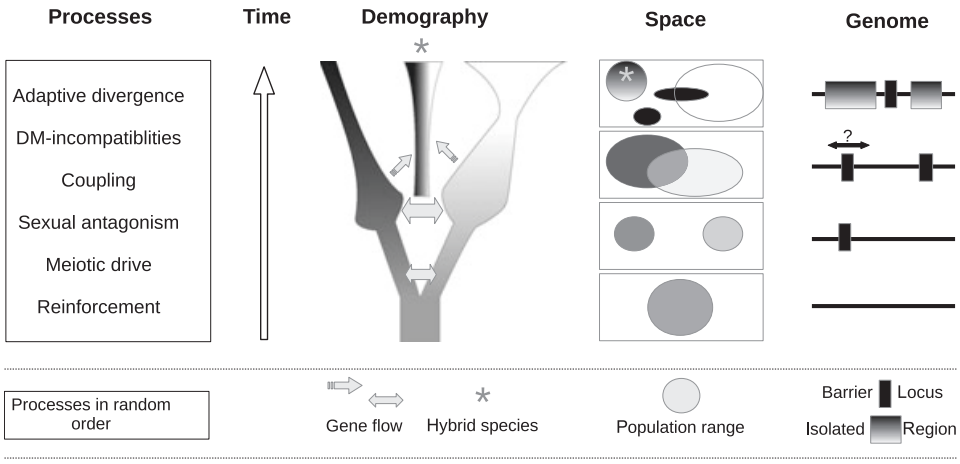

Fig. 1 Speciation is a multi-level process unfolding through time and space. Populations are subjected to demographic processes and are repeatedly redistributed in space. Novel ecological opportunities can arise, and periods of physical separation will alternate with periods of gene flow. It is quite conceivable that different mechanisms are acting during the different phases of the divergence process. From a genetic perspective, barrier loci that contribute to reduction in gene flow between diverging genomes may, if conditions are right, accumulate gradually, extend or combine their effects until the diverging genomes are eventually sealed off from each other and will not mix any further. Introgression may favour divergence or hybridization may generate new, isolated populations.

ways for tackling them. First, we consider alternative outcomes: (i) The development of barriers to gene flow and the factors that promote fission rather than fusion of hybridizing populations and (ii) The contribution that hybridization makes to adaptive divergence and to the origin of new hybrid species. We then discuss the genetic and genomic foundations of these divergent processes, and finally, we look at some of their longer-term consequences.

Hybridization and the development of genetic barriers to gene flow

Barriers to gene exchange might accumulate during periods when gene flow does not occur due to spatial isolation or physical obstacles to dispersal. However, it is common for populations that have developed incomplete reproductive barriers to be in contact at some stage of divergence, often due to range change, allowing the opportunity for gene flow between them. A critical question in speciation is whether, under these conditions, initial divergence breaks down or barriers to gene flow are enhanced and promote speciation. Theory suggests that this will largely depend on the overall antagonism between selection and recombination among diverging loci (Felsenstein, 1981). For incompletely isolated populations to progress towards speciation, associations among the loci that influence isolation must build up (Smadja & Butlin, 2011). This implies that gene flow is further reduced either at individual barrier loci or across a greater fraction of the genome through associations with these loci (we define 'barrier loci' as those under divergent selection or that contribute to reduced hybrid fitness or to assortative mating). Loci that do not contribute to reproductive isolation or are not closely linked to loci that confer some degree of isolation are likely to introgress between hybridizing populations (Barton & Bengtsson, 1986). This raises the important question of what patterns of genomic differentiation we expect to see between diverging taxa: How many genomic regions differentiate during speciation? How small are regions where divergence significantly exceeds the genomic average (sometimes called islands, continents or signatures of divergence in the genome, see Turner *et al.*, 2005; Nosil *et al.*, 2009; Michel *et al.*, 2010)? How are regions of exceptional divergence dispersed around the genome? We suggest that recent discussions of these issues in the context of ecological speciation would benefit from closer attention to well-established cline theory.

What does cline theory teach us about the development of isolating barriers?

Cline theory provides a framework for understanding the dynamics of reproductive barriers in the face of gene flow. Single-locus barriers to gene flow are rarely absolute and protect only closely linked loci from introgression. Associations between very many barrier loci, spread across chromosomes and likely to be involved in multiple traits, are required to allow significant portions of the genome to diverge on each side of a consensus cline (Barton & Hewitt, 1985). Recombination will

break down these associations, whereas selection limits introgression and maintains them. The strength of associations is therefore determined by the balance between the two, quantified by a coupling coefficient S/R (Barton, 1983; Baird, 1995; Kruuk *et al.*, 1999), in which selection (S), is totalled over barrier loci and recombination (R) is the total map length between barrier loci. High coupling maintains associations and consequently a strong barrier in the long term, favouring independent adaptation despite hybridization. In contrast, with low coupling, barrier loci act independently of one another and are ineffective in keeping populations isolated (Fig. 2a) (Barton, 1983; Baird, 1995).

Under certain conditions, barriers to gene flow can be enhanced over time (Navarro & Barton, 2003; Barton & de Cara, 2009). Clines at endogenous barrier loci (where selection results from intrinsic incompatibilities) are not constrained to occur at environmental transitions; they are expected to move towards and coincide in areas of lower population density (Hewitt, 1975; Barton, 1979). Clines can also move and become coincident due to asymmetrical fitness of parental genotypes (Barton & Turelli, 2011). When different endogenous clines meet and overlap, they are expected to become coupled and then these multiple clines move together in space. Such moving tension zones will be trapped by natural barriers to dispersal (Barton, 1979) or will couple with local adaptation clines that are geographically stabilized by selection and therefore become localized (Fig. 2b, Bierne *et al.*, 2011). Spatially coupled barriers increase the number of loci contributing to *S* at their new joint position, which in turn sharpens clines (Clarke, 1966), increases barrier strength and makes long-term maintenance of the hybrid zone and of the differentiation between populations more likely (Barton, 1983).

The effect of spatial structure in favouring such a coupling process by generating sufficient linkage disequilibrium to associate unlinked loci when clines overlap has been known for some time (Slatkin, 1975; Endler, 1977; Barton, 1983). Such increases in coupling may be considered steps towards speciation, as they lead to increasingly independent evolutionary trajectories of the taxa on either side of the accumulated barrier. Indeed, spatial coupling is part of a more general phenomenon which includes the build-up of reproductive barriers through linkage disequilibrium between adaptive and assortative mating loci (Felsenstein, 1981). An analogous process can also operate within a single panmictic population, though requiring some combination of strong selection, tight linkage and multiplicative fitness effects (Barton & de Cara, 2009). A current challenge is to integrate these ideas with those about the strengthening of barriers between locally adapted populations that are an important component of the current ecological speciation literature (e.g. Via, 2009; Feder *et al.*, 2012).

Mechanisms that can enhance coupling

From the theory briefly outlined above, one can think of mechanisms that can catalyse speciation as those that enhance the coupling of a system: (i) mechanisms that reduce recombination (*R*), (ii) mechanisms that maximize selection at the genome scale (*S*) and (iii) mechanisms that make clines overlap and prevent their movements, bringing both endogenous and exogenous selection together.

(i) Coupling is more efficiently maintained with reduced recombination, which can arise due to segregating inversions (Noor *et al.*, 2001; Navarro & Barton, 2003; Kirkpatrick & Barton, 2006) or other modifiers of recombination (e.g. genomic divergence due to transposable elements that suppress recombination in hybrids; see below). (ii) Epistasis among barrier loci would lead to higher *S* than under additivity; gene expression patterns in hybrids can be consistent with this type of epistasis

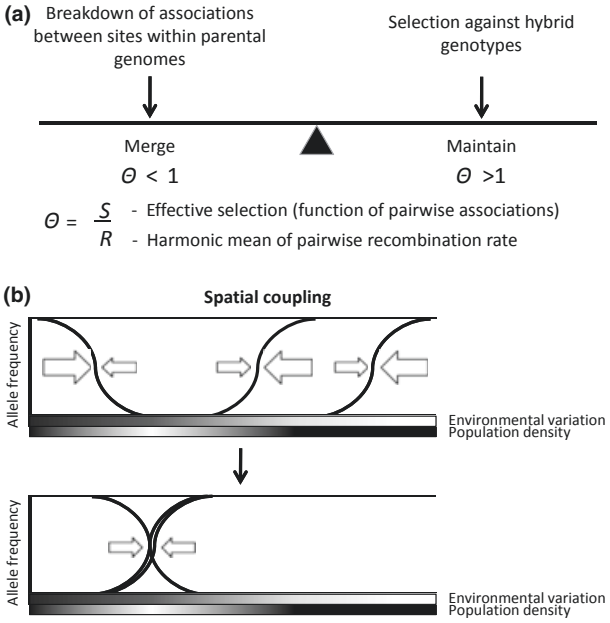

Fig. 2 (a) The outcome of hybridization, leading to fusion of populations or to a stable or increasing barrier to gene exchange, depends on the balance between selection and recombination, which can be expressed as a coupling coefficient, Θ. (b) Spatial coupling of clines. Coupling depends on the genetic architecture of incompatibility selection and on population density and environmental variation. Upper panel: Incompatibility clines can move because of asymmetric fitness effects (one parental genotype is fitter) or because of a gradient in population density. Arrows illustrate this movement as asymmetric-effective migration rates, with asymmetry due to either demography or selection. Lower panel: Increased coupling in space arises from the tendency of clines to attract one another and then to move together to regions of low population density, environmental transitions or both. The shaded areas illustrate the variation in the environment and population density.

(see below). (iii) Moving clines can be spatially stabilized by physical barriers to dispersal or by local adaptation. All these mechanisms can act independently or in concert to build up genetic barriers. It should be emphasized that the effect of ecologically driven divergent selection is two-fold: it fuels the populations with divergently selected barrier loci, and it contributes to anchoring, at environmental boundaries, clines for barrier loci that do not interact directly with the environment (endogenous loci, e.g. Dobzhansky–Muller incompatibilities, assortative mating genes; Bierne *et al.*, 2011).

Coupling new ecological adaptations with old intrinsic barriers, an alternative interpretation of seemingly rapid speciation events

Coupling may play a more important role during rapid adaptive population subdivision than is appreciated. The accumulation of intrinsic genetic incompatibilities is often thought to occur too slowly to explain emblematic examples of ecological speciation (e.g. recent host shifts in phytophagous insects or pathogens, Rundle & Nosil, 2005). This is because ecological adaptation has been shown to evolve on a short timescale, even in the absence of geographical isolation, despite the comparatively long waiting time for mutations that could cause incompatibilities between populations to accumulate in appreciable numbers (see Kondrashov, 2003; Gavrilets, 2004). However, coupling theory shows that pre-existing intrinsic incompatibilities in a tension zone can be recruited to enhance ecological barriers between populations (Bierne *et al.*, 2011). This coupling recruitment process is different from the usual view of reinforcement of premating isolation (Barton & de Cara, 2009). Coupling can build up associations between loci that contribute to any kind of barrier (pre- or post-zygotic, endogenous or exogenous), including pre-existing barrier loci segregating within one of the populations. This could explain the strikingly deep coalescences often observed at exceptionally differentiated loci between populations in different habitats (Schulte *et al.*, 1997; Pogson, 2001; Colosimo *et al.*, 2005; Wood *et al.*, 2008). Some recent host shifts in phytophagous insects (corn borer, Malausa *et al.*, 2005; maggot fly, Michel *et al.*, 2010) might well result from a new adaptive polymorphism that contributes to the host shift coming into association and coupling with incompatibility loci from a cryptic pre-existing tension zone. If this is the case, then in these systems ecology should probably not be thought of as the initial catalytic agent of speciation, but rather a subsequent ingredient enhancing further build-up of reproductive barriers.

New directions in the study of genetic barriers to gene flow

Theory demonstrates that it is possible for multiple barriers to accumulate, or couple, even without spatial isolation. Yet we do not know, either from theory or from accumulated empirical data, whether it is a common evolutionary outcome for reproductive isolation to be enhanced when incompletely isolated populations are in contact. It is unclear to what extent initially divergent populations will become further isolated under conditions of gene flow, stay at the current level of isolation or become less distinct. Evidently, the likelihood of these alternative outcomes will be shaped by the fluctuations in geographical and demographic conditions over time because phylogeographical history strongly influences the nature of the interacting populations and the circumstances of their contact (Hewitt, 2011). The final outcome whereby two species are completely isolated, in the sense that neutral loci are expected to diverge, may occur long after genomically localized divergence was established at multiple, coupled barrier loci. Since introgression and time can easily erase the history of populations at most neutral markers (Grahame *et al.*, 2006; Roberts *et al.*, 2010; Marshall *et al.*, 2011), the history of incipient speciation should ideally be reconstructed using data from barrier loci, accounting for the potential action of selection on these loci (Williamson *et al.*, 2005). A considerable empirical challenge is to move from simply identifying such loci (both endogenous and locally adapted) via analyses of the patterns of genomic divergence (genome scans), QTL mapping or genome-wide association studies to determining sources of selection, measuring their interactions and inferring their temporal sequence of accumulation.

Although cline theory provides expectations for the behaviour of a high-dimensional system (populations, evolutionary processes, genomes, time, space, demography, etc., see Fig. 1), it is an ongoing challenge, even with the relative ease of producing genomic data, to connect nucleotide variation to phenotypes of individuals and tie these to the evolutionary dynamics of populations. To understand the build-up of a genetic barrier, one might capitalize on situations in which associations between the various components of reproductive isolation differ. This can be the case between multiple transects across a single hybrid zone (Szymura & Barton, 1991; Yanchukov *et al.*, 2006; Nolte *et al.*, 2009; Teeter *et al.*, 2010) or replicated combinations of the same lineages at different locations (Riginos & Cunningham, 2005; Butlin *et al.*, 2008; Simard *et al.*, 2009; Bernatchez *et al.*, 2010; Hohenlohe *et al.*, 2010; Caputo *et al.*, 2011). At a larger timescale, the comparison of barriers among multiple taxa with different divergence times can provide insights into the sequential accumulation of barrier loci in a genome (Nadeau *et al.*, 2012). Experimental evolution is an alternative way of studying the accumulation of barrier loci in a controlled environment (Dettman *et al.*, 2007). If known, the number and effects of potential barrier loci and their rates of introgression between populations can provide a basis for analysis. The physical linkage and statistical associations

among barrier loci in hybrids are also key to understanding the dynamics of further development of isolating barriers. High-resolution comparative linkage maps for the divergent populations can indicate whether chromosomal rearrangements are likely to play a role. Likewise, estimates of recombination rates and pairwise associations between putative barrier loci in hybrids and the potential for blocks of ancestry and disequilibria to be retained in hybrids (Baird, 1995) are crucial for understanding the dynamics of progress towards speciation. The theoretical basis of such analyses is best developed for cline theory, but needs to be extended more fully to other geographical scenarios (Fitzpatrick *et al.*, 2009) and to intermittent hybridization.

How often is hybridization a source of adaptive variation that may contribute to speciation?

Incomplete barriers to gene flow retard the exchange of adaptive variation very little. Therefore, one possible outcome of hybridization may be the introgression of selectively favoured alleles from one population into another. This can bring together new adaptive combinations of alleles, which arose in different populations, in much the same way as sexual reproduction within populations leads to the production of combinations of alleles that may provide the basis for adaptive evolution. In this section, we argue that introgression could have important implications for the origin of species.

Consider the following:

1 Hybridization among species is reasonably common on a per-species basis, even though usually very rare on a per-individual basis. About 10–30% of multicellular animal and plant species hybridize regularly. Among those that do hybridize, between 1 in 100 and 1 in 10 000 individuals are hybrids when in sympatry (Mallet, 2005).
2 Mutations are rare, around 10^{-8} to 10^{-9} per generation per base pair. Thus, it is likely to take considerable time for novel adaptations to evolve via mutation and natural selection within a species (depending on the population size).
3 Hybridization among species can act as an additional, perhaps more abundant, source of adaptive genetic variation than mutation (Grant & Grant, 1994; Kim & Rieseberg, 1999; Arnold & Martin, 2009; Whitney *et al.*, 2010; Kunte *et al.*, 2011). For example, in Darwin's finches, 'New additive genetic variance introduced by hybridization is estimated to be two to three orders of magnitude greater than that introduced by mutation' (Grant & Grant, 1994). This process is often referred to as 'adaptive introgression' (a somewhat misleading term because, whereas hybridization and introgression can lead to adaptive evolution, the initial hybridization itself is unlikely to be adaptive and is often selected against).
4 Adaptation is thought to be the most important process driving divergence during speciation (Coyne & Orr, 2004; Sobel *et al.*, 2010; Servedio *et al.*, 2011). Barriers to gene exchange between species, including assortative mating, ecological divergence and Dobzhansky–Muller incompatibilities, can all be driven by adaptation. Assortative mating can be a result of sexual selection, social organization, reinforcement or a by-product of adaptation to different habitats (Ritchie, 2007; Seifert, 2010; Sobel *et al.*, 2010). Divergence in ecology occurs almost exclusively under selection. Recently characterized Dobzhansky–Muller incompatibilities in *Drosophila* have been shown to be driven by strong positive selection, although this may not derive from adaptation to the external environment (Orr *et al.*, 2004).
5 Closely related species tend to hybridize more often. In particular, species in rapidly diversifying adaptive radiations may be particularly prone to hybridization (Price & Bouvier, 2002; Seehausen, 2004; Gourbière & Mallet, 2010).

Taken together, these points suggest that hybridization and introgression, via their role in adaptation, are likely to contribute to speciation, especially in rapidly speciating taxa.

The importance of adaptive introgression in speciation will depend on the nature of adaptive variation. In species with very large populations (e.g. *Homo sapiens* and *Drosophila melanogaster*), every possible DNA substitution may arise even within one generation. However, not all species have such large populations, and some classes of adaptive variation may be uncommon even in large populations. Complex adaptations consisting of many genetic changes, for example, will be more rarely encountered than simple mutations. QTL mapping has shown that adaptive traits often consist of multiple loci, spread throughout the genome (McKay & Latta, 2002; Albert *et al.*, 2008). Hybridization has the potential to introduce large sets of new alleles at multiple unlinked loci simultaneously, although strong nonadditive selection may be needed to maintain these sets. Modular, cassette-like variation (e.g. multiple substitutions in a single gene or a set of linked coding genes and their regulatory elements) (Kim *et al.*, 2008), the components of which have been tested previously by natural selection on their original genetic backgrounds, may be exchanged. In *Heliconius*, transfer of mimetic patterns across species boundaries requires introgression of complex alleles at multiple loci (Heliconius Genome Consortium, 2012), allowing the rapid acquisition of a genetic architecture that would be difficult to evolve by sequential accumulation of mutations. Repeated introgression is particularly effective in introducing polygenic variation because it will generate multilocus genotypes that remain in transitory linkage disequilibrium, persisting for several generations after each hybridization event.

A large fraction of introgressed variation is likely to be deleterious, and many hybridization events may have no long-term impact. However, when large numbers of hybridizations occur among closely related species, there is more chance that some will contribute to adaptation and speciation. This is expected to depend very much on ecological opportunity. The existence of opportunities for hybrid populations is seemingly demonstrated by the high frequency of speciation events produced by allopolyploidy in plants (but see below – Allopolyploid Speciation). The abundant genotypes produced by recombination in hybrids should facilitate further exploration of ecological niches different from those of the parents.

Hybridization leading to a new taxon, distinct from both parent species (but with no increase in ploidy), is variously called homoploid hybrid speciation or recombinational speciation (Mallet, 2007; Mavarez & Linarez, 2008; Abbott *et al.*, 2010) (see Fig. 3 and next section). It is usually argued that this process is rare (Rieseberg, 1997), but promotion of adaptive divergence as a result of introgression may be much more common and have the potential to lead to increased reproductive isolation between populations. Therefore, it is critical that these processes are separated, both conceptually and empirically. However, detecting potential adaptive introgression is difficult. It should become easier with new genomic techniques which may show that its frequency has been underestimated in the past. Introgressed genetic variation can enhance the ability to coexist and promote invasiveness (Prentis *et al.*, 2008), and thus help to enlarge the range of a hybrid population substantially. There is likely to be a positive feedback between hybridization and speciation (Seehausen, 2004): hybridization may increase the rate of speciation, and the resulting diversity of closely related species may then provide more opportunities for hybridization. Introgression and hybrid speciation could therefore contribute to the positive feedback of diversity on diversification (Emerson & Kolm, 2005). Systematic tests which conclusively distinguish introgressed alleles from shared polymorphisms are needed, extending beyond cases where there are initial phenotypic clues (such as in butterfly wing patterns) and specifically addressing the role of introgression in adaptive radiation.

Homoploid hybrid speciation

As mentioned above, one potential outcome of hybridization and admixture is homoploid hybrid speciation, which does not involve ploidy changes in the hybrid (Mallet, 2007; Mavarez & Linarez, 2008; Abbott *et al.*, 2010). A causative, creative role of hybridization is the key feature distinguishing hybrid speciation from neutral admixture of multiple parental genomes. Novel combinations of parental alleles must have contributed to the establishment and persistence of a new population that maintains its distinctness by means of reproductive barriers with both parents. This outcome is what distinguishes hybrid speciation from adaptive introgression. The crucial line of evidence for hybrid speciation is therefore to identify unique hybrid traits that cause isolating barriers, although extensive genomic admixture can also be an important indicator of the process. *Helianthus* sunflowers are at the highly admixed end of a continuum where the hybrid genomes comprise major contributions from both parental taxa and are now isolated from both parents. In contrast, hybrid speciation in *Heliconius* butterflies involves adaptive introgression of just one or a few loci that are incorporated into a divergent genetic background and play a direct role in barriers to gene flow (Heliconius Genome Consortium, 2012). In both cases, evidence that hybridization has played a key role was obtained through experimental re-creation of hybrid phenotypes in the laboratory (Rieseberg *et al.*, 2003; Mavarez *et al.*, 2006). Whereas these systems stand out as hallmark examples, the question arises as to how frequently hybrid speciation occurs and which genotypic and phenotypic signatures remain? Mixed ancestry in the genome of a new taxon is an important signal of hybrid speciation, but it is hard to distinguish from ancestral polymorphism or continued gene exchange and alone is not a sufficient criterion. Admixture measures should ideally be combined with trait-based studies that connect admixture with the origin of reproductive barriers, such as the

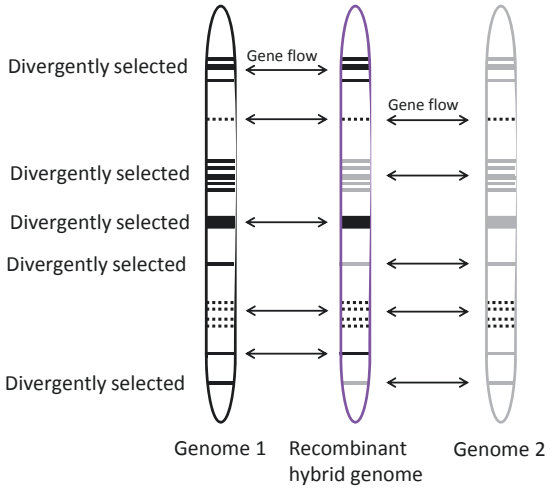

Fig. 3 Adaptive introgression and hybrid speciation. Divergently selected loci (depicted as black and grey solid lines) in two populations can be combined by recombinant hybridization. The resulting hybrid combination can potentially be adaptive and favoured in a new habitat and can give rise to an independent hybrid taxon (hybrid speciation), or it can allow one population to evolve further, replacing the original genome (adaptive introgression). Globally adaptive variation as well as neutral variation (both depicted as broken lines) can be exchanged between all populations via gene flow through hybridization.

identification of alleles underlying specific wing pattern elements in *Heliconius* (Salazar *et al.*, 2010).

When comparing examples, it is important to consider the ages of the hybrid taxa and whether they are proceeding along divergent evolutionary trajectories in order to separate stages in the process. Analyses of hybrid swarms or young hybrid taxa can play an important role in elucidating the first steps towards hybrid species (Nolte & Tautz, 2010). Although such taxa may not, in the end, give rise to well-differentiated hybrid species, they can facilitate testing key predictions from models of hybridization and hybrid speciation (Buerkle *et al.*, 2000; Barton, 2001). For example, hybrid populations most likely originate following secondary contact in newly available habitat (after expansion from refugia or artificial introduction) but may be most likely to evolve into hybrid species when a new ecological space is available that is not utilized by the parental taxa. Exogenous selection can then maintain the distinct hybrid taxon even though initial barriers to gene exchange with the parents are not complete. Case studies of recently emerged hybrid taxa, such as *Cottus* fishes (Stemshorn *et al.*, 2011), Italian sparrows (*Passer italiae*; Elgvin *et al.*, 2011; Hermansen *et al.*, 2011), Appalachian swallowtail butterflies (Kunte *et al.*, 2011) and Oxford ragwort (*Senecio*; James & Abbott, 2005; Brennan *et al.*, 2012), show that they remain distinct even though reproductive barriers are not absolute. In Oxford ragwort, the hybrid population has colonized a new environment geographically isolated from those occupied by its parents whereas, in the other three cases, reproductive barriers are sufficient for hybrid taxa to coexist parapatrically (Nolte *et al.*, 2006) or even sympatrically with parental forms (Hermansen *et al.*, 2011; Kunte *et al.*, 2011). Nevertheless, additional evidence should be sought for a direct role of hybrid allelic combinations in barriers to gene flow. Progress in this direction has been made in the analysis of the very recent hybrid origin of Lonicera flies (*Rhagoletis mendax* × *zephyria*; Schwarz *et al.*, 2005, 2007), where hybrid traits governing host selection have emerged rapidly and simultaneously caused significant reproductive isolation (allowing the hybrid to persist in sympatry with both parents).

There are numerous study systems in which admixture has occurred at some point in the past. Examples include radiations of fishes such as crater lake cichlids (Schliewen & Klee, 2004), sharpfin silversides (Herder *et al.*, 2006) and the postglacial radiation of whitefishes (Bernatchez, 2004; Hudson *et al.*, 2011). Convincing evidence for ancient admixture has been found in all of these systems, but further evidence is needed for a direct role of hybridization in creating reproductively isolated populations or accelerating diversification. Fixed genomic blocks derived from different parental populations can indicate a hybrid genetic architecture that has evolved because it confers a fitness advantage and creates a reproductive barrier (Fig. 3). Great potential for future studies lies in analyses of the structure of hybrid genomes, particularly the size and distribution of blocks derived from alternative ancestors (Barton, 1983; Baird, 1995). However, fixation of blocks from different parents will also occur through genetic drift (Ungerer *et al.*, 1998; Buerkle & Rieseberg, 2008), and this scenario must be excluded before evidence for hybrid speciation is accepted. Although modelling of the decay of linkage disequilibrium in admixed genomes (Pool & Nielsen, 2009) and the fixation of ancestral blocks (Buerkle & Rieseberg, 2008) have been employed to study hybrid speciation, such methods have yet to be applied to a wide range of hybrid taxa, and further development of these methods is critical. Inferring the evolutionary significance of hybrid genetic architecture in speciation may become more problematic the further back in time the event lies because drift and selection become harder to distinguish.

Together with the age of the hybrid species itself, the level of divergence between the parental taxa is another important consideration for homoploid hybrid speciation studies (as it is for allopolyploid speciation: Paun *et al.*, 2009; Buggs *et al.*, 2009). When divergence is low, there may be little chance of major novelties arising in hybrids but, when divergence is high, intrinsic incompatibility may prevent successful hybridization. Crossing experiments with cichlid fish provide support for more divergent populations being more likely to generate novel trait combinations (Stelkens & Seehausen, 2009). Thus, an important challenge in studies of hybrid speciation is to ask whether there is an 'optimal' genetic distance for homoploid hybrid speciation (Arnold *et al.*, 1999; Gross, 2012).

Allopolyploid speciation

Polyploidy, which results in species containing three or more homologous chromosome sets rather than the two in their diploid ancestors, is an important mechanism in hybrid speciation because it creates a strong, though often incomplete, postzygotic reproductive barrier between a hybrid and its parents. While common in only some animals (Mable *et al.*, 2011), polyploidy is of major significance in plant evolution with the latest estimates indicating that all extant flowering plants have polyploidy in their ancestry (Jiao *et al.*, 2011), whereas 15% of angiosperm and 31% of fern speciation events directly involve polyploidy (Wood *et al.*, 2009). Two types of polyploids are normally recognized: autopolyploids in which chromosome sets are derived from the same species and allopolyploids that contain chromosome sets from different species as a consequence of interspecific hybridization. This classification is oversimplistic (Stebbins, 1971), as it draws a somewhat arbitrary division through a continuum of degrees of divergence between parents involved in crossing and

polyploid formation. Allopolyploidy is considered to be more common in nature than autopolyploidy (Coyne & Orr, 2004; although see Soltis *et al.*, 2007), but despite its obvious importance, much remains unknown about the process and its consequences (Soltis *et al.*, 2010). This is particularly true with regard to the establishment of allopolyploid species in the wild.

Well-established allopolyploid species often occur in habitats where their diploid relatives are not found (Brochmann *et al.*, 2004; Paun *et al.*, 2011). It is feasible, therefore, that ecological divergence is an important driver of allopolyploid establishment, enabling a new allopolyploid species to escape the minority-type disadvantage resulting from intermating with a parent (Levin, 1975), additional negative effects of interploidal gene flow (Chapman & Abbott, 2010) and possible competitive disadvantages in parental habitats. Determining the role of hybridization *per se* vs. subsequent ecological selection on the hybrid genotype is important for understanding the relative importance of hybridization vs. selection in the establishment of allopolyploids, as it is for homoploid hybrid populations (see above). Allopolyploids are often geographically widespread, occupying open habitats created by climatic, human or other disturbances (Stebbins, 1984; Brochmann *et al.*, 2004). They frequently exhibit greater vigour and homoeostatic buffering relative to their diploid relatives, making them well suited for colonizing new habitats (Grant, 1981). There are several mechanisms, such as fixed heterozygosity, that may explain the advantages allopolyploids display under such conditions (Levin, 2002; Hegarty & Hiscock, 2007).

The recent finding that many newly formed allopolyploids exhibit considerable genomic and transcriptomic variation relative to their parents (Doyle *et al.*, 2008; Hegarty & Hiscock, 2008) opens the way to examine possible links between the nature of such variation, ecological divergence and speciation (Parisod, 2012). Paun *et al.* (2011) recently used cDNA-amplified fragment length polymorphism (cDNA-AFLP) to examine gene expression differences between two diploid orchid (*Dactylorhiza*) species and three derivative allotetraploids that differed markedly in ecology, geography and morphology from each other. Certain transcriptomic differences between the five species were correlated with particular eco-climatic variables, suggesting they could be adaptive. Going beyond association, it will be necessary to demonstrate a direct link between regulatory networks affected by alterations to gene expression and ecological divergence to show that such differences are adaptive. Moreover, it will be necessary to distinguish between the impacts of changes occurring at the time of origin of an allopolyploid and during subsequent evolution on both adaptation and reproductive isolation (Ramsey & Schemske, 2002).

Despite the likely importance of ecological divergence in allopolyploid speciation, there is surprisingly no direct evidence that it originates at the time of origin of an allopolyploid species. This contrasts with the position for homoploid hybrid speciation (Gross & Rieseberg, 2005; Abbott *et al.*, 2010). In seeking experimental evidence to determine whether ecological divergence accompanies or follows allopolyploid speciation, we could focus on the few species known to have originated within the last 100 years or so (Abbott & Lowe, 2004), particularly those that can be resynthesized artificially [e.g. *Senecio cambrensis, Tragopogon mirus* and *T. miscellus* (Hegarty *et al.*, 2005; Tate *et al.*, 2009)]. Synthetics of each of these species exhibit considerable genomic and transcriptomic variation relative to their parents, providing a source of novelty on which selection could act (Hegarty *et al.*, 2008; Buggs *et al.*, 2011). Comparisons of fitness between synthetics and parental types transplanted into sites occupied by the wild form of allopolyploid would be one approach to test whether ecological divergence accompanied the origin of these neo-allopolyploids.

It has been suggested that over the longer term, polyploidy may set the stage for rapid diversification, perhaps even explaining the 'abominable mystery' of the origins of angiosperm diversity (De Bodt *et al.*, 2005). Evidence for multiple ancient polyploidization events in the genomes of plants whose chromosomes appear to be diploid seems to favour this view (Blanc *et al.*, 2003; Jiao *et al.*, 2011), as does the frequency of polyploidy in island radiations (Murray & de Lange, 2011) and the theoretical expectation that gene duplication provides raw material for evolution (Lynch & Conery, 2000). However, this view is contradicted by apparently lower diversification rates of polyploids compared to their diploid relatives within genera (Mayrose *et al.*, 2011), and the predictive success of models in which polyploidization is a neutral, one-way process (Meyers & Levin, 2006; Mayrose *et al.*, 2011). The role of allopolyploidy as a driver of plant diversification thus remains an open question. In neo-allopolyploids, multiple origins are common (Soltis & Soltis, 1993), forming independent lineages that might merge to generate polyploid populations with high genetic diversity (Soltis & Soltis, 2000; Holloway *et al.*, 2006) or follow independent evolutionary trajectories leading to separate species (Werth & Windham, 1991). The latter has not yet been demonstrated in natural species (Soltis & Soltis, 2009), but patterns of chromosomal change found in independent lineages of the recent allopolyploid *Tragopogon miscellus* may create incompatibilities that promote speciation (Lim *et al.*, 2008; Chester *et al.*, 2012).

Diverse genetic mechanisms underlie novel phenotypes in hybrids

Hybridization can lead to very different evolutionary outcomes, as discussed above, but what are the genetic mechanisms underlying these alternatives? Hybrid attributes that reduce fitness and those that increase it

are generally treated as qualitatively different phenomena (e.g. 'incompatibilities' vs. 'evolutionary novelty'). However, both describe the appearance of potentially fitness-related phenotypic traits in hybrids that lie outside the parental distributions, be it in fecundity, physiology, morphology or behaviour. The very same genetic mechanisms can underlie novel transgressive phenotypes whether their fitness effects are positive or negative: in both cases, they are due to the creation of genetic combinations that have not been tested by selection in the parental populations. Determining the mechanisms that cause these phenotypes to appear will aid understanding of the impact of hybridization on the speciation process.

Two classes of mechanism might be considered. First, alleles of additive effect may not all be fixed in the same direction between diverging populations, especially if selection is weak (Orr, 1998). Some hybrid genotypes then fall outside the parental distribution (+++− x −−−+ can generate ++++ or −−−−). Second, new phenotypes may result from interactions (dominance or epistasis) between alleles fixed independently in different populations. Dobzhansky–Muller incompatibilities, where these interactions have negative consequences, have dominated research on the genetics of speciation, and the focus has tended to be on simple two-locus incompatibilities mediated through protein–protein interactions. In fact, both classes of mechanism can be interpreted much more broadly, and the last decade of research has started to reveal a wider variety of genetic mechanisms underlying novel hybrid phenotypes, including genome restructuring, duplication/deletion (Oka's model, see e.g. Nei & Nozawa, 2011), alterations in the timing and levels of gene expression, epigenetic effects and transposon activation (Landry *et al.*, 2007; Doyle *et al.*, 2008; Masly *et al.*, 2006; Michalak, 2009; references in Ainouche & Jenczewski, 2010). Dissecting these mechanisms will help to understand why hybridization sometimes generates new adaptive phenotypes, how incompatibilities accumulate over time and whether incompatibilities are likely to break down or not when exposed to gene flow and recombination.

The proximate causes of extensive phenotypic novelty in hybrids lie in differences between the contributing genomes that, when combined, have novel effects. Divergence in the regulatory architecture of genes may be particularly likely to produce correlated, genome-wide responses to hybridization and may occur quickly following isolation. The extent of novel expression patterns in the first few generations following hybridization often exceed what can be expected from simple reshuffling of pairwise epistatic interactions (Ranz *et al.*, 2004). In particular, regulatory genes are fast-evolving (Castillo-Davis *et al.*, 2004) and evolve in a compensatory fashion within complex networks, increasing the probability of epistatic effects after hybridization (Johnson & Porter, 2000; Birchler & Veitia, 2010) and leading to one-to-many or many-to-many interactions rather than the classic one-to-one Dobzhansky–Muller incompatibilities. Structural variation between species, including chromosomal organization, gene duplication or loss and transposable element distribution, can also produce substantial phenotypic effects and directly impact recombination rate and reproductive compatibility with parental species (Rieseberg, 2001; Nei & Nozawa, 2011). Differences in genome structure may induce further restructuring (with possible phenotypic consequences) after recombination of the hybrid genomes (Gaeta & Pires, 2010).

Divergence in transposable element complements can occur rapidly and can have profound consequences following hybridization. The merging of divergent genomes in F1 hybrids may result in quantitative or qualitative mismatches between interspersed transposable elements and their maternally transmitted siRNA repressors (Comai *et al.*, 2003; Bourc'his & Voinnet, 2010). Such miss-regulation can induce the activation of specific transposable elements and promote both restructuring and epigenetic re-patterning throughout the hybrid genome (Parisod *et al.*, 2010). Although massive mobilization of transposable elements inducing mutation bursts may lead to low hybrid fitness in extreme cases (e.g. hybrid dysgenesis in *Drosophila*; Blumenstiel & Hartl, 2005), more limited reactivation may promote moderate transposition and result in structural polymorphism that suppresses recombination at homologous loci (e.g. recombinationally inert haplotypes in maize; He & Dooner, 2009). Activation of transposable elements induced by hybridization may thus play a pivotal role during speciation by triggering genome-wide variation in functional genes (e.g. stably altering expression through sequence disruption or epigenetic changes in the vicinity of insertion sites; Hollister *et al.*, 2011) or strongly modifying recombination patterns across the genome, with potential consequences for barriers to gene flow (Ungerer *et al.*, 2006).

These various genetic mechanisms underlying transgressive hybrid phenotypes differ in a number of attributes that may have important implications for the evolutionary dynamics of populations produced through hybridization. Miss-regulation of gene expression may be expected to produce new phenotypes immediately upon genome merging, perhaps more readily than protein–protein interactions, with further variants emerging over time as recombination produces novel combinations of interacting genetic elements. Accordingly, the emergence of novel variation is likely to be an ongoing process, with different phenotypes being exposed to natural selection over successive generations. Moreover, the mechanisms that change genome structure and those that alter genome functions might be expected to impact different aspects of speciation and to contribute in qualitatively different

ways to the evolutionary dynamics of hybridization. Structural changes are expected to contribute primarily to barriers to gene flow, as chromosomal restructuring that restores reproductive function within the hybrid population also likely induces incompatibility with the parental forms (as in allopolyploidy). In contrast, functional changes can have a wide array of effects on every aspect of the phenotype, playing some role in barriers if they reduce fitness (Ortiz-Barrientos *et al.*, 2007) and being important in generating fitness-enhancing evolutionary novelty (Ni *et al.*, 2008; Edelist *et al.*, 2009), a critical prerequisite for ecological differentiation and competitive success in incipient hybrid species. Whether there is a predictable shift from fitness enhancement to fitness reduction with increasing divergence between interacting species, as regulatory network differences, transposable elements, etc. begin to exceed the limits of complementarity or rapid recovery in hybrids, is an open question that could profitably be addressed with experimental systems or controlled studies within particular groups. A better understanding of the mechanisms contributing to hybrid phenotypes may help to resolve some areas of disagreement over the role of hybridization in the speciation process. If gene miss-regulation is indeed a common source of incompatibilities, as is suggested both by classic studies of hybrid unfitness (Wittbrodt *et al.*, 1989) and by more recent work in yeast and *Drosophila* (Anderson *et al.*, 2010; Araripe *et al.*, 2010; Kao *et al.*, 2010), a network-based modelling approach that can accommodate the complex patterns of epistasis typical of regulatory networks may perform significantly better than two-locus models in predicting evolutionary outcomes (e.g. Porter & Johnson, 2002; Palmer & Feldman, 2009; see sections Hybridization and the Development of Genetic Barriers to Gene Flow and Homoploid Hybrid Speciation).

At the molecular level, we still know relatively little about how these mechanisms work outside of model organisms. Gaining insights into the nature of the multiple genetic elements involved in speciation and hybridization, and including more precise analysis of molecular aspects of phenotypic evolution, is an important task that will substantially increase our ability to identify what is occurring when divergent genomes interact. This is becoming more tractable in nonmodel organisms, with the rapid advances in next-generation sequencing technologies (e.g. Wolf *et al.*, 2010b). Finally, although we are beginning to appreciate the impact of genome changes on phenotypic variation, linking this to fitness remains a critical challenge (Barrett & Hoekstra, 2011). The hypothesis that particular genetic mechanisms influence the outcome of hybridization via their effect on phenotypes has been tested rigorously in very few systems (e.g. Edelist *et al.*, 2009; Tirosh *et al.*, 2009; Groszmann *et al.*, 2011; Arnold *et al.*, 2012). Experimental approaches would be particularly valuable in integrating laboratory results with natural hybridization events.

Consequences of reinforcement

In the sections above, the emphasis has been on hybridization's direct effects on speciation. Hybridization can give rise to new recombinant populations that become divergent enough from other populations to form new species. Alternatively, hybridization may either break down existing barriers or favour the evolution of stronger barriers to gene exchange that might ultimately finalize speciation. Yet, as we describe below, hybridization can play an additional, *indirect* role in speciation, by setting the stage for new speciation events.

Given selection against unfit hybrids, traits that generate enhanced prezygotic isolation could evolve where populations are in contact (i.e. reinforcement may occur; Dobzhansky, 1940). Although extensively debated and often controversial, recent theoretical and empirical work indicates that reinforcement can generate increased prezygotic isolation (Servedio & Noor, 2003; Coyne & Orr, 2004), but may not complete speciation (e.g. Bímová *et al.*, 2011). Continued work is still needed to assess its overall contribution to speciation, its frequency and when it is likely to result in complete isolation. Here, we evaluate possible indirect consequences of reinforcement. Reinforcement may result in divergence between populations inside and outside zones of contact with an interacting taxon, leading to three possible outcomes.

First, consider two incompletely isolated taxa, A and B, with partial range overlap. Because hybrids have reduced fitness, reinforcement may lead to divergence between A and B in the region of overlap. The extent of divergence between A and B will depend in part on gene flow into the region of overlap (sympatry) from populations outside the area of overlap (allopatry), where mating traits are under different selection pressures. At the same time, gene flow out from the overlap populations may cause divergent phenotypes to spread into the regions of each taxon where they do not overlap. The balance between these effects can produce an inverse cline (Antonovics, 2006 and references therein; Bímová *et al.*, 2011). Simple models suggest that the leakage of traits that evolve within the hybrid zones into allopatric populations will only be local (Caisse & Antonovics, 1978; Sanderson, 1989) unless driven by an additional form of selection. Reinforcement within the hybrid zone might stall, rather than increase further, if the build-up of linkage disequilibrium is counteracted by gene flow or recombination from nonselected individuals outside the zone (e.g. Bímová *et al.*, 2011; see reviews by Servedio & Noor, 2003; Coyne & Orr, 2004). Consequently, the degree to which reinforcement drives divergence between populations inside and outside the hybrid zone within

taxon A or B depends on (i) the extent to which reinforcement drives divergence between A and B in the first place within the area of overlap, (ii) the extent of gene flow from overlap populations into the remainder of the distributions of A and B and (iii) other selection pressures operating on traits that influence assortment. The balance of these factors may mean that reinforcement results in little or no divergence between the taxa, or among populations within the taxa.

If gene flow within taxon A (or B) is limited, for example in a patchy environment, a second outcome of reinforcement may be the evolution of reproductive traits in overlap populations that are so divergent from those outside the area of contact that individuals with the alternative trait types are less likely to reproduce (Howard, 1993; see also reviews, discussion, and references therein by Pfennig & Pfennig, 2009, 2010; Ortiz-Barrientos *et al.*, 2009; Hoskin & Higgie, 2010). Consequently, this can lead to the initiation of reproductive isolation between sympatric and allopatric populations of taxon A (or B), which can ultimately lead to speciation (Howard, 1993; Pfennig & Pfennig, 2009, 2010; Ortiz-Barrientos *et al.*, 2009; Hoskin & Higgie, 2010; for theoretical treatments see Pfennig & Ryan, 2006; McPeek & Gavrilets, 2006). For example, Jaenike *et al.* (2006) showed that, between two sympatric species of *Drosophila*, strong hybrid inviability not only selected for discrimination against heterospecifics but also, as a side product, led to discrimination against conspecifics from allopatric populations. Similarly, Svensson *et al.* (2006) found that strong divergent sexual selection was accompanied by a significant decrease in female matings with conspecifics from other populations. Trade-offs in fitness between assortative mating and sexual selection within populations may enhance the divergence between populations (e.g. Pfennig & Pfennig, 2005; for further discussion, see Pfennig & Pfennig, 2009, 2010; Ortiz-Barrientos *et al.*, 2009; Hoskin & Higgie, 2010). These trade-offs may be emphasized when a stepwise change in environmental conditions coincides with the boundary of the region of range overlap. Other factors that favour reinforcement in the first place (e.g. strong selection, linkage between fitness and mating traits) may also foster divergence between overlap and allopatric populations, underscoring the potential for reinforcement-mediated speciation to be autocatalytic in nature.

A third possibility occurs where taxa A and B have multiple independent areas of overlap. If traits evolve differently in response to a given heterospecific among geographically distinct overlap populations, or if a given species encounters and undergoes reinforcement differently with several distinct species across its range, such conspecific populations may become reproductively isolated from one another (Howard, 1993; Pfennig & Pfennig, 2009; Hoskin & Higgie, 2010; e.g. Hoskin *et al.*, 2005; Lemmon, 2009). Such diversity in the outcome of reinforcement is especially likely when reinforcement may operate on a multitude of traits (McPeek & Gavrilets, 2006; Pfennig & Ryan, 2006; Lemmon, 2009). As an example of the former scenario, Hoskin *et al.* (2005) found that premating isolation between two different populations of rainforest tree frogs resulted from unequal divergence in mate preferences in their separate contact zones with an alternative tree frog population.

Evaluating these possibilities is both an empirical and a theoretical challenge. How often do the relevant circumstances arise that lead to these outcomes? How likely are the various types of divergence to persist in the face of gene flow and thereby ultimately result in new species? How likely is gene flow between overlap and nonoverlap regions, particularly where they are ecologically distinct? To answer these questions, comparisons of reproductive traits are needed among populations as well as between taxa. Of particular value are data that: (i) identify the reproductive traits and trait values that are differentially favoured within and outside the range overlap; (ii) measure fitness consequences of trait variation in both regions to identify sources of selective trade-offs, if any; (iii) evaluate whether trait divergence impacts reproductive success and (iv) determine whether increased genetic differentiation has evolved between regions, independent of the direct effects of hybridization (for a similar set of criteria, see Hoskin & Higgie, 2010). Regarding the last goal, it is important to note that divergence may sometimes be detected using neutral markers (e.g. Svensson *et al.*, 2004; Rice & Pfennig, 2010), but not always (e.g. Hoskin *et al.*, 2005; Jaenike *et al.*, 2006; Thibert-Plante & Hendry, 2009; see also Hoskin & Higgie, 2010). In addition to empirical studies, theoretical work would be useful for addressing these issues. Finally, future work should incorporate an explicit consideration of other sources of divergent selection that could drive similar patterns and therefore be mistaken for population divergence that arises indirectly from reinforcement (*sensu* Rundle & Schluter, 1998; see also Coyne & Orr, 2004; Pfennig & Pfennig, 2009; cf. Hoskin & Higgie, 2010). For example, ecological factors (e.g. resource competition, abiotic conditions) differing between overlap and nonoverlap regions may be as important for population differentiation as selection driven by hybridization avoidance (e.g. Etges *et al.*, 2009; see also Price, 1998; Coyne & Orr, 2004; Rundle & Nosil, 2005; Price, 2008; Sobel *et al.*, 2010; Pfennig & Pfennig, 2010; Hoskin & Higgie, 2010 and references therein).

The ideas above are not new (see, for example, Howard, 1993; Price, 1998; and references above), but they have received relatively little investigation, possibly because attention has focused on the process of reinforcement itself. Our goal here is to highlight the need to extend consideration of reinforcement to include its consequences. Moreover, this discussion

reflects one of our original points: that hybridization between two populations typically occurs in a complex spatial and temporal context. The outcomes of interactions in different parts of the range of a species may vary, depending on both the environmental and the genetic conditions locally. This creates divergence but the net effect is hard to predict: hybridization may accelerate diversification, as described above for both the case of reinforcement and the case of adaptive introgression, but it need not. More empirical evidence is required.

Concluding remarks

Historically, hybridization has been viewed primarily as a countervailing process to speciation. Secondary contact zones with extensive gene flow may remain stable for thousands of generations, and much of the genome of the interacting species may become mixed. Nevertheless, variation distinguishing the populations is usually maintained and may be built upon or recruited through coupling with other barriers to gene flow. This may also set the scene for reinforcement, and barriers to gene exchange may become stronger and more widespread genomically. Alternatively, populations may fuse. As highlighted above, the factors determining these different outcomes remain poorly understood. Hybridization can also play a more diverse role in promoting speciation. It may provide the raw material for adaptive divergence or initiate new hybrid populations, potentially leading to speciation. Again, the impact of factors such as existing levels of divergence and ecological opportunity on these outcomes requires further study. Both reinforcement and hybrid speciation may generate positive feedback that accelerates diversification. The genomic signatures of hybridization and introgression will be investigated (theoretically and empirically) more fully now that the incidence of hybridization during speciation is better appreciated. However, it is striking that, after so much study, we are still poorly equipped to tackle a fundamental problem such as how to estimate the proportion of hybridization events that have led to speciation, and we cannot yet predict whether a hybridization event will be favourable to speciation or not. In the mid-20th century, Edgar Anderson was the greatest proponent of the importance of hybridization in evolution (Anderson, 1949; Anderson & Stebbins, 1954), which led to light-hearted ridicule by some colleagues. As Warren H. Wagner (relayed by Michael Arnold, pers. comm.) once said, 'We used to make fun of Edgar Anderson by saying that he was finding hybrids under every bush. Then we realized that even the bushes were hybrids'. With genomic tools, we are beginning to understand that the evolutionary importance of hybridization may even exceed Anderson's expectation.

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