REVIEW AND SYNTHESIS

Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification

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Abstract

Adaptive diversification is driven by selection in ecologically different environments. In absence of geographical barriers to dispersal, this adaptive divergence (AD) may be constrained by gene flow (GF). And yet the reverse may also be true, with AD constraining GF (i.e. 'ecological speciation'). Both of these causal effects have frequently been inferred from the presence of negative correlations between AD and GF in nature – yet the bi-directional causality warrants caution in such inferences. We discuss how the ability of correlative studies to infer causation might be improved through the simultaneous measurement of multiple ecological and evolutionary variables. On the one hand, inferences about the causal role of GF can be made by examining correlations between AD and the potential for dispersal. On the other hand, inferences about the causal role of AD can be made by examining correlations between GF and environmental differences. Experimental manipulations of dispersal and environmental differences are a particularly promising approach for inferring causation. At present, the best studies find strong evidence that GF constrains AD and some studies also find the reverse. Improvements in empirical approaches promise to eventually allow general inferences about the relative strength of different causal interactions during adaptive diversification.

Keywords

Adaptive divergence, demography, dispersal, divergent selection, ecological diversification, gene flow, positive feed-back, reproductive isolation.

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INTRODUCTION

A long-standing debate in the study of adaptive diversification concerns the interaction between diversifying selection and homogenizing gene flow (e.g. Mayr 1963; Jain & Bradshaw 1966; Antonovics 1968; Ehrlich & Raven 1969; Endler 1973; Jackson & Pounds 1979; Slatkin 1987; Morjan & Rieseberg 2004). Contributing heavily to this debate have been empirical studies that examine associations among environmental differences, adaptive divergence (AD, genetically based phenotypic differences that improve local fitness), dispersal (movement of individuals between populations) and gene flow (GF, movement of genes between populations). In particular, many studies of natural populations have noted negative associations between AD and either dispersal or GF. These associations have then been used to infer either that (i) GF constrains AD (e.g. Storfer *et al.* 1999; Langerhans *et al.*

2003; Hendry & Taylor 2004) or (ii) AD constrains GF (e.g. Smith *et al.* 1997; Lu & Bernatchez 1999; Schneider *et al.* 1999). Our main goal is to consider difficulties associated with such causal inferences and to suggest some solutions.

The need for careful evaluation of cause and effect arises from the variety of ways in which AD and GF can interact. First, dispersal and/or GF may *promote* AD through the introduction of genetic variation, the spread of advantageous alleles, non-random dispersal and demographic benefits (Levins 1964; Slatkin 1987; Gomulkiewicz *et al.* 1999; Richards 2000; Forde *et al.* 2004; Holt *et al.* 2004; Tallmon *et al.* 2004; Garant *et al.* 2005; Postma & van Noordwijk 2005; Perron *et al.* 2007). Second, GF may *constrain* AD by reducing the independence of gene pools that would otherwise diverge owing to selection in ecologically different environments (Mayr 1963; Antonovics 1968; Endler 1973, 1977; Slatkin 1987; Hendry *et al.* 2001;

Lenormand 2002). As an aside, the above two effects sometimes lead to the expectation that an intermediate level of dispersal is optimal for adaptation (e.g. Gomulkiewicz *et al.* 1999; Kawecki & Ebert 2004). Third, AD can reduce GF through the evolution of reproductive isolation (i.e. 'ecological speciation,' Schluter 2000). As one example, threespine stickleback (*Gasterosteus aculeatus*) adapted to different environments show limited GF as a result of selection against migrants and hybrids, combined with positive assortative mating based on the divergent traits (McKinnon & Rundle 2002).

We here focus on the second and third effects described above – because of the recent flush of work attempting to infer causation from negative association between AD and GF. At the outset, it seems valuable to formally confirm verbal arguments that AD and GF can each negatively influence the other. We use a quantitative genetic model (Appendix S1) to show that (i) variation in dispersal can lead to negative correlations between AD and GF, and (ii) variation in the magnitude of ecological differences can have the same effect (Fig. 1). These results confirm that negative correlations between AD and GF do not, in themselves,
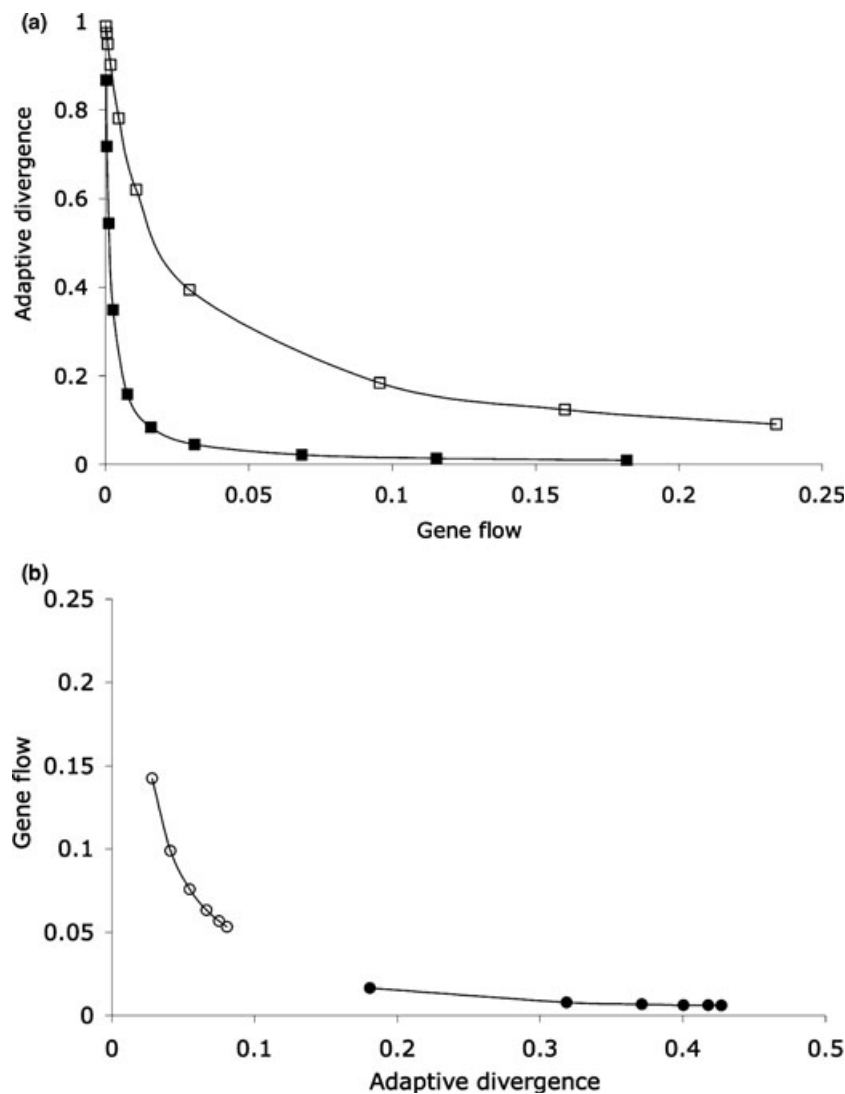

Figure 1 Example scenarios from a simple quantitative genetic island–mainland model (Hendry 2004) illustrating the relationship between adaptive divergence and gene flow that results from (a) varying the number of dispersers between populations in the presence of either weak (solid squares) or strong (open squares) stabilizing selection, or (b) varying the ecological contrast between populations in the presence of either low (solid circles) or high (open circles) numbers of dispersers. In (a) gene flow is varied by altering the number of dispersers from the mainland to the island and in (b) adaptive divergence is varied by altering the environmental difference between the mainland and the island (as difference in the respective phenotypic optima). Note that the gap in the lines between high and low number of dispersers is a consequence of the parameter values chosen for illustration. For further details see the Appendix S1 and Hendry 2004.

allow inferences about which is the cause and which the effect.

Our main goal is to evaluate empirical methods for *inferring the arrow of causality* between AD and GF in natural populations. Our paper thus forms a bridge between recent reviews that focus primarily on one causal pathway (GF to AD: Lenormand 2002; Garant *et al.* 2007) or the other (AD to GF: Rundle & Nosil 2005; Hendry *et al.* 2007). In particular, we illustrate how understanding ecologically driven diversification requires a clear understanding of both causal pathways. We first summarize some of the main ecological and evolutionary forces that influence diversification (Fig. 2), and then discuss how best to reveal the action of these forces in nature. We focus primarily on discrete populations, rather than clinal scenarios, because of the diverse, but diffuse, recent work in the former context. We will argue that the greatest inferential power can be achieved through a simultaneous

consideration of multiple ecological and evolutionary forces, as well as through experimental manipulations in nature. We close by considering the inferences drawn from study systems where to date the best inferential methods have been applied. These studies consistently find support for GF constraining AD, and often also for the reverse. More work of an integrated nature is needed, however, before we can ascertain the generality of these initial observations.

A SUMMARY OF CAUSAL EFFECTS AND INTERACTIONS

We start by summarizing the main causal pathways that promote or constrain AD (Fig. 2a). On the promoting side, populations that occupy increasingly different environments should experience increasing divergent selection (path 1 in Fig. 2a) and should therefore undergo greater AD (path 2).
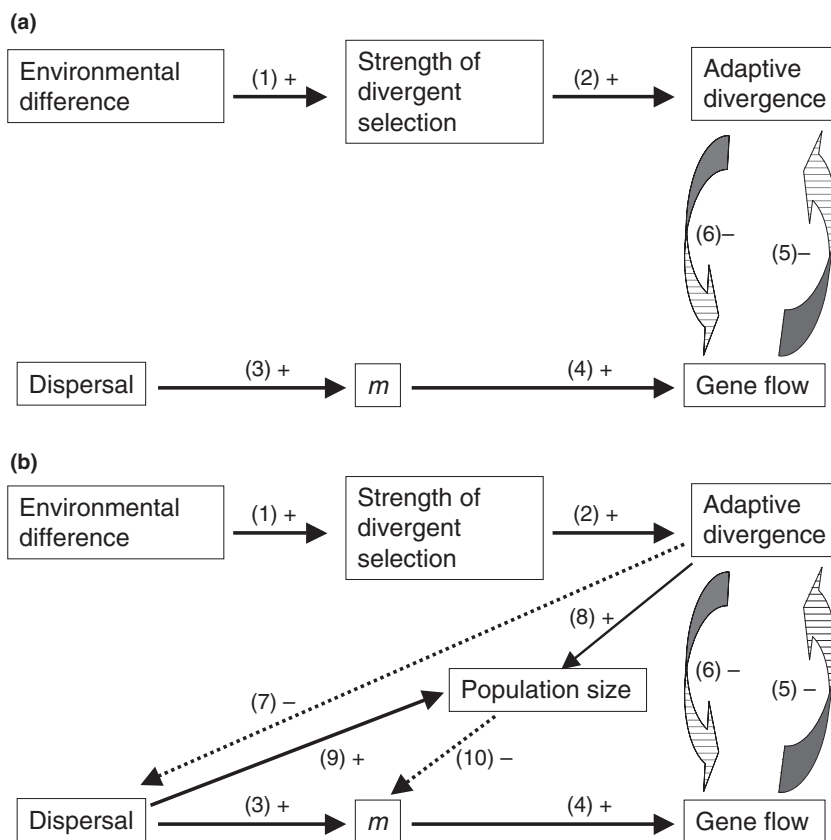

Figure 2 A schematic representation of some of the main factors influencing the relationship between adaptive divergence and gene flow. Solid lines indicate a positive relationship and '+' symbols indicate an increase in the direction of the arrow, whereas hatched lines indicate a negative relationship and '−' symbols indicate a decrease in the direction of the arrow. The symbol '*m*' represents the proportion of immigrants into a population. Path 5 indicates that gene flow can reduce adaptive divergence, whereas path 6 indicates that adaptive divergence can reduce gene flow through ecological speciation. (a) What we consider main effects and (b) the additional relationships arising from dispersal and population size. The numbers relate to pathways discussed in the text.

On the constraining side, an increasing number of individuals dispersing between environments should increase the proportion of immigrants (m, path 3), which should increase GF (path 4) and therefore reduce AD (path 5). Paths 2 and 5 thus represent the classically recognized tension between diversifying selection and homogenizing GF, or the 'migration-selection balance' (e.g. Haldane 1948; Mayr 1963; Jain & Bradshaw 1966; Ehrlich & Raven 1969; Slatkin 1973; Felsenstein 1976). We next incorporate the idea that AD can reduce GF via the evolution of reproductive isolation (path 6 = ecological speciation, Schluter 2000). Paths 5 and 6 thus represent the bi-directional arrow of causality that makes it hard to infer cause and effect between AD and GF.

This simple framework (Fig. 2a) immediately suggests some interesting feedback loops (Rice & Hostert 1993; Hendry *et al.* 2001; Crespi 2004; Hendry 2004). For instance, a decrease in dispersal should reduce GF, which should permit an increase in AD, which should further reduce GF (ecological speciation), which should allow more AD, and so on until some potential equilibrium (or quasi-equilibrium). Working the other way, an increase in dispersal should increase GF, which should reduce AD, which might further increase GF, and so on until perhaps some other equilibrium. These feedbacks then suggest the possibility of alternative stable states, such as near-complete adaptation vs. near-complete maladaptation (e.g. Ronce & Kirkpatrick 2001; Holt *et al.* 2004), species fission vs. fusion, or perhaps a stable tension at some other intermediate point.

Many additional complexities can be layered onto this simple framework, and we take here up some that are especially important to our later discussions (Fig. 2b). The first set of complications arises via the potential evolution of dispersal. When AD reduces the fitness of migrants between environments (Nagy & Rice 1997; Hendry 2004; Nosil 2004; Nosil *et al.* 2005), increasing AD should favour the evolution of reduced dispersal (path 7 in Fig. 2b; Billiard & Lenormand 2005; Fraser & Bernatchez 2005). An interesting feedback loop emerges here because the evolution of reduced dispersal will decrease GF (Fig. 2b) and thereby allow increased AD. This increase in AD may favour further evolutionary reductions in dispersal, although reduced dispersal decreases the proportion of the population under selection. These complicated effects are ripe for theoretical examination.

Another set of complications emerges through the effects of demography. First, increasing AD may increase population sizes (path 8) when better-adapted populations grow faster or have higher equilibrium abundances (Kirkpatrick & Barton 1997; Tufto 2001; Lenormand 2002). This effect is most likely when density dependence is weak (Gomulkiewicz *et al.* 1999; Saccheri & Hanski 2006; Kinnison & Hairston 2007; Kokko & López-Sepulcre 2007). Second, increasing dispersal may directly increase the size of

recipient populations (path 9) and may thereby reduce the negative effects of small population size (Holt *et al.* 2004). If these demographic effects lead to changes in the relative size of interacting populations (i.e. asymmetries in population size), then GF may change – because relatively larger populations will experience relatively lower immigration *rates* (m) for a given *number* of immigrants (path 10; Holt & Gomulkiewicz 1997; Tufto 2001; Hendry 2004). This change may result in lower GF (path 4), increased AD (path 5) and further feedback loops. For example, increasing AD that increases local population size may reduce GF – and thereby further increase AD. It is worth noting here that asymmetries in dispersal and population size between environments can lead to an asymmetric equilibrium, whereby adaptation is primarily to only one of the two environments (e.g. Holt & Gaines 1992; Kawecki 2000; Ronce & Kirkpatrick 2001; Kawecki & Holt 2002; Kisdi 2002).

We have sketched only some of the major forces influencing diversification, with the goal to summarize the major effects, generate interesting hypotheses and the following exploration of empirical methods. It is important to recognize, however, that we have not included many other effects, such as evolutionary changes in genetic variation (Guillaume & Whitlock 2007), frequency-dependent selection and co-evolutionary dynamics (Nuismer *et al.* 2000; Thompson 2005). It is also possible that environmental differences directly impact dispersal even in the absence of AD – e.g. if individuals within populations 'imprint' on their local conditions. We hope that theoretical models can ultimately be used to examine these and other interactions in an integrated framework.

CORRELATIVE APPROACHES

Most studies of interactions between AD and GF in nature have used simple correlative approaches. We therefore begin our discussion with such simple designs before turning to alternatives. Note that we avoid lengthy lists of citations to studies that have employed the least effective designs – instead reserving space for particularly informative and robust methods. Note also that all of the studies focus on systems where AD is expected, and may therefore ignore cryptic genetic divergence and cryptic reproductive isolation, which may well be very important in nature.

Some studies focus on a single population with a phenotype unexpected for its environment, inferring a constraining role for GF simply for this reason. This inference is obviously improved by confirming that the observed deviation is in the direction of nearby populations experiencing a different environment, and further by determining whether GF may be sufficiently high to cause the inferred constraint. It is also important to confirm,

however, that the unexpected phenotype is not simply the result of unaccounted selection. Ideally, all of this information would then be incorporated into theoretical models designed to test whether the observed phenotypic deviation is consistent with the measured parameters (King & Lawson 1995; Hendry *et al.* 2001; Moore *et al.* 2007).

An improvement to the above single-population approach can be to sample multiple pairs of populations in divergent environments. Studies adopting this approach often find that AD is lower for population pairs that exchange more genes, a pattern used to infer that AD constrains GF (e.g. Smith *et al.* 1997; Gíslason *et al.* 1999; Lu & Bernatchez 1999) or that GF constrains AD (e.g. Storfer *et al.* 1999; Langerhans *et al.* 2003; Hendry & Taylor 2004). These analyses are strongest when the population pairs are evolutionarily independent and numerous, which has been the case for few studies to date. And, of course, these correlations cannot by themselves illuminate cause and effect.

Despite their limitations, correlative studies are likely to remain common, and so we now consider correlative methods that show the greatest potential for causal inferences. For the sections that follow, it is important to remember that dispersal and GF are not the same thing (Kawecki & Ebert 2004). For example, GF can be higher than dispersal when populations are inbred – because immigrants may have higher fitness than residents (Ingvarsson & Whitlock 2000; Ebert *et al.* 2002). Alternatively, dispersal can be higher than GF owing to selection against migrants and hybrids (Hendry *et al.* 2000; Hendry 2004; Nosil *et al.* 2005). Therefore, estimates of dispersal cannot be used as surrogates for GF nor *vice versa*.

AD to GF inferred from environmental differences and GF

The problem with drawing causal inferences from correlations between AD and GF is the bi-directional arrow of causality that links them (e.g. paths 5 and 6 in Fig. 2). One solution might therefore be to design a test with at least one uni-directional arrow. For example, GF may rarely *cause* environmental differences, and so we might infer that AD constrains GF when population pairs that occupy more divergent environments show lower GF. Exceptions to uni-directional causality in such comparisons may occur if environmental differences are determined by (i) competition that changes with dispersal (e.g. density- or frequency-dependence, Gomulkiewicz *et al.* 1999), or (ii) co-evolution of the population and the 'environment' (e.g. predator-prey or host-parasite interactions, Thompson 2005). As long as these effects are not particularly strong, a negative correlation between environmental differences and GF implies

that environmental differences drive divergent selection, which influences AD, which influences GF – this last effect being the one we wish to infer.

A typical application of the above approach tests whether GF (estimated from genetic markers) decreases as populations occupy increasingly different ecological environments. A number of studies have found this very result (e.g. Smith *et al.* 1997; Schneider *et al.* 1999; Ogden & Thorpe 2002; Rolán-Alvarez *et al.* 2004; Grahame *et al.* 2006), whereas others have not (e.g. Hendry & Taylor 2004; Crispo *et al.* 2006). These conflicting outcomes suggest that divergent selection does not inevitably reduce GF, that factors other than AD more strongly influence variation in GF (e.g. geographical features, arbitrary sexual selection, drift, cryptic divergence or isolation), or that parameter estimates are not always reliable. In hopes of reducing the latter possibility, we now consider issues related to the estimation of environmental differences and GF.

For environmental differences, a key is to examine the specific ecological variables that determine selection on the traits of interest. This is not always straightforward and, as in so many cases, detailed knowledge of the organism's natural history is critical. One might also formally quantify divergent selection on the traits, although accurate estimates of selection are logistically difficult (Kingsolver *et al.* 2001; Hereford *et al.* 2004; Hersch & Phillips 2004). Even with accurate estimates, it is important to remember that divergent selection depends not only on the environmental difference but also on the amount of GF. That is, increasing GF leads to stronger selection – because populations are held farther from their respective optima (García-Ramos & Kirkpatrick 1997; Bolnick & Nosil 2007). In fact, path 2 (Fig. 2) could be redrawn with bi-directional causality, again complicating interpretations of cause and effect. Thus even if selection is quantified, it remains important to assess the important environmental differences. In general, selection and adaptation are a function of both the environment and the phenotypic distribution. Inferences would therefore best be drawn by constructing adaptive landscapes – although this has been exceptionally rare for natural populations (Schluter 2000).

Accurate estimates of GF are equally important but many problems arise here also. For instance, most studies use neutral genetic markers to estimate 'historical' GF – but no consensus exists as to the best such method (Slatkin & Barton 1989; Beerli & Felsenstein 1999; Whitlock & McCauley 1999; Abdo *et al.* 2004). Estimating historical GF from genetic markers also assumes that the populations are at equilibrium, which can take some time to achieve depending on effective population size (Whitlock & McCauley 1999). Moreover, most studies estimate the 'effective number of migrants' ($N_e m$), even though it is the *rate* of immigration (m) that most directly influences AD

(Kirkpatrick & Barton 1997; Hendry *et al.* 2001; Tufto 2001; Lenormand 2002). Unfortunately, estimating m is even more difficult than estimating $N_e m$ because the former also requires the estimation of N_e (Wang 2005). Another complication is that historical GF will vary among neutral loci depending on their linkage to loci under selection (e.g. Kelly 2000; Emelianov *et al.* 2004; Gavrilets 2004, pp. 147–148; Grahame *et al.* 2006). These difficulties might encourage the use of assignment methods to estimate 'contemporary' GF (Hauser *et al.* 2006; Waples & Gaggiotti 2006). These estimates, although useful, are more relevant to dispersal (Berry *et al.* 2004) than to GF, and so one is again left with the problem of measuring GF. Somewhere between 'historical' and 'contemporary' GF, linkage disequilibrium can be used to estimate GF that has occurred in the recent past, as has been demonstrated for clines and hybrids zones (e.g. Mallet *et al.* 1990). At present, we suggest it is most valuable to measure GF using multiple methods and to look for correspondence among them. In doing so, it is important to recognize that the accuracy is maximized, and bias reduced, by analyzing many loci and carefully assessing outliers. Overall, relationships among dispersal, contemporary GF, and historical GF (as well as their estimators) are still unclear, calling for additional theoretical and empirical work.

AD to GF inferred from reproductive barriers

Adaptive divergence is thought to constrain GF through the evolution of reproductive isolation ('ecological speciation', Schluter 2000). One might therefore test for reproductive barriers between populations adapted to ecologically different vs. similar environments. Barriers that might evolve owing to ecological differences include reproductive timing (e.g. Silvertown *et al.* 2005; Antonovics 2006), positive assortative mating (Rundle *et al.* 2000; Nosil *et al.* 2002; Boughman *et al.* 2005), natural selection against migrants (Nagy & Rice 1997; Hendry 2004; Nosil 2004; Nosil *et al.* 2005) and natural selection against hybrids (Nagy 1997; Via *et al.* 2000; Rundle 2002; Gow *et al.* 2007). These and many other studies have demonstrated that AD can cause the evolution of particular reproductive barriers – but what are the consequences for overall GF in nature?

One reason for caution when extrapolating from specific reproductive barriers to GF is that effects on different potential barriers may offset each other. One possible example comes from Trinidadian guppies (*Poecilia reticulata*). In particular, brightly coloured males from low-predation populations above waterfalls that move into less-colourful high-predation populations below waterfalls will have lower survival (increased susceptibility to predators) but possibly higher mating success (increased attractiveness to females) than residents. Effects of environmental differences on one

potential barrier (natural selection disfavours migrants) may thus be offset by the effects on another barrier (mate choice favours migrants), potentially leading to no net effect on GF (Crispo *et al.* 2006). More studies of interactions between AD and GF should therefore examine multiple reproductive barriers (e.g. McGraw & Antonovics 1983; Via *et al.* 2000; Ramsey *et al.* 2003; Nosil 2007).

GF to AD inferred from dispersal and AD

Bi-directional causality might here be avoided by testing for a negative correlation between AD and the potential for dispersal (e.g. geographical distance). This should work because the *potential* for dispersal is unlikely to be influenced by AD, thus achieving uni-directional causality in the statistical test (caveats are discussed below). Indeed, several such studies have found a negative correlation between the potential for dispersal and phenotypic divergence (e.g. Sandoval 1994a,b; Langerhans *et al.* 2003). This approach depends, however, on appropriate estimates of AD and dispersal, subjects to which we now turn.

Estimating AD might seem straightforward – simply measure phenotypic differences – but inevitable complications arise (Kawecki & Ebert 2004). First, it is important to carefully ascertain which traits are subject to consistent divergent selection – and therefore of interest with respect to a constraint. Second, a simple correlation between phenotypic divergence and GF does not necessarily reveal the extent of trait maladaptation in a given population, because this also requires knowledge about the optimum phenotype (Estes & Arnold 2007; Moore *et al.* 2007). Third, analyses based on a subset of relevant traits will not reveal the constraint on overall adaptation (i.e. the migration load: García-Ramos & Kirkpatrick 1997; Lenormand 2002), which requires measurements of fitness itself. Fourth, phenotypic differences may reflect phenotypic plasticity rather than genetic differences (Pigliucci 2001), and plasticity may even be favoured by GF (Sultan & Spencer 2002). Moreover, environmental and genetic effects on traits may offset each other in nature, leading to apparent phenotypic similarity among populations despite underlying adaptive genetic divergence (counter-gradient variation, Conover & Schultz 1995). In such cases, selection might, for example, act more strongly against immigrants than would be expected from a comparison of phenotypes among wild populations. For all of these reasons, it is important to isolate adaptive genetic differences from phenotypic effects (Kawecki & Ebert 2004), as has recently been done in some elegant long-term studies of AD and GF in the great tit (*Parus major*; Garant *et al.* 2005; Postma & van Noordwijk 2005). Arguments for a GF constraint on AD can be further strengthened by studying variation at specific loci under selection, or linkage disequilibrium between loci, as has been

done in some clinal studies (e.g. Mallet *et al.* 1990; Lenormand & Raymond 2000).

Estimating dispersal is notoriously difficult – because it is usually impossible to survey all potential sites and because point estimates may not reflect long-term patterns (Koenig *et al.* 1996). Because these problems and their solutions (increased sampling effort and temporal replication) are well known, we will not dwell on them further. Instead, we focus on assessing the potential for dispersal, such as the distance between sites (Langerhans *et al.* 2003), barriers between sites (Bertness & Gaines 1993; Crispo *et al.* 2006), relative population sizes (Sandoval 1994a; Dias & Blondel 1996; Nosil 2004) and dispersal vectors (Bohonak 1999). Here, it is important to verify that these variables really do influence dispersal as predicted – and this is not always the case (Moore *et al.* 2007). It is also important to consider possible covariation between dispersal (or the potential for dispersal) and environmental factors that might influence AD. For example, more distant sites may also be more ecologically different, which could cause a negative correlation between phenotype and distance as a result from selection instead of GF (Moore *et al.* 2007). Moreover, when distant populations are smaller, distance-based reductions in the *number* of immigrants may not translate into similar reductions in the *rate* of immigration (e.g. Antonovics 1976).

GF to AD inferred through other correlations

Several other correlations can help provide evidence that GF constrains AD. First, one can test whether divergent selection is positively correlated with GF – while also controlling for variation in environmental differences. The reason is that increasing GF holds populations farther from their local optima, and therefore maintains stronger selection, as has recently been shown for *Timema* walking sticks (Bolnick & Nosil 2007). Second, one can test whether the phenotypes of populations are correlated with the relative phenotype and frequency of immigrants, such as has recently been shown for great tits (Garant *et al.* 2005; Postma & van Noordwijk 2005).

Integrated correlative studies

Given the great number and complexity of interactions, the best way to approach correlative work is to quantify as many of the relevant factors as possible, with those in Fig. 2 as a reasonable starting point. This integrated approach can be illustrated by work on *Timema* walking sticks adapted to different host plants (e.g. Nosil *et al.* 2002, 2008; Nosil 2004, 2007; Nosil & Crespi 2004; Bolnick & Nosil 2007). For many populations, data were collected on environmental differences (host plant type), divergent selection (phenotypic changes within a generation), phenotypic divergence (morphology and colouration), premating isolation (mate choice and selection against migrants and hybrids), GF (measured using mtDNA and AFLP markers) and the potential for dispersal (geographic isolation and the relative sizes of adjacent patches of host plants). With these data, it has been possible to show that, for colouration at least, environmental differences promote divergent selection (path 1), divergent selection promotes AD (path 2), an increase in the potential for dispersal increases GF (path 3 to path 4 – dispersal itself has not been measured), GF decreases AD (path 5) and AD leads to the evolution of reproductive barriers that likely limit GF (path 6). All of the above effects were found also for other morphological traits, except that AD in morphology did not seem to influence mating isolation. Genetic evidence further suggests that AD constrains GF, at least to some extent, also in nature (Nosil *et al.* 2008).

For this and other integrated studies, statistical analyses based on path models and structural equations might prove useful (Shipley 2002). Different causal hypotheses could be specified in alternative models and standardized coefficients from the best models used to infer the strength of each pathway. Such models have already been used, albeit rarely, in studies quantifying the role of divergent selection on AD (e.g. Johnson 2002), but apparently not yet in combination with GF. Effectively implementing such models is not trivial, because it requires the accurate estimation of numerous parameters in many populations. Yet, with rigorous sampling on particularly suitable model systems at least, such integrated statistical models of the various factors influencing adaptive diversification might ultimately allow rigorous insight.

Demography: the final frontier

Very few studies have explicitly incorporated demography into the study of interactions between AD and GF, with an exception being Hanski & Saccheri (2006). We argue that this should be performed more often – given the many potential influences of population size (Holt & Gomulkiewicz 1997; Kirkpatrick & Barton 1997; Gomulkiewicz *et al.* 1999; Kawecki 2000; Kawecki & Holt 2002; Lenormand 2002; Holt *et al.* 2004; Saccheri & Hanski 2006; Kinnison & Hairston 2007), which we next summarize in hopes of motivating further inclusion in correlative studies.

As explained earlier, AD and dispersal can both influence population size, which can then influence GF and feed back on AD. In addition, population size can influence the *relative* contributions of dispersal and AD to GF. For example, the relative contribution of selection against migrants to reducing GF will decrease as the number of immigrants decreases relative to the number of residents (Hendry 2004).

When the proportion of immigrants is small, AD may therefore have little direct effect on GF.

Other demographic effects are also possible. For example, high immigration may hold population size above the carrying capacity, thus causing negative density dependence, reductions in mean population fitness and declines in adaptation (Gomulkiewicz *et al.* 1999; Kawecki 2000). Alternatively, immigration may facilitate adaptation owing to positive density dependence (i.e. Allee effects, Holt *et al.* 2004) and by sustaining populations until adaptation can occur (Holt & Gomulkiewicz 1997). Incorporating demography into correlative studies may not be easy, but the above summary suggests that the potential payoffs are high.

EXPERIMENTAL EVOLUTION IN NATURAL POPULATIONS

Experimental manipulations of AD and GF is a powerful way to infer causation, as has been shown in many laboratory studies (Endler 1977; Rice & Hostert 1993; Cuevas *et al.* 2003; Forde *et al.* 2004; Swindell & Bouzat 2006). If we are to infer causation in nature, however, such studies need to be done on natural populations. At present, we know of only one experimental manipulation of dispersal that was aimed at testing the constraining role of GF (Riechert 1993; Riechert & Hall 2000; Riechert *et al.* 2001). This experiment was motivated by the observation that spiders (*Agelenopsis aperta*) from environments with different predation pressures differed in anti-predator behaviour – except in one population. To test whether GF was the cause of the phenotypic deviation, Riechert (1993) constructed drift fences that reduced dispersal between the environments. A single generation later, the formerly deviant population had evolved appropriate antipredator behaviour – thus confirming the original constraint imposed by GF. This rare manipulation convincingly demonstrated the constraining role of GF on AD in nature, but more such studies are clearly needed.

Testing the opposite causal pathway (AD to GF) can involve the experimental manipulation of selection, coupled with the monitoring of AD and GF in subsequent generations. For example, populations can be introduced into new ecological environments, and then periodically examined for evidence that increasing AD reduces GF. For example, native insects adapting to introduced host plants often show substantial reproductive isolation from their ancestors on native plants (e.g. Feder *et al.* 1990). Other work on introduced populations suggests that GF between ancestral and descendent populations can be reduced after only dozens of generations (Hendry *et al.* 2000, 2007; Sheldon & Jones 2001). Additional opportunities to examine how contemporary adaptation influences GF (or GF influences AD)

are manifold given the large number of organisms introduced to new environments (Reznick & Ghalambor 2001). An important next step would be to design and implement such studies with the expressed intent of measuring the rate at which reproductive isolation evolves and GF decreases through time.

ANY GENERALITIES?

We have mainly focused on ways to improve causal inferences, but it also seems appropriate to attempt some initial conclusions about the causal interaction between AD and GF in nature. For this, we focus on a few study systems that have been examined with the best inferential methods (Table 1) to infer whether AD constrains GF or GF constrains AD. We again focus on studies of discrete populations/environments (except where they are combined with studies of clinal variation and for the classical clinal work on *Anthoxantum odoratum* adapting to mine tailings). These systems represent only a small subset of all studies on AD and GF, but they have (in our opinion) done the best job of demonstrating the arrow of causality – especially when they have explicitly addressed both causal pathways. As in many clinal studies (Lenormand 2002), we find consistent and clear evidence that GF constrains AD in nature (Table 1). These inferences are often only qualitative, but theoretical models applied to water snakes (King & Lawson 1995; Hendry *et al.* 2001), lake and stream stickleback (Hendry *et al.* 2002; Moore *et al.* 2007) and *Timema* walking sticks (Bolnick & Nosil 2007) have confirmed that estimated levels of GF and other parameters are indeed consistent with the observed AD. These results therefore belie the old expectation (Ehrlich & Raven 1969) that rates of GF in nature are too low to have much of an effect on AD.

Evidence for the opposite causal pathway (AD to GF) is also present but is restricted mostly to the demonstration that AD generates particular reproductive barriers (Schluter 2000; Rundle & Nosil 2005). Studies that have directly tested whether AD reduces GF *in nature* are rare (but see Gow *et al.* 2007), and we therefore argue that the jury is still out on whether AD can have as large effects on GF as the reverse.

CONCLUSIONS

Negative correlations between phenotypic differences and gene flow can arise if adaptive divergence constrains gene flow or if gene flow constrains adaptive divergence. We suggest that the first of these causal pathways might be inferred by testing for negative correlations between environmental differences and gene flow. The second causal pathway might be inferred by testing for negative correla-

Table 1 A selected set of empirical study systems explicitly examining causal relationships between adaptive divergence (AD) and gene flow (GF) in natural populations

Species	Inference	n	GF estimate	Adaptive trait/reproductive barriers (yes/n.a.)	Selective factor	Other factors estimated	Type of study	Reference
Anthoxantum odoratum	GF constrains AD, AD constrains GF	8	Geographic distance	Metal tolerance, morphology/yes	Soil type	h, s	Corr	Antonovics (1968), McNeilly & Antonovics (1968), Antonovics & Bradshaw (1970), Antonovics (2006)
Agelonopsis aperta	GF constrains AD	5	Genetic	Behaviour/yes	Predation, habitat quality	dr, s	Corr/exp	Riechert (1993), Riechert et al. (2001), Riechert & Hall (2000)
Timema cristinae	GF constrains AD, AD constrains GF	12–27	Geographic isolation, relative population size, genetic	Cryptic colour, morphology/yes	Host plant, predation	h, N, s	Corr/exp	Sandoval (1994a,b), Bolnick & Nosil (2007), Nosil (2007) (and references therein, Nosil et al. (2008)
Stilbosis quadricustatella	AD constrains GF	2–6	Geographic distance, genetic	Survival/n.a.	Host plant	dr, s	Corr	Mopper et al. (1995, 2000)
Osmerus mordax	GF constrains AD, AD constrains GF	2 pairs	Genetic	Morphology, life-history/n.a.	Trophic polymorphism	opt*, s*	Corr	Saint-Laurent et al. (2003)
Gasterosteus aculeatus	GF constrains AD	3 + 1 transect, 4 pairs	Genetic	Morphology/n.a.	Habitat type	h, N*, opt*, s*	Corr	Hendry et al. (2002), Hendry & Taylor (2004), Moore et al. (2007)
Nerodia sipedon	GF constrains AD	6	Geographic distance, genetic	Colour/n.a.	Predation	dr, h, s, N	Corr	King & Lawson (1995) (and references therein)

The column 'Inference' indicates the support for GF constraining AD or AD constraining GF based on the components measured. Shown are the number of sampling sites or populations (n), the metric by which GF was estimated, the adaptive traits that were examined, whether or not reproductive barriers were found, and the expected selective factor driving the divergence. Other factors that were estimated are indicated: strength of selection (s), the expected difference in optima (opt) determined by comparing divergence in the presence and absence of GF, population sizes (N), actual dispersal rates (dr) and genetic basis (h). Type of study refers to correlations between AD and GF (corr) and actual manipulations of GF and/or selection (exp) to infer causality. (See text for further details).
*Indirect estimate, n.a., not examined.

tions between the potential for dispersal and adaptive divergence. Overall, the greatest inferential power in correlative studies is gained by simultaneously measuring and integrating multiple ecological and evolutionary factors, including environmental differences, divergent selection, dispersal, gene flow, adaptive divergence, reproductive barriers and population size (Fig. 2b). A good knowledge of the natural history of the study system is clearly essential. Particularly robust inferences may emerge from appropriate experimental manipulations of dispersal (e.g. increasing or decreasing movement between populations) and selection (e.g. introductions or environmental manipulations) in nature. At present, there appears to be more qualitative evidence for the constraining role of gene flow on adaptive divergence than for vice versa. However, much more integrated studies of exemplary model systems are needed before we can make rigorous inferences about generalities in nature.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 Island-mainland model illustrating the relationship between adaptive divergence and gene flow.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1461-0248.2008.01176.x.

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