

REVIEW

Conceptual issues in local adaptation

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Abstract

Studies of local adaptation provide important insights into the power of natural selection relative to gene flow and other evolutionary forces. They are a paradigm for testing evolutionary hypotheses about traits favoured by particular environmental factors. This paper is an attempt to summarize the conceptual framework for local adaptation studies. We first review theoretical work relevant for local adaptation. Then we discuss reciprocal transplant and common garden experiments designed to detect local adaptation in the pattern of deme \times habitat interaction for fitness. Finally, we review research questions and approaches to studying the processes of local adaptation – divergent natural selection, dispersal and gene flow, and other processes affecting adaptive differentiation of local demes. We advocate multifaceted approaches to the study of local adaptation, and stress the need for experiments explicitly addressing hypotheses about the role of particular ecological and genetic factors that promote or hinder local adaptation. Experimental evolution of replicated populations in controlled spatially heterogeneous environments allow direct tests of such hypotheses, and thus would be a valuable way to complement research on natural populations.

Keywords

Adaptive deme formation, adaptive evolution, coevolution, heterogeneous environments, gene flow, metapopulations, natural selection, population differentiation, reciprocal transplant, review.

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INTRODUCTION

The forces of natural selection often vary in space, resulting in genotype \times environment interactions for Darwinian fitness. In the absence of other forces and constraints such *divergent selection* should cause each local population (deme) to evolve traits that provide an advantage under its local environmental conditions (which we refer to as its habitat), regardless of the consequences of these traits for fitness in other habitats. What should result, in the absence of other forces and constraints, is a pattern such that resident genotypes in each deme would have on average a higher relative fitness in their local habitat than genotypes originating from other habitats. This pattern and the process leading to it is local adaptation (Williams 1966). Local adaptation may be hindered by gene flow, confounded by genetic drift, opposed by natural selection due to temporal environmental variability, and constrained by lack of genetic variation or by the genetic architecture of underlying traits. Thus, although divergent natural selection is the driving force, these other forces, in particular gene flow, are integral aspects of the process of local adaptation. Because of those

other forces, the pattern of local adaptation is not a necessary outcome of evolution under spatially divergent selection.

We reserve the term ‘local adaptation’ for patterns and processes observed across local populations of the same species connected, at least potentially, by dispersal and gene flow. This emphasizes the tension between the potentially differentiating effect of natural selection and the homogenising effect of gene flow. For convenience, throughout this paper we refer to the local populations as demes, and to the entire spatially structured population (i.e. a set of demes) as metapopulation. However, extinction obliterates locally adapted gene pools, so extinction-colonization dynamics, which is the defining feature of Levins-type metapopulations (Hanski 1999), is unfavourable to local adaptation. Furthermore, the concept of local adaptation is not restricted to a patchy environment. The demes may be discrete units in well-delimited habitat patches, or may represent arbitrary sampling units in a continuous species range. Similarly, the spatial variation in the environment may be discrete, with several distinct habitat types, or it may consist of continuous environmental gradients, whereby a ‘habitat’ represents the conditions at a given point of the gradient.

The study of local adaptation is obviously within the realm of studying adaptation in general, but there are some specific aspects. Generally, an adaptation is a phenotypic feature which is functionally designed by past natural selection, and which improves Darwinian fitness relative to alternative features (Williams 1966). Thus, studying adaptation would require considering the historical aspect, i.e. a comparison between derived, adapted populations and their presumably less adapted ancestors (e.g. Korona 1996; Travisano & Rainey 2000). This is usually not possible. The study of local adaptation offers the more feasible alternative of comparison between local populations, which have evolved under different conditions. In the absence of divergent (i.e. spatially heterogeneous) natural selection, genetic differentiation in fitness-related traits is expected to be obliterated by gene flow. Therefore, local adaptation in a set of demes connected by gene flow must be due to ongoing (or very recent) natural selection related to differences in environmental conditions experienced by different demes. In contrast, traits that are unconditionally adaptive will tend to become fixed within the species. Once a trait has become genetically fixed, it may continue to be expressed even if an environmental change causes it to lose its advantage or become detrimental (Stearns 1994). Therefore, in local adaptation studies it is often possible to identify the selective forces at work, while in classical adaptation studies this may not be possible anymore (Williams 1993). This has made local adaptation studies a paradigm for testing hypotheses about adaptations thought to be favoured by specific environmental factors (Reznick & Ghalambor 2001). Examples include life history evolution in response to predation (e.g. Reznick & Endler 1982), geographic variation in diapause strategies (e.g. Bradford & Roff 1995), reproductive phenology on alternative host species (e.g. Filchak *et al.* 2000), or types of cues used in spatial learning depending on the stability of the environment (e.g. Girvan & Braithwaite 1998).

Several other aspects of local adaptation make its study particularly interesting in the general context of adaptive evolution in natural populations. First, gene flow hinders local adaptation. Therefore, the existence of a pattern of local adaptation despite gene flow certifies to the strength of natural selection imposed by particular environmental factors. Second, it is sometimes possible to infer the age of a deme from geological or historical data; this allows one to estimate the rate of adaptive evolutionary change (e.g. Stearns 1983; Gomi & Takeda 1996). Third, local adaptation has been recognized as an important mechanism maintaining genetic variation (reviewed by Felsenstein 1976; Hedrick *et al.* 1976; Hedrick 1986). Finally, a number of scenarios for allopatric and sympatric speciation (reviewed by Schluter 2001; Turelli *et al.* 2001; Via 2001) assign local adaptation a crucial role in initiating the divergence of incipient species.

This paper is an attempt to review conceptual issues relevant for local adaptation studies. It is not intended as a summary of the existing local adaptation literature; we use selected examples to illustrate specific points. In the next section, we briefly review population genetic theory relevant to local adaptation. Then we discuss reciprocal transplant and common garden experiments designed to detect the pattern of local adaptation in the pattern of deme \times habitat interaction for fitness. Finally, we review the research questions and approaches to study the processes of local adaptation. We conclude with a call for studies directly addressing the predictions of the theory as to how much local adaptation should be expected under what circumstances.

THEORY OF LOCAL ADAPTATION

Models of adaptive divergence

A large body of theoretical literature is concerned with the interplay between spatially divergent selection and gene flow, and its effect on adaptive evolution. Although much of that work has been motivated by other questions (e.g. maintenance of genetic polymorphism, evolution of specialization, dispersal, or phenotypic plasticity) and often even does not mention local adaptation, it has yielded important predictions concerning local adaptation, providing theoretical underpinning of local adaptation studies. In this section, we briefly review the predictions of those studies relevant for local adaptation.

Genotype \times environment interaction for fitness is an obvious pre-requisite for local adaptation. Of several forms such an interaction can take, the most important for local adaptation is antagonistic pleiotropy, whereby the alleles have opposite effects on fitness in different habitats. Such antagonistic pleiotropy implies that no single genotype is superior in all habitats, leading to trade-offs in adaptation to different habitats. Beginning with Levene (1953) a number of authors (reviewed in Felsenstein 1976; Hedrick *et al.* 1976; Hedrick 1986) have shown that spatial heterogeneity facilitates maintenance of polymorphism that shows such antagonistic pleiotropy, provided that density-dependence (population regulation) operates within demes (Christiansen 1975; Pimm 1979; Karlin & Campbell 1981). Density-dependence operating independently in different demes favours rare alleles that improve fitness in a habitat, in which most individuals perform poorly. This is a form of frequency-dependent selection, which helps to maintain polymorphism, even when the average fitness of the heterozygote is below that of both homozygotes (underdominance). In the less likely case of population regulation operating at the level of the global (meta-)population (known as 'hard selection'), a single locus polymorphism will

not be maintained (protected) by selection alone unless there is overdominance for fitness averaged over the habitats (Dempster 1955; Christiansen 1975; Karlin & Campbell 1981). Population genetics theory often contrasts 'hard' with 'soft' selection. The latter assumes an extreme form of population regulation, such that the reproductive output of each local population is fixed, no matter how well or poorly the population is adapted (Christiansen 1975). In reality, population regulation is likely to fall somewhere between the 'hard' and 'soft' extremes, and models with intermediate population regulation show that the more 'soft'-like is population regulation, the more favourable are the conditions for maintenance of protected polymorphism (Pimm 1979; Christiansen 1985; Wilson & Turelli 1986; Holsinger & Pacala 1990). Maintenance of polymorphism in continuously varying environments has been studied by models of clinal variation (e.g. Slatkin 1973, 1978; Barton 1999).

Protected polymorphism in a heterogeneous environment may be maintained even if dispersal results in complete mixing of the gene pool. However, in such a case demes will not differentiate genetically, i.e. there will be no local adaptation. Thus, restricted gene flow is a pre-requisite for local adaptation. Restricted gene flow (due to low passive dispersal or active habitat choice) also makes the conditions for maintenance of polymorphism more favourable (e.g. Maynard Smith 1966). The conditions for maintenance of polymorphism are more favourable for loci with large effects; such loci also show greater differentiation of allele frequencies under divergent selection (Hedrick *et al.* 1976). Furthermore, alleles with strong effects are less likely to be lost by drift (Crow & Kimura 1970). Therefore, loci with large effects on fitness should disproportionately contribute to local adaptation (Macnair 1991). This is indeed the case in the classic examples of local adaptation of plants to sites contaminated with heavy metals (reviewed in Macnair 1987, 1991).

Nonetheless, many fitness-related characters likely to play a role in local adaptation show polygenic variation. In contrast to single-locus models, the theory of polygenic traits under divergent selection remains relatively unexplored. Most theory relevant for local adaptation concentrates on the evolution of ecological specialization, assuming a trade-off in fitness across habitats mediated by a quantitative trait or traits (reviewed in Futuyma & Moreno 1988; Jaenike 1990; Fry 1996). Models developed under this heading usually take an ESS approach (Maynard Smith 1982), assuming continuous variation in the focal trait, and aiming to identify an evolutionarily stable state, i.e. a phenotypic composition of the population, which makes it impossible for genotypes with other phenotypes to invade when rare. Three extremes define the range of possible evolutionarily stable states: (i) a single generalist phenotype

showing a similar degree of adaptation to all habitats; (ii) a single specialist phenotype optimally adapted to one habitat (usually the habitat that is most frequently encountered or of highest quality) and poorly adapted to other habitats; and (iii) a set of specialist phenotypes each maximizing fitness in one habitat type. Local adaptation requires an outcome close to (iii). Because it also requires limited gene flow, we limit our attention to models that consider limited dispersal. The evolution of divergent specialized phenotypes in such models results from selection at equilibrium being effectively disruptive (Day 2000). Of course, in a sexual population the evolution of such divergent specialized phenotypes will be prevented by recombination (unless there is very strong assortative mating). Instead, in a sexual population such disruptive selection will tend to maintain polymorphism at a greater number of loci, and thus promote differentiation between demes living in different habitats (Spichtig & Kawecki 2004).

The evolutionarily stable state predicted by the ESS models is often a discontinuous function of parameters (Brown & Pavlovic 1992; Kisdi 2002). Spichtig & Kawecki (2004) observe similar sharp transitions in their sexual polygenic model, where a small increase in dispersal rate can result in large differences in the number of polymorphic loci and the amount of equilibrium genetic variance. Population differentiation corresponding to local adaptation is promoted by low dispersal and strong selection (Brown & Pavlovic 1992; Day 2000; Kisdi 2002; Spichtig & Kawecki 2004). However, if selection is very strong (i.e. fitness falls off very quickly as the phenotype deviates from the local optimum), intermediate genotypes have low fitness in all habitats. This makes it difficult for a population initially adapted to one habitat to invade other habitats and evolve into a set of locally adapted demes, promoting the stability of an 'asymmetric' equilibrium with a single phenotype specialized on one habitat (Day 2000; Kawecki 2000, 2003; Ronce & Kirkpatrick 2001; Kisdi 2002). At such an equilibrium the population has a source-sink structure, characterized by asymmetric gene flow (Holt & Gaines 1992; Dias 1996), which makes it difficult for alleles improving adaptation in a sink habitat to spread (Holt & Gaines 1992; Kawecki 1995; Holt 1996). Therefore, the conditions for local adaptation mediated by polygenic traits are most favourable when selection in habitat 1 against genotypes well adapted to habitat 2 and vice versa is strong, but selection against intermediate (recombinant) genotypes is moderate. If selection against intermediate genotypes is weak, intermediate generalist phenotypes are likely to be favoured, leading to loss of genetic variance and little differentiation (Spichtig & Kawecki 2004). If it is too strong, the population is likely to be trapped in a source-sink situation with little differentiation among demes (in a single-locus model this case corresponds to loss of polymorphism

because of excessive marginal underdominance for fitness averaged between habitats; Christiansen 1974). An asymmetric equilibrium is also promoted by differences in size and quality of habitats, so local adaptation is most likely when such differences are small (Kawecki 1995, 2000, 2003; Ronce & Kirkpatrick 2001; Kisdi 2002).

In addition to gene flow, other forms of selection may act against local adaptation. In particular, temporal variation in natural selection favours generalist phenotypes (e.g. Kisdi 2002). Furthermore, temporal fluctuations in habitat quality favour increased dispersal (unless the fluctuations are strongly positively correlated across habitats; e.g. Levin *et al.* 1984), and thus act against local adaptation. In contrast, spatial environmental heterogeneity favours reduced dispersal and habitat fidelity (e.g. Hastings 1983), which make conditions for local adaptation more favourable. Such feedbacks lead to coadaptation between dispersal rates and traits involved in habitat adaptation (Kisdi 2002). They have also been implicated in the evolution of host races in herbivorous insects (Diehl & Bush 1989).

Finally, it should be noted that environmental heterogeneity favours the evolution of adaptive phenotypic plasticity. In the absence of costs of and constraints on plasticity, a genotype that in each habitat produces the locally optimal phenotype would become fixed in all demes. Adaptive phenotypic plasticity would thus lead to adaptive phenotypic differentiation, but without underlying genetic differentiation. The failure of the metapopulation to evolve such ideal plasticity is thus a pre-requisite for local adaptation.

To summarize, ecological factors predicted to promote local adaptation include: low gene flow (i.e. low dispersal or strong habitat fidelity), strong selection against genotypes optimally adapted to other habitats but moderate selection against intermediate genotypes (most likely under moderate differences between habitats with respect to traits under selection), little temporal variation in the forces of selection, small differences between habitats in size and quality (e.g. the amount of resources), and costs of or constraints on adaptive plasticity. We know much less about the effects of genetic architecture, as even the models with explicit genetics typically assume a simplistic genetic architecture (additivity and unlinked loci). One may expect that loci with large effects may be more important – polymorphism is more easily maintained and greater allele frequency differentiation is possible. Similarly, one could conjecture that linkage will be favourable for local adaptation as it reduces the power of recombination to break up locally adapted gene combinations (for a simple model see Dickinson & Antonovics 1973). Further theoretical work specifically addressing local adaptation mediated by polygenic traits with more complex genetic architecture is needed to substantiate these conjectures and generate new predictions.

Local adaptation in host-parasite systems

Divergent selection will often be imposed by the biotic environment, i.e. other organisms with which the focal population interacts. Biological environments evolve, and they may coevolve specifically in response to adaptation in the focal species. In recent years increasing attention has been paid in particular to local adaptation driven by interaction between hosts and parasites (broadly defined to include pathogens, parasitoids, ectoparasites and small herbivores). This may reflect the current general interest in parasites, but also we note that some conditions favouring local adaptation are particularly likely to be satisfied in host–parasite systems: selection imposed on parasites by host defences is strong, and parasites often impose strong selection on their hosts, the role of phenotypic plasticity and maternal effects appears comparatively small, and single genes have often strong effects. Thus, host–parasite systems may be particular rewarding models for studying local adaptation in general (Thompson 1994; Morand *et al.* 1996; Gandon & Van Zandt 1998; Kaltz & Shykoff 1998).

Possibly the most important aspect of local adaptation in two antagonistic species is the relative rate at which they (co)evolve. Most theoretical studies concentrate on two extremes of a continuum of relative rates. We summarize here some of their conclusions, but it should be kept in mind that natural systems usually fall somewhere between the extremes.

One end of the continuum is characterized by small parasites, which attack large long lived hosts and form demes on single host individuals (e.g. some insects on trees; Edmunds & Alstad 1978; Karban 1989). The large number of generations on single hosts, large population sizes, and replenishment of genetic variation by occasional immigrants tend to favour local adaptation of parasite demes to individual hosts (scenario known as adaptive deme formation; Edmunds 1973; Edmunds & Alstad 1978). From the perspective of the parasite deme the environment is rather constant – the genotype of a single host individual does not change, and changes of its phenotype are negligible. However, adaptation to a particular host individuals becomes useless after its death because each host is a unique habitat patch. New demes are founded by immigration from older demes and young demes are thus not expected to be locally adapted (e.g. Mopper *et al.* 2000). Parasites forming locally adapted demes on single host individuals are expected to favour host outbreeding, which improves the chances that the offspring will be genetically different from their parents, and thus more resistant to parasites adapted to their parents. Likewise, if genetically related hosts are similar from the parasite's perspective, long distance dispersal of host offspring should be favoured (Augsburger 1984; Packer & Clay 2000).

At the other extreme are host–parasite systems in which a parasite has only one generation on a given host individual and its offspring disperse to colonize other hosts. In this case each deme of the parasite is under selection to become adapted to the host deme it encounters, and vice versa. Thus, in a spatial setting the genotypic composition of a deme of one antagonist defines the ‘habitat’ for the local deme of the other antagonist. Furthermore, local adaptation of the parasite will be counteracted by local adaptation of the host. The coevolutionary feedback creates frequency-dependent selection with a time lag, and models predict unstable dynamics with constantly changing patterns, sometimes the host and sometimes the parasite being more locally adapted. Whether the host or the parasite should be more likely to show local adaptation depends mainly on the inflow of new genetic variation, i.e. mutation, migration, and recombination, and their relationship to population size (Gandon *et al.* 1996; Gandon & Michalakis 2002). These factors are usually not independent from each other. Long living species tend to have larger body sizes, smaller population size and reproduce sexually, while small organisms (viruses, bacteria, protozoa) often have huge population sizes, short generation times and reproduce usually without genetic recombination. As parasites are typically the much smaller of the two antagonists, they are often the one with the presumably higher evolutionary potential. This has led to the conventional wisdom, that parasites are ahead in the coevolutionary arms race, and that they should therefore be locally adapted more often than their hosts (Hamilton *et al.* 1990). However, parasites are often asexual while their hosts reproduce sexually, which can accelerate host evolution. Furthermore, dispersal propensities of hosts and parasites play an important role for the evolutionary potential of a deme (Gandon *et al.* 1996). Thus, whether hosts or parasites become locally adapted will depend on the biology of the systems (Kaltz & Shykoff 1998).

DETECTING LOCAL ADAPTATION

Local adaptation should be manifested in improved fitness of each deme in its own habitat. Most empirical studies focus, at least initially, on detecting local adaptation in the pattern of mean fitness shown by a set of demes across a set of habitats in a reciprocal transplant or common garden ‘explant’ experiment. In this section, we briefly summarize the design of such experiments, and discuss their analysis from the viewpoint of testing for local adaptation. We emphasize two main points. First, the key comparison from the viewpoint of local adaptation is between the relative fitness of ‘local’ vs. ‘immigrant’ genotypes within each test habitat, not between the performance of a given genotype ‘at home’ and ‘away’. Second, the unit of biological

replication is a deme, and more than two demes need to be studied to distinguish the pattern of local adaptation from other forms of deme \times test habitat interactions. At the end, we discuss a couple of technical issues relevant to fitness measurement and minimization of maternal effects.

Reciprocal transplant and common garden experiments

Demonstrating the pattern of local adaptation in the deme \times test habitat interaction for fitness requires an experiment in which samples of genotypes from the local deme and some other deme(s) are directly compared under the same environmental conditions (in the same habitat). Ideally, this would be done in the field, by transplanting individuals originating from different demes between the original habitats, from which the demes were sampled. Such reciprocal transplants will often be impossible for practical, ethical or legal reasons. An alternative is to re-create the essential properties of different habitats in the laboratory (or greenhouse, experimental plots, etc.) while controlling for other factors, and test samples from different demes there. We refer to this as ‘common garden’ (or ‘explant’) approach. It is often used in studies of local adaptation in parasites, where the genetic composition of local host populations is assumed to be the environmental factor essential for parasite adaptation (Karban 1989; Lively 1989; Thrall *et al.* 2002). Other examples include testing local adaptation of cladocerans to different levels of salinity (Weider & Hebert 1987) or growing samples of soil bacteria on media based on soil extracts from their original localities (Belotte *et al.* 2003). In addition to practical advantages, this approach directly tests the role of a particular environmental factor as an agent of divergent selection driving local adaptation (see below). The downside is that local adaptation to habitat differences neglected in the experiment may confound the results. For example, a particular genotype may perform better than others in all experimental habitats because it happens to be best adapted to the general laboratory conditions. Similarly, an experiment designed to mimic a specific environmental difference may neglect a key factor. For example, if demes of a plant living in warmer climate invest more in defence because of a stronger herbivore pressure, they may turn out to have inferior seed yield at all temperatures if the assay is carried out in the absence of herbivores.

Predictions

There has been some controversy concerning the specific pattern of deme \times test habitat interaction for fitness which should be considered diagnostic of local adaptation in reciprocal transplant or common garden experiments. Two criteria have been proposed. The ‘local vs. foreign’ criterion emphasizes the comparison between demes within habitats:

in each habitat the local deme is expected to show higher fitness than demes from other habitats. In contrast, the ‘home vs. away’ criterion emphasizes the comparison of a deme’s fitness across habitats: local adaptation would be said to occur if each deme had a higher fitness in its own habitat (at home) than in other habitats (away).

Of course, as the number of demes and habitats increases, these idealized criteria will be increasingly unlikely to hold in each habitat or for each deme, so they need to be re-formulated in statistical terms. In general statistical terms, local adaptation implies a specific form of deme \times habitat interaction: mean deme fitness should be systematically higher for the ‘sympatric’ deme \times habitat combinations (i.e. a deme is tested in its habitat of origin) than in the remaining, ‘allopatric’ cases. However, existence of the predicted ‘sympatric vs. allopatric’ contrast is not sufficient to conclude about local adaptation. This is illustrated with a hypothetical two-habitat example in Fig. 1: the average difference between the ‘sympatric’ and ‘allopatric’ cases is identical in all panels, yet panel (d) suggests a very different conclusion about local adaptation than panel (a). The degree to which the ‘local vs. foreign’ criterion is satisfied is thus indicated by the magnitude of this ‘sympatric vs. allopatric’ contrast relative to variation in overall deme performance (averaged across habitats). In turn, the ‘home vs. away’ criterion is quantitatively addressed by the magnitude of the ‘sympatric vs. allopatric’ contrast relative to variation in intrinsic

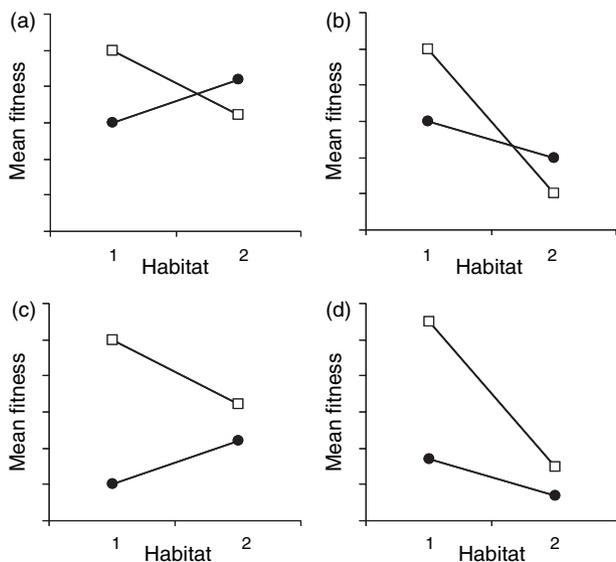


Figure 1 Hypothetical patterns of deme \times habitat interaction for fitness. Squares: the average of demes originating from habitat 1; circles: the average of demes originating from habitat 2. The patterns in panels (a) and (b) satisfy the ‘local vs. foreign’ criterion. The patterns in panels (a) and (c) satisfy the ‘home vs. away’ criterion. The average difference between the ‘sympatric’ and ‘allopatric’ deme–habitat combinations is the same in all graphs.

habitat quality (averaged over demes tested in it). Thus, the two criteria are not independent, but also not equivalent.

The two criteria will often be simultaneously satisfied (as in Fig. 1a), and it is certainly worth examining both (Gandon & Van Zandt 1998; Kaltz *et al.* 1999; Thrall *et al.* 2002; Belotte *et al.* 2003). However, we do not agree with the proposition that they are equally relevant for testing the pattern of local adaptation. Rather, we believe that the ‘local vs. foreign’ criterion should be regarded as diagnostic for the pattern of local adaptation. This criterion is directly relevant to the driving force of local adaptation – divergent natural selection – which acts on genetic differences in relative fitness within each habitat. The ‘local vs. foreign’ criterion addresses the efficacy of divergent selection relative to other evolutionary processes. In contrast, the ‘home vs. away’ criterion confounds the effects of divergent selection with intrinsic differences in habitat quality. Survival or fertility of a genotype optimally adapted to a poor-quality habitat may still increase following a transplant to a resource-rich habitat, although in the poor habitat this genotype is favoured (i.e. has higher relative fitness than other genotypes) while in the rich habitat it would be outcompeted by other genotypes. In contrast to genetically-based differences among demes in overall performance, which are a product of evolution, differences in intrinsic habitat quality are a property of the environment. Theory of local adaptation predicts evolutionary changes in the metapopulation, but not changes in the environment. We, therefore, propose that a pattern that satisfies the ‘local vs. foreign’ but not ‘home vs. away’ criterion (like that in Fig. 1b) offers as much support for local adaptation, as a pattern that satisfies both criteria (like that in Fig. 1a). In contrast, a pattern that satisfies the ‘home vs. away’ but not the ‘local vs. foreign’ criterion (Fig. 1c) implies that some demes consistently outperform others in all habitats, in contrast to what the theory predicts.

Nonetheless, detection of a pattern like in Fig. 1c invites follow-up studies to explain it. It still does suggest an imprint of natural selection imposed by the local conditions in each habitat. It may even represent a case of local adaptation masked by experimental artefacts, such as non-genetic differences between individuals originating from different demes (e.g. different sensitivity to handling, maternal effects or different infection status; see below), or accidental pre-adaptation of some demes to the general common garden environment. Or, it may reflect genuine genetic differentiation because of other processes, like differential inbreeding of demes, a beneficial mutation spreading through the metapopulation (already fixed in some demes and still absent in others). It may also indicate that different demes have reached alternative ‘adaptive peaks’ of different ‘height’ (Goodnight 2000). The existence of such pattern is highly relevant for other issues, e.g.

evolution of dispersal or a virulence of a novel parasite. Nonetheless, we believe that such a pattern does not on its own provide convincing evidence for local adaptation.

Replication

Both criteria described above require that 'sympatric' deme-habitat combinations have higher fitness than the 'allopatric' ones. However, demes may be genetically differentiated for reasons other than divergent selection (e.g. drift, migration or history). Combined with genotype \times environment interaction, any genetic differentiation among demes will produce some deme \times habitat interaction, which may accidentally cause the expected 'sympatric vs. allopatric' difference. Therefore, independent of the issues discussed in the preceding section, it is desirable to show that the 'sympatric vs. allopatric' difference is unlikely to be explained by deme \times test habitat interaction unrelated to local selection. This requires replication at the level of the deme; if only two demes are studied, these two interaction terms are impossible to separate. The lack of replication prevents one from concluding that the differentiation is because of divergent selection, rather than chance events in the demes' history. Of course, with only two demes under study convincing evidence for driving role of divergent selection can still come from a detailed study of natural selection, dispersal, and the pattern of genetic differentiation between the demes (see the following section). Nonetheless, replication at the level of the deme is needed to demonstrate local adaptation on the basis of the fitness pattern alone.

There are two basic ways in which local adaptation studies can be replicated at the level of the deme, depending on whether *a priori* knowledge or a hypothesis exists about environmental factors relevant for the divergent selection that drives local adaptation.

The first approach (which we refer to as 'parallel local adaptation') is to classify the habitats in several (usually two) clearly defined and reproducible types of habitat, based on differences in a factor or factors hypothesized to be relevant for differential selection underlying local adaptation. Examples include normal vs. contaminated soils (McNeilly 1968), ruderal vs. agricultural habitats (Leiss & Müller-Schärer 2001), or different host species (Via 1991). Several replicate demes originating from each habitat type are sampled and tested in each habitat type. These demes could be sampled independently (e.g. Leiss & Müller-Schärer 2001), or paired between habitat types based on geographic proximity (e.g. Berglund *et al.* 2004). Most studies in this category involve a common-garden assay in a controlled environment, but some have been done with reciprocal transplant in the field (e.g. Via 1991). Because the focus in 'parallel local adaptation' is on the specific ecological factors which define habitat types, the main effect of the habitat is treated from

the statistical viewpoint as fixed (type I) factors (Sokal & Rohlf 1981, section 8.6). In contrast, the demes included in the study will usually be treated as a sample of all demes evolved in the focal habitat types; one would usually want to generalize the findings to other demes. This perspective implies that deme should be treated as random (type II) statistical factor (Sokal & Rohlf 1981, section 8.7).

The second approach, which we refer to as 'unique local adaptation', does not make an assumption about the ecological factor(s) behind divergent selection. Instead, the habitat of each deme is considered unique. Multiple demes can be sampled, and the fitness of each deme tested in its own and at least two other (away) habitats (e.g. Lively 1989; Roy 1998; Kaltz *et al.* 1999, 1999). From the statistical viewpoint, the habitats included in the study are thus a random sample of all habitats, suggesting that both deme and habitat should be treated as a random (type II) factors. Assaying each deme in more than one 'away' habitat allows one to split the deme \times habitat interaction into a component because of the 'sympatric vs. allopatric' contrast, and the residual component not related to local adaptation. This residual deme \times test habitat interaction forms the baseline for testing the significance of the 'sympatric vs. allopatric' contrast (Kaltz *et al.* 1999; Thrall *et al.* 2002).

These two basic approaches – 'parallel' and 'unique' local adaptation – can be modified in several ways. The geographic or spatial distance between the habitat of origin and the test habitat can be incorporated as an explanatory variable (for various designs and statistical approaches see e.g. Ebert 1994; Kaltz *et al.* 1999; Joshi *et al.* 2001; Thrall *et al.* 2002; Belotte *et al.* 2003). Similarly, one can measure an 'ecological distance' between habitats along an environmental axis defined by quantitative environmental parameters (e.g. Rice & Mack 1991; Lively & Jokela 1996). Finally, one could combine the 'parallel' and 'unique' approaches in a single design, simultaneously testing for 'parallel' local adaptation to a specific environmental factor defining broad habitat types (transplants across habitat types), and for 'unique' local adaptation to sites within each habitat type (transplants across sites within each habitat type).

Finally, we would like to reiterate that local adaptation as defined above is not a property of individual populations, but of a set of demes (i.e. a metapopulation). Nonetheless, it may be of interest to identify subsets of demes that do show a pattern of local adaptation vs. those that do not, especially if these subsets can be characterized by specific properties such as history, spatial arrangement, habitat size, spatial isolation, or deme size or age (see below).

Fitness measurement

Testing for local adaptation requires estimates of fitness, so the question arises, how should fitness be measured? There

is no simple general answer to this question. Of course, the ideal would be a measure of performance that corresponds most closely to fitness relevant in the process of adaptation. However, this requires detailed knowledge of the system under investigation, which will often not be available, and even if it is, the ideal performance measure may be impractical or too costly. Ultimately, the measure used will usually reflect a compromise between what is ideal and what is doable, as judged by the researcher based on the knowledge of the biology of the system.

There are three basic approaches to estimating fitness in local adaptation studies. First, a direct way of assessing fitness involves staging competition between or among genotypes sampled from different demes and measuring their genetic contribution to the next generation (Capaul & Ebert 2003). Alternatively, all genotypes may be tested against a common 'tester' genotype (e.g. Kraaijeveld & Godfray 1997). Such experiments mimic natural selection and is most relevant for species which typically encounter intra-specific competition in their natural environment. Its applicability will usually be limited by the availability of deme-specific genetic markers that would allow to trace the genetic contribution to the next generation.

The second approach is to measure the population growth rate (the Malthusian parameter or net reproductive rate) of each deme in a given habitat. This seems most appropriate for organisms that often go through phases of exponential growth with little intraspecific competition. For micro-organisms, for which no genetic markers are available the population growth rate will often be the only fitness-related phenotype that can be easily measured (e.g. Belotte *et al.* 2003), but this approach has also been applied to other short-lived organisms, such as aphids (Via 1991). An analogous measure for parasites is the number of secondary infections resulting from a primary infection (epidemiological R_0 , Anderson & May 1991), which estimates the rate at which infection will spread in a susceptible host population. Assuming certain underlying models, the Malthusian parameter or R_0 can also be estimated from fitness components such as age-specific survival and fertility or epidemiological parameters (Anderson & May 1991; Stearns 1992).

The third and most common approach is to use one or more individual traits as measures of performance. Examples range from major fitness components like juvenile survival or fecundity (e.g. Mopper *et al.* 2000; Leiss & Müller-Schärer 2001), through life history traits such as age at first reproduction or, for a parasite, infectivity (e.g. Lively 1989; Mopper *et al.* 1995; Kaltz *et al.* 1999), to traits such as body size, root growth or number of leaves (e.g. Gomez-Mestre & Tejedo 2003; Berglund *et al.* 2004) or resistance to local parasites and herbivores (e.g. Roy 1998). A working assumption behind using such traits as measures of performance is that they are monotonically related to

fitness, i.e. are under directional selection in all demes. However, fitness-related traits are often under stabilizing selection and/or are trade-off with other fitness components (reviewed in Roff 1992; Stearns 1992). In this case, different intermediate trait values may be optimal in different locations; treating these traits as measures of performance (e.g. the bigger the fitter) may be misleading. However, if the habitat-specific optima of a trait are known, the degree to which each deme matches the optimum for its habitat is highly informative about local adaptation (e.g. Dias & Blondel 1996). Some traits (e.g. juvenile survival or parasite infectivity) will in general be more often under directional selection than others (e.g. root size, phenological traits or parasite virulence). In any case careful consideration should be given to the relationship of a given performance trait to actual fitness. This relationship can often be verified through measurements of selection gradients (see below).

Minimizing non-genetic effects

Local adaptation is about genetic differentiation, so care should be taken to minimize non-genetic effects such as differences in handling, plasticity and maternal effects. If individuals transplanted from a different deme are simply released into a habitat and let compete with the local population, they may do poorly because of the stress due to the transplant procedure or environmental change. Maternal effects may induce plastic responses adaptive in the maternal environment and thus mimic local adaptation in the absence of genetic differences (e.g. Agrawal *et al.* 1999). In other cases, effects due to maternal environment may obscure the pattern of local adaptation, e.g. by improving performance of offspring produced in more productive habitats (for an example, see Stanton & Galen 1997). Such confounding effects will be eliminated (or at least minimized) if samples from all demes are maintained (acclimated) under common environmental conditions for two or three generations before their fitness is measured. Ideally, each sample would be acclimated under the conditions under which its fitness is to be measured. This is often possible if the fitness assays take place in the laboratory, but will usually be impractical if fitness is measured in the field (but see Karban 1989). Therefore, most reciprocal transplant experiments involve transplanting offspring of lines maintained for one or a few generations in single common laboratory (or greenhouse, etc.) environment (e.g. Leiss & Müller-Schärer 2001). This common environment may induce some maternal effects, but this will not be a problem unless these maternal effects affect fitness of genotypes originating from different demes very differently.

Another source of systematic non-genetic differences in performance between demes could be infection with

parasites, if experimental individuals originating from some demes are more parasitized than others. If infection is easily transmitted to the offspring the differences may persist over several generations of acclimation in a common garden. The problem can be addressed by clearing the infection with medication (e.g. Little & Ebert 2000) or by estimating the level of infection in different demes and correcting for it in the analysis (e.g. Osnas & Lively 2004).

STUDYING PROCESSES OF LOCAL ADAPTATION

Demonstrating a pattern consistent with local adaptation certifies to the power of divergent selection relative to gene flow and other evolutionary processes. However, this pattern in itself tells us little about the underlying processes themselves. Furthermore, a metapopulation may fail to show genetic differentiation for fitness traits because of lack of divergent selection, too much gene flow, or temporal variation in selection, which favours generalist genotypes. It may also show a pattern of differentiation other than that predicted by local adaptation. This could be due to drift or history. More recently established demes may show poorer adaptation to their habitat than older ones. A beneficial mutation may be spreading in the metapopulation, being already fixed in some demes (which thus would show higher fitness) while absent in others. Finally, different demes may have reached alternative 'adaptive peaks' of different 'height', i.e. if they have evolve alternative epistatic gene combinations that result in different fitness. Thus, the absence of the pattern does not necessarily mean that the process of divergent natural selection is not operating. Studying the processes driving, hindering, and interacting with, local adaptation would help to understand why local adaptation is apparent in some metapopulations but not in others. This involves asking questions and testing hypotheses about ecological factors responsible for divergent natural selection, traits under selection and their genetic architecture, gene flow, and processes affecting immigrants (such as heterosis or outbreeding depression). In this section, we address some of these questions and the empirical approaches used to address them.

Agents of and traits under divergent selection

Because divergent natural selection is the driving force of local adaptation, studying the traits under divergent selection and the ecological factors responsible for it ('agents' of divergent selection) is an essential step towards understanding of local adaptation. As mentioned in the introduction, local adaptation offers a rewarding context to study the link between traits under and agents of selection. In this subsection, we review the approaches to this issue.

Many studies of the pattern of local adaptation are designed with a particular hypothesis concerning the agents of divergent selection and particular traits in mind. These can be often inferred from the knowledge of the biology of the organism and the characteristics of its environment (e.g. Dias & Blondel 1996). Common garden experiments explicitly test the role of the factor that differs between treatments – other factors are kept constant. Thus a pattern consistent with local adaptation automatically implicates the focal factor(s) as agent(s) of divergent selection. An agent can be defined as precisely as the concentration of a particular heavy metal ion (Berglund *et al.* 2004), or as vaguely as the composition of a soil extract (Belotte *et al.* 2003). In the latter case, as well as in those involving reciprocal transplants testing 'unique local adaptation', narrowing down the agents of divergent selection requires testing more specific hypotheses. Such hypotheses may be based on educated guesses and on analysis of correlations between deme fitness and characteristics of the local habitats.

Agents of divergent selection are causally coupled with traits under selection. Given that essentially any phenotypic characteristic of an organism can be defined as a trait, the issue of traits under selections has many layers. For example, soil contamination with copper salts will obviously select for copper tolerance. The interesting question is, what changes in other traits – biochemical, physiological, morphological, life history, etc. – does evolution of copper tolerance entail? Thanks to intensive research we know a lot about those mechanistic bases of heavy metal tolerance in plants, as well as about underlying genetics (Macnair 1993; Hall 2002), but for most other traits involved in local adaptation this knowledge is much more rudimentary.

Traits mediating local adaptation should show genetically based phenotypic differences between demes evolved in different habitats, the phenotype being understood broadly to include physiological and biochemical characteristics and patterns of gene expression. However, not all genetically based phenotypic differences between demes must be adaptive. Instead they may represent the costs of adaptive traits, mediated by pleiotropic effects of underlying genes. They may also be because of genetic hitchhiking of genes linked to those favoured by divergent selection. Finally, such differences may be produced by processes not related to local adaptation (such as drift or evolution of alternative coadapted gene combinations).

One way to identify traits under divergent selection involves analysis of multivariate fitness gradients within local habitats. A fitness gradient (usually based on partial regression) estimates the relationship between a trait and fitness while controlling for other traits. This approach has been widely used to describe natural selection in natural populations (Lande & Arnold 1983; Mitchell-Olds & Shaw

1987; Brodie *et al.* 1995). In the context of local adaptation we are interested in *differences* in fitness gradients between habitats (e.g. Kalisz 1986; Petit & Thompson 1998; Caruso 2001). Being based on correlations, the fitness gradient approach has some serious limitations. For example, apparent selection on a particular trait may in fact reflect a correlation with another trait, which is under selection but has not been included in the analysis (for discussion of this and other limitations see Wade & Kalisz 1990; Willis 1996; Stinchcombe *et al.* 2002). Despite these limitations, analysis of fitness gradients can provide important insights into the nature of selection, especially if it is combined with other approaches, and if its interpretation is guided by the knowledge of the biology of the species.

The analysis of fitness gradients can also help to identify the agents of selection: differences between habitats in fitness gradients should be correlated with differences in the environmental factors causing divergent selection (Wade & Kalisz 1990). Again, because of correlational character this approach has limitations. In particular, an apparent relationship between the fitness gradient of a trait and an environmental factor may be because of another factor, which causes selection on the trait and is correlated with the first factor, but has not been included in the analysis. For example, some aspects of plant adaptation to ore mining sites seem to have been selected by water deficit or release from competition, rather than by the presence of the heavy metals in the soil (Macnair 1987). Therefore, it is desirable to verify such correlational evidence from reciprocal transplants with common garden experiments controlling for all but the focal factor(s) (e.g. Petit & Thompson 1998) or manipulation of the focal factor in the field (e.g. Mauricio & Rausser 1997).

Some problems with correlational character of fitness gradient analysis can be circumvented by manipulative approaches to measuring selection. Experimental manipulation of the phenotype and measuring its consequences for fitness has been extensively applied in studies of optimal clutch size in birds (Lack 1966) and of sexually selected traits (reviewed in Andersson 1994). This approach is being increasingly used to test adaptive nature of traits in general (reviewed by Schmitt 1999; Sinervo 1999), and of phenotypic plasticity in particular (reviewed by Schmitt *et al.* 1999). It has rarely been applied to study local adaptation (but see, e.g. Callahan & Pigliucci 2002; Tremblay *et al.* 2003). The main limitation of this approach is technical – few traits are as easy to manipulate as clutch size. Recent advances in genetic techniques allow genetic manipulation of the phenotype in a few model organisms, which can also be used to study adaptation (Tatar 2000); to our knowledge this approach has not been applied to local adaptation.

A suspected coupling between agents of divergent selection and particular traits can also be verified with

experimental evolution. This approach involves exposing experimental populations of common origin to controlled environments over a number of generations and following their evolutionary changes. It was used, e.g. to support the role of temperature as an agent of selection responsible for latitudinal clines in body size in *Drosophila* (Partridge *et al.* 1994). The relevance of the experimental evolutionary response for local adaptation to natural environments can be verified by testing their fitness in the natural habitats which the laboratory regimes were supposed to imitate.

Finally, local adaptation requires that spatial variation in selection is substantially greater than temporal variation. Both theoretical arguments (Gillespie 1973) and experimental data (reviewed in Kassen 2002) show that temporal variation in selection favours generalist genotypes and thus hinders local adaptation. Thus irrespective of the approach used to study divergent selection, it is desirable to address the issue of its constancy through time.

Genetics of local adaptation

Once the traits under divergent selection have been identified, one would like to learn about their genetic bases. The genetics, as well as the cellular mechanisms, of heavy metal tolerance in plants are relatively well understood, no doubt partially because this adaptation typically involves one or a few major loci (Macnair 1993; Hall 2002). Most ecologically relevant traits are, however, affected by many segregating loci and show a large non-genetic variability. Identification of genes responsible for divergence in such traits (QTL mapping) requires large sample sizes and is labour-intensive (for methodology of QTL mapping see Lynch & Walsh 1998, part II). For examples of QTL mapping in the context of local adaptation see Hurme *et al.* (2000); Calboli *et al.* (2003b) or Verhoeven *et al.* (2004). Candidate loci involved in local adaptation can also be identified with genetic approaches, without measuring a phenotype (other than bands on a gel). In particular, latitudinal clines and other forms of spatial variation correlated with environmental factors are often observed for allele frequencies at allozyme loci (reviewed in Eanes 1999). Chromosomal location of candidate loci for local adaptation can also be suggested by the loss of variation at linked neutral marker loci (selective sweeps, Schlotterer 2002). The role of candidate loci in local adaptation can be verified by demonstrating differential survival or reproduction of genotypes (e.g. Lenormand *et al.* 1998; Schmidt & Rand 2001).

Even if the genes responsible for local adaptation remain unknown, useful information about the genetic aspects of traits mediating local adaptation can be learned with the methods of quantitative genetics. First, how much additive genetic variation for this trait exists and how is it distributed

within vs. among demes? The former indicates the ability of the trait to respond to selection (for estimation see Falconer & Mackay 1996; Lynch & Walsh 1998, part III). The latter, which can be quantified as Q_{st} (Merila & Crnokrak 2001; McKay & Latta 2002), measures the degree of genetic differentiation of quantitative traits between populations. Traits under strongest divergent selection are expected to have the highest Q_{st} , which is another way of identifying traits mediating local adaptation.

Second, what is the genetic architecture of diverged traits? Is it mostly because of few major loci or are many loci with small effects involved? What are the patterns of dominance and epistatic interaction between loci? Are the traits affected by genetically-based maternal effects? These questions can be addressed with the analysis of crosses between genotypes originating from different demes (for methods see Lynch & Walsh 1998, Chapter 9). This approach has been used, e.g. to demonstrate contribution of dominance and epistasis to divergence in photoperiodism in a mosquito (Hard *et al.* 1992) and wing size in *Drosophila* (Gilchrist & Partridge 1999).

Third, do these traits show phenotypic plasticity and is it adaptive? As discussed above, adaptive plasticity may be seen as an evolutionary alternative to local adaptation. However, plasticity may also be a maladaptive by-product of environmental influences on physiology. Selection will counteract such maladaptive plasticity, so local adaptation will in this case be manifested as reduction of phenotypic differences between demes living in different habitats. Selection will thus be divergent at the genetic level, but not at the phenotypic level, and will thus not be detectable as a difference in fitness gradients. This type of local adaptation has been termed countergradient variation (Conover & Schultz 1995). Examples include the evolution of faster intrinsic growth or development to compensate for the physiological effect of lower ambient temperatures in poikilotherms (reviewed in Arendt 1997), and a similar evolutionary adjustment of a temperature-dependent environmental sex determination mechanism (Conover & Heins 1987).

Fourth, are the traits mediating local adaptation involved in genetically-based trade-offs with other traits relevant for fitness? Such trade-offs may be due to pleiotropy or linkage of underlying genes (reviewed in Roff 1992). Their understanding is essential for the interpretation of selection patterns. Many fitness-related traits like the number and quality of offspring are positively correlated with fitness, but trade off with each other, so the effective selection on them is stabilizing. Also, many specific adaptations, like heavy metal tolerance or herbivore resistance, have physiological costs. In environments where these adaptations are not needed these costs translate into a fitness disadvantage (e.g. Strauss *et al.* 2002), and thus are the reason why such

adaptations are local rather than global. Although the pattern of differentiation between demes often suggests such costs, they may be elusive to pinpoint (e.g. Harper *et al.* 1997, 1998).

Finally, cases of parallel local adaptation provide a context to study the issue of repeatability of evolution. Will different demes adapt to the same agent of selection in the same way, or the response will involve different traits and genes? The results are mixed. Schat *et al.* (1996) reported that populations of *Silene* on mining sites in Ireland and Germany independently evolved heavy metal tolerance based on the same loci. Similarly, much of latitude-related difference in wing size in two independently evolved clines in *Drosophila* seems to map to the same QTL (Calboli *et al.* 2003b). However, other studies found that parallel latitudinal clines in *Drosophila melanogaster* wing size show different underlying genetic architectures (Gilchrist & Partridge 1999), and that some of them are mostly due to differences in cell number while others in cell size (Zwaan *et al.* 2000; Calboli *et al.* 2003a). The issue remains open, and detailed studies of parallel local adaptation can provide important insights.

Gene flow and other processes

Divergent selection is the driving force of local adaptation, but the outcome depends on the interaction between divergent selection and other forces of evolution, in particular gene flow. Therefore, quantitative estimates of gene flow provide important insights in the process of local adaptation. Gene flow is usually estimated indirectly based on differentiation at (presumably) neutral genetic marker loci. The methods and underlying models, as well as their advantages and limitations have been repeatedly reviewed (Neigel 1997; Bossart & Prowell 1998; Ouborg *et al.* 1999; Sork *et al.* 1999; Paetkau *et al.* 2004; for an application in a context of local adaptation see, e.g. Mopper *et al.* 2000; Brown *et al.* 2001). This approach relies on more or less sophisticated models of population structure; in particular in most cases it is assumed that the metapopulation is at a drift – gene flow equilibrium. Reliability of the estimates can be strongly affected if the assumptions are violated (e.g. Neigel 2002; Burczyk & Chybicki 2004).

Genetic differentiation at marker loci is often inversely related to the geographic distance among populations, an observation termed *isolation by distance*. In the context of local adaptation gene flow may be, however, confounded by asymmetric dispersal among demes and by selective processes acting on the fate of immigrants.

Asymmetric gene flow occurs when migration among demes is not random or a function of distance, but if the likelihood to receive immigrants from certain demes is unproportionally higher than the likelihood to receive migrants from other demes (e.g. Watkinson 1985; Dias

et al. 1996; Stanton & Galen 1997). In the most extreme cases, this can lead to a source-sink population structure, in which the sink populations may not be able to evolve local adaptation because they are flooded with migrants, while the source populations receive comparatively few migrants (reviewed by Kawecki 2004). Such asymmetry may be caused by ecological factors such as habitat productivity, edge effects or predominant wind direction.

Another reason for deviations from a simple isolation by distance pattern is habitat choice. Genetic variation for habitat choice automatically becomes non-randomly distributed among habitats, and can cause gene flow to be greater between distant patches of similar habitats than between neighbouring patches of different habitats. Such divergent habitat choice is very favourable for local adaptation, however, an association between genes for habitat preference and habitat-specific performance will tend to be broken down by recombination (Felsenstein 1981). Therefore, in local adaptation studies in animals capable of active dispersal it is desirable to address divergent habitat preference and the genetic correlation between preference and performance (e.g. Via 1999; Filchak *et al.* 2000; Hawthorne & Via 2001).

Gene flow is, however, not only a function of dispersal, but also of the success of the migrants in their new habitat. A number of other evolutionary processes will affect the fate of migrants and their offspring, and thus influence the effective gene flow. From the viewpoint of local adaptation it is thus of great interest to study the details of immigration and the subsequent introgression of immigrant genes into the gene pool of the local deme. Genes 'flow' from one deme to another 'packaged' in migrating individuals or propagules (seeds, spores, pollen, etc.), and all genes carried by a propagule initially share the same fate. Because local adaptation is characterized by the inferiority of immigrants relative to locals, effective gene flow is reduced by the presence of locally adapted residents. If immigrants go through several generations of asexual reproduction before they introgress, this effect is amplified and gene flow may be drastically reduced (De Meester *et al.* 2002). In sexuals recombination will over generations dissociate the fate of neutral markers from genes under selection, a process slowed down by linkage.

A different mechanism influencing gene flow is related to the fact that immigrants are usually rare, so their fitness may be biased by frequency-dependent selection. For example, if different demes have evolved different sexually selected ornament-preference systems, immigrant males will be discriminated against by local females; given that most females will be local, this will create sexual selection against immigrants. The converse is also possible, as females may show preference for males they perceive as unusual (e.g. Sinnock 1970; Ball *et al.* 2000). In general, frequency

dependent selection will tend to obscure local adaptation (if it favours rare genotypes), or to create an appearance of one (if it discriminates against rare genotypes). This calls for studies designed to disentangle local adaptation from frequency dependent selection (e.g. Roy 1998).

Furthermore, emigrants will often not be a representative sample of their deme. In species with contest competition poorer competitors will often be more likely to emigrate because they are unable to gain a territory or a breeding site in their native habitat (e.g. Serrano *et al.* 2003). If these individuals are also likely to be inferior in other habitats, this will lead to a pattern of inferiority of immigrants to the residents not related to local adaptation. If the inferiority is transmitted to the offspring (genetically or through maternal effects), the disadvantage to the immigrants genes will extend beyond the first-generation immigrants. Migrants will also often differ from non-migrants with respect to traits directly related to dispersal; the difference may be genetic or reflect plasticity. Thus, detailed study of immigrants and their fate may provide insights relevant for local adaptation.

Finally, the effective gene flow will not only depend on the dispersal rate and the performance of immigrants, but also on the fitness of their offspring and later descendants. Because immigrants are rare relative to the local genotypes, most of their offspring will originate from mating with the locals, and most of those 'hybrids' will themselves backcross with the local genotypes. The performance of those intermediate 'hybrid' genotypes has important consequences for local adaptation (see the theory section above). There are three general reasons why fitness of such hybrid genotypes may deviate from a simple average of the two parental genotypes. First, the phenotype for the traits mediating local adaptation may deviate from the mid-parent value because of dominance and epistatic interactions (see above); even if it does not, the effects on habitat-specific fitness will not be additive if the relationship between the phenotype and fitness is not linear (e.g. Hartfield & Schluter 1999). Second, offspring of immigrants may enjoy hybrid vigour (heterosis). Hybrid vigour usually reflects complementation of recessive deleterious mutations whose frequency differentiated between demes due to drift. Hybrid vigour favours immigrant genes and thus magnifies the effective gene flow (e.g. Ebert *et al.* 2002). Third, hybrid genotypes, especially those of second and later generations, may suffer from outbreeding depression not related to traits under divergent selection. Outbreeding depression is expected if, due to their initial genetic makeup or historical contingencies, the parental demes have evolved alternative coadapted gene combinations (i.e. reached alternative 'adaptive peaks'; Goodnight 2000). Breakdown of those beneficial epistatic interactions would reduce the fitness of recombinant genotypes, especially in the second and following generation of immigrant offspring backcrossed into the local gene pool.

Outbreeding depression will thus cause selection against immigrant genes for reasons unrelated to local adaptation. Outbreeding depression is often observed in crosses among demes, sometimes at small spatial scales (Burton 1990; Armbruster *et al.* 1997; Fenster & Galloway 2000). These considerations can be addressed by including hybrid genotypes in reciprocal transplant or common garden experiments (e.g. Hartfield & Schluter 1999; Via *et al.* 2000; for an approach to analysis see O'Hara Hines *et al.* 2004).

PERSPECTIVES: TESTING HYPOTHESES

How much local adaptation should we expect and under what circumstances? As summarized above, the existing theory makes some predictions concerning these questions; others will hopefully be derived. Ultimately one would like these predictions to be tested. Such tests might involve a comparative approach. For example, some plant species have adapted to sites contaminated with heavy metals, while others did not despite being abundant in the surrounding habitat. A comparison of those two groups of species, controlled for phylogeny (Harvey & Pagel 1991), could help to identify attributes of life history, mode of dispersal, physiology, and other characteristics increasing the likelihood of becoming locally adapted. An alternative approach involves a comparison of sets of demes within a metapopulation. If many demes are studied, the degree to which individual demes show local adaptation can be correlated with their characteristics such as size, age, demography, isolation, and habitat quality. In the spirit of this approach Mopper *et al.* (2000) have demonstrated that older demes of a leafmining lepidopteran (those on older trees) show more pronounced local adaptation to their individual host trees than young demes, despite being less differentiated from one another in neutral markers. Both these approaches are correlational and are thus prone to confounding effects not measured in the study; the latter also may suffer from non-independence of data. One potential solution would be experiments manipulating some local habitats or demes in the field (e.g. by changing the dispersal rate or habitat productivity) and following the evolutionary changes relative to unmanipulated control demes. This has, to our knowledge, not been applied to study local adaptation.

An alternative approach to testing the predictions of local adaptation theory is experimental local adaptation. It involves experimental evolution of replicated experimental metapopulations each consisting of two or more demes living in different experimental habitats and connected by controlled 'migration'. This approach allows one to study the processes of local adaptation in real time, and to test directly the effects of differences in ecological variables

(deme size, habitat quality, dispersal rate and pattern, etc.) on the evolutionary outcome. Using this approach Cuevas *et al.* (2003) have beautifully demonstrated how the degree of local adaptation of an RNA virus to three types of host cells gradually declines with increasing migration rate. Several other experimental evolution studies compared populations evolving in a spatially heterogeneous environment with high gene flow against populations evolving in single habitats or in temporarily varying environments (reviewed by Kassen 2002). A similar approach has been used to study the effects of properties of metapopulation structure on metapopulation and community dynamics (e.g. Davis *et al.* 1998; Thrall *et al.* 2003). We believe that the experimental evolution approach to local adaptation offers great potential to compensate for limitations of studies of natural metapopulations.

CONCLUSION

Heritable phenotypic differentiation between local populations has long attracted the attention of naturalists and evolutionary biologists, and at least since Darwin it has usually been assumed to be adaptive. This assumption has only begun to be put to rigorous test in recent decades, with the emergence of the concept of local adaptation. In this paper, we attempted to review the conceptual framework for studies of local adaptation. This framework has many facets, reflecting the complexity of local adaptation being the outcome of interactions between natural selection, gene flow, and other evolutionary processes. We believe that furthering our understanding of local adaptation requires comprehensive studies of the processes of local adaptation in natural populations, supplemented with further theoretical developments and studies involving experimental local adaptation in model systems in controlled environments.

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