Moving beyond Common-Garden and Transplant Designs: Insight into the Causes of Local Adaptation in Species Interactions

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ABSTRACT: Theoretical and empirical studies of local adaptation in species interactions have increased greatly over the past decade, yielding new insights into the conditions that favor local adaptation or maladaptation. Generalizing the results of these studies is difficult, however, because of the different experimental designs that have been used to infer local adaptation. Particularly challenging is comparing results across empirical studies conducted in a common laboratory or garden environment with results of those conducted using transplants in natural environments. Here we develop simple and easily interpretable mathematical expressions for the quantities measured by these two different types of studies. Our results reveal that common-garden designs measure only a single component of local adaptation—the spatial covariance between the genotype frequencies of the interacting species—and thus provide only a partial description of local adaptation. In contrast, reciprocal-transplant designs incorporate additional terms that measure the contribution of spatial variability in the ecological environment. Consequently, the two types of studies should yield identical results only when local adaptation is caused by spatial variability in the genotype frequencies of the interacting species alone. In order to unify these disparate approaches, we develop a new methodology that can be used to estimate the individual components of local adaptation. When implemented in an appropriate experimental system, this partitioning allows the examination of fundamental questions such as the relative proportion of local adaptation attributable to interactions between species or to the abiotic environment.

Keywords: coevolution, gene flow, geographic mosaic, host-parasite, Red Queen.

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Interactions between species play an important role in the ecological and evolutionary dynamics of the component species. For instance, parasites can regulate host population densities (Hudson et al. 1992; Ebert et al. 2000), favor evolutionary shifts in host ploidy or gene expression (Nuismer and Otto 2004, 2005; Oswald and Nuismer 2007), and, in some cases, drive the evolution of increased rates of sexual reproduction (Howard and Lively 1998, 2002; Peters and Lively 1999; Otto and Nuismer 2004; Gandon and Otto 2007). To a large extent, the impact of interacting species on ecological and evolutionary dynamics depends on the degree to which the component species are locally adapted to one another (Kaltz and Shykoff 1998; Otto and Michalakis 1998; Thompson et al. 2002; Kawecki and Ebert 2004; Lively et al. 2004; Thompson 2005; Gandon and Otto 2007). Consequently, numerous mathematical models have been developed to explore the factors responsible for local adaptation between interacting species, yielding several predictions (e.g., Gandon et al. 1996; Lively 1999; Parker 1999; Gandon 2002; Gandon and Michalakis 2002; Nuismer 2006; Ridenhour and Nuismer 2007). At the same time, local adaptation has been studied empirically in a wide variety of systems, with results that provide support for at least some of the theoretical predictions (Kaltz et al. 1999; Parker 1999; Lively et al. 2004; Morgan et al. 2005). Nevertheless, the robustness of this agreement between theoretical and empirical studies is unclear because of differences in the methodology used to estimate local adaptation across studies.

A key methodological difference that distinguishes studies of local adaptation is whether a common-garden or transplant design is used. Common-garden studies estimate local adaptation by measuring fitness components of

the focal species (e.g., probability of infection, probability of survival, number of offspring produced) in interactions with nonfocal species individuals drawn from either local or foreign habitats within a common laboratory, garden, or greenhouse environment (e.g., Lively 1989; Ballabeni and Ward 1993; Ebert 1994; Lively and Jokela 1996; Imhoof and Schmid-Hempel 1998; Kaltz and Shykoff 1998; Kaltz et al. 1999; Oppliger et al. 1999; Laine 2005). The primary advantage of these studies is that they minimize the contribution of environmental variation to fitness and thus may more efficiently isolate the contribution of genetic variation. In some cases, particularly when both interacting species are mobile, these studies may also be easier to conduct. In contrast, transplant studies estimate local adaptation by measuring components of fitness for samples of individuals that have been moved between natural environments (e.g., Parker 1985, 1989; Kaltz and Shykoff 1998; Carlsson-Graner et al. 1999; Laine 2007; Ridenhour and Nuismer 2007). Consequently, these studies incorporate not only the contribution of genetic variation to fitness—as do common-garden studies—but also the contribution of environmental variation.

Although it is currently difficult to make quantitative statements regarding the importance of environmental variation for species interactions, numerous studies exist that show that it may be important, at least in some cases. For instance, plant resistance to pathogens may depend on availability of essential nutrients (Springer et al. 2007); probability of snail infection by trematodes may depend on snail condition (Dybdahl and Krist 2004); pathogen virulence may depend on the density of conspecific competitors (Lively et al. 1995; Bedhomme et al. 2005); and benefits of mutualistic interaction may depend on nutrient availability (Heath and Tiffin 2007). This well-documented environmental variation suggests that common-garden and transplant studies may not generally yield identical results. In line with this expectation, the single empirical study of which we are aware that has compared results of common-garden and transplant studies within the same system found that the two types of studies yielded different results (Laine 2007).

From a theoretical perspective, too, there is some evidence that common-garden and transplant designs can yield qualitatively different conclusions for local adaptation (Ridenhour and Nuismer 2007). Specifically, using a model of spatially structured interactions mediated by quantitative traits, Ridenhour and Nuismer (2007) found that local adaptation measured using a transplant design was generally greater than local adaptation measured using a common-garden design. However, these theoretical results were derived under relatively specific genetic assumptions, preventing the formation of any general conclusions regarding the expected relationship between results of common-garden and transplant studies.

Taken together, these empirical and theoretical results suggest that common-garden and transplant studies may not generally yield identical insights into the magnitude of local adaptation in species interactions. To quantify this suggestion, we develop very general mathematical models that allow us to predict when, if ever, we expect common-garden and transplant studies to yield equivalent results. By rewriting expressions for local adaptation as a series of statistical moments, our results provide fresh insight into which abiotic and biotic factors contribute to local adaptation as measured by the two experimental designs. These results lead to a novel empirical approach to studying local adaptation that can be used to partition local adaptation into its underlying causes.

The Model

We consider a very general model of a spatially structured interaction between two species that occurs over a landscape consisting of N habitats or populations. Although we will refer to one of these species as the "host" and one as the "parasite" for clarity, our results are quite general and apply equally well to other forms of interspecific interaction, such as mutualism or competition. We assume that some arbitrary component of host and parasite fitness is measured in a local adaptation study (e.g., seed set, infection success, mortality), with a value determined by the abiotic environment and interspecific interactions. Each species S is assumed to have an arbitrary number (n_S) of genotypes that contribute to the measured fitness component. Specifically, we will assume that the fitness of an individual of species S with genotype i in an encounter with an individual of the interacting species with genotype j in patch k is given by

$$W_{S,i,j,k} = \kappa_{S,i,k} + \alpha_{S,i,j,k}. \quad (1)$$

The first term in equation (1), $\kappa_{S,i,k}$, is the contribution of the abiotic (and assumed temporally constant) environment to the measured fitness component of an individual of species S with genotype i in population k, and thus it incorporates genotype (G) × environment (E) interactions on fitness as a result of the heterogeneity of the abiotic environment. This term also implicitly incorporates interactions with other nonfocal species. The second term in equation (1), $\alpha_{S,i,j,k}$, measures the effect on the measured fitness component of an individual of species S and genotype i of an encounter with an individual of the interacting species with genotype j within population k; this term is generally positive for the parasite and negative for the host (for a mutualistic interaction, this term would be

positive for both species; for a competitive interaction, it would be negative for both species). This second term thus allows for G × G × E interactions on fitness as a result of the heterogeneity of both the biotic and the abiotic environment.

Equation (1) makes no assumptions regarding host and parasite genetics, allowing for any ploidy level, number of loci, pattern of pleiotropy, and number of alleles. Nor does it make assumptions regarding the genetic basis of parasite resistance in the host, allowing for any of the commonly used models of host resistance, such as gene-for-gene or inverse matching alleles (table 1). In addition, equation (1) allows the abiotic component of the measured fitness component ($\kappa_{S,i,k}$) to change across space, as has been documented for many traits such as flowering time (Hall and Willis 2006), drought tolerance (Knight et al. 2006), coloration (Camin and Ehrlich 1958), and cost of parasite resistance (Fornoni et al. 2004). Finally, equation (1) is sufficiently general to allow the fitness consequences of interactions between particular pairs of host and parasite genotypes ($\alpha_{S,i,j,k}$) to vary across space. This may occur any time the probability of infection, or fitness consequences of infection (virulence), varies spatially because of factors extrinsic to the genotypes of the interacting species. Plausible scenarios include spatial variation in encounter rates (Little and Ebert 2000; Craig et al. 2007), density of competing species (Lively et al. 1995), and availability of essential nutrients (Springer et al. 2007). Thompson's geographic mosaic theory of coevolution explicitly incorporates this form of variation as the basis for selection mosaics and intermingled coevolutionary hot and cold spots (Nuismer et al. 1999; Thompson 2005; Gomulkiewicz et al. 2007).

General Expressions for Local Adaptation

We define local adaptation in species S (Δ_S) as the difference between the expected value of a fitness component measured for individuals in their native populations ($\overline{W}_{S,\text{home}}$) and the expected value of the same fitness component when measured for individuals in foreign populations ($\overline{W}_{S,\text{foreign}}$) or in all populations ($\overline{W}_{S,\text{global}}$):

$$\Delta_{S,\text{foreign}} = \overline{W}_{S,\text{home}} - \overline{W}_{S,\text{foreign}} \tag{2a}$$

or

$$\Delta_{S,\text{global}} = \overline{W}_{S,\text{home}} - \overline{W}_{S,\text{global}}, \tag{2b}$$

where positive values indicate local adaptation and negative values indicate local maladaptation. These two measures of local adaptation have been termed "without replacement" and "with replacement" by Morgan et al. (2005) and have been shown to be related to one another in the following way: $\Delta_{S,\text{global}} = \Delta_{S,\text{foreign}}[N/(N-1)]$. We will see later that although definition (2b) is less often used than (2a), it greatly facilitates comparisons of local adaptation across studies and is thus strongly recommended. Throughout our calculations, we assume that a fully reciprocal design is used.

Assuming that species interactions occur at random, equation (1) can be used to calculate the expected values of the fitness components that appear in equations (2). Specifically, the expected value of the fitness component for species S individuals in their native populations is given by

$$\overline{W}_{S,\text{home}} = \frac{1}{N}\sum_{k=1}^{N}\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}}[X_{i,k}Y_{j,k}(\kappa_{S,i,k} + \alpha_{S,i,j,k})], \tag{3}$$

where $X_{i,k}$ is the frequency of genotype i in the focal species S within patch k and $Y_{j,k}$ is the frequency of genotype j in the interacting species S^* within patch k. The expected value of the fitness component for species S individuals in foreign populations is

$$\overline{W}_{S,\text{foreign}} = \frac{1}{N(N-1)}\sum_{l=1}^{N}\sum_{k=1\neq l}^{N}\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}}[X_{i,l}Y_{j,k}(\kappa_{S,i,k} + \alpha_{S,i,j,k})], \tag{4}$$

Table 1: Absolute fitness increment/decrement to a parasite or host individual of genotype i in an encounter with an individual of the interacting species with genotype j in habitat k ($\alpha_{S,i,j,k}$) for the case of two haploid loci

	Host genotype							
	AB ($X_{\text{AB},k}$)		Ab ($X_{\text{Ab},k}$)		aB ($X_{\text{aB},k}$)		ab ($X_{\text{ab},k}$)	
Parasite genotype	Consequence for parasite	Consequence for host	Consequence for parasite	Consequence for host	Consequence for parasite	Consequence for host	Consequence for parasite	Consequence for host
AB ($Y_{\text{AB},k}$)	$\gamma_{P,k}$	$-\gamma_{H,k}$	$\gamma_{P,k}$	$-\gamma_{H,k}$	$\gamma_{P,k}$	$-\gamma_{H,k}$	$\gamma_{P,k} + \xi_{P,k}$	$-\gamma_{H,k} - \xi_{H,k}$
Ab ($Y_{\text{Ab},k}$)	0	0	$\gamma_{P,k}$	$-\gamma_{H,k}$	$\xi_{P,k}$	$-\xi_{H,k}$	$\gamma_{P,k}$	$-\gamma_{H,k}$
aB ($Y_{\text{aB},k}$)	0	0	$\xi_{P,k}$	$-\xi_{H,k}$	$\gamma_{P,k}$	$-\gamma_{H,k}$	$\gamma_{P,k}$	$-\gamma_{H,k}$
ab ($Y_{\text{ab},k}$)	$\xi_{P,k}$	$-\xi_{H,k}$	0	0	0	0	$\gamma_{P,k}$	$-\gamma_{H,k}$

Note: If the interaction is mediated by a gene-for-gene mechanism, all ξ values are equal to 0, whereas if the interaction is mediated by an inverse matching-alleles model, all γ values are equal to 0. Genotype frequencies are in parentheses. See "General Expressions for Local Adaptation" for parameter definitions.

where $X_{i,l}$ is the frequency of genotype i in the focal species within patch l and $Y_{j,k}$ is the frequency of genotype j in the interacting species S^* within patch k. The expected value of the fitness component for species S individuals in all populations is

$$\overline{W}_{S,\text{global}} = \frac{1}{N^2}\sum_{l=1}^{N}\sum_{k=1}^{N}\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}}[X_{i,l}Y_{j,k}(\kappa_{S,i,k} + \alpha_{S,i,j,k})]. \quad (5)$$

Equations (4) and (5) can be substituted into equations (2) and, in conjunction with equation (3), can be used to yield expressions for local adaptation in species S. Although exact, these expressions offer little insight into the factors that contribute to local adaptation. In the next section, we show how rewriting these expressions for local adaptation as a series of statistical moments offers a new perspective.

A Moment-Based Expression for Local Adaptation in Transplant Studies

Appendix A in the online edition of the *American Naturalist* shows how the summations of the previous section can be rewritten as a series of statistical moments. Ultimately, this yields the following expression for local adaptation in species S, assuming a reciprocal-transplant study:

$$\Delta_S = \psi\Bigg\{\sum_{i=1}^{n_S} C(X_i, \kappa_{S,i}) + \sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}}\left[\bar{\alpha}_{S,i,j}C(X_i, Y_j) + \overline{Y}_j C(X_i, \alpha_{S,i,j}) + C(X_i, \alpha_{S,i,j}, Y_j)\right]\Bigg\}, \quad (6)$$

where $\psi = 1$ if local adaptation is calculated "with replacement" (as assumed in eqq. [2b] and [5]) and $\psi = N/(N-1)$ if local adaptation is calculated "without replacement" (as assumed in eqq. [2a] and [4]; Morgan et al. 2005), $\bar{x}$ indicates the expectation of the variable x across populations, $C(x, y)$ is the covariance between the variables x and y across populations, and $C(x, y, z)$ is the expectation of the quantity $(x - \bar{x}) \times (y - \bar{y}) \times (z - \bar{z})$ across populations.

The first term in equation (6) measures the degree to which the focal species is locally adapted to the abiotic environment and thus quantifies the contribution of statistical interactions between focal species genotype and abiotic fitness (a form of G × E interaction) to local adaptation. Specifically, this term measures the spatial covariance between the frequency of genotype i in the focal species (X_i) and its fitness with respect to the abiotic environment ($\kappa_{S,i}$). If the abiotic environment is spatially homogenous, this value is equal to 0 and does not contribute to local adaptation. If, however, the abiotic environment is spatially variable but temporally constant, classical population genetic theory shows that, at equilibrium, this covariance will take a positive value determined largely by the strength of selection relative to the rate of gene flow (e.g., Fisher 1950; Endler 1973; Slatkin 1973). Thus, to the extent that spatial variability in the abiotic environment is common and rates of gene flow are not too high, this term should increase levels of local adaptation in both host and parasite.

While the first term of equation (6) measures local adaptation to the abiotic environment, the second term reflects local adaptation to the genotype frequency distribution of the interacting species and thus measures the contribution of statistical interactions between focal species genotype and nonfocal species genotype (a form of G × G interaction) to local adaptation. More precisely, this term measures the spatial covariance between host and parasite genotype frequencies. This covariance should be positive for the parasite any time parasite genotypes are found more frequently than expected with host genotypes that confer the largest fitness benefit to the parasite. It should be positive for the host any time host genotypes are found more frequently than expected with parasite genotypes that reduce their fitness the least. In the next section, we show that it is this term alone that is measured by common-garden studies.

The third term in equation (6) measures local adaptation to the fitness consequences of interactions with the focal species and thus measures the contribution of statistical interactions between focal species genotype and the fitness consequence of species interactions (a form of G × E interaction) to local adaptation. More precisely, this term measures the spatial covariance between genotype frequencies in the focal species and the fitness consequences of interactions between genotypes. This term should be positive for the focal species if the frequency of genotype X_i tends to be above or below average in those habitats/regions where the fitness consequences to this genotype of interacting with the other species are larger or smaller than average, respectively. This would be the case for the host if, for instance, the frequency of host genotype X_i tended to exceed its spatial average in regions where parasite virulence also exceeds its spatial average.

The fourth and final term in equation (6) measures joint local adaptation of the focal species to the fitness consequences of interactions and the genotype frequency distribution of the interacting species. Thus, this term

measures the contribution of statistical interactions between focal species genotype, nonfocal species genotype, and the fitness consequence of species interactions (a form of G × G × E interaction) to local adaptation. This term should be positive for the parasite any time parasite genotypes tend to match those host genotypes they can best infect within those habitats where successful infection results in the greatest fitness increase to the parasite. Similarly, this term should be positive for the host any time host genotypes match those parasite genotypes they can best resist within those habitats where infection results in the greatest fitness loss to the host.

Unlike the first two terms, the last two terms in equation (6) take nonzero values only when there is spatial variation in the fitness consequences of interspecific interactions ($\alpha_{S,i,j,k}$). Consequently, these last two terms take nonzero values only when the selection mosaics and coevolutionary hot and cold spots central to Thompson's geographic mosaic theory are present (Thompson 2005; Gomulkiewicz et al. 2007). An additional consideration is that the fourth term in equation (6) takes nonzero values only when there are more than two populations (see app. D in the online edition of the *American Naturalist*).

In summary, equation (6) shows that local adaptation, as measured using a reciprocal-transplant design, incorporates the contributions of (1) adaptation to the abiotic environment, (2) adaptation to the genotype frequency distribution of the interacting species, (3) adaptation to the fitness consequences of interactions, and (4) joint adaptation to the fitness consequences of interactions and the genotype frequency distribution of the interacting species.

The Special Case of Common-Garden Studies

Common-garden studies proceed by infecting host populations with either local or foreign parasites in a common environment. Measuring fitness components in a common environment minimizes spatial variation in the abiotic component of genotypic fitness ($\kappa_{i,k}$) and in the fitness consequences of interactions between host and parasite genotypes ($\alpha_{i,j,k}$). For instance, in those cases where parasite virulence has been shown to be affected by density of competitors (Lively et al. 1995; Bedhomme et al. 2005), using a common-garden experiment eliminates the possibility that parasite virulence (measured by host $\alpha_{i,j,k}$ terms) varies as a function of spatial variation in the density of competitors. In this case, $\kappa_{i,k}$ and $\alpha_{i,j,k}$ take values that depend on environmental conditions of the test environment, yielding a particularly simple expression for local adaptation in species S:

$$\Delta_S = \psi\left[\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}} \alpha_{S,i,j,T} C(X_i, Y_j)\right], \tag{7}$$

where $\alpha_{S,i,j,T}$ measures the fitness consequences of interactions between host and parasite genotypes within the common test environment T; all other terms are as defined previously. Expression (7) demonstrates that local adaptation inferred from common-garden designs is simply a measure of the spatial covariance between host and parasite genotype frequencies (S. Gandon and S. L. Nuismer, unpublished manuscript).

Comparing expressions (6) and (7) shows that the value of local adaptation estimated using a common-garden design may be quite different from that measured using a transplant design. In the following sections, we identify conditions under which both types of studies yield identical results and develop a new methodology that allows the individual components of local adaptation to be estimated independently.

When do Common-Garden and Transplant Studies Yield Identical Results?

Although it might seem that transplant and common-garden studies should yield identical results over a broad set of conditions, comparison of equations (6) and (7) shows that this may not generally be the case. In fact, the two types of studies can yield identical results only if the first, third, and fourth terms of equation (6) sum to 0 or all have 0 values. Even though the first explanation could occur in isolated cases, it seems unlikely that these three terms would generally sum to 0 despite having individual nonzero values. In contrast, it is easy to see how all three terms could have zero values. Specifically, this would occur anytime there was no spatial variation in the fitness of focal species genotypes with respect to the abiotic environment and no spatial variation in the fitness consequences of interactions between genotypes of the interacting species. As the spatial scale of the local adaptation study decreases, this would seem to become increasingly likely.

Even if the sum of the first, third, and fourth terms in equation (6) is equal to 0, it is not guaranteed that the results of transplant and common-garden studies will be equivalent. This is because the second term in equation (6), which measures the contribution of statistical associations between host and parasite genotype frequencies to local adaptation, is weighted by the spatial average of the fitness consequences of interactions in the natural environment (represented by the matrix $\bar{\alpha}_{S,i,j}$). In contrast, the sole term in equation (7), which measures the contribution of statistical associations between host and par-

asite genotype frequencies in common-garden experiments, is weighted by the fitness consequences of interactions between genotypes of the interacting species within the test environment (represented by the matrix $\alpha_{S,i,j,T}$). Thus, for the two types of studies to yield identical results, experimental conditions used for the common-garden experiment must be fortuitously—or painstakingly—aligned with average conditions in the natural environment. Finally, if conducted "without replacement" as in equation (2a), the two types of studies will yield identical results only if the same number of populations is used in each, because of the factor of $N/(N-1)$ multiplied by the estimate of local adaptation in equations (6) and (7).

Given that quantitative agreement between the two designs is unlikely, it is worth exploring the conditions required for the two types of experiments to yield qualitatively similar conclusions, such as whether a species is locally adapted or maladapted. In most cases, a common-garden design should show one species to be locally adapted and the other to be locally maladapted, simply because the entries of $\alpha_{S,i,j,k}$ that appear in equation (7) should generally have opposite signs in host and parasite (table 1). In contrast, a transplant experiment can reveal that both species are simultaneously locally adapted because of the extra terms appearing in equation (6), which capture the variability in fitness consequences that may exist in the natural environment.

We evaluated conditions under which common-garden and transplant studies would yield qualitatively different conclusions, using multilocus simulations of a coevolving host-parasite interaction. The details of these simulations are provided in appendix B in the online edition of the *American Naturalist*, and the C++ source code is available upon request. As expected from our analytical expressions, simulations show that when spatial variation in the abiotic environment and fitness consequences of interactions is absent, both types of studies yield identical results if the common garden faithfully replicates the fitness consequences of interactions in the natural environment (fig. 1*A*). If the fitness consequences of interspecific interactions (α) vary spatially but those of the abiotic environment (κ) do not, such that the second, third, and fourth terms of equation (6) are nonzero, we find that the two types of studies generally yield qualitative, but not quantitative, agreement (fig. 1*B*). If, in contrast, the abiotic environment varies spatially but the fitness consequences of interspecific interactions do not, such that the first term of equation (6) is nonzero, results of the two studies may differ qualitatively at some points in time (fig. 1*C*). Finally, if both the abiotic environment and fitness consequences of interactions vary spatially, such that the first through fourth terms of equation (6) are all nonzero, the two types of studies may yield results that differ wildly and consistently over time (fig. 1*D*).

Moving beyond Reciprocal-Transplant and Common-Garden Studies: A General Approach for Partitioning the Causes of Local Adaptation

Our results demonstrate that local adaptation in natural populations is composed of a series of statistical moments, each measuring the contribution of an easily understood statistical interaction (i.e., G × E, G × G, and G × G × E). Because the relative magnitude of these different statistical interactions has important consequences for evolutionary and coevolutionary dynamics (e.g., Thompson 2005; Nuismer 2006; Ridenhour and Nuismer 2007), it is important to be able to estimate their contributions independently. Although these components have been estimated directly in some systems through experimental manipulations of interacting species genotypes and physical environment (e.g., Johnson and Agrawal 2005; Heath and Tiffin 2007), such studies have generally not been performed for the full range of naturally occurring genetic variation or at the scale relevant to local adaptation—multiple populations. In this section, we present an experimental design and accompanying mathematical expressions that can be used to estimate any of the components of local adaptation that appear in equation (6) in a way that takes into account the full complement of genetic diversity both within and across populations.

The key to this methodology is to perform replicate common-garden experiments in different habitats. From a statistical perspective, the methodology we propose equates to using a full factorial design where the factors are focal species population of origin, nonfocal species population of origin, and habitat in which fitness components are measured. For the case of only two study populations, the methodology is particularly straightforward and can be easily illustrated (fig. 2). Practically speaking, implementing the design shown in figure 2 entails measuring a fitness component W for a sample of η focal species individuals drawn at random from each of the eight experimental treatments. The η random samples can be used to calculate eight expected values of the fitness component for the focal species. We define the expected value of this fitness component as $\bar{\omega}_{S,i,j,k}$, where i is the focal species population of origin, j is the nonfocal species population of origin, and k is the habitat in which the fitness component is measured (fig. 2).

In some empirical systems (e.g., Thompson and Pellmyr 1992; Pellmyr et al. 1996) it is possible to estimate a fitness component attributable to species interactions independent from a fitness component attributable to the abiotic

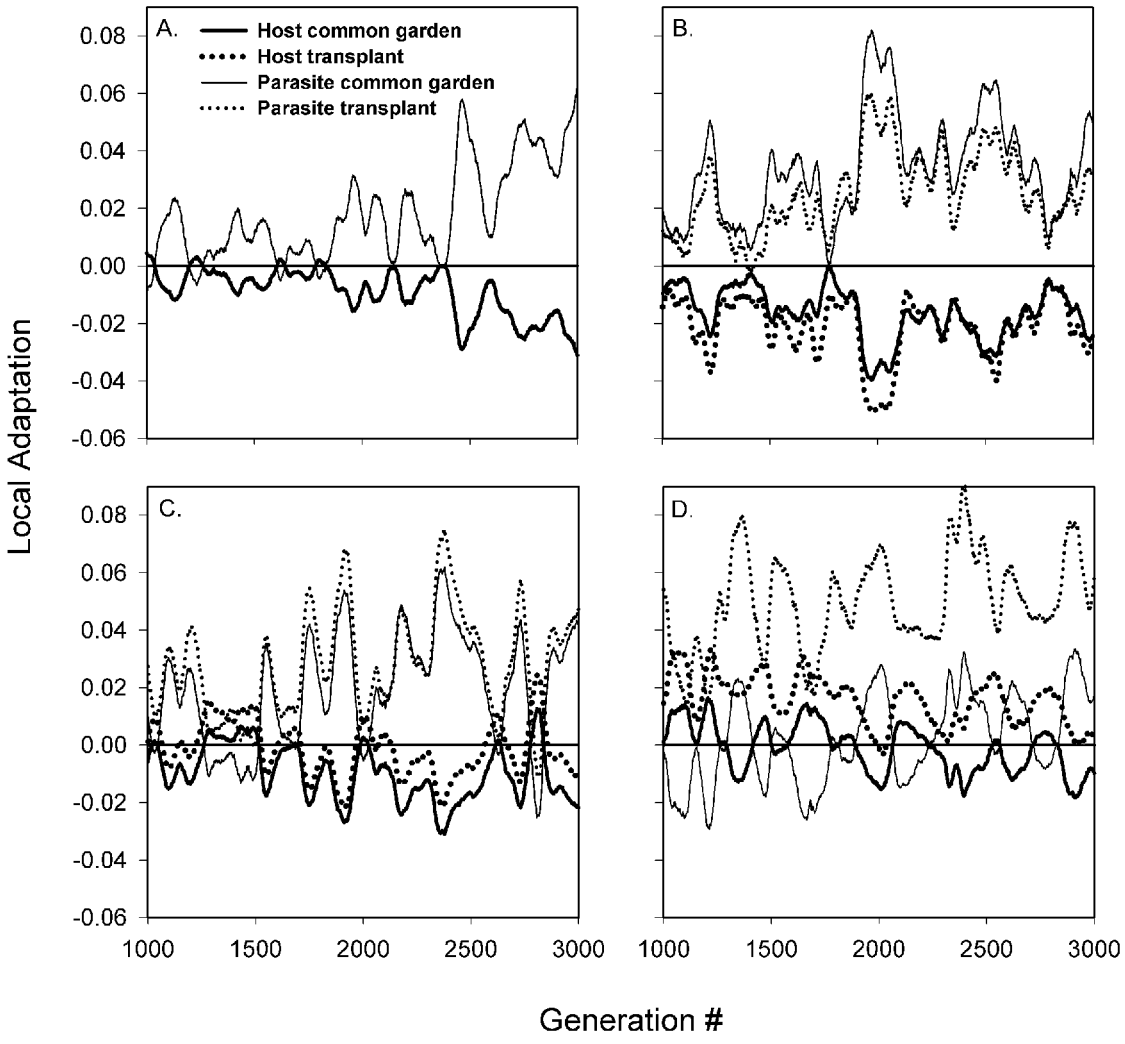

Figure 1: Host and parasite local adaptation measured "without replacement" using equation (2a) for transplant (*dotted lines*) and common-garden (*solid lines*) designs and four different scenarios. In *A*, the abiotic environment induces no selection, and the fitness consequences of interactions are spatially homogenous. In *B*, the abiotic environment induces no selection, but the fitness consequences of interactions vary across space (biotic selection mosaic). In *C*, the abiotic environment induces spatially variable selection, and the fitness consequences of interactions are spatially homogenous (abiotic selection mosaic). In *D*, the abiotic environment induces spatially variable selection, and the fitness consequences of interactions vary spatially (biotic and abiotic selection mosaic). Each species was composed of 10 populations, each containing 3,000 host and 3,000 parasite individuals. All populations were assumed to be equally connected by gene flow at a rate of 1×10^{-5} in the host and 1×10^{-4} in the parasite. In all cases, infection/resistance was mediated by an inverse matching-alleles model with two diallelic loci, recombination occurred at rate 0.1, mutation occurred at rate 1×10^{-6}, $\bar{\xi}_H = 0.1$, and $\bar{\xi}_P = 0.2$. *A*, $\sigma^2_{\xi,H} = 0$, $\sigma^2_{\xi,P} = 0$, $\tau_{\min,H} = 0$, $\tau_{\min,P} = 0$, $\tau_{\max,H} = 0$, and $\tau_{\max,P} = 0$; *B*, $\sigma^2_{\xi,H} = 0.005$, $\sigma^2_{\xi,P} = 0.001$, $\tau_{\min,H} = 0$, $\tau_{\min,P} = 0$, $\tau_{\max,H} = 0$, and $\tau_{\max,P} = 0$; *C*, $\sigma^2_{\xi,H} = 0$, $\sigma^2_{\xi,P} = 0$, $\tau_{\min,H} = -0.01$, $\tau_{\min,P} = -0.02$, $\tau_{\max,H} = 0.01$, and $\tau_{\max,P} = 0.01$; *D*, $\sigma^2_{\xi,H} = 0.005$, $\sigma^2_{\xi,P} = 0.001$, $\tau_{\min,H} = -0.01$, $\tau_{\min,P} = -0.02$, $\tau_{\max,H} = 0.01$, and $\tau_{\max,P} = 0.01$. A complete description of the simulations and definitions of parameters are provided in appendix B in the online edition of the *American Naturalist*.

environment. In these cases, all components of local adaptation appearing in equation (6) can, in principle, be estimated using the mathematical formulas presented in table 2 and derived in appendix C in the online edition of the *American Naturalist*. For systems where the fitness contributions of species interactions and the abiotic environment cannot be adequately disentangled, a less complete, although still insightful, decomposition of local adaptation can be made (app. D). In both cases, the statistical significance of the estimated components of local adaptation can be determined through bootstrapping if the fitness components have been estimated from a sufficient sample of individuals.

Although the methodology we outline here allows new

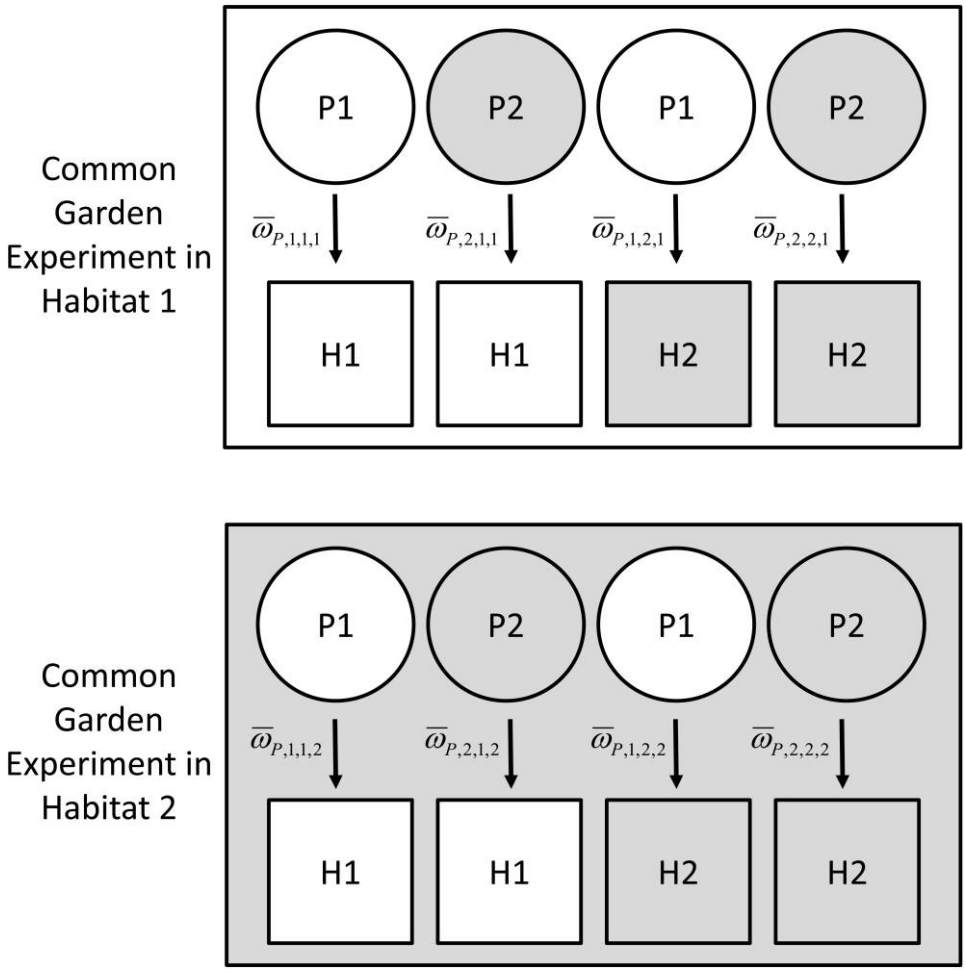

Figure 2: New methodology for estimating components of local adaptation illustrated for a simple host-parasite interaction where the contributions of the abiotic and biotic environments to parasite fitness can be estimated independently and only two populations are studied. Parasite (P) individuals are moved into one of four different experimental treatments, represented by squares occupied by host (H) individuals within each of the two habitats. Only those parasite individuals added to the experimental treatments are allowed to interact with host individuals within the experimental treatment, necessitating some form of exclosure. Parasite fitness components are measured for η individuals in each treatment to yield estimates for the expected values of fitness components $\bar{\omega}_{P,i,j,k}$, where i is parasite population of origin, j is host population of origin, and k is the habitat in which the fitness component is measured. Note that because this example utilizes only two populations, the estimated value of $\sum_{i=1}^{ns}\sum_{j=1}^{ns} C(X_j, \alpha_{S,i,j}, Y_j)$ will always equal 0. See "General Expressions for Local Adaptation" for parameter definitions.

insights into the causes of local adaptation, it also imposes significant methodological hurdles not present in standard reciprocal-transplant or common-garden designs. First and foremost, the new methodology requires that both focal and nonfocal species can be effectively moved between habitats and that the nonfocal species can be excluded from study "plots" in each habitat. This is likely to form an insurmountable hurdle in some study systems. Second, because the complete design requires N^3 different cross-infection treatments to estimate all components of local adaptation in a single species, investigations are likely to be limited to studies of only two (requiring eight treatments) or three (requiring 27 treatments) populations. However, if only two populations are used, the value estimated for the fourth term in equation (6) will always be 0, irrespective of its true value in the metapopulation as a whole (app. C). Moreover, as pointed out by Kawecki and Ebert (2004), using only two populations creates the possibility that local adaptation can be inferred as a result of purely stochastic processes.

Discussion

We have developed simple moment-based expressions for the values of local adaptation estimated using transplant

Table 2: Formulas for estimating components of local adaptation in those systems where the fitness contributions of the abiotic environment and species interactions can be estimated independently

Component to be estimated	Biological interpretation	Formula
$\sum_{i=1}^{n_S} C(X_i, \kappa_{S,i})$	Adaptation to the abiotic environment	$(1/N^2)\sum_{k=1}^{N} \bar{k}_{S,k,k} - (1/N^3)\sum_{k=1}^{N}\sum_{l=1}^{N} \bar{k}_{S,k,l}$
$\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}} \bar{\alpha}_{S,i,j} C(X_i, Y_j)$	Adaptation to the genotype frequency distribution of the interacting species	$(1/N^2)\sum_{k=1}^{N}\sum_{l=1}^{N} \bar{a}_{k,k,l} - (1/N^3)\sum_{k=1}^{N}\sum_{l=1}^{N}\sum_{m=1}^{N} \bar{a}_{k,l,m}$
$\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}} \overline{Y}_j C(X_i, \alpha_{S,i,j})$	Adaptation to the fitness consequences of species interactions; a form of Thompson's "selection mosaic"	$(1/N^2)\sum_{k=1}^{N}\sum_{l=1}^{N} \bar{a}_{k,l,k} - (1/N^3)\sum_{k=1}^{N}\sum_{l=1}^{N}\sum_{m=1}^{N} \bar{a}_{k,l,m}$
$\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}} C(X_i, \alpha_{S,i,j}, Y_j)$	Joint adaptation to the fitness consequences of species interactions and the genotype frequency distribution of the interacting species; a form of Thompson's "selection mosaic"	$(1/N)\sum_{k=1}^{N} \bar{a}_{k,k,k} - (1/N^2)\sum_{k=1}^{N}\sum_{l=1}^{N}(\bar{a}_{k,l,l} + \bar{a}_{l,l,k} + \bar{a}_{l,k,l}) + (2/N^3)\sum_{k=1}^{N}\sum_{l=1}^{N}\sum_{m=1}^{N} \bar{a}_{k,l,m}$

Note: The parameter $\bar{k}_{i,j}$ is the expected fitness of a focal species individual from habitat i when grown in habitat j, and the parameter $\bar{a}_{i,j,k}$ is the expected fitness consequence of species interactions between focal species individuals from habitat i, in encounters with nonfocal species individuals from habitat j, when the interaction occurs in habitat k. Unless more than two populations are studied, the term $\sum_{i=1}^{n_S}\sum_{j=1}^{n_{S^*}} C(X_i, \alpha_{S,i,j}, Y_j)$ will always yield an estimate of 0, irrespective of its true value in the metapopulation as a whole.

and common-garden designs. Our results show that the two approaches are likely to yield different results, with the difference largely determined by the extent to which the fitness consequences of interactions between species are determined by environmental variability rather than simply by host and parasite genetics. If environmental variation is substantial, common-garden studies estimate only the portion of local adaptation attributable to spatial covariation between host and parasite genotype frequencies, and thus they may provide inaccurate estimates of local adaptation as a whole. In contrast, if environmental variation is insignificant, local adaptation is driven entirely by the spatial covariation between host and parasite genotype frequencies, and common-garden studies could, in principle, yield results identical to those of transplant studies. Even if this is the case, however, our results show that unless the common-garden study faithfully replicates the average natural environment, the two types of studies will yield different results. This may explain why Laine (2007) found different results in transplant and common-garden studies.

Although empirical studies exist that demonstrate significant environmental variation for the fitness consequences of interspecific interactions (Lively et al. 1995; Dybdahl and Krist 2004; Osnas and Lively 2004; Johnson and Agrawal 2005; Mitchell et al. 2005; Heath and Tiffin 2007), it is unknown how much this variation contributes to local adaptation. Consequently, the extent to which common-garden studies accurately reflect levels of total local adaptation in the field cannot be determined a priori. Thus, common-garden studies should not be used as the sole means of estimating local adaptation if the goal is to understand overall levels of local adaptation. Common-garden studies, do, however, provide an effective means of isolating the spatial covariation between host and parasite genotype frequencies, which, at least in some cases, is a reflection of the extent to which parasites track their hosts over evolutionary time (Dybdahl and Lively 1998; Lively and Dybdahl 2000; Dybdahl and Storfer 2003). Because such tracking has long been thought to be an important prerequisite for the Red Queen hypothesis for the evolution of sex (Otto and Michalakis 1998; Lively et al. 2004), common-garden studies can be used quite effectively in this context.

Our results also lead to a new methodology that allows components of local adaptation to be estimated, providing novel opportunities for evaluating the extent to which spatial variation in the fitness consequences of interactions contributes to local adaptation. Specifically, our results show that this methodology can be used to partition local adaptation into three components: (1) adaptation to the environment, reflecting the contribution of G × E interactions; (2) adaptation to the genotype frequency distribution of the interacting species, reflecting the contribution of G × G interactions; and (3) joint adaptation to the fitness consequences of interactions and the genotype frequency distribution of the interacting species, reflecting the contribution of G × G × E interactions (app. D). In those systems where the fitness contributions of species interactions (α) and the abiotic environment (κ) can be disentangled, local adaptation can be partitioned further, with G × E interactions split into interactions between

focal species genotype and abiotic environment (G × κ) and interactions between focal species genotype and the fitness consequences of species interactions (G × α; app. C; table 2).

The ability to estimate the various components of local adaptation independently provides new opportunities to gain insight into the relative importance of various forces thought to drive local adaptation in natural populations. For instance, the methodology we outline can be used to evaluate the relative importance of adaptation to the biotic versus abiotic environment. Similarly, it can be used to evaluate the importance of the selection mosaics (G × E and G × G × E) that define Thompson's geographic mosaic theory relative to the G × G interactions on which the bulk of traditional coevolutionary theory is predicated (reviewed in Thompson 2005). Finally, the new methodology makes it possible to evaluate the importance of environmental variation for various fitness components. For instance, it should be possible to use the new methodology to assess whether environmental variation is greater for a fitness component such as infection success relative to other components such as virulence. This information is critical for developing predictive models of coevolution.

Although we have developed our new methodology for the case of only two factors—a single interacting species and a single dimension of the abiotic environment—extension to an arbitrary number of factors is straightforward from a theoretical perspective. Consequently, it should, in principle, be possible to use our methods to address fundamental questions such as the proportion of local adaptation explained by different members of a biological community or by different dimensions of the abiotic environment. An important caveat, however, is that all relevant components of local adaptation can be estimated only if the number of populations studied is equal to or greater than the number of factors thought to mold local adaptation and to potentially be statistically associated. If these methodological hurdles can be overcome, it will be possible to greatly increase our understanding of the key abiotic and biotic factors driving local adaptation in natural populations.

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