

Environmental Effects on the Expression of Quantitative Trait Loci and Implications for Phenotypic Evolution

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Organisms in natural populations experience environmental heterogeneity over a range of temporal and spatial scales, and this heterogeneity has significant evolutionary implications. By affecting patterns of selection and the expression of genetic variation, environmental heterogeneity can play an important role in determining the evolutionary dynamics of phenotypic traits and the maintenance of genetic variation. Although mapping quantitative trait loci (the loci that underlie continuously varying quantitative traits) has a long history in agricultural and applied studies, the technique has only recently been applied to evolutionary studies. This application has made it possible to identify the specific loci underlying trait variation in different environments, to measure environmental variation in natural selection on those loci, and to test assumptions of models regarding the maintenance of genetic variation under environmentally heterogeneous selection. Here we review recent studies that have examined interactions between quantitative trait loci and ecologically relevant environments to address evolutionary questions.

Keywords: antagonistic pleiotropy across environments, evolutionary constraints, locus-specific selection, QTL mapping, QTL–environment interactions

Organisms in natural populations experience heterogeneous environments in which local conditions vary over both temporal and spatial scales. These heterogeneous environments exert correspondingly heterogeneous natural selection on quantitative traits, and different traits may be targeted in different environments. The magnitude of evolutionary response to selection in heterogeneous environments depends on the trait heritability in each environment, as well as on the genetic covariances among traits within and across environments. The evolution of multiple quantitative traits is formalized in Lande's equation (1979),

$$\Delta\bar{z} = G\beta,$$

in which β is the vector of selection gradients representing direct selection on each trait, G is the matrix of trait variances and covariances, and $\Delta\bar{z}$ is the vector of changes in trait means in the next generation. However, G may not always be constant, because measures of trait variances and covariances depend strongly on the conditions in which these estimates are made (Falconer and Mackay 1996), indicating that variation at some loci may be available to selection only in some environments. Moreover, although Lande's equation enables statistical predictions of phenotypic evolution, it provides no insight as to the identity of loci targeted by selection.

It therefore remains unclear how selection at specific loci varies across environments and how the expression of allelic variation at individual loci differs across environments. Such information is needed if we are to understand the genetic mechanisms underlying adaptive evolution in heterogeneous natural conditions.

The extent of environmental heterogeneity is important not only to predicting phenotypic evolution but also to understanding the genetic parameters of population, such as the equilibrium levels of genetic variation in natural populations. One hypothesis states that the introduction of new alleles through mutation will counterbalance the erosion of genetic variation resulting from purifying selection (Hedrick 1986, Barton and Turelli 1989). However, estimates of selection and mutation rates in some species suggest that observed levels of genetic variation are higher than can be explained solely by the balance of mutation and selection (Houle 1992, Mackay et al. 1995, Nuzhdin et al. 1995). A

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second hypothesis, which is not necessarily inconsistent with the first, suggests that environmental heterogeneity may act to preserve genetic variation (Felsenstein 1976, Gillespie and Turelli 1989, Prout and Savolainen 1996). More specifically, the complexity and the severity of some physical environments are expected to impose performance trade-offs such that genotypes differ in rank across environments and no single genotype has the highest fitness in all environments (figure 1a). The potential for environmental heterogeneity to maintain genetic variation depends on additional factors, including dominance relationships (i.e., the fitness of heterozygous versus homozygous individuals) (Felsenstein 1976). The scale of environmental heterogeneity and the frequency with which alternative environments are encountered are also relevant to the maintenance of genetic variation. The hypothesis that environmental heterogeneity may preserve genetic variation has been the subject of intensive theoretical and empirical study because of the central importance of heritability to a trait's evolutionary potential, but evidence for trade-offs is rare (Nuzhdin et al. 1995, Gurganus et al. 1998, Vieira et al. 2000, Weinig et al. 2003a).

Performance trade-offs have most commonly been tested at the genotypic level. That is, phenotypic variation (or variation in fitness) expressed by a given genotype across environments is depicted as the genotypic "norm of reaction" (figure 1). Significant differences among reaction norms may arise when the genotypic rank order shifts across environments (figure 1a) or when genotypes differ in the magnitude of their response to different environments (figure 1b). The relative contribution of shifts in magnitude and shifts in rank can be evaluated in part by estimating the across-environment genetic correlation, r_{GE} which quantifies changes

in the trait value across environments (Falconer and Mackay 1996). When r_{GE} is less than zero for a component of fitness, shifts in rank order and performance trade-offs exist, which may act to protect genetic polymorphisms (Via and Lande 1987). Studies using quantitative genetic approaches to characterize across-environment genetic correlations at the genotypic level have typically not shown a significantly negative value for r_{GE} . In contrast, more recent studies at the level of individual loci have shown that many loci have antagonistic additive effects across environments (Nuzhdin et al. 1995, Gurganus et al. 1998, Vieira et al. 2000, Weinig et al. 2003a). The discrepancy between studies at the genotypic and locus-specific levels probably arises from the fact that genetic correlations estimate the sum phenotypic effect of all contributing loci and, therefore, can conceal considerable heterogeneity in the effects of individual loci. The difference in resolution between these studies underscores the importance of characterizing the loci that underlie quantitative traits and environment-specific genetic expression.

The expression of quantitative variation and of genotype-environment interactions is also relevant to current debates within evolutionary ecology regarding the evolution of adaptive strategies. For instance, if performance trade-offs exist between environments, a single genotype with the highest fitness may evolve within each of two alternative environments. This adaptive strategy is commonly referred to as *ecological specialization*. However, the evolution of specialization depends on several additional features of the population, including the frequency of alternative habitats and the scale of gene flow relative to the patterns of dispersal into these habitats. If gene flow is coarse-grained with respect to environmental heterogeneity (i.e., if individuals are likely to experience only one microsite

in their lifetime and their progeny are likely to disperse into similar conditions), then selection is predictable and favors specialization (Levins 1963, Sultan and Spencer 2002). In fine-grained environments where the likelihood of dispersal into alternative environments is high, genotypes capable of modifying their phenotype in response to local conditions will experience a fitness advantage. Such genotypes have long been described as *phenotypically plastic* (Bradshaw 1965).

A primary focus of evolutionary biologists is the effort to understand the genetic mechanisms of adaptation, which includes identifying the loci that contribute to plasticity or ecological specialization. By definition, this task involves characterizing the degree to which individual loci differ in their phenotypic effects across environments. This topic is equally relevant for conservation biologists, because an understanding of site-specific selection histories and mechanisms of

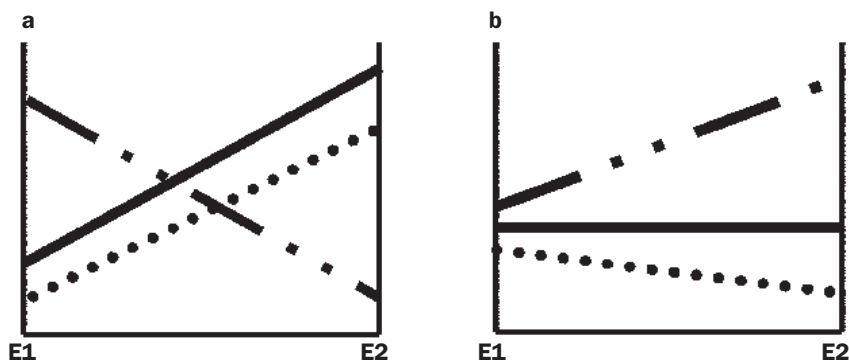


Figure 1. Norms of reaction, or responses of alternative genotypes to alternative environments. The lines represent different genotypes encountering alternative environments, E1 and E2. Both panels show significant genotype-environment interaction, which results either from shifts in genotypic rank order (panel a) or genotypic differences in the magnitude of environmental response (panel b). Panel a also illustrates the environmental heterogeneity model for the maintenance of genetic variation, whereby genetic variation is preserved when different phenotypes are favored in each of two or more different environments, and the ability to express an adaptive phenotype in one environment is negatively genetically correlated with the ability to express the alternative phenotype favored in the second environment.

adaptation is helpful when reintroducing individuals to an extirpated or threatened population.

Understanding how specific genes interact with the environment is critical to determining gene function and evolution. This topic has received recent attention in the plant community through the “Arabidopsis 2010” project, which aims to characterize the function of the 30,000 predicted genes in *Arabidopsis thaliana*. The genetic characterization of development (i.e., the identification of loci contributing to a developmental pattern or process) has traditionally proceeded using experimental genetic tools, such as induced loss-of-function mutants or transgenic organisms that over-express gene products of interest. From the perspective of a decade ago, it would appear that developmental biologists have rapidly characterized the genetic basis of many developmental paths. However, considering the number of genes currently annotated relative to the number of genes predicted on the basis of open reading frames, it is clear that only a small fraction of genes have been characterized. One possible reason for the relatively small proportion of genes that have been described is that mutational screens are biased toward genes of large qualitative effect because of the relative ease with which such mutations are visibly identifiable and tracked in segregation analyses (Mackay 2001). This bias might contribute to the large number of predicted, but as yet unknown, genes. The controlled growth conditions commonly used in mutant screens may also mask the effects of some induced mutations; at the quantitative-genetic level, genotype-by-environment variance is predicted to be larger in harsher environments (Khondrashov and Houle 1994), suggesting that gene function may be clear only in complex, ecologically relevant settings. Classic molecular-genetic studies of gene function also fail to provide information regarding selection (i.e., the history of selection at a given gene and the contribution to population differentiation by the past or current forces of selection at that locus). Traditional mutant screens and the use of controlled growth environments are fundamental to determining which loci are necessary to the expression of a normal phenotype. Yet characterizing gene function and evolution requires an understanding of how these loci affect variation in natural populations, which in turn requires the integration of genetic resources into ecological studies.

One means to investigate the evolutionary questions raised above is quantitative trait locus (QTL) mapping (i.e., mapping the loci that underlie continuously varying quantitative traits), followed by investigating environmental interactions in segregating progenies. Because of space limitations, it is impossible to review here all of the relevant research, particularly in the agricultural literature. Our primary aim is to examine recent empirical studies from the evolutionary literature that have successfully addressed questions of evolutionary dynamics. As an introduction to these studies, we first give a brief overview of QTL mapping techniques (for a more thorough review of QTL mapping and cloning, see Mackay [2001] and Remington and colleagues [2001]).

Estimating locus-specific effects on the evolution of quantitative traits

Phenotypic variation in quantitative traits arises from the segregation of alleles at multiple loci, with each allele having a relatively small effect. As described above, evolutionary ecologists have relied primarily on quantitative-genetic approaches to predict the evolution of quantitative traits (Falconer and Mackay 1996). Although these approaches have proved useful, the identity of the individual loci underlying genotypic differences remains largely unclear. By combining genetic tools and field experiments in relevant ecological settings, it is possible to test whether the expression of variation at specific QTLs is environment-specific and whether selection in different environments acts on different loci. For instance, segregating progenies have been used as tools for mapping the genes that underlie agronomically desirable traits in cultivars (Doebley 1992) and, more recently, for gene discovery by molecular biologists (Lister and Dean 1993, Alonso-Blanco et al. 1998, Wilson et al. 2001). These tools are also of increasing interest to plant evolutionary biologists interested in diverse questions, including life-history strategies (Mitchell-Olds 1996, Ungerer et al. 2002, Weinig et al. 2003a), mating systems, local adaptation and speciation (Bradshaw et al. 1995, Chul and Rieseberg 2001, Fishman et al. 2002), and the evolution of resistance to herbivore damage (Kliebenstein et al. 2002).

Recombinant inbred lines (RILs) are one type of segregating progeny. These lines are typically generated by crossing two homozygous parents that differ phenotypically in the trait of interest (although even individuals with similar trait means will probably segregate variation that is masked by the presence of alleles with positive and negative effects at contributing loci). The resulting F_1 generation is mated *inter se* (among themselves) and the F_2 sublines are bred to homozygosity by producing seed via self-fertilization for eight or more successive generations. After the selfing generations, the resulting progeny are again homozygous at most loci, but they possess alleles from both parents and therefore show a greater range of genetic variation than either parent. The RILs also express a greater range of phenotypic variation than either of the two parental populations from which they are derived, enhancing the potential to estimate phenotypic selection.

Because many genes underlie the expression of quantitative traits, it is impossible to determine the effect of a given QTL using segregation analysis. Rather, marker loci that are polymorphic between the two parents can be used to develop a linkage map that distinguishes specific genomic regions affecting a given trait. Variation at any given marker locus will be associated with variation in the focal trait, if the marker locus is physically linked to the genetic locus. The most common mapping analysis, composite interval mapping, evaluates the null hypothesis that the intervening region between any two marker loci possesses a QTL, while statistically controlling for the effects of loci segregating elsewhere in the genome (Basten et al. 1994). Tests for significant QTLs thus compare the effects of many marker loci simultaneously and

provide a genomewide screen for contributing loci. By definition, QTL mapping involves a large number of independent tests (i.e., individual tests of association between a marker interval and the quantitative trait). The significance threshold (commonly $P = 0.05$ for a single marker) must be adjusted downward to account for the large number of tests. Permutation analyses are used to make this adjustment (Doerge and Churchill 1996). One additional, and perhaps obvious, caveat is that a QTL is detectable only when the two parents used to construct the mapping population have different alleles at that QTL. Moreover, the number of QTLs that can be detected and the estimated size of their effects are highly sensitive to sample size (i.e., the number of RILs or of independent backcross or F_2 lines) (Beavis 1994), in part because distinguishing each of two linked QTLs depends on recombination between the loci, and because the likelihood of detecting rare recombination events increases with the number of lines sampled. The number of significant QTLs therefore estimates the minimum number of contributing loci.

While some QTLs affect the quantitative trait regardless of environmental background, others may have environment-specific phenotypic expression. Environment-specific effects, which are detected as QTL–environment interactions in ANOVA (analysis of variance; Fry et al. 1998) or in maximum-likelihood analyses, can arise from changes in either the direction or the magnitude of a QTL's effect across environments (as in figures 1a and 1b, respectively, for genotypic values). Recombinant inbred lines facilitate tests of environmental interactions because siblings within an RIL are genetically identical, and genotypes can therefore be grown and compared across diverse experimental settings. Such replication is not possible in other mapping populations, such as backcross or F_2 progenies, unless the organism can be cloned. QTL–environment interactions have received much recent attention because of their relevance to the range of evolutionary hypotheses discussed above. These interactions are relevant to the questions of how far selection at individual loci differs across environments, of whether the expression of genetic variation at specific loci differs across environments, and of whether spatial or temporal heterogeneity may act to preserve variation.

QTL mapping is linkage mapping based on molecular markers, and the presence of significant QTLs therefore refers to genomic regions rather than genetic loci. By localizing a QTL to a genomic region, mapping is one first step toward genetic characterization of a quantitative trait. Genetic characterization may be unimportant for some evolutionary studies, such as those testing for differences and similarities in the loci targeted by selection in different environments, as well as for applied studies seeking to identify genomic regions affecting agronomically desirable traits and to conduct marker-based selection for crop improvement. On the other hand, genetic characterization is important to some evolutionary questions, such as how to distinguish pleiotropy from physical linkage and how to identify the genetic basis of evolutionary constraints. Identifying the genetic locus within a

QTL also provides evolutionary ecologists with the means to determine which genetic loci contribute to phenotypic evolution in natural populations and provides molecular biologists with a tool for gene discovery beyond the traditional mutagenic ones. The identification of underlying genetic loci will largely be limited to genetic model systems, that is, organisms that have been the subject of intensive developmental studies and therefore possess a range of “positional candidate loci” (loci of known function that lie in the physical region bounded by the QTL). Even in genetic models, identifying the genetic locus is complicated by the fact that a QTL may span several hundred genetic loci (Ungerer et al. 2002). Differentiating among the large number of positional candidate loci is a time-consuming process, although there are several recent examples of successful QTL cloning (Remington et al. 2001).

Empirical analysis of QTL–environment interactions in simulated growth settings

In a series of groundbreaking experiments, Mackay and colleagues (1995) have had excellent success over the past two decades both in testing several of the evolutionary hypotheses described above and in beginning to characterize the genes that underlie quantitative traits in *Drosophila melanogaster* using simulated natural environments. In natural settings, *D. melanogaster* may experience variation in temperature and ethanol levels because they rely on fruit in varying stages of senescence as a food source. Larvae of this species are likely to encounter variable fly densities as a consequence of varying oviposition patterns. Mackay and colleagues have given particular attention to the role that heterogeneity in diverse settings plays in maintaining genetic variation. As discussed above, models for the maintenance of genetic variation by spatial heterogeneity require at a minimum that selection favors alternative alleles across spatial microsites (Hedrick 1986); such shifts in allelic rank order have been referred to as “trade-offs” or “antagonistic pleiotropy across environments” (Fry et al. 1998). There is variable evidence for this type of pleiotropy from studies in *Drosophila*, depending on the environments and traits studied. Fry and colleagues (1998) found that different QTLs determined reproductive performance in RILs of *Drosophila* that were raised across combinations of temperature and ethanol environments, but there was no evidence of across-environment antagonistic pleiotropy. A second study in *Drosophila* found that a single QTL had antagonistic effects on sensory bristle number in flies' growth across temperature settings (Gurganus et al. 1998); the parental allele that increased bristle number in males at 18 degrees Celsius ($^{\circ}\text{C}$) decreased female bristle number at 29 $^{\circ}\text{C}$ relative to the alternative parental allele. Because only 1 marker out of 19 that affected bristle number in at least one temperature environment exhibited this mode of QTL–environment interaction (Gurganus et al. 1998), antagonistic pleiotropy does not appear to be a mechanism for maintaining variation in these traits.

Additional studies with *Drosophila*, testing for longevity QTLs, have detected antagonistic pleiotropy across larval densities and temperatures. Alleles at QTLs that increased female life span at high larval densities reduced life span at low densities (Leips and Mackay 2000), consistent with the hypothesis that environmental heterogeneity might maintain genetic variation. A similar pattern of antagonistic pleiotropy was observed at 10 out of 17 markers across temperature and starvation treatments (Vieira et al. 2000). Despite the presence of many loci with antagonistic effects, it is worth noting that genetic correlations estimated across environments and across sexes were not significantly negative, underscoring the fact that the sum effects of all contributing loci can conceal significant heterogeneity in the behavior of individual loci. Interestingly, antagonistic pleiotropy at the QTL level tends to appear more commonly in traits closely related to fitness, rather than phenotypic traits such as morphology. Antagonistic pleiotropy across environments has been found in a few crop studies, again in traits related to fitness (i.e., QTLs for pollen competitive success in corn and for seed oil and protein content in soybean across planting years; Brummer et al. 1997, Sari-Gorla et al. 1997). Although the number of studies is very small, the pattern is consistent with what would be expected as a result of variable selection (Falconer and Mackay 1996). A further possibility is that ongoing selection will favor modifier alleles that reduce trade-offs associated with antagonistic pleiotropy.

Leips and Mackay (2000), Vieira and colleagues (2000), and Nuzhdin and colleagues (1995) also found evidence of “sexually antagonistic pleiotropy” (i.e., reversals of allelic effect between male and female flies), in which alleles that increased female life span acted to reduce male life span. These findings constitute an extension of Williams’s (1957) original hypothesis regarding the evolution and genetic origin of senescence, in which alleles that have favorable effects on survival or fecundity early in life have detrimental effects later in life (and vice versa), and the balance of selection across life-history stages, or across sexes, protects polymorphisms at longevity loci (Vieira et al. 2000). Moreover, because males and females are present in any viable fly population, sexually antagonistic pleiotropy acts to maintain genetic variation, in much the same way that pleiotropy acts to maintain variation across heterogeneous environments.

Empirical studies estimating QTLs in ecologically relevant natural settings

The use of genetic models in evolutionary studies has been criticized on the basis that model organisms have uninteresting or unknown ecology. However, these organisms often derive from diverse natural settings and possess unique natural histories amenable to field study. *Arabidopsis thaliana*, for instance, exhibits variable germination patterns across its geographic range, presumably as a bet-hedging strategy against environmental heterogeneity (Venable 1985). Plants within a single population may germinate in fall or spring, and subsequently flower and complete their life cycle either the

following spring (for those plants with fall germination) or in the same season in which they germinated (Napp-Zinn 1985). Most North American populations have been characterized as winter annuals (Nordborg and Bergelson 1999), in which all seedlings germinate in fall, overwinter as rosettes, and flower and set seed in the spring (figure 2a). However, plants in some populations at northern latitudes in North America complete their life cycle in a single season: Individuals germinate, flower, and set seed in approximately four months, either in spring or fall (figures 2b, 2c). These two alternative life-history strategies should be selectively advantageous when the likelihood of overwinter mortality is severe. Where winters are predictably mild and summers harsh (i.e., sites at southern latitudes), the life histories of winter annuals are selectively advantageous, because these plants attain a large size at reproduction. *Arabidopsis* is therefore well suited ecologically to test how selection at specific QTLs differs across environments, as well as how the expression of variation at specific loci differs across seasonal environments.

A recent field study mapped QTLs for the expression of life-history and architectural traits, as well as components of fitness, in natural seasonal environments experienced by

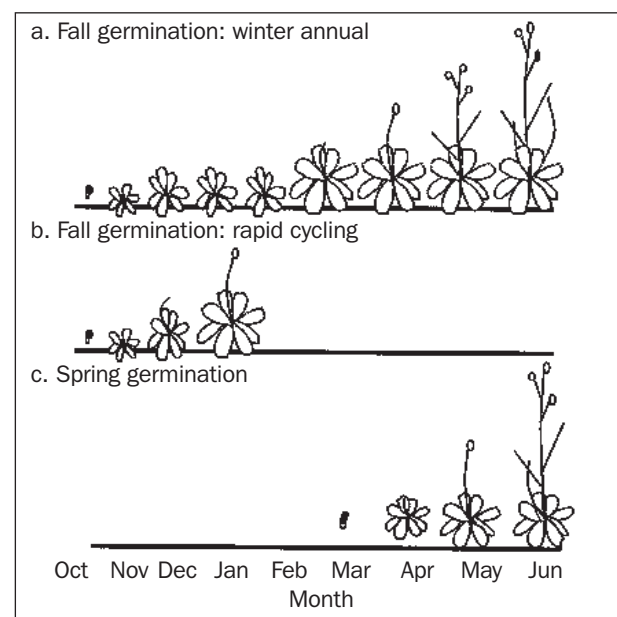


Figure 2. Life-history strategies of *Arabidopsis thaliana*. Most North American populations have been characterized as winter annuals, but alternative life histories evolve depending on the selective pressures encountered by a given population. (a) At the southern edge of *A. thaliana*’s range distribution, plants are commonly winter annuals, which germinate in the fall, overwinter as rosettes, and then flower and set seed the following spring. In northern locations, some plants may be winter annuals, but others have a rapid cycling strategy in which seeds (b) germinate and complete their life cycle before winter or (c) germinate, flower, and set seed in a single spring, before the onset of hot summer temperatures.

A. thaliana, with the aim of evaluating ecological hypotheses regarding the evolution of life histories and the genetic basis of adaptive phenotypic evolution. In this experiment, RILs of *A. thaliana*, developed from a cross between the Landsberg *erecta* (*Ler*) and Columbia ecotypes, were planted into natural seasonal settings (fall and spring plantings in Rhode Island and North Carolina). Fitness was estimated both in terms of survivorship to reproduction and in terms of fruit production, which is strongly correlated with seed number (Westerman and Lawrence 1970). Consistent with hypotheses regarding the evolution of life histories in different regions, spring germinants attained higher fitness at the northern Rhode Island site, while winter annuals had higher fitness at the southern North Carolina site (Weinig et al. 2003a). RILs differed significantly in fitness and in the expression of diverse life-history traits within any given environment, but analysis of the variance components revealed that genetic variation for all traits was explained primarily by interactions of RIL with specific combinations of site and season (a significant RIL–season–site interaction) (Weinig et al. 2002, 2003a). The significance of the three-way interaction suggests either that different genes determine a trait's phenotypic expression across the seasonal environments or that alleles at contributing loci vary in their environmental sensitivity.

Consistent with the quantitative genetic analyses, most traits showed significant QTL–season or QTL–site interactions. Most noteworthy is that the *ERECTA* marker, an induced phenotypic mutation in *Ler*, was associated with large phenotypic effects on flowering time in some, but not all, environments (Weinig et al. 2002). If *ERECTA* rather than a linked gene underlies the QTL, then the environmental interaction indicates that even a major developmental mutation may have no phenotypic effect in certain natural environments and thus may be sheltered from natural selection in those environments.

QTL–environment interactions for fecundity and survivorship may be viewed as evidence for heterogeneous selection, because components of fitness contribute directly to genetic representation in successive generations. In the field study described above, selection did in fact act at different loci both in the different seasonal environments and across the geographic regions (Weinig et al. 2003a). In all but one setting (fall in North Carolina), significant QTLs for fecundity were detected, and significant QTL–environment interactions were observed. Thus, different loci determine genetic representation in successive generations of different germination cohorts. The presence of QTL–season interactions, like that of QTL–site interactions, demonstrates that allelic variation at some loci is available to selection only in certain environments; it also indicates both that phenotypic evolution may be relatively slowed in some populations, and that phenotypic evolution may occur by way of alternative genetic pathways even when the initial populations are genetically identical. The hypothesis that equilibrium levels of genetic variation are higher in populations with dimorphic life histories than in those with a fixed fall annual life history remains to be tested.

Studies in ecologically relevant settings may also be used to test for the presence of evolutionary constraints, including antagonistic pleiotropy or genetically based trade-offs. In the *Arabidopsis* experiment described above, QTL–season interactions were also expressed as life-history trade-offs within and across seasonal settings. For instance, the allele from the Columbia parent at two QTLs had positive effects on overwinter survival but detrimental effects on growth rate in the fall seasonal cohort in North Carolina. Interestingly, this trade-off was absent at the Rhode Island site. These QTLs may therefore be viewed as underlying the expression not only of negative correlations and life-history trade-offs that act as evolutionary constraints but also of environment-specific genetic trade-offs. Different environmental conditions (e.g., sites) might yield different correlated responses to selection, because the effects of individual loci on the expression of life-history traits vary according to environment. Given that selection acts to increase growth rate in at least some years, the antagonistic fitness effects of alleles at this locus may also act to preserve genetic variation.

Combined field and controlled-environment studies

Studies combining information from controlled and natural settings can provide insights into the ecological relevance of known developmental paths. Functional studies in *Arabidopsis* have shown that long-day conditions activate a developmental pathway (the photoperiod pathway) that is the primary determinant of flowering time, and considerable effort has been devoted to the genetic characterization of this pathway (Koornneef et al. 1998, Weigel 1998). However, genes identified by studies of mutant or transgenic plants in controlled experimental settings may not be the primary loci controlling variation in flowering time in natural populations.

Comparisons of QTLs for flowering time that were detected in controlled photoperiod settings and in natural seasonal field settings provide a means to test the ecological relevance of photoperiod loci. In combination with the field study in *Arabidopsis* discussed above, replicate plants from the same RILs were grown in controlled phytotron conditions under short (10-hour) and long (14-hour) photoperiod conditions designed to reflect the day lengths experienced by plants in natural seasonal populations. Plants in fall germination cohorts experience short days at the time of flowering, whereas spring cohorts experience long days. As expected, some QTLs detected under long days in the phytotron also affected flowering time in the spring seasonal cohort, but not in the fall cohort, supporting the hypothesis that genetic variation in the photoperiod developmental pathway is an important determinant of flowering time in natural settings (Weinig et al. 2002). A similar pattern was seen in comparisons of QTLs for flowering time in the fall seasonal cohort and in plants exposed to short days in the phytotron. However, for most QTLs, the effects of environmental interactions on flowering time were highly significant across the phytotron and field growth environments. Several QTLs detected in the controlled environments (short or long days) had little effect on

flowering date in the associated natural seasonal cohort. Expression of allelic variation at these photoperiod-related loci was apparently overridden by some other environmental factor in the field. Thus, although the photoperiod pathway is known to override the other known flowering-time pathways, the observed QTL–environment interactions across controlled and field settings indicate that variation at some loci in this developmental path are comparatively unimportant to the expression of life-history traits and hence to fitness in natural settings.

QTL–environment interactions also resulted from the difference between natural and controlled settings: A large number of QTLs for flowering time were detected in the field but not in the phytotron. Although the genetic and environmental mechanisms that mediate flowering in the field remain unclear, the large number of QTLs specific to the field demonstrates that the genetic characterization of developmental paths under controlled conditions provides only an incomplete picture of the ecologically relevant developmental loci.

QTL–QTL epistasis and evolutionary potential

A few studies have detected evidence of QTL–QTL epistasis, in which the phenotypic effect of variation at one marker locus is reversed depending on the allele present at a second interacting locus (Shook and Johnson 1999, Leips and Mackay 2000, Weinig et al. 2003a). Such allelic interactions are analogous to antagonistic pleiotropy across environments, in that the genetic background may behave in a similar manner to modify the availability of genetic variation to selection and actively preserve genetic variation. Detection of QTL–QTL epistasis is strongly limited by the number of lines in the mapping population, as tests of all marker interactions (often $n > 10,000$; Borevitz et al. 2002) rapidly deplete power. Better characterization of such interactions awaits studies with larger mapping populations.

The presence of QTL–QTL interactions is relevant to questions regarding mechanisms of adaptation. In self-fertilizing species such as *A. thaliana*, natural selection discriminates among families or lineages, thus acting on epistatic variance. Moreover, when dispersal is limited, populations may be genetically structured such that an individual's performance depends not only on its own fitness but also on that of its neighbors (Wright 1931, Goodnight 1985, Wade and Goodnight 1998). In this scenario, allelic combinations are preserved across generations, such that rare outcrossing events and the attendant production of novel, favorable allelic combinations may accelerate evolutionary responses to selection. Although there is considerable debate as to the contribution of epistasis to phenotypic evolution in natural populations (Coyne et al. 1997, Wade and Goodnight 1998), epistatic variance could accelerate progress in breeding programs. Breeders typically select at levels higher than the individual, planting monocultures of a single genotype and perpetuating those that have the highest stand yield. Studies of QTL–QTL epistasis may help to determine the genetic

potential for evolutionary response to such multilevel selection, both artificial and natural.

Limitations of sampling in QTL mapping studies

The QTL mapping studies in *Drosophila*, corn, and soy described above are consistent with models regarding the maintenance of genetic variation by spatial heterogeneity. However, attention must be given to environmental and genetic sampling methods, and additional lines of evidence are necessary to determine the relevance of these mechanisms in natural populations. Although QTL mapping studies have detected reversals in the direction of allelic effects across environments, it is worth noting that the expression of antagonistic pleiotropy is likely to depend on the severity of the sampling conditions, as more benign conditions may reduce genotype–environment interactions (Khondrashov and Houle 1994). That said, ecological relevance is desirable, and sampling conditions should approximate the range of natural (or relevant agricultural or growth) settings. Finally, spatial heterogeneity protects genetic polymorphisms only if allelic effects on the focal trait reverse ranking across environments and if heterozygotes are less sensitive than homozygotes to environmental variation (Gillespie and Turelli 1989). As described above, RILs allow testing of QTL–environment interactions, as lines can be replicated across environments. However, researchers must use segregating progenies other than RILs to test the relative effects of heterozygous versus homozygous classes.

Genetic sampling is also relevant to almost all evolutionary questions that are addressed using segregating progenies. As described above, a QTL is detectable only when the parents from which the mapping population is developed possess alternative alleles, and the number of QTLs mapped in any two individuals will provide only a sample of the total number of loci affecting the trait. The magnitude of this shortcoming depends on the distribution of allelic effects in the parental population. In principle, if many alleles segregate at all contributing loci, then mapping any pair of individuals drawn at random from the population should give similar results, provided that the experimental design enables detection of QTLs whose effects are small. However, if only two alleles segregate at a locus and one is rare, the likelihood of capturing alternative alleles in any two individuals is low, leading to a variable number of QTLs and to potentially variable estimates of effect size among maps. One study mapped a similar set of phenotypic traits under identical, replicable growth conditions in two mapping populations of *A. thaliana* (Ungerer et al. 2002). Because the molecular markers were largely nonoverlapping between the two mapping populations, only coarse comparisons of significant QTLs could be made. A QTL near the induced *ERECTA* mutation was a significant determinant of several traits in both mapping populations, but QTLs unique to each mapping population were also common.

Conclusions

Studies mapping QTLs for diverse phenotypic traits are proceeding in a range of species. Many of the evolutionary questions that can be addressed in these studies overlap between model and nonmodel systems, including questions related to the genetic basis of adaptation, the maintenance of genetic variation, the genetic origin of evolutionary constraints, and the evolution of adaptive strategies. Models have the advantage that genetic tools are typically easier to develop; for instance, the presence of two genomic sequences in *Arabidopsis* (the *Ler* and Columbia accessions) facilitates the identification of likely marker polymorphisms and the development of a linkage map. Such systems also have the advantage that positional candidates of known function can be identified within a QTL; in the absence of such information, the genetic loci underlying a QTL are more challenging to characterize. Models must be chosen carefully, with consideration of their ecology and of their consistency with the evolutionary hypotheses to be tested. *Arabidopsis* is proving to be an excellent system in which to investigate selection in seasonal settings, as well as the genetic loci targeted by disease (e.g., Bergelson et al. 2001) and by herbivory (e.g., Mitchell-Olds 2001, Weinig et al. 2003b). Mapping tools are rapidly being developed in diverse species, providing the opportunity to investigate evolutionary dynamics in a greater range of ecological settings.

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