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The Adaptive Evolution of Plasticity: Phytochrome-Mediated Shade Avoidance Responses[1]

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SYNOPSIS. Many plants display a characteristic suite of developmental ''shade avoidance'' responses, such as stem elongation and accelerated reproduction, to the low ratio of red to far-red wavelengths (R:FR) reflected or transmitted from green vegetation. This R:FR cue of crowding and vegetation shade is perceived by the phytochrome family of photoreceptors. Phytochrome-mediated responses provide an ideal system for investigating the adaptive evolution of phenotypic plasticity in natural environments. The molecular and developmental mechanisms underlying shade avoidance responses are well studied, and testable ecological hypotheses exist for their adaptive significance. Experimental manipulation of phenotypes demonstrates that shade avoidance responses may be adaptive, resulting in phenotypes with high relative fitness in the environments that induce those phenotypes. The adaptive value of shade avoidance depends upon the competitive environment, resource availability, and the reliability of the R:FR cue for predicting the selective environment experienced by an induced phenotype. Comparative studies and a reciprocal transplant experiment with *Impatiens capensis* provide evidence of adaptive divergence in shade avoidance responses between woodland and clearing habitats, which may result from population differences in the frequency of selection on shade avoidance traits, as well as differences in the reliability of the R:FR cue. Recent rapid progress in elucidating phytochrome signaling pathways in the genetic model *Arabidopsis thaliana* and other species now provides the opportunity for studying how selection on shade avoidance traits in natural environments acts upon the molecular mechanisms underlying natural phenotypic variation.

INTRODUCTION

In coarse-grained heterogeneous environments, the ability of a genotype to develop different phenotypes in response to environmental cues of future selective conditions may be an important performance trait. If such phenotypic plasticity results in accurate matching of phenotype to environment, it may result in high relative fitness across the range of ecological conditions an organism experiences. Plasticity is therefore often assumed to be adaptive. However, to determine the relationship between plasticity and fitness, it is necessary to determine the optimal phenotype in each relevant environment, by measuring selection on the plastic traits of interest. If plasticity is adaptive, we expect to observe that the phenotype induced by each experimental environment will confer high fitness in that environment relative to alternative phenotypes (Dudley and Schmitt, 1996; Huey *et al.*, 1999). The problem with testing this prediction is that plasticity often prevents the expression of ''inappropriate'' phenotypes within an environment under natural conditions. Consequently, it is impossible to determine how selection would have acted on unexpressed alternative phenotypes in that environment. Thus, testing the adaptive plasticity hypothesis presents a challenge for experimentalists.

Measuring natural selection on plastic traits in multiple environments, and on plasticity itself, calls for a combination of approaches. Usually it is necessary to replicate the same genotypes across environments and measure trait expression as well as fitness in each environment. To test ecological hypotheses about the adaptive value of plasticity, it is important to manipulate specific environmental factors known to influence the expression of the trait (Wade and Kalisz, 1990; Dudley and Schmitt, 1996; Schmitt *et al.*, 1999). However, to measure the natural pattern and frequency of selection on plastic traits may require a ''phytometer'' approach in which experimental individuals or genotypes are exposed to ambient environmental variation (*e.g.*, Kingsolver *et al.*, 2001; Kingsolver and Gomulkiewicz, 2003; Huber *et al.*, 2004). In all cases, it is important to determine the relationship between phenotype and fitness in each environment. If sufficient phenotypic variation is expressed within environments, phenotypic selection analysis (*e.g.*, Lande and Arnold, 1983; Brodie *et al.*, 1995) may be a useful tool. Genotypic selection analysis (Rausher, 1992; Mauricio and Mojonnier, 1997; Stinchcombe *et al.*, 2002) is even more valuable for experimental studies of selection on plasticity, because in coarse-grained environments, reaction norms are properties of genotypes, not individuals, and selection on plasticity can only be measured at the genotype level. By replicating genotypes within and across environments and performing selection analyses on genotypic values, we can also ask whether this adaptive plasticity is costly. If so, we expect that plastic genotypes will suffer reduced fitness relative to less plastic genotypes expressing the same trait mean within an environment (Van Tienderen, 1991; DeWitt *et al.*, 1998). Genotypic se-

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lection analysis also avoids bias due to environmental correlations caused by ecological factors simultaneously affecting the measured traits and fitness (Stinchcombe *et al.*, 2002). Direct selection on plasticity may also be measured using a path analysis of selection on genotypic means incorporating environmental frequencies and transition probabilities (Scheiner and Callahan, 1999).

Studies of selection on naturally expressed variation in multiple environments may offer insight into patterns and causes of selection on plastic traits. However, there is an important limitation: plasticity will create a different range of phenotypes, and thus a different opportunity for selection, within each environment (Kingsolver, 1995*b*; Dudley, 1996). It is impossible to determine how selection would have acted on alternative phenotypes that are not expressed within an environment. If there is a history of strong selection toward the optimal reaction norm, there may be little remaining genetic or phenotypic variation around the trait optimum within any environment, and consequently little opportunity for current selection. In this case, it is useful to extend the range of expressed phenotypes within an environment in order to measure selection on alternative phenotypes that normally would not be expressed (*e.g.,* Nylin *et al.*, 1996; Kingsolver, 1995*a*, 1996; Schmitt *et al.*, 1995, 1999; Dudley and Schmitt, 1996; Baldwin, 1998). This extension may be accomplished by direct manipulation of a trait (*e.g.*, Kingsolver, 1996), by manipulation of the environmental cue that elicits expression of alternative phenotypes (Kingsolver, 1995*a*; Dudley and Schmitt, 1996, by application of regulatory substances that mediate a developmental pathway to elicit alternative phenotypes (*e.g.*, Van Hinsberg, 1997; Baldwin, 1998; Cipollini and Schultz, 1999), or by genetic manipulation of specific loci involved in environmental cue perception and transduction, or regulated by specific signal transduction pathways (Schmitt *et al.*, 1995, 1999; Pigliucci and Schmitt, 1999; Feder, 1999). These approaches are powerful because they allow extension of the phenotype beyond the naturally occurring range. In some cases phenotype manipulation can be combined with phenotypic or genotypic selection analysis to distinguish direct selection on specific plastic traits from selection on correlated manipulated traits or other fitness consequences of the manipulation (Dudley and Schmitt, 1996).

If a plastic response is shown to be adaptive, the next challenge is to understand how it will evolve in natural populations. Adaptive plasticity can evolve only if it is genetically variable within populations, and selection response may be constrained by genetic correlations or tradeoffs within or across environments (Via and Lande, 1985; Gomulkiewicz and Kirkpatrick, 1992). Assuming that the genetic potential for response to selection exists within a population, adaptive plasticity is more likely to evolve toward the optimal reaction norm if the environmental cue triggering the plastic response reliably predicts the future selective environment (Moran, 1992; Kingsolver and Huey, 1998; De Jong, 1999; Tufto, 2000; Sultan and Spencer, 2002) and if maintaining the capacity for plasticity is not costly (Van Tienderen, 1991; DeWitt *et al.*, 1998). The evolutionary outcome will also depend upon the frequency of different selective environments, as well as the extent of gene flow in structured populations (Van Tienderen, 1991; De Jong, 1999; Tufto, 2000; Sultan and Spencer, 2002). Thus, to understand the evolution of adaptive plasticity, we must not only measure natural selection on plastic traits across the range of natural environments encountered by the study organism, but also evaluate the predictability and frequency of those environments.

Here we use phytochrome-mediated ‘‘shade avoidance’’ responses of plants to crowding and vegetation shade as a model system for investigating these issues. We begin by describing the underlying biology and ecological context of shade avoidance responses. Second, we review recent empirical tests of the hypothesis that these responses are a form of adaptive plasticity. Third, we discuss factors that may shape the adaptive evolution of plastic shade avoidance in natural environments. Specifically, we ask: Are shade avoidance responses costly? What is the frequency of different selective environments in the wild? Fourth, we review comparative and experimental evidence for adaptive divergence in shade avoidance responses within and among species. Finally, we suggest directions for further research.

SHADE AVOIDANCE RESPONSES IN PLANTS: A MODEL SYSTEM

As any gardener who has waited too long to thin the seedlings knows, crowding in many plant species elicits a suite of developmental responses, such as stem and petiole elongation, branch suppression, and accelerated flowering (Smith, 1982; Schmitt and Wulff, 1993). The environmental cue eliciting these responses is well known. Shade avoidance is triggered by the reduced ratio of red to far red wavelengths (R:FR) transmitted through or reflected from green vegetation due to selective absorption of visible wavelengths by chlorophyll (Smith, 1982). In open environments, R:FR reflected from neighbors is an accurate signal of neighbor proximity (Ballaré *et al.*, 1990; Smith *et al.*, 1990; Gilbert *et al.*, 2001) and is likely to be an accurate indicator of the present and future competitive environment. In such habitats, there is a steep and predictable vertical gradient of light availability (Huber and Wiggerman, 1997; Weinig, 2000*a*). For example, R:FR in a dense clearing population of the North American annual species *Impatiens capensis* (‘‘jewelweed’’ or ‘‘touch-me-not’’) can range from approximately 0.2 at ground level to 1.2 above the stand, correlated with an over 100-fold increase in photosynthetically active radiation (unpublished data, S.A. Dudley and J.S.). However, R:FR is a less accurate cue of neighbor competition in woodland habitats or other closed-canopy environments because it is reduced by transmission through the overhead foliage (Morgan and Smith, 1979; Dudley and Schmitt, 1995; Weinig, 2000*a*). For example, during the summer growth season for *Impatiens capensis* in a deciduous woodland site, mean R:FR is approximately 0.2 both at ground level and above the understory canopy (unpublished data, S.A. Dudley and J.S.)

The molecular, physiological, and developmental mechanisms of R:FR cue perception and developmental response are also well characterized. Environmental variation in R:FR is sensed by the phytochrome family of photoreceptors, which switch reversibly between R and FR-absorbing forms and interact with signaling pathways to produce a sensitive, graded plastic response to the range of R:FR typical of vegetation shade (Smith, 1982, 1995). Phytochrome-mediated signal transduction pathways are currently the subject of intense investigation by molecular biologists and developmental geneticists (Smith, 2000; Quail, 2002; Nagy and Schafer, 2002; Schlichting and Smith, 2002; Halliday and Fankhauser, 2003), providing the potential for elucidating the genetic mechanisms underlying shade avoidance responses. The biochemical and physiological mechanisms underlying shade avoidance responses are also well studied (Smith, 1995, 2000; Morelli and Ruberti, 2000). There is also growing evidence for quantitative genetic variation in plasticity to light quality, crowding and vegetation shade both within and between natural plant populations, suggesting the evolutionary potential for adaptive evolution (Skálová and Krahulec, 1992; Dudley and Schmitt, 1995; Van Hinsberg and Van Tienderen, 1997; Donohue and Schmitt, 1999; Donohue *et al.*, 2000*b*, 2001; Maloof *et al.*, 2001; Botto and Smith, 2002; Pigliucci *et al.*, 2003).

Phytochrome-mediated shade avoidance responses are also an ideal model system for investigating the evolution of adaptive plasticity because testable ecological hypotheses exist for their adaptive significance in natural populations. In particular, it has been assumed that stem elongation in response to low R:FR reflected from neighbors is adaptive because it allows plants to develop a phenotype appropriate for the competitive environment that they experience (Casal and Smith, 1989; Schmitt and Wulff, 1993; Ballaré *et al.*, 1990). This hypothesis is ecologically reasonable. For example, in many species stem elongation responses to low R:FR may confer an advantage in dense vegetation by increasing the ability to compete for light (Schmitt *et al.*, 1995; Dudley and Schmitt, 1996; Huber and Wiggermann, 1997; Huber *et al.*, 1998; Weinig, 2000*a*). On the other hand, inappropriate stem elongation at low density may be disadvantageous due to opportunity costs of investing in stems rather than resource-capturing organs such as leaves or roots (Maliakal *et al.*, 1999), as well increased risk of mechanical failure (Schmitt and Wulff, 1993; Casal *et al.*, 1994; Niklas, 1995; Cipollini and Schultz, 1999). If R:FR is an inaccurate indicator of neighbor proximity, or if elongation in response to low R:FR does not result in greater light interception, plants will suffer the costs of allocating resources to stem elongation at the expense of leaves and roots, without incurring the benefits. The adaptive value of plasticity to R:FR may thus be diminished beneath an overhead foliage canopy, where the R:FR cue may be a less reliable signal of neighbor competition and the shade avoidance response cannot enable plants to escape overstory shade (Morgan and Smith, 1979; Dudley and Schmitt, 1995; Weinig, 2000*a*, *b*).

In the genetic model species *Arabidopsis thaliana*, a short-lived annual rosette plant of open, disturbed areas, phytochrome-mediated shade avoidance responses may have a different kind of adaptive value. In this species, vegetative internodes do not elongate, and most of the photosynthetic leaf area is invested in basal rosettes, which may be overtopped by growing vegetation as spring progresses (Scheiner and Callahan, 1999; Callahan and Pigliucci, 2002) Plants of this species respond to low R:FR by reproducing at an earlier developmental stage (Pigliucci and Schmitt, 1999; Dorn *et al.*, 2000). This accelerated reproduction in response to vegetation shade may enhance the probability of producing seeds under deteriorating conditions. On the other hand, for plants in the open with a low risk of mortality, later flowering at a larger size may enhance fruit production (Dorn *et al.*, 2000).

ARE SHADE AVOIDANCE RESPONSES ADAPTIVE?

Phytochrome-mediated shade avoidance responses have long been assumed to be adaptive in the physiological literature (*e.g.*, Morgan and Smith, 1979; Smith, 1982; Casal and Smith, 1989), but only recently has this hypothesis been explicitly tested. To do so, it is necessary to measure selection on a range of phenotypes both under conditions that normally elicit shade avoidance responses as well as under noninductive environmental conditions. This has been done in model systems and wild species by genetic and physiological manipulation (Schmitt *et al.*, 1995; Pigliucci and Schmitt, 1999), by manipulating the R:FR cue to induce alternative phenotypes (Dudley and Schmitt, 1996), and by measuring genotypic selection on naturally expressed variation within manipulated environments (Dorn *et al.*, 2000).

In model species, it is possible to take advantage of genetic tools such as mutants and transgenic constructs to test the adaptive significance of shade avoidance responses. For example, transgenic tobacco plants in which plastic elongation responses to neighbors were blocked by overexpression of phytochrome A suffered a reduction in fitness when grown in competition with normally elongating wild type (Schmitt *et al.*, 1995). Thus, phytochrome-mediated stem elongation is advantageous for crowded plants, as predicted by the adaptive plasticity hypothesis. Conversely, phytochrome-B-deficient mutants of *Brassica rapa* (Schmitt *et al.*, 1995) and *Arabidopsis thaliana* (Pigliucci and Schmitt, 1999) constitutively expressing the shade avoidance phenotype had lower fitness relative to plastic wild type in low density, high R:FR environments, as predicted if the shade avoidance phenotype is disadvantageous at low density. Ballaré and Scopel, (1997) also demonstrated a decrease in fecundity of the *Arabidopsis phyB* mutant relative to wild type at low density. Paradoxically, they also observed a decrease in fecundity of this mutant from intermediate density to high density, which they attributed to the fact that the mutants were ''blinded'' to R:FR cues and thus unable to adjust their morphology to their position in the canopy. Physiological manipulation, such as application of exogenous gibberellic acid (GA) to phenocopy the phytochrome-mediated shade avoidance response to low R:FR (Van Hinsberg, 1997; Cipollini and Schultz, 1999), is also useful. For example, Cipollini and Schultz (1999) showed that bean plants treated with GA to induce stem elongation suffered reductions in biomass and reproduction compared with control plants grown in the same environment. This result suggests that there is an opportunity cost to expression of the elongated phenotype.

There is also evidence that phytochrome-mediated shade avoidance responses can be adaptive in wild species under natural conditions. Dudley and Schmitt (1996) manipulated the inductive R:FR cue to test the adaptive value of phytochrome-mediated stem elongation responses to crowding in *Impatiens capensis*. They grew seedlings at high density under a FR removal pretreatment that raised R:FR, thus blocking perception of neighbors and suppressing stem elongation responses, and a neutral shade pretreatment that exposed plants to the same level of photosynthetically active radiation but allowed normal elongation responses to crowding. Elongated and cue-suppressed seedlings were then transplanted back into the source population at both high and low density. Individual seedlings were marked, measured, and censused throughout the growing season for estimates of lifetime reproduction. Elongated plants had higher relative fitness than cue-suppressed plants at high density, but lower relative fitness than cue-suppressed plants at low density, as predicted by the adaptive plasticity hypothesis.

This result tells us that the suite of plastic responses elicited by the R:FR cue is adaptive, but it is insufficient to determine whether density-dependent selection was acting directly on the trait of particular interest, plant height. To do so, Dudley and Schmitt (1996) measured phenotypic selection on height in an analysis of covariance, with the initial R:FR treatment included as a class variable. This analysis revealed that the fitness advantage of the elongated phenotype at high density could be completely explained by direct selection on plant height. In contrast, the fitness disadvantage observed for elongated plants at low density was not due to selection on height per se, but to some other intrinsic cost of the shade avoidance phenotype associated with other unmeasured correlated characters.

Phenotype manipulation also allows us to explore possible mechanisms for this cost of expression. For example, in a greenhouse experiment, *I. capensis* plants displaying induced elongation responses to neighbors allocated proportionately less to roots relative to leaf area or shoot biomass than non-elongated cue-suppressed plants, suggesting that expression of the shade avoidance phenotype may result in greater vulnerability to drought stress (Maliakal *et al.*, 1999). If costs of expression depend upon soil water availability, then the net benefit of expressing shade avoidance traits could be less in dryer sites. Huber *et al.* (2004) examined the impact of microenvironmental variation on the relative fitness of elongated and non-elongated *I. capensis* plants within the site of a natural population. Seedlings of 8 inbred lines from the population were pretreated with either high or low R:FR to induce alternative phenotypes. Pairs of elongated and non-elongated seedlings from each line were then planted into 50 randomly selected microsites within a 40 × 40 m plot in the source population, and monitored for survivorship and lifetime fruit production. The mean fitness of elongated relative to non-elongated plants was calculated over the 8 R:FR pretreatment pairs for each microsite as the mean of $\log(w_e/w_n)$ where w_e is the lifetime fruit production (adjusted for effects of germination date) of the elongated plant and w_n is the adjusted lifetime fruit production of the non-elongated plant. Path analysis demonstrated a direct positive effect of microsite soil water availability on the relative fecundity of the elongated phenotype within that microsite, controlling for microsite seedling density and light availability. Thus, the relative advantage of expressing shade avoidance traits declined with increasing water stress, as predicted, suggesting that the adaptive value of shade avoidance responses to R:FR may be lower in dry habitats.

To examine the adaptive value of phytochrome-mediated response to vegetation shade in *Arabidopsis thaliana*, Dorn *et al.* (2000) manipulated irradiance and light spectral quality and measured genotypic selection on natural variation expressed within different environments. They grew 36 inbred lines derived from four natural New England populations in a greenhouse in four treatments: full sun (high irradiance, high R:FR), neutral shade (low irradiance, high R:FR), and simulated foliage shade (low irradiance, low R:FR), and high density. This design dissected responses to foliage shade into components due to irradiance (full sun *vs.* neutral shade) and to R:FR (neutral shade *vs.* foliage shade). Genotypic selection was measured on plastic traits in each environment to test whether the observed direction of plasticity was adaptive. Plants responded to simulated foliage shade (*i.e.,* to the low R:FR cue combined with reduced light resource availability) by reproducing at an earlier developmental stage and smaller size than in full sun. In simulated foliage shade, selection favored early reproduction, whereas in full sun later reproduction at a larger size was favored. Plasticity to reduced light availability in the absence of the R:FR cue was maladaptive for several traits, due to a maladaptive reduction in growth rate under low irradiance. However, phytochrome-mediated responses to the R:FR cue were generally in the adaptive direction, that is, toward early reproduction at a small rosette size under low R:FR compared with neutral shade. Thus adaptive plasticity to R:FR served to ameliorate maladaptive resource-mediated plasticity to low light availability under foliage shade. However, there was no evidence for adaptive plasticity to density.

In contrast, Callahan and Pigliucci (2002) observed selection for earlier bolting both in the open and under grass shade in *A. thaliana* grown in pots outdoors in Tennessee, and a path analysis detected no selection on plasticity of bolting date (Scheiner and Callahan, 1999). However, these plants were planted in late winter, and appear to have bolted somewhat later than fall-germinating plants overwintering normally in the source populations. As a result of this delayed phenology, the natural climatic conditions the plants experienced may have selected for early flowering regardless of competition treatment. Delayed flowering in open conditions will only be adaptive if the season is long enough for plants to translate their larger size into fruit production, and plastic shade avoidance may only be favored when mortality is delayed in open conditions.

ARE SHADE AVOIDANCE RESPONSES COSTLY?

Assuming that the inductive cue is a reliable predictor of the selective environment, we would expect reaction norms to evolve toward expression of the optimal phenotype in every environment in the absence of physiological costs of plasticity or genetic constraints. However, if maintaining the capacity for plasticity is costly, the optimal plastic response will be a compromise between the reaction norm expressing the optimal phenotype in each environment and the reaction norm with the lowest cost (Van Tienderen, 1991). To measure such costs requires measuring direct selection on plasticity controlling for selection on the trait expressed within an environment (Van Tienderen, 1991; DeWitt *et al.*, 1998; Scheiner and Berrigan, 1998). In coarse-grained environments, however, selection on plasticity cannot be measured at the individual level because each individual expresses only one phenotype. Experimental manipulations in which genotypes are replicated across environments are therefore necessary to test for costs of plasticity and determine the optimal reaction norm (Van Tienderen, 1991; DeWitt *et al.*, 1998). To test for maintenance costs, plasticity is measured as the difference in genotype means between environments. Genotype mean relative fitness in an environment is then regressed on the genotype trait mean within the same environment and the genotype plasticity (Scheiner and Berrigan, 1998). In this multivariate selection analysis, a negative selection coefficient for plasticity indicates a cost, independent of selection on the trait mean.

A priori, maintenance of phytochrome signaling pathways does not seem likely to incur large physiological costs, and thus far the evidence that such costs will constrain the adaptive evolution of shade avoidance responses is weak at best. Dorn *et al.* (2000) tested for costs of adaptive plasticity to R:FR for four life history traits in four environments and detected a cost of plasticity in only one of sixteen tests. In contrast, a direct benefit of plasticity to R:FR, independent of trait means (*i.e.*, a positive selection coefficient for plasticity), was apparent for two traits under neutral shade conditions. Donohue *et al.* (2000*a*) detected weakly significant costs of plasticity to density for first internode length, a shade avoidance trait, in woodland environments, and a direct benefit of plasticity in a low density, open environment. However, direct selection on plasticity was not detectable when other traits were included in a multivariate selection model, suggesting that the apparent costs or benefits may have been due to selection on correlated characters. In both of these studies the range of genotypic variation was extended by including lines from more than one population, and it is very likely that the few ''costs of plasticity'' detected are attributable to population differences in shade tolerance or vigor under the experimental conditions, rather than intrinsic physiological costs of plasticity per se.

WHAT IS THE FREQUENCY OF DIFFERENT SELECTIVE ENVIRONMENTS?

The frequency of selective environments is a crucial variable in models of the evolution of reaction norms (*e.g.,* Van Tienderen, 1991; Gomulkiewicz and Kirkpatrick, 1992; Via *et al.*, 1995; De Jong, 1999; Tufto, 2000; Sultan and Spencer, 2002). Yet few investigators have attempted to measure the actual distribution of selective environments in the wild (Weis and Gorman, 1990; Scheiner and Callahan, 1999; Kingsolver *et al.*, 2001; Arnold and Peterson, 2002), prompting the assertion of Arnold and Peterson (2002) that ''many plasticity studies may be exercises in ecological fantasy.'' What then, can we say about patterns of selection on shade avoidance traits in the real world?

To address this question, (Huber *et al.* (2004) used a phytometer approach combined with phenotype manipulation to examine microgeographic variation in selection on induced shade avoidance responses in a woodland population of *Impatiens capensis*. As described above, seedlings pretreated with high or low R:FR to induce or suppress expression of the elongated shade avoidance phenotype were planted out into 50 randomly selected microsites within the source population in a woodland site, and scored for lifetime fruit production. The distribution of relative fitness of the elongated phenotype was roughly bell-shaped, with selection strongly favoring elongated or nonelongated plants in a few extreme microsites, but not acting strongly on elongation traits in the most common microsites. Probably within these intermediate microsites stabilizing selection favored an intermediate phenotype, although the experiment, employing extreme phenotypes, was not designed to detect such selection.

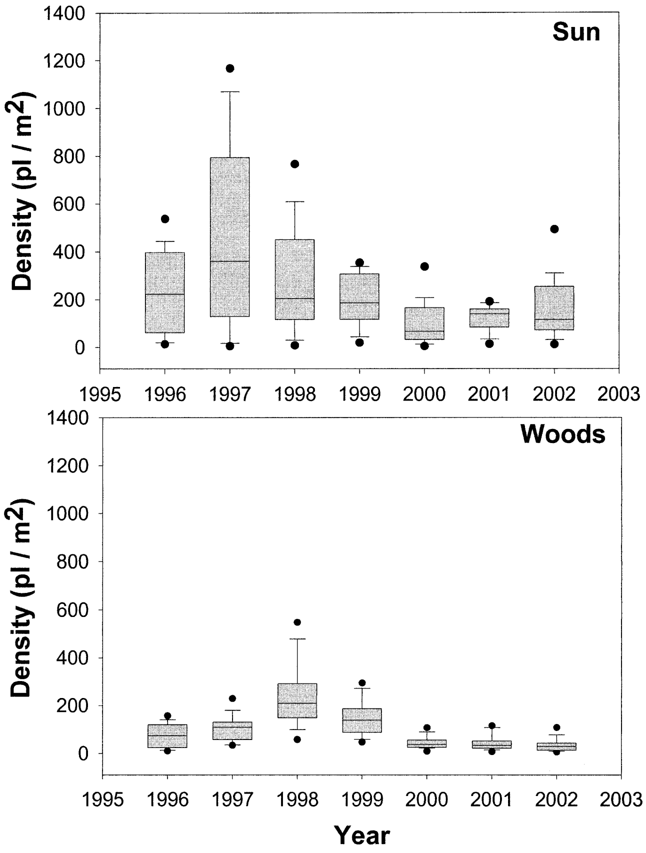

FIG. 1. Box plots of variation in early season seedling density within and between woodland and clearing populations of *Impatiens capensis* in 1996–2002 (unpublished data, M.S.H). Boxes show median and 25% and 75% quartiles, bars 10% and 90%, and dots 5% and 95% percentiles.

The number of naturally occurring *Impatiens* seedlings within microsites was a surprisingly poor predictor of selection on the shade avoidance phenotype, probably because the range of seedling densities was especially low in the site during 2000, the year of this experiment (Figs. 1). Rather, the best predictor of microenvironmental selection on shade avoidance traits was microsite soil moisture, probably due to drought-dependent opportunity costs of elongation as discussed above.

How ecologically realistic were experimental density manipulations designed to test for density-dependent selection on shade avoidance traits in *Impatiens capensis* in this site (Dudley and Schmitt, 1996; Donohue *et al.*, 2000*a*) and in a nearby clearing (Donohue *et al.*, 2000*a*)? Examination of data on naturally occurring seedling densities in the woodland site from 1996–2002 M.S.H., unpublished data; Fig. 1) indicates that the 5 cm spacing (470 seedlings/m^2) used as ‘‘maximum natural density’’ in that site by Donohue *et al.* (2000*a*) occurred as a rare environment in only one of seven years, and the 3-cm (1,305 seedlings/m^2) spacing used by Dudley and Schmitt (1996) was never observed in this site. Thus, emerging *Impatiens* seedlings in this site rarely experience strong intraspecific competition (although they may experience interspecific competition with later emerging species later in the summer). Plants in this site are also subject to temporally heterogeneous drought stress (Heschel and Hausmann, 2001), which may select against expression of the shade avoidance phenotype (Huber *et al.*, 2004). In contrast, densities of emerging seedlings in a nearby clearing population were much higher from 1996–2002 and much more variable within and between years (Fig. 1). In this site, seedling densities $\geq$ 400/m^2 were relatively common in three out of seven years. The 3-cm spacing used as a high density treatment by Dudley and Schmitt (1996), and as a ‘‘maximum density’’ of Donohue *et al.* (2000*a*) in the clearing site, is somewhat extreme for that site, but similar to the maximum density observed in one year in that site, and much lower than the mean density of 2,564 seedlings/m^2 observed in a self-thinning population in a nearby clearing by Schmitt *et al.* (1987). Thus, strong density dependent selection on shade avoidance traits appears to be a plausible scenario for *I. capensis* in open habitats. Moreover, soil moisture in the clearing population is uniformly high compared with the woodland site (Heschel and Hausmann, 2001), suggesting that drought-related costs of expressing the shade avoidance phenotype will be much less important in this site. These data, taken together with the greater reliability of the R:FR cue in open environments, suggest that selection is more likely to favor the shade avoidance phenotype in the open site than in the woodland site.

IS THERE EVIDENCE FOR ADAPTIVE DIVERGENCE?

Comparative studies reveal greater responsiveness to R:FR in species or populations from open habitats compared with closed-canopy habitats, as predicted if the adaptive value of shade avoidance responses depends upon the reliability of this cue (Morgan and Smith, 1979; Corré, 1983; Dudley and Schmitt, 1995; Weinig, 2000*b*). In *Impatiens capensis,* genotypes from a clearing population display greater elongation responses to low R:FR (Dudley and Schmitt, 1995) and crowding (Donohue *et al.*, 2000*b*, 2001) than woodland genotypes under common garden conditions, suggesting local adaptive differentiation in shade avoidance traits. Similarly, cornfield populations of *Abutilon theophrasti* are less responsive to R:FR than populations from weedy habitats (Weinig, 2000*b*), as predicted from the relative reliability of the cue for predicting the fitness consequences of elongation in these two environments. Moreover, stoloniferous clonal species exhibit reduced sensitivity of internodes and increased sensitivity of petioles to simulated foliage shade compared with erect congeners, in which the roles of these structures as horizontal and vertical spacers are reversed (Huber, 1996; Huber *et al.*, 1998). In each case, the vertical structure, which is likely to benefit from enhanced light interception by elongating in a predictable steep vertical gradient in light availability (Huber and Wiggerman, 1997; Huber *et al.*, 1998), was more plastic than the horizontal structure, for which the benefits of elongation are less predictable in a horizontally heterogeneous canopy (*e.g.,* Skálová *et al.,* 1999).

Ecological arguments and comparative studies (Morgan and Smith, 1979) strongly suggest that the divergénce in shade avoidance traits observed for several species between open and closed-canopy habitats (Dudley and Schmitt, 1995; Weinig, 2000*b*) may be a product of adaptive evolution. However, to test directly for local adaptation and understand how selection on specific traits contributes to adaptive divergence, it is necessary to perform a multivariate selection analysis in each site within a reciprocal transplant design (Bennington and McGraw, 1995). Thus, to detect adaptive divergence in shade avoidance responses requires measuring density-dependent selection on shade avoidance traits in the closed-canopy and open sites of the divergent populations.

Donohue *et al.* (2000*a*, *b*, 2001) used this approach to test for adaptive differentiation in plasticity to density in *Impatiens capensis*. Inbred lines from nearby woodland and open sites were planted reciprocally into the source populations at low density and at a high density chosen to approximate the maximum density naturally occurring in each site. Genotypic selection analysis revealed strong density-dependent selection in the open site, favoring genotypes with longer internodes at high density and with short internodes at low density. Thus, plasticity of these traits to density was adaptive in the open site. In contrast, in the woodland site, plasticity of internode length did not confer an advantage. A thought experiment calculating selection on plasticity at different simulated frequencies of high and low density revealed that plasticity of internode length would be favored in the open site even when the frequency of low density environments was very low, because of the high contribution of this favorable environment to overall genotype fitness. In contrast, plasticity of internode length was uniformly maladaptive in the woodland site. These results are consistent with the hypothesis that the observed population differentiation in plasticity is adaptive. When flowering date and size traits were included in a multivariate selection model, the selection gradients for internode length were not significant at low density in either site, indicating that selection did not act directly on elongation at low density, but indirectly on correlated characters. The fitness advantage of the woodland genotypes at high density in their home site could not be completely explained by selection on the measured traits, suggesting that additional traits were also important for local adaptation in this site.

In contrast, Callahan and Pigliucci (2002) found little evidence for adaptive differentiation in plasticity in a reciprocal transplant between populations of *Arabidopsis thaliana* from sites that differed in the degree of late season shading by encroaching grass. Selection favored early bolting at a large rosette size in both sites, and populations did not differ in plasticity of bolting time to vegetation shade. However, without knowing how selection would have acted in the absence of competition in each site, it is difficult to know whether adaptive divergence in plasticity would be expected. All of the microsites in the shaded site experienced late season shade, but so did two thirds of microsites in the open site (Scheiner and Callahan, 1999). These unique data on the frequency of environments actually experienced by *A. thaliana* in the wild are a major strength of this study.

FUTURE DIRECTIONS

How does selection act on continuous reaction norms?

Clearly environmental variation in both the R:FR cue and the competitive environments predicted by that cue is continuous, but thus far most evolutionary studies of shade avoidance responses have focused on selection in a few discrete environments. Likewise, manipulation of shade avoidance traits, although a valuable experimental tool, has been used to produce discrete, extreme phenotypes, whereas in nature responses to R:FR (Smith, 1982) and neighbor proximity (Ballaré *et al.,* 1990; Gilbert *et al.*, 2001) are continuous, graded responses, and intermediate phenotypes are probably common. Differences in continuous reaction norms to R:FR between species from open and woodland habitats (Morgan and Smith, 1979) have been attributed to adaptive evolution as discussed above. How, then, do continuous reaction norms to R:FR and density evolve in variable natural environments?

This problem can be approached within an emerging theoretical framework for analyzing the evolution of ''function-valued'' or ''infinite dimensional'' traits (Gomulkiewicz and Kirkpatrick, 1992; Kingsolver *et al.*, 2001; Kingsolver and Gomulkiewicz, 2003). Reaction norms of such traits can be described as functions of a continuous environmental variable (in this case density or R:FR). Genetic variation in function-valued traits can be described by a genetic covariance function representing genetic variances and covariances among trait values across all possible pairs of environments. An important advantage of the infinite dimensional approach is that it allows one to model the genetic variances and covariances among trait values for unobserved environmental states that fall between the experimental environments, providing additional statistical power for detecting genetic constraints. The impact of selection across a range of environments can be described by a continuous selection gradient function (Gomulkiewicz and Kirkpatrick, 1992). Together, the genetic covariance function and the selection gradient function can be used to predict the evolutionary response to selection on continuous reaction norms (Gomulkiewicz and Kirkpatrick, 1992; Kingsolver *et al.*, 2001) just as the traditional G matrix and selection gradient vector can be used to predict the evolutionary response of correlated traits (Lande and Arnold, 1983).

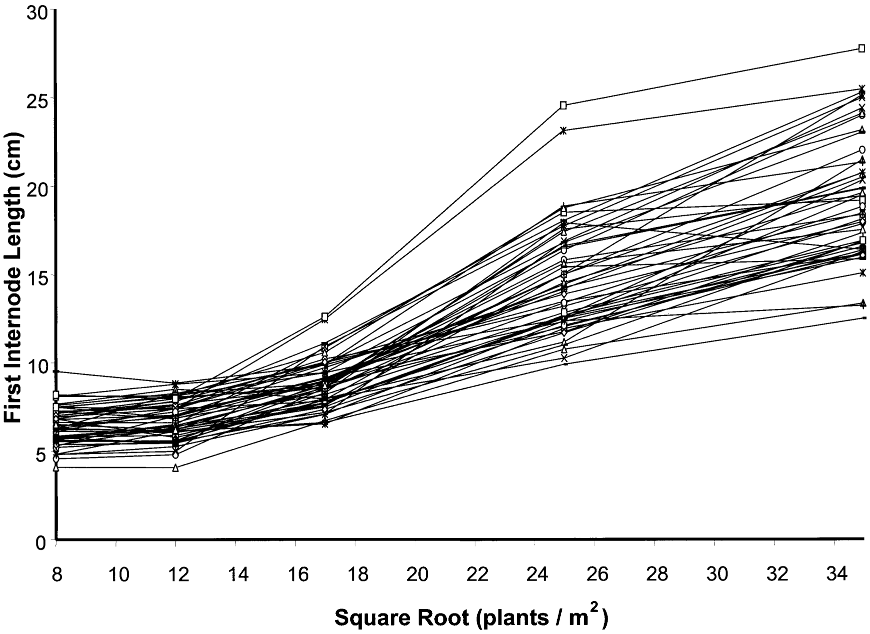

FIG. 2. Reaction norms of first internode length, an elongation trait, to a density gradient in a greenhouse experiment using recombinant inbred lines of *Impatiens capensis* from a cross between parental lines from woodland and clearing populations (unpublished data, J.R.S., M.S.H., and J.S.).

We have observed dramatic genetic variation in continuous reaction norms to density in *Impatiens capensis* (unpublished data, J.R.S., M.S.H., and J.S.; Fig. 2). The next step is to estimate the selection gradient function across the range of natural selective environments. Ultimately, by integrating our estimates of the genetic variance covariance function and the selection function, it will be possible to predict the evolutionary response of shade avoidance traits across the range of naturally occurring density environments.

How does natural selection on shade avoidance responses act at the molecular level?

Shade avoidance responses and other plant photomorphogenic responses are especially interesting for studies of the adaptive evolution of plasticity because the underlying molecular mechanisms are rapidly being elucidated (*e.g.,* Smith, 1995, 2000; Quail, 2002; Schlichting and Smith, 2002). The members of the phytochrome gene family have been identified (*e.g.,* Mathews and Sharrock, 1997) and their distinct but overlapping functions characterized through analysis of mutants and transgenic constructs (Quail *et al.,* 1995; Smith, 1995, 2000; Smith and Whitelam, 1997; Whitelam and Devlin, 1997; Halliday and Whitelam, 2003). Studies of molecular evolution in the phytochrome family indicate that the photosensory domain of phytochrome A evolved rapidly under positive selection early in the history of the angiosperms, suggesting functional divergence (Mathews *et al.,* 2003). Within the angiosperms, there is evidence that the regulatory domain involved in signal transduction is evolving more rapidly than the photosensory domain, possibly associated with acquisition of novel functions (Alba *et al.,* 2000). There has been rapid progress in dissecting the phytochrome signaling pathways (Nagy and Shaffer, 2002; Quail, 2002). In particular, microarray analysis of expression profiles is now being used to dissect phytochrome-regulated transcriptional networks (Tepperman *et al.,* 2001; Wang *et al.,* 2002). Molecular biologists are also becoming increasingly interested in investigating natural variation in phytochrome signaling (Yanovsky *et al.,* 1997; Maloof *et al.,* 2000, 2001; Botto and Smith, 2002; Borevitz *et al.,* 2002) and there is now evidence for natural allelic polymorphism in photoreceptor genes, as well as demonstrated phenotypic effects (Howe *et al.,* 1998; Maloof *et al.,* 2001; El Assal *et al.,* 2001).

This explosion of information about the genetic basis and physiological mechanisms of shade avoidance responses, together with the availability of genetic and genomic tools, provide an exciting opportunity for evolutionary biologists. The opportunity now exists to address questions such as: What are the effects of allelic variation in phytochrome signaling pathways on patterns of light-regulated gene expression and on shade avoidance phenotypes? How does this variation interact with environmental signals to influence the expression of shade avoidance traits under natural conditions? How does natural selection act on this allelic variation, and how is it mediated through selection on gene expression, physiology, and morphology? The combination of mechanistic information with an understanding of the environmental factors inducing and selecting on shade avoidance traits in nature will make it possible to study the adaptive evolution of shade avoidance responses from the level of molecular mechanisms through gene expression and physiological performance to ecological consequences.

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