

# The Evolution of Plant Ecophysiological Traits: Recent Advances and Future Directions

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**P**lants exhibit enormous ecophysiological and functional diversity, which underlies variation in growth rates, productivity, population and community dynamics, and ecosystem function. The broad congruence of these variations with climatic and environmental conditions on local, regional, and global scales has fostered the concept that plant ecophysiological characteristics are well adapted to their local circumstances. For example, the repeated occurrence of plants with CAM (Crassulacean Acid Metabolism) photosynthesis and succulent leaves or stems in severely water-limited environments, and the independent evolution of these traits in numerous plant lineages, provides compelling evidence of the physiological evolution of these water-conserving traits under the influence of natural selection (Ehleringer and Monson 1993). Similarly, studies of the evolution of heavy metal tolerance confirm that natural selection may cause rapid ecophysiological evolution in just a few generations, leading to local adaptation in populations just a few meters apart (Antonovics et al. 1971).

NEW RESEARCH ADDRESSES NATURAL SELECTION, GENETIC CONSTRAINTS, AND THE ADAPTIVE EVOLUTION OF PLANT ECOPHYSIOLOGICAL TRAITS

Many ecophysiological traits—considered here as all aspects of resource uptake and utilization, including biochemistry, metabolism, gas exchange, leaf structure and function, nutrient and biomass allocation, canopy structure, and growth—are likely to influence fitness and undergo adaptive evolution. Traits affecting the assimilation and use of resources such as carbon, water, and nutrients directly influence plant growth. Patterns of resource allocation to growth, reproduction, defense, and stress tolerance are also likely to be under strong selection. Phenotypic plasticity, the expression of different phenotypes by

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the same genotype in response to environmental variation, also affects plant function and, hence, fitness in diverse environments. However, only recently have plant biologists directly studied selection in natural populations. Moreover, until the last few years, little was known about the genetic basis for evolutionary change in ecophysiological traits, or about genetic, developmental, or phylogenetic constraints on the evolutionary response to natural selection.

The authors of this article presented research findings and discussed future directions in evolutionary plant ecophysiology in a symposium at the 1998 annual meeting of the Ecological Society of America. This article presents an overview of advances in the field and addresses a number of questions regarding the evolution of ecophysiological traits:

- How are ecophysiological traits related to fitness? How do patterns of natural selection for these traits vary with the environment?
- What is the genetic basis for ecophysiological traits? How much genetic variation for these traits exists in natural populations? How do genetic and developmental constraints influence the adaptive evolution of ecophysiological traits?
- What is the evidence for adaptive evolution of ecophysiological traits in natural populations? Do closely related populations or species demonstrate ecophysiological divergence, and do their differences reflect patterns of environmental variation as expected on functional grounds?
- What are the macroevolutionary patterns in ecophysiological traits, viewed from a phylogenetic perspective? Do these patterns suggest constraints on ecophysiological evolution or do they suggest high evolutionary lability and frequent convergence?

### ***The adaptive value of ecophysiological traits***

An ecophysiological trait can be considered adaptive if it has a direct impact on fitness in natural environments. Here we examine the successes and limitations of varied perspectives from which this problem has been addressed.

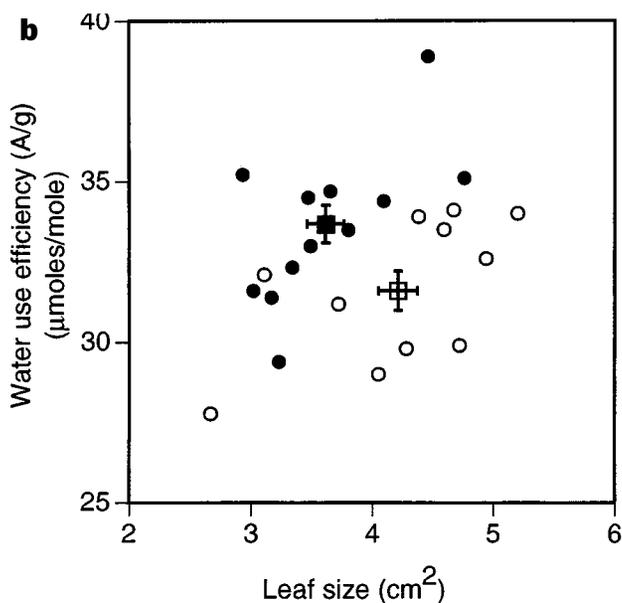
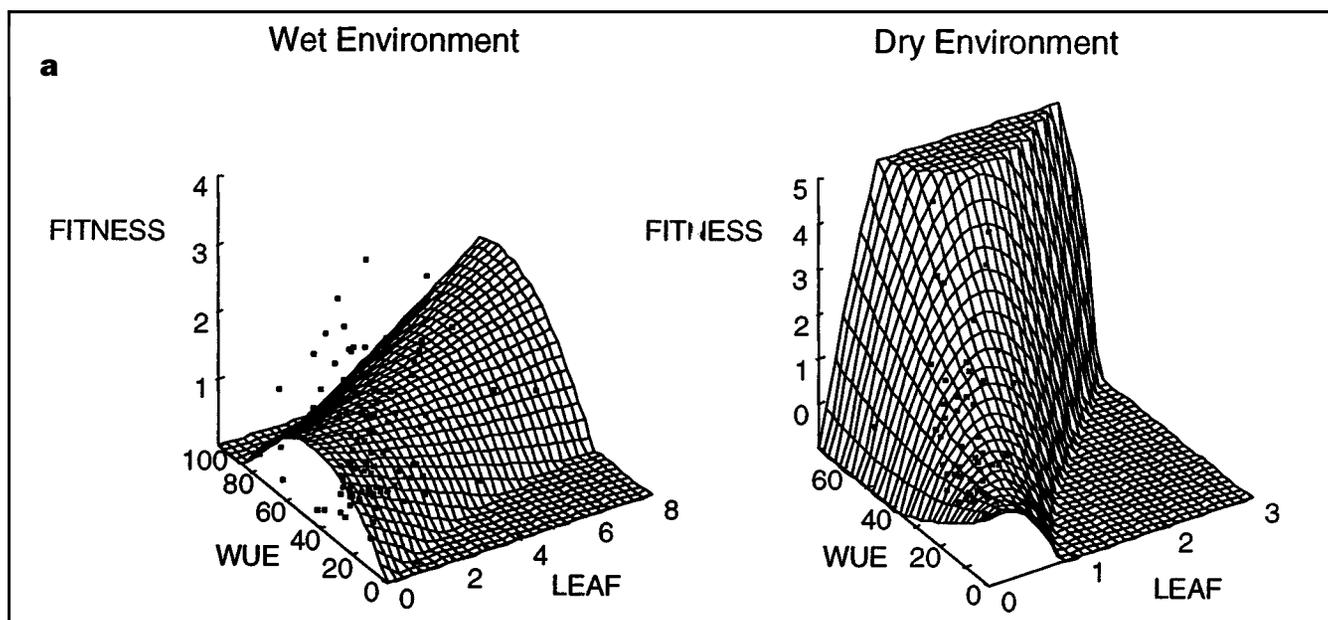
**Phenotypic selection analysis.** The most straightforward evidence for the adaptiveness of ecophysiological traits is the observation of correlations between traits and fitness in natural populations, but this approach has proven problematic. For example, direct correlations between photosynthetic rates and fitness are rarely observed in natural populations (e.g., Farris and Lechowicz 1990 and references). Moreover, even when correlations are observed, it is difficult to determine whether individual traits contribute directly to variation in fitness or whether these relationships reflect indirect selection via

correlated traits. For example, a study of *Plantago lanceolata* found a significant positive correlation between photosynthetic capacity and reproductive dry weight, but correlations were also observed for corm diameter, number of leaves, leaf size, specific leaf weight, and transpiration rate (Tonsor and Goodnight 1997). Thus, it is impossible to say that variation in photosynthetic rate, per se, contributed directly to individual fitness.

In these situations, when many interacting traits may influence fitness, multivariate selection analysis (Lande and Arnold 1983) provides a powerful tool for detecting selection on ecophysiological traits. This method involves measuring a suite of traits, as well as a measure of fitness (e.g., lifetime seed production) on individual plants. Direct selection on each trait is measured as a partial regression coefficient in a multiple regression of relative fitness on all measured traits. If causal relationships among traits are known, path analysis can also be used to determine the direct and indirect effects of each trait on fitness (Kingsolver and Schemske 1991).

The first selection study on plant ecophysiological traits was conducted by Farris and Lechowicz (1990), who measured selection on ecophysiological, phenological, morphological, and growth traits in a *Xanthium* population grown under uniform experimental conditions. They created an experimental population in the greenhouse that maximized genetic variation and then planted seedlings into a garden, preventing any correlation between genotype and microenvironment that could confound the results. Twelve traits on each plant were measured at different times during the season, and path analysis was used to integrate the functional relationships among traits into the selection analysis. The researchers found that seedling emergence time, height and branch growth rates, and water-use efficiency all influenced total vegetative biomass, which in turn was strongly correlated with fitness, illustrating that these traits were under selection.

Similar methods have been applied successfully in several recent studies. One of the most important results of such research is the demonstration that patterns of phenotypic variation, its genetic basis, and natural selection vary in different environmental conditions. For example, in the sand-dune annual *Cakile edentula*, high water-use efficiency and intermediate leaf sizes were correlated with fitness in an environment with low water availability, while larger leaf sizes were favored in an environment with high water availability (Figure 1; Dudley 1996a). In the low-water environment, the finding that genotypes with intermediate leaf size had the highest fitness illustrates the importance of nonlinear approaches in selection studies. This pattern indicates the occurrence of stabilizing selection, in which intermediate trait values are favored, in contrast with directional selection, which favors lower or higher values of a trait. In addition, genetic differentiation between populations derived from low- and high-water



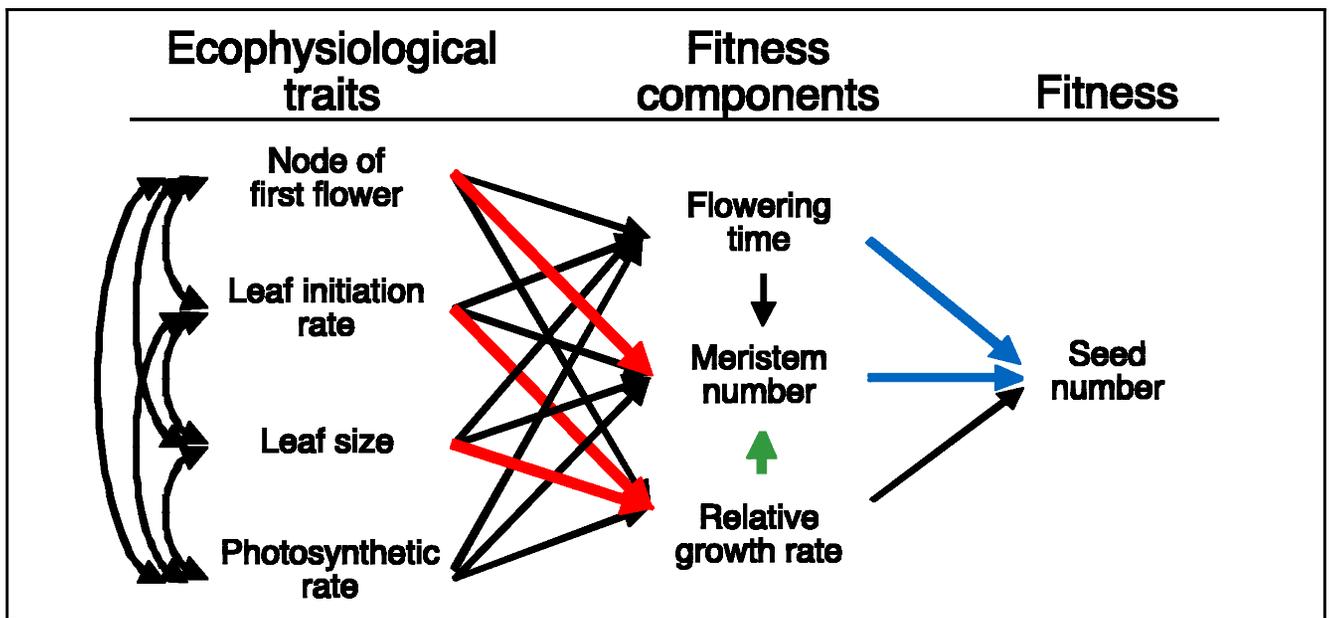
**Figure 1. Phenotypic selection analysis.** (a) Phenotypic selection analysis for leaf size and water-use efficiency in *Cakile edentula*, measured in two contrasting environments. In the wet environment, plants with large leaves and intermediate water-use efficiency exhibit the highest fitness, while in the dry environment, small leaves and high water-use efficiency are favored. These results illustrate the importance of using nonlinear analysis to detect patterns of stabilizing selection (in which intermediate trait values are favored). (b) Scatterplot of family means for leaf size and water-use efficiency for populations derived from a wet site (open circles) and a dry site (closed circles), and grown in a common environment to evaluate genetic differences in ecophysiological traits. The dry-site population had higher water-use efficiency and smaller leaf size (squares with error bars show population means), consistent with predictions of the selection analysis. Reprinted from Dudley (1996a, 1996b), with permission.

environments mirrored the selection results: The low-water population had higher water-use efficiency and smaller leaf size when lines from both populations were raised in a common greenhouse environment (Dudley 1996b).

In another example, Evans (1998) grew field-collected sibships of *Townsendia annua* in a factorial experiment with high and low levels of both water and nutrients to examine patterns of selection (i.e., the strength and the sign [positive versus negative] of correlations between ecophysiological traits and fitness) in contrasting environments. She examined both nitrogen-use efficiency and water-use efficiency, measured using carbon:nitrogen ratios and carbon isotope discrimination, respectively (see Ehleringer 1991 for a discussion of carbon isotope methods). The adaptive value of water-use efficiency changed with environment: In the low-nutrient treatments, fitness

(measured as flower production) was higher in individuals with lower water-use efficiency, whereas in the high-water and high-nutrient treatment, this pattern was reversed. There was also a positive correlation between nitrogen-use efficiency and flower production in all treatments. Changes in patterns of selection in contrasting environments, illustrated in these studies, provide the basis for differentiation and local adaptation of populations.

To understand the functional mechanisms underlying changing patterns of selection, it is valuable to examine how different traits interact to influence fitness in contrasting environments. For example, populations of the annual *Polygonum arenastrum* are genetically variable in physiology (photosynthetic rates, water-use efficiency), in development (node position of the first flowering meristem, leaf production rate), and in morphology (leaf size;



**Figure 2.** Path analysis illustrating how functional traits affect fitness in *Polygonum arenastrum*. Low-level traits include those related to development (node of first flowering, leaf initiation rate), morphology (leaf size), and leaf gas exchange (photosynthetic rate per unit leaf area). Fitness components include traits related to life history (flowering time), growth in biomass (relative growth rate), and architecture (total meristem number). Double-headed arrows between traits indicate that they are correlated; single-headed arrows indicate that the trait at the base of the arrow causally affects the trait at the tip of the arrow. The path coefficient (not shown), which measures the effect of one trait on another, may be positive or negative. For example, the node of first flowering has a positive effect on flowering time because plants that begin flowering at a distal (high) node flower late. Conversely, leaf initiation rate has a negative effect on flowering time because plants that initiate leaves (and nodes) rapidly attain a reproductive size earlier than plants with slow rates of leaf initiation. The magnitude and sign of paths can also change among environments.

To understand the pattern of selection on traits, and how the patterns differ among environments, 20 genotypes of *P. arenastrum* were grown in replicate in three environments differing in water availability (high, medium, and low), and functional traits, fitness components, and fitness were measured on each plant. Path coefficients were estimated for the path diagram for each environment. The thick, colored arrows in the path diagram are paths whose coefficients differ significantly in magnitude or sign among environments (see text). For example, leaf initiation rate has a strong positive effect on relative growth rate in drier (low, medium) environments but no effect in a wet environment. Differences in the pattern of selection among environments are due to differences in the effect of functional traits on fitness components (red paths), in relationships among fitness components (green path), and in the effects of fitness components on fitness (blue paths). From Monica Geber, Cornell University (unpublished data).

Geber and Dawson 1990, 1997). In addition, the traits are genetically correlated, so that early-flowering genotypes initiate their first flower at a basal node and have small leaves, high photosynthetic rates, and low water-use efficiency, while the converse is true of late-flowering genotypes. To determine whether selection might favor different suites of traits in different environments, greenhouse studies examined the influence of these traits on plant fitness (seed number) in environments that differed in water availability (Figure 2).

Results of path analysis suggest that selection on the functional traits (physiology, development, and morphology) can be accounted for by the effect of these traits on components of fitness, such as life history (date of flowering), growth (plant relative growth rate), and architecture (meristem number). Environmental differences in patterns of selection on functional traits can be attributed to differences in the effects of these traits on fitness components, in the relationships among fitness components, and in the effects of fitness components on fitness (Figure 2; Monica Geber, Cornell University, unpublished data). For example, selection favors a more basal node of first flowering in drier environments because early-flowering plants have higher fitness under drought while flowering time does not affect fitness under well-watered conditions. Selection favors a higher leaf initiation rate in dry compared with wet environments because in dry environments, a high leaf initiation rate leads to a high relative growth rate, and a high relative growth rate results, in turn, in plants with many meristems. By contrast, the links between leaf initiation rate, relative growth rate, and meristem number are weaker or absent under well-watered conditions. The results of this experiment point to the complex relationships that link development and physiology to fitness and the variable nature of these relationships across

environments. Understanding the interactions among multiple traits and their consequences for plant performance remains one of the outstanding challenges in evolutionary ecophysiology.

**Manipulating genotypes.** The ability to manipulate expression of individual genes has provided new insights into the ecological and evolutionary consequences of variation in ecophysiological traits. Directed mutagenesis and transgenic manipulation (both knockout and overexpression mutants) allow researchers to generate variation beyond the range observed in natural populations while maintaining a constant genetic background (Schmitt et al. 1999). For example, in studies of tobacco plants, an antisense version of the gene coding for the small subunit of Rubisco was inserted, resulting in a range of genotypes with variable amounts of the Rubisco protein, which is responsible for carbon fixation in photosynthesis. These genotypes, which were otherwise identical, exhibited significant differences in Rubisco enzyme levels, photosynthetic rates, responses to light levels, specific leaf area, biomass allocation, and relative growth rates (Stitt and Schulze 1994). The cascade of effects resulting from this simple genetic manipulation dramatically illustrates how changes at the molecular level can influence ecophysiological characteristics at many levels of organization, as well as whole plant performance.

Several studies have employed transgenic plants to test the hypothesis that traits conferring physiological resistance to herbicides, pathogens, or herbivores also incur costs in performance or fitness (Bergelson and Purrington 1996). For example, Arntz et al. (1998) tested the fitness effects of resistance to the herbicide atrazine, using transgenically modified *Amaranthus hybridus* with a resistance allele inserted into the chloroplast genome. Individuals with the atrazine resistance allele, in an otherwise-identical genetic background, had reduced photosynthetic electron transport resulting in reductions in photosynthetic rates of 15%–25%. In the absence of the herbicide, resistant individuals were less fit than wild-type plants, particularly in a high-density competitive environment, supporting the hypothesis that increased photosynthetic rate results in increased fitness. In studies of transgenically modified *Arabidopsis*, resistance to the herbicide chlorsulfuron also conferred a cost in lifetime seed production, but there was no apparent cost of resistance to the antibiotic kanamycin (Purrington and Bergelson 1997). Analysis of the mechanisms of resistance and their associated physiological effects (e.g., Purrington and Bergelson 1999) may reveal why resistance confers a fitness cost in some cases and not others. An understanding of these variable costs will enrich studies of physiological tradeoffs and ecophysiological evolution.

Transgenic plants hold enormous promise for testing hypotheses in evolutionary and ecological physiology, but their use comes with some cautionary notes. Transformation technologies often introduce mutations in addition to

the intended insertion event; moreover, the effect of the transgene may depend on where it is inserted and on the overall genetic background, reflecting epistatic interactions (De Block 1993, Purrington and Bergelson 1999). Furthermore, constructs that alter the physiological phenotype in ways that are not observed in natural populations may have limited value in unraveling the ecological and evolutionary consequences of natural patterns of genetic or phenotypic variation. Genetic manipulation is potentially useful to study the adaptive role of individual traits, but in many cases, a genetic change results in multiple phenotypic effects (pleiotropy) that may influence fitness in different ways (De Block 1993). Although such effects are realistic as mimics of the natural mutation process, the functional role of individual traits will be interpreted incorrectly if pleiotropic effects are not detected or examined. An understanding of the genetic and developmental mechanisms underlying such pleiotropic and epigenetic effects will play an increasingly important role in understanding evolutionary change in functionally important traits.

**Manipulating phenotypes.** In some systems, the adaptive value of individual ecophysiological traits can be assessed by direct experimental manipulation. A classic example is the study of leaf pubescence in the genus *Encelia*. Physical removal of pubescence from the leaf surface demonstrated that pubescence plays a direct role in leaf energy and water balance by reducing both energy absorption and water loss (Ehleringer et al. 1976). Complementary studies of intraspecific and interspecific variation in pubescence demonstrated correlations with climate that were consistent with this functional role (Ehleringer et al. 1981, Sandquist and Ehleringer 1997, 1998; see below).

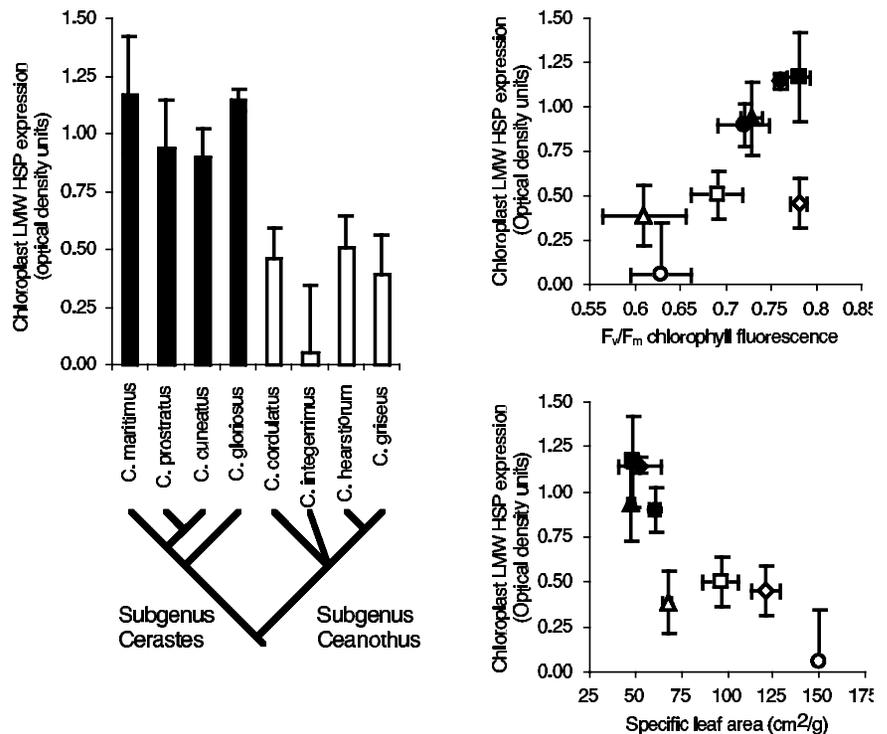
Direct manipulation of this sort is most feasible with external morphological traits, and it has also been applied to the study of inflorescence and flower morphology (e.g., Fishbein and Venable 1996). In studies of biochemical and physiological processes at the cellular and intracellular levels, experimental manipulation is a standard technique. For example, Heckathorn et al. (1998) studied isolated chloroplasts with and without the presence of heat shock proteins (hsps) to elucidate the proteins' role in protecting the photosystem from heat stress (see box page 984). However, manipulation of physiological traits in intact plants is much more difficult, and this type of experimentation may be possible only for a small subset of ecophysiological and functional traits.

**Manipulating virtual plants: Computer simulations.** Computer models of plant function allow manipulation of individual traits, assessment of the consequences, and tests of the interactions among multiple traits. Such models have played a prominent role in studies of canopy, root, and clonal architecture. For example, Niklas (1988) modeled light interception on a single shoot in relation to phyllotaxy, leaf shape, leaf angles, and internode lengths.

### Box 1. Heat shock proteins: A model system for the study of ecophysiological evolution

One exciting area of advance in the past 10 years is in the study of heat shock proteins and their functions at the biochemical, physiological, and ecological levels (Coleman et al. 1995). In plants, low molecular weight heat shock proteins (lmw hsp) are rapidly induced in response to heat shock and other environmental stresses. The lmw hsp genes are located in the nucleus, and in response to stress, the proteins are synthesized and transferred to the chloroplast and mitochondria. In an elegant experiment, Heckathorn et al. (1998) isolated chloroplasts, so that the nuclear-encoded proteins could not be synthesized, and exposed them to heat shock. In the absence of the lmw hsp, photosystem II experienced heat damage and electron transport levels declined. The subsequent addition of purified hsp rapidly restored photosynthetic activity, demonstrating that these heat shock proteins protect the electron transport chain from damage and may contribute to repair of damaged photosystem proteins.

Variation in patterns of hsp production may help explain the evolution of broad variation in thermotolerance among plant species. Some evidence suggests that thermotolerant species such as desert cacti allocate a far greater proportion (several orders of magnitude) of leaf protein to chloroplast hsp than do thermosensitive species (Downs et al. 1998). The relative allocation of protein to chloroplast hsp is also highly correlated with photosynthetic thermotolerance of horticultural varieties of tomato (Heckathorn et al. 1999), populations of the annual plant species *Chenopodium album* (Heckathorn et al. 1999), and species of the perennial shrub genus *Ceanothus* (Knight and Ackerly in press; see figure above). Given the strong correlation between chloroplast hsp production and thermotolerance, and given that all plants tested to date have the machinery to make chloroplast hsp, why do all plants not produce large quantities of hsp, conferring greater thermotolerance? One possibility is that production of chloroplast heat shock proteins is physiologically costly in terms of their nitrogen requirement; nitrogen limitation reduces the relative allocation of protein to chloroplast hsp in corn and tomato and reduces photosynthetic thermotolerance (Heckathorn et al. 1996). Together, these results suggest that although hsp may be required for physiological function, especially in the presence of heat shock and other stresses, variation in their production may reflect tradeoffs in allocation of protein between hsp and photosynthetic proteins, and optimization of the benefits of thermotolerance with the physiological costs of hsp production. Because of the detailed work on the molecular biology of hsp, their conservation throughout plant evolution, and their significance with respect to plant tolerance of environmental stresses, hsp represent a model trait for the study of optimizing selection and ecophysiological evolution.



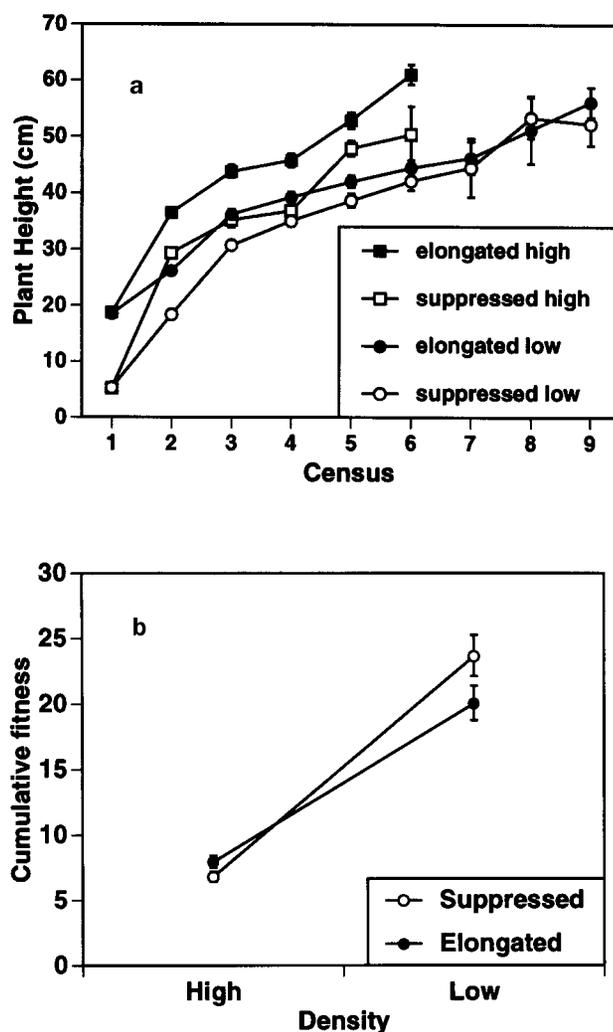
**Variation in expression of low molecular weight hsp, chlorophyll fluorescence, and specific leaf area among eight species of the shrub genus *Ceanothus*.** (a) Hsp expression levels, in response to a 4-hour, 45°C heat shock, are significantly higher in four closely related species in subgenus Cerastes, compared with species of subgenus Ceanothus. (b) Hsp expression levels are positively correlated with  $F_v/F_m$  chlorophyll fluorescence, consistent with the function of hsp in protecting photosystem II from damage (see text). (c) Hsp expression is also negatively correlated with specific leaf area (SLA, the ratio of leaf area to mass). Species with high hsp expression and low SLA (i.e., thick leaves) also show a suite of physiological traits related to greater drought tolerance. Reprinted from Knight and Ackerly (in press), with permission.

By comparing different combinations of these parameters, he was able to show that phyllotaxy has a very strong effect on efficiency of light interception in rosette plants but that the effect greatly diminishes when leaves are vertically displaced by elongated internodes. This study illustrates the power of simulations to explore many different trait combinations and examine how variation in one trait, in this case internode elongation, can dramatically alter the functional significance of other characteristics.

Pearcy and Yang (1996) have developed a more-detailed model (YPLANT) incorporating light interception patterns; biomass investments in internodes, petioles, and leaves; and leaf physiology to estimate both the costs of canopy construction and resulting carbon gain. This model helps reveal the consequences of allocational tradeoffs, such as biomass investment in leaves to increase canopy area versus in petioles to reduce self-shading (Pearcy and Valladares 1999). Models of this type make it possible to examine the function of hypothetical plants with a wide variety of different trait combinations, many of which may never occur in nature (or occur only in the fossil record; see Niklas 1997), and thus provide critical comparisons between the performance of observed phenotypes and possible alternatives that may have been eliminated by natural selection.

**Testing the adaptive plasticity hypothesis.** Much of the phenotypic variation in plant populations reflects the direct effects of environment on plant growth and development, that is, phenotypic plasticity. Plasticity is observed at all levels of organization. For example, plants grown in low versus high light exhibit differences in biochemical and physiological aspects of photosynthesis, leaf anatomy and morphology, allocation and canopy structure, and whole plant growth (Givnish 1988). Plasticity may range from complex and apparently adaptive developmental responses mediated by signal transduction pathways (e.g., phytochrome-mediated stem elongation in dense stands; Schmitt and Wulff 1993) to apparently passive responses to biophysical properties of the environment (e.g., reduced growth of rhizomes in low-temperature soils; MacDonald and Lieffers 1993). In some cases, ecophysiological plasticity may contribute to homeostasis of growth and fitness in variable environments. Phenotypic plasticity creates a challenge in the analysis of the adaptive value of a trait because plants in different environments will also be phenotypically distinct. Thus, it is difficult to separate the contributions of trait variation and environmental variation to relative fitness.

The manipulation of genotypes and phenotypes (either real or simulated) provides one solution to this dilemma. The adaptive plasticity hypothesis predicts that phenotypes produced in contrasting environments will be more fit in their respective environments when compared with alternative phenotypes (Schmitt et al. 1999). Testing this hypothesis requires a factorial experiment in which the fitness of each phenotype is assessed in each environment.



**Figure 3.** Test of the adaptive plasticity hypothesis in *Impatiens capensis*. To test the adaptive value of stem elongation, elongated and suppressed plants were experimentally induced and then planted in mixture at different densities to test their relative fitness at low and high density. (a) Because stem elongation is highly plastic with respect to density, the plants responded to the experimental environments. At high density, suppressed plants grew rapidly in height, although they remained shorter than the high-density elongated plants throughout the experiment. Elongated plants at low density exhibited reduced height growth, and were eventually the same height as the low-density suppressed plants. (b) Despite these adjustments, the adaptive plasticity hypothesis was confirmed by a crossover in cumulative reproductive output, a measure of relative fitness: Elongated plants had higher fitness at high density, and suppressed plants had higher fitness at low density. From Dudley and Schmitt (1996), with permission.

For example, Dudley and Schmitt (1996) combined phenotype manipulation with selection analysis to test the adaptive value of phytochrome-mediated stem elongation in dense stands of *Impatiens capensis*.

Based on functional considerations, it is predicted that at high density, stem elongation will be favored because the tallest plants will receive the most light, while at low density, excess investment in height growth would result in opportunity costs for reproduction, as well as a more unstable stem. These predictions were tested by experimentally inducing plants to produce either elongated or suppressed stems by manipulating the ratio of R:FR wavelengths experienced by seedlings during development. (The R:FR ratio of light is perceived by the light-sensitive phytochrome molecule, and low R:FR ratios, which occur in vegetation shade or high-density stands, promote stem elongation.) These seedlings were then planted out in the field in high and low densities. As predicted, the elongated plants exhibited higher fitness at high density, while suppressed plants were more fit at low density (Figure 3). Selection analysis (based on correlations between fitness and functional traits) suggested that increased stem height relative to leaf size was favored in high density but not low density, supporting the hypothesis that the stem elongation response contributes to these fitness differences, and is adaptive (Dudley and Schmitt 1996).

Genetic manipulation can also be used to test the adaptive plasticity hypothesis. For example, Schmitt et al. (1995) tested the adaptive value of phytochrome-mediated stem elongation responses to neighbors using genetically modified plants lacking these plastic responses. Transgenic tobacco plants in which plastic elongation responses were blocked showed a reduction in fitness when grown at high density with normally elongating wild type. Conversely, phytochrome-deficient mutants of *Brassica rapa* constitutively expressing the elongated phenotype had lower fitness relative to the plastic wild type at low density. These results confirm that phytochrome-mediated stem elongation is advantageous for crowded plants but maladaptive at low density; they thus demonstrate that plasticity for stem elongation is adaptive across this range of environments.

Computer models can also be used to assess the performance of different phenotypes across a range of environments. Ackerly and Bazzaz (1995) conducted a "virtual" experiment evaluating plants with different crown structures across a range of light environments. Using the YPLANT model (Pearcy and Yang 1996, see above), they reconstructed the crown structure and leaf display of tropical tree seedlings growing in different treefall gap environments, and evaluated their light interception capacity using hemispherical canopy photographs taken directly above each plant. Light interception capacity was then evaluated in alternative microsites by repeating this analysis for each plant using canopy photographs from other treefall gaps of different size and orientation. The results showed that for most individuals, the adjustments in leaf

display (principally leaf angle) in response to the asymmetry of their light environment enhanced light interception compared with what it would have been in other localities in the forest.

These studies of the performance of alternative phenotypes in contrasting environments are analogous to reciprocal transplant studies of genetically divergent populations (see below), and provide valuable insights into the adaptive value of the responses of individual plants to their environments.

### **The genetic basis of ecophysiological evolution**

Phenotypic analyses, as discussed above, highlight the potential for natural selection on ecophysiological traits. Studies of the genetic basis of ecophysiological variation are critical to understanding the potentials and limitations for adaptive evolutionary change and the nature of the genetic mechanisms underlying contemporary ecophysiological diversity.

**Genetic variation for ecophysiological traits.** For natural selection to cause evolutionary change in ecophysiological traits, genetic variation for those traits must exist within natural populations. Adaptive differentiation among populations (see below) demonstrates that sufficient genetic variation was present in the past to permit divergent responses to selection. However, measuring such variation in natural populations has proved to be a substantial challenge because of the large sample sizes required for quantitative genetics and the sensitivity of ecophysiological traits to fine-grained environmental variability. Experiments in controlled conditions are critical for revealing physiological differences among genotypes. Using quantitative genetic techniques, heritability estimates can be calculated to describe the proportion of total variation associated with either additive or overall genetic differences among individuals (narrow- and broad-sense heritability, respectively). Heritability estimates range from 0 to 1, with higher levels indicating that a trait has greater potential to respond rapidly to selection. Heritability estimates for ecophysiological traits vary widely, ranging from less than 0.1 for instantaneous photosynthetic rate and water-use efficiency (Dudley 1996b, Tonsor and Goodnight 1997), to 0.20–0.81 for carbon isotope discrimination, an integrated index of water-use efficiency (Schuster et al. 1992, Donovan and Ehleringer 1994), and to 0.66–0.94 for measures of biochemical and stomatal limitations on photosynthesis measured under carefully controlled conditions (Geber and Dawson 1997).

Although heritability has been addressed in only a handful of studies, evidence suggests that instantaneously measured physiological traits have lower heritabilities than traits that reflect integrated ecophysiological processes over time, such as carbon isotope discrimination. This difference probably reflects the increased variability of instantaneous traits caused by environmental fluctuations

and developmental effects (e.g., leaf age), whereas these factors are integrated over longer time scales in traits such as carbon isotope discrimination. If we could measure integrated photosynthetic rates over the lifetime of each leaf and each individual under controlled conditions, genetic differences among individuals might become more apparent. Moreover, it is important to note that heritability does not necessarily relate to the evolutionary significance of a trait, since low heritability may reflect strong prior selection that has eliminated genetic variation in a trait of adaptive importance.

**Genetic constraints on adaptive evolution.** While heritable variation provides the raw material for adaptive evolution, a variety of genetic factors may limit evolutionary responses to selection. The most direct constraint is the absence of appropriate genetic variation. For example, the evolution of heavy metal tolerance in contaminated soils has been observed in populations of a variety of plant species (Antonovics et al. 1971). However, in a series of five *Agrostis capillaris* populations growing along a utility line, where the soils beneath each pylon were heavily contaminated by zinc, variable levels of zinc tolerance were observed. In one population, tolerance had failed to evolve. Greenhouse experiments showed no genetic variation for zinc tolerance in this population even though it was located less than 1 km from other populations in which such variation did occur and tolerance had evolved (Alhiyaly et al. 1993). The lack of appropriate genetic variation for adaptive response to selective pressures may be frequent in natural populations, and its role in understanding patterns of adaptive evolution deserves greater attention (Bradshaw 1991).

Plant mating systems and life histories will influence the transmission of heritable variation and may also impose significant constraints on adaptive evolution. In particular, asexual reproduction, which prevents genetic recombination, is relatively common in flowering plants (Briggs and Walters 1997). While asexuality does not diminish the efficacy of selection (it may actually promote rapid responses by selective “sweeps” of favorable genotypes), it will limit the generation of new and potentially adaptive genotypes through recombination. The very long life span of some woody plants, and the prolongation of genetic longevity in clonal plants through vegetative reproduction, will also reduce the potential for rapid response to selection in many plant species.

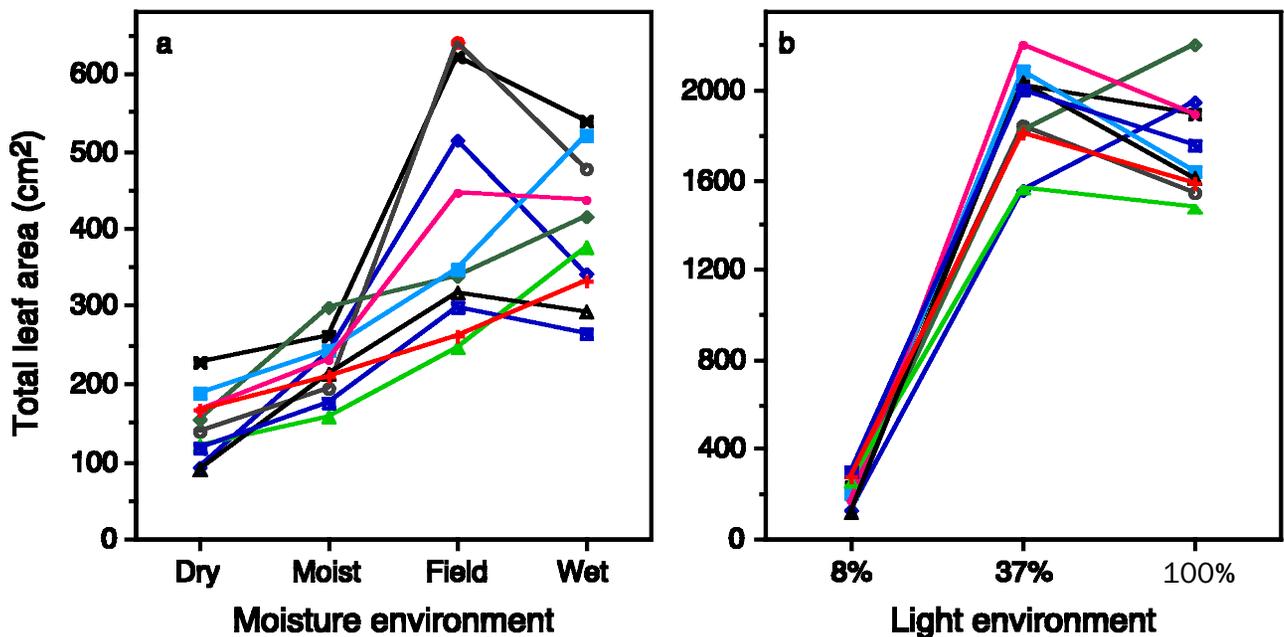
When natural selection acts on two or more traits simultaneously, evolutionary responses may be limited by genetic correlations that reflect genetic, physiological, and/or developmental constraints. For example, Dudley (1996b) observed positive genetic correlations (i.e., between the mean values for different genotypes) between stomatal conductance and photosynthetic rate and between leaf size and water-use efficiency in *Cakile edentula*. She also detected a genetic tradeoff between stomatal conductance and water-use efficiency, indicating

that selection to enhance one trait will lead to a decline in the other. In populations of the annual plant *Polygonum arenastrum*, there is genetic variation in, and covariation between, developmental (node position on the main stem of the first flowering meristem), morphological (leaf size), and ecophysiological traits (photosynthetic rates, water-use efficiency, electron transport activity; Geber and Dawson 1990, 1997). Genotypes that flower first at a higher node on the main stem have larger leaves and lower rates of gas exchange than those that start flowering at lower nodes.

The expression of genetic variation and covariation also changes with environmental conditions. For example, in *Townsendia annua*, Evans (1998) observed significant family–nutrient treatment interactions for nitrogen- and water-use efficiency, suggesting genetic variation in the response to nutrient availability. Genetic correlations between water- and nitrogen-use efficiencies also changed with environment. At higher nitrogen availability, water-use efficiency was positively correlated with nitrogen-use efficiency, but at lower nitrogen levels, there was a negative correlation between these traits. This change in the tradeoff in resource-use efficiency suggests that the action of genes governing pleiotropic effects changes with environment. Together, these three examples of genetic correlations demonstrate that ecophysiological traits cannot necessarily respond independently to selection. Strong selection on one trait may result in correlated evolutionary responses in others, and genetic correlations between traits with opposing effects on fitness may constrain their responses to selection.

Pleiotropic linkages between traits may also constrain responses to selection. For example, one of the first reported etiolation mutants of *Arabidopsis*, which lacked the normal stem elongation response in darkness, was also found to have green roots (Chory et al. 1989). Apparently, the absence of chlorophyll in roots is itself a developmental response to darkness that is regulated in part by the same gene as the etiolation response. If, in a particular environment, selection favored a mutation at this locus reducing the etiolation response, the linkage between etiolation and root development might limit the response to selection because of the energy costs of unnecessary chlorophyll production in roots.

**Genotype by environment interactions: Phenotypic plasticity.** Several recent studies have shown the existence of genetic diversity for the responses of ecophysiological traits across diverse environments, that is, genetic variation in patterns of phenotypic plasticity. In statistical terms, variation in plasticity appears as a significant genotype–environment interaction in analysis of variance, or as significant genetic variation in measures of plastic responses. Variation in ecophysiological plasticity has been observed among both populations (e.g., Emery et al. 1994, Dudley and Schmitt 1995) and genotypes within populations (e.g., Schmitt 1993, Sultan and Bazzaz 1993a, 1993b,



**Figure 4. Genetic variation in phenotypic plasticity.** Patterns of individual plasticity (reaction norms) for whole-plant leaf area in the widespread annual *Polygonum persicaria*. Each line depicts the response of a single genotype, based on four to six clonal replicates grown in each environment. Panels A and B show the responses of the same ten genotypes grown along gradients of moisture or light availability. These patterns exemplify how the amount of genetic variation in a trait depends upon environmental conditions; for example, on the moisture gradient, there is greater variation among genotypes under field capacity conditions than in the dry or moist environments. In addition, the relative ranking of different genotypes changes with environment, and no single genotype exhibits highest performance in all environments within or across gradients. As a result, selection in variable environments may result in the maintenance of genetic variability. From Sultan and Bazzaz (1993a, 1993b), with permission.

1993c). The observation of widespread genotype–environment interaction for ecophysiological traits in natural plant populations suggests that the genetic potential often exists for the pattern or amount of adaptive plasticity to evolve in response to variable selection in heterogeneous environments.

Variation in plasticity among genotypes may have further effects on evolutionary responses to selection in natural populations. If reaction norms (i.e., patterns of phenotypic expression) converge in one environment, then heritability, and thus the potential for response to selection, will be lower in that environment than in other environments where greater genetic variation is expressed. If reaction norms for performance traits cross, so that different genotypes are favored in different environments, then environmental heterogeneity may contribute to the maintenance of genetic variation. Sultan and Bazzaz (1993a, 1993b, 1993c) compared norms of reaction for naturally occurring genotypes of *Polygonum persicaria* along resource gradients (Figure 4). Although the genotypes differed significantly for many traits, these differences varied from one environment to another, and most genotypes exhibited appropriate phenotypic responses that reduced the deleterious effects of stressful environments. Such complex patterns of plasticity variation may permit diverse

genotypes to be maintained in populations inhabiting variable environments.

Phenotypic plasticity may also evolve as an individual characteristic in response to fine-grained environmental heterogeneity (Lloyd 1984). For example, leaves developing in sun and shade within the canopy of a single plant, or below and above the water level in the case of heterophyllous aquatic plants, often exhibit physiological and structural differences. Plants that produce these divergent leaf forms may have greater fitness than an individual able to produce only one type or the other. However, although Winn (1996) documented the existence of genetic variation for within-individual variation in leaf traits, no fitness differences were detected in association with this variation in fine-grained plasticity (Winn 1999b). The expression of phenotypic plasticity within individuals (e.g., in different leaves or at different developmental stages) is extremely important in plants because of their modular growth and indeterminate development, and studies of the evolution of plasticity at this level merit greater attention in the future (Winn 1999a).

These studies of genetic variation reflect a fruitful synthesis of the technical approaches of ecophysiology with the statistical methods of population biology and evolutionary genetics. The strength of this quantitative

approach is that it permits the study of genetic variation and selective dynamics based on phenotypic data in experimental and natural populations. However, this is also its limitation because the specific genes responsible for heritable variation are not examined.

**QTL mapping and candidate genes.** One of the most exciting advances in recent years, which addresses this limitation, is the use of tools from molecular and developmental genetics to examine the genetic basis of phenotypes at a mechanistic level. Two of the most important approaches in this research are the study of candidate genes controlling a trait of interest, and mapping of quantitative trait loci (QTL) to search for unknown or multiple loci affecting trait expression.

Techniques for mapping QTL have recently been used to examine genes involved in plant responses to different light environments. For example, Dorn and Mitchell-Olds (unpublished data) used hybrid segregates of a cross between two natural *Arabidopsis thaliana* ecotypes to map several loci involved in plasticity of flowering time and rosette leaf number in response to differences in spectral light quality. Yanovsky et al. (1997) used recombinant inbred lines of a cross between two *Arabidopsis* ecotypes to map two QTL associated with naturally occurring variation in signal transduction of very-low-fluence responses. These responses, which are mediated by phytochrome A, include seedling germination and de-etiolation in response to brief pulses of very dim light. Once a quantitative trait locus has been mapped, candidate gene studies may be used to determine whether it is linked with a particular locus, using mutant and transgenic approaches.

### **Adaptive differentiation of populations and species**

The study of evolutionary processes within populations, as discussed above, is critical to understand the potentials and limitations for adaptive evolution. Variation in patterns of selection on genetically variable traits in different environments provides the conditions for adaptive divergence of conspecific populations. Comparative studies of ecophysiological variation between populations and species have long served as a powerful tool for examining these patterns of adaptive evolution.

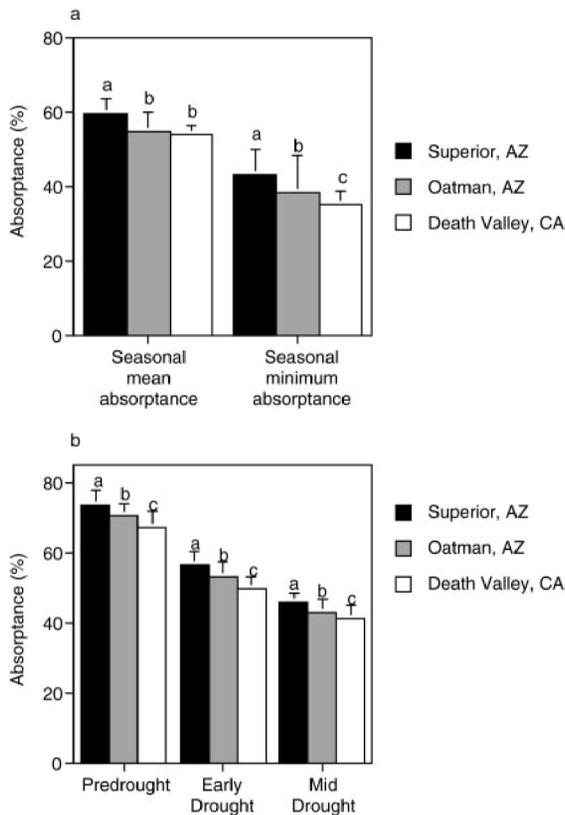
**Ecophysiological variation among populations and species.** The study of genetic differentiation among plant populations, based on common garden experiments and reciprocal transplants, was pioneered by Tureson (1922) in Sweden and Clausen and colleagues in North America (Clausen et al. 1940). Since then, numerous studies have documented evolutionary divergence in ecophysiological and life history traits, including photoperiod and temperature responses, specific leaf area, photosynthetic rate, water-use efficiency, leaf size, allocation, etc. (Early studies were reviewed in Hiesey and Milner 1965; recent examples include McGraw and Antonovics 1983, Gurevitch

1992, Monson et al. 1992, Sawada et al. 1994, Dudley and Schmitt 1995, Dudley 1996b.)

Reciprocal transplant experiments test the adaptive nature of population divergence. In these experiments, genotypes from two contrasting environments are grown in their native site and in the site of the other population to compare their function and performance. Higher fitness of genotypes in their native environment, when compared to genotypes transplanted from contrasting environments, provides evidence of local adaptation of populations to their respective environments. It is important to recognize that reciprocal transplants do not address the adaptive significance of individual ecophysiological traits. Instead, they test whether the entire suite of traits that has differentiated between populations confers a performance advantage in the local environment. Combining reciprocal transplants with selection analyses makes it possible to examine the underlying causes of fitness variation across contrasting environments (e.g., Jordan 1992, Bennington and McGraw 1995).

Divergence among populations provides the material for speciation and differentiation among closely related species. For example, over a broad rainfall gradient in the deserts of southwestern North America, leaf pubescence in populations of *Encelia farinosa* declined as mean water availability increased (Sandquist and Ehleringer 1997). Variation among populations in both pubescence and carbon isotope discrimination persisted when they were grown in common environments differing in water availability, indicating a genetic basis for variation in these traits (Figure 5; Sandquist and Ehleringer 1998). This genetic variation in ecophysiological function within *E. farinosa* parallels similar variation found among species within the genus *Encelia* (Ehleringer et al. 1981), demonstrating both intraspecific and interspecific adaptation.

The interface between intraspecific and interspecific variation provides a critical link between microevolutionary processes within populations and larger patterns of diversification and adaptation. For example, both within and among species of *Helianthus*, variation in the relative proportions of saturated and unsaturated fatty acids in seed oils parallels climatic differences in the species' ranges. Experimental studies suggest that within *H. annuus*, seed oil composition influences germination performance, producing a tradeoff between the timing of germination at low temperature and the rate of growth at high temperature (Linder 2000). This tradeoff suggests that the interspecific biogeographical patterns in seed oil composition of *Helianthus* species result from selection within each lineage for optimal seed oil composition in its local environment. By studying local adaptation of species with broad geographic distributions, it may be possible to explain how selection has influenced the microevolution of seed oil composition at the level of individual genes. Patterns revealed at this level can then be compared with genetic differences among species with



**Figure 5. Variation in leaf traits among populations. Leaf absorbance values (mean + 1 sd) from (a) field and (b) common garden studies of three *Encelia farinosa* populations. The variation in absorbance values among populations is correlated with mean annual precipitation in the region from which they were collected: Superior, AZ, 453 mm  $y^{-1}$ ; Oatman, AZ, 111 mm  $y^{-1}$ ; Death Valley, CA, 52 mm  $y^{-1}$ . The consistency of the differences observed in the field and in a common garden, in which plants were grown under the same conditions and then measured at periodic intervals during a drought treatment, demonstrates that there is a genetic basis for the variation among populations. (Different letters within each comparison indicate significantly different means based on arcsin transformations;  $P < .05$ .) See Sandquist and Ehleringer (1997, 1998) for experimental details.**

different geographic ranges. Such an approach would indicate whether physiologically important traits become adapted to different environments by the same genetic mechanisms in independently evolving lineages.

Patterns of phenotypic plasticity also evolve in the course of species differentiation, and related species may express different characteristic plastic responses to environmental conditions (Sultan 1995). Because adaptive plasticity influences environmental tolerance, such differences may contribute to differences in the range of environments that

species inhabit in the field. For example, four closely related species of *Polygonum* differed in the magnitude, direction, and timing of plasticity for such key functional traits as photosynthetic rate, proportional biomass allocation, leaf size and total area, and root morphology and total length (Sultan et al. 1998, Bell and Sultan 1999). These differences in patterns of plasticity were broadly consistent with differences in the species' ecological distributions: for example, genotypes of *P. persicaria* (a species found in both open and moderately shaded sites) more than doubled allocation to leaf tissue in low versus high light, while shaded individuals of *P. hydropiper* (a species excluded from shaded sites) increased leaf biomass allocation less than half as much (Figure 6). In some cases, the *Polygonum* species differed in the timing as well as the amount of plasticity. For instance, *P. persicaria* plants showed significantly faster as well as more pronounced spatial redeployment of roots to the soil/air interface in response to soil flooding than plants of *P. cespitosum*, which does not occupy flood-prone sites (Bell and Sultan 1999). These results suggest that differences in plasticity may be an important aspect of adaptive diversity among plant species.

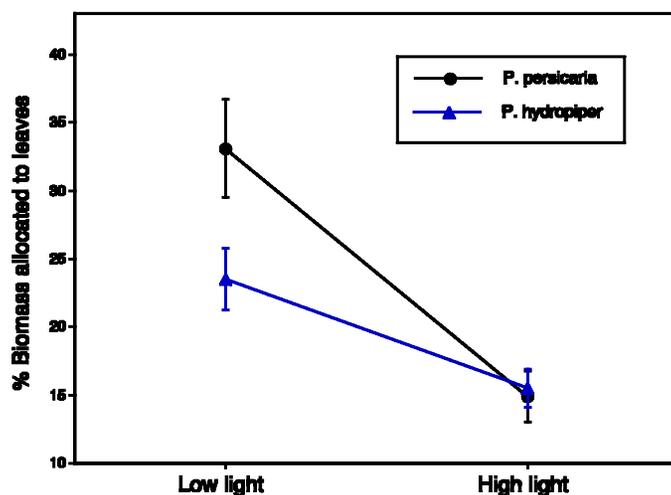
**Ecophysiological variation in a phylogenetic context.** Comparative studies of interspecific variation, such as the examples just mentioned, have long played a central role in the study of adaptation and the examination of evolutionary constraints and patterns of macroevolution. In the context of species' evolutionary relationships, adaptive variation has been addressed from two complementary perspectives, focusing on divergent versus convergent evolution. Differentiation among closely related species occupying different habitats provides evidence of adaptive divergence (e.g., Robichaux et al. 1990). The study of closely related species pairs (e.g., congeneric annual versus perennial grasses; Garnier and Laurent 1994) is a particularly powerful method to detect statistically repeatable patterns of divergence. Alternatively, phenotypic similarity among distantly related species occupying similar habitats demonstrates evolutionary convergence and provides powerful evidence for the action of selection (as in the repeated independent evolution of  $C_4$  and CAM photosynthesis).

Explicit consideration of phylogenetic relationships allows us to address a variety of novel questions regarding patterns of ecophysiological evolution, such as reconstruction of the sequence and direction of evolutionary changes in complex traits. For example, the genus *Flaveria* has three species with the  $C_3$  photosynthetic pathway, eight species with  $C_4$  photosynthesis, and nine species with varying levels of intermediate characteristics ( $C_3$ - $C_4$ ). Based on the phylogenetic distribution of these traits, it appears that even within this one genus, the eight  $C_4$  species result from three independent origins of this pathway, at least two of which started with the transition to  $C_3$ - $C_4$  intermediates (Figure 7; Monson 1996).

Dawson et al. (1998) are studying ecophysiological variation in *Schiedea*, an endemic plant genus that has radiated throughout the Hawaiian islands from a single colonization event. As plants of this group colonized drier habitats, evolutionary transitions to the woody habit and smaller leaves have been accompanied by changes in plant hydraulic architecture (e.g., vulnerability to cavitation under drought), water-use efficiency, and plant carbon balance. As in studies of the Hawaiian silversword alliance (Robichaux et al. 1990), the evolution of ecophysiological traits in *Schiedea* illustrates that functional diversification and adaptive radiation appear to have occurred simultaneously. Mapping of functional traits onto the phylogenetic tree for these species will provide rigorous tests of evolutionary hypotheses about the origin of the possible ecophysiological adaptations; the underlying cause-and-effect relationships; the trajectories, modes, and tempos of functional changes; and alternative explanations to “adaptive evolution” for the origin of ecophysiological characteristics and their maintenance in nature.

Lechowicz and Wang (1998) have conducted one of the few studies evaluating phenotypic plasticity in a phylogenetic context (see also Pigliucci et al. 1999). In a study of 16 species of North American spruce growing in ambient and elevated CO<sub>2</sub> and low and high water availability, they found that interspecific variation in many morphological and ecophysiological traits was not associated with the species’ phylogenetic relationships. However, relative growth rate, which is the outcome of interactions among many ecophysiological traits, showed consistent evolutionary trends across species. Perhaps most interesting, relative growth rate was also less plastic across environments than the many ecophysiological traits underlying variation in growth, but the levels of plasticity in growth rate did not themselves show any pattern of phylogenetic constraint. Evolution of function in extant spruces has apparently involved different patterns of diversification in the mean value of traits affecting growth and in the plastic expression of these traits in differing environmental regimes.

Comparative analyses of interspecific variation are frequently invoked to test adaptive hypotheses based on correlations among different traits or between traits and environments (e.g., variation in pubescence in *Encelia* species along rainfall gradients; Ehleringer et al. 1981). In the past 15 years, there has been widespread attention to the conceptual and statistical issues associated with comparative analyses in the context of phylogenetic relationships. In particular, it has been argued that phylogenetic relationships among species create a hierarchical data structure that prevents closely related species from being used as independent data points. Nonindependence leads to inflated type I errors in testing the significance of correlations (i.e., increased chance of rejecting a null hypothesis when, in fact, there is no relationship), suggesting that claims about adaptation derived from interspecific correlations

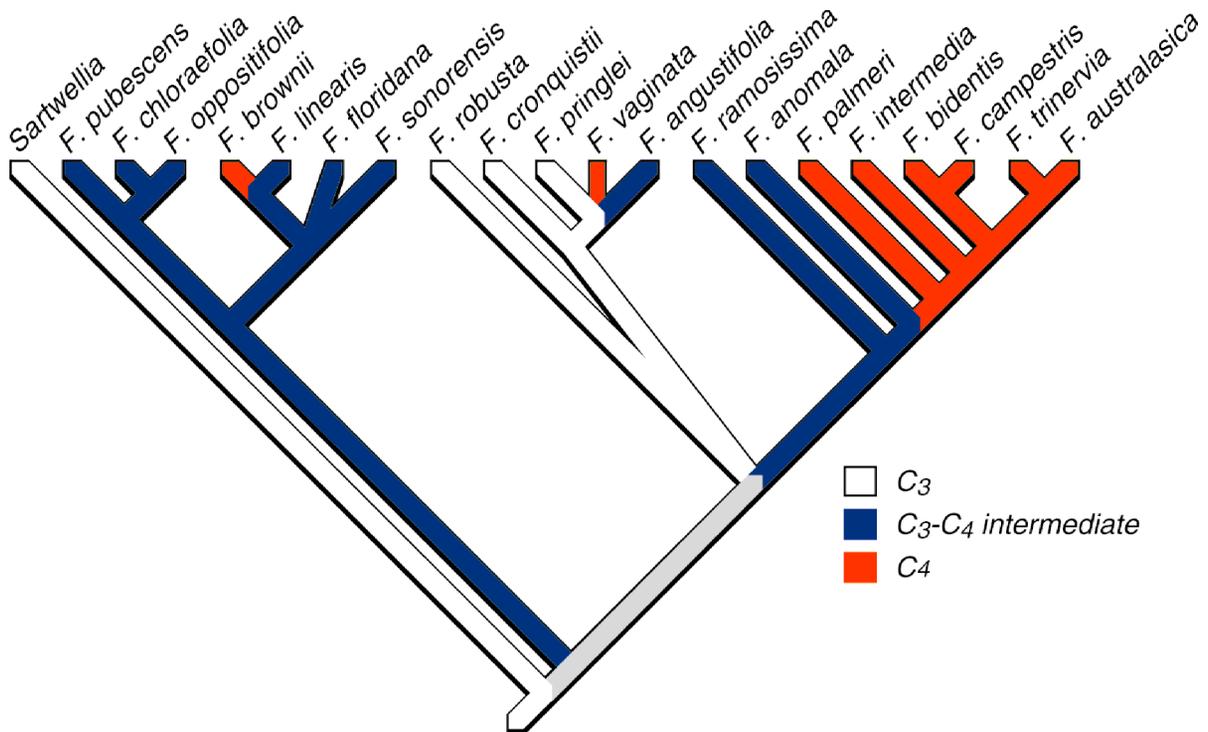


**Figure 6. Plasticity in closely related species. Plasticity for proportional biomass allocation to leaves in low (20%) light versus high (100%) light in *Polygonum persicaria* and *P. hydropiper*. *P. persicaria* occurs in both moderately shaded and open habitats, while *P. hydropiper* is excluded from shaded sites. Means  $\pm$  2 standard errors are shown based on 48 plants per species per light treatment, over a range of three moisture  $\times$  two nutrient levels within each light treatment (Sonia E. Sultan and Amity M. Wilczek, unpublished data).**

between traits and environment should be reexamined (Kelly and Beerling 1995).

The method of phylogenetic independent contrasts, based on differences in traits between related taxa rather than on the trait values themselves, provides a powerful tool for evaluating trait correlations in a phylogenetic context (Figure 8; see Ackerly 1999). For example, Reich et al. (1999) have demonstrated significant and consistent correlations among leaf functional traits, including life span, specific leaf area, assimilation rates, and nitrogen concentration, across a broad range of conifers and angiosperms inhabiting diverse environments. Ackerly and Reich (1999) found that these relationships were robust from a phylogenetic perspective, that is, correlations observed in the species data were also significant using independent contrasts (Figure 8). However, they also examined the correlation between leaf size and leaf life span and found a strong, negative correlation in the interspecific data. This suggests the rather unexpected result that smaller-leaved species also have longer-lived leaves. The use of independent contrasts showed that this correlation was due entirely to the single divergence in each of these traits between conifers and angiosperms, with no correlation between these traits within each group, and no significant association overall.

In a review of comparative studies of plant functional traits, Ackerly (1999) found that there does not appear to be any consistent bias in trait correlations or regression slopes when comparing phylogenetic and nonphylogenetic



**Figure 7.** Evolution of  $C_4$  photosynthesis in *Flaveria* (Asteraceae; Monson 1996). The colors along each branch of the phylogeny represent a hypothesized reconstruction of the evolution of photosynthetic pathways based on phylogenetic parsimony methods (i.e., the reconstruction that requires the fewest evolutionary transitions leading to the observed present-day distribution of photosynthetic types). The hatched bar indicates an uncertain reconstruction. If this branch is inferred to be  $C_3$ , there are three independent origins of  $C_3$ – $C_4$  intermediate pathways (including *F. angustifolia*); alternatively, if this branch is reconstructed as  $C_3$ – $C_4$  intermediate, there is one origin of  $C_3$ – $C_4$  and one subsequent reversal to  $C_3$ . The  $C_4$  pathway is inferred to have evolved independently three times, and in at least two of these cases, the  $C_3$ – $C_4$  type represented an intermediate evolutionary stage. A more recent molecular phylogeny of *Flaveria*, for 12 of the 20 species shown here, suggests at least two independent origins of  $C_4$  photosynthesis (Kopriva et al. 1996). Redrawn with permission from Monson (1996).

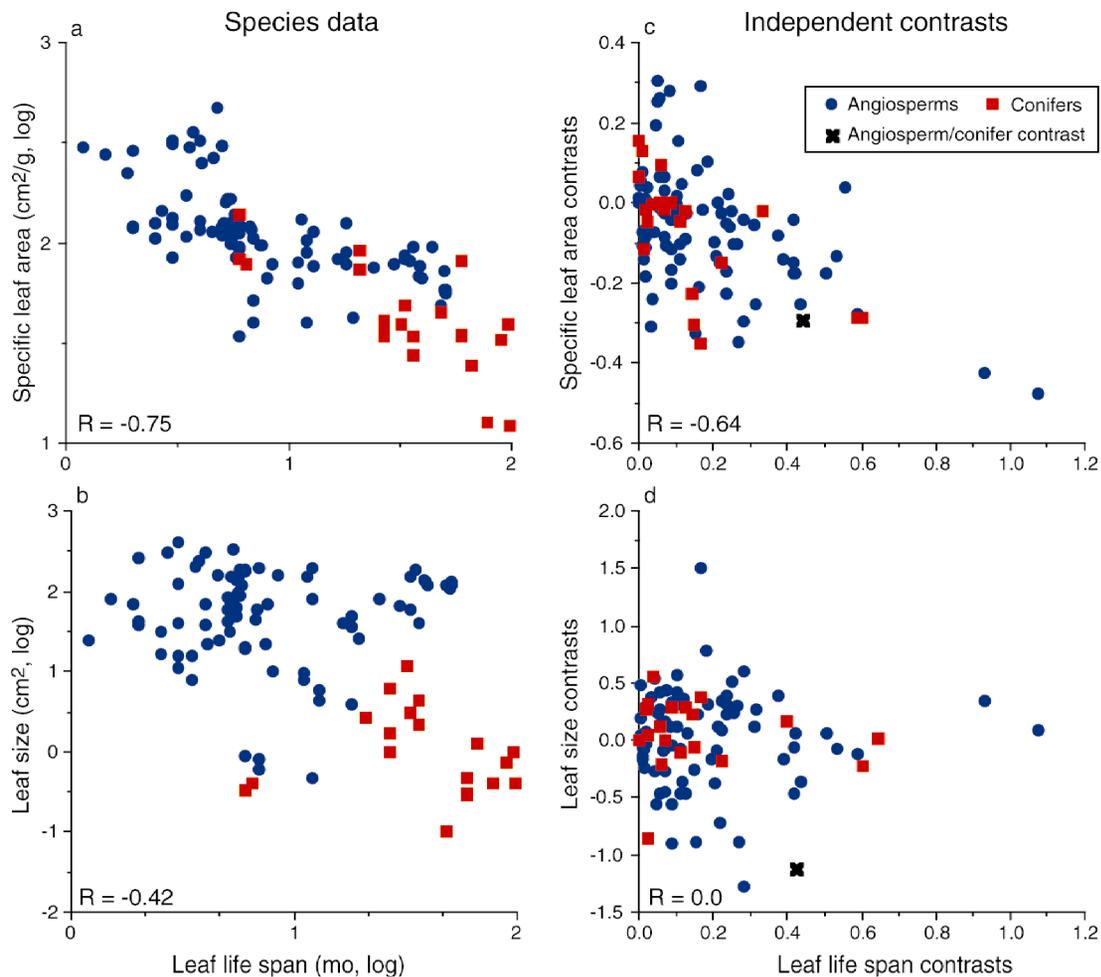
analyses, although the two methods at times lead to quite divergent results (as in the above example of leaf size and life span). Quantitative analysis of levels of convergent evolution (a high level of convergence for a trait indicates that closely related species are highly divergent, while distantly related species are most phenotypically similar) demonstrated that when traits are highly convergent, analyses of trait correlations based on interspecific patterns are most similar to results based on phylogenetically structured independent contrasts. However, these methods produce different results when convergence is low, as in the leaf size example, in which two major phylogenetic groups of species (conifers versus angiosperms) were markedly divergent. This result accords with the long-held view that studies of convergent traits provide particularly valuable and robust insights in the study of adaptive evolution.

### Future directions

In this article, we have reviewed recent advances in evolutionary ecophysiology of plants, focusing on the analysis of adaptive value of ecophysiological traits, the genetic basis of ecophysiological variation, and the evidence for adaptive differentiation among populations and species.

Enormous progress has been made in recent decades, reflecting advances in molecular biology, phylogenetics, and related fields, and the application of refined experimental and quantitative evolutionary approaches. We have also sought to highlight the technical and conceptual difficulties that accompany these approaches. No single approach will succeed in unraveling the complexities of the evolutionary process, and a recognition of their respective limitations is critical to gaining complementary perspectives on these problems. A comprehensive and integrated approach to ecophysiological evolution is particularly critical as global environmental changes impose new selective pressures on plant populations and species (e.g., Bazzaz et al. 1995, Potvin and Tousignant 1996). Several areas are particularly promising for research in the next decade:

- Analysis of the genetic architecture of ecophysiological traits, including identification of molecular markers for trait variation and the study of candidate genes responsible for naturally occurring variation in ecophysiological traits and plasticity in these traits; once identified, these genes would open up further opportunities for



**Figure 8. Analysis of correlations among leaf traits using independent contrasts.** Scatterplots of leaf life span versus specific leaf area (a, c) and leaf life span versus leaf size (b, d). Left-hand panels show correlations for 90 species of angiosperms (blue circles) and 22 conifers (red squares), based on the species values themselves (from Reich et al. 1999; leaf size data, Peter B. Reich, University of Minnesota, unpublished data). The right-hand panels show the corresponding patterns for phylogenetic independent contrasts (Ackerly and Reich 1999). Each contrast represents the difference in the trait values between two related taxa, and reflects an independent evolutionary divergence. The "X" indicates the contrast between angiosperms and conifers, reflecting the basal divergence in each of the three traits between these two major lineages. Correlations of independent contrasts provide an estimate of correlated changes in the evolution of the two traits. For leaf life span versus specific leaf area, the strong correlations among extant taxa (a) reflect a significant pattern of correlated evolutionary change (c). In contrast, for leaf life span versus leaf size, the correlation among species (b) is due to the divergence between angiosperms and conifers (d), whereas there is no correlation between these traits within each group and no evidence of correlated evolutionary changes overall. Modified from Ackerly and Reich (1999), with permission.

- mutant and transgenic studies of ecophysiological variation and covariation among multiple genes and traits.
- Elucidation of the developmental cascades that link changes at the molecular and biochemical level to changes in physiology, resource allocation, and performance.
- Development of general models of plant function, addressing the interactions among traits that influence survival, growth, and reproduction, and how these lead to diversification of suites of traits in contrasting environments.
- Integrated studies of model systems for ecophysiological evolution across multiple levels of organization (e.g., phytochrome-mediated responses, heat shock proteins, or seed oil composition), linking genetic variation, intrapopulation selection, and intraspecific and interspecific differentiation across contrasting environments.
- Long-term studies of selection in natural populations, in order to understand the role of temporal fluctuations and rare events on long-term evolutionary dynamics.
- Comparative study of ecophysiological evolution in closely related species, examining variation at genetic

and phenotypic levels, and mapping evolutionary changes onto phylogenies to understand the sequence and direction of evolutionary transitions.

- Evolutionary responses to changing environments, especially the effects of global change on plant populations and the influence of evolutionary changes on community and ecosystem responses to climate change.

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## References cited

- Ackerly DD. 1999. Phylogeny and the comparative method in plant functional ecology. Pages 391–413 in Press M, Scholes JD, Barker MG, eds. *Physiological Plant Ecology*. Oxford: Blackwell Scientific.
- Ackerly DD, Bazzaz FA. 1995. Seedling crown orientation and interception of diffuse radiation in tropical forest gaps. *Ecology* 76: 1134–1146.
- Ackerly DD, Reich PB. 1999. Convergence and correlations among leaf size and function in seed plants: A comparative test using independent contrasts. *American Journal of Botany* 86: 1272–1281.
- Alhiyal S, McNeilly T, Bradshaw AD, Mortimer AM. 1993. The effect of zinc contamination from electricity pylons: Genetic constraints on selection for zinc tolerance. *Heredity* 70: 22–32.
- Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* 7: 1–85.
- Arntz AM, DeLucia EH, Jordan N. 1998. Contribution of photosynthetic rate to growth and reproduction in *Amaranthus hybridus*. *Oecologia* 117: 323–330.
- Bazzaz FA, Jasienski M, Thomas SC, Wayne P. 1995. Microevolutionary responses in experimental populations of plants to CO<sub>2</sub>-enriched environments: Parallel results from two model systems. *Proceedings of the National Academy of Sciences of the United States of America* 92: 8161–8165.
- Bell DL, Sultan SE. 1999. Dynamic phenotypic plasticity for root growth in *Polygonum*: A comparative study. *American Journal of Botany* 86: 807–819.
- Bennington CC, McGraw JB. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. *Ecological Monographs* 65: 303–323.
- Bergelson J, Purrington CB. 1996. Surveying patterns in the cost of resistance in plants. *American Naturalist* 148: 536–558.
- Bradshaw AD. 1991. Genostasis and the limits to evolution. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 333: 289–305.
- Briggs D, Walters SM. 1997. *Plant Variation and Evolution*. 3rd ed. Cambridge (UK): Cambridge University Press.
- Chory J, Peto C, Feinbaum R, Pratt L, Ausubel F. 1989. *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell* 58: 991–999.
- Clausen J, Keck DD, Hiesey WM. 1940. *Experimental Studies on the Nature of Species I. Effect of Varied Environments on Western North American Plants*. Washington (DC): Carnegie Institute of Washington. Publication No. 520.
- Coleman JS, Heckathorn SA, Hallberg RL. 1995. Heat-shock proteins and thermotolerance: Linking molecular and ecological perspectives. *Trends in Ecology and Evolution* 10: 305–306.
- Dawson T, Sakai A, Weller S. 1998. Physiology, phylogeny and adaptation in Hawaiian plants. Page 8. *Ecological Society of America, 83rd Annual Meeting, Abstracts*.
- De Block M. 1993. The cell biology of plant transformation: Current state, problems, prospects, and the implications for plant breeding. *Euphytica* 71: 1–14.
- Donovan LA, Ehleringer JR. 1994. Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *American Journal of Botany* 81: 927–935.
- Downs CA, Heckathorn SA, Bryan JK, Coleman JS. 1998. The methionine-rich low molecular weight chloroplast heat shock protein: Evolutionary conservation and accumulation in relation to thermotolerance. *American Journal of Botany* 85: 175–183.
- Dudley SA. 1996a. Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypotheses. *Evolution* 50: 92–102.
- \_\_\_\_\_. 1996b. The response to differing selection on plant physiological traits: Evidence for local adaptation. *Evolution* 50: 103–110.
- Dudley SA, Schmitt J. 1995. Genetic differentiation in morphological responses to simulated foliage shade between populations of *Impatiens capensis* from open and woodland sites. *Functional Ecology* 9: 655–666.
- \_\_\_\_\_. 1996. Testing the adaptive plasticity hypothesis: Density-dependent selection on manipulated stem length in *Impatiens capensis*. *American Naturalist* 147: 445–465.
- Ehleringer J. 1991. 13C/12C fractionation and its utility in terrestrial plant studies. Pages 187–200 in Coleman DC, Fry B, eds. *Carbon Isotope Techniques*. San Diego: Academic Press.
- Ehleringer J, Björkman O, Mooney HA. 1976. Leaf pubescence: Effects on absorbance and photosynthesis in a desert shrub. *Science* 192: 376–377.
- Ehleringer J, Mooney HA, Gulmon SL, Rundel PW. 1981. Parallel evolution of leaf pubescence in *Encelia* in coastal deserts of North and South America. *Oecologia* 49: 38–41.
- Ehleringer JR, Monson RK. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* 24: 411–439.
- Emery RJN, Reid DM, Chinnappa CC. 1994. Phenotypic plasticity of stem elongation in 2 ecotypes of *Stellaria longipes*: The role of ethylene and response to wind. *Plant, Cell and Environment* 17: 691–700.
- Evans A. 1998. How do tradeoffs in resource use efficiencies arise? Page 9. *Ecological Society of America, 83rd Annual Meeting, Abstracts*.
- Farris MA, Lechowicz MJ. 1990. Functional interactions among traits that determine reproductive success in a native annual plant. *Ecology* 71: 548–557.
- Fishbein M, Venable L. 1996. Evolution of inflorescence design: Theory and data. *Evolution* 50: 2165–2177.
- Garnier E, Laurent G. 1994. Leaf anatomy, specific mass, and water content in congenic annual and perennial grass species. *New Phytologist* 128: 725–736.
- Geber MA, Dawson TE. 1990. Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant. *Oecologia* 85: 153–158.
- \_\_\_\_\_. 1997. Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. *Oecologia* 109: 535–546.
- Givnish TJ. 1988. Adaptation to sun and shade: A whole plant perspective. *Australian Journal of Plant Physiology* 15: 63–92.
- Gurevitch J. 1992. Differences in photosynthetic rate in populations of *Achillea lanulosa* from two altitudes. *Functional Ecology* 6: 568–574.
- Heckathorn SA, Poeller GJ, Coleman JS, Hallberg RL. 1996. Nitrogen availability alters patterns of accumulation of heat stress-induced proteins in plants. *Oecologia* 105: 413–418.

- Heckathorn SA, Downs CA, Sharkey TD, Coleman JS. 1998. The small, methionine-rich chloroplast heat shock protein protects photosystem II electron transport during heat stress. *Plant Physiology* 116: 439–444.
- Heckathorn SA, Downs CA, Coleman JS. 1999. Small heat-shock proteins protect electron transport in chloroplasts and mitochondria during stress. *American Zoologist* 39: 865–876.
- Hiesey WM, Milner HW. 1965. Physiology of ecological races and species. *Annual Review of Plant Physiology* 16: 203–213.
- Jordan N. 1992. Path analysis of local adaptation in two ecotypes of the annual plant *Diodia teres* Walt. (Rubiaceae). *American Naturalist* 140: 149–165.
- Kelly CK, Beerling DJ. 1995. Plant life form, stomatal density, and taxonomic relatedness: A reanalysis of Salisbury (1927). *Functional Ecology* 9: 422–431.
- Kingsolver JG, Schemske DW. 1991. Path analyses of selection. *Trends in Ecology and Evolution* 6: 276–280.
- Knight C, Ackerly DD. In press. Correlated evolution of chloroplast heat shock protein expression in closely related plant species. *American Journal of Botany*.
- Kopriva S, Chu C-C, Bauwe H. 1996. Molecular phylogeny of *Flaveria* as deduced from the analysis of nucleotide sequences encoding the H-protein of the glycine cleavage system. *Plant, Cell and Environment* 19: 1028–1036.
- Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* 36: 1210–1226.
- Lechowicz M, Wang ZM. 1998. Comparative ecology of seedling spruces: A phylogenetic perspective on adaptation. Page 16. *Ecological Society of America, 83rd Annual Meeting, Abstracts*.
- Linder CR. 2000. Adaptive evolution of seed oil composition. *American Naturalist* 156: 442–458.
- Lloyd DG. 1984. Variation strategies of plants in heterogeneous environments. *Biological Journal of the Linnean Society* 21: 357–385.
- MacDonald SE, Lieffers VJ. 1993. Rhizome plasticity and clonal foraging of *Calamagrostis canadensis* in response to habitat heterogeneity. *Journal of Ecology* 81: 769–776.
- McGraw JB, Antonovics J. 1983. Experimental ecology of *Dryas octopetala* ecotypes. I. Ecotypic differentiation and life-cycle stages of selection. *Journal of Ecology* 71: 879–897.
- Monson RK. 1996. The use of phylogenetic perspective in comparative plant physiology and developmental biology. *Annals of the Missouri Botanical Garden* 83: 3–16.
- Monson RK, Smith SD, Gehring JL, Bowman WD, Szarek SR. 1992. Physiological differentiation within an *Encelia farinosa* population along a short topographic gradient in the Sonoran Desert. *Functional Ecology* 6: 751–759.
- Niklas KJ. 1988. The role of phyllotactic pattern as a “developmental constraint” on the interception of light by leaf surfaces. *Evolution* 42: 1–16.
- \_\_\_\_\_. 1997. Adaptive walks through fitness landscapes for early vascular land plants. *American Journal of Botany* 84: 16–25.
- Pearcy RW, Valladares F. 1999. Resource acquisition by plants: The role of crown architecture. Pages 45–66 in Press M, Scholes JD, Barker MG, eds. *Advances in Physiological Plant Ecology*. Oxford: Blackwell Scientific.
- Pearcy RW, Yang W. 1996. A three-dimensional crown architecture model for assessment of light capture and carbon gain by understory plants. *Oecologia* 108: 1–12.
- Pigliucci M, Cammell K, Schmitt J. 1999. Evolution of phenotypic plasticity: A comparative approach in the phylogenetic neighborhood of *Arabidopsis thaliana*. *Journal of Evolutionary Biology* 12: 779–791.
- Potvin C, Tausignant D. 1996. Evolutionary consequences of simulated global change: Genetic adaptation or adaptive phenotypic plasticity. *Oecologia* 108: 683–693.
- Purrington C, Bergelson J. 1997. Fitness consequences of genetically engineered herbicide and antibiotic-resistance in *Arabidopsis thaliana*. *Genetics* 145: 807–814.
- \_\_\_\_\_. 1999. Exploring the physiological basis of costs of herbicide resistance in *Arabidopsis thaliana*. *American Naturalist* 154: S82–S91.
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999. Generality of leaf trait relationships: A test across six biomes. *Ecology* 80: 1955–1969.
- Robichaux RH, Carr GD, Liebman M, Pearcy RW. 1990. Adaptive radiation of the Hawaiian silversword alliance (Compositae-Madiinae): Ecological, morphological, and physiological diversity. *Annals of the Missouri Botanical Garden* 77: 64–72.
- Sandquist DR, Ehleringer JR. 1997. Intraspecific variation of leaf pubescence and drought adaptation in *Encelia farinosa* associated with contrasting desert environments. *New Phytologist* 135: 635–644.
- \_\_\_\_\_. 1998. Intraspecific variation of drought adaptation in brittlebush: Leaf pubescence and timing of leaf loss vary with rainfall. *Oecologia* 113: 162–169.
- Sawada S, Nakajima Y, Tsukuba M, Sasaki K, Hazama Y, Futatsuya M, Watanabe A. 1994. Ecotypic differentiation of dry matter production processes in relation to survivorship and reproductive potential in *Plantago asiatica* populations along climatic gradients. *Functional Ecology* 8: 400–409.
- Schmitt J. 1993. Reaction norms of morphological and life-history traits to light availability in *Impatiens capensis*. *Evolution* 47: 1654–1668.
- Schmitt J, Wulff R. 1993. Light spectral quality, phytochrome, and plant competition. *Trends in Ecology and Evolution* 8: 47–51.
- Schmitt J, McCormac AC, Smith H. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *American Naturalist* 146: 937–953.
- Schmitt J, Dudley SA, Pigliucci M. 1999. Manipulative approaches to testing adaptive plasticity: Phytochrome-mediated shade avoidance responses in plants. *American Naturalist* 154: S43–S54.
- Schuster WS, Phillips SL, Sandquist DR, Ehleringer JR. 1992. Heritability of carbon isotope discrimination in *Gutierrezia microcephala* (Asteraceae). *American Journal of Botany* 79: 216–221.
- Stitt M, Schulze ED. 1994. Does Rubisco control the rate of photosynthesis and plant growth? An exercise in molecular ecophysiology. *Plant, Cell and Environment* 17: 465–487.
- Sultan SE. 1995. Phenotypic plasticity and plant adaptation. *Acta Botanica Neerlandica* 44: 363–383.
- Sultan SE, Bazzaz FA. 1993a. Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. *Evolution* 47: 1009–1031.
- \_\_\_\_\_. 1993b. Phenotypic plasticity in *Polygonum persicaria*. II. Norms of reaction to soil moisture and the maintenance of genetic diversity. *Evolution* 47: 1032–1049.
- \_\_\_\_\_. 1993c. Phenotypic plasticity in *Polygonum persicaria*. III. The evolution of ecological breadth for nutrient environment. *Evolution* 47: 1050–1071.
- Sultan SE, Wilczek AM, Bell DL, Hand G. 1998. Physiological response to complex environments in annual *Polygonum* species of contrasting ecological breadth. *Oecologia* 115: 564–578.
- Tonsor SJ, Goodnight CJ. 1997. Evolutionary predictability in natural population: Do mating system and nonadditive genetic variance interact to affect heritabilities in *Plantago lanceolata*? *Evolution* 51: 1773–1784.
- Turesson G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* 3: 211–350.
- Winn AA. 1996. Adaptation to fine-grained environmental variation: An analysis of within-individual leaf variation in an annual plant. *Evolution* 50: 1111–1118.
- \_\_\_\_\_. 1999a. The functional significance and fitness consequences of heterophylly. *International Journal of Plant Sciences* 160: S113–S121.
- \_\_\_\_\_. 1999b. Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia*. *Journal of Evolutionary Biology* 12: 306–313.
- Yanovsky MJ, Casal JJ, Luppi JP. 1997. The VLF loci, polymorphic between ecotypes *Landsberg erecta* and *Columbia*, dissect two branches of phytochrome A signal transduction that correspond to very-low-fluence and high-irradiance responses. *Plant Journal* 12: 659–667.