

Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*)

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Summary

1. Given the rapid and reversible impact of the environment on plant ecophysiological traits, it has been speculated that such traits may have low heritabilities, high phenotypic plasticity and experience selection which is highly variable.
2. We estimated heritabilities, phenotypic plasticity and natural selection on five ecophysiological traits (carbon : nitrogen (C : N) ratio, leaf water content, specific leaf area (SLA), trichome density and water use efficiency (WUE)) of swamp milkweed *Asclepias incarnata*. In a 2-year field experiment, we studied 49 full-sibling families in a common garden and assessed phenotypic and genotypic selection.
3. Consistent evidence indicated selection for increased leaf trichome density and reduced WUE. Less strong evidence suggested selection for reduced SLA and leaf water content, and there was no selection on C : N of leaves.
4. Although the ecophysiological traits showed many phenotypic and genetic correlations, we found little evidence that these correlations would constrain adaptive evolution.
5. To evaluate the phenotypic plasticity of the same traits, we conducted a separate growth chamber experiment and found evidence that leaf water content, SLA and C : N responded to environmental conditions (herbivory, water stress, nutrient addition).
6. *Synthesis.* We have demonstrated natural selection on ecophysiological traits and their probable response to selection with a genotypic selection analysis. We cautiously reject the notion that these traits lack heritable variation and that genetic correlations among them constrain their response to selection. Thus, plants such as *A. incarnata* appear capable of adaptive responses in their ecophysiological traits.

Key-words: carbon : nitrogen ratio, drought tolerance, evolution of plant physiology, leaf water content, phenotypic plasticity, phenotypic selection, responses to environmental change, specific leaf area, trichome, water use efficiency

Introduction

Spatial and temporal variation in environmental conditions imposes natural selection on adaptive traits (Conner & Hartl 2004). In addition, global change is occurring at a rapid and unprecedented pace, exposing organisms to a host of novel biotic and abiotic conditions (Inouye 2000; Walther *et al.* 2002; Humphries & Baldwin 2003). Organisms can respond adaptively to these changes by maintaining physiological function despite altered conditions, inducing phenotypes via phenotypic plasticity and/or evolving by natural selection (Geber & Dawson 1993; Ward & Kelly 2004; Parmesan 2006).

The impact of environmental change may be especially acute for those species with restricted geographical ranges or those limited to specific abiotic conditions. For example, the swamp milkweed *Asclepias incarnata* occurs across North America, but is restricted to wetland sites where it grows in small populations (Woodson 1954; Ivey *et al.* 1999). We used this species as a model to predict the potential response of a habitat-restricted species to environmental change. To do so, we assessed the current variation in phenotypic traits exhibited by swamp milkweed, the heritable basis of these traits and their potential to respond to natural selection (Etterson & Shaw 2001).

Although a tremendous amount of research has focused on the ecophysiological responses of plants to environmental

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variability, remarkably little is known about the adaptive evolution of these traits (Dudley 1996; Ackerly *et al.* 2000; Arntz & Delph 2001; Geber & Griffen 2003; Caruso *et al.* 2005; Heschel & Riginos 2005; Sherrard & Maherali 2006; Donovan *et al.* 2007). Given the rapid and reversible impact of the environment on plant ecophysiological traits, it has been speculated that such traits may have low heritability and high phenotypic plasticity, and experience highly variable selection (Arntz & Delph 2001). To assess the possible evolutionary response of swamp milkweed to environmental change, we measured the heritability and phenotypic plasticity of several ecophysiological traits, as well as natural selection on these traits. In addition, we measured phenotypic and genetic correlations between the traits to estimate potential barriers to adaptive evolution (Caruso *et al.* 2005).

Our goal was to explore a suite of traits related to diverse ecophysiological functions. We thus measured leaf carbon : nitrogen (C : N) ratio, leaf water content, specific leaf area (SLA), trichome density and water use efficiency (WUE) as integrated proxies of ecophysiological function (Reich *et al.* 1997; Meziane & Shipley 2001). For example, C : N correlates with photosynthetic rates and plant nutrient status (Lambers *et al.* 1998). Water content is associated with allocation to cell walls and leaf density (Garnier & Laurent 1994). Trichomes have been implicated in many plant functions, including as a barrier against water loss and evapotranspiration (Woodman & Fernandes 1991). Determining the selection on and integration of these traits allowed us to rigorously test for the potential of plant ecophysiological traits to evolve.

This study represents one of the more rigorous analyses of natural selection on plant ecophysiological traits for two reasons. First, because the heritability and selection aspects of this 2-year study were conducted in the field, plants were exposed to a full complement of natural stresses and their growth was not controlled by artificial environments (Conner *et al.* 2003; Reznick & Ghelambor 2005). Second, we employed both phenotypic and genotypic selection analyses because traditional estimates of phenotypic selection (Lande & Arnold 1983) may be biased due to microsite differences that cause trait values and fitness to covary (Rausher 1992). Although sample size is reduced with genotypic selection analysis (because it employs family means rather than individual values), this approach is more appropriate for understanding responses to selection (Rausher 1992; Stinchcombe *et al.* 2002). In addition, ecophysiological traits are thought to be highly plastic to environmental conditions, so a genotypic selection analysis is particularly appropriate because family means average over phenotypic values which may be affected by microenvironment.

Methods

Asclepias incarnata is a native perennial plant that reproduces via hermaphroditic flowers (Woodson 1954; Ivey *et al.* 1999). Unlike some milkweeds, individual plants do not propagate vegetatively. Above-ground growth dies back each autumn, and new stems (often more than one) are produced each spring from the root stock. Seeds

from a single fruit of swamp milkweed are typically full-siblings because a single pollen sac (pollinium) sires all the seeds in a fruit (Gold & Shore 1995). In August 2004, we collected seeds at Montezuma National Wildlife Refuge at the north end of Cayuga Lake, New York from plants occurring at least 5 m apart along a transect through a marsh. One fruit was collected from each of 65 plants; the seeds from each fruit represent full-siblings of a single family ($n = 65$ families).

HERITABILITY AND SELECTION EXPERIMENT

In March 2005, seeds from the 65 full-sibling families were germinated and 6–10 plants from each family were grown individually in 500 mL pots for 1 month in a growth chamber. In late May, plants were transplanted to a completely randomized common garden (1 m spacing between plants) in Ithaca, New York, two miles from the Cornell University campus. The field site was a wet marshy habitat dominated by cattails. The natural vegetation was lightly weeded to allow establishment, but the site was not otherwise disturbed. *Asclepias incarnata* does not occur naturally at this site, but does grow nearby. Due to transplant mortality, we used 49 of the full-sibling families in this analysis, including only those families with at least three surviving plants ($n = 292$ total plants, on average, six plants survived per family). Transplant mortality was independent of genetic family (G -test, $P \gg 0.1$, data not shown).

In July 2006, we measured five traits which are integrated proxies of ecophysiological functions: leaf C : N, leaf water content, SLA, trichome density and WUE. In the field, we harvested a leaf disc (28 mm²) from the tip of the youngest fully-expanded leaf on each plant and placed each disk in a sealed plastic tube in a cooler with ice. We weighed the leaf discs (μg) on the day of collection (fresh mass) and after 24 h of drying at 40 °C (dry mass). We calculated water content as the ratio of water mass (fresh–dry mass) to the fresh mass of the leaf disc. We counted trichomes on both sides of each leaf disc under a dissection microscope and divided this number by disc area to estimate trichome density. We assessed SLA as the area per dry mass of the leaf disc. Two fully expanded leaves from each plant were also harvested, dried and ground for isotopic measurement of carbon isotope ration ($\delta^{13}\text{C}$), and total percent carbon and nitrogen at the Cornell University Stable Isotope Laboratory. $\delta^{13}\text{C}$ can provide an estimate of WUE (the amount of carbon fixed per unit of water transpired) integrated over the lifetime of the leaf. More negative $\delta^{13}\text{C}$ values correspond to lower WUE (Farquhar *et al.* 1989).

Few plants set seed in 2005. At the natural time of seed maturation in 2006 (September), we harvested all fruits from each plant and weighed them fresh. Fresh fruit mass correlates with seed number ($n = 49$, $r = 0.395$, $P = 0.005$) and total dry seed mass ($n = 49$, $r = 0.880$, $P < 0.001$). Thus, we used fruit mass as an estimate of fitness, incorporating both seed number and mass.

EVOLUTIONARY ANALYSES

We employed one-way ANOVA to test for genetic variation in the five ecophysiological traits and fruit mass. Family was the only factor included in the model (random effect). We calculated a full-sibling heritability for each of the traits as [$2 \times$ variance component of family/total variance] (estimated in SAS Proc Mixed, Littell *et al.* 1996). Full-sibling heritabilities approach narrow sense heritabilities when all genetic variance is additive, and are typically lower than broad sense heritabilities (Roff 1997). Low

heritabilities (due to high environmental variation) do not necessarily indicate low genetic variation. Therefore, we also calculated coefficients of genetic variation or 'evolvability' for each trait as $100 \times \sqrt{(\text{family variance component} \times 2)/(\text{mean trait value})}$. Evolvabilities better estimate a population's ability to respond to selection than do heritabilities because they are not affected by environmental variation (Houle 1992, J.K. Conner, personal communication).

Selection gradients measure the strength of direct adaptive selection, after correcting for correlations among the measured traits (Conner & Hartl 2004). We employed the standard phenotypic selection analysis that uses multiple regression ($n = 292$) (Lande & Arnold 1983) and a modified method (genotypic selection) that uses family means for all traits ($n = 49$) (Rausher 1992). In contrast, selection differentials estimate the strength of total selection, including both direct and indirect selection caused by correlations; these were calculated as Pearson product moment correlations between each trait and fitness. Relative values of the estimates of fitness [value/mean] were regressed on standardized estimates for the traits [(value-mean)/SD]. Selection gradients and differentials were estimated with models containing the linear terms only; estimates of quadratic selection gradients, which estimate curvature of the fitness function, were not significant and were thus eliminated from the model. Selection coefficients were measured using relative values of untransformed fitness, while P -values were generated from $\log(x + 1)$ -transformed data to improve normality of the residuals (J. K. Conner, personal communication).

In addition to correlations between each trait and fitness, we also present both phenotypic and genetic (family mean) Pearson correlations between all trait pairs (SYSTAT version 9). These correlations help elucidate the selection gradient results and identify possible synergisms or constraints in trait responses to natural selection (Conner & Hartl 2004; Caruso *et al.* 2005). Some correlations between traits were expected. For example, SLA can be positively correlated with water content because leaf dry mass is an important component of both measures. Nonetheless, our null hypothesis was that there would be no correlations between traits because none of these correlations is statistically inevitable. To control for Type I error, we used a binomial expansion test to examine whether the number of significant correlations we found could have occurred by chance (Zar 1996). Our six traits yielded a total of 15 pairwise correlations, and thus two significant ($P < 0.05$) correlations could have been found by chance ($P = 0.135$, binomial expansion test).

PHENOTYPIC PLASTICITY EXPERIMENT

To test for the phenotypic responses of swamp milkweed to changing environmental conditions, we conducted a growth chamber experiment.

Each of the environments we tested (soil moisture, nutrient availability and herbivory) varies considerably in space and time and has been predicted to change with anthropogenic influences (Geber & Dawson 1993; Parmesan 2006). The seeds used were randomly sampled from the same stock as that used in the field experiment (total sample size = 22 plants). We measured the same five ecophysiological traits on plants that experienced one of four environments within the growth chamber (12 h of daylight, 26 °C (day) and 22 °C (night)); (i) Pots (500 mL) in the 'control' environment had 100% potting soil (Metro-Mix, Sun Gro Horticulture, Bellevue, WA), *ad libitum* water and one application of fertilizer (N : P : K 21 : 5 : 20 150 p.p.m. N) 10 days after the experiment began. The three other environments were identical to the control in all respects except for one manipulation each. (ii) A 'dry' environment was achieved by mixing 50% potting soil with 50% perlite (an inert rocky substance that increases drainage). For pots without plants, the soil in the dry environment held 13% less water than the controls immediately after watering ($n = 10$ of each, $P < 0.001$) and 23% less water than the control soil after 6 days without watering ($P < 0.001$). (iii) The high 'nutrient' environment was fertilized weekly starting 10 days after the experiment began. (iv) The 'herbivory' environment received a newly hatched monarch butterfly (*Danaus plexippus*) caterpillar when each plant was at the four leaf stage. The caterpillars fed until each plant had lost 25% of their leaf tissue. Ten days after caterpillars were removed from plants in the herbivory environment (when plants were *c.* 39-days-old) leaf tissue was harvested. Traits were measured as in the field experiment.

Analysis was initially conducted as a one-way MANOVA with all five traits included as multivariate response variables. This was followed by univariate analyses to identify the individual traits that responded. We present trait means for each environment by trait combination, as well as the maximum percent difference (= plasticity) for each trait among the four environments.

Results

HERITABILITY AND SELECTION EXPERIMENT

Heritability values were generally low, but SLA, $\delta^{13}\text{C}$ and C : N values were significantly different from zero (Table 1). Another measure of the potential response to natural selection, evolvability, showed values that were substantial for all traits except water content, and were particularly high for trichome density and fruit mass (Table 1).

Overall, the phenotypic selection analysis revealed directional selection gradients (i.e. direct selection after accounting for

Table 1. Range of variation, heritability, evolvability and phenotypic plasticity estimates for five ecophysiological traits and fruit mass of swamp milkweed, *Asclepias incarnata*. The first three columns are calculated from full-sib families in a field common garden, with range of variation reflecting the lowest to highest family means. Plasticity is given as the maximum percent difference from the lowest to the highest mean value across the four growth chamber environments. Significance tests from ANOVAs indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bold values are statistically significant at $P < 0.05$

Plant trait	Range of variation	Heritability	Evolvability	Plasticity (%)
Trichome density (hairs/cm ²)	416–728	0.050	6.85	94.7
% Water	69.18–75.84	0.041	0.77	17.8**
SLA (mm ² /mg)	15.26–19.78*	0.136*	4.50*	43.9*
WUE ($\delta^{13}\text{C}$)	–28.35– –26.43***	0.332***	1.51***	2.6
C : N	15.20–21.80*	0.158*	5.98*	126.3***
Log($x + 1$) fruit mass (g)	1.03–3.45	0.037	10.96	NA

Table 2. Phenotypic and genotypic selection gradients (β) for five plant ecophysiological traits (standardized) and relative fitness (fruit mass) of swamp milkweed, *Asclepias incarnata*. Bold values are statistically significant at $P < 0.05$

	Phenotypic β	P	Genotypic β	P
Trichomes	0.120	0.006	0.081	0.010
% Water	-0.044	0.108	-0.064	0.019
SLA	-0.138	0.002	0.104	0.478
WUE ($\delta^{13}\text{C}$)	0.026	0.118	-0.132	0.013
C : N	-0.060	0.329	-0.060	0.203

correlations) for reduced SLA and increased trichome density ($F_{5,286} = 8.617$, $P < 0.001$, $R^2 = 0.131$) (Table 2). In the genotypic analysis, which used the 49 full-sibling families as the unit of replication, directional selection was also found for increased trichome density, reduced leaf water content and reduced WUE ($F_{5,43} = 4.279$, $P = 0.003$, $R^2 = 0.332$) (Table 2). However, there was no evidence for selection on SLA in the genotypic analysis. Neither analysis indicated selection on C : N. The substantial increase in the R^2 values from phenotypic to genotypic analyses suggests high random error in our measurements, and points to genotypic values being more reliable.

Selection differentials estimate the strength of total selection, including both direct selection and indirect selection caused by correlations between traits. The genotypic estimates of total selection (differentials, Table 3) were completely consistent with estimates of direct selection (gradients, Table 2), suggesting little evidence for constraints on adaptation caused by genetic correlations. For both phenotypic and genetic analyses, the observed number of significant correlations (9 out of 15 and 6 out of 15, respectively) (Table 3) is highly unlikely to have occurred by chance (binomial expansion test, $P < 0.001$).

We found a strong positive phenotypic and genetic correlation between SLA and water content, and phenotypic selection differentials on both traits were significant. However, when we accounted for trait correlations with multiple regressions, only SLA was significant. Thus, the phenotypic selection differential that predicts reduced water content was likely to be driven by indirect selection (i.e. via correlations between water content, SLA and trichome density). In contrast, there

was genotypic selection in both the gradient and differential analysis to reduce water content, and only weak evidence for selection on SLA. Although water content and SLA are correlated, they show slightly different patterns of selection and heritability. Thus, it is difficult to reconcile which trait is the true target of selection. Although trichome density and SLA were negatively, phenotypically correlated (Table 3), selection on these two traits persisted in both gradient and differential analysis. In other words, it appears that both traits were direct targets of selection.

In the plasticity experiment, the environmental treatments caused phenotypic responses in the ecophysiological traits overall (MANOVA: Wilks' $\lambda = 0.154$, $F_{15,39} = 2.527$, $P = 0.010$). This was most evident in the effects on leaf water content, SLA and C : N (univariate ANOVAs: leaf water content, $F_{3,18} = 4.854$, $P = 0.012$; trichome density, $F_{3,18} = 1.994$, $P = 0.151$; SLA, $F_{3,18} = 3.328$, $P = 0.043$; $\delta^{13}\text{C}$, $F_{3,18} = 2.200$, $P = 0.123$; C : N $F_{3,18} = 8.571$, $P < 0.001$) (Table 1, Fig. 1).

Discussion

The most robust result of our selection analysis on ecophysiological traits of swamp milkweed was the positive selection on trichome density. Although we did not find significant heritability for this trait, its response to both phenotypic and genotypic selection (Table 2), its substantial evolvability and its relatively low degree of plasticity (Table 1) all suggest that trichome density may respond to selection. We emphasize that the high variance of our data set was likely to have contributed to the low heritabilities. Such joint assessments of heritability and selection from field experiments have rarely been conducted, although it is well known that comparable data from controlled environments may be misleading (Conner *et al.* 2003; Reznick & Ghalambor 2005). Knowledge of selection on plant ecophysiological traits is particularly scarce, despite the importance of such traits for resource acquisition and allocation.

Trichomes are known to mediate interactions between plants and the biotic and abiotic environment. For example, insect herbivores imposed natural selection for increased trichomes in *Arabidopsis* spp. (Mauricio & Rausher 1997; Karkkainen *et al.* 2004; Loe *et al.* 2007). In other studies, selection on trichomes was related to their ecophysiological

Table 3. All pairwise Pearson product moment correlations between five plant ecophysiological traits (standardized) and relative fitness (fruit mass) of swamp milkweed, *Asclepias incarnata*. Above the diagonal are genetic correlations ($n = 49$) and below are phenotypic correlations ($n = 292$). The vertical column 'Fitness' indicates the phenotypic selection differentials, while the horizontal row 'Fitness' indicates selection differentials from the genotypic analysis. Bold values are statistically significant at $P < 0.05$

	Fitness	Trichomes	% Water	SLA	WUE ($\delta^{13}\text{C}$)	C : N
Fitness		0.209*	-0.045*	0.163	-0.200*	-0.105
Trichomes	0.159***		0.051	0.111	-0.027	-0.076
% Water	-0.120***	-0.164**		0.420**	0.129	-0.205
SLA	-0.177***	-0.284***	0.554***		0.264	-0.402**
WUE ($\delta^{13}\text{C}$)	0.031	-0.051	0.013	0.026		-0.353*
C : N	-0.004	0.067	-0.315***	-0.292***	-0.262***	

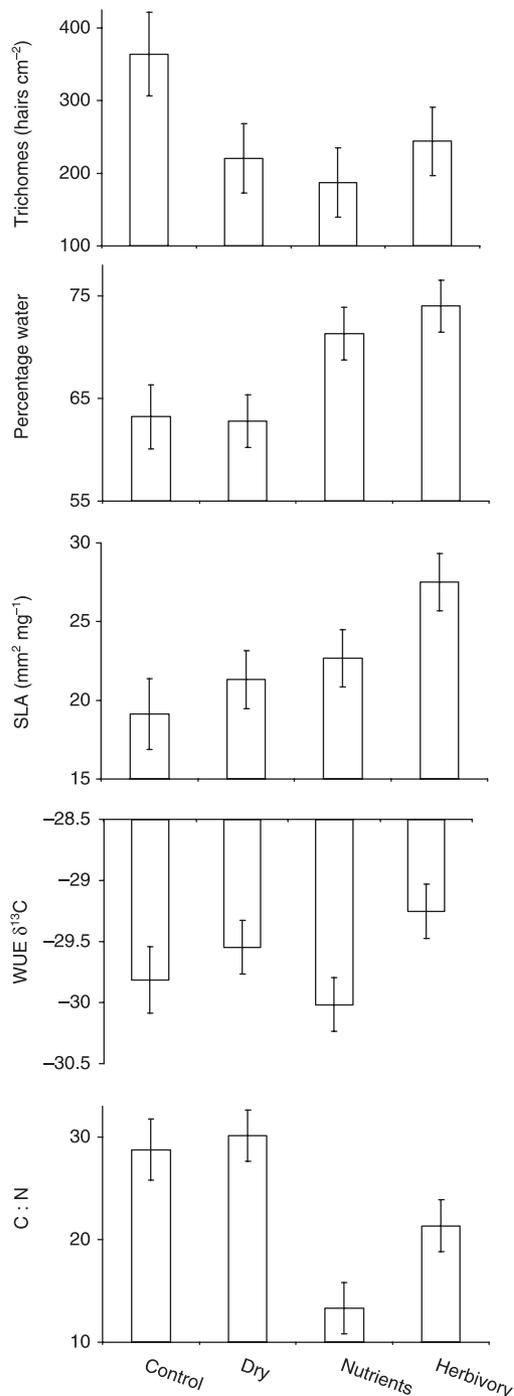


Fig. 1. Phenotypic plasticity in five ecophysiological traits of swamp milkweed *Asclepias incarnata*. Shown are least squares means and SE. WUE, water use efficiency and SLA, specific leaf area. Neither WUE or trichomes were significantly affected by environment (see Results for details).

function via water relations. For example, trichomes of *Encelia farinosa* vary intraspecifically along a moisture gradient. These trichomes reduce the absorbance of solar radiation which alters photosynthesis, WUE and drought tolerance (Sandquist & Ehleringer 1998, 2003a,b). Given that leaf herbivores were exceedingly rare in the 2 years of this study

(< 5% damage on nearly all plants; Agrawal, unpublished data), and the first growing season was dry (40% less precipitation in July and August than the 30 year average), we speculate that drought could have been the causal agent of selection. Observationally, it was clear that plants experienced stress in 2005, as indicated by early leaf fall and minimal growth. However, 2006 was a wet year (68% higher precipitation than normal), so if the selection was driven by the 2005 drought, the effects of this drought must have persisted into the next year.

We also found genotypic selection for reduced WUE, shown by both the selection gradient and differential analyses. This result is consistent with the wet conditions of 2006. Recent studies of selection on (or breeding for) WUE reported that WUE often affects plant productivity in drought conditions; however, these effects varied widely from positive to negative (Condon *et al.* 2004; Heschel *et al.* 2004; Donovan *et al.* 2007). For example, Heschel *et al.* (2005) suggest that higher WUE is favoured when drought affects plants late in the growing season, but that lower WUE can be favoured when plants experience drought early in the season. Thus, an understanding of both the costs and benefits of WUE will be required to predict its response to environmental variability. Our estimate of WUE was an indirect integrated measure (carbon isotope ratio, $\delta^{13}\text{C}$) and several physiological mechanisms including photosynthetic rate and stomatal conductance were likely to be responsible for the $\delta^{13}\text{C}$ results (Farquhar *et al.* 1989; Sandquist & Ehleringer 2003a; Sherrard & Maherali 2006). Nonetheless, significant heritability (Table 1), lack of phenotypic plasticity (Table 1), and substantial genotypic selection (Table 2) indicate that WUE may evolve in response to natural selection. This is somewhat in contrast with our results for water content, which also show negative genotypic selection but no significant heritability, very low evolvability and strong phenotypic plasticity (Tables 1 and 2).

Our phenotypic and genotypic selection analyses on the five traits of swamp milkweed were not completely congruent. The former indicated selection for reduced SLA but the latter indicated selection for reduced water content and WUE. Genetic correlations between SLA and WUE are variously reported as negative or non-significant for other species (Caruso *et al.* 2005; Monclus *et al.* 2005; Omami *et al.* 2006). Although they are not all statistically significant in our experiment, we found all of the phenotypic and genetic correlations between SLA, water content, and WUE to be positive (Table 3). Thus, consistent selection against these traits is indicative of their integrated nature. Although it was clear that the ecophysiological traits measured were weakly correlated with each other (Table 3), the targets of selection were also mostly clear in multiple regression analyses. For example, although the C : N ratio of leaves was phenotypically and genetically correlated with other traits, C : N was never under significant direct or total selection (Table 3). C : N was also the most phenotypically plastic trait by far, suggesting that adjusting C : N to the environment may be important and feasible, especially under spatially or temporally variable conditions.

CONSTRAINTS ON THE EVOLUTION OF ECOPHYSIOLOGICAL TRAITS

The adaptive evolution of plant traits could be limited by many factors including low heritabilities and genetic correlations among the traits. We did find low heritabilities for all traits (Table 1). These results are influenced by large error variances, which likely reflect substantial environmental heterogeneity in the field. Despite the non-significant heritabilities, trichome density and fruit mass showed reasonable evidence for genetic variation (as measured by evolvability; Table 1). Furthermore, the significant genotypic selection analyses suggest indirectly that underlying heritable variation is likely. Thus we cautiously reject the notion that a lack of heritable variation would pose a barrier to response to selection for the traits measured in this study.

Ecophysiological traits often rapidly respond to the microenvironment and it has been thought that trait plasticity may reduce estimates of heritability (Arntz & Delph 2001). This logic is based on heritability being calculated as genetically based variation divided by total phenotypic variation in a trait; as plasticity increases total phenotypic variation, heritability estimates will necessarily be lower for more plastic traits compared to less plastic traits. Indeed, we found significant phenotypic plasticity for water content, SLA and C : N (Table 1). Plasticity of ecophysiological traits has been widely demonstrated in other species (Sultan *et al.* 1998), and heritability and plasticity of these traits may differ among environments. For example, *Polygonum persicaria* showed substantial plasticity for WUE, and this plasticity varied among populations with different moisture regimes (Heschel *et al.* 2004). Thus, it is increasingly recognized that heritability and plasticity are not independent, and may show complex relationships in the evolutionary process.

Genetic correlations between traits may also constrain an adaptive response to selection. This is especially true when two positively correlated traits are selected for in opposing directions (or when negatively correlated traits are selected for in the same direction). Alternatively, there might be selection for trait integration (i.e. synergisms rather than constraints). Many plant ecophysiological traits appear to be strongly integrated. For example, Reich *et al.* (1999) have shown patterns of strong positive correlations among leaf traits such as SLA, leaf nitrogen and photosynthetic rate. These traits may be expected to evolve in a correlated fashion in response to habitat variation (Chapin *et al.* 1993; Reich *et al.* 1999). Indeed, the current genetic and phenotypic data on swamp milkweed supports this view with a strong positive correlation between SLA and leaf nitrogen (data not shown). Despite significant genetic correlations between traits, correlated traits did not experience opposing selection in our analyses. We found consistent patterns between direct and total genotypic selection, suggesting little evidence for constraints on adaptation caused by genetic correlations (Tables 2 and 3).

Conclusion

We have presented phenotypic and genetic evidence for selection on plant ecophysiological traits from a field experiment. Such measures from field experiments are relatively rare, despite the importance of ecophysiological traits in resource acquisition and allocation, and the potential importance of selection on these traits as a result of environmental change. Our estimates of heritabilities were low and we found some evidence for phenotypic plasticity in our ecophysiological traits. However, our data, particularly from the genotypic selection analysis, indicate that a response to natural selection is likely. We found little evidence that correlated traits experience opposing selection, and thus genetic correlations are unlikely to constrain adaptive evolution of these traits (Table 3).

There has been some controversy in the literature as to whether plant species will be able to physiologically adapt to environmental change (Kelly & Levin 1997; Bone & Farres 2001; Steinger *et al.* 2007). Whether such a response could prevent local extinction depends on the amount of heritable variation in a population (and therefore the speed with which it may adapt), especially when selection pressure is strong (Etterson & Shaw 2001). More field estimates of heritability and selection on ecophysiological traits are needed to resolve this issue. Our data directly bear on this debate, indicating that *A. incarnata* could evolutionarily respond to selection in its ecophysiological traits.

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