Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia*?

A. A. WINN

Department of Biological Science, Florida State University, Tallahassee, Florida, USA

Keywords:

Dicerandra linearifolia;
natural selection on leaf traits;
phenotypic plasticity;
seasonal variation;
temperature adaptation.

Abstract

Empirical studies of phenotypic plasticity have often relied on the plausibility that a plastic response to the environment would increase fitness in order to diagnose the response as adaptive. I conducted a test of the hypothesis that seasonal variation in leaf traits is an adaptive response to seasonal variation in environmental conditions faced by the annual plant *Dicerandra linearifolia*. This species exhibits variation in leaf morphology and anatomy in response to temperature that is consistent with the expectations for adaptive plasticity. I examined variation in the size, thickness and density of stomata of leaves that develop in summer and winter and used analysis of phenotypic selection during winter and summer seasons to test the hypothesis that seasonal variation in these traits is adaptive. Regression analyses of estimated dry mass (as a proxy for fitness) on leaf traits revealed no evidence supporting the adaptive hypothesis. Selection favoured individuals with large and thick leaves in both winter and summer, and density of stomata had little or no effect on estimated relative fitness in any season. Correspondence between seasonal variation in leaf thickness and density of stomata and expectations for adaptive plasticity appears to be purely fortuitous. Seasonal variation in leaf traits may persist simply because there is no selection against individuals in which these traits vary. My results underscore the importance of definitive tests of the hypothesis of adaptation to distinguish adaptive plasticity from neutral or nonadaptive phenotypic plasticity.

Introduction

The ability of a single genotype to produce different phenotypes may constitute a significant means of adaptation to variation in the environment, but observation of plasticity in a phenotypic trait does not necessarily indicate adaptation. Because adaptive and nonadaptive phenotypic plasticity have different evolutionary causes and consequences, distinguishing which patterns of plasticity are adaptive is critical to identifying empirical systems in which we can further our understanding of how adaptive phenotypic plasticity evolves. Despite the importance of this distinction, many empirical studies of putatively adaptive phenotypic plasticity (e.g. Sultan & Bazzaz, 1993a–c; Andersson & Shaw, 1994; Pigliucci & Schlichting, 1995; Sultan, 1996; Winn, 1996a,b) rely heavily on the plausibility that a plastic response is adaptive rather than on definitive tests of the hypothesis of adaptation (but see Weis & Gorman, 1990; Kingsolver, 1995a,b; Schmitt *et al*., 1995; Dudley & Schmitt, 1996). In this paper, I demonstrate that a very plausible scenario for adaptive response of leaf traits to seasonal variation in temperature is not supported by a test of the hypothesis of adaptation. This result calls into question the wisdom of reliance on plausibility arguments to identify adaptive plasticity.

One reason for reliance on plausibility to diagnose adaptive plasticity is that logistics of the experiments necessary to demonstrate that a plastic response is adaptive can be daunting. Analysis of phenotypic selection can be used to distinguish between adaptive and nonadaptive plasticity (van Tienderen, 1990, 1991; Weis & Gorman, 1990; Kingsolver, 1995a,b; Schmitt *et al*., 1995; Dudley & Schmitt, 1996). If different values of a

Correspondence: A. A. Winn, Department of Biological Science, Florida State University, Tallahasse, FL 32306–1100, USA.
E-mail: winn@ bio. fsu. edu

trait confer the greatest fitness in different environments and if the appropriate phenotypes are produced in each environment, then plasticity in the trait is adaptive. If the optimum value of a trait is the same in two or more environments, then homeostasis is favoured, and variation in the trait is not adaptive. Tests of the hypothesis that a plastic response is adaptive require measuring the phenotypes and relative fitnesses of genetic replicates exposed to a range of environments in the field. This can be a considerable undertaking.

For modular organisms, difficulties often inherent in tests of the hypothesis that a plastic response is adaptive can be reduced considerably because the modules that make up an individual develop sequentially, creating repeated opportunities for small-scale spatial and temporal variation in the environment to affect module phenotype in a single individual (Winn, 1996a). Consequently, plastic response of traits of modules can be described for a single individual that is exposed to different environments, eliminating the need for genetic replicates. Many plants exhibit within-individual variation in leaf size, shape and physiology (see, for example, Lee & Richards, 1991; Evans, 1992; Dong, 1993; de Kroon & Hutchings, 1995; Winn, 1996a,b). Such variation can arise from phenotypic plasticity in response to fine-grained variation in the environment and could be a means of adaptation to variation in the environment at a fine spatial and/or temporal scale (Evans, 1992; Dong, 1993; Winn, 1996a,b).

The annual plant *Dicerandra linearifolia* exhibits seasonal variation in leaf morphology and anatomy that is consistent with the expectations for adaptive plasticity in these traits. As a consequence of their nearly year-long life cycle, individuals of this species in their natural habitat must be able to photosynthesize at air temperatures ranging over 20 °C. Leaves that develop at 18 °C in growth chambers are larger in area and thicker in cross-section and have a lower density of stomata than leaves of the same individuals that develop at 30 °C (Winn, 1996a,b). Studies of the effects of leaf traits on photosynthetic efficiency in several species support the hypothesis that larger, thicker leaves with fewer stomata are more efficient at low temperature and that smaller, thinner leaves with more stomata are more efficient at high temperatures (Jones, 1983; Huner, 1985; Korner *et al.*, 1989). Thus the hypothesis that seasonal, within-individual variation in leaf traits is adaptive for *D. linearifolia* is plausible. There is also significant additive genetic variation for the pattern of variation in leaf traits (Winn, 1996a), so if a particular pattern is favoured by natural selection, response to selection is possible in this species.

In this paper I describe the phenotypic traits of leaves of *Dicerandra linearifolia* produced in winter and summer, and I test the hypothesis that characteristics of leaves of *D. linearifolia* that develop in the winter are favoured by selection under the environmental conditions that prevail in winter and that those of leaves produced in summer are favoured during the summer months. Multivariate regression methods are used to quantify the relationships of several leaf traits to plant growth and survival during winter and summer for 2 years in the field. These analyses constitute tests of the hypothesis that seasonal variation in leaf traits is adaptive for this species.

Materials and methods

Dicerandra linearifolia (Lamiaceae) is an annual plant of open, sandy habitats that is endemic to north-west Florida and adjacent regions of Georgia and Alabama. My study population was in sandhills habitat in the St Marks National Wildlife Refuge in Wakulla County, Florida. Seeds of this species germinate in December, and flowers are produced in October. Consequently individuals must be able to photosynthesize at both winter and summer temperatures, which can range from 14 °C to 34 °C (mean monthly maxima) over this time period at the study site.

Field methods

In December 1992 and January 1993 I marked and numbered 245 naturally occurring seedlings of *D. linearifolia* as they emerged. All seedlings within a defined area (10 m × 15 m) were included in the sample. This area was enlarged, and a total of 848 more seedlings were added to the sample by June. To quantify characteristics of leaves produced during winter, I measured the length and thickness of a leaf produced at the first node by each marked individual in February. For a subset of these plants ($n = 97$), I also measured the density of stomata on the leaves. Leaf thickness was measured from cross-sections taken from the centres of leaves viewed under a dissecting microscope fitted with an ocular micrometer. Density of stomata was measured from impressions of leaf surfaces made in clear nail enamel (described by Winn, 1996a). I delayed the destructive measurement of leaf thickness and density of stomata until leaves showed signs of senescence in order to minimize influence of leaf removal on plant performance. I measured the length, thickness and stomatal density of a second leaf (the most recent fully expanded leaf) produced in July to quantify characteristics of leaves produced during summer. All measurements were of fully mature leaves.

At the end of winter (late March), the beginning of summer (late May), and the end of summer (early September), I measured the stem height and counted all leaves on each marked individual. At each of these times, I made similar measurements on 25 nearby, unmarked individuals, which were then harvested, dried and weighed to the nearest 0.001 mg. For each date, I carried out a multiple regression of plant dry weight on stem height and number of leaves of the harvested plants.

I used the regression equation and measurements of stem height and leaf number of marked individuals to estimate the dry weights of the marked plants at the end of winter and at the beginning and end of summer. In November the flowers of all surviving individuals were counted. These individuals were harvested, dried and weighed to the nearest 0.01 mg.

In 1994, transplanted seedlings rather than naturally occurring plants were measured because they were easier to relocate and measure without disturbance to surrounding vegetation. Seeds were taken from plants that had been collected as seedlings from the study population during the previous January and had been raised in the greenhouse. These seeds were germinated in an unheated greenhouse in individual compartments filled with soil collected from the field site. The time of emergence (days since planting) was recorded for each seedling.

In January 1994, seedlings from the greenhouse were planted 10 cm apart in 11 blocks (2 m × 1 m) of 96 seedlings per block. Seedlings were distributed among blocks only to make them more easily accessible for measurement. Individuals from all blocks were combined for all analyses. Locations of the blocks were chosen haphazardly with the requirement that they appear to consist of suitable habitat for *D. linearifolia*. All 11 blocks were contained in an area 20 m on a side. Disturbance to the soil and surrounding vegetation was kept to a minimum. Seedlings had just begun to expand their first true leaves at the time of transplanting and were similar in size and developmental stage to nearby, naturally occurring seedlings.

Leaf length and width, leaf thickness and density of stomata were measured for a leaf produced at the first node in February and for a second leaf (the most recent fully expanded leaf) produced in July. At each sampling time, a regression of leaf area on leaf length and width was constructed from leaves collected from nearby unmarked individuals. These regressions were used to estimate areas of measured leaves from their lengths and widths. Using regressions constructed from unmarked plants as in 1993, I estimated plant dry weight at the end of the winter and at the beginning and end of the summer. Regression equations for predicting dry weight were constructed as described above for the 1993 study.

Analyses

Several different regression analyses were used to quantify the pattern of natural selection on leaf traits during the winter and summer of each year of the study and to test for the expected functional relationship of each trait to individual survival and growth. The dependent variable in these regressions was an estimate of individual relative fitness. The fitness of an individual in a season was estimated as (survival) × (increase in dry mass), where survival was scored as one for individuals that survived the season and zero for those that died during the season. Dry mass was estimated from regressions. Relative fitness was calculated as individual fitness/average fitness for all plants. Individuals that died were assigned a fitness of zero.

To determine whether dry weight was a reasonable proxy for fitness, I regressed total flower number on actual final dry weight for the 1993 sample. Because seeds are dropped from the plants as they mature over several weeks, it was not possible to compare dry weights with total seed production. I also compared the mean estimated dry weights of individuals that did and did not survive from one census to the next for two census intervals (one in winter and one in summer) in each year. Estimated dry weights were log transformed prior to this analysis so that they would meet assumptions of normality and homogeneity of variances. Means were compared in a two-sample *t*-test.

Separate regressions of estimated relative fitness on leaf traits were calculated for winter and summer data. Relative fitness for the winter analyses was calculated on the basis of dry weights estimated at the end of the winter. Relative fitness for the analysis of summer selection was calculated from the difference between estimated dry weights at the beginning and end of the summer. I used the increase in dry weight over the summer rather than the dry weight at the end of the summer to estimate fitness for the summer analyses because I was interested in the relationship between leaf traits and the component of fitness that is determined during the summer. In 1993, the estimated increase in dry weight and the total dry weight at the end of the summer were highly correlated ($r = 0.969$, $n = 395$), and using the total dry weight to estimate relative fitness gave the same results as increase in dry weight over the summer.

Multiple regressions of estimated relative fitness on leaf traits were calculated separately for each season, and the partial regression coefficients were examined to test the hypothesis that selection favoured different leaf traits during different seasons. Selection on each trait during each season was quantified as the partial regression coefficient (β) in the regression of estimated relative fitness on the set of leaf traits (Lande & Arnold, 1983). These regression coefficients are equivalent to the selection gradients of Lande & Arnold (1983). I also quantified stabilizing/disruptive selection on each trait as one half of the coefficient of the squared value of the trait in regressions of estimated relative fitness on trait values and the squared values of the traits (Lande & Arnold, 1983).

I regressed my estimates of individual relative fitness on the set of leaf traits measured in each season of 1993 and the leaf traits plus time of emergence in each season of 1994. Density of stomata was omitted from the winter 1993 analysis because measurements were only available for a subset of individuals for which leaf length and thickness had been measured ($n = 55$).

I further dissected the relationships between plant performance and leaf traits in separate regressions of survival and growth on the leaf traits measured in each season. For estimates of the effects of leaf traits on survival, the dependent variable in the multiple regression was zero for individuals that did not survive the season and one for those that did survive. The coefficients from these analyses measure viability selection on leaf traits and timing of emergence. No analysis was conducted for the summer of 1994 because only five individuals for which I had all leaf measurements died during the summer interval.

For estimates of the direct effects of leaf traits on growth, the dependent variable in the multiple regression was estimated relative fitness, but only individuals that had survived the selection interval were included in these analyses. Coefficients from these analyses for summer data are estimates of fecundity selection. Those from winter analyses estimate a combination of fecundity and viability selection because some individuals that survive the winter will die before the end of the summer, when flowering and seed set begin.

It is possible that correlations between the values of traits expressed in different seasons might influence estimates of selection coefficients. To account for the possibility that correlations in the expression of leaf traits across seasons might obscure patterns of selection, I also carried out a multiple regression of relative fitness on all leaf traits measured in both seasons. For each year of the study, I regressed final estimated relative fitness on the leaf traits measured in both seasons (plus emergence time in 1994). These analyses included only individuals for which I had measures of all leaf traits in both seasons.

To preserve the evolutionary interpretation of all selection coefficients, I transformed neither trait distributions nor relative fitnesses to meet the assumptions for parametric regression. Because relative fitness was not normally distributed, a jackknifing procedure was applied to test for statistical significance of regression coefficients (Mitchell-Olds & Shaw, 1987). Linear selection coefficients were standardized by multiplication of each partial regression coefficient by the standard deviation of the corresponding trait. Stabilizing/disruptive selection coefficients were multiplied by the variance rather than standard deviation of the trait.

Results

Regressions constructed to estimate plant dry weight explained between 85 and 95% of the variation in plant dry weight (Table 1). Actual plant dry weight at harvest explained 87% of the variation in flower number for marked plants that produced flowers in 1993 ($n = 413$, $P < 0.001$). For each census interval examined, the average dry weight estimated at the beginning of the interval was significantly greater for those individuals that survived the interval than for those that died during the interval (Table 2).

Table 1 The proportion of variance in plant dry weight explained by a multiple regression on stem height and number of leaves at each of three times during each of the 2 years of the study. The sample size was 25 for each analysis, and all regressions were highly significant ($P < 0.001$).

Time of analysis	1993	1994
End of winter	0.857	0.904
Beginning of summer	0.944	0.903
End of summer	0.946	0.912

Leaves produced in the winter were consistently significantly smaller in size and had significantly lower densities of stomata than leaves produced in the summer (Table 3). Leaf thickness also differed significantly for leaves produced during different seasons, but the pattern of differences was not consistent for the two years. In 1993, leaves produced in the winter were significantly thicker, and in 1994, leaves produced in the summer were significantly thicker (Table 3).

Selection gradients

Standardized selection gradients from multiple regression analyses indicated consistent selection favouring individuals with larger leaves in summer and in winter during both years of the study (Table 4). For leaf size, the selection gradients were as great as 0.6 standard deviation units and indicated significant positive direct effects of leaf size on relative fitness during the winter and summer of both years (Table 4). For leaf thickness, three of the four selection gradients were positive, and all were less than 0.1 standard deviation unit. Only the positive selection gradient for leaf thickness in winter of 1993 was significantly different from zero (Table 4).

There was a significant negative selection gradient for density of stomata in the winter of 1994 (Table 4). This relationship is consistent with the functional hypothesis that a low density of stomata is favoured at low temperatures. Density of stomata was not significantly related to relative fitness during the summer of 1993 or

Table 2 Back-transformed mean weights (mg) of plants that did and did not survive from one census to the next in the winter and summer in each of two years. Statistics are from *t*-tests comparing means for plants that did and did not survive.

	Dead (*n*)	Alive (*n*)	T	d.f.	*P*
1993					
Winter	2.92 (262)	4.17 (317)	6.98	577	<0.001
Summer	4.46 (103)	9.06 (214)	6.41	315	<0.001
1994					
Winter	1.40 (150)	1.57 (455)	4.67	603	<0.001
Summer	3.85 (59)	6.28 (398)	6.33	455	<0.001

Table 3 Mean values ± SD (n) for traits of fully mature leaves produced in the field in the summer and winter of 1993 and 1994. Within each year, means for the same trait differed significantly between seasons ($P < 0.001$) as determined by t-tests.

	Leaf length (mm)	Leaf thickness (mm)	Density of stomata (mm^{-2})
1993			
Winter	6.61 ± 2.24 (557)	0.162 ± 0.039 (600)	56.8 ± 18.8 (97)
Summer	15.90 ± 7.50 (450)	0.134 ± 0.021 (452)	76.2 ± 21.5 (336)
1994			
Winter	7.72 ± 1.81 (495)	0.143 ± 0.026 (489)	77.9 ± 22.7 (495)
Summer	28.66 ± 7.48 (392)	0.160 ± 0.022 (390)	93.0 ± 21.2 (391)

Table 5 Coefficients from multiple regression of survival (0 or 1) on leaf traits during the winter and summer of 1993 and the winter of 1994. Too few individuals died during the summer of 1994 to permit a meaningful analysis.

	Leaf size	Leaf thickness	Density of stomata	Time of emergence	n	r^2
1993						
Winter	0.031^{**}	0.604	–	–	416	0.028
Summer	0.001	−0.566	−0.001	–	206	0.029
1994						
Winter	0.120	1.467^{*}	0.004	0.001	397	0.021

– trait not measured. $^{*}P < 0.05$. $^{**}P < 0.01$.

1994. The selection gradients for time of emergence were not significant for either season (Table 4).

I detected weak but significant positive stabilizing/disruptive selection gradients (not reported) in the multiple-regression analyses for leaf thickness (standardized coefficient = 0.025) in the winter of 1994 and for leaf area (standardized coefficient = 0.030) in the summer of 1994. These results indicate that the relationship between each of these traits and relative fitness was accelerating over at least some portion of the range of the trait values.

Almost all of the coefficients for viability selection were low and nonsignificant (Table 5), indicating that most mortality was not selective with respect to leaf traits or timing of emergence. Analysis of these same data with potentially more powerful logistic regressions (not shown) gave the same results. Only one of the viability selection coefficients for leaf size was significant, and the magnitude of this coefficient was only about one-tenth of that of the corresponding selection gradient (Table 5). The only other significant coefficient in the analyses of viability selection indicated strong positive selection for leaf thickness during the winter of 1994. Although this coefficient was nearly 1.5 standard-deviation units, the regression model explained only 2% of the total variation in survival (Table 5).

Table 4 Standardized selection gradients, sample sizes and proportions of the variance in estimated relative fitness explained from multiple regression of estimated relative fitness on leaf traits (leaf traits plus time of emergence in 1994) in summer and winter of 1993 and 1994.

	Leaf size	Leaf thickness	Density of stomata	Time of emergence	n	r^2
1993						
Winter	0.206^{***}	0.034^{*}	–	–	343	0.412
Summer	0.483^{***}	0.082	−0.065	–	187	0.455
1994						
Winter	0.358^{***}	0.072	-0.055^{*}	−0.046	341	0.429
Summer	0.602^{***}	−0.030	−0.013	0.014	310	0.865

– trait not measured. $^{*}P < 0.05$. $^{***}P < 0.001$.

The analyses of selection excluding individuals that died gave results similar to those of the analysis including all individuals. The magnitude of selection gradients was smaller for all traits in all seasons when dead individuals were excluded (compare Table 4 and Table 6), but the amount of variance in relative fitness explained was consistently greater for the analyses including only survivors. The selection gradient for leaf thickness for the summer of 1993 was not significant in the full analysis (Table 4) but was significantly positive when only survivors were included (Table 6). In contrast, the selection gradient for leaf thickness for the winter of 1993, which was significantly positive in the analysis of all plants (Table 4), was not significant in the analysis excluding dead individuals (Table 6).

In both 1993 and 1994, the only significant selection gradients in the regression of final estimated relative fitness on all leaf traits expressed in both seasons were for summer leaf size (Table 7). The selection gradients for leaf length in 1993 and leaf area in 1994 were both positive.

Discussion

Leaves of *D. linearifolia* differ in morphology and anatomy depending on whether they are produced in the winter or

Table 6 Coefficients from multiple regression of estimated relative fitness on leaf traits including only those individuals that survived to the end of the interval over which selection was measured.

	Leaf size	Leaf thickness	Density of stomata	Time of emergence	n	r^2
1993						
Winter	0.144^{***}	0.012	–	–	373	0.461
Summer	0.326^{***}	0.060^{***}	−0.042	–	192	0.690
1994						
Winter	0.178^{***}	0.009	-0.020^{*}	-0.036^{***}	346	0.491
Summer	0.245^{***}	0.00004	−0.004	0.006	289	0.870

– trait not measured. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

Table 7 Coefficients from multiple regression of estimated relative fitness on leaf traits measured in winter and summer for each of two years.

	Winter leaf traits			Summer leaf traits					
	Leaf size	Leaf thickness	Density of stomata	Leaf size	Leaf thickness	Density of stomata	Time of emergence	*n*	r^2
1993	0.016	0.056	–	0.490***	0.072	–0.026	–	133	0.530
1994	0.028	–0.003	0.002	0.614***	–0.026	–0.015	0.008	236	0.854

– trait not measured. ***$P < 0.001$.

the summer. The lower density of stomata observed during the winter of both years and the greater thickness of winter leaves in 1993 are consistent with past observations of within-individual variation in this species (Winn, 1996a,b) and with expectations for adaptive seasonal variation in leaf phenotype (Huner, 1985; Korner *et al.*, 1989). The pattern of seasonal variation in leaf thickness in 1994 opposed both the pattern observed in 1993 and the expectation for adaptive seasonal variation. The reason for the reversal between years in the direction of seasonal change in leaf thickness could be related to seedlings' having germinated in the field in 1993 and in the greenhouse in 1994. Although seeds were germinated on field soil and the greenhouse was unheated, seedlings were kept well watered in the greenhouse, and temperatures were probably less extreme there than in the field. These conditions might have influenced the thickness of leaves produced soon after transplanting.

The larger size of leaves produced in summer than in winter in both years of the study conflicts with the expected pattern for adaptive variation in leaf size and with the pattern observed in two previous studies conducted in growth chambers (Winn, 1996a,b). Because plants were quite small in the winter, when they produced their first true leaves, it is likely that they were constrained to produce relatively small leaves at this time. In past studies, which consistently found smaller leaf size for plants grown at high temperature (Winn, 1996a,b), leaves produced at low temperature were measured after plants had attained a much larger total size than they had at the time first true leaves were measured in the present study.

The differences I observed between the pattern of seasonal variation in leaf size and thickness in the field and those observed under artificial conditions in past studies (Winn, 1996a,b) argue for caution in extrapolating the results of observations and analyses of phenotypic plasticity in the lab to field environments.

Is seasonal variation in leaf traits adaptive?

Although some patterns of seasonal variation in leaf phenotype were consistent with adaptive explanations, measurements of natural selection on leaf traits provided no support for the hypothesis that different leaf phenotypes are favoured in different seasons for *D. linearifolia*. The results of analysis of selection suggest that large leaves are consistently favoured in both winter and summer and that selection on leaf thickness and on density of stomata is weak and intermittent (Table 4). There was no evidence to suggest that the optimum value of any leaf trait differed between the winter and summer seasons. The conclusion that seasonal variation in leaf traits is not adaptive was also supported by the multiple regression of relative fitness on all leaf traits measured in both seasons (Table 7).

Several factors suggest that lack of statistical power did not limit my ability to detect selection favouring seasonal variation in leaf traits. Sample sizes for most analyses were reasonably large. For multiple regressions within seasons, sample sizes were 187–416. In addition, my analyses were sensitive enough to detect significant selection coefficients as small as 0.034 standard-deviation units (Table 4). Finally, the consistency between years in the patterns and magnitudes of selection coefficients also supports the reliability of my analyses of natural selection on leaf traits.

The results of the selection analyses are at odds with the expectations for the adaptive consequences of leaf traits at different temperatures. Past studies have demonstrated that photosynthetic efficiency or plant performance at higher temperatures is enhanced by smaller, thinner leaves with a greater density of stomata and that high performance at lower temperatures is associated with large, thick leaves with lower stomatal densities (Jones, 1983; Huner, 1985; Korner *et al.*, 1989). I found no evidence to support this scenario for *D. linearifolia*. My analysis of viability selection (Table 5) suggested that the traits I measured have little effect on whether or not an individual survives over a season. I also found no support for hypotheses that the functional consequences of leaf traits for growth are different at different temperatures, even when I eliminated potentially nonselective mortality by analysing only plants that survived to the end of each season (Table 6). My failure to detect the expected effects of leaf traits on performance does not necessarily conflict with our general understanding of functional consequences of leaf traits at different temperatures. It is likely that the range of variation in leaf traits expressed within this species is too small for the expected functional consequences to be manifest. My results do suggest that the observed within-individual variation in leaf traits in *D. linearifolia* is not an adaptation to seasonal variation in temperature.

The correspondence between patterns of seasonal variation in some leaf traits of *D. linearifolia* observed in this study and the expectations for functional consequences of leaf traits appears to be fortuitous. It seems likely that efficient photosynthesis at temperatures differing by as much as 20 °C would require different leaf traits, but for *D. linearifolia*, seasonal variation in the traits I measured does not appear to be important in determining performance at different temperatures. Other traits not measured in this study, such as cell-membrane composition or concentrations and chemical structures of pigments and photosynthetic enzymes, may also vary seasonally in a pattern that could facilitate effective photosynthesis over a wide range of temperature (Berry & Raison, 1981; Weis & Berry, 1988).

Why do leaf traits vary seasonally?

The lack of evidence that selection favours different leaf sizes, thicknesses and stomatal densities at different times of the year raises the question of why these traits vary seasonally in *D. linearifolia*. Selection consistently favoured large leaf size and the observation that smaller leaves are produced in the winter is almost certainly the result of small total plant size at that time. It appears that some constraint prevents individuals from producing a constant, optimally large leaf size. Direct selection on leaf thickness and density of stomata was consistently weak or absent, suggesting that these traits had little influence on fitness. Seasonal differences in stomatal density and leaf thickness may be passive consequences of development under different environmental conditions or at different developmental stages. Such variation may persist simply because there is little or no selection against individuals in which these traits vary among leaves.

Past studies of *D. linearifolia* have shown that plants grown at a constant temperature exhibit differences in traits of leaves produced early and late in development that parallel the differences between leaves produced in response to low and high temperatures (Winn, 1996b). The absolute growth rate of a plant is likely to be lower both at lower temperature and when the individual is small. Seasonal differences in leaf thickness and density of stomata may be passive consequences of the effect of growth rate on patterns of leaf development that persist because there is no selection against them.

The literature is full of examples of traits of organisms that vary depending on the environment. Much of this phenotypic plasticity may not be adaptive (cf. Weis & Gorman, 1990; Schmitt *et al.*, 1995). Observation of a pattern of plastic variation in a trait that is consistent with expectations for adaptive variation is not sufficient to demonstrate that plasticity is adaptive. Passive effects of the environment on traits that have negligible effects on fitness are likely to evolve very differently from adaptive responses to environmental variation. In order to identify appropriate empirical systems to test our ideas for how adaptive phenotypic plasticity evolves, we must test the hypothesis that the plasticity being examined is indeed adaptive.

Acknowledgments

Comments from J. Travis on an early draft led to substantial improvements in the paper. J. Hentz and J. Travis provided valuable advice regarding statistical analysis. T. Mitchell-Olds generously shared his program FREE-STAT to run the jackknifing procedures. Cheerful and adept assistance in the field and lab were provided by K. Chodyla, J. Ernst, D. Kean, and D. Spinelli. This work was supported by NSF award DEB-9221107.

References

Andersson, S. & Shaw, R.G. 1994. Phenotypic plasticity in *Crepis tectorum* (Asteraceae): genetic correlations across light regimens. *Heredity* **72**: 113–125.

Berry, J.A. & Raison, J.K. 1981. Responses of macrophytes to temperature. In: *Physiological Plant Ecology. I. Responses to the Physical Environment. Encyclopedia of Plant Physiology New Series, Vol. 12A* (O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler, eds), pp. 277–338. Springer-Verlag, New York.

Dong, M. 1993. Morphological plasticity of the clonal herb *Lamiastrum galeobdolon* (L.) Ehred. & Polatschek in response to partial shading. *New Phytol.* **124**: 291–300.

Dudley, S.A. & Schmitt, J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am. Nat.* **147**: 445–465.

Evans, J.P. 1992. The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. *Oecologia* **89**: 265–276.

Huner, N.P.A. 1985. Morphological, anatomical, and molecular consequences of growth and development at low temperature in *Secale cereale* L. CV. Puma. *Am. J. Bot.* **72**: 1290–1306.

Jones, H.G. 1983. *Plants and Microclimate.* Cambridge University Press, Cambridge, UK.

Kingsolver, J.G. 1995a. Viability selection on seasonally polyphenic traits: wing melanin pattern in western white butterflies. *Evolution* **49**: 932–941.

Kingsolver, J.G. 1995b. Fitness consequences of seasonal polyphenism in western white butterflies. *Evolution* **49**: 942–954.

Korner, K., Neumayer, M., Menendez-Riedl, S.P. & Smeets-Scheel, A. 1989. Functional morphology of mountain plants. *Flora* **182**: 353–383.

de Kroon, H. & Hutchings, M.J. 1995. Morphological plasticity in clonal plants: the foraging concept reconsidered. *J. Ecol.* **83**: 143–152.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Lee, D.W. & Richards, J.H. 1991. Heteroblastic development in vines. In: *The Biology of Vines* (F. E. Putz & H. A. Mooney, eds), pp. 205–243. Cambridge University Press, Cambridge, UK.

Mitchell-Olds, T. & Shaw, R.G. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**: 1149–1161.

Pigliucci, M. & Schlichting, C.D. 1995. Ontogenetic reaction norms in *Lobelia siphilitica* (Lobeliaceae): response to shading. *Ecology* **76**: 2134–2144.

Schmitt, J., McCormac, A.C. & Smith, H. 1995. A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *Am. Nat.* **146**: 937–953.

Sultan, S.E. 1996. Phenotypic plasticity of offspring traits in *Polygonum persicaria*. *Ecology* **77**: 1791–1807.

Sultan, S.E. & Bazzaz, F.A. 1993a. Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. *Evolution* **47**: 1009–1031.

Sultan, S.E. & Bazzaz, F.A. 1993b. Phenotypic plasticity in *Polygonum persicaria*. II. Norms of reaction to soil moisture and the maintenance of genetic diversity. *Evolution* **47**: 1032–1049.

Sultan, S.E. & Bazzaz, F.A. 1993c. Phenotypic plasticity in *Polygonum persicaria*. III. The evolution of ecological breadth for nutrient environment. *Evolution* **47**: 1050–1071.

van Tienderen, P.H. 1990. Morphological variation in *Plantago lanceolata*: the limits of plasticity. *Evol. Trends Plants* **4**: 35–43.

van Tienderen, P.H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**: 1317–1331.

Weis, E. & Berry, J.A. 1988. Plants and high temperature stress. In: *Plants and Temperature* (S. P. Long & F. I. Woodward, eds), pp. 329–346. Society of Experimental Biology. The Company of Biologists, Cambridge, UK.

Weis, A.E. & Gorman, W.L. 1990. Measuring selection on reaction norms: an exploration of the *Eurosta-Solidago* system. *Evolution* **44**: 820–831.

Winn, A.A. 1996a. The contributions of programmed developmental change and phenotypic plasticity to within-individual variation in leaf traits in *Dicerandra linearifolia*. *J. Evol. Biol.* **9**: 737–752.

Winn, A.A. 1996b. Adaptation to fine-grained environmental variation: an analysis of within-individual leaf variation in an annual plant. *Evolution* **50**: 1111–1118.

Received 14 January 1998; accepted 4 March 1998