ECOPHYSIOLOGY

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Variation in carbon isotope discrimination in relation to plant performance in a natural population of *Cryptantha flava*

Abstract Few studies of phenotypic selection have focused on physiological traits, especially in natural populations. The adaptive significance of plant water-use efficiency, the ratio of photosynthesis to water loss through transpiration, has rarely been examined. In this study, carbon isotopic discrimination, Δ, an integrated measure of water-use efficiency, was repeatedly measured in juveniles and adults in a natural population of the herbaceous desert perennial *Cryptantha flava* over a 4-year period and examined for plasticity in Δ, consistency between years in values of Δ, and evidence for selection on Δ phenotypes. There was significant concordance in Δ values among the 4 years for adult plants and significant correlations in Δ values measured in different years for juveniles and adults combined. The wettest year of the study, 1998, proved an exception because Δ values that year were not correlated with Δ values in any other year of the study. Consistency in Δ measured on the same plants in different years could indicate genotypic variation and/or consistency in the water status of the microhabitats the plants occupied. Two forms of plasticity in Δ were also evident; mean seasonal values were correlated with precipitation the preceding autumn, and Δ values also declined with plant size, indicating increasing water-use efficiency. Phenotypic selection was evident because in the first year of the study juvenile plants that would survive until year five averaged lower Δ values than did those that failed to survive. During the driest year, 2000, Δ was significantly negatively correlated with adult plant size, measured as the number of leaf rosettes, but the negative relationship between Δ and the number of flowering stalks, a more direct measure of fitness, was not significant. These results suggest that the direction of phenotypic selection on Δ changes as plants grow.

Keywords *Cryptantha flava* · Integrated water-use efficiency · Phenotypic plasticity · Phenotypic selection · Selection on WUE

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Introduction

The vast majority of studies examining phenotypic selection on quantitative traits, across all taxa, have focused on morphological characters, with behavioral and physiological characters seriously under represented (Endler 1986; Kingsolver et al. 2001). In plants, studies relating physiological performance to direct or indirect components of fitness are also biased toward annuals and are often set in common gardens that may or may not involve manipulation of environmental conditions (Geber and Dawson 1990; Dudley 1996a, b; Heschel et al. 2002, Heschel 2005). There is a recognized need for studies evaluating the ecological physiology of plants in the context of natural selection. Kingsolver et al. (2001) cited the great need for studies of natural selection on physiological traits in general, and Geber and Griffen (2003) called for more studies relating heritable variation in plant physiological traits to fitness, particularly in long-lived species.

Here, we identify sources of variation in water-use efficiency, the ratio of photosynthesis to transpirational water loss, within a natural population of the herbaceous desert perennial *Cryptantha flava* (Boraginaceae) and evaluate phenotypic selection on that variation. Because stomata simultaneously allow diffusion of CO_2

into the leaf and diffusion of water vapor out of the leaf, photosynthetic carbon gain comes at the expense of water loss and potential desiccation. The optimal balance between photosynthesis and transpiration under different environmental conditions has interested plant ecologists for decades. By one view, natural selection should maximize water-use efficiency in dry environments (WUE; Cowan 1982; Field et al. 1983; Schuster et al. 1992; Ehleringer 1993), but it has also been suggested that even in desert plants, selection should operate to maximize carbon assimilation—not necessarily water conservation (Gibson 1998). Indeed, in many cases, high photosynthetic rates occur together with high transpiration rates and low WUE (Geber and Dawson 1990, 1997; Arntz and Delph 2001; Geber and Griffen 2003; Heschel and Riginos 2005).

Ideally, WUE is expressed as the ratio of total carbon gained to total water lost over the course of the growing season, but such measurements are difficult to obtain, especially under field conditions. WUE based on instantaneous rates of photosynthesis and transpiration is easier to measure but highly variable, subject to current and past environmental conditions. An alternative method for C_3 plants, and the one applied here, uses the ratio of the naturally occurring carbon isotopes ($^{13}C/^{12}C$) in plant tissue as an integrative measure of water-use efficiency over the period in which the carbon was assimilated. This is possible because $^{13}C/^{12}C$ and WUE both depend on the leaf intercellular concentration of CO_2, c_i. A low c_i reflects either a high efficiency of carboxylation, or more commonly, a low stomatal conductance, which reduces the diffusion of water molecules away from the water-saturated intercellular spaces more strongly than it reduces diffusion of CO_2 into the leaf (Larcher 2003). The carbon isotopic ratio in plant tissues also depends on c_i because carboxylating enzymes naturally discriminate against CO_2 molecules carrying the heavier isotope, and discrimination, expressed as the value Δ, increases with c_i (Farquhar et al. 1989).

Sources of intraspecific variation in Δ are both environmental and genetic. Relevant environmental conditions affecting Δ include leaf temperature, leaf-to-air vapor pressure differences, solar irradiance, and soil water conditions (Dawson et al. 2002). One type of evidence for genetically based variation in Δ is provided by correlations between Δ values measured on the same plants in different years (Donovan and Ehleringer 1994; Damesin et al. 1998), although such results could also reflect consistent differences in the microhabitats the plants occupy. Other, more convincing evidence for genotypic variation includes maintained differences in Δ when plants from different populations are grown under common conditions (Comstock and Ehleringer 1992; Zhang et al. 1993; Dudley 1996a, b) and direct estimates of genetic variance or heritability (Geber and Dawson 1990; Schuster et al. 1992; Donovan and Ehleringer 1994). The value Δ can also vary with the plant's stage of development

(Donovan and Ehleringer 1991; Cavender-Bares and Bazzaz 2000) and in dioecious species with plant gender (Dawson and Ehleringer 1993).

Despite an understanding of factors causing variation in Δ, few studies have directly examined whether selection operates on Δ by evaluating the relationship between Δ and plant performance in natural populations. In one such study with the desert shrub *Encelia farinosa*, individuals with high values of Δ were less likely to survive under root competition but exhibited increased productivity in the absence of competition (Ehleringer 1993). Investigations with other woody species have not always found relationships between Δ and survival or growth (Donovan and Ehleringer 1994; Pennington et al. 1999). For herbaceous species, relationships between Δ and plant performance traits have been documented under experimental field conditions (Geber and Dawson 1990; Dudley 1996a, b; Heschel et al. 2002; Heschel and Riginos 2005), but similar studies of natural populations are apparently lacking.

Our evaluation of the significance of variation in Δ was part of a broader, 5-year study with *C. flava* investigating how plants of different developmental stages respond physiologically and demographically to drought. Consequently, some plants were subjected to artificially created drought in either year 2 or 3 while others experienced ambient precipitation every year. A natural drought in year 4 proved more severe than our experimental treatments. This design allowed us to search for consistency in values of Δ measured on the same plants over 4 years with changing soil water conditions. While controlling for the developmental stage of the plant, we also examined the relationships between Δ and survival, growth and reproduction for evidence of phenotypic selection on Δ.

Materials and methods

The study system

Cryptantha flava grows in sandy soils from central Wyoming, throughout eastern Utah, and into northern Arizona and New Mexico. Although considered herbaceous (Cronquist et al. 1984), a plant consists of one to many basal leaf rosettes supported by a branched woody underground stem (caudex) connected to a single taproot. The narrow, nearly vertical, oblanceolate leaves (6.0–9.0 cm long) first appear in mid-April. New leaves are produced and old ones die throughout the spring and early summer growing season (Casper et al. 2001), and new rosettes arise from axillary buds. A rosette meristem may continue to produce leaves for several years. In early spring, some rosettes bolt to produce flowering stalks bearing well-developed, alternate cauline leaves and terminal inflorescences, with the first flowers opening by mid-May. Flowering stalks die when seeds ripen in mid-June to early July, and the remaining leaves on vegetative rosettes senesce a few weeks later.

Mortality of vegetative rosettes between growing seasons is particularly common in larger plants and in unfavorable years (Casper 1996). New leaves may appear in response to late summer rains (Casper et al. 2001), but most growth and all reproduction occur in the spring. Dead flowering stalks and vegetative rosettes remain attached to the plant and are apparent for at least a year. Few individuals survive more than 15 years (B.B. Casper, unpublished).

The study population is located adjacent to Red Fleet State Park in Uintah County in northeastern Utah at 1,730 m elevation (40° 30′N, 109° 22′ 30″E), where vegetation is dominated by sagebrush, *Artemisia tridentata* Nutt., rabbitbrush, *Chrysothamnus nauseosus* (Pallas) Britt., and the small tree *Juniperus osteosperma* (Torr.) Little.

Climatic data

Ambient precipitation was measured at the field site during the growing seasons of 1998 and 1999 in order to calculate the amount of rainfall excluded by the rainout shelters and is reported in Casper et al. (2001). Climatic data from the Maeser 9 weather station, located ∽ 18 km SW of the site, at 1,950 m elevation, (Western Regional Climate Center, http://www.wrcc.dri.edu) were used to compare precipitation during the years of the study to long-term climatic means.

Experimental design

In early spring 1997, we marked 18 5×5 m study plots in six blocks of three plots with at least a meter between plots and usually several meters between blocks. In each block, a rainout shelter covered one plot in 1998 and another in 1999. The remaining six plots received ambient precipitation all years. Between March 1 and June 17, 1998, sheltered plots were only covered during precipitation events by unrolling 6×6 m (allowing a 0.5 m overhang to reduce edge effects) opaque canvas tarpaulins over metal frames, which were slanted from 1 m to 2.5 m in height to allow runoff. The covers affected plot microclimate for the limited time they were in place, but were not in place for several unforeseen rains. For plots sheltered in 1999, the tarpaulin was replaced with stationary polyethylene roofing material that transmits both photosynthetically active (400–700 nm) and infrared radiation (Reynolds et al. 1999); the roofing material was left in place continuously between March 1 and May 23.

For our study of drought effects on plant performance and carbon isotope ratios, three large, adult plants (> 12 rosettes) without dead vegetative rosettes, which would have indicated shrinkage from a previously larger size, and at least three juvenile plants (≤ 12 rosettes) were chosen arbitrarily in each plot in 1997. While the absolute ages of plants could not be known,

juveniles were developmentally younger than large plants and easily distinguished from older plants that had shrunk in size. Most juveniles had never flowered. All plants were growing in the open away from shrubs and were marked with short flags and buried metal tags, which could be relocated with metal detectors. Additional juvenile plants were selected in 1999 to replace those that had died or grown out of the small size category. We followed the growth, flower stalk production and survival of these individuals until 2001, and until 2000, we annually measured carbon isotope ratios in leaf tissue. These same plants were also the subjects of several physiological measurements from 1997 to 2000, including instantaneous measures of mid-day photosynthesis and stomatal conductance, taken twice during the growing season, in late May or early June and again in July.

Leaf carbon isotope measures

Two to four leaves were collected from each living marked plant in June, dried, and ground to a fine powder in liquid nitrogen. Carbon isotope ratios (δ, $^{13}CO_2/^{12}CO_2$) were determined relative to standards by mass spectrometry at the Stable Isotope Ratio Facility for Ecological Research (SIRFER) at the University of Utah, Salt Lake City, UT, or using the mass spectrometry facility at the University of Maryland (Department of Geology). Fifty samples were used to cross-calibrate measurements from both facilities. The Maryland samples were analyzed using a continuous flow isotope ratio mass spectrometer (IRMS; Isoprime, Micromass, UK). Gas samples were obtained via combustion on a CHN analyzer (Eurovector, UK) and fed directly to the IRMS. Carbon isotope ratio values were converted to discrimination values (Δ) by the equation (Farquhar et al. 1989):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p/1000},$$

where, δ_a = carbon isotope ratio of CO_2 in the atmosphere (assumed to be -8 parts per mil, $^o/_{oo}$) and δ_p = measured carbon isotope ratio of the plant tissue. Lower values of Δ indicate higher water-use efficiency.

Variation in Δ

Variation in Δ was analyzed several ways in order to explore the relationship between seasonal precipitation and Δ, the year-to-year consistency in Δ with respect to other plants, and the relationship between Δ and survival, growth and reproduction. Because the more effective artificial drought treatment of 1999 caused a nearly significant reduction in Δ ($p < 0.07$; I.N. Forseth unpublished), we also separated plants by drought treatment in our analyses when appropriate (i.e., sheltered in 1998, sheltered in 1999, or controls).

First, for plants never sheltered, we looked for correlations between annual mean values of Δ and the amount of precipitation during each of four seasons: the spring of the same year Δ was measured (March to May), the preceding summer (June to August), the preceding autumn (September to November), and the preceding winter (December to February).

Second, in order to identify variation that might be contributed by plant genotype, we looked for year-to-year consistency in Δ values for the same plants sampled repeatedly in different years. Controlling for developmental stage, we focused on plants that were adults (> 12 rosettes) when first identified in 1997 and also survived until 2000. Plants within each treatment group were ranked by their Δ values each of the 4 years and the Kendall coefficient of concordance, a non-parametric statistic, was used to test for similarity in the rankings of the plants among years (Siegel 1956).

We also looked for direct correlations between measurements of Δ made on the same individuals in two different years. These between-year comparisons included both juveniles and adults because their separate analyses did not qualitatively differ. We first compared only unsheltered plants, excluding any plants that received an artificial drought treatment in either of the 2 years. For example, for the 1997–1998 comparison we excluded plants that were sheltered in 1998. For the 1997–2000 comparison, we were able to include all plants that survived the 4 years because no plants were sheltered in either 1997 or 2000. Additionally, for plants sheltered in 1998, we compared Δ values between 1997–1998 and 1998–1999. Similarly, for plants sheltered in 1999, we compared Δ values between 1998–1999 and 1999–2000.

Plant performance and Δ

We searched for a relationship between the probability of plant survival and Δ values measured at the start of the study. For plants identified in 1997, we classified plants by size (juveniles versus adults) and survival category (whether they survived until 2001 or died during the study). We then examined 1997 Δ values as a function of size and survival category (both fixed effects) using a two-way ANOVA. Upon finding that the 1997 measure of Δ differed between survival categories in juveniles, we calculated the selection differential (i) for Δ as the difference between the mean Δ value for all juveniles and that for the survivors divided by the square root of the variance in Δ for the survivors. Since individuals differed in the amount they reproduced before they died, the selection differential was adjusted (i*) for the variance in relative fitness using the procedure in Endler (1986). For these juveniles identified in 1997, relative fitness was measured as the total number of flowering stalks a given individual produced between 1997 and 2001, divided by the mean for this group of plants.

We used the same ANOVA model to determine whether instantaneous mid-day photosynthesis (A) or WUE (A/g), measured either early (mid-May to June) or late (July) in the 1997 growing season, differed similarly to Δ . Maximum daily photosynthetic rates (A) and stomatal conductance (g) were measured on fully exposed leaves from 1000 h to 1400 h MST approximately bi-weekly. One to three leaves from a vegetative rosette were enclosed in a 0.25 l chamber of a LiCor 6200 closed gas exchange system (LiCor, Inc. Lincoln, NE, USA), and photosynthetic rate was calculated from the depletion of CO_2 in the chamber over time. Leaf area was calculated by multiplying the length×width of the enclosed portion of each leaf.

Finally, we asked whether plant size, growth or reproduction varied with Δ . We separately examined juveniles and adults, in order to control for developmental stage, and restricted our analyses to the years 1997 and 2000 only. These years differed greatly in ambient precipitation but since no plants received artificial drought treatment, we were able to include all plants of the appropriate size in our analyses. We focused on possible correlations between Δ in year t and several demographic traits: (1) total rosettes in year t, (2) relative growth rate between year t and year $t+1$ (and between year $t-1$ and year t, if available), and for adult plants, (3) the number of flowering stalks and reproductive effort in year t. Relative growth rate (RGR) is defined as the [(number of rosettes in year $t+1$)−(the number of rosettes in year t)]/(the number of rosettes in year t), while reproductive effort (RE) = the number flowering stalks/total number of rosettes.

Results

Climate

From 1997 to 1999, winter and spring precipitation at the Maesar, UT, weather station was above the 30-year mean (Western Regional Climate Center, http://www.wrcc.dri.edu). The shelters excluded 60% of total precipitation from March through June in 1998 and over 86% during the same period in 1999 (Casper et al. 2001). Ambient precipitation between October 1999 and February 2000 was 70% below the 30-year mean, resulting in dry soils for the initiation of plant growth in spring 2000; precipitation in May and June that year was also 46% below the 30-year mean. This resulted in a more severe drought during the 2000 growing season than that caused by rainout shelters in the two preceding years.

Variation in Δ

Reflecting differences in precipitation regimes, mean Δ values for all plants in the study varied greatly among years, with the lowest in 2000, the year of severe natural drought, and the highest in 1998, the year of most
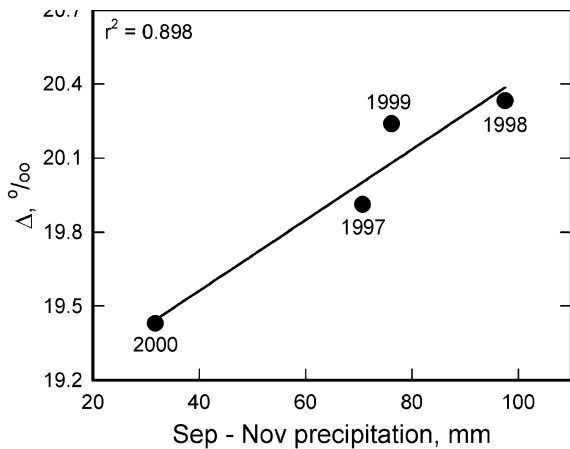
Fig. 1 Relationship between mean Δ values and precipitation the preceding autumn (September–November) for plants that were never sheltered. Data points are labeled with the year Δ was measured

rainfall (Fig. 1). The Δ values for plants that were never sheltered were more highly correlated with precipitation in the preceding autumn, September–November (Fig. 1), than in any other season.

Two different analyses showed some consistency among years in Δ values measured repeatedly on the same plants. For adult plants that lived until 2000 there was concordance in ranked Δ values for the group of plants ($n = 14$) that were sheltered in 1998 (Kendall Coefficient of Concordance; $W = 0.58$, $r_{sav} = 0.44$, $P < 0.01$) and for those plants sheltered in 1999 ($n = 16$; $W = 0.55$, $r_{sav} = 0.40$, $P < 0.01$). Plants in the control plots showed weak, nearly significant concordance in Δ values over the 4 years ($n = 12$; $W = 0.44$, $r_{sav} = 0.25$, $P < 0.06$).

When Δ values measured on the same individuals in different years were examined using correlation analysis, values of Δ were significantly ($p < 0.05$) correlated among all years except 1998, with correlation coefficients (r) consistently around 0.5. Values of Δ measured in 1998 were not correlated with those of any other year.

Plant performance and Δ

Roughly the same proportion of juveniles identified in 1997 survived until 2001 (41/55) as did adults (36/52), but Δ values were related to the probability of survival only in juveniles. When 1997 Δ values are analyzed as a function of size (juveniles vs. adults) and survival category (whether they lived until 2001 or not), the interaction term is significant (Table 2). Juveniles that survived until 2001 had higher Δ values, indicating lower WUE, than juveniles that died before 2001 and higher values than surviving adults (LSD Test, $P < 0.05$; Table 3). Before adjustment for relative fitness, the selection differential (i) in these juveniles was 0.16. The selection differential after adjustment for variance in relative fitness (i^*) was 0.22.

Table 1 Significant ($P < 0.05$) correlation coefficients (r) for Δ values measured on the same plants in different years

	1998	1999	2000
A. Unsheltered both years			
1997	NS(67)	0.51 (57)	0.50 (87)
1998		NS (25)	NS (53)
1999			0.54 (71)
B. Sheltered in 1998			
	1998	1999	
1997	NS (33)		
1998		NS (25)	
C. Sheltered in 1999			
		1999	2000
1998		NS (32)	
1999			0.53(41)

Table 2 Mean squares and P-values from ANOVA examining 1997 values of Δ and three instantaneous gas exchange parameters (A, g, A/g) measured in July 1997

Effect	Δ	A	g	A/g
Size	0.76	340.49**	0.04	400.2
Survival category	0.09	16.94	0.01	36.3
Size × Survival category	1.46**	91.77	0.00	691.4*
Error	0.24	59.88	0.04	159.4

Plant size ($\leq$ 12 rosettes or > 12 rosettes) and survival category (surviving until 2001 or not) are both treated as fixed effects in the ANOVA model. Error df = 103 for Δ and 68 for the other variables. Significance level indicated by asterisks; *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$

Instantaneous mid-day gas exchange measurements made late in the 1997 season showed similar patterns in WUE as that indicated by values of Δ. Mid-day A/g was lowest in juveniles that survived until 2001 but they differed significantly only from those in surviving adult plants (Tables 2, 3). Mid-day A was likewise lowest in surviving juveniles, differing significantly from both categories of adult plants but not from juveniles that failed to survive until 2001. In ANOVA, these differences between juveniles and adults are reflected in a significant main effect of size for A and a significant interaction between size and survival category for A/g. Late season measures of g did not differ between size or survival categories ($P > > 0.05$). There were no differences as a function of size or survival category ($P > > 0.05$) for A, g, or A/g measured early in the 1997 growing season.

Examining juveniles and adults separately, there were no strong relationships between Δ and measures of plant size, growth or reproduction in either 1997 or 2000. Juveniles did not exhibit significant correlations between Δ and total rosettes or RGR either year ($P > 0.15$). For adult plants, there was a negative correlation between Δ and total rosettes in 2000. The slope of the regression relationship, β, between the standardized values of Δ and the relative number of total rosettes was -0.26, $P < 0.05$, $n = 59$. When the dependent value is a measure of fitness in such a regression, β is the selection

Table 3 Mean (SE) 1997 Δ values and late season (July) gas exchange (A, g, A/g) as a function of plant size (≤ 12 rosettes = juveniles, >12 rosettes = adults) and whether they survived until 2001 or not

	Δ	A (µmol m^{-2} s^{-1})	g (mol m^{-2} s^{-1})	A/g
Juveniles				
Surviving	20.22[a] (0.08)	18.49[a] (1.52)	0.42 (0.04)	46.59[a] (2.48)
Dying	19.89[b] (0.13)	22.00[a,b] (2.33)	0.44 (0.06)	54.86[a,b] (3.81)
Adults				
Surviving	19.77[b] (0.08)	25.67[b] (1.58)	0.46 (0.04)	58.44[b] (2.58)
Dying	19.97[a,b] (0.12)	24.27[b] (2.33)	0.50 (0.06)	53.25[a,b] (3.81)

Values with the same superscript are not significantly different ($P < 0.05$)

differential. Also for adults in 2000, there was a trend toward a significant regression between standardized values of Δ and the relative number of flowering stalks ($\beta = -0.22$), but it was not significant ($P < 0.10$). For adult plants, there was no relationship between Δ and RE in 2000, RGR from 1999 to 2000, or RGR from 2000 to 2001, using either linear or quadratic regression. There was no relationship between Δ and any measure of size, growth or reproduction in 1997.

Discussion

Phenotypic plasticity

Our measures of Δ and plant performance over several years illustrate both phenotypic plasticity in measures of Δ and selection on Δ phenotypes. Plasticity in Δ includes year-to-year variation, which tracks precipitation, and developmental plasticity because the lower Δ values found in older plants is not explained by selective mortality in younger stage classes. In fact, just the opposite occurs. Plants first identified as juveniles in 1997 that survived the 5 years of the study had higher Δ values in 1997 than did juveniles that failed to survive the 5 years. In *C. flava*, as in *Chrysothamnus nauseosus* (Donovan and Ehleringer 1991), an individual plant must develop greater WUE as it ages. According to the instantaneous mid-day gas exchange data, this change in Δ with plant size could be due, in part, to older plants maintaining a higher photosynthetic rate but similar stomatal conductance late in the growing season.

The strong relationship between Δ measured in a particular year and precipitation in the preceding autumn (September–November) has at least two possible explanations. First, this may confirm physiological plasticity in response to late season rainfall. We have documented the production of new leaves in *C. flava* following substantial rains in late August and early September, when plants would be dormant in many years (Casper et al. 2001). Finding higher Δ values following years of particularly heavy autumn precipitation is consistent with significant assimilation under the cooler autumn temperatures. If this is the explanation, it must also mean that carbon assimilated during the preceding autumn figures importantly in spring leaf construction. Alternatively, the relationship between Δ

and autumn precipitation may simply indicate the contribution of autumn precipitation to recharging soil water that will support new growth the following spring.

Selection on Δ

Two conditions must be met in order for natural selection to be operating on Δ ; there must be a correspondence between Δ phenotypes and plant fitness, and there must be a genetic basis to variation in Δ (Geber and Griffen 2003). The first condition is met by the selective mortality of juveniles with lower Δ values. The resulting selection differential of 0.22, when adjusted for relative fitness, is above the median of 0.16 reported by Kingsolver et al. (2001) for previously published studies examining phenotypic selection on quantitative traits. As in many studies, our data are truncated in that we did not measure lifetime reproduction. Selection in another form occurred during the drought year of 2000, when there was a tendency for adults with lower Δ values to have higher reproductive output, a relationship that might have improved with a larger sample size. Together these results suggest that the direction of selection on Δ changes as plants age (Donovan and Ehleringer 1991).

The mid-day gas exchange measures in late season 1997 allow us to make some judgments about selection on photosynthesis and transpiration, the two components of WUE. The fact that photosynthesis also did not differ in the two survival categories of juveniles is contrary to the idea that selection operates to maximize photosynthesis instead of WUE. Likewise, a comparison of photosynthesis and WUE in the different size and survival categories does not suggest that these parameters are negatively related. These conclusions must be qualified, however, because instantaneous gas exchange measures present limited information. Plants could also differ in the onset or duration of the diurnal or seasonal periods over which they are photosynthetically active. Late season photosynthesis is much reduced from the maximum photosynthetic rates measured in *C. flava*, which can exceed 40 µmol m^{-2} s^{-1} (Forseth et al. 2001), comparable to rates in desert annuals (Gibson 1998).

In adult plants, the strength of selection on Δ seems to vary with soil water availability. The negative correlation we found in adult plants between Δ and total

rosettes and the nearly significant correlation between Δ and total flower production only in 2000 suggests either that individuals capable of higher WUE are more likely to exhibit higher performance under drought stress (Dudley 1996a, b) or that the carbon sinks, in part caused by flowers and fruits (Paul and Foyer 2001), increase assimilation proportionately more than transpiration in such years. The latter would mean that low Δ values are a manifestation of large plant size and/or high reproductive output rather than the cause of them. In this case, our measures of Δ were limited to those individuals for which we measured instantaneous gas exchange, and measures of Δ and performance in larger numbers of plants are needed to evaluate the relationship between Δ and reproduction more fully.

Genetic basis to Δ

The concordance in ranked values of Δ and the large number of significant correlations between Δ values measured on the same plants in different years supports the idea that there is a genetic basis to variation in Δ in *C. flava* such that genotypes are consistent in their expression of Δ with respect to each other, but such results could also indicate consistent differences in the microhabitats the plants occupy (Donovan and Ehleringer 1994). The lack of correlation between Δ values in 1998, the wettest year, and those of any other year suggests that any genotypic variation in WUE is obscured under conditions of greater soil water availability, again supporting the idea that selection on WUE may be stronger in dry years.

Data collected in this study of phenotypic selection well illustrate why documenting genotypic selection in natural populations is difficult. In *C. flava*, as found in other perennial species, Δ values change as plants grow or age (Donovan and Ehleringer 1991; Cavender-Bares and Bazzaz 2000), and so genotypic variation in Δ and phenotypic selection need to be examined in long-term studies of the same individuals. Spatial and temporal environmental heterogeneity together with strong phenotypic plasticity may also obscure any genetically based variation in Δ (Donovan and Ehleringer 1994). In this study, both the expression of Δ and the occurrence of correlations between Δ and plant performance traits varied among years. Additionally, there is the possibility for selection on traits correlated with Δ or indirect selection if fitness and Δ respond to the same environmental variables (Rausher 1992; Arntz and Delph 2001; Geber and Griffen 2003). For example, if large plants exhibit lower Δ values simply because of the particular gas exchange properties of large plants, then selection may well operate, instead, on other traits that directly influence plant size and reproduction. Similarly, the lower values of Δ in juveniles that failed to survive could have been symptomatic of water stress.

Studies of selection on physiological traits incur particular problems. Especially because WUE is a composite of two physiological parameters, transpiration and photosynthesis, correlations between these components or with other physiological traits may particularly limit our ability to interpret the significance of any apparent selection on Δ (Geber and Dawson 1997; Gibson 1998; Arntz and Delph 2001). Physiological studies often have small sample sizes because of the instrumentation necessary to make physiological measurements, and large sample sizes are needed to gain the statistical power necessary to detect selection of the magnitude often reported for natural populations (Kingsolver et al. 2001). Future studies relating variation in physiology to fitness should take all these factors into consideration.

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