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## Carbon isotope discrimination differences within and between contrasting populations of *Encelia farinosa* raised under common-environment conditions

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**Abstract** Previous studies of the desert shrub *Encelia farinosa* have shown variation of morphological and physiological integration that appears to match environmental differences among populations. Such findings led us to ask if there is a genetic basis for such differentiation that may be related to physiological control of intercellular CO<sub>2</sub> concentrations as indicated by carbon isotope discrimination ( $\Delta$ ) values, and if genetic variance for  $\Delta$  is detectable within populations. Under common environment conditions,  $\Delta$  values were compared between two populations of *E. farinosa* from desert regions with contrasting rainfall patterns: Superior, Ariz., a region with high annual rainfall and droughts of short duration, and Oatman, Ariz. a region with lower annual rainfall and longer drought periods. Superior plants had consistently greater mean  $\Delta$  values than Oatman plants across a broad range of soil water potentials, indicating that there is a genetic basis for  $\Delta$  variation between these populations. At the intrapopulation level only Oatman plants showed detectable genetic variance of  $\Delta$  based on: (1) consistent individual-rank values for  $\Delta$  among soil-drought stages, and (2) evidence of heritable genetic variance for  $\Delta$  during one drought stage. No genetic variance in  $\Delta$  was evident for the Superior population. It is hypothesized that the high spatio-temporal heterogeneity of water availability at Oatman may facilitate the maintenance of genetic variance for carbon isotope discrimination within this population. Both the inter- and intra-population level findings suggest that selection associated with rainfall and drought has resulted in genetic divergence of the physiological factors involved in  $\Delta$  determination for these populations. There appears to be strong differences of water-use and carbon-gain strategies among populations,

and broader functional breadth among plants in the habitat of greatest environmental heterogeneity.

**Keywords** Carbon isotope discrimination · Drought response · *Encelia farinosa* · Environmental heterogeneity · Genetic variation

### Introduction

Carbon isotope discrimination ( $\Delta$ ) of C<sub>3</sub> plants depends on the ratio of internal-to-ambient CO<sub>2</sub> concentrations ( $c_i/c_a$ ) during photosynthesis (Farquhar et al. 1989; Vogel 1993). While  $c_a$  is constant over a broad range of environmental conditions,  $c_i$  can change greatly as a result of physiological responses to the environment; thus,  $\Delta$  values of C<sub>3</sub> plants can be closely correlated with environmental variation. However, there may also be marked differences in  $\Delta$  values due to genetic factors. For example, comparisons among species within the same community have demonstrated life-form based, interspecific differences of  $\Delta$  – longer-lived species having consistently lower values relative to shorter-lived species (Ehleringer and Cooper 1988; Schuster et al. 1992; Huc et al. 1994). Other studies have shown intraspecific genetic determination for  $\Delta$  in crop cultivars (see Hall et al. 1993; Richards and Condon 1993), weeds (Geber and Dawson 1990, 1997) and community dominants (e.g., Johnson et al. 1993; Rundel and Sharifi 1993; Flanagan and Johnsen 1995; Ares et al. 2000). Although  $\Delta$  variation is often associated with fitness-related characters, and a selective basis for differentiation within and among species is probable given the consistency of  $\Delta$  variation among life forms and genotypes, only a handful of studies have examined genetically-based variation of  $\Delta$  within the context of natural selection factors (Geber and Dawson 1990; Comstock and Ehleringer 1992a; Donovan and Ehleringer 1994; Zhang and Marshall 1995; Guehl et al. 1995; Ares et al. 2000).

Consistent differences of  $\Delta$  means among species are so prevalent that some reviews have proposed it might be

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useful as an indicator of a metabolic 'set-point' (Ehleringer 1993a; Marshall and Monserud 1996); the set point being a result of integrated physiological processes that function to maintain a relatively constant  $c_i/c_a$  across two or more time periods or environments. If so, variation in  $\Delta$  should be strongly associated with selective factors that influence the physiological determinants of  $c_i/c_a$ . Testing this hypothesis requires examination of  $\Delta$  variation using a population genetic approach.

To address the selective basis and physiological determinants of  $\Delta$  variation in the desert perennial *Encelia farinosa* Gray (*Asteraceae*), we examined the genetic and environmental components of variance between and within two populations raised under common environment conditions. This species occupies a very broad range over which there is extreme environmental variability, especially for water availability, and water, being the resource most limiting to desert plant growth (Le Houerou et al. 1988; Comstock and Ehleringer 1992b), should play a crucial selective role in the physiological variation of this species. For this investigation, one population, Oatman, originated from a dry habitat (ca. 100 mm year<sup>-1</sup> mean annual rainfall) whereas the other, Superior, was from a more mesic environment (ca. 450 mm year<sup>-1</sup>).

These contrasting environments appear to have influenced divergence of a suite of traits associated with water-use and productivity in *E. farinosa*. Leaf retention into drought is longer, and leaf pubescence density, which is associated with reduced water consumption, is greater for plants of the more-arid population, Oatman (Sandquist and Ehleringer 1997, 1998). In contrast, Superior plants have greater instantaneous photosynthesis and stomatal conductance rates – except when drought is most severe (Sandquist and Ehleringer 1997, 1998). Thus, when raised under similar ambient conditions, it was predicted that plants from the Superior population would have higher  $\Delta$  values, resulting from the greater  $c_i/c_a$  values that typically accompany higher rates of stomatal conductance, whereas plants from Oatman should have lower  $\Delta$  (and  $c_i/c_a$ ) values reflecting the mechanisms for greater water conservation.

In addition, it was hypothesized that intrapopulation variance of  $\Delta$  would correspond to the degree of spatio-temporal heterogeneity of water availability within a site. Where water availability is more unpredictable (i.e., Oatman), plants displaying high  $\Delta$  values may have a fitness advantage in high rainfall years or microhabitats, while low- $\Delta$  plants may have greater relative fitness in poorer years or microhabitats. Similar climatic variability can promote genetic differentiation and maintain polymorphisms for traits such as flowering-time (Fox 1989) and seed morphology (Venable et al. 1995) in desert annuals. If similar selection processes are influencing physiological trait variation within *E. farinosa* populations, then greater  $\Delta$  variance would be expected in environments where variability of rainfall and drought are most extreme.

## Materials and methods

### Seed collection sites

*Encelia farinosa* (brittlebush) is an obligate-outcrossing desert perennial shrub with a normal life span of 10–25 years. In the northern part of its distribution this species spans a broad rainfall gradient, ranging from <50 mm year<sup>-1</sup> to >450 mm year<sup>-1</sup> (Turner et al. 1995; Sandquist and Ehleringer 1998). Two populations along this gradient were chosen as seed sources for the present study. The drier site, Oatman, Arizona (34°55'N, 114°25'W, 427 m), is in a region of low rainfall (115±59 mm year<sup>-1</sup>, mean annual rainfall ±SD;  $n=51$  years for all climate data) with moderate drought lengths (91±65 days, mean duration of longest annual rain-free period ±SD). The wetter site, Superior, Arizona (33°46'N, 111°05'W, 914 m) is in a region with higher rainfall (432±138 mm year<sup>-1</sup>, mean ±SD) and shorter drought duration (43±13 days, mean ±SD). The coefficient of variation (CV; calculated as standard deviation divided by mean) for annual rainfall at these sites and the CV of longest annual drought were used as two measures of heterogeneity with respect to water availability. The former represents the unpredictability of rainfall amount received each year; the latter is a measure of the year-to-year unpredictability of drought duration. Both indices were greater for the drier site (51% and 71% respectively) than for the wetter site (32% and 30% respectively) – a pattern typically seen in comparisons of spatio-temporal heterogeneity of water availability in arid regions (MacDonald 1956; Ehleringer 1994; Frank and Inouye 1994). This variation in unpredictability should bear significantly on selection related to drought response in *Encelia farinosa*.

### Common garden study 1

Seeds from up to 20 plants of each population were germinated in September 1990 at the University of Utah greenhouse and transplanted as seedlings to a common garden plot at the Desert Botanical Garden (DBG) of Phoenix, Arizona, on 31 March 1991. Low germination of Superior seeds resulted in only 10 usable seedlings for this population; 20 seedlings were used from the Oatman population. All plants were planted in an unblocked random array within a 12-plant ×12-plant matrix (144 m ×144 m) on native desert alluvium soil. To fill out the matrix, 121 other *E. farinosa* seedlings of the same age were transplanted at the same time. All individuals were separated by 2 m and none of the 30 experimental plants were planted on the matrix border. Seedlings were watered daily for 2 weeks and then watered once monthly through August 1991.

Predawn water potential ( $\psi_{pd}$ ), the ratio of internal- to ambient- $\text{CO}_2$  concentration ( $c_i/c_a$ ) and stomatal conductance ( $g$ ) were measured (see below) approximately every 45 days from March to October 1992. Leaf samples (ca. 5 per plant) for carbon isotope analyses were collected on these same dates except in the first month (March). Carbon isotope ratio values were determined for all 10 plants of the Superior population, and for a subset of 10 randomly chosen plants of the Oatman population – the same plants were used for each monthly measurement. Comparisons from this study provided an estimate of the within and between population variability of fully matured *E. farinosa* plants grown in a natural setting.

### Common garden study 2

In the second study, 6 individuals each from 10 Oatman families and 5 Superior families were raised in a common environment at the University of Utah Research Garden (URG) to estimate intrapopulation genetic variances of  $\Delta$ . Field-collected seeds taken from one maternal plant constituted a 'family'. The relatedness of individuals within each family could therefore range from half-sibling to full-sibling.

Seeds were germinated in spring 1992 in the University of Utah greenhouse and transplanted in mid-June to 55-l pots containing coarse sand; one plant per pot. Plants were randomly arrayed in an open field at URG and watered to pot capacity every day for 1 month (predrought). At the end of this month, samples of the most recently matured leaves ( $N=2-3$  leaves per plant) were collected for isotope analyses. Plants were then subjected to three incremental drought stages. In the 'early-drought' stage, watering was reduced to once every 5 days for 3 weeks (natural precipitation provided an addition input of 2 mm). On the last day of this stage leaf samples were collected for carbon isotope analyses (as above). Water input during the second drought stage (mid-drought) came from one manual watering (to pot capacity) on day 1, and light rain showers on five of the next 15 days (total precipitation = 18.5 mm). Plants then received no water for the following 20 days, after which leaves were collected for isotope analyses (as above). The 'late-drought' stage was terminated after one week due to unusually low minimum temperatures, which damaged the majority of leaves.

### Carbon isotope discrimination

Leaves collected on each date were always of the two most recently matured cohorts. These leaves were bulked into a single sample per plant and oven dried at 70°C for 72 h. Dried leaves were ground with mortar and pestle into a fine powder, from which 1.5–2.5 mg subsamples were used for carbon isotope analyses.

Carbon isotope ratios ( $\delta$ ) were determined relative to the Pee Dee Belemnite standard using a Delta S mass spectrometer (Finnigan-MAT, San Jose, Calif.) at the Stable Isotope Ratio Facility for Environmental Research, University of Utah, Salt Lake City, Utah. Carbon isotope ratio values were converted to discrimination values ( $\Delta$ ) by the equation,

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p) \quad (1)$$

where  $\delta_p$  is the carbon isotope ratio of the plant, and  $\delta_a$  is the carbon isotope ratio of  $\text{CO}_2$  in the atmosphere ( $-0.008$  or  $-8\text{‰}$ ). The standard per mil ( $\text{‰}$ ) notation is used throughout this text.

The  $\Delta$  value of a  $\text{C}_3$  plant is related to the ratio of intercellular-to-ambient  $\text{CO}_2$  concentration ( $c_i/c_a$ ) based on the model

$$\Delta = a + (b - a)c_i/c_a \quad (2)$$

where  $a$  (4.4‰) is discrimination due to diffusion differences between  $^{13}\text{CO}_2$  and  $^{12}\text{CO}_2$ , and  $b$  (27‰) is net carboxylation discrimination due to the greater affinity for  $^{12}\text{CO}_2$  relative to  $^{13}\text{CO}_2$  by ribulose 1,5 bisphosphate carboxylase-oxygenase (Farquhar et al. 1989). Overall, the  $\Delta$  value of a leaf reflects the time-integrated average of  $c_i/c_a$  values present during leaf construction and photosynthate production. Variation of  $c_i/c_a$  is influenced by changes in stomatal conductance ( $g$ ) and biochemical processes associated with carbon assimilation. The value of  $c_i/c_a$  will decrease as stomatal conductance decreases or as biochemical activity increases. For the most part, differences in  $\Delta$  values reflect variation in these physiological mechanisms, however, the actual  $\Delta$  value for a leaf can depart from the relationship in Eq. 2 if a significant amount of stored carbon (assimilated at an earlier time) is translocated to the leaf.

### Field measurements of $c_i/c_a$ , stomatal conductance, and $\psi_{pd}$

Instantaneous measurements of internal-to-ambient  $\text{CO}_2$  concentrations ( $c_i/c_a$ ) and stomatal conductance ( $g$ ) were made only in the interpopulation study (DBG common garden), using a LiCor 6200 portable gas analyzer (LiCor, Lincoln, Neb.). Measurements were made during peak daily photosynthetic activity. All leaves measured were mature and in the exposed upper canopy. Predawn plant water potential values ( $\psi_{pd}$ ), also measured for only the DBG study, were taken as an indicator of soil water potential and baseline plant water status ( $N=1-3$  stems per plant). Measurements

were made prior to sunrise with a Scholander-type pressure chamber (PMS, Corvallis, Ore.).

### Statistical analyses

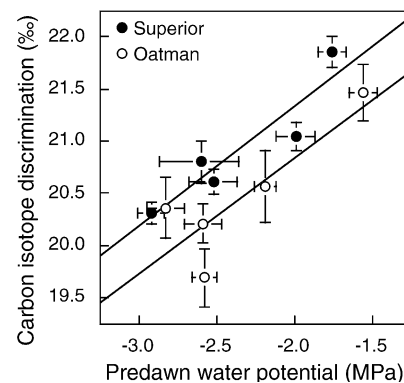
All statistical analyses were executed with JMP 3.1 statistical package (SAS 1994). Unless otherwise noted, standard methods of Model II analysis of variance were used. For interpretation of interpopulation or interfamilial seasonal differences, a repeated measures analysis of variance (ANOVAR) was employed. Only values of  $\psi_{pd}$  required transformations; a common-log transformation of the absolute value of  $\psi_{pd}$  was used since  $\psi_{pd}$  values are negative.

## Results

### Interpopulation differences of $\Delta$

At the Desert Botanical Garden,  $\psi_{pd}$  values decreased from March through October, but season-long mean  $\psi_{pd}$  did not differ among populations (ANOVAR:  $df=1$ ,  $F=0.04$ ,  $P=0.84$ ) indicating that plants from both populations experienced similar water availability throughout the study period. There was a positive relationship between  $\psi_{pd}$  and carbon isotope discrimination for both populations (Fig. 1), and no differences were detected between the slopes of these relationships ( $df=1$ ,  $F=0.12$ ,  $P=0.74$ ). However, Superior plants had significantly higher mean  $\Delta$  values than Oatman plants at any given  $\psi_{pd}$  (analysis of covariance: population effect  $df=1$ ,  $F=8.74$ ,  $P=0.004$ ).

Lower  $\Delta$  values are indicative of decreased  $c_i/c_a$  ratios – which appears to have resulted from a reduction of stomatal conductances ( $g$ ) in response to the drop in water availability for both populations (Table 1). Indeed, there was a consistent positive relationship between  $g$  and  $c_i/c_a$  for both populations, except in July when the  $c_i/c_a$  values were relatively high for the corresponding values of  $g$  (Fig. 2). This discontinuity may have resulted from the extremely high leaf temperatures (mean=41.6°C) and low

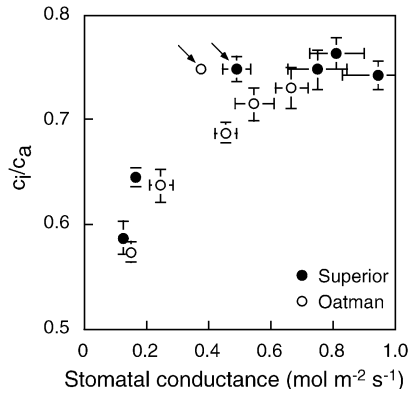


**Fig. 1** Relationships between predawn water potential and carbon isotope discrimination for *Encelia farinosa* plants from the arid Oatman population and wetter Superior population grown in a common environment (Desert Botanical Garden, Ariz.). Values shown are means  $\pm 1\text{SE}$

**Table 1** Monthly mean values for predawn water potential, stomatal conductance, internal to ambient CO<sub>2</sub> ratio and carbon isotope discrimination of Oatman and Superior plants raised in a common environment at Desert Botanical Garden, Phoenix, Ariz.

	Oatman				Superior			
	$\Psi_{pd}$	$g_{max}$	$c_i/c_a$	$\Delta$	$\Psi_{pd}$	$g_{max}$	$c_i/c_a$	$\Delta$
March	-1.20 (0.05)	0.667 (0.05)	0.73 (0.02)	na	-1.25 (0.05)	0.812 (0.09)	0.76 (0.02)	na
April	-1.56 (0.09)	0.546 (0.06)	0.71 (0.02)	21.47 (0.27)	-1.76 (0.09)	0.749 (0.09)	0.75 (0.02)	21.85 (0.15)
May	-2.19 (0.08)	0.456 (0.04)	0.69 (0.01)	20.56 (0.34)	-1.99 (0.12)	0.945 (0.11)	0.74 (0.01)	21.05 (0.13)
July	-2.83 (0.12)	0.376 (0.03)	0.75 (0.01)	20.36 (0.29)	-2.52 (0.16)	0.492 (0.05)	0.75 (0.01)	20.61 (0.12)
Sept.	-2.59 (0.13)	0.246 (0.04)	0.64 (0.02)	20.21 (0.19)	-2.61 (0.22)	0.166 (0.02)	0.64 (0.01)	20.80 (0.20)
Oct.	-2.58 (0.08)	0.149 (0.01)	0.57 (0.01)	19.69 (0.28)	-2.93 (0.08)	0.124 (0.02)	0.59 (0.02)	20.31 (0.11)

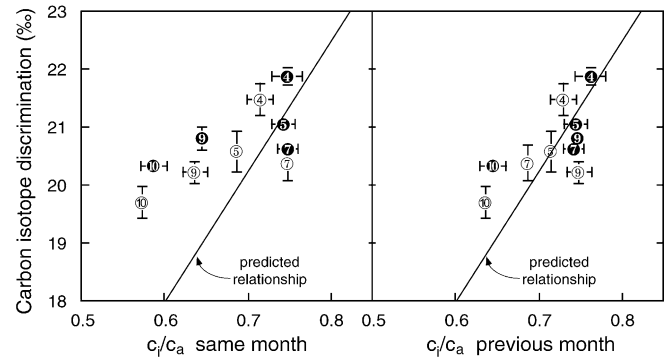
Values are presented as mean (1SE).  $\Psi_{pd}$ =predawn water potential (MPa),  $g_{max}$ =stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>),  $c_i/c_a$ =internal-to-ambient CO<sub>2</sub> ratio, and  $\Delta$ =carbon isotope discrimination (‰).  $n=20$  and 10 plants for Oatman and Superior, respectively



**Fig. 2** Relationship between stomatal conductances and the ratio of internal to ambient CO<sub>2</sub> concentrations ( $c_i/c_a$ ) for *Encelia farinosa* plants from the arid Oatman population and wetter Superior population grown in a common environment (Desert Botanical Garden, Ariz.). July values, noted by arrows, appear to depart from the general relationship due to high water stress at this period. Values shown are means $\pm$ 1SE

$\Psi_{pd}$  values (mean=-2.72 MPa) in this month—conditions that often lead to a biochemical disruption of CO<sub>2</sub> assimilation, which would cause relatively higher internal CO<sub>2</sub> concentrations. Nonetheless, stomatal conductance values were consistently higher for Superior plants during each month (except when  $\Psi_{pd}$  values were very low; Table 1). Thus, the greater  $\Delta$  values for Superior plants can be explained by higher overall stomatal conductance during periods of carbon assimilation.

Mean values of measured  $c_i/c_a$  were plotted against the mean  $\Delta$  values for leaves collected on the same day (Fig. 3a). These values were compared to the expected relationship between  $c_i/c_a$  and  $\Delta$  as predicted by Eq. 2 (line in Fig. 3a). The measured values of  $c_i/c_a$  versus  $\Delta$  are close to the predicted relationship during spring months but considerably removed from the theoretical  $c_i/c_a$ - $\Delta$  relationship in September and October (Fig. 3a). These two disparities may indicate that a high proportion of the carbon in these leaves came from CO<sub>2</sub> assimilated earlier in the month, or translocated from storage carbon (see also Smedley et al. 1991). Indeed, the relationship between  $\Delta$  values and  $c_i/c_a$  measured in the previous month more closely approximates the theoretical relationship between  $\Delta$  and  $c_i/c_a$  (Fig. 3b). The discrepancy



**Fig. 3** Carbon isotope discrimination ( $\Delta$ ) of *Encelia farinosa* leaves versus, ratio of internal to ambient CO<sub>2</sub> concentration ( $c_i/c_a$ ) measured in the same month that leaves were collected (left) and  $c_i/c_a$  measured in the month prior to leaf collections (right). All plants were raised at the Desert Botanical Garden, Ariz. Each symbol represents mean values  $\pm$ 1SE for plants ( $N=10$ ) of the Superior population (leaves collected in April, 4 against black; May, 5 against black; July, 7 against black; September, 9 against black or October, 10 against black) and of the Oatman population (leaves collected in April, ringed 4; May, ringed 5; July, ringed 7; September, ringed 9; October, ringed 10). Also shown is a line depicting the predicted relationship between  $\Delta$  and  $c_i/c_a$  based on the equation:  $\Delta=a+(b-a)c_i/c_a$

remained greatest, however, for later months. For example, the  $\Delta$  values for October leaves predicted  $c_i/c_a$  values that are even greater than those actually measured in the previous month of September (Superior: 0.70 vs 0.64 respectively,  $t=6.28$ ,  $P<0.001$ ; Oatman: 0.68 vs 0.64 respectively,  $t=1.50$ ,  $P=0.08$ ).

#### Intrapopulation differences of $\Delta$

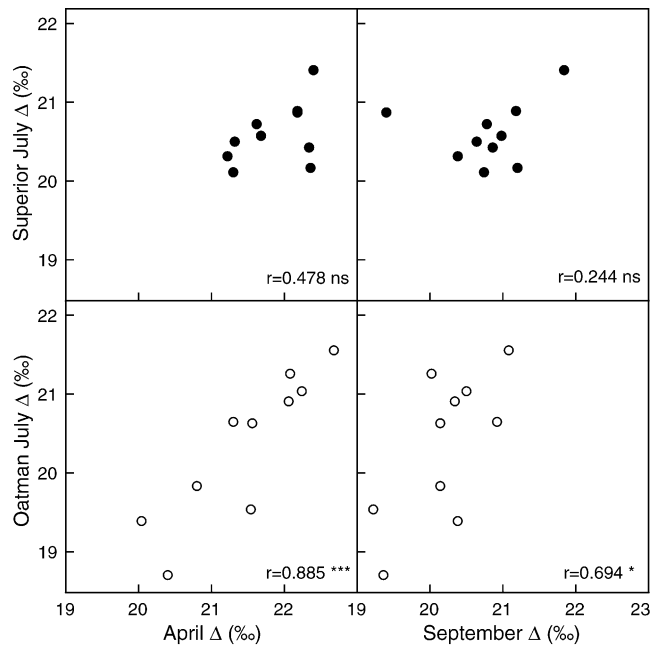
Comparisons within each population at the Desert Botanical Garden showed that carbon isotope discrimination was positively correlated among drought stages in eight of ten comparisons for the Oatman population, but in only 3 of 10 comparisons for Superior plants (Table 2). Two periods during which plant responses must accommodate major environmental changes are: (1) summer drought – from the low water stress of early spring to the high water stress of late summer, and (2) monsoon

**Table 2** Correlation matrix among dates for carbon isotope discrimination by *Encelia farinosa* plants from Superior (above diagonal) and Oatman (below diagonal). Plants were raised under

	April	May	July	September	October
April	–	0.6705*	0.4784	0.3119	0.7476*
May	0.8427**	–	0.6103	0.2236	0.4418
July	0.8853***	0.9300***	–	0.2444	0.5133
September	0.3948	0.6860*	0.6936*	–	0.6409*
October	0.4296*	0.6764*	0.7356*	0.9349***	–

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

common environment conditions at the Desert Botanical Garden in Phoenix, Ariz. Values are Pearson product-moment coefficients.  $n=10$  plants per population



**Fig. 4** Correlations of seasonal carbon isotope discrimination values ( $\Delta$ ) for *Encelia farinosa* from Superior (upper panels) and Oatman (lower panels) raised at Desert Botanical Garden, Ariz. Left panels depict correlations for changes during the premonsoon period (April–July), right panels show correlations for premonsoon to post-monsoon months (July–September). Correlation coefficients and significance probabilities are shown in the lower right portion of each panel. See Table 2 for complete correlation matrix for all dates

commencement – when summer rainfall alleviates summer drought. In both comparisons, Superior plants showed no significant correlation among periods whereas Oatman plants had strong positive correlations (Fig. 4; Table 2). Thus, Oatman plants had similar response patterns thereby maintaining their relative ranks through these transition periods whereas Superior plants either had different responses or the variance among individuals was too narrow to detect rank maintenance.

To further evaluate these patterns, we examined intrapopulation differences of individual relationships between  $\Delta$  and water status ( $\log\psi_{pd}$ ), and included the plant-by- $\log\psi_{pd}$  effect, which corresponds to the slope differences or similarities among plants in the population. The water potential effect on  $\Delta$  was significant for both populations

**Table 3** Estimates of variance components, intraclass correlations and heritabilities (broad-sense) for carbon isotope discrimination ( $\Delta$ ) at two stages of drought for Superior and Oatman *Encelia farinosa* plants grown in a common environment

	Variance components <sup>a</sup>		Intraclass correlation	Heritability range <sup>b</sup>
	Family ( $V_g$ )	Total ( $V_p$ )		
<b>Superior</b>				
Early drought	0.007	0.227	n.s.	–
Mid-drought	0.047	0.300	n.s.	–
<b>Oatman</b>				
Early drought	0.016	0.173	n.s.	–
Mid-drought	0.055	0.156	0.354*	0.71–1.42

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

<sup>a</sup>  $V_g$  genetic variance based on maternal families,  $V_p$  total (phenotypic) variance

<sup>b</sup> Heritabilities are broad-sense; heritability range is given based on estimates for half-sib relatedness (maximum value) and full-sib relatedness (minimum value)

(Superior:  $df=9$ ,  $F=21.98$ ,  $P < 0.001$ ; Oatman:  $df=9$ ,  $F=7.66$ ,  $P=0.01$ ), but neither population showed a significant plant-by- $\log\psi_{pd}$  interaction (Superior:  $df=9$ ,  $F=0.90$ ,  $P=0.54$ ; Oatman:  $df=9$ ,  $F=0.39$ ,  $P=0.93$ ), indicating that the slopes of individual plant responses were similar, on average. The plant effect was not significant for Superior ( $df=9$ ,  $F=1.28$ ,  $P=0.29$ ), but was significant for Oatman plants at a probability level of 0.08 ( $df=9$ ,  $F=1.96$ ,  $P=0.08$ ). These results concur with those of the correlation analysis in that Oatman plants had high variability among individuals and equivalent responses to  $\psi_{pd}$  whereas no mean differences of  $\Delta$  were found among individuals of Superior.

Genetic variances for carbon isotope discrimination were determined from the Utah Research Garden study based on intraclass correlations (maternal family constitutes the class). Because no information exists about sires (pollen donors) heritabilities ( $h^2$ ) are in the broad sense. The potential range of heritability values were calculated by estimating maximum  $h^2$  value based on assumptions of half-sib relatedness among all progeny and negligible epistatic effects, and minimum  $h^2$  value based on full-sib relatedness among all progeny (Falconer 1989).

Oatman plants had a significant intraclass correlation for  $\Delta$  values during early drought but not at mid-drought (Table 3). The maximum heritability of 1.42 is impossibly

high, indicating that relatedness of progeny within a family is probably closer to full-sibling, on average. Superior plants showed no significant correlation at either drought stage (Table 3). Small sample sizes and the cooler and wetter environment at URG precluded a more robust analysis of genetic variances for these populations. Nonetheless, the significant genetic variance for Oatman  $\Delta$  does correspond to the DBG findings associated with intrapopulation  $\Delta$  variation.

## Discussion

### Genetic basis of $\Delta$ variation

Two populations of *Encelia farinosa* from contrasting climates were used to examine intraspecific genetic variability for carbon isotope discrimination and the physiological correlates that may result in such variation. When raised in common environments, plants from the more-arid Oatman region had consistently lower  $\Delta$  values than plants of the wetter Superior region, and the differences were maintained over a broad range of  $\psi_{pd}$  values (Fig. 1). These results coincide with the lower rainfall and longer drought-length at Oatman relative to Superior, and indicate that the  $\Delta$  differences between these populations result from a genetic basis (Table 1; see also Sandquist and Ehleringer 1997).

Previous studies have also found differences in  $\Delta$  among plants from environments with contrasting water availability, but the results have not always been consistent (e.g., Rundel and Sharifi 1993; Zhang et al. 1993, Lauteri et al. 1997). This may be because carbon isotope discrimination is associated with many physiological traits, and the importance of any of these traits, in a fitness context, might vary among environments and species (Donovan and Ehleringer 1994).

For desert plants, geographic variation of physiological function is often ascribed to selection resulting from the relationship between water availability and the tradeoff between carbon gain and water loss (Ehleringer 1993a). Where water is most limited, water-use strategies that reduce water loss should confer better survival whereas more liberal use of water, associated with greater instantaneous productivity, might be favorable where rainfall is relatively high and drought duration short (see also Cohen 1970; Orians and Solbrig 1977; Dawson and Ehleringer 1993). The  $\Delta$  variation found between Oatman and Superior plants in the present study agrees with this prediction. Variation found in other traits associated with water-use and carbon gain in *E. farinosa* also concurs with this hypothesis. For example, Sandquist and Ehleringer (1997, 1998) have shown that leaf pubescence of *E. farinosa*, which helps reduce transpirational demand, is always greater for Oatman plants than for Superior plants.

At the intrapopulation level, phenotypic and genotypic variances were high for Oatman plants and there was evidence of genetically based variation in  $\Delta$  (Table 3).

Such variation was not found in the Superior plants (Table 3). Although any differences within a common environment imply genetic variation, this conclusion should still be interpreted cautiously (Nilsen and Semons 1997). However, the consistency of results from both the URG and DBG common environment studies strongly suggests that the genetic differences found are tenable.

### Maintenance of $\Delta$ variation within *E. farinosa*

Genetic variance in  $\Delta$  has been found among cultivars of many crop species (e.g., Hall et al. 1993; Johnson et al. 1993), and in comparisons of wild plant populations from environments with different levels of stress (e.g., Comstock and Ehleringer 1992a; Zhang et al. 1993). Results from the present study suggest that evolutionary changes have given rise not only to differences in mean  $\Delta$  between populations but also to deviation in the variance of  $\Delta$  within populations.

An extreme degree of spatio-temporal heterogeneity could maintain greater within-population trait variation as compared to populations from regions with lower environmental heterogeneity (Orians and Solbrig 1977; Ellner and Hairston 1994). At the site with less predictable water availability, Oatman, relatively wet periods or microsites might occur often enough to maintain genotypes that have greater fitness in wet environments – those that operate at higher  $c_i/c_a$  values and thus have higher  $\Delta$  values. Other plants, which operate at lower  $c_i/c_a$  values and have lower  $\Delta$  values, are generally those with greater survival during drier years, through longer droughts, and within drier microsites (Ehleringer 1993a).

Indeed, high phenotypic variance in  $\Delta$  appears to be common in *E. farinosa* of drier habitats. Ehleringer (1993b) found relatively high variance in  $\Delta$  values for one population of *E. farinosa* (also in the Oatman region), and showed that individuals of this population maintained their  $\Delta$  rank over a prolonged drought. We found a similar pattern for our Oatman population in the DBG study (Table 2). In conjunction with evidence from the UTG study these patterns suggest that variation in  $\Delta$  at Oatman is related to high genetic variation, which may be maintained by the highly heterogeneous nature of this region.

The same patterns were not found for plants of the more mesic Superior region (Table 2). These plants had higher  $\Delta$  values, less variance and no consistent rank correlation between drought periods (Tables 2, 3). Ehleringer (1993b) found that *E. farinosa* plants with higher  $\Delta$  values were more likely to die during drought, but had greater productivity in the absence of competition or water stress. This association (also found in other species, Geber and Dawson 1990) suggests that higher water availability and lower drought stress at Superior should favor plants with greater  $\Delta$  values. In addition, the Superior site has lower spatio-temporal heterogeneity in water availability. As such, stabilizing selection for high  $\Delta$  values may be relatively strong and consistent enough

to reduce variation (Falconer 1989), to the extent that rank maintenance of  $\Delta$  values, or 'set point' differences (sensu Ehleringer 1993b) are undetectable.

In summary, we found genetic divergence for mean carbon isotope discrimination values between the Oatman and Superior populations of *E. farinosa*, and this divergence may be related to differences in amount and timing of water availability at these locations. Plants from a relatively arid and unpredictable environment (Oatman) had lower  $\Delta$  values and greater variances which stem, in part, from greater genetic variance. Higher  $\Delta$  values and lower variances were observed for the population where rainfall is greater and more predictable (Superior). For these populations,  $\Delta$  variation seems to reflect the functional integration of traits associated with a strategy of perseverance in the arid and unpredictable environment, versus a strategy of greater instantaneous productivity in the region where water availability is greater and more predictable. Furthermore, there appears to be greater breadth of function present within the more unpredictable habitat, where greater heterogeneity may result in the persistence of broader genotypic variation.

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