

Phenotypic selection on leaf water use efficiency and related ecophysiological traits for natural populations of desert sunflowers

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Abstract Plant water-use efficiency (WUE) is expected to affect plant fitness and thus be under natural selection in arid habitats. Although many natural population studies have assessed plant WUE, only a few related WUE to fitness. The further determination of whether selection on WUE is direct or indirect through functionally related traits has yielded no consistent results. For natural populations of two desert annual sunflowers, *Helianthus anomalous* and *H. deserticola*, we used phenotypic selection analysis with vegetative biomass as the proxy for fitness to test (1) whether there was direct and indirect selection on WUE (carbon isotope ratio) and related traits (leaf N, area, succulence) and (2) whether direct selection was consistent with hypothesized drought/dehydration

escape and avoidance strategies. There was direct selection for lower WUE in mesic and dry *H. anomalous* populations, consistent with dehydration escape, even though it is the longer lived of the two species. For mesic *H. anomalous*, direct selection favored lower WUE and higher N, suggesting that plants may be “wasting water” to increase N delivery via the transpiration stream. For the shorter lived *H. deserticola* in the drier habitat, there was indirect selection for lower WUE, inconsistent with drought escape. There was also direct selection for higher leaf N, succulence and leaf size. There was no direct selection for higher WUE consistent with dehydration avoidance in either species. Thus, in these natural populations of two desert dune species higher fitness was associated with some combination direct and indirect selection for lower WUE, higher leaf N and larger leaf size. Our understanding of the adaptive value of plant ecophysiological traits will benefit from further consideration of related traits such as leaf nitrogen and more tests in natural populations.

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Introduction

In deserts habitats, plant growth and reproduction are limited by temporal and spatial availability of water and nutrients (Noy-Meir 1973). Thus, ecophysiological traits related to carbon, water and nutrient relations are expected to be under selection in these habitats (Chapin et al. 1993; Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003; Wright et al.

2003). Although natural selection has been confirmed for morphological and reproductive traits in natural populations, relatively few studies have focused on physiological traits (Kingsolver et al. 2001; Geber and Griffen 2003). We test expectations for natural selection (*sensu* Lande and Arnold 1983) on leaf-level water-use efficiency (WUE) and related leaf traits in natural populations of two desert sunflower species.

At the broadest scale, WUE is the ratio of whole plant carbon gained (whole plant photosynthesis, biomass or yield) to whole plant transpirational water loss (Briggs and Shantz 1913; Blum 2005). At the leaf level, WUE is the ratio of photosynthetic carbon gain to transpirational water loss. Because whole plant biomass gain and water loss are very difficult to measure, two leaf-level proxies of WUE have generally been used for sampling large numbers of native plants: instantaneous A/g (photosynthesis/stomatal conductance) and A/g integrated over leaf life-time from the leaf carbon isotope ratio ($\delta^{13}\text{C}$, see [Materials and methods](#)) (Ehleringer 1993a; Donovan and Ehleringer 1994a, 1994b; Dudley 1996; Heschel et al. 2002; Ludwig et al. 2004; Casper et al. 2005; Heschel and Riginos 2005).

Most expectations for variation in WUE have focused on stomatal regulation because stomatal closure (lower g) decreases transpiration more than A , thus increasing WUE (Cowan 1982; Ehleringer 1993b). If increased WUE is mainly due to lower g , then higher WUE comes at the cost of lower A and productivity (Cohen 1970; Ludlow 1989; Chaves et al. 2003; McKay et al. 2003). On this basis, higher WUE is expected to be favored in arid habitats when decreasing water loss allows the plant to avoid dehydration and extend water use over a longer time period. This strategy is effective when a plant has exclusive use of that soil moisture and can use that moisture later if not used now. However, if the soil moisture can be used by other plants or is otherwise lost via evaporation or percolation, then an alternative strategy is expected: use the water to grow as quickly as possible even if at a reduced efficiency. Thus, plants may escape dehydration by growing fast and completing reproduction prior to or early during the water availability decline. This “live fast, die young” strategy could be achieved with higher A due to higher g , ultimately lowering WUE. Several studies of annuals have suggested that within species, trade-offs may exist between dehydration avoidance traits such as higher WUE and later flowering time, and dehydration escape traits such as fast growth, lower WUE and earlier flowering (Geber and Dawson 1990, 1997; Stanton et al. 2000; McKay et al. 2003; but see Sherrard and Maherali 2006).

There are several factors, in addition to stomatal regulation, that can directly or indirectly alter WUE. For instance, WUE can vary due to the amount of leaf N investment in photosynthetic capacity. A plant with higher leaf N and photosynthetic capacity can achieve higher A and higher WUE, independent of changes in g and transpiration (Field et al. 1983; Field and Mooney 1986; Wright et al. 2003). Since N availability is variable and usually limiting in arid habitats, expectations for selection on WUE should also consider N availability and use. The observation that many plants in hot dry habitats have small leaves led to the suggestion that a smaller individual leaf size is favored in water-limited habitats (Jones 1992; Lambers et al. 1998; Nobel 1999). A potential mechanism is that under sunny conditions, a smaller leaf is generally cooler due to a smaller boundary layer and more convective cooling. The cooler leaf has a lower leaf-to-air vapor pressure deficit, less transpirational water loss for a given g and A , and thus a higher WUE (Nobel 1999). Higher leaf succulence may also interact with WUE since it is often associated with plants in arid and saline habitats. The simultaneous analysis of these interacting traits can reveal which one(s) actually confer a fitness advantage.

Phenotypic selection analysis (Lande and Arnold 1983; Brodie et al. 1995) provides a means of testing expectations for selection on individual traits and related suites of traits. Selection differentials (S) are a measure of covariance between an individual trait and relative fitness, and thus comprise direct selection on a trait and indirect selection resulting from direct selection on other, correlated traits. Selection gradients (β) come from a multiple regressions, and thus partition direct selection on a trait from indirect selection for the traits included in the analysis. While several studies have assessed the relationship between WUE and some measure of fitness for annuals (Dudley 1996; Heschel et al. 2002; Ludwig et al. 2004; Sherrard and Maherali 2006) and perennial species (Ehleringer 1993a; Donovan and Ehleringer 1994a, 1994b; Casper et al. 2005), only a few have used the multivariate approach to sort out whether the selection is direct on WUE or indirect via related traits (Dudley 1996, Ludwig et al. 2004). This is important because direct phenotypic selection should predict the long-term evolutionary change, given that there is genetic variation for WUE in native plants (Geber and Dawson 1990; Schuster et al. 1992; Donovan and Ehleringer 1994b; Monclus et al. 2005), and provided that genetic correlations with other traits under selection do not serve as constraints. For example, WUE estimated as $\delta^{13}\text{C}$ is genetically correlated with other leaf physiological

traits, growth traits and flowering time, all of which could also be under direct selection (Geber and Dawson 1990; McKay et al. 2003; Monclus et al. 2005; Caird et al. 2006). Including both leaf $\delta^{13}\text{C}$ estimates of WUE and leaf N as a proxy for photosynthetic capacity in the selection analysis allows us to roughly separate out fitness variation associated with stomatal regulation and N investment.

We measured selection on leaf WUE, N, leaf size and succulence in natural populations of two closely related desert annual species. *Helianthus anomalous* and *H. deserticola* are desert annual sunflowers of hybrid origin from the same ancestral parental cross (Schwarzbach and Rieseberg 2002, Gross et al. 2003) that differ in phenology and are endemic to habitats differing in water and nutrient availability (Rosenthal et al. 2005a; Ludwig et al. 2006). We asked the following questions: (1) is there direct and indirect selection on integrated leaf WUE, N, area and succulence and (2) is direct selection on WUE consistent with hypothesized drought/dehydration escape and avoidance strategies.

Materials and methods

Study species and populations

The study species, *H. anomalous* and *H. deserticola*, are annual sunflowers of recent homoploid hybrid origin (170,000–63,000 ybp) from the same ancestral parent species: *H. annuus* and *H. petiolaris* (Schwarzbach and Rieseberg 2002, Gross et al. 2003). The two hybrid study species occur in the desert southwest US, in habitats that are more extreme than those of their ancestral parent species (Schwarzbach et al. 2001; Gross et al. 2003; Rosenthal et al. 2005a). The active dune habitat of *H. anomalous* has higher water availability and lower N than the adjacent stabilized dune habitats of *H. deserticola* (Rosenthal et al. 2005a). Phenologically, *H. anomalous* generally flowers and sets seed later than *H. deserticola* (Rieseberg et al. 2003, Rosenthal et al. 2005b). Previous comparisons of leaf ecophysiological traits in greenhouse studies and field experimental gardens have demonstrated that *H. anomalous* is always more succulent and tends to have larger leaves with lower N concentration, but that the species do not consistently differ in WUE (Schwarzbach et al. 2001; Rosenthal et al. 2002; Rieseberg et al. 2003; Brouillette et al. 2006).

The three study populations were located in central Utah, USA, at the Little Sahara Recreation Area (39°41'N, 112°22'W) managed by the Bureau of Land Management. The area has a cold desert climate with

an average rainfall of 297 mm (24-year average, 1979–2003) received predominantly in winter and spring. The study was conducted in a relatively dry year. Precipitation in the 8 months prior to the study was 41% of average, and precipitation during the study was 19% of average (Ludwig et al. 2004). Within Little Sahara, *H. anomalous* grows on actively moving sand dune complexes with low vegetation cover, and *H. deserticola* grows on older stabilized dunes with relatively high vegetation cover (Gross et al. 2004; Rosenthal et al. 2005a). The “mesic” population of *H. anomalous* was located on a relatively wet site adjacent to the experimental garden described in Ludwig et al. (2004). The “dry” population of *H. anomalous* occurred on a windy dune ridge that was found to have lower soil moisture content (see Results). The *H. deserticola* population occurred on the dune slope to the leeward side of the dry *H. anomalous* population. All three populations were within 500 m of each other. Both species germinated April through May. From 31 May to 6 June 2002, we set up a 10 × 30 m² study plot within each population and marked the study plants: 127 and 123 plants in the mesic and dry *H. anomalous* sites, respectively (all available plants in the plots), and 142 *H. deserticola* plants (every third available plant in the plot). We did not study additional *H. deserticola* populations because they had insufficient individuals (approximately <20) for selection analyses.

On 18 June 2002, we took five soil cores in each population plot. From each core, samples were collected from the soil surface and at 25, 50, 75 and 100 cm depth. For each sample, a portion was used to analyze gravimetric water content, and the remainder was dried at 60°C for nutrient analyses. Soils were analyzed for N by dry combustion with a Carlo Erba NA 1500 elemental analyzer (Milan, Italy). Soils were analyzed for total P with an acid persulfate digestion (Nelson 1987) followed by colorimetric analysis with a continuous flow analyzer. The three study population sites were compared for soil moisture and nutrient content using ANOVAs (site and depth main effects), followed by post hoc Tukey's means comparisons (SAS 2001).

Trait, survival and growth measurements

Plants were measured for growth, reproductive status and survival approximately every 3 weeks, and the number of buds, flowers and seed heads were counted. If dry seed heads were present, they were collected for reproductive biomass, but the seeds had generally already dispersed. If a plant was dead (i.e., all leaves desiccated) then all reproductive (buds, flowers, seed heads and any remaining seeds) and vegetative (stem

and leaf) biomass was collected. The census of reproductive status was not frequent enough to include the date of first flowering as a trait in the selection analysis.

Leaf measurements were made on 7–12 June for mesic *H. anomalus* ($n = 114$), dry *H. anomalus* ($n = 114$) and *H. deserticola* ($n = 135$), and on 10–11 July for mesic *H. anomalus* ($n = 103$) and dry *H. anomalus* ($n = 84$). The decrease in mesic and dry *H. anomalus* sample size from June to July was largely due to mortality. The *H. deserticola* population was not sampled again in July because 80% of the plants had senesced. The samples sizes reflect the number of live plants that had ≥ 4 leaves. The youngest fully expanded leaf was collected between 6.00 and 8.00 a.m., when maximally hydrated, and temporarily stored in a sealed bag in a cooler until leaf wet biomass and leaf size (individual leaf area, CID, Inc., Pullman, WA) were measured within 4 h. Leaves were then dried at 60°C until constant biomass. Leaf succulence was calculated as (wet biomass – dry biomass)/leaf area (Jennings 1976). The leaves were then individually ground and analyzed for N concentration (mg N/g dry biomass) (Carbo Erba NA 1500) and leaf carbon isotopic composition (leaf $\delta^{13}\text{C}$, Finnegan, continuous flow mass spectrometer, Bremen Germany). Leaf $\delta^{13}\text{C}$ provides an integrated measure of leaf intercellular CO_2 concentration (c_i) over the lifetime of the leaf. Integrated c_i is, in turn, a relative measure of integrated instantaneous WUE, provided leaf temperatures are similar (Farquhar et al. 1989; Ehleringer et al. 1992; Ehleringer 1993b). A higher (less negative) leaf $\delta^{13}\text{C}$ reflects higher WUE.

On 5 September, all surviving plants were harvested for aboveground biomass. For each plant with leaf trait data, including those that died before harvest, all biomass collected during the summer or at final harvest was sorted into vegetative biomass (stem and leaf) and reproductive biomass (buds, flowers, seed heads and any remaining seeds), dried at 60°C, and weighed. We used vegetative biomass as the measure of relative fitness, based on the expectation that leaf traits impact fitness primarily through vegetative biomass (Ehleringer and Clark 1988; Dudley 1996). As a measure of fitness, biomass incorporates both survivorship and fecundity because plants that died younger should be smaller and plants that are larger should produce more seeds. While seed production is a better measure of fitness for annuals, we were not able to measure that for our study. First, the harvest in September truncated flower initiation and seed maturation for *H. anomalus* because it initiated flowering much later than *H. deserticola*. Additionally, seed heads were not collected frequently enough to collect all seeds. There was a

significant correlation between vegetative and reproductive biomass for each population ($r^2 = 0.93^{***}$, 0.90^{***} and 0.39^{***} for mesic *H. anomalus*, dry *H. anomalus* and *H. deserticola*, respectively). There was also a significant correlation between vegetative biomass and the number of reproductive units (buds, flowers and seed heads) for each population ($r^2 = 0.90^{***}$, 0.96^{***} and 0.67^{***} for mesic *H. anomalus*, dry *H. anomalus* and *H. deserticola*, respectively).

Analyses of plant traits

Differences in mean trait values between *H. deserticola* and *H. anomalus* populations were tested with a Mann–Whitney U test because in several cases the variance in trait values was not equal for both species. Differences in mean trait values between the two *H. anomalus* populations were also tested with a Mann–Whitney U test.

For each of the three natural populations (mesic *H. anomalus*, dry *H. anomalus* and *H. deserticola*), we did phenotypic selection analyses. For each selection analysis, the trait data were standardized to a mean of 0 and a variance of 1, with no further transformations applied. Vegetative biomass, the dependent variable, was relativised to population means: i.e., mean of 1. Standardized linear selection differentials (S) were estimated for each leaf trait and vegetative biomass as the slope of the regression between the standardized trait and relative fitness (Lande and Arnold 1983; Conner 1998). The residuals of the regression analyses were not normally distributed, so significance levels were determined by a bootstrap analysis with 10,000 estimates (Dixon 2000). Ninety-five percent confidence intervals were then estimated from the bootstrap distribution using the percentile.

Standardized linear selection gradients (β) were estimated for the leaf traits with multiple regressions with relative fitness as the dependent variable and standardized traits as independent variables (Lande and Arnold 1983). We first analyzed selection gradients for just the June traits, to parallel analyses of *H. anomalus* transplants in the nearby experimental garden during the same year (Ludwig et al. 2004). Then, since we also had July leaf traits for the mesic and dry *H. anomalus* populations, we analyzed the selection gradients for the additional July data in two different ways. Because the June and July data are for the same plants in each population (except plants lost to mortality), we first analyzed the selection gradients for each of the populations with June and July data entered as separate traits in the same multiple regression. This analysis accounts for the expected correlations between the June and

July traits, and thus we feel that it is the most appropriate way to incorporate the additional July data. Then we analyzed the July data in a separate analysis that excluded the June data, which provides an alternative estimate of direct selection as if plant traits were only measured later in the growing season.

For the June traits and July traits analyzed separately, non-linear selection gradients (γ) were estimated using a general linear model with relative fitness as the dependent variable and all trait values as linear terms, but with all quadratic terms (non-linear selection), and all two-way interactions between traits (correlational selection) included. The residuals of the multiple regression analyses and linear models were not normally distributed. The significance of the linear and non-linear selection gradients was determined with a bootstrap analysis with 10,000 estimates and 95% confidence intervals estimated from the bootstrap distribution using the percentile. Additional pair-wise contrasts of populations for selection gradients in June were done with population as an additional term in the general linear model, with significance again assessed with bootstrap analysis with 10,000 estimates. Path diagrams are used to summarize the selection gradients (paths from standardized traits to relative above-ground vegetative biomass) and correlations among traits (Pearson's product moment correlations).

Results

Helianthus anomalous populations

The soil moisture content was higher in the slope *H. anomalous* site as compared to the ridge *H. anomalous* site, justifying their designation as mesic and dry, respectively ($F = 3.25$, $df = 2$, 65 , $P = 0.04$) (Fig. 1). Within each site, soil water content generally increased with depth ($F = 42.7$, $df = 1$, 65 , $P < 0.001$). The *H. anomalous* sites did not differ in soil N (site $F = 20.9$; $df = 2$, 75 ; $P = 0.001$, mesic and dry means comparison $P > 0.05$) or soil P (site $F = 2.7$; $df = 2$, 75 ; $P = 0.07$).

The mesic and dry *H. anomalous* populations initiated flowering at approximately the same time and had similar leaf traits in June (Tables 1, 2; Fig. 2). By July, however, the two *H. anomalous* populations differed for traits in a manner consistent with the lower soil moisture availability at the dry site. The dry *H. anomalous* had smaller leaves, lower succulence and higher WUE (less negative $\delta^{13}\text{C}$). The populations also differed in phenology as the season progressed. Most of the mesic *H. anomalous* (70.1%) were still alive and flowering at final harvest on 5 September, and 22.8% died before

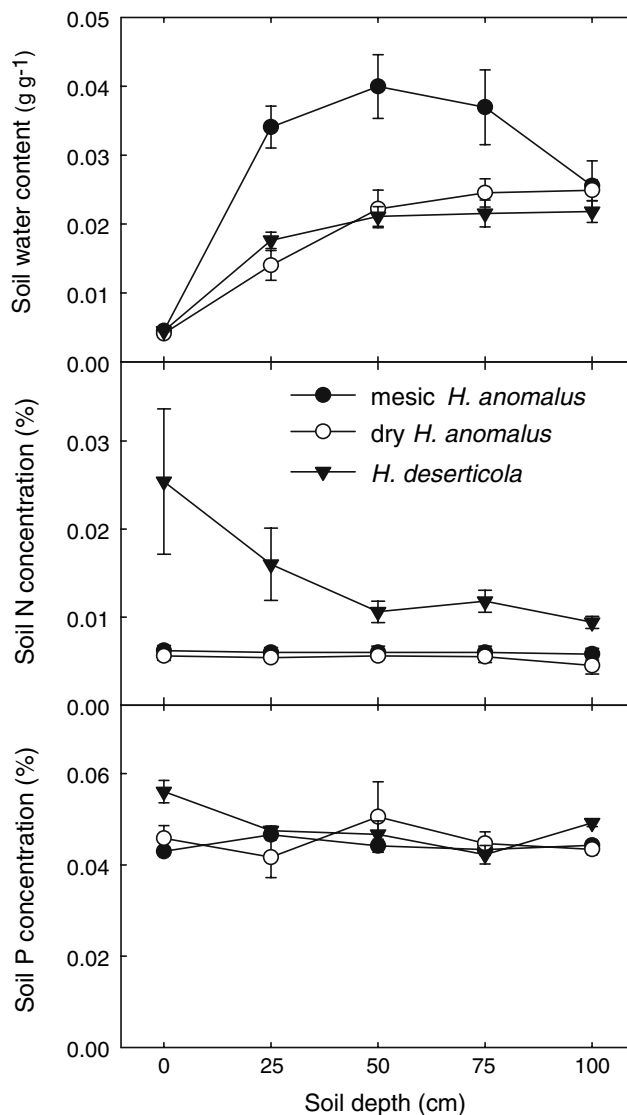


Fig. 1 Soil water content and soil N and P concentrations (± 1 SE, $n = 5$) at five different depths in naturally occurring populations of *Helianthus anomalous* (mesic and dry) and *H. deserticola*

flowering. The dry *H. anomalous* had only 39.0% still alive and flowering at harvest, and 29.3% died before flowering. At senescence or final harvest, the number of reproductive units (buds, flowers and seed heads) was higher for mesic (11.3 ± 15.2 mean \pm SD; range 0–93) than for dry *H. anomalous* (4.2 ± 15.8 ; range 1–151).

Direct selection for higher WUE would be consistent with a dehydration avoidance strategy. The β (selection gradients, direct linear selection) were not positive and significant, indicating that there was no direct linear selection for higher WUE in the dry *H. anomalous* population for June and July data considered separately (Fig. 3) or for the analysis of June and July data combined (Fig. 4). The selection differentials (S , sum of direct and indirect selection) also indicated no

Table 1 Demographic fate of plants in *Helianthus anomalous* (mesic and dry) and *H. deserticola* populations at Little Sahara Dunes, UT, USA, during the 2002 study

	Mesic <i>H. anomalous</i>	Dry <i>H. anomalous</i>	<i>H. deserticola</i>
Dead before flower initiation	22.8%	29.3%	1.4%
Senesced after flowering	3.2%	30.9%	97.2%
Flowering at final harvest	70.1%	39.0%	1.4%
Budding at final harvest	3.9%	0.0%	0.0%
Vegetative (no buds or flowers) at final harvest	0.0%	0.8%	0.0%

Plants in naturally occurring populations were marked between 31 May and 6 June, and harvested when they died during the season or at final harvest 5 September

Table 2 Mid-season leaf traits and subsequent final biomass for naturally occurring populations of *H. anomalous* (mesic and dry) measured in June and July and *H. deserticola* measured in June

	Mesic <i>H. anomalous</i>		Dry <i>H. anomalous</i>		<i>H. deserticola</i> June	June species effect (Z-value)	<i>H. anomalous</i> population difference June (Z-value)	<i>H. anomalous</i> population difference July (Z-value)
	June <i>n</i> = 114	July 103	June 114	July 82				
Leaf size (cm ²)	7.24 (4.19)	8.15 (4.48)	8.23 (4.54)	5.54 (3.58)	3.92 (1.73)	-9.45***	-1.70	-5.06***
Leaf succulence (mg/cm ²)	44.8 (4.8)	45.1 (6.0)	46.3 (6.4)	41.8 (6.5)	35.2 (4.3)	-14.17***	-1.75	-4.05***
Leaf N concentration (mg/g)	34.1 (6.00)	29.6 (6.7)	35.0 (5.6)	28.4 (4.8)	32.2 (4.88)	-3.65***	-0.85	-0.95
Leaf $\delta^{13}\text{C}$ June (‰)	-26.62 (0.98)	-26.20 (1.01)	-26.6 (0.97)	-25.52 (1.15)	-27.12 (0.95)	-4.65***	-1.01	-3.91***
Final vegetative biomass (g)	7.84 (11.4)	8.56 (11.49)	2.28 (3.11)	2.72 (3.54)	0.59 (0.50)	-11.57***	-5.08***	-4.62***
Final reproductive biomass (g)	2.40 (3.28)	2.64 (3.36)	0.41 (1.31)	0.58 (1.52)	0.12 (0.11)	-2.01*	-8.44***	-7.45***

Presented are means with SD in parentheses. Differences between *H. anomalous* and *H. deserticola* plants and between the two *H. anomalous* populations were tested with a Mann–Whitney *U* test. Bold text indicates statistically significant differences (**P* < 0.05, ***P* < 0.01, ****P* < 0.001)

total selection for higher WUE in the mesic or dry *H. anomalous* (Fig. 3).

Direct selection for lower WUE would be consistent with a dehydration escape strategy. There was direct selection for lower WUE (β negative and significant) in mesic *H. anomalous* in June ($\beta = -0.27^{**}$; Fig. 3). Pair-wise population comparisons for June indicated that the mesic β for WUE tended to be stronger than the dry β (*P* = 0.06). For June and July combined data, both mesic and dry *H. anomalous* populations demonstrated direct selection for lower WUE in July ($\beta = -0.23^*$ and -0.36^{**} , respectively; Fig. 4). There was also non-linear (stabilizing) selection for WUE for mesic *H. anomalous* in July, i.e., intermediate WUE favored ($\gamma = -0.16^*$; Table 3).

There was direct selection for higher N in the mesic *H. anomalous* in June ($\beta = 0.45^{***}$) and July ($\beta = 0.37^{***}$) (Fig. 3) and in June and July combined

data ($\beta = 0.29^{**}$ and 0.35^{**} ; Fig. 4). For the dry *H. anomalous*, there was non-linear (stabilizing) selection for leaf N in June, i.e., intermediate N favored ($\gamma = -0.36^*$; Table 3) and direct linear selection for higher leaf N for July ($\beta = 0.34^{**}$). Pair-wise population comparisons of β for June indicated that the direct selection for greater leaf N in mesic *H. anomalous* was significantly greater than that for dry *H. anomalous* (*P* = 0.02). In addition, there was correlative selection involving leaf N. For mesic *H. anomalous* in both June and July, plants that had a higher leaf N and larger leaf size were more fit, but leaf N made less difference for smaller leaves (Table 3; Fig. 5). In the dry *H. anomalous*, selection also favored plants that had leaf high N at high leaf WUE, but leaf N made little difference at a lower WUE (Table 3; Fig. 5). For both mesic and dry *H. anomalous*, in both June and July, leaf WUE was positively correlated with leaf N (Fig. 3).

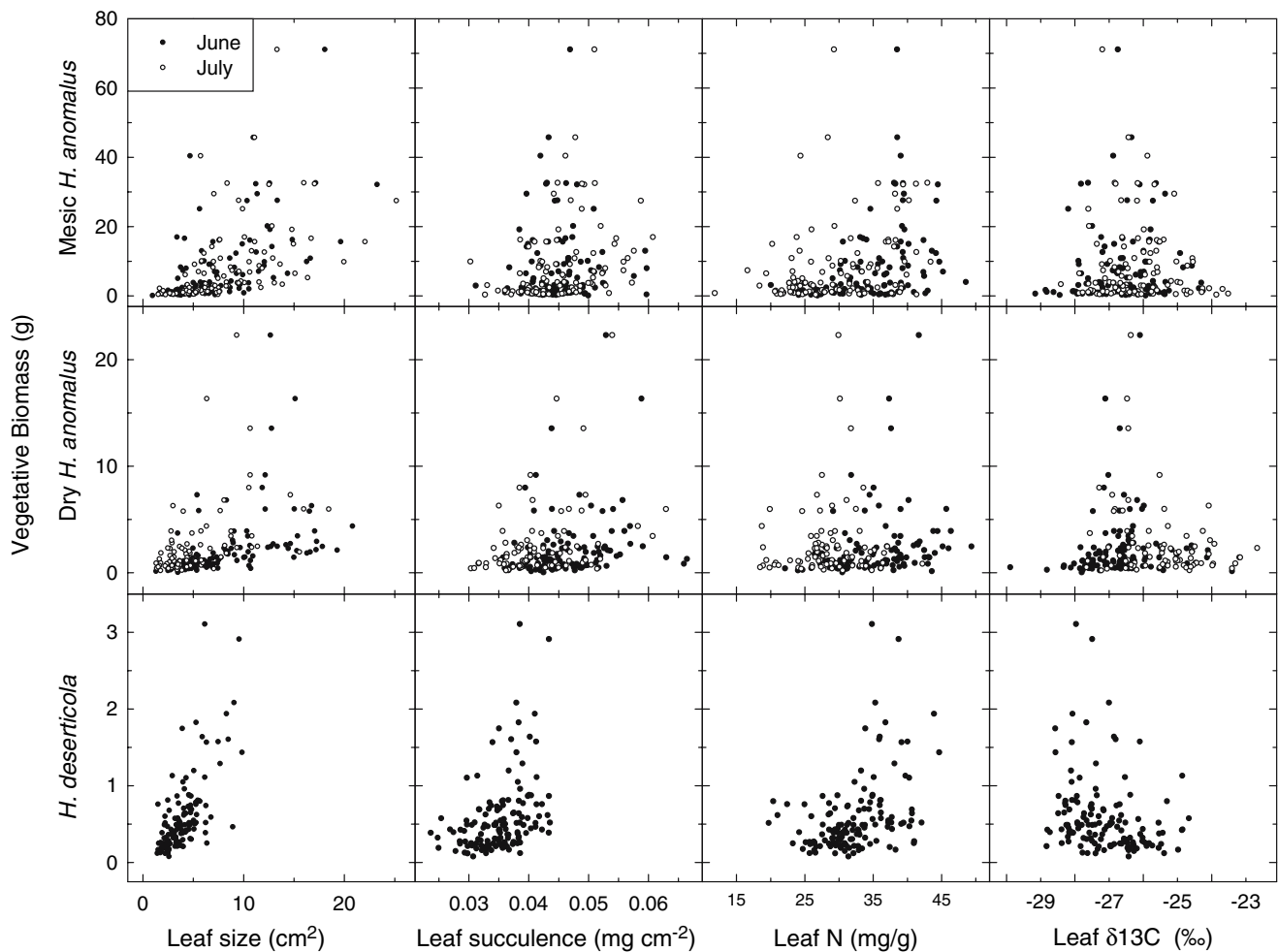


Fig. 2 Relationship of leaf traits to aboveground biomass for naturally occurring populations of *H. anomalus* (mesic and dry) measured in June and July and *H. deserticola* measured in June: individual leaf size, succulence, N concentration and $\delta^{13}\text{C}$. Leaf $\delta^{13}\text{C}$ provides an estimate of integrated instantaneous water-use

There was direct selection for larger leaf size in both populations for June and July and for June and July combined data (Figs. 3, 4). For leaf succulence, there was no direct selection for June data, but there was direct selection for higher leaf succulence in mesic *H. anomalus* in July (Fig. 3) and for June and July data combined (Fig. 4). In addition, there was correlative selection involving leaf succulence. For mesic *H. anomalus* in July, plants that had a lower leaf WUE and higher leaf succulence were more fit, but leaf succulence made little difference for higher WUE leaves (Table 3; Fig. 5).

Helianthus deserticola population

The soil moisture content for the *H. deserticola* site was lower than that of mesic *H. anomalus* and not different from that of dry *H. anomalus* ($F = 3.25$; $df = 2, 65$;

efficiency (WUE): greater (less negative) leaf $\delta^{13}\text{C}$ reflects greater WUE. These trait data were then standardized, and aboveground biomass was relativized, for analysis presented in Table 3 and Figs. 3, 4 and 5

$P = 0.04$) (Fig. 1). A concurrent study assessed soil particle size and soil moisture retention curves and demonstrated that the *H. deserticola* site had lower plant water availability than either *H. anomalus* site (Rosenthal et al. 2005a). The soil N concentration was higher in the *H. deserticola* site than either *H. anomalus* site (site $F = 20.9$; $df = 2, 75$; $P = 0.001$, means comparison *H. deserticola* to each *H. anomalus* site $P < 0.05$), but there was no difference in site soil P (site $F = 2.7$; $df = 2, 75$; $P = 0.07$).

Helianthus deserticola had different leaf traits and an accelerated phenology as compared to *H. anomalus*. In June, *H. deserticola* leaves were smaller, less succulent, lower in N concentration and lower in WUE (more negative $\delta^{13}\text{C}$) than those of *H. anomalus* (Table 2; Fig. 2). On 26 June, 69% of the *H. deserticola* had initiated buds, in contrast to only 3–5% of *H. anomalus* (both populations). At final harvest on 5 September,

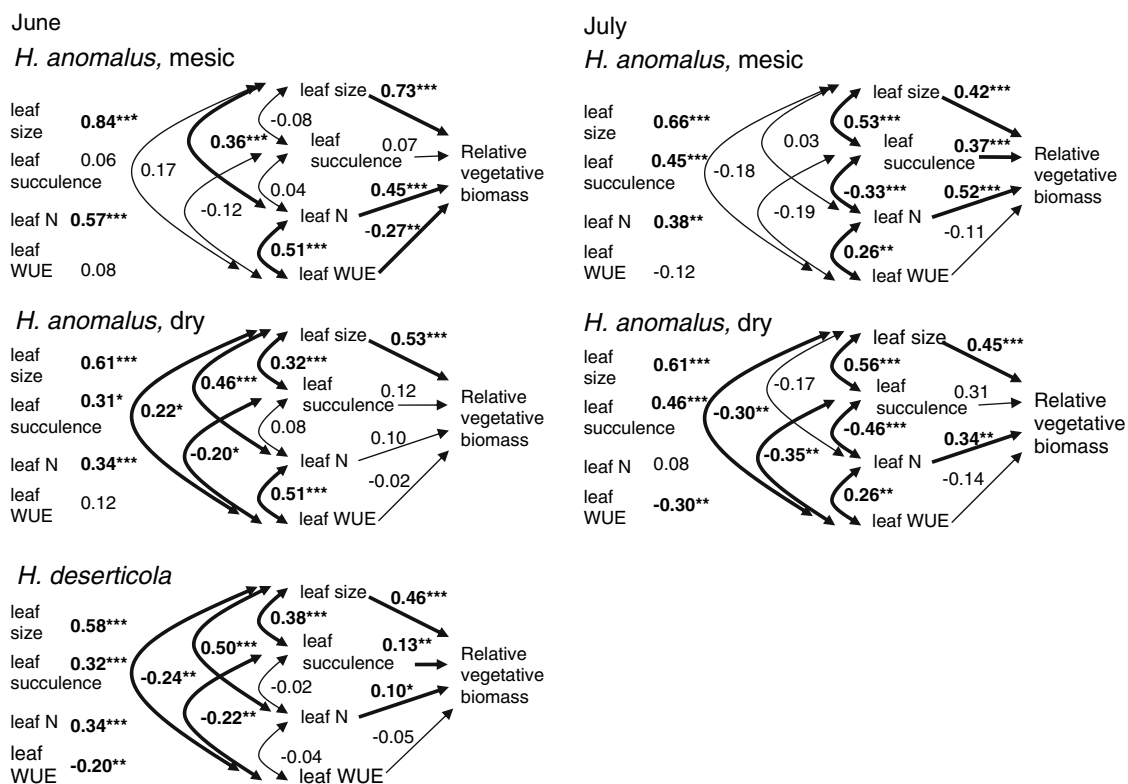


Fig. 3 Path diagrams summarizing the effects of leaf size, succulence, N concentration and WUE (all standardized) and vegetative biomass (relative fitness) for naturally occurring populations of *H. anomalous* (mesic and dry) measured in June and July, and *H. deserticola* measured in June. Selection differentials (S , on left after trait names), selection gradients (β , on right as paths leading

to relative vegetative biomass) and correlations among traits (curved double headed arrows) are presented for all three populations. Bold text and arrows indicate statistically significant difference from 0 ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Sample sizes: mesic *H. anomalous* June, $n = 114$; July, $n = 103$; dry *H. anomalous* June, $n = 114$; July, $n = 82$; *H. deserticola* June, $n = 135$

only 2 (1.4%) *H. deserticola* plants were still alive and flowering, and 97.2% had flowered and senesced. At senescence or final harvest, the number of reproductive units (buds, flowers and seed heads) was 2.8 ± 1.9 (range 0–10) for *H. deserticola*.

The accelerated phenology of *H. deserticola* was not associated with direct selection for lower WUE (Fig. 3). However, S was significant ($-0.20***$), indicating indirect selection on WUE, mediated through negative correlations with leaf size and succulence, both of which were under direct positive selection. There was also direct selection for greater leaf N. Pair-wise population comparisons of β for June indicated that selection for leaf N in *H. deserticola* ($P = 0.01$) was significantly less than that for mesic *H. anomalous*. For *H. deserticola*, leaf N concentration was not correlated with WUE (Fig. 3).

Discussion

In these natural populations of *H. anomalous* and *H. deserticola*, we found both direct (β) and total selection

(S , indirect + direct selection) for lower WUE, but no direct selection for higher WUE. For mesic and dry populations *H. anomalous*, there was direct selection for lower WUE, consistent with a dehydration escape strategy, even though it is the longer lived of the two species. For the shorter lived *H. deserticola* in the drier habitat, there was only indirect selection for higher WUE. Thus, selection was not always consistent with expectations based on water availability and phenology.

Other studies have detected direct (β) selection for lower WUE. Lower WUE and drought escape were inferred for artificial populations of *Impatiens capensis* planted into a habitat with an early season drought, based on direct selection for higher g , but not A , although WUE itself was not analyzed (Heschel and Riginos 2005). Total selection for lower WUE (estimated from $\delta^{13}C$) was also demonstrated for juvenile plants of an herbaceous desert perennial, *Cryptantha flava*, with fitness assessed as survival after 5 years in a natural population (Casper et al. 2005). For the mesic *H. anomalous* population that we studied, we suggest that the concurrent direct selection for lower WUE

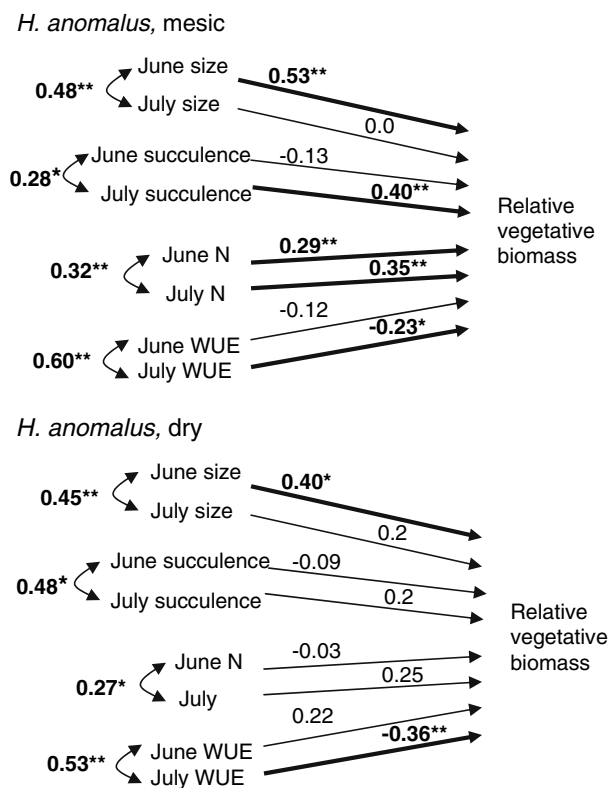


Fig. 4 Path diagrams summarizing the effects of June and July traits combined in the same analysis for leaf size, succulence, N concentration and WUE (all standardized) and vegetative biomass (relative fitness) for plants sampled on both dates for two naturally occurring *H. anomalus* populations: mesic ($n = 102$) and dry ($n = 81$). Selection gradients (β , on right as paths leading to relative vegetative biomass) and correlations among traits (curved double headed arrows) are presented for each population. Bold text and arrows indicate statistically significant difference from 0 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Selection differentials for the June and July traits are found in Fig. 3

and higher leaf N in the more mesic environment may reflect adequate water availability through deep rooting and greater N acquisition. Our reasoning is that by including both leaf $\delta^{13}\text{C}$ and N in our multivariate analyses we partitioned any effect of N on WUE. So the WUE β predominately reflected stomatal regulation and indicated that higher g was favored. This higher g probably increased transpirational water loss, which could increase bulk flow delivery of mobile nutrients such as nitrate to the plant roots (Smith 1991; McDonald et al. 2002). The selection for higher leaf N was also found for a population of *H. anomalus* transplanted into the desert dune site during the same year as this natural population study (Ludwig et al. 2004). Greater leaf N may indicate a greater rooting volume, efficiency of N uptake and use, or allocation to photosynthesis.

We did not find any direct selection or total selection for higher WUE in *H. anomalus* that would be

consistent with a dehydration avoidance strategy. This was unexpected, particularly for the dry *H. anomalus*, because those plants were widely dispersed and had few neighbors, so that any of the water known to be stored in the dunes throughout the growing season (Rosenthal et al. 2005a) and not used by the plant early in the season should have been available for later use (Cohen 1970). Sherrard and Maherali (2006) also found no selection on WUE (measured as A/g) in an experimental drought treatment, although they did find selection for higher WUE in the well watered treatment. In contrast, other experimental studies imposing a dry treatment found direct and/or total selection for higher WUE. Dudley (1996) found significant total selection ($S = 0.66$) and direct selection ($\beta = 0.51$) for higher WUE for the dune annual *Cakile edentula* transplanted into a drier site and no selection in a wetter site. Heschel et al. (2002) also found significant total selection ($S = 0.27$) for higher WUE for the annual *Impatiens capensis* transplanted into a site with a later growing season drought, but direct selection was not analyzed. For an *H. anomalus* population transplanted into the desert dune site during the same year at this study, there was indirect selection for higher WUE mediated through leaf N and area, but no direct selection (Ludwig et al. 2004). For the desert perennial *C. flava* adults in a natural population, total selection tended to favor higher WUE, opposite the pattern for juveniles (Casper et al. 2005). Thus, there are mixed results supporting selection for higher WUE for plants where dehydration avoidance was expected.

Helianthus deserticola was shorter lived and had a lower population average for WUE as compared to *H. anomalus*, suggestive of a “live fast, die young” annual that should be able to escape seasonal drought (Geber and Dawson 1990, 1997; McKay et al. 2003; Heschel and Riginos 2005). However, *H. deserticola* did not show evidence of direct selection for the lower WUE expected to be associated with this strategy. Instead, in June it responded more like the drier *H. anomalus* by exhibiting weaker selection on leaf N than mesic *H. anomalus*, and no direct selection on WUE.

What might account for the differences between these studies? First, it should be noted that the timing and method of WUE measurements may affect the results. The use of instantaneous A/g as a proxy for WUE (Dudley 1996; Heschel et al. 2002; Sherrard and Maherali 2006) reflects a narrower window of time than seasonally integrated WUE estimated from leaf $\delta^{13}\text{C}$ (this study, Ludwig et al. 2004; Casper et al. 2005). Casper et al. (2005) found that agreement between estimates of selection on WUE from instantaneous A/g and $\delta^{13}\text{C}$ differed depending on when A/g was measured.

Table 3 Non-linear selection (γ) and correlational selection gradients of leaf traits for naturally occurring populations of *H. anomalous* (mesic and dry) measured in June and July, and *H. deserticola* measured in June

	Mesic <i>H. anomalous</i>		Dry <i>H. anomalous</i>		<i>H. deserticola</i>
	June	July	June	July	June
	<i>N</i> = 114	103	114	82	135
Leaf size \times leaf size	-0.09	-0.15	-0.21	-0.28	0.08
Leaf succulence \times leaf succulence	-0.09	-0.02	-0.01	-0.01	0.02
N concentration \times N concentration	0.1	-0.05	-0.36*	0.02	-0.01
WUE \times WUE	-0.02	-0.16*	-0.09	-0.01	0.05
Leaf size \times leaf succulence	-0.12	0.06	-0.05	0.38	0.10
Leaf size \times N concentration	0.48*	0.27*	0.18	0.32	-0.06
Leaf size \times WUE	-0.13	-0.13	-0.21	-0.26	0.03
Leaf succulence \times N concentration	0.04	0.08	0.17	0.21	-0.06
Leaf succulence \times WUE	-0.08	-0.24*	0.02	-0.09	-0.08
N concentration \times WUE	-0.11	-0.03	0.40*	-0.20	-0.01

Bold text indicates significantly different from 0, determined from bootstrap analyses ($*P < 0.05$)

Second, it is possible that the choice of traits included in the multivariate analysis affects the estimate of direct selection on WUE. For example, if leaf N is not included in the multivariate analysis for mesic *H. anomalous*, then the β for WUE is no longer significant (results not presented). However, for this study, in no case did leaving leaf N out of the analysis result in an apparent increase in fitness explained by WUE (i.e., β becoming significant). Thus, the inclusion of N cannot explain the differences in direct selection on WUE detected for *H. anomalous* in this study and *C. edentula* (Dudley 1996). Finally, consideration of differences in nutrient availability may change the expectations for selection on WUE (Wright et al. 2003). In habitats where nutrients are equally or more limiting than water, as has been found for *H. anomalous* (Ludwig et al. 2006), plants may benefit from wasting water to get N. In order to fully understand the importance of WUE as a trait, further discussions are needed about which traits are appropriate for inclusion in tests of the adaptive significance of WUE. We suggest that leaf nitrogen should be included more often, and where possible, the date of first flowering (Stanton et al. 2000; Heschel and Riginos 2005; Sherrard and Maherali 2006).

Although we focused on leaf WUE, we also assessed leaf size and succulence because of their potential interactions with WUE. We did not find any selection for the smaller leaf size that has been suggested to be favored in water-limited habitats (Nobel 1999) and was found in the dry population of *C. edentula* (Dudley 1996), but not the dry *I. capensis* (Heschel and Riginos 2005). We only observed selection for larger leaf size in both species in this natural population study. For the *H. deserticola*, the lack of selection for smaller leaves

may be because the leaves are relatively small and further reducing the boundary layer would not provide a substantial leaf temperature and thus WUE benefit. The *H. anomalous* leaves are larger, but they have a petiole that allows them to twist the leaf away from direct sunlight when water is limiting, and promotes fluttering with the slightest wind, which likely reduces the boundary layer. Alternatively, the size of the most recently fully expanded leaf may increase with overall plant size or age for these species, and thus may reflect earlier germination or some other parameter. This may overwhelm any advantage in higher WUE associated with smaller individual leaf size for these species.

We found direct linear selection for greater leaf succulence only for the mesic *H. anomalous* in July and for *H. deserticola*. This is surprising because *H. anomalous* leaf succulence is consistently greater than that of *H. deserticola* and both of their ancestral parent species, suggesting that leaf succulence has been an essential adaptation to the *H. anomalous* habitat (Rosenthal et al. 2002; Gross et al. 2004; Ludwig et al. 2004). We suggest two explanations that merit further study. First, the consistently greater succulence of *H. anomalous* may be due to an advantage that it provides under only some conditions, as suggested by the significant direct selection for greater succulence and correlational selection of succulence and WUE only in July. Second, the species differences may be due to indirect selection through correlation with some measured or unmeasured traits, as suggested by the significant positive S for succulence in the dry *H. anomalous*. Further investigation is needed of genetic correlations that may promote or constrain the evolutionary response leaf succulence and the other traits (Geber and Griffen 2003; Rosenthal et al. 2005b).

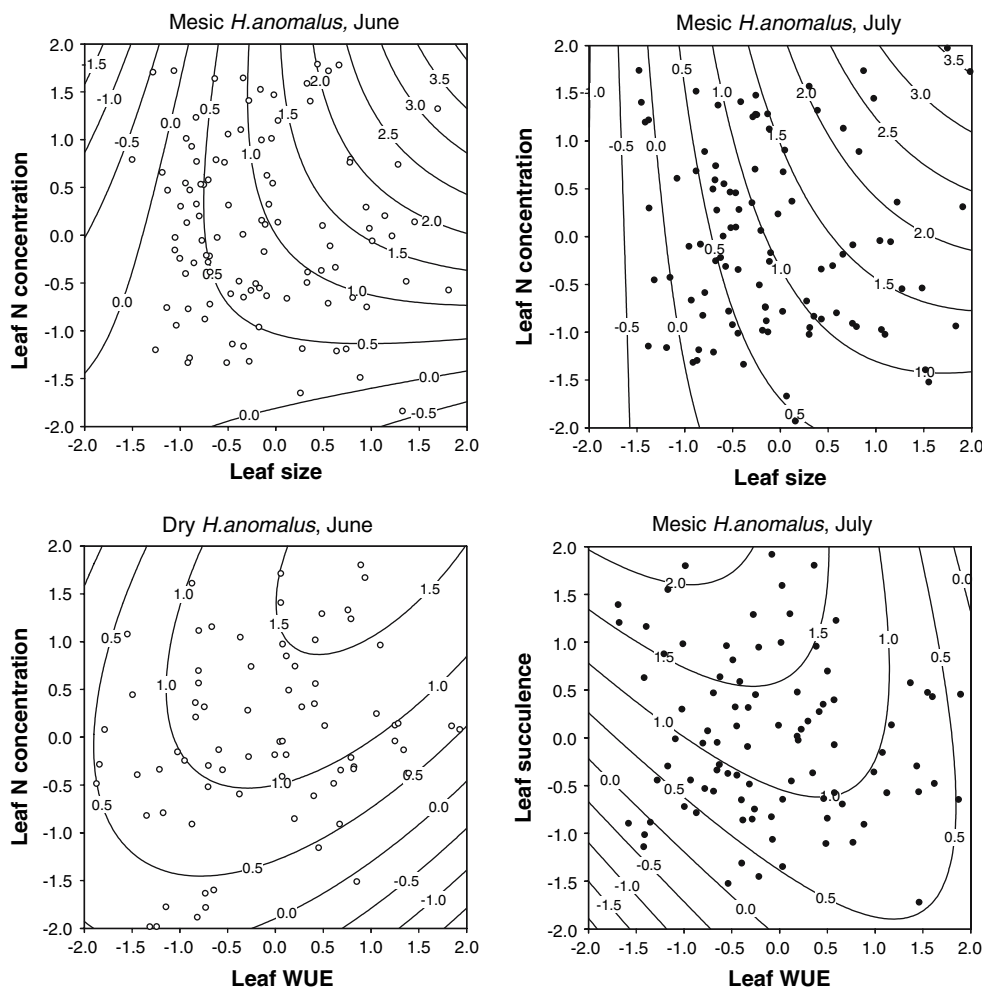


Fig. 5 Two-dimensional representations of correlative selection for pairs of traits of *H. anomalus* (see Table 3). On the x and y axes, the standardized trait values are shown from –2 until +2 stan-

dard deviations from the population mean (0). Relative fitness is represented by the *isoclines*

Overall, our study demonstrates significant direct and total selection on each ecophysiological trait (WUE, N, leaf size and succulence) in at least one of the natural populations studied, although selection was not always consistent with expectations based on water availability and phenology. Lower WUE was not favored by direct selection in the habitat where early drought is associated with the “live fast, die young” strategy of *H. deserticola*, but was favored in the *H. anomalus* habitat where nutrients are co-limiting or more limiting than water (Ludwig et al 2006). A lower WUE may be needed to establish deep rooting and avoid dehydration, and to assist in N capture through increased transpiration. *Helianthus anomalus* appears to maximize the likelihood of continued access to soil water with extensive rooting to the deeper soil layers where water remains available throughout the growing season (Rosenthal et al. 2005a; Ludwig et al. 2006). Under these growth conditions, there is a large reproductive

payoff for *H. anomalus* in the fall, with the potential for hundreds of flowers and seed heads produced as compared to many fewer in the *H. deserticola* that reproduce and senesce earlier as water availability declines in the stabilized dune habitat.

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