

SELECTION ON LEAF ECOPHYSIOLOGICAL TRAITS IN A DESERT HYBRID *HELIANTHUS* SPECIES AND EARLY-GENERATION HYBRIDS

FULCO LUDWIG,^{1,2} DAVID M. ROSENTHAL,^{1,3} JILL A. JOHNSTON,^{1,4} NOLAN KANE,^{5,6} BRIANA L. GROSS,^{5,7}
CHRISTIAN LEXER,^{5,8} SUSAN A. DUDLEY,^{9,10} LOREN H. RIESEBERG,^{5,11} AND LISA A. DONOVAN^{1,12}

¹Department of Plant Biology, 2502 Plant Sciences Building, University of Georgia, Athens, Georgia 30602-7271

³E-mail: davidr@plantbio.uga.edu

⁵Department of Biology, Indiana University, Bloomington, Indiana 47405

⁶E-mail: nkane@indiana.edu

⁷E-mail: brgross@indiana.edu

⁸E-mail: c.lexer@rbgkew.org.uk

⁹Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1 Canada

¹⁰E-mail: sdudley@mcmaster.ca

¹¹E-mail: lriesebe@bio.indiana.edu

¹²E-mail: donovan@plantbio.uga.edu

Abstract.—Leaf ecophysiological traits related to carbon gain and resource use are expected to be under strong selection in desert annuals. We used comparative and phenotypic selection approaches to investigate the importance of leaf ecophysiological traits for *Helianthus anomalus*, a diploid annual sunflower species of hybrid origin that is endemic to active desert dunes. Comparisons were made within and among five genotypic classes: *H. anomalus*, its ancestral parent species (*H. annuus* and *H. petiolaris*), and two backcrossed populations of the parental species (designated BC2ann and BC2pet) representing putative ancestors of *H. anomalus*. Seedlings were transplanted into *H. anomalus* habitat at Little Sahara Dunes, Utah, and followed through a summer growing season for leaf ecophysiological traits, phenology, and fitness estimated as vegetative biomass. *Helianthus anomalus* had a unique combination of traits when compared to its ancestral parent species, suggesting that lower leaf nitrogen and greater leaf succulence might be adaptive. However, selection on leaf traits in *H. anomalus* favored larger leaf area and greater nitrogen, which was not consistent with the extreme traits of *H. anomalus* relative to its ancestral parents. Also contrary to expectation, current selection on the leaf traits in the backcross populations was not consistently similar to, or resulting in evolution toward, the current *H. anomalus* phenotype. Only the selection for greater leaf succulence in BC2ann and greater water-use efficiency in BC2pet would result in evolution toward the current *H. anomalus* phenotype. It was surprising that the action of phenotypic selection depended greatly on the genotypic class for these closely related sunflower hybrids grown in a common environment. We speculate that this may be due to either phenotypic correlations between measured and unmeasured but functionally related traits or due to the three genotypic classes experiencing the environment differently as a result of their differing morphology.

Key words.—Carbon isotope discrimination, fitness, leaf area, nitrogen, succulence, water-use efficiency.

Received July 8, 2004. Accepted September 9, 2004.

In desert habitats, soil water and nutrient limitations affect plant success on both ecological and evolutionary time scales (Noy-Meir 1973; Comstock and Ehleringer 1992; Danin 1996; Smith et al. 1997). Comparative studies of related species and selection analysis of phenotypic variation within species (natural selection sensu Lande and Arnold 1983) have been used to assess the adaptive value of leaf ecophysiological traits (Chapin et al. 1993; Dudley 1996; Ackerly et al. 2000; Aerts and Chapin 2000; Arntz and Delph 2001; Grime 2001; Ackerly and Monson 2003; Geber and Griffen 2003; Reich et al. 2003). However, the plant systems appropriate for phenotypic selection analysis rarely provide the opportunity to compare current selection to long-term evolutionary outcomes and known ancestors. We combine these approaches to test hypothesized adaptive value of leaf ecophysiological traits and the role that selection on these traits may have played in hybrid speciation by comparing an annual species

of diploid hybrid origin to its ancestral parents and early generation hybrids putatively similar to its ancestors.

Helianthus anomalus is part of an annual sunflower complex that is a classic example of diploid or homoploid hybrid speciation (i.e., no increase in ploidy; Arnold 1997; Rieseberg 1997). *Helianthus annuus* and *H. petiolaris*, the ancestral parents of *H. anomalus*, also gave rise to two other hybrid species: *H. deserticola* and *H. paradoxus* (Rieseberg 1991; Rieseberg et al. 1991). All three hybrid species are endemic to habitats that are ecologically distinct and appear to be more stressful than those of their ancestral parents. When new species arise via diploid hybrid speciation, there is no immediate reproductive isolation as a by-product of chromosome doubling, so there must be other mechanisms of isolation (Rieseberg et al. 1999). If the hybrid species' specialization to extreme habitats occurred early in the speciation process, the resulting spatial and ecological isolation would have facilitated the speciation process (Buerkle et al. 2000). Rieseberg has proposed that extreme traits likely arose in early generation hybrids through complementary gene action or transgressive segregation (Rieseberg et al. 1999, 2003). Thus, hybrids may escape to new habitats by virtue of transgressive trait states that are adaptive in a new habitat.

Phenotypic and genomic comparisons of ancient and syn-

² Present address: CSIRO Plant Industry, Private Bag No. 5, Wembley, Western Australia 6913, Australia; E-mail: fulco.ludwig@csiro.au.

⁴ Present address: Department of Plant Biology, University of Minnesota, St. Paul, Minnesota 55108; E-mail: john6388@umn.edu.

thetic hybrid sunflowers support the hypothesis that new gene combinations and extreme (transgressive) traits in early-generation hybrids played a role in ecological isolation (Rieseberg et al. 2003). Morphological, physiological, and life-history traits that are extreme in the hybrid species can also be transgressive in artificial hybrids (Schwarzbach et al. 2001; Rosenthal et al. 2002; Lexer et al. 2003a; Rieseberg et al. 2003), and some of the leaf level traits have been shown to be under selection in the habitat of the hybrid species (e.g., leaf succulence, area, and sodium concentration; Lexer et al. 2003a; Gross et al. 2004). However, these studies did not measure selection on the natural hybrid species itself. The present hybrid species phenotype is the outcome of selection on the suite of traits and the genetic variances and covariance, which may differ between the artificial hybrids and the hybrid species. The evolutionary response to selection includes both direct and indirect components, so that a trait may evolve because it is under selection or because it is correlated with another trait under strong selection (Lande and Arnold 1983).

The sand dune habitat of *H. anomalus* differs markedly from that of its ancestral parent species. The active dunes are unstable, nitrogen limited, and store more water than adjacent stabilized areas, although access to that water requires root growth into deeper soil regions (Pavlik 1980; Bowers 1982; Danin 1996; Gibson 1998; Rosenthal 2004). Selection on leaf traits such as area, succulence, nitrogen concentration and water-use efficiency (WUE) is expected to be influenced by water and nitrogen limitations (Ehleringer 1985; Ehleringer and Clark 1988; Danin 1996; Dudley 1996; Geber and Dawson 1997; Heschel et al. 2002). Higher leaf succulence (water content per unit leaf surface), as found in *H. anomalus* relative to either ancestral parent species, may contribute to drought tolerance (Jennings 1976; Schwarzbach et al. 2001; Rosenthal et al. 2002; Rieseberg et al. 2003). Leaf area, leaf nitrogen, and leaf WUE (photosynthetic carbon gain per transpirational water loss) are physiologically linked to each other, as well as total carbon gain and water use. A smaller leaf area is generally associated with a small boundary layer that reduces leaf temperature and transpirational water loss for a given stomatal conductance. Leaf nitrogen is also a major determinant of the biochemical capacity of photosynthesis (Field and Mooney 1986; Wright et al. 2003). Plants can increase leaf WUE by either increasing leaf nitrogen invested in photosynthetic capacity or by decreasing stomatal conductance, only the latter of which decreases water loss. Dudley (1996) tested the prediction that phenotypic selection would favor higher WUE in drier habitats for the dune annual *Cakile endulata*. She found that dry dune sites favored higher instantaneous WUE and intermediate leaf size, whereas neither WUE nor leaf size was selected in the wet dune site (Dudley 1996). Heschel et al. (2002) also detected selection for greater instantaneous WUE in populations of *Impatiens capensis*. We expect leaf area, succulence, nitrogen, and WUE to be under selection for annual sunflowers in the desert dune habitat of *H. anomalus*.

We used the comparative approach and phenotypic selection analysis to investigate the importance of leaf traits for *H. anomalus* success in desert dune habitats and the role that these traits may have played in hybrid speciation. Seedlings of *H. anomalus*, its ancestral parents (*H. annuus* and *H. pe-*

tiolaris), and two early-generation hybrid populations (BC2ann and BC2pet) were transplanted into the desert sand dune habitat of *H. anomalus*. We focused on leaf traits (area, succulence, nitrogen, WUE), phenology, and vegetative biomass (relative fitness). We ask the following questions. Which ecophysiological and phenological traits are transgressive for *H. anomalus* relative to ancestral parents in the dune habitat? Does current selection in *H. anomalus* favor the transgressive leaf traits? Is selection on the hybrid backcross populations similar to current selection in *H. anomalus*, or could it result in evolution toward the current *H. anomalus* phenotype?

MATERIALS AND METHODS

Study Species

Helianthus anomalus Blake (sand sunflower) is a diploid hybrid species that diverged between 116,000 and 160,000 yrs ago from the ancestral parents *H. annuus* L. (common sunflower) and *H. petiolaris* Nutt. (prairie sunflower; Rieseberg 1991; Rieseberg et al. 1996). Recent DNA analysis showed that *H. anomalus* probably had multiple origins (Schwarzbach and Rieseberg 2002). It has a relatively small geographic distribution in Utah and northern Arizona in the southwestern United States, restricted to active desert sand dunes (Schwarzbach et al. 2001). Both ancestral parent species, *H. annuus* and *H. petiolaris*, have a much wider distribution and are common in the central and western United States. *Helianthus annuus* prefers mesic, clay-based soils, whereas *H. petiolaris* grows on relatively drier, sandier soils. However, the two species overlap and form hybrid zones in some areas.

Achenes (one-seeded fruits, hereafter called seeds) of *H. anomalus*, *H. annuus*, and *H. petiolaris* were collected from natural populations: ANO1244, Little Sahara Recreation Area, Utah; ANN1295, Hanksville, Utah; and PET1277, Page, Arizona, respectively. The BC2 populations were generated by crossing a single *H. annuus* individual with a single *H. petiolaris* individual to generate F₁ individuals. These were then backcrossed to a different *H. petiolaris* from the same population to produce BC₁, and these individuals were backcrossed to different *H. petiolaris* parent to produce the BC₂ (designated BC2pet; for more crossing details see Lexer et al. 2003a; Rieseberg et al. 2003 online supplemental materials). The BC2ann were produced in a similar manner except that the F₁ individuals were backcrossed twice with *H. annuus*. Thus, each backcross population had one parent of the introgressing species and three parents of the recurrent species. This BC₂ design was necessitated by the obligate outcrossing breeding system and a concurrent goal to compare quantitative trait loci (QTLs) for traits in the field and the greenhouse (Rieseberg et al. 2003; Lexer et al. 2003b). Although use of a limited number of parent plants potentially limits the genetic variation included in the backcross populations, the genomic composition of *H. anomalus* was predicted from the QTL analysis of these synthetic hybrids, indicating that the backcross populations do contain appropriate genetic variation (Rieseberg et al. 2003). *Helianthus anomalus*-like multitrait phenotypes have also been found in both backcross populations (Rosenthal 2004).

Field Site

The field experiment was conducted in central Utah, at Little Sahara Recreation Area (281.3°N, 112.3°W) managed by the U.S. Bureau of Land Management. The area has a cold desert climate with an average rainfall of approximately 297 mm (24-year average, June 1979 to August 2003). The experiment was conducted in a relatively dry year. Precipitation was 83 mm for the 8 months prior to the study (September–April), 41% of the cumulative average for these months. During the experiment (May–August 2002) rainfall was 18 mm, 19% of average.

The experimental garden was located within the Jericho picnic area on active sand dunes typical of *H. anomalus* habitat. The presence of *H. anomalus* skeletons from previous years and the germination of naturally occurring seedlings in 2002 demonstrated the suitability of the habitat for *H. anomalus*. Plant cover was 10% and other species growing near the garden were *Psoralidium lanceolatum* var. *stenotachys* (Pursh.) Rydb. (dune scurfpea) and *Stipa hymenoides* R. & S. (Indian ricegrass). The soil was very sandy and poor in nutrients: total phosphorus = 0.030 ± 0.007 (SD) % and total nitrogen = 0.005 ± 0.001 %. However, soil moisture was generally available at deeper soil depths. For example, on 18 June, gravimetric soil moisture was 3.4% at 25-cm depth and increased to 5.8% at 100-cm depth (soil water potentials of -0.35 and -0.01 MPa, respectively).

Seed Germination and Seedling Transplantation

Seed germination was initiated on 3–9 April 2002 at Indiana University, following the protocol of Schwarzbach et al. (2001) and Gross et al. (2004). *Helianthus anomalus* are difficult to germinate and a brief (1-h) exposure to 1000 ppm gibberellic acid (GA) was required to break dormancy. Although sustained applications of GA applied to seeds and seedlings can affect plant morphology (Cabin et al. 1997; Taiz and Zeiger 1998), the lack of a major morphological effect on traits of interest in our study has been substantiated by comparison of studies where GA was used (Schwarzbach et al. 2001; Rosenthal et al. 2002) and was not used (L. Donovan, pers. comm.) to break *H. anomalus* dormancy. These studies, although done in different years, used the same greenhouses, cultivation techniques (pot size, soil, and light and irrigation regimes), and high-nutrient treatment. The *H. annuus*, *H. petiolaris*, and *H. anomalus* differences for leaf succulence, leaf area, specific leaf area, and plant height at maturity were generally the same with or without using GA to induce germination, indicating that use of GA to break dormancy did not bias results for the traits of interest.

After germination, seedlings were individually transplanted into $6 \times 6 \times 10$ -cm biodegradable pots with a 1:1 sand:soil mixture. Seedlings were grown in a greenhouse and received 2/3 strength 20:20:20 (N:P:K) fertilizer before transport from Indiana to Utah on 8–10 May. Although starting the seedlings in the greenhouse has the disadvantage of initial growth under nonfield conditions, it was necessary to ensure germination and sufficient sample size and had the advantage of minimizing potentially confounding effects of emergence variation. In Utah, seedlings were maintained outside to acclimatize to local temperature, humidity, and ultra violet con-

ditions. On 14 May, seedlings were transplanted into dune *H. anomalus* habitat and watered daily for 12 days. The experimental garden was 14×14 m and divided into 12 rows, with a 0.75-m aisle between each row. Each of the 12 rows in the study was a block. Each row contained three contiguous sections of BC2ann, BC2pet, and *H. anomalus*, and between these sections there were additional individuals of *H. annuus*, *H. petiolaris*, and *H. anomalus*. A few volunteers of naturally occurring *H. anomalus* and other dune plants germinated in the experimental garden during the study and were removed.

Trait, Survival, and Growth Measurements

Measurements were initiated on all plants on 20 May. Results are presented only for plants suitable for leaf trait sampling on 14 June (see criteria below): $n = 20$ *H. annuus*, 14 *H. petiolaris*, 130 *H. anomalus*, 114 BC2ann, and 104 BC2pet. Although these plants were only a subset of the study plants (83–88% for the three species, and 53% of each of the BC₂ populations), the demographic fate of the subset sampled for leaf traits (results below) paralleled that of the remaining plants (data not presented).

After measuring initial plant height and stem diameter on 20 May, plants were checked for mortality three times a week for the first 10 weeks and once a week thereafter until the final harvest. Aboveground biomass was collected for dead plants. During the first 8 weeks of the experiment, plant height (from the ground to the top of the stem or bottom of bud) was measured every week. Stem diameter was measured 2 cm above the ground every 2 weeks. The date of first flower was recorded, along with height and number of buds at first flower. To determine total seed production and reproductive biomass for each individual, seed heads were collected as they matured (three times per week until 14 July and weekly thereafter). Number of vegetative days was recorded for each plant. In flowering plants, the number of vegetative days was equal to days to first flower. For nonflowering plants, the number of vegetative days was equal to days to death (without reproducing) or final harvest (6 September).

Leaf traits were assessed on 14–15 June on each live plant that had at least four leaves produced after transplant. One mature (fully expanded) leaf was collected from each plant between 0600 and 0800 h (when maximally hydrated) and temporarily stored in a sealed bag in a cooler until leaf wet biomass and leaf area were measured with a leaf area meter (CID, Inc., Pullman, WA) within 4 h. Thereafter plants were dried at 70°C for at least 48 h and dry biomass was determined. Leaf succulence was calculated as $([\text{wet mass} - \text{dry mass}]/\text{leaf area})$; Jennings 1976). Specific leaf area was calculated as leaf area/leaf dry mass. The leaves were then individually ground and analyzed for nitrogen on a mass basis per dry leaf biomass (mg/g, Carbo Erba NA 1500 elemental analyzer, Milan, Italy) and leaf carbon isotopic composition (leaf $\delta^{13}\text{C}$, measured on a continuous flow mass spectrometer, Finnegan, Bremen, Germany). We also explored the use of leaf nitrogen/area and specific leaf area instead of nitrogen/mass in the selection analysis (Wright et al. 2003), but present nitrogen/mass because it explains more of the variation and is more biologically relevant in this context. Leaf $\delta^{13}\text{C}$ provides an integrated measure of leaf intercellular CO₂ con-

centration (CI) over the lifetime of the leaf. Integrated CI is, in turn, a relative measure of integrated instantaneous WUE, provided leaf temperatures are similar (Farquhar et al. 1989; Ehleringer et al. 1992; Donovan and Ehleringer 1994). Greater (i.e., less negative) leaf $\delta^{13}\text{C}$ reflects greater WUE.

On 6 September, all surviving plants were harvested for aboveground biomass. All plants were sorted into stem, leaf, and reproductive biomass (bud, flower, and seeds) and dried for 48 h at 60°C. Biomass components were then weighed and the number of seeds counted. Aboveground vegetative biomass includes stem and leaf biomass.

Data Analyses

Genotypic class comparisons.—Traits were compared among the five study genotypic classes with a one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test (PROC GLM, SAS 8.02, SAS Institute 2001). Block was initially incorporated into the analysis, but later dropped because it became apparent during the experiment that the blocking did not capture the primary environmental gradient at the site, as confirmed by its nonsignificance. Moreover, because genotypic classes were grouped within blocks, the environmental gradient could have resulted in a biased estimate of genotype means when block and block X genotype were estimated. For the genotypic class comparisons, vegetative biomass (aboveground), reproductive biomass, and the number of buds at first flower were log transformed to better meet ANOVA assumptions of normality of residuals and homoscedasticity of variance. Traits were considered to be transgressive if the average value for *H. anomalus* was significantly higher or lower than both *H. annuus* and *H. petiolaris*.

Selection Experiments for BC2ann and BC2pet and Helianthus anomalus.—Phenotypic selection analysis was used to determine the relationship of traits to performance and plant fitness (Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Brodie et al. 1995). The covariance of an individual trait and plant fitness is a selection differential (S) and is interpreted as net (indirect and direct) selection. The multiple regression coefficient of one of several traits to fitness is a selection gradient (β) and is interpreted as direct selection on an individual trait. An important caveat for all selection analyses is that estimates of selection gradients assume that all correlated traits under selection have been incorporated into the analyses. For the selection analysis of each genotypic class, the leaf traits were standardized to a mean of zero and a variance of one, with no further transformations applied. Vegetative biomass, the dependent variable, was relativized to the population means: that is, a mean of 1.0. Vegetative biomass was used as the measure of relative fitness instead of some measure of reproduction for two main reasons. First, *H. anomalus* reproduction was truncated by the final harvest. Second, data analysis at the end of the study indicated that we had two groups of *H. anomalus* that differed in leaf shape and phenology. Given that the *H. anomalus* seed source was one population, and that the difference in phenology was associated with a group that was germinated later to supplement germination, the difference was most likely due to differences in postgermination and preplanting environment

such as light quality and quantity cues. The use of vegetative biomass as relative fitness eliminates any potential confounding effect of morphotype and truncated reproduction for *H. anomalus*. The *H. anomalus* groups did not differ for relationships among leaf traits and the relationships of these traits to vegetative biomass (SAS, PROC GLM, analysis of covariance and homogeneity-of-slopes models using standardized traits and relative fitness, results not presented). For all plants from the selection analyses, including many plants only part way through reproduction, the correlations between vegetative biomass and reproductive biomass were $r = 0.56$, 0.73 , and 0.75 for *H. anomalus*, BC2ann, and BC2pet, respectively (Pearson correlations, $P < 0.001$ for all). When restricted to more mature plants, that is, at least half of the reproductive units were mature seed heads at final harvest, the correlation coefficient improved to 0.90 for *H. anomalus* ($n = 11$, $P < 0.001$), but did not change for the other two classes.

Standardized linear selection differentials (S) were estimated for the four focal leaf traits and vegetative biomass as the covariance between standardized traits and relative fitness (PROC CORR; Lande and Arnold 1983). Selection differentials were considered significantly different from zero if the Spearman rank correlation coefficient between the trait and relative fitness differed significantly from zero (Lande and Arnold 1983). Standardized linear selection gradients (β) were estimated for the four leaf traits using multiple regressions with relative fitness as the dependent variable (PROC GLM). Nonlinear selection gradients were then estimated from regressing relative fitness on linear and quadratic terms. Because residuals from the regression analyses were not normally distributed, the significance of the selection gradients was tested using a bootstrap analysis with 10,000 estimates (Dixon 2000). Ninety-five percent confidence intervals were then calculated from the bootstrap distribution using the percentile. Selection gradients were compared among genotypic classes, again using a bootstrap analysis with 10,000 estimates. The trait selection gradients and Pearson correlations among the traits (SAS, PROC CORR) are presented in path diagrams for *H. anomalus*, BC2ann, and BC2pet.

RESULTS

Comparison of *Helianthus anomalus* to Ancestral Parents

For leaf traits, *H. anomalus* had a higher leaf succulence and a lower leaf nitrogen than either of its ancestral parents, *H. annuus* and *H. petiolaris* (Table 1). *Helianthus anomalus* leaf area was not different from *H. annuus* but was larger than *H. petiolaris*. *Helianthus anomalus* WUE (as interpreted from leaf $\delta^{13}\text{C}$, see Materials and Methods) was similar to both ancestral parents. *Helianthus anomalus* also differed in some phenological and demographic traits from its ancestral parents. The *H. anomalus* plants that initiated flowering (61%) did so at least two weeks later than *H. annuus* and *H. petiolaris*, and had more buds at first flower than *H. annuus* (Table 1). Looking at the number of days that plants were vegetative, which takes into consideration all plants (those that flowered, died before flowering, or remained nonflowering), the delayed phenology of *H. anomalus* is even more pronounced. *Helianthus anomalus* also had more above-

TABLE 1. Genotypic class comparison of traits (mean \pm SD) of *Helianthus annuus*, *H. petiolaris*, *H. anomalous*, and backcross populations BC2ann and BC2pet ($n = 20, 14, 130, 114$, and 104), except traits related to first flower ($n = 13, 12, 80, 100$, and 100) and individual seed mass ($n = 7, 11, 46, 69$, and 56 plants, respectively). Plants were transplanted into the desert dune native habitat of *H. anomalous* for the summer 2002 growing season. Leaf $\delta^{13}\text{C}$ provides an estimate of integrated instantaneous water-use efficiency (see Materials and Methods): greater (less negative) leaf $\delta^{13}\text{C}$ reflects greater water-use efficiency. ABGR is aboveground biomass at senescence or harvest on 6 September. Different letters indicate significant species differences tested with a one-way ANOVA, followed by Duncan's test. The *H. anomalous* phenotype is scored as transgressive if significantly higher or lower than both *H. annuus* and *H. petiolaris*.

Trait	<i>H. annuus</i>	<i>H. petiolaris</i>	<i>H. anomalous</i>	BC2ann	BC2pet	<i>P</i>	<i>H. anomalous</i> phenotype
Height when leaf traits sampled, 14–15 June (cm)	24.8 \pm 7.3 ^a	17.3 \pm 8.6 ^b	13.3 \pm 5.7 ^c	15.9 \pm 6.9 ^{bc}	14.5 \pm 6.8 ^{bc}	<0.0001	–transgressive
Leaf area (cm ²)	5.19 \pm 1.41 ^b	4.16 \pm 1.38 ^c	5.38 \pm 2.30 ^{ab}	6.32 \pm 2.62 ^a	3.57 \pm 1.39 ^c	<0.0001	<i>annuus</i> like
Specific leaf area (cm ² /g)	107.2 \pm 12.5 ^b	97.7 \pm 11.4 ^c	91.8 \pm 14.0 ^c	96.2 \pm 13.2 ^c	116.9 \pm 19.4 ^a	<0.0001	<i>petiolaris</i> like
Leaf succulence (g/cm ²)	0.027 \pm 0.002 ^d	0.035 \pm 0.004 ^b	0.050 \pm 0.005 ^a	0.032 \pm 0.005 ^c	0.034 \pm 0.003 ^b	<0.0001	+transgressive
Leaf N (mg/g)	27.39 \pm 6.41 ^a	25.03 \pm 5.57 ^{ab}	20.07 \pm 4.93 ^c	22.83 \pm 5.88 ^{bc}	25.45 \pm 7.54 ^{ab}	<0.0001	–transgressive
Leaf $\delta^{13}\text{C}$ (‰)	–26.81 \pm 0.89 ^a	–26.77 \pm 0.58 ^a	–27.15 \pm 1.06 ^{ab}	–27.27 \pm 0.77 ^b	–27.85 \pm 0.90 ^c	<0.0001	<i>annuus/petiolaris</i> like
Percent flowering during study	65%	86%	61%	88%	96%		
Height at first flower	33.51 \pm 6.27 ^a	36.79 \pm 14.47 ^a	36.73 \pm 15.13 ^a	27.97 \pm 8.34 ^b	20.63 \pm 6.10 ^c	<0.0001	<i>annuus/petiolaris</i> like
Buds at first flower	2.38 \pm 1.80 ^b	3.08 \pm 1.478 ^{ab}	5.33 \pm 5.96 ^a	2.29 \pm 1.14 ^b	2.24 \pm 0.72 ^b	<0.0001	<i>petiolaris</i> like
Days to first flower	52.54 \pm 9.86 ^b	50.50 \pm 23.62 ^b	68.01 \pm 25.05 ^a	46.23 \pm 13.94 ^b	28.53 \pm 14.42 ^c	<0.0001	+transgressive
Vegetative days	49.1 \pm 10.8 ^b	54.4 \pm 24.1 ^b	78.5 \pm 27.8 ^a	47.8 \pm 15.6 ^b	29.9 \pm 15.9 ^c	<0.0001	+transgressive
Reproductive days	23.1 \pm 26.3 ^c	45.8 \pm 32.2 ^b	27.3 \pm 29.6 ^c	37.1 \pm 25.1 ^{bc}	72.66 \pm 28.1 ^a	<0.0001	<i>annuus</i> like
Survival days	72.2 \pm 29.4 ^c	100.2 \pm 26.1 ^a	105.8 \pm 19.3 ^a	84.9 \pm 24.1 ^b	102.6 \pm 22.8 ^a	<0.0001	<i>petiolaris</i> like
ABGR vegetative biomass (g)	1.24 \pm 1.03 ^b	1.88 \pm 1.90 ^b	5.64 \pm 6.92 ^a	0.93 \pm 0.62 ^b	1.29 \pm 1.40 ^b	<0.0001	+transgressive
Reproductive biomass (g)	0.24 \pm 0.26 ^b	0.48 \pm 0.15 ^{ab}	0.73 \pm 1.31 ^a	0.43 \pm 0.47 ^{ab}	0.32 \pm 0.27 ^{ab}	0.02	<i>petiolaris</i> like
Seed biomass per seed (mg)	2.57 \pm 0.94 ^c	1.57 \pm 0.78 ^d	4.55 \pm 2.0 ^a	3.47 \pm 1.23 ^b	2.21 \pm 0.68 ^{cd}	<0.0001	+transgressive
Demographic fate:							
Dead before flowering	35%	14%	21%	11%	4%		
Senesced after flowering	40%	14%	2%	57%	24%		
Flowering at harvest	25%	72%	59%	31%	72%		
Buds but no flowers at harvest	0%	0%	9%	1%	0%		
Uncommitted to reproduction at harvest	0%	0%	9%	0%	0%		

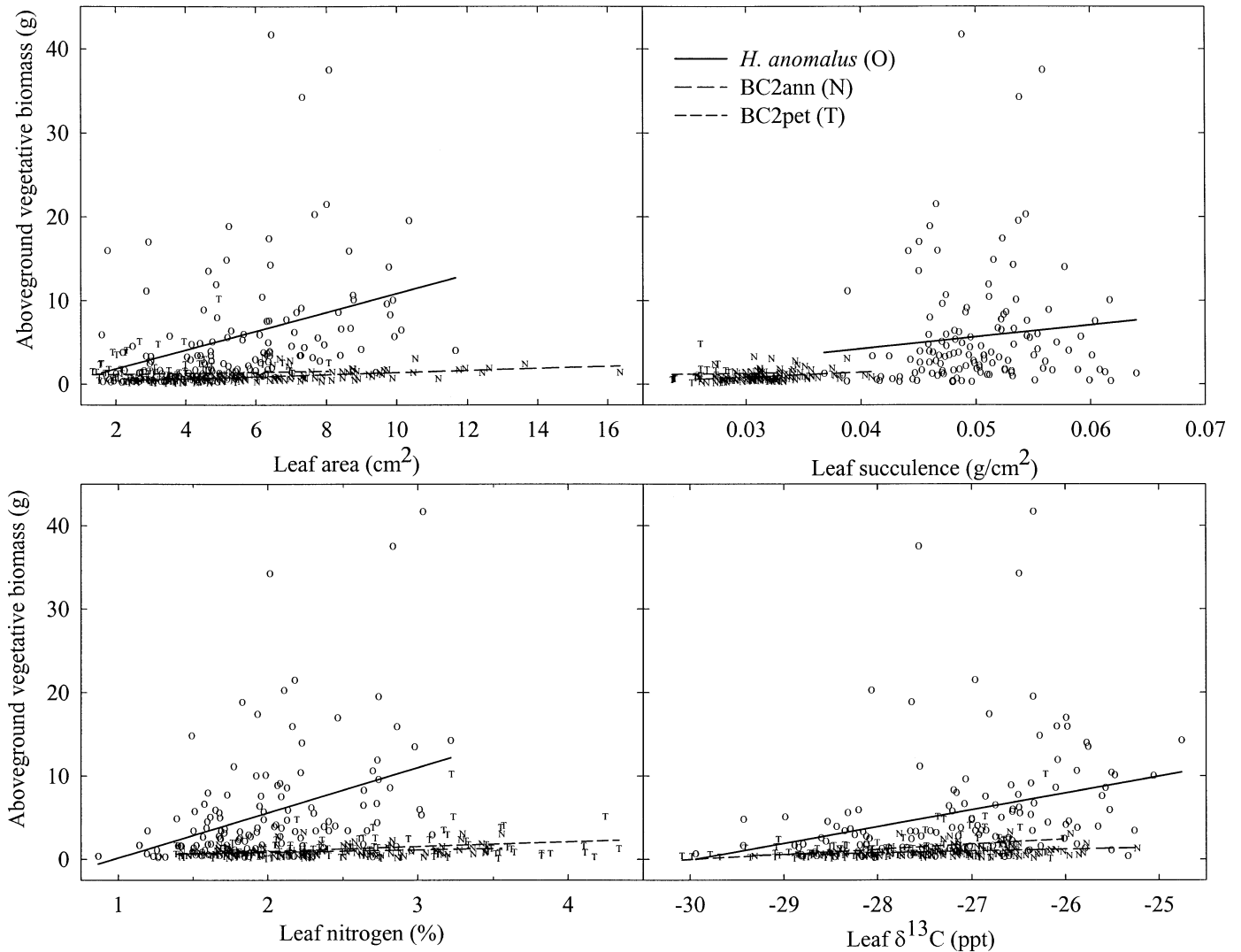


FIG. 1. Relationship of leaf traits to aboveground vegetative biomass for BC2ann ($n = 114$), BC2pet ($n = 104$), and *Helianthus anomalus* ($n = 130$). Lines are linear regressions using the least squares method for each genotypic class. Leaf $\delta^{13}\text{C}$ provides an estimate of integrated instantaneous water-use efficiency (see Materials and Methods): greater (less negative) leaf $\delta^{13}\text{C}$ reflects greater water-use efficiency.

ground vegetative biomass and bigger seeds than *H. annuus* and *H. petiolaris*.

Comparison of Early-Generation Hybrids to Parental Species

The early-generation hybrid populations were similar to the parent species for many traits (Table 1). BC2ann was not different from *H. annuus* and *H. petiolaris* for six of 15 and nine of 15 traits, respectively. The BC2pet population was not different than *H. annuus* and *H. petiolaris* for five of 15 and nine of 15 traits, respectively. However, BC2ann did have a larger leaf area, lower WUE, and a shorter height at first flower than *H. annuus* and *H. petiolaris*. BC2pet had a higher specific leaf area, lower WUE, and a shorter height at first flower and flowered sooner and for a longer interval than *H. annuus* and *H. petiolaris*. For all traits, the traits value ranges

for the BC2s overlapped with those of *H. annuus* and *H. petiolaris*.

Comparison of *Helianthus anomalus* to Early-Generation Hybrids, BC2ann, and BC2pet

Helianthus anomalus had greater leaf succulence than either BC2 population (Table 1). For leaf area, *H. anomalus* was not different from BC2ann but larger than BC2pet. For leaf nitrogen, *H. anomalus* was not different from BC2ann but was lower than BC2pet. For leaf WUE, *H. anomalus* was not different from BC2ann but was higher than BC2pet. However, the range of leaf trait values in BC2 individuals largely overlapped with that of *H. anomalus*, with the exception of leaf succulence (Table 1, Fig. 1). Only the most succulent BC2ann overlapped with the least succulent *H. anomalus*. *Helianthus anomalus* plants achieved much larger aboveground vegetative biomass than either BC₂ population.

Phenologically, *H. anomalus* remained vegetative longer and produced more buds at first flower than BC2pet and BC2ann plants (Table 1). Although BC2pet flowered earliest, they also had the largest proportion of plants still flowering at final harvest. BC2ann initiated flowering after BC2pet and before *H. anomalus*, but had the largest proportion of plants that flowered and then senesced. Most of the BC2pet and BC2ann exhibited continual flowering so that those plants flowering earlier produced more total reproductive units over the growing season (data not shown). Of the *H. anomalus* plants that flowered, later flowering was associated with more buds at first flower and total reproductive units, a more determinate-like pattern of flowering. At final harvest, *H. anomalus* was the only genotypic class that still had a large number of living plants (18%) that had not yet flowered (Table 1). Many of these nonflowering *H. anomalus* were relatively large (5.10 ± 5.35 g vegetative biomass for plants budding at harvest, and 4.77 ± 4.71 g for nonreproductive plants) and would likely have flowered if the study had continued longer.

Selection Analyses for Leaf Traits of Helianthus anomalus, BC2ann, and BC2pet

Linear selection differentials (S, encompasses indirect and direct selection) were significant for leaf area, succulence, nitrogen, and WUE for BC2ann and *H. anomalus*, and for leaf nitrogen and WUE for BC2pet (Fig. 2). However, many of the traits were highly correlated, consequently fewer of the selection gradients (β , direct selection) were significant. The leaf trait selection gradients differed by genotypic class. For *H. anomalus*, there was selection for greater leaf area and leaf nitrogen (β , Fig. 2). For BC2ann, selection favored greater leaf area and leaf succulence. For BC2pet, selection favored greater leaf nitrogen and WUE. Comparing two genotypic classes at a time, selection gradients in *H. anomalus* did not differ from either BC2ann or BC2pet. However, BC2ann and BC2pet differed for leaf succulence ($P < 0.05$) and leaf WUE ($P < 0.01$) selection gradients. There was no nonlinear or correlational selection for *H. anomalus*, BC2ann or BC2pet ($P > 0.05$ for all).

Selection on leaf traits in the BC2s favored the phenotype of *H. anomalus* in two instances. In the first case, the *H. anomalus* mean leaf succulence was higher than that of BC2ann and greater leaf succulence was favored in BC2ann. In the second case, *H. anomalus* WUE was greater than that of BC2pet, and greater WUE was favored in BC2pet (Table 1, Fig. 2). Selection in the BC2s did not necessarily favor the phenotype of *H. anomalus*. Leaf nitrogen was lower in *H. anomalus* than in the BC2pet, but greater leaf nitrogen was favored in BC2pet, opposite the direction of *H. anomalus*. Leaf nitrogen was positively correlated with leaf WUE in all genotypic classes, positively correlated with leaf area in *H. anomalus* and BC2ann, and negatively correlated with leaf succulence in BC2pet (Fig. 2).

DISCUSSION

Transgressive Traits

Trait differences for species from different habitats have often been used to infer adaptations to particular habitats. In

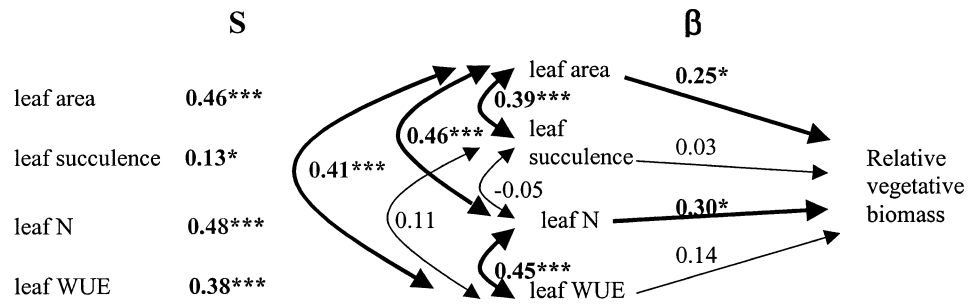
the desert dune habitat, some *H. anomalus* traits are transgressive, that is, significantly greater or smaller than those of both ancestral parents. *Helianthus anomalus* was positively transgressive for leaf succulence, days to first flower, seed mass, and aboveground vegetative biomass and negatively transgressive for leaf nitrogen. Some of these field results agree with previous greenhouse studies of well-watered and fertilized plants (Schwarzbach et al. 2001; Rosenthal et al. 2002). Both in the field and in the greenhouse, *H. anomalus* was positively transgressive for leaf succulence and seed mass and not transgressive for specific leaf area or WUE. However, there were some differences between the greenhouse and field that can be attributed to genotype \times environment interactions since the same seed sources were used for all of the studies. For example, *H. anomalus* was negatively transgressive for leaf nitrogen only in the field, and negatively transgressive for leaf area only in the greenhouse. Compared to the greenhouse studies, plants in the experimental garden experienced lower and patchier nutrient availability, declining soil moisture in shallow soils, lower humidity, higher light and disturbance, and much greater fluctuations in temperature. The trait expressions in the field are more relevant for inferring adaptation to the dune habitat. From the transgressive traits in the field, we would have expected stabilizing selection or directional selection toward *H. anomalus* phenotype for leaf succulence and leaf nitrogen in the dune habitat.

Current Selection in Helianthus anomalus

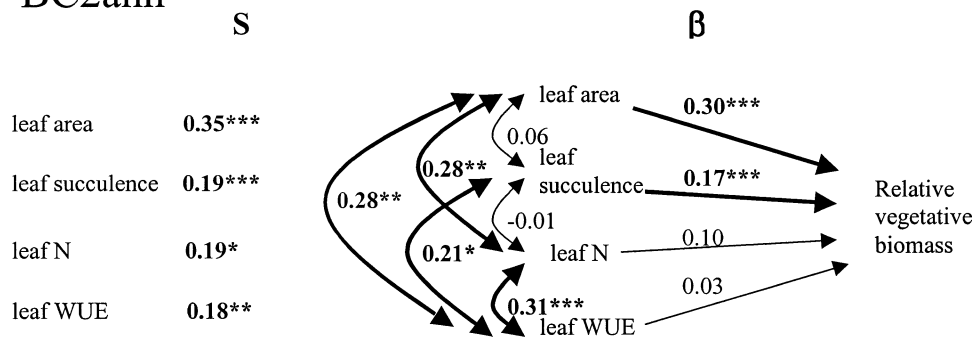
For *H. anomalus*, selection favored larger leaf area and greater leaf nitrogen. This selection was not consistent with the directional or stabilizing selection on leaf succulence and leaf nitrogen that would have been predicted from the transgressive traits of *H. anomalus* relative to ancestral parents. One consideration is that the current phenotype of the parental species may differ from that of the ancestral phenotypic state. We know of no other studies that have looked at selection on leaf ecophysiology and morphology of an extant species in its home habitat within the context of known ancestral phenotypes. However, other relevant studies have looked at selection on ecotypes or hybrids put back into source habitats (e.g., Jordan 1991; Dudley 1996; Nagy 1997; Heschel et al. 2002; Lexer et al. 2003a; discussed below) comparable to the BC₂ populations in our study. The selection on leaf traits for *H. anomalus* in the garden was similar to that found for nearby naturally occurring *H. anomalus* populations that were followed concurrently (F. Ludwig, pers. comm.).

Current Selection in the BC2s

We expected phenotypic selection on BC2ann and BC2pet, putatively similar to *H. anomalus* ancestors, to be either similar to current selection in *H. anomalus* or result in evolution toward the current *H. anomalus* phenotype. However, the action of selection depended greatly on the genotypic class. In BC2ann, selection favored greater leaf area and leaf succulence. In BC2pet, selection favored greater leaf nitrogen and WUE. Only the selection for greater succulence in BC2ann and greater WUE in BC2pet would result in evo-

H. anomalous

BC2ann



BC2pet

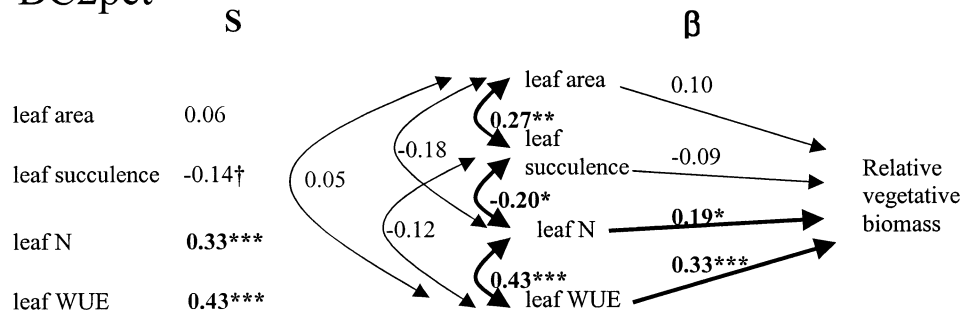


FIG. 2. Path analysis of effects of leaf traits (standardized) on relative aboveground vegetative biomass (fitness estimate) for *Helianthus anomalous* ($n = 130$), BC2ann ($n = 114$), and BC2pet ($n = 104$) transplanted into the desert sand dune habitat of *Helianthus anomalous*. Paths from traits to vegetative biomass are selection gradients (β). Two-headed arrows between traits are Pearson correlations. Selection differentials (S) for the same leaf traits are presented to the left of the path analyses. Leaf water-use efficiency (WUE) is estimated from leaf $\delta^{13}\text{C}$ (see Materials and Methods). Bold type indicates value is significantly different from zero, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and † indicates trend ($0.05 \leq P \leq 0.10$, see Materials and Methods).

lution toward the current *H. anomalous* phenotype. The selection for greater succulence for BC2ann in the *H. anomalous* habitat parallels the finding of selection for greater leaf succulence for BC2pet in the saline habitat where *H. paradoxus* was positively transgressive for succulence (Lexer et al. 2003a). These results support the hypothesis that transgressive segregation for succulence and subsequent selection may have contributed to the adaptation of *H. anomalous* ancestors to the desert dune habitats. However, in this and parallel studies with other *Helianthus* hybrid species from the same ancestral parents (Lexer et al. 2003a; Gross et al. 2004) not

all of the current selection was consistent with native phenotypes in that habitat. In this study, greater succulence was not favored in BC2pet and lower leaf nitrogen was not favored in any of the genotypic classes.

Studies with other genera have also put hybrids or mixtures of ecotypes back into source habitats to examine whether selection on ecophysiological or related traits is consistent with the short-term evolutionary differentiation of the native phenotype. For two studies that included leaf morphologies, selection on hybrids was sometimes, but not always, consistent of the native phenotypes in the source habitats (Jordan

1991; Nagy 1997). For WUE, two other studies found that selection depended on the environment (Dudley 1996; Heschel et al. 2002), but again was not necessarily consistent with the native phenotype (Heschel et al. 2002).

The variation in selection that would apparently hinder the evolutionary trajectory of hybrids toward the *H. anomalus* phenotype seems at first glance to be inconsistent with the strong and uniform selection suggested by multiple origins of *H. anomalus* (Schwarzbach and Rieseberg 2002). However, the present-day patterns of phenotypic selection may not be consistent with the long-term outcome of evolution of ecotypes or species for a number of reasons. One general caveat is that temporal variation in selection over seasonal, annual, and longer-term (historical) time scales likely varies due to fluctuations in climate, community changes, and human influences (Jordan 1991; Geber and Griffen 2003). This study was conducted in a relatively dry year compared to long-term weather records. Also, the estimated origin of *H. anomalus* as a species was 116,000–160,000 generations (years) ago, and the climate at that time is unknown. An additional general caveat is that lack of heritable genetic variation for traits is another possible constraint on the response to phenotypic selection. However, this does not seem likely for the study traits given that genetic variation has been demonstrated for many of these traits, and there is no strong evidence for a trade-off between strength of selection and heritability for these traits (Arntz and Delph 2001; Geber and Griffen 2003). The genetic covariances are more likely to have constrained the response to phenotypic selection in this study. Substantial linkage and/or pleiotropy has been found for QTLs underlying these and other traits differentiating the parental species (Rieseberg et al. 2003), indicating that genetic correlations likely do constrain the outcome of phenotypic selection in this system. Additionally, fertility selection plays an important role in establishing hybrid genomic composition and may also constrain phenotypic selection (Rieseberg et al. 1996).

Understanding the Variation in Phenotypic Selection

The significant differences between BC2ann and BC2pet for selection for succulence and WUE are surprising given that the BC2ann and BC2pet were genetically very similar to each other (sharing two of four parents) and to *H. anomalus* (Rieseberg et al. 2003), and that they were all grown in a common environment. One potential explanation for the variation in phenotypic selection is that it results from the common problem of selection analyses; that spurious selection gradients may be caused when a measured trait is correlated with another unmeasured trait that was under strong selection (Lande and Arnold 1983). It is certainly possible that the genotypic class differences in measured phenotypic selection reported here were caused by genotypic class differences in correlations among measured and unmeasured traits. This is consistent with the different correlations between measured traits among the genotypic classes. However, because vegetative biomass was used as a measure of fitness for selection on carbon-acquisition traits, it is possible to identify potential candidate traits to explore this possibility. The most likely

traits to differ in their function and trade-offs with other traits are leaf nitrogen and leaf succulence.

Leaf nitrogen appears to be a key trait for all of the *Helianthus* genotypic classes in desert dune environment, but the results are somewhat contradictory. Comparatively, *H. anomalus* had the lowest leaf nitrogen of the genotypic classes, but selection favored greater leaf nitrogen in *H. anomalus* and BC2ann. Among genotypic classes, *H. anomalus* had the lowest leaf nitrogen in the field, and a low aboveground growth rate compared to *H. annuus* when grown under high-resource conditions in the greenhouse (Schwarzbach et al. 2001; D. Rosenthal, pers. comm.). This suggests that *H. anomalus* grows successfully with a lower leaf nitrogen by having a slow maximum growth rate and efficiently using nitrogen that it acquires (Grime and Hunt 1975; Van der Werf et al. 1993; Poorter and Evans 1998; Aerts and Chapin 2000). Within *H. anomalus*, a higher leaf nitrogen may reflect a higher nitrogen availability or greater success at gaining this limiting nutrient due to belowground traits. In any case, the greater leaf nitrogen was apparently invested in photosynthetic capacity leading to greater WUE, which has also been shown in water-stressed commercial sunflowers (Fredeen et al. 1991). However, greater leaf level WUE does not necessarily mean less water used, because greater WUE can be achieved by greater stomatal closure (less water loss) or greater nitrogen investment in photosynthetic capacity (greater carbon gain given same amount of water loss), or some combination thereof (Donovan and Ehleringer 1994; Wright et al. 2003). Photosynthetic response curves and scaling to whole-plant WUE (growth/whole plant water use) and nitrogen use efficiency (growth/whole plant nitrogen) are needed to determine how *H. anomalus* trades off water and nutrient use. Incorporating such trade-offs into future selection analyses may resolve the apparently different selection on the BC2ann and BC2pet.

Given that desert dunes generally have a higher water availability than other desert habitats (Pavlik 1980; Rosenthal 2004), why was greater succulence favored in one of the backcross populations, and why is *H. anomalus* much more succulent than either ancestral parent? Greater leaf succulence may provide water storage capacity, as for succulent cacti that disconnect themselves from the environment and use internally stored water when dry (Nobel and Cui 1992; Smith et al. 1997). Greater water storage may compliment or be an alternative strategy to high WUE during dry periods. Alternatively, greater succulence may be associated with greater leaf toughness or some other unmeasured trait that is favored. *Helianthus anomalus* leaves did appear to be less abraded and abused by periodic wind/sand storms as compared to the other species. Based on specific leaf area, *H. anomalus* leaves were among the thickest or densest compared to the other genotypic classes. However, specific leaf area did not explain any additional variation in fitness when included in addition to or as an alternative to leaf area in the multivariate analyses. Further investigation will be needed to establish any direct or indirect advantage of greater succulence.

It is also possible that the differences in phenotypic selection among the genotypic classes may be explained by the three genotypic classes experiencing the environment differ-

ently. Desert annuals are generally thought to use the drought escape strategy (i.e., grow fast and reproduce early), as compared to a more dehydration avoidance strategy generally described for desert perennials (i.e., more conservative resource use and slower growth; Ludlow 1989; Gibson 1998; Stanton et al. 2000). However, within annuals, there can be genetically based differences for suites of demographic and ecophysiological traits, resulting in a spectrum from drought escape to avoidance (Geber and Dawson 1990; Ackerly et al. 2000; Stanton et al. 2000; McKay et al. 2003). Earlier flowering genotypes with lower WUE are consistent with the live fast–die young strategy (Geber and Dawson 1997; McKay et al. 2003). Comparing genotypic classes in our study, BC2pet flowered earlier and had the lowest WUE. Overall, compared to its ancestral parental species and their early-generation hybrids, *H. anomalus* appears to have a more conservative drought avoidance strategy. Based on trait observations in this study, we hypothesize that *H. anomalus* succeeds by: (1) efficient use of leaf nitrogen regardless of water availability, but shifting selection on WUE depending on water availability; (2) slow aboveground growth and biomass accumulation, with flexibility to tolerate burial and excavation; and (3) delayed reproduction after maximizing resource accumulation to make many large seeds. Based on excavations, we hypothesize that *H. anomalus* invests carbon and nitrogen into larger tap and lateral roots to access patchy nutrients and deep soil moisture and to spatially avoid drought throughout the growing season. In contrast, other desert annual *Helianthus* may be selected for the live fast–die young strategy due to higher nitrogen availability and a less reliable source of seasonal water availability (Rosenthal 2004).

This study was limited to seedling growth and survival for well-established, large seedlings beyond the stage of having several true leaves. Additional selection is likely taking place during germination and early seedling growth. Ongoing studies in the dune habitat indicate that seedling germination and survival is much greater for *H. anomalus* than either parental species (F. Ludwig, pers. comm.). More information on seed and early seedling traits will further our understanding of *H. anomalus* ecological and evolutionary success in the desert dune environment.

ACKNOWLEDGMENTS

We thank J. Lance, B. Brewer, S. Peglow, and S. Scarborough for assistance with field and lab work, and P. Chu for bootstrapping. We also thank F. Clegg and Bureau of Land Management; Little Sahara Recreation Area for use of the field site, and Utah State University for use of Tintic Field Station. This project was funded by National Science Foundation grant IBN-0131078 to LAD and National Institutes of Health grant GM59065 to LHR.

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Corresponding Editor: M. Geber