

## TRANSGRESSIVE CHARACTER EXPRESSION IN A HYBRID SUNFLOWER SPECIES<sup>1</sup>

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Diploid hybrid lineages often are ecologically distinct from their parental species. However, it is unclear whether this niche divergence is typically achieved via hybrid intermediacy, a mixture of parental traits, and/or the evolution of extreme (transgressive) morphological and ecophysiological features. Here we compare an extensively studied hybrid sunflower species, *Helianthus anomalus*, with its putative parents, *H. annuus* and *H. petiolaris*, for 41 morphological and 12 ecophysiological traits. *Helianthus anomalus* was morphologically intermediate for one trait (2.4%), parental-like for 23 traits (56.1%), and transgressive for 17 traits (41.5%). For ecophysiological traits, *H. anomalus* was not significantly different from one or both parents for nine traits (75%), and was transgressive for the remaining three (25%). Thus, *H. anomalus* appears to be a mosaic of parental-like and transgressive phenotypes. Although the fitness effects of the transgressive characters are not yet known, many of these characters are consistent with adaptations reported for other sand dune plants. Genetic studies are currently underway to ascertain whether these extreme characters arose as a direct byproduct of hybridization or whether they evolved via mutational divergence.

**Key words:** adaptations; carbon isotope ratios; *Helianthus*; hybridization; photosynthesis; speciation; transgressive segregation; water-use efficiency.

A requirement for a new diploid hybrid species to survive and not be outperformed by its parental species is niche separation between parent and hybrid lines (Lewontin and Birch, 1966; Grant, 1981; Templeton, 1981; Schluter, 1998; Buerkle et al., in press). Empirical data support this hypothesis and identify most stabilized hybrids as ecologically different from their parents (Abbott, 1992; Arnold, 1997; Rieseberg, 1997). A major question that has not yet been studied in detail concerns the origin of the adaptations that allow derived hybrid lineages to colonize new habitats.

Four possibilities have been suggested. First, it is possible that the habitats are intermediate between those of the parental species and thus most easily colonized by hybrids. Even habitats that appear divergent relative to those of the parental species may actually be intermediate with respect to the morphological and ecophysiological parameters required. Second, it may be that a mixture of parental traits is required to colonize the new habitat. For example, a hybrid derivative of a salt-tolerant, shade species and a freshwater, sun species might be able to colonize a saline, sunny habitat. Third, hybridization may somehow generate extreme or transgressive phenotypes that enable colonization of habitats that are truly extreme or novel relative to that of either parent. The latter hypothesis has received recent support from quantitative trait locus (QTL) studies (reviewed in Rieseberg, Archer, and Wayne, 1999), which indicate that transgressive phenotypes are an expected consequence of hybridization between genetically divergent lineages. Finally, it may be that the morphological and physiological adaptations required for niche colonization have arisen through mutational divergence following hybrid lineage formation. However, this would require hybrid lineage estab-

lishment in the absence of niche divergence—an unlikely scenario according to theoretical models (McCarthy, Asmussen, and Anderson, 1995; Buerkle et al., in press).

We have initiated a series of ecological and genetic studies to distinguish among these explanations with respect to the colonization of sand dune habitats by a well-characterized hybrid sunflower species, *Helianthus anomalus*. This paper reports on the results of the first of these studies, in which we quantified morphological and ecophysiological differences that distinguish *H. anomalus* from its putative parental species, *H. annuus* and *H. petiolaris*. We ask whether morphological and ecophysiological traits exhibited by *H. anomalus* are intermediate, parental-like, or extreme when compared to its parents. We also ask whether extreme characters that are identified are consistent with other known adaptations to sand dune habitats.

Both parental species, *H. annuus* and *H. petiolaris*, are widespread taxa (Fig. 1) that mainly grow in disturbed sites. *Helianthus petiolaris* typically occurs at higher elevations than *H. annuus*, but there are many locations where they overlap. They appear to have different ecological preferences as well, with *H. annuus* preferring heavier more mesic soils and *H. petiolaris* more xeric sandy habitats (Heiser, 1947). The two parental species are not sister taxa, but occur in divergent clades (Fig. 2) based on morphological evidence (Schilling and Heiser, 1981), nuclear ribosomal DNA (Rieseberg, 1991), and chloroplast DNA (Rieseberg et al., 1991).

*Helianthus anomalus* is a rare endemic species adapted to sand dune and swale habitats in Utah and northern Arizona (Heiser, 1958; Heiser et al., 1969; Rieseberg, 1991). Based on its occurrence in sand dune habitats, it frequently has been cited as potentially being more drought tolerant than either parental species and thus a candidate to improve cultivated sunflower germplasm (Heiser, 1978; Thompson, Zimmerman, and Rogers, 1981; Nabhan and Reichhardt, 1983). In addition, much work has been done to describe and characterize the two parental species separately or together, although cultivated

<sup>1</sup> Manuscript received 14 October 1999; revision accepted 20 April 2000.

The authors thank Michele Arntz, Diana Wolf, and three anonymous reviewers for comments on the manuscript; Andy Tull and Mark Zimmerman for excellent plant care; and Meredith Phillips for help with the measurements. This work was supported by NSF grant DEB-9806290.

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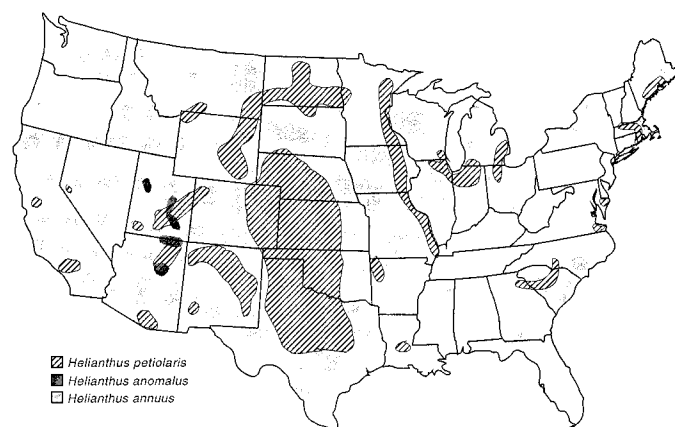


Fig. 1. Distribution map of *Helianthus petiolaris*, *H. annuus*, and *H. anomalus* (redrawn from Rogers, Thompson, and Seiler, 1982).

lines of *H. annuus* were often used instead of wild accessions (Heiser, 1947, 1961; Sobrado and Turner, 1983a, b, 1986). However, the rare hybrid species never has been comparatively studied with its parents under controlled conditions.

Hence, we quantified species differences under common greenhouse conditions. We grew the plants under well-watered and fertilized conditions and compared the three wild species for many of the morphological and ecophysiological (photosynthesis, stomatal and cuticular conductance, water-use efficiency) characters that have been known or suggested to differ among them. In addition, to investigate possible differences in drought tolerance, the species were compared for ecophysiological responses to one short-term water stress treatment.

## MATERIALS AND METHODS

We used achenes from wild populations of *Helianthus annuus* from Utah, jct. I-15 and Hwy. 6, exit 261, east side (Rieseberg no. 1286); *H. petiolaris* from Utah 1/4 mi E of Zion park entrance on Hwy. 9 (Rieseberg no. 1287); and *H. anomalus* from Utah, 26.5 mi S of Hanksville on Hwy. 95 (Rieseberg no. 1273). Achenes were collected from a large number of individuals (>50) for each species and pooled. We only chose one population as a species representative for the following reasons: (1) There are only small genetic differences within species (Rieseberg, Carter, and Zona, 1990; Rieseberg and Seiler, 1990; Rieseberg, 1991). Interspecific genetic identities are at least 96% for chloroplast DNA and isozyme data. (2) Morphological variation within species is also clearly smaller than between species (Heiser 1954, 1961; Heiser et al., 1969).

The greenhouse study was conducted at the University of Georgia, Athens campus, starting 25 August 1998. About 50 randomly selected achenes from each species were germinated in petri dishes. The fruit wall was incised with a razor blade, and the achenes were placed on moist paper tissue containing 1% (w/v) gibberellic acid in sterilized distilled water. Previous experiments had shown that without any special treatment the percentage of seed germination was low. After 2 d, the fruit wall was removed, and the seeds kept at 4°C in the dark. The paper substrate was changed daily to avoid fungal growth. The cold treatment helped to synchronize the germination of all three species, holding back the seeds that germinated earlier. After 5 d, roots started growing, and the petri dishes were placed in a sunny place for 2 d. After 1 wk, 20 individuals of each species were planted (day 0) in 25 cm diameter plastic pots containing a 3:1 mixture of sand/fritted clay (Turface, Profile Products LLC, Buffalo Grove, Illinois, USA) and randomly assigned to a randomized block design with four blocks, and five individuals of each species per block. Except for the duration of the drought experiment, the plants were watered twice daily and fertilized every 4th d. High-intensity metal halide

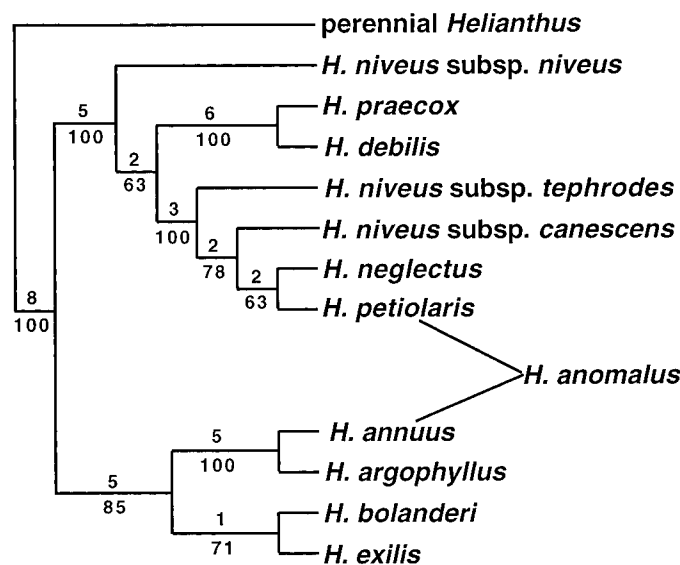


Fig. 2. Phylogenetic tree for *Helianthus* section *Helianthus* based on combined chloroplast DNA and nuclear ribosomal DNA data (redrawn from Rieseberg, 1991). Numbers of character state changes are given above branches, and bootstrap percentages are given below branches.

lamps supplemented sunlight for 16 h per day. Several plants did not survive the early seedling stage and were subsequently excluded from measurements. In all, 9–15 individuals of each species were analyzed.

**Morphological measurements**—Starting the 3rd d after planting, total plant height was measured on a 2-d cycle for the first 17 d after planting. Thereafter, height was measured on a 4-d cycle, with the last measurement taken on day 93. Side branches eventually overtop the main stem and in those cases the tallest branch was measured. Leaves were counted on the same days as height measurements until day 57, at which time the last individual had terminated the main stem with the production of a flower head. Only leaves along the main stem, including the cotyledons, were counted, and we used this measurement as one indicator for early plant growth. Growth curves were plotted for plant height and leaf number.

Under well-watered conditions, a mature leaf of each individual was chosen and measurements were taken of its wet mass, length and width of leaf blade, leaf petiole length, and leaf area (LI-COR 3100 area meter, LI-COR Inc., Lincoln, Nebraska, USA). The leaves were dried at 60°C, weighed again, and then used for chemical analyses (see below). When the first signs of a terminal flower head appeared, the time (in days after planting) was recorded. All of the study species produced multiple flower heads, and the number of heads was counted on day 62. When all disk flowers of a head had opened, the first three heads per individual were measured for head diameter and phyllary (bracts surrounding the head) number, length, width, and area. Leafy phyllaries, which are larger and more leaf-like than the rest, and present in some individuals, were also counted. Additionally, the number of ray flowers per head and ligule lengths and widths were measured.

Disk flowers were hand-pollinated daily (with a bulked mix of pollen from all individuals of the same species) until all flowers had opened, and the heads were harvested and dried at the end of the season. The number of disk flowers and achenes were counted, and seed set was calculated as a proportion of the total number of disk flowers. The achenes were weighed and measured for length, thickness, and width. The ratio of width and thickness was used as an index for shape where a value of 1 indicates a round cross section and a value <1 indicates a more flattened shape.

On day 93, stem diameter 2 cm above the ground and the distance between the ground and the first branch were measured. After harvesting on day 100, soil was gently removed from the roots by washing. The length and width of the total root system were used to estimate volume explored, and the angle

of lateral roots relative to horizontal was determined. The taproot, if present, was measured for length. Since the plants were not pot bound, these root measurements provide an approximate descriptor of root morphology.

**Ecophysiological measurements**—On day 50 the photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), and internal  $\text{CO}_2$  concentrations ( $c_i$ ) of one fully mature leaf of comparable age (at about the same height of plant) were measured with a LI-COR 6400 portable photosynthesis system (LI-COR Inc.; ambient  $\text{CO}_2$  concentration 350 ppm, air temperature  $26^\circ\text{C}$ , photosynthetically active radiation  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ ). The difference between leaf and air temperature in the cuvette was also recorded. Measurements were made on well-watered plants between 0930 and 1400, and preliminary measurements indicated no midday stomatal closure during this time. Instantaneous  $c_i$  was used as a comparative estimate of instantaneous water-use efficiency (WUE) since ambient  $\text{CO}_2$  concentration, leaf temperature, and  $\Delta w$  (leaf-to-air vapor pressure deficit) were similar for all individuals (Ehleringer, Phillips, and Comstock, 1992; Donovan and Ehleringer, 1994). A higher  $c_i$  reflects lower or less conservative instantaneous WUE. Photosynthetic nitrogen use efficiency (PNUE) was calculated from  $A$ , leaf nitrogen (by micro-Dumas combustion technique, Carlo Erba, Milan, Italy) and specific leaf area (Field, Merino, and Mooney, 1983; Field and Mooney, 1986).

Leaf carbon isotope ratios ( $\delta^{13}\text{C}$ ) were measured using continuous flow mass spectrometry (University of Georgia Stable Isotope/Soil Biology Laboratory) in order to estimate leaf-lifetime integrated  $c_i$  and WUE (Farquhar, Ehleringer, and Hubick, 1989; Donovan and Ehleringer, 1994). A more negative  $\delta^{13}\text{C}$  reflects a lower or less conservative integrated WUE.

Cuticular conductance was measured on day 85 for well-watered plants. Starting at 0800 one fully expanded leaf per plant was harvested, weighed immediately, placed with its petiole in beakers of water, and kept in a dark room to reach full hydration. After 3 h the leaves were removed from the beaker, petioles sealed with parafilm, and the leaves were weighed. Leaves were then placed in a dark box filled with silica gel desiccant to induce complete stomatal closure. Weight measurements were repeated every 30 min for the next 270 min to determine cuticular conductance, calculated as the rate of water loss per unit leaf area of leaf blade.

The plants were subjected to a short-term drought by withholding water starting on day 56. When each plant reached leaf wilting point (visible turgor loss), we measured  $A$ ,  $g_s$ , and  $c_i$  of a wilted leaf (leaves of comparable age were chosen), and excised leaf water potential (pressure chamber; PMS, Corvallis, Oregon, USA). The plants were watered immediately after the measurements were taken to avoid fatal water stress situations.

**Data analysis**—Data were analyzed with two-way analysis of variance (ANOVA) with block and species as independent variables. Significant mean differences between species were determined with an LSD multiple range test. We corrected for the unequal sample sizes by reducing power based on the smaller sample size. We did not find any block effects and therefore block statistics are not further reported. When a character differed significantly among species, the character was reported as transgressive (negative or positive) if the value for *H. anomalus* significantly exceeded the parental values. Otherwise the character pattern of *H. anomalus* relative to the parental species was reported as intermediate, *H. petiolaris*-like, or *H. annuus*-like.

A mean growth curve for each species was plotted and one-way ANOVA was used to detect differences between the means for each measuring date. Since multiple tests were conducted,  $P$  values were Bonferroni corrected (Rice, 1989). Differences for cuticular conductances were calculated by comparing the mean slope for the water loss curves for each species using one-way ANOVA.

## RESULTS

**Morphological characters**—We compared *H. annuus*, *H. petiolaris*, and the hybrid species *H. anomalus* for 41 morphological characters: 31 single characters and ten combined characters. Our study revealed significant species differences

for 40 of the 41 characters (Table 1). The only character that did not show any species effect was the length of the taproot.

Although *H. anomalus* comprised morphological characters from both parents, only one character (2.4% of all morphological characters) was intermediate, achene thickness (Table 1). *H. anomalus* was *H. petiolaris*-like (i.e., not significantly different from *H. petiolaris*) for 17 characters (41.5%), and *H. annuus*-like for five characters (12.2%). One character did not differ for all three species (2.4%). A total of 17 characters (41.5%) were transgressive: 12 negatively transgressive and five positively transgressive.

Plant growth, measured as increase in height and leaf number, differed with developmental stage (Fig. 3a, b, c). *Helianthus annuus* had the greatest height for a large part of the entire season, but did not significantly differ from *H. petiolaris* between days 37 and 77. *Helianthus anomalus* did not initially differ from *H. petiolaris*, but between days nine and 17 it was significantly taller than *H. petiolaris* (Fig. 3a;  $P < 0.01$ ). All three species terminate their main stem with a flower head before the plants branch, so branches eventually overtop the main stem. *Helianthus petiolaris* produced side branches earlier than the other two species, resulting in an increase in growth rate starting at  $\sim$ day 34 vs.  $\sim$ day 44 in *H. anomalus* and *H. annuus* (Fig. 3b). Eventually, *H. annuus* outgrew *H. petiolaris* and was the tallest species by the end of the experiment, while *H. anomalus* was clearly the shortest (height at day 93; Table 1).

*Helianthus annuus* produced more leaves along the main stem than the other two species (Table 1, Fig. 3c). This is in accordance with the overall faster growth rates of *H. annuus*. However, *H. petiolaris* produced a terminal inflorescence earlier and grew more side branches with additional heads than *H. annuus* (Table 1), however, all three species show the same overall branching pattern.

*Helianthus anomalus* was positively transgressive for water content per leaf area, indicating more succulent leaves (Table 1).

Almost all individuals produced a taproot, but the extent of lateral roots did vary, with *H. anomalus* producing the fewest and shortest lateral roots, and *H. annuus* displaying a well-developed and extended lateral root system. *H. petiolaris* was very similar to *H. annuus*, but differed by overall volume of soil explored by roots (Table 1).

**Ecophysiological characters**—We compared *H. annuus*, *H. petiolaris* and the hybrid species *H. anomalus* for 12 ecophysiological characters: eight during well-watered conditions, and four during short-term drought. There were significant species differences for eight of the ecophysiological characters (Table 2). *Helianthus anomalus* was *H. petiolaris*-like for five characters (41.7% of all ecophysiological characters) and three characters (25.0%) were positively transgressive.

Under well-watered conditions, there were no species differences for leaf  $A$ ,  $g_s$ , nitrogen content, and PNUE (Table 2). Instantaneous WUE (from instantaneous  $c_i$ ) was positively transgressive for *H. anomalus* and leaf-lifetime integrated WUE (from  $\delta^{13}\text{C}$ ) was *H. petiolaris*-like. The differences between air and leaf temperature (leaf  $<$  air) and cuticular conductance were also positively transgressive for *H. anomalus* (Table 2). When plants were subjected to a short-term drought and reached leaf wilting point, the *H. anomalus* characters of  $A$ ,  $g_s$ ,  $c_i$ , and leaf water potential were all *H. petiolaris*-like (Table 2).

TABLE 1. Morphological character expression in *H. annuus*, *H. petiolaris*, and in the hybrid derivative *H. anomalus* (mean  $\pm$  1 SE).<sup>1</sup>

Trait	<i>H. annuus</i>	<i>H. anomalus</i>	<i>H. petiolaris</i>	F, P for taxa	Hybrid phenotype
Total plant height at day 93 (m)	2.15 $\pm$ 0.08 <sup>a</sup>	1.2 $\pm$ 0.11 <sup>b</sup>	1.80 $\pm$ 0.65 <sup>c</sup>	27.01, <0.0001	neg. transgressive
Ligule number	22.26 $\pm$ 0.78 <sup>a</sup>	10.70 $\pm$ 0.57 <sup>b</sup>	14.20 $\pm$ 0.45 <sup>c</sup>	89.16, <0.0001	neg. transgressive
Ligule length (cm)	4.33 $\pm$ 0.15 <sup>a</sup>	2.88 $\pm$ 0.16 <sup>b</sup>	3.01 $\pm$ 0.06 <sup>b</sup>	42.52, <0.0001	petiolaris-like
Ligule width (cm)	1.36 $\pm$ 0.03 <sup>a</sup>	1.13 $\pm$ 0.06 <sup>b</sup>	1.07 $\pm$ 0.05 <sup>b</sup>	12.21, 0.0001	petiolaris-like
Ligule shape (length/width)	3.19 $\pm$ 0.11	2.58 $\pm$ 0.13	2.89 $\pm$ 0.13	5.56, 0.0080	see <sup>2</sup>
Phyllary length (cm)	18.75 $\pm$ 0.93 <sup>a</sup>	17.45 $\pm$ 0.88 <sup>a</sup>	13.02 $\pm$ 0.51 <sup>b</sup>	16.97, <0.0001	annuus-like
Phyllary width (cm)	9.22 $\pm$ 0.31 <sup>a</sup>	2.26 $\pm$ 0.17 <sup>b</sup>	3.01 $\pm$ 0.15 <sup>c</sup>	288.52, <0.0001	neg. transgressive
Phyllary area (mm <sup>2</sup> )	94.23 $\pm$ 5.17 <sup>a</sup>	28.20 $\pm$ 2.81 <sup>b</sup>	25.00 $\pm$ 2.29 <sup>b</sup>	115.71, <0.0001	petiolaris-like
Phyllary shape (length/width)	2.05 $\pm$ 0.11 <sup>a</sup>	8.05 $\pm$ 0.61 <sup>b</sup>	4.49 $\pm$ 0.27 <sup>c</sup>	69.45, <0.0001	pos. transgressive
Phyllary numbers	31.10 $\pm$ 0.55 <sup>a</sup>	18.52 $\pm$ 0.91 <sup>b</sup>	20.11 $\pm$ 0.59 <sup>b</sup>	104.85, <0.0001	petiolaris-like
Number of leafy phyllaries per head	0.31 $\pm$ 0.08 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	1.13 $\pm$ 0.12 <sup>b</sup>	18.30, <0.0001	annuus-like
Flower head diameter (cm)	3.53 $\pm$ 0.74 <sup>a</sup>	1.42 $\pm$ 0.07 <sup>b</sup>	1.95 $\pm$ 0.05 <sup>c</sup>	285.69, <0.0001	neg. transgressive
Flower head initiation (days after planting)	44.38 $\pm$ 1.93 <sup>a</sup>	43.90 $\pm$ 3.55 <sup>a</sup>	34.27 $\pm$ 0.99 <sup>b</sup>	8.12, 0.0013	annuus-like
Number of heads/plant (62 d after planting)	6.69 $\pm$ 1.03 <sup>a</sup>	9.70 $\pm$ 2.91 <sup>a</sup>	37.53 $\pm$ 4.78 <sup>b</sup>	24.23, <0.0001	annuus-like
Number of flowers per head	330.08 $\pm$ 10.28 <sup>a</sup>	69.60 $\pm$ 6.06 <sup>b</sup>	153.93 $\pm$ 6.67 <sup>c</sup>	252.28, <0.0001	neg. transgressive
Number of leaves along main stem at day 65	25.69 $\pm$ 1.16 <sup>a</sup>	18.60 $\pm$ 0.99 <sup>b</sup>	19.53 $\pm$ 0.78 <sup>b</sup>	15.04, <0.0001	petiolaris-like
Leaf length (cm)	20.75 $\pm$ 1.04 <sup>a</sup>	9.69 $\pm$ 0.51 <sup>b</sup>	10.29 $\pm$ 0.36 <sup>b</sup>	78.32, <0.0001	petiolaris-like
Leaf width (cm)	15.86 $\pm$ 1.07 <sup>a</sup>	3.79 $\pm$ 0.30 <sup>b</sup>	3.78 $\pm$ 0.23 <sup>b</sup>	109.60, <0.0001	petiolaris-like
Leaf area (cm <sup>2</sup> )	197.46 $\pm$ 49.89 <sup>a</sup>	23.90 $\pm$ 2.57 <sup>b</sup>	23.53 $\pm$ 1.89 <sup>b</sup>	139.26, <0.0001	petiolaris-like
Leaf shape (length/width)	1.37 $\pm$ 0.09 <sup>a</sup>	2.63 $\pm$ 0.14 <sup>b</sup>	2.82 $\pm$ 0.14 <sup>b</sup>	37.95, <0.0001	petiolaris-like
Leaf petiole length (cm)	18.30 $\pm$ 1.06 <sup>a</sup>	3.39 $\pm$ 0.32 <sup>b</sup>	5.63 $\pm$ 0.45 <sup>c</sup>	123.59, <0.0001	neg. transgressive
Leaf length/petiole length	1.18 $\pm$ 0.09 <sup>a</sup>	3.06 $\pm$ 0.28 <sup>b</sup>	1.97 $\pm$ 0.14 <sup>c</sup>	27.92, <0.0001	pos. transgressive
Leaf dry mass (mg)	942.0 $\pm$ 84.7 <sup>a</sup>	79.0 $\pm$ 11.0 <sup>b</sup>	86.1 $\pm$ 7.5 <sup>b</sup>	96.63, <0.0001	petiolaris-like
Leaf wet mass (mg)	6109.2 $\pm$ 489.9 <sup>a</sup>	929.0 $\pm$ 128.6 <sup>b</sup>	727.3 $\pm$ 62.1 <sup>b</sup>	108.04, <0.0001	petiolaris-like
Leaf dry mass per area (mg/cm <sup>2</sup> )	4.71 $\pm$ 0.19 <sup>a</sup>	3.56 $\pm$ 0.15 <sup>b</sup>	3.66 $\pm$ 0.38 <sup>b</sup>	18.14, <0.0001	petiolaris-like
Leaf wet weight per area (mg/cm <sup>2</sup> )	30.64 $\pm$ 0.52 <sup>a</sup>	41.18 $\pm$ 2.07 <sup>b</sup>	30.86 $\pm$ 0.72 <sup>a</sup>	27.32, <0.0001	pos. transgressive
Leaf succulence <sup>3</sup>	25.94 $\pm$ 0.43 <sup>a</sup>	37.62 $\pm$ 1.98 <sup>b</sup>	27.21 $\pm$ 0.69 <sup>a</sup>	34.62, <0.0001	pos. transgressive
Stem diameter (mm)	15.74 $\pm$ 0.58 <sup>a</sup>	5.89 $\pm$ 0.70 <sup>b</sup>	10.13 $\pm$ 0.95 <sup>c</sup>	34.32, <0.0001	neg. transgressive
Distance from ground to first branch (cm)	37.31 $\pm$ 2.12 <sup>a</sup>	23.2 $\pm$ 2.75 <sup>b</sup>	21.93 $\pm$ 2.39 <sup>b</sup>	12.63, 0.0001	petiolaris-like
Achene length (mm)	4.62 $\pm$ 0.09 <sup>a</sup>	6.59 $\pm$ 0.20 <sup>b</sup>	3.99 $\pm$ 0.05 <sup>c</sup>	143.29, <0.0001	pos. transgressive
Achene width (mm)	2.85 $\pm$ 0.04 <sup>a</sup>	1.71 $\pm$ 0.05 <sup>b</sup>	1.80 $\pm$ 0.03 <sup>b</sup>	280.40, <0.0001	petiolaris-like
Achene thickness (mm)	1.74 $\pm$ 0.05 <sup>a</sup>	1.50 $\pm$ 0.04 <sup>b</sup>	1.04 $\pm$ 0.02 <sup>c</sup>	93.82, <0.0001	intermediate
Achene shape (width/thickness)	1.65 $\pm$ 0.05 <sup>a</sup>	1.14 $\pm$ 0.07 <sup>b</sup>	1.74 $\pm$ 0.22 <sup>a</sup>	37.15, <0.0001	neg. transgressive
Number of achenes per head	102.92 $\pm$ 14.67 <sup>a</sup>	11.50 $\pm$ 3.23 <sup>b</sup>	57.93 $\pm$ 8.73 <sup>c</sup>	16.45, <0.0001	neg. transgressive
Achene mass (mg)	6.10 $\pm$ 0.77 <sup>a</sup>	7.62 $\pm$ 0.54 <sup>a</sup>	2.66 $\pm$ 0.20 <sup>b</sup>	22.70, <0.0001	annuus-like
Seed set (seeds/number of head flowers)	0.31 $\pm$ 0.04 <sup>a</sup>	0.17 $\pm$ 0.06 <sup>b</sup>	0.36 $\pm$ 0.04 <sup>a</sup>	3.76, 0.0331	neg. transgressive
Angle of lateral roots	2.08 $\pm$ 1.46 <sup>a</sup>	18.50 $\pm$ 4.09 <sup>b</sup>	12.67 $\pm$ 2.28 <sup>b</sup>	9.75, 0.0004	petiolaris-like
Total root mass length (cm)	11.04 $\pm$ 0.58 <sup>a</sup>	6.45 $\pm$ 1.07 <sup>b</sup>	7.93 $\pm$ 0.45 <sup>b</sup>	11.51, 0.0001	petiolaris-like
Total root mass width (cm)	20.15 $\pm$ 0.82 <sup>a</sup>	5.05 $\pm$ 1.33 <sup>b</sup>	11.10 $\pm$ 0.51 <sup>c</sup>	73.58, <0.0001	neg. transgressive
Volume of soil explored by roots (cm <sup>3</sup> )	224.0 $\pm$ 17.4 <sup>a</sup>	42.6 $\pm$ 15.5 <sup>b</sup>	89.8 $\pm$ 7.9 <sup>c</sup>	45.63, <0.0001	neg. transgressive
Taproot length (cm)	3.08 $\pm$ 0.79 <sup>a</sup>	1.95 $\pm$ 0.68 <sup>a</sup>	2.43 $\pm$ 0.40 <sup>a</sup>	0.75, 0.4810	n.s.

<sup>1</sup> P values in boldface indicate significant differences among taxa, whereas letters after means indicate significant differences in means as estimated with the LSD multiple range test.

<sup>2</sup> Ligule shape of *H. anomalus* is significantly different from *H. annuus*, but not from *H. petiolaris*; *H. petiolaris* is not different from both *H. anomalus* and *H. annuus*.

<sup>3</sup> Calculated as wet mass per area minus dry mass per area.

## DISCUSSION

### *Morphological and ecophysiological character expression—*

As alluded to in the introduction to this paper, there are several possible explanations for the ability of a hybrid species to colonize a habitat different from that preferred by either parental species. These include the possibility that (1) the new habitat is intermediate between that of the parental species in terms of critical morphological and ecophysiological parameters and thus favorable to intermediate hybrids, (2) that hybridization has created new combinations of parental traits and/or (3) extreme phenotypes that facilitate adaptation to novel or extreme habitats, or (4) that habitat divergence has evolved gradually via the fixation of favorable mutations. Therefore, one major question to be answered by this paper was whether morphological and ecophysiological traits displayed by the hybrid species *H. anomalus* are intermediate, parental-like, or extreme when compared to its parents. The answer is clear. *Helianthus*

*anomalus* is a mosaic of parental-like and transgressive phenotypes, of which a considerable proportion is transgressive. However, our data cannot discriminate between hybridization and mutational divergence as explanations for the origin of the extreme traits.

The proportion of transgressive traits reported for *H. anomalus* (~38% for morphological and ecophysiological traits combined) is considerably higher than the average reported for nine hybrid species (16%) in a recent review of morphological character expression in hybrids (Rieseberg and Ellstrand, 1993). Likewise, a higher proportion of characters in *H. anomalus* are parental-like (~60%) than that generally reported for hybrid species (48.7%). In contrast, most other hybrid species express a larger proportion of intermediate phenotypes (35.3%) than reported here for *H. anomalus* (~2%). One possible reason for this difference is that many of the "hybrid species" included in the Rieseberg and Ellstrand (1993) review were experimentally synthesized; perhaps natural selec-

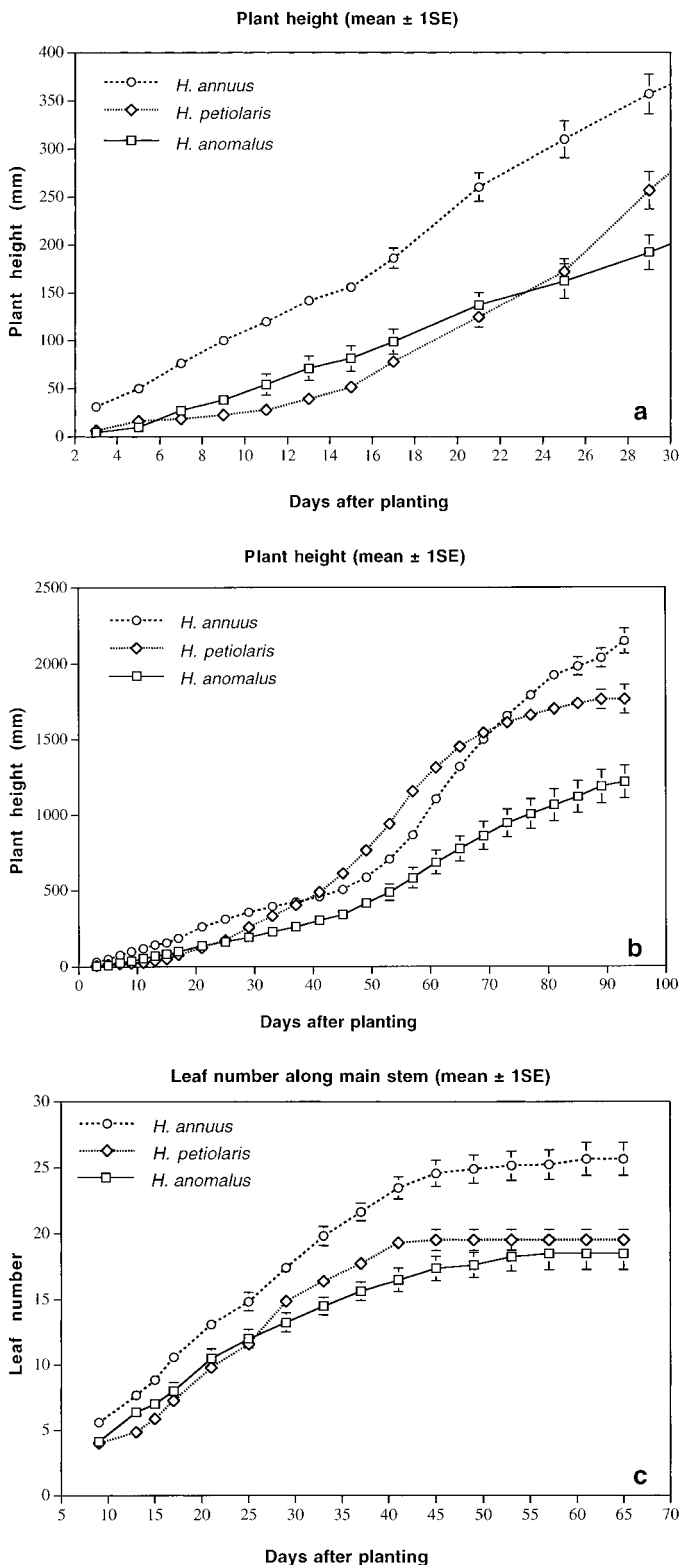


Fig. 3. (a) Growth curves (mean ± 1 SE) measured as plant height for the three species during the first 30 d after planting, (b) growth curves measured as plant height for the entire season, and (c) growth curves measured as leaf number along the main stem.

tion is most likely to favor the establishment of only the most divergent hybrid phenotypes. Clearly, more data for naturally occurring hybrid species will be needed to decide whether this is a general outcome of hybrid speciation.

One interesting aspect of our study concerns the fact that more morphological/ecophysiological characters are *H. petiolaris*-like and likewise most transgressive characters exceed the values for *H. petiolaris* and not *H. annuus*. This is even more remarkable since the genetic composition of the hybrid species does not differ significantly from a ~1:1 ratio of *H. petiolaris* and *H. annuus* genetic material (Ungerer et al., 1998). Possibly, ecological selection has driven *H. anomalus* in the direction of *H. petiolaris* with respect to morphological and ecophysiological traits (*H. anomalus* is most similar to *H. petiolaris* with respect to habitat preference), whereas fertility selection may have favored genetic material from *H. annuus* (Rieseberg et al., 1996).

**Ecological interpretation of traits**—A second major question is whether the transgressive characters as well as the new parental-like character combinations displayed by *H. anomalus* can be considered adaptations to sand dune habitats. Below we provide arguments in favor of adaptive function for many of these characters or character combinations, fully realizing that in the absence of comparative fitness data for different habitat/trait combinations these arguments are speculative. Nonetheless, by using the available literature on sand dune adaptation to interpret the morphological and ecophysiological characteristics of *H. anomalus*, we believe that some valuable, albeit preliminary, conclusions can be made.

**Morphological traits**—*Helianthus anomalus* is most similar to *H. petiolaris* in terms of leaf characters (Table 1). However, *H. anomalus* leaves have a tendency toward succulence and store much more water per leaf area than both parental species (Table 1). Increased leaf thickness previously has been associated with an increased vigor of buried plant parts in a dune grass species (Yuan, Maun, and Hopkins, 1993). *Helianthus anomalus* often can be found partially buried in its natural habitat. The succulent leaves might be able to better withstand sand abrasions as was shown for the grass species, although this remains to be tested. *Helianthus anomalus* also maintains a larger temperature difference between air and leaf than its parents. Thus it appears to be able to more effectively keep its leaves cool, possibly avoiding the high sand and air temperatures characteristic of sand dunes (Hegazy and El Amry, 1998).

The hybrid species has some of the largest achenes of all wild annual sunflower species (Heiser et al., 1969) and large size correlated with large internal nutrient storage can facilitate rapid early root growth (Wulff, 1986; Chen and Maun, 1999) and the tapping of water reservoirs. Dittmer (1959) comparatively studied the root system of 13 sand dune plants and found that most have dominant taproots and few lateral roots. Our crude root data indicate that *H. anomalus* had fewer lateral roots (measured as volume of soil explored by roots) than its parents, but differences in taproot length were not significant (Table 1). However, in wild populations *H. anomalus* does tend to have a more well-developed taproot and fewer lateral roots than the parental species (Schwarzbach, personal observation).

In regard to dispersal strategies dune plants behave largely like island endemics and often exhibit adaptations that reduce

TABLE 2. Ecophysiological trait expression in *H. annuus*, *H. petiolaris*, and *H. anomalus* (mean  $\pm$  1 SE)<sup>1</sup>.

Trait	<i>H. annuus</i>	<i>H. anomalus</i>	<i>H. petiolaris</i>	F, P for taxa	Hybrid phenotype
Ecophysiology under well-watered conditions					
Photosynthesis ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	35.9 $\pm$ 0.7 <sup>a</sup>	34.9 $\pm$ 1.3 <sup>a</sup>	37.0 $\pm$ 0.7 <sup>a</sup>	1.22, 0.309	n.s.
Stomatal conductance ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	1.66 $\pm$ 0.07 <sup>a</sup>	1.69 $\pm$ 0.19 <sup>a</sup>	1.62 $\pm$ 0.07 <sup>a</sup>	0.23, 0.794	n.s.
Cuticular conductance ( $\text{mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ )	0.045 $\pm$ 0.006 <sup>a</sup>	0.161 $\pm$ 0.027 <sup>b</sup>	0.051 $\pm$ 0.006 <sup>a</sup>	18.2, <b>0.001</b>	pos. transgressive
Internal CO <sub>2</sub> concentration (ppm)	245 $\pm$ 2 <sup>b</sup>	253 $\pm$ 4 <sup>a</sup>	244 $\pm$ 2 <sup>b</sup>	3.69, <b>0.036</b>	pos. transgressive
Air-leaf temperature ( $^{\circ}\text{C}$ )	1.53 $\pm$ 0.08 <sup>b</sup>	1.83 $\pm$ 0.10 <sup>a</sup>	1.44 $\pm$ 0.09 <sup>b</sup>	4.26, <b>0.023</b>	pos. transgressive
Nitrogen (%)	4.78 $\pm$ 0.58 <sup>a</sup>	5.62 $\pm$ 0.42 <sup>a</sup>	5.00 $\pm$ 0.23 <sup>a</sup>	0.76, 0.475	n.s.
Carbon isotope ratio (ppt)	-30.26 $\pm$ 0.20 <sup>a</sup>	-32.11 $\pm$ 0.28 <sup>b</sup>	-31.59 $\pm$ 0.22 <sup>b</sup>	15.4, <b>0.001</b>	petiolaris-like
PNUE ( $\mu\text{mol CO}_2\cdot\text{mol N}^{-1}\cdot\text{s}^{-1}$ )	288.6 $\pm$ 58.1 <sup>a</sup>	279.8 $\pm$ 39.2 <sup>a</sup>	304.9 $\pm$ 22.3 <sup>a</sup>	0.06, 0.944	n.s.
Ecophysiology at leaf wilting point					
Water potential (MPa)	-1.15 $\pm$ 0.06 <sup>a</sup>	-0.99 $\pm$ 0.06 <sup>b</sup>	-0.81 $\pm$ 0.02 <sup>b</sup>	11.8, <b>0.001</b>	petiolaris-like
Photosynthesis ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	20.3 $\pm$ 3.5 <sup>a</sup>	8.7 $\pm$ 1.7 <sup>b</sup>	11.3 $\pm$ 2.7 <sup>b</sup>	5.02, <b>0.019</b>	petiolaris-like
Stomatal conductance ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	0.42 $\pm$ 0.14 <sup>a</sup>	0.07 $\pm$ 0.02 <sup>b</sup>	0.11 $\pm$ 0.04 <sup>b</sup>	4.95, <b>0.019</b>	petiolaris-like
Internal CO <sub>2</sub> concentration (ppm)	187 $\pm$ 21 <sup>a</sup>	119 $\pm$ 17 <sup>b</sup>	108 $\pm$ 26 <sup>b</sup>	4.04, <b>0.037</b>	petiolaris-like

<sup>1</sup> P values in boldface indicate significant differences among taxa, whereas letters after means indicate significant differences in means as estimated with the LSD multiple range test.

the possibility of long-distance dispersal. The relatively high rate of endemism on active dunes suggests that dune endemics are likely to be propagated by dispersal units that are not easily blown off the dunes, but remain in the habitat to which they are best adapted (Bowers, 1982; Keddy and Keddy, 1984). The dispersal units typically are relatively large and heavy (Bowers, 1996) and often have a shape that allows them to roll on the sand or be dragged by the wind over small distances (Darin, 1991). Consistent with these predictions, *H. anomalus* has heavy seeds that are nearly cylindrical in shape with an almost round cross section, which may allow them to roll on the sand. In contrast, both parents have flattened achenes (achene shape; Table 1). This adaptive interpretation of seed size and shape is also supported by the fact that the only other annual *Helianthus* species on dunes, *H. niveus* subsp. *tephrodes* (Fig. 2), has achenes that approach those of *H. anomalus* in size and shape (Heiser et al., 1969). This observation led Heiser et al. (1969) to suggest that these two taxa might be closely related. However, phylogenetic work (Fig. 2) did not support this hypothesis, and the similarities are more parsimoniously interpreted as resulting from parallel evolution.

**Ecophysiological traits**—All three southwestern annual sunflower species examined in this study share characteristics common to many annual species in arid environments: high photosynthetic rates, relatively high stomatal conductances, and adaptations for optimal regulation of their energy budget (Toft and Percy, 1982; Gibson, 1998). Although *H. anomalus* has often been described as a xerically adapted species that might exceed the drought tolerance performance of its parents (e.g., Thompson, Zimmerman, and Rogers, 1981), this description presents two challenges. First, tolerance to drought is a complex trait, making it difficult to decipher which traits may be contributing to greater avoidance or tolerance of water stress (Blum, 1996). Second, water limitation may not be the major limiting factor for sand dune habitats where *H. anomalus* is found. We will discuss each of these factors in turn.

Plant traits involved in adaptation to water limitations can be related to either drought avoidance or drought tolerance at less negative or more negative water potentials (Blum, 1996; Nilsen and Orcutt, 1996; Gibson, 1998). Given the available data on *Helianthus* (wild and cultivated) and other desert and sand dune annuals, it is likely that greater drought tolerance

of *H. anomalus* would fall into the category of drought tolerance at less negative water potentials and involve traits that reduce water loss or enhance water accumulation (Pavlik, 1980; Sobrado and Turner, 1983a, c, 1986; Gibson, 1998; García-Mora, Gallego-Fernandez, and García-Novo, 1999). One trait related to water loss is WUE (photosynthetic carbon gain per unit transpirational water loss), which is under strong genetic control (Guy, Warne, and Reid, 1989; Martin et al., 1989; Donovan and Ehleringer, 1994; Handley et al., 1997). Plants adapted to prolonged water limitations generally have a higher or more conservative WUE (Nilsen and Orcutt, 1996; Gibson, 1998). Estimating WUE at two different time scales, the species ranked as *H. anomalus* < *H. petiolaris* = *H. annuus* for instantaneous WUE, and *H. anomalus* = *H. petiolaris* < *H. annuus* for leaf-lifetime integrated WUE. Thus, under well-watered conditions, *H. anomalus* has a less conservative water-use strategy that is inconsistent with greater tolerance of prolonged water limitation.

Low leaf cuticular conductance is also thought to be an adaptation to more xeric habitats because it minimizes water loss through the cuticle when stomata are closed (Quisenberry, Roark, and McMichael, 1982; Hamerlynck and Knapp, 1996). However, *H. anomalus* had a cuticular conductance much higher than that of either parental species (Table 2).

The ability to close stomata and conserve water in response to declining leaf water potentials may prolong survival through short-term drought episodes (Pezeshki and Chambers, 1986; Sparks and Black, 1999). When exposed to severe short-term water limitation, as indicated by reaching wilting point, *H. anomalus* and *H. petiolaris* closed their stomata to a much greater extent than *H. annuus* (Table 2), potentially indicating a greater ability to tolerate short-term water deficits likely associated with sandy habitats. However, *H. anomalus* did not exceed *H. petiolaris* in this capacity. Thus, the water loss characters examined to date fail to support the suggestion that *H. anomalus* is more drought tolerant than its parental species.

Although xeric in appearance, dune habitats may not be more xeric than the habitats of the parental species as has been suggested previously. Water is usually not the limiting factor for growth in dunes if the plants can manage to tap below-ground water resources. The ecological literature suggests that the primary environmental factors limiting plant growth and existence on dunes are moving sand and low nutrient levels

(Pemadasa and Lovell, 1975; Pavlik, 1980; Bowers, 1982; Moreno-Casasola, 1986; Danin, 1991; Maun, 1998). This is a paradoxical situation since the fast growth would be advantageous for outgrowing the disturbance of sand burial, whereas slow growth is more often advantageous in nutrient-limited habitats (Grime, 1977; Chapin, 1991; Chapin, Autumn, and Pugnaire, 1993). We found that under well-watered high-nutrient conditions, *H. anomalous* grew more slowly and was shorter than the parental species (Fig. 3) even though it did not differ from its parents for A, g<sub>s</sub>, PNUE, and leaf nitrogen content (Table 2). These growth data are consistent with the average field heights of these species, *H. annuus* > *H. petiolaris* > *H. anomalous* (Heiser et al., 1969; Thompson, Zimmerman, and Rogers, 1981). We are currently exploring the hypothesis that the inherently slower growth rate of *H. anomalous* is associated with greater ability to tolerate low-nutrient conditions (Grime, 1977; Chapin, 1991; Chapin, Autumn, and Pugnaire, 1993).

**Conclusions**—We were able to identify many characters that were transgressive for *H. anomalous* and that may have allowed this hybrid species to quickly adapt to its new and unique habitat. Surprisingly, the bulk of these differences were found for morphological characters rather than ecophysiological characters. However, measurements in the natural habitat of these taxa are required to verify our greenhouse results. For example, soil nutrient composition and carbon isotope ratios for wild populations are currently under study. Additionally, a greenhouse experiment that includes stress factors other than water stress will be conducted in the near future.

Furthermore, we found that a larger number of parental-like and transgressive characters are *petiolaris*-like or are exceeding values for *petiolaris*, although the genomic composition for *H. anomalous* does not significantly differ from a 1:1 ratio of *H. annuus* and *H. petiolaris* genome parts (Ungerer et al., 1998). As described above, we believe that the selection for morphological and ecophysiological characters is possibly largely driven by ecological habitat requirements, whereas genomic composition is a result of a combination of ecological and fertility selection.

We are currently mapping quantitative trait loci (QTLs) for a large number of the ecophysiological and morphological traits that were included in this comparative study and hope to get some insights about the genetic bases of transgressive characters in this species. We also plan to examine experimental hybrids between *H. annuus* and *H. petiolaris* to determine whether they exhibit transgression for the same traits that are transgressive in *H. anomalous*. This comparison will allow us to distinguish between the possibility that some differences between the hybrid and its parents stem from selection acting on variation generated by transgressive segregation vs. variation generated by mutation since the origin of *H. anomalous*. Additionally, these artificial hybrids will be raised in the natural habitat of *H. anomalous* to determine the selective value of individual QTLs and transgressive genotypes. Then the genomic composition of the most fit artificial hybrid could be compared with the natural hybrid, and we shall determine whether the most transgressive phenotypes for a specific trait provide the greatest selective advantage. Although we are currently not able to predict the outcome of these projected studies, the experiments mentioned do represent an important step toward understanding the possible role of transgressive segregation in adaptation and speciation.

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