

PHENOTYPIC DIFFERENTIATION BETWEEN THREE ANCIENT HYBRID TAXA AND THEIR PARENTAL SPECIES

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The primary requirement for a new diploid species to arise via hybridization is ecological divergence from its parental species. Ecological divergence protects the nascent hybrid species from competition with its progenitor species and may contribute to reproductive isolation. However, the means by which hybridization might facilitate the necessary adaptive transitions are poorly understood. Here, we report the results of a glasshouse experiment in which 42 morphological and ecophysiological traits were measured in three hybrid sunflower species (*Helianthus anomalus*, *Helianthus deserticola*, and *Helianthus paradoxus*) and their parental species (*Helianthus annuus* and *Helianthus petiolaris*). A surprisingly high proportion of traits were extreme relative to the parental species (24%, 20%, and 39% of traits in *H. anomalus*, *H. deserticola*, and *H. paradoxus*, respectively). Most of the extreme traits have previously been reported in the literature as adaptations to dune (*H. anomalus*), high-desert (*H. deserticola*), or high-salt (*H. paradoxus*) habitats. We propose that hybridization has contributed to ecological divergence largely via the generation of extreme traits in segregating hybrids, a commonly observed phenomenon called “transgressive segregation.”

Keywords: adaptation, ecological divergence, *Helianthus*, hybridization, photosynthesis, speciation, sunflower, transgressive segregation.

Introduction

There are two primary mechanisms by which new species may arise via hybridization: allopolyploidy and diploid, or “homoploid,” hybrid speciation (Grant 1981). Allopolyploid speciation is theoretically straightforward because reproductive isolation arises as an instantaneous by-product of chromosomal doubling. In contrast, there is no universal mechanism by which new homoploid hybrid derivatives become reproductively isolated from their parental species. Computer simulations of the process indicate that reproductive isolation of the incipient homoploid hybrid species may arise through ecological divergence, spatial isolation, and/or the fixation of a recombinant karyotype (McCarthy et al. 1995; Buerkle et al. 2000). Karyotypic divergence is well documented for several natural hybrid species (Randolph 1966; Gallez and Gottlieb 1982; Rieseberg et al. 1995b; Hirai et al. 2000), and at least some degree of spatial isolation is a consistent feature of this process (Rieseberg 1991; Arnold 1993; Wang and Szmidt 1994; Sang et al. 1995; Taylor et al. 1996; Allan et al. 1997; Wolfe et al. 1998; Brochmann et al. 2000; Maki and Murata 2001). However, evidence of ecological isolation derives largely from ad hoc observations that hybrid species occur in habitats that differ from those of their parental species (although see Schwarzbach et al. 2001; Welch and Rieseberg 2002).

For ecological differences to contribute to hybrid speciation, they must arise very early in the speciation process (Buerkle

et al. 2000), presumably as a consequence of hybridization itself. Because hybrids combine alleles from their parental species, they traditionally are assumed to be phenotypically intermediate. However, this need not be the case. Extreme phenotypes are frequently reported in segregating hybrid populations from controlled crosses (Rieseberg et al. 1999a). Such transgressive segregation might facilitate the acquisition of ecological isolation and can have a variety of genetic causes (de Vicente and Tanksley 1993). Most frequently, transgression appears to arise from the complementary action of genes that are dispersed between the parental species (Rieseberg et al. 1999a), although overdominance (de Vicente and Tanksley 1993) and epistasis (Monforte et al. 1997) also contribute.

The production of transgressive phenotypes provides a straightforward explanation for the ecological divergence of hybrid lineages, but other explanations are possible as well. For example, a combination of parental traits might lead to novel ecological preferences, even if none of the traits are individually transgressive (Johnston et al. 2001). This appears to have happened in *Iris* (Burke et al. 2000), in which the homoploid hybrid species *Iris nelsonii* (shade/freshwater) appears to be recombinant for ecological tolerances that differentiate its parental species (shade/saltwater and sun/freshwater). Alternatively, it is possible that a given hybrid habitat is truly intermediate and requires no special genetic explanation for its colonization by hybrids.

All of the above explanations assume that hybridization had a causative role in niche divergence. However, it may be that the ecological differences characteristic of most hybrid species arose through mutational divergence and were incidental to hybrid origin (the null hypothesis).

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The annual sunflowers of the genus *Helianthus* are ideal for studies of the ecological divergence of hybrid lineages specifically and of homoploid hybrid speciation more generally. Molecular phylogenetic studies have identified three species in this group (*Helianthus anomalus*, *Helianthus deserticola*, and *Helianthus paradoxus*) that are stabilized diploid hybrid derivatives of two widespread species, *Helianthus annuus* and *Helianthus petiolaris* (Rieseberg et al. 1990; Rieseberg 1991). The hybrid species appear to have been derived independently from the parental species, providing a replicated natural hybrid speciation experiment. Moreover, the three hybrid species occur in what appear to be the three most extreme habitats that annual sunflowers occupy: *H. anomalus* occurs on sand dunes, *H. deserticola* occurs on the desert floor, and *H. paradoxus* occurs in brackish, saline marshes (Heiser et al. 1969). We are attempting to exploit this unique situation to understand how hybridization might facilitate major adaptive transitions.

The specific purpose of this study is to provide a detailed assessment of morphological and ecophysiological differences among the three hybrid sunflower species and their parental species. Differences are interpreted with respect to the habitats in which the species are found, although we recognize that these interpretations must necessarily be preliminary in the absence of experimental evidence demonstrating the adaptive value of particular trait differences.

Material and Methods

Plants

The five *Helianthus* species analyzed are diploid ($n=17$), self-incompatible annuals, all native to North America. *Helianthus annuus* and *Helianthus petiolaris*, the parental species, are widespread, polytypic taxa with similar geographic distributions. Both are abundant in the central and western United States and are sporadic eastward. *Helianthus annuus* is found in clay-based mesic soils, whereas *H. petiolaris* occurs in drier, sandier soils. Hybridization occurs when these two habitats are juxtaposed, which is frequent, but the two species retain their genetic integrity because of the synergistic action of several reproductive barriers (Rieseberg et al. 1995a, 1999b; Schwarzbach et al. 2001). Schemske (2000) claims that hybridization is unlikely to have played a significant role in the evolution of *Helianthus* because many present-day hybrid swarms are associated with human disturbance. However, hybrid swarms often occur in naturally disturbed habitats such as floodplains or buffalo wallows (L. H. Rieseberg, unpublished observation). Moreover, buffalo, which appear to have been the primary dispersal agent for annual sunflowers (Asch 1993), likely created ample disturbed habitat for hybrid formation long before humans colonized North America. Finally, detection of several unique mutations in each of the hybrid species (Rieseberg et al. 1990, 1991; Rieseberg 1991; Schilling et al. 1998; Ungerer et al. 1998) implies both a prehistoric origin of the hybrid species and ancient hybridization between their parents.

The three hybrid taxa have much narrower geographic distributions than their parental species (Heiser et al. 1969). *Helianthus anomalus* is known only from a few sand dune habitats in northern Arizona and Utah. *Helianthus deserticola*

occurs on the floor of the Great Basin Desert but has only been reported from a small number of localities in Nevada, Utah, and northern Arizona. Likewise, *Helianthus paradoxus* is restricted to several brackish marshes in western Texas and New Mexico.

Achenes (one-seeded fruits) were collected from one population of each species: *H. annuus* (ANN 1295; Highway 24, one-fourth mile north of Hanksville, Utah), *H. anomalus* (ANO 1282; Highway 160, 6 mi east of Mexican Water, Ariz.), *H. deserticola* (DES 1270; Frontage Road, 6 mi east of Toquerville, Utah), *H. paradoxus* (PAR 1370; south side of Interstate 40, one-fourth mile east of Exit 85, Grants, N.Mex.), and *H. petiolaris* (PET 1277; Highway 89, boundary of Glenn Canyon Recreation Area, Kane County, Utah). For each population, achenes were derived from 30–100 maternal plants collected along a transect at irregular intervals. Achenes from all heads from a given population were pooled, and a subset was arbitrarily chosen for propagation at the University of Georgia greenhouses. We felt that the analysis of a single population per species was justified because most genetic and morphological variation is found within rather than among populations of annual sunflower species (Heiser 1954, 1961; Rieseberg et al. 1990, 1991).

Achenes were germinated following the protocol of Schwarzbach et al. (2001), and 20 seedlings from each population were planted in 25-cm-diameter plastic pots containing a 3 : 1 mixture of sand/fritted clay (Turface, Profile Products, Buffalo Grove, Ill.). This mixture does not contain any nutrients or NaCl, which allowed us to add them in controlled quantities. Pots were arrayed in a randomized block design in the University of Georgia greenhouses, with 10 blocks and two individuals of each species per block. Each block also included 40 individuals from a BC₂ population between *H. annuus* and *H. petiolaris*, which is the focus of an ongoing quantitative trait locus (QTL) study. Plants were watered twice each day and fertilized with a time-release microencapsulated complete fertilizer (Osmocote Plus, Scotts-Sierra Horticultural Products, Marysville, Ohio). In addition, 8 d after planting, plants were treated with 5.0 mM NaCl twice weekly for 3 wk because previous studies indicated that *H. paradoxus* responds to salt stress by accumulating sodium in its leaves (Welch and Rieseberg 2002). The very dilute NaCl treatment employed was high enough to ensure detection of this response but low enough so that the remaining species were unaffected. Some plants died early in the experiment and were excluded from analyses. In all, 20 individuals of *H. annuus*, 11 of *H. anomalus*, 16 of *H. deserticola*, 18 of *H. paradoxus*, and 17 of *H. petiolaris* were analyzed.

Morphological and Ecophysiological Measurements

Twenty-four morphological traits were measured (table 1), including seed and seedling traits, vegetative and floral traits, and traits relating to plant architecture and/or timing of developmental stages. The term “phyllaries” refers to the leaflike bracts that underlie flowering heads, and ligules are the single florets found on ray flowers. Flower characters were measured on flowers where $\geq 75\%$ of the florets were mature. Leaf characters (leaf area, leaf shape, petiole length, leaf succulence) were measured on a fully expanded nonsenescent leaf from

Table 1
Traits Measured in Five Annual Sunflower Species

Trait	Units	Abbreviation
Morphological traits:		
Average leaf toughness	Relative scale	LFTOUG
Branch number at harvest	(Count)	BRNUM
Days until budding	d	BUDDAY
Days until first floret	d	FLODAY
Disk diameter	mm	DISKDIA
Flower number at 65 d after planting	(Count)	FLRNUM
Height 7 d after planting (week 1)	cm	HGT1
Height at final harvest	cm	HARHGT
Height at first branch	cm	BRHGT
Hypocotyl length at 96 h	mm	HYPOLG
Initial seed weight	mg	SEEDW
Leaf area	cm ²	LFAREA
Leaf shape (length/width)	mm/mm	LSHAP
Leaf succulence ([wet wt – dry wt]/leaf area)	mg/cm ²	LSUC
Ligule length	mm	LIGLGTH
Ligule width	mm	LIGWDTH
Number of ligules (ray flowers)	(Count)	LIGNUM
Petiole length	mm	PETLEN
Phyllary number	(Count)	PHYNUM
Phyllary shape (length/width)	mm/mm	PHYSHAP
Specific leaf area	cm ² /g	SLA
Stem diameter at 2 cm above ground	mm	STEMDIA
Relative growth rate ([height at week 1 – height at week 5]/d)	cm/d	RGR
Relative root hair density (1 = not dense; 5 = very dense)	Relative scale	RTHAIR
Ecophysiological traits:		
Leaf boron concentration	ppm	B
Leaf calcium concentration	ppm	Ca
Leaf carbon (g C/g leaf)	g/g	LFC
Leaf carbon isotope ratio	ppt	DEL13C
Leaf carbon/nitrogen ratio	g/g	CNRAT
Leaf intercellular CO ₂ concentration	ppm	CI
Leaf magnesium concentration	ppm	Mg
Leaf manganese concentration	ppm	Mn
Leaf nitrogen isotope ratio	ppt	DEL15N
Leaf nitrogen per unit area	mmol/m ²	LFNAREA
Leaf nitrogen per unit leaf mass (g N/g leaf)	g/g	LFNG
Leaf nitrogen per unit leaf mass (mmol N/g leaf)	mmol/g	LFNMOL
Leaf phosphorus concentration	ppm	P
Leaf photosynthesis	μmol/m ² /s	PHOTO
Leaf potassium concentration	ppm	K
Leaf sodium concentration	ppm	Na
Leaf stomatal conductance	mmol/m ² /s	COND
Photosynthetic nitrogen use efficiency	μmol CO ₂ /mol N/s	PNUE

each individual. Leaf area was measured using a LI-COR 3100 area meter (LI-COR, Lincoln, Nebr.), and average leaf toughness was estimated with a push dynamometer (McCormick Fruit Tech, Yakima, Wash.).

Measurements were also taken to detect possible differences between these species in ecophysiological traits, such as photosynthetic rate, water and nitrogen use efficiency, and nutrient status. Over a 2-wk period beginning 47 d after planting, we measured the photosynthetic rate, stomatal conductance, and internal CO₂ concentration of a single mature leaf from each plant using a LI-COR 6400 portable photosynthesis system (CO₂ concentration = 360 ppm; air temperature = 26°C; photosynthetically active radiation = 1500 μmol m⁻² s⁻¹). Pre-

vious measurements indicated that *Helianthus* did not exhibit midday stomatal closure (Schwarzbach et al. 2001); therefore, all measurements were taken between 10:30 A.M. and 4:30 P.M. on clear sunny days. As in Schwarzbach et al. (2001), internal CO₂ concentration was employed as an estimate of instantaneous water use efficiency (WUE) (Farquhar et al. 1989; Donovan and Ehleringer 1994). Efficiency over the entire growing season (integrated WUE) was determined from stable carbon isotope ratios (δ¹³C) of dried leaf material (continuous flow mass spectrometry; University of Georgia Stable Isotope Soil Biology Laboratory), where more negative δ¹³C values are indicative of reduced integrative WUE.

To detect differences in nutrient status and use, a mature

leaf was air dried, and one portion was analyzed for percentage of N and C by Dumas combustion with a Carlo Erba NA 1500 elemental analyzer (Milan, Italy). Photosynthetic nitrogen use efficiency (NUE) was calculated as the ratio of assimilation and N content per unit area of leaf (Field et al. 1983; Donovan et al. 1996). Acid extracts (Sah and Miller 1992) from leaf tissue collected the day after the final 5 mM NaCl applications were analyzed for B, Ca, K, Mg, Mn, Na, and P on an inductively coupled plasma-atomic emission spectrophotometer.

Data Analysis

Traits were analyzed using three methods. First, the overall phenotypic similarity among individuals and species across all 42 traits was graphically assessed by principal components analysis (PCA) of character \times character correlations (Sokal and Rohlf 1995). Traits were standardized (but not transformed) before analysis by dividing the centered variable by its standard deviation to ensure that all variables contributed equally to the analysis. The PCA also allowed us to appraise the extent of phenotypic correlations among the traits analyzed. Second, two-way ANOVA were used to test for effects of "species" and "block" on each of 41 traits (root-hair length was not tested because variation was categorical rather than continuous). Third, Kruskal-Wallis comparisons were made for each trait to provide a nonparametric test of species differences. A significance level of 5% was employed for the latter two approaches. Because multiple comparisons were made, least significant difference methods (Sokal and Rohlf 1995) were employed to identify significant pairwise combinations. All significance levels were corrected using the sequential Bonferroni method (Rice 1989).

Results

Principal Components Analysis

PCA of the 42 morphological and ecophysiological traits easily differentiated three of the five species under investigation (figs. 1, 2). However, there was some overlap between *Helianthus petiolaris* and *Helianthus deserticola* for factors 1 and 2, which account for 44.4% of the total phenotypic variation (this is a typical value when many traits are analyzed). Factor 3 (10.1% of phenotypic variation) provided fairly good separation between the two species, but it was not complete. The lack of complete separation between *H. petiolaris* and *H. deserticola* for the first four factors (figs. 1, 2) is puzzling, given that the two species differ significantly for 14 traits (table 2). A possible explanation for this is that some traits differentiating the two species have high loadings for factors not presented graphically in figures 1 and 2. Indeed, this is the case for leaf potassium, which is loaded most heavily for factor 5. Also, it should be noted that *H. deserticola* and *H. petiolaris* are isolated by a very strong sterility barrier (first generation hybrids average 6.7% pollen viability), indicating that they are good species (Chandler et al. 1986).

Overall, factor 1 was most effective in discriminating among the five species. It is noteworthy that both *Helianthus anomalus* and *Helianthus paradoxus* are intermediate between their

parental species for this factor (fig. 1) but not for factors 2–4. Traits contributing most strongly to the separation observed for factor 1 include all three plant height traits (height at final harvest, at first branch, and 7 d after planting), seed weight, stem diameter, ligule number, disk diameter, leaf shape, specific leaf area, density of root hairs, and flower number (fig. 1).

Factor 2 served mostly to differentiate *H. paradoxus* from the other species. *Helianthus paradoxus* occurs in habitats with high soil salt concentrations and most of the traits that contribute to its separation are typical of halophytic species (fig. 1). These include leaf succulence, reduced rates of photosynthesis and plant growth, high concentrations of leaf Na, Mn, and Mg, and low concentrations of leaf K (fig. 1; table 2).

The species occurring in the most xeric habitats, *H. anomalus*, *H. deserticola*, and *H. petiolaris*, were best differentiated by factor 3 (fig. 2), primarily on the basis of seed weight (*H. anomalus* has very large seeds) and the shape of leaves and phyllaries. Factor 4 was unsuccessful at differentiating any of the species (fig. 2).

Suites of characters that differentiated each taxon tended to be fairly strongly correlated, as can be visually assessed in figures 1 and 2. For example, many of the size traits that differentiate *Helianthus annuus* from the other species in factor 1 are correlated, as are many of the traits separating *H. paradoxus* from the other taxa in factor 2. Note that these are phylogenetic correlations and thus may not have a developmental or functional basis.

To detect developmental or functional correlations, we also searched for character correlations within each species. The strongest correlations are presented in figure 3 and generally make sense from a developmental or functional standpoint. For example, in all of the species except *H. paradoxus*, stomatal conductance (COND) and internal CO₂ concentration (CI) are tightly correlated. This relationship is expected since the former largely controls the latter. Similar, very tight correlations are observed between petiole length (PETLEN) and leaf area (LFAREA), days until budding (BUDDAY) and days until flowering (FLODAY), and ligule width (LIGWIDTH) and ligule length (LIGLGTH). A developmental explanation for each of these correlations is obvious. Finally, various measures of leaf carbon and leaf nitrogen tend to be correlated in some way in each of the five species, indicating a strong coupling between nitrogen use and photosynthetic carbon acquisition.

Statistical Tests of Species Differences

Although the data were analyzed using both parametric (ANOVA) and nonparametric (Kruskal-Wallis comparisons) methods, we have chosen to present results from the Kruskal-Wallis analysis for two reasons. First, Kruskal-Wallis comparisons are robust to variation in sample sizes and to deviations from a Gaussian distribution, both of which characterized this data set, whereas ANOVA is not. Second, the results generated from the ANOVA and Kruskal-Wallis tests are essentially identical.

The parental species, *H. annuus* and *H. petiolaris*, were significantly different for 19 of the 23 morphological traits and eight of the 18 ecophysiological traits tested (table 2). Not

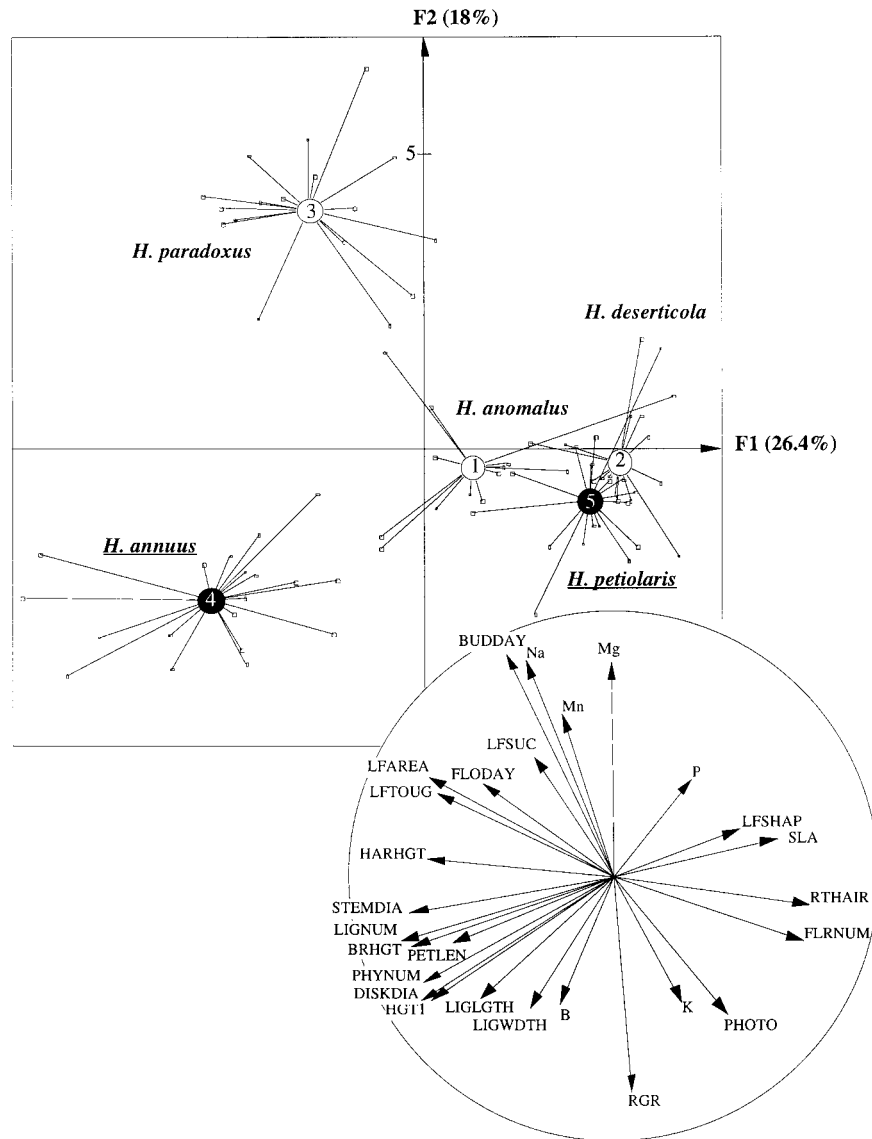


Fig. 1 Principal components analysis (PCA) of morphological and ecophysiological data for five wild sunflower species (factor 1 \times factor 2). The centroid of the phenotypic distribution of each species is indicated by a circled number (black number on white = hybrids; white number on black = parental species) to which each individual of that species is connected by lines. The circle below the PCA shows the traits that contributed to separation in each direction. Arrows closest to the edge of the circle indicate traits with the highest character loadings. Traits with arrows in the same direction and close to the edge of the circle are highly correlated. Abbreviations of traits follow table 1, whereas abbreviations for taxa follow table 2.

surprisingly, many of these traits have high loadings for factor 1, which best separates the two taxa in the PCA as discussed previously (fig. 1).

Trait differences between the three hybrid species and their parents also make sense when compared to the positions of taxa in the PCA. *Helianthus paradoxus*, for example, is intermediate between the parental species for only nine of 41 traits (22%; table 3), extreme or transgressive for 16 traits (39%), and similar to one or the other parental species for the remaining 16 traits. *Helianthus deserticola* and *H. anomalous* are less strongly differentiated from the parental species, with

a total of eight (19.5%) and 10 (24.4%) transgressive traits, respectively (table 3). The two species also are similar in terms of the number of traits they share with the parental species (26 traits for *H. deserticola* and 21 for *H. anomalous*) and the number of intermediate traits (seven and 10, respectively). As suggested by the PCA, *H. deserticola* is similar to *H. petiolaris* for a much larger number of traits (17) than for *H. annuus* (1). A similar trend is observed for *H. anomalous* and *H. paradoxus*, but the bias toward *H. petiolaris* is not as pronounced.

The total number of transgressive traits may be slightly inflated resulting from the presence of functional or develop-

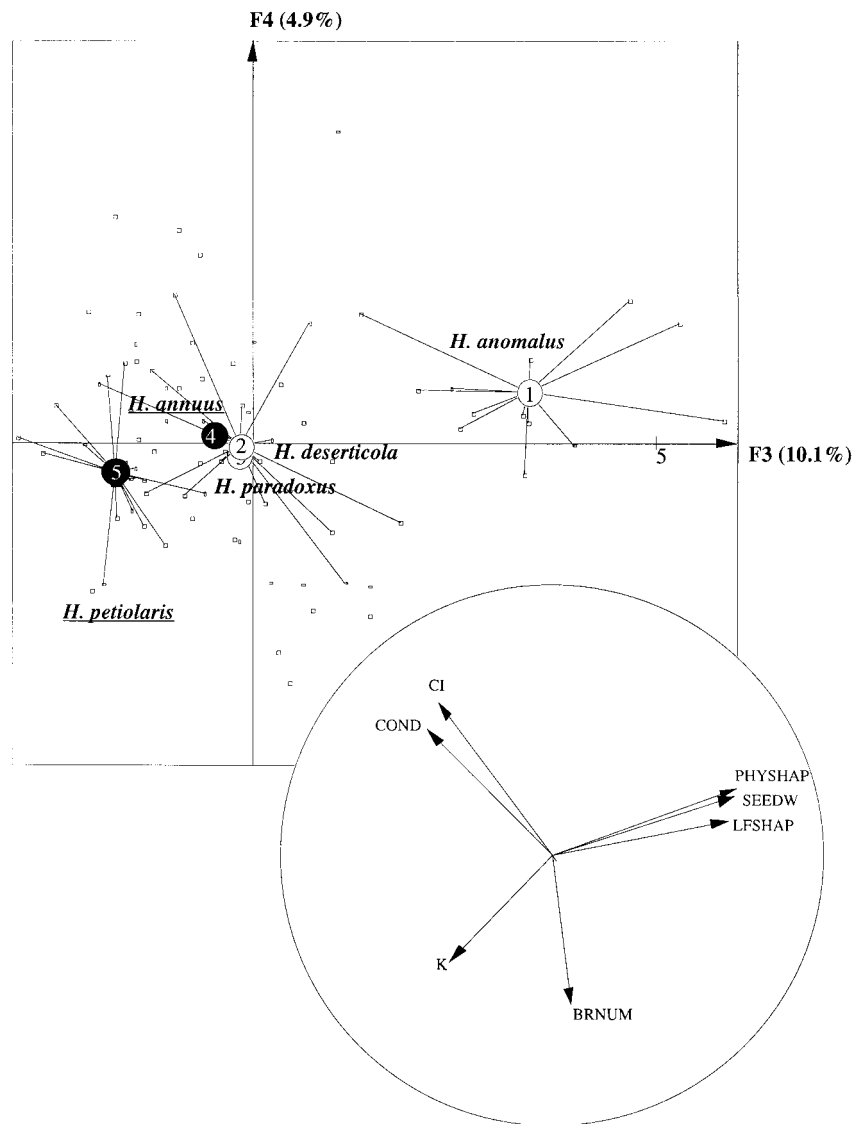


Fig. 2 Principal components analysis of morphological and ecophysiological data for five wild sunflower species (factor 3 \times factor 4). Interpretive details are as in fig. 1. Data points for *Helianthus annuus* and *Helianthus paradoxus* overlap considerably and therefore are not connected to centroids by lines.

mental correlations. For example, BUDDAY and FLODAY are highly correlated (fig. 3) and transgressive in *H. deserticola* and *H. paradoxus* (table 2). However, there are essentially no changes in the proportion of traits that are transgressive when intraspecific correlations are considered.

Discussion

Character Expression in Ancient Hybrid Species

Theory indicates that for new diploid species to arise via hybridization, they must diverge ecologically from their parental species (Templeton 1981; McCarthy et al. 1995; Buerkle et al. 2000). Otherwise, the new hybrid lineages are likely to

be overcome by competition and/or gene flow with their parental species (Wolf and Rieseberg 2001). Thus, a major prediction from theory is that hybrid neospecies will differ from their parents for traits that allow them to occupy divergent habitats. This prediction is satisfied for the hybrid species analyzed herein. Not only do they occur in unique habitats in nature, but they each have a suite of morphological and ecophysiological traits that distinguishes them from their parental species, even when grown under common garden conditions (figs. 1, 2; table 2).

Another prediction from theory is that for hybrid speciation to be successful, the requisite ecological differences must arise very early in the speciation process (Buerkle et al. 2000). Given the very short window available for ecological divergence, hy-

bridization seems more likely to serve as the immediate source of selectable genetic variation than mutation. Unfortunately, these issues cannot be directly assessed with this data. Nonetheless, we can examine the kinds of phenotypic changes that accompanied ecological divergence. Our data indicate that the hybrid sunflower species are not “intermediate” with respect to morphological and ecophysiological traits. Rather, they are mosaics of intermediate, parental-like, and extreme traits. Indeed, up to 39% of the traits assayed were significantly transgressive (table 3). This pattern of character expression implies that generation of extreme traits through transgressive segregation likely played a critical role in the speciation process, although the null hypothesis of mutational divergence cannot be ruled out.

In the future, segregating hybrid populations between the two parental species, *Helianthus annuus* and *Helianthus petiolaris*, will be studied to determine whether the extreme phenotypes characterizing the ancient hybrid taxa can be accounted for by hybridization. If so, the same segregating populations will be used to estimate the genetic basis for interspecific transgression.

Comparison with Previous Studies

This investigation largely replicates an earlier study of character expression in one of the three hybrid species, *Helianthus anomalus* (Schwarzbach et al. 2001). Although the suite of characters analyzed in the two studies is not identical, overlap exists between them, making direct comparisons possible. Overall, 38% of traits in *H. anomalus* were reported to be transgressive by Schwarzbach et al. (2001), compared to 24.4% in this study. However, the proportion of parental-like traits is similar between the two studies. To better understand the differences between the two studies, we examined character expression on a trait-by-trait basis. Only 12 of 24 traits (50%) examined in both studies were classified as the same in terms of character expression. Although the concordance between the two studies is somewhat greater than the 20% expected by chance (there are five possible categories of character expression), it does not exactly inspire confidence in the repeatability of our results. Closer scrutiny of the data, however, revealed that, in some instances, trait values actually exhibit a similar relationship among the two studies, but significance thresholds were narrowly missed in one of the studies, possibly because of differences in sample size. On the other hand, there are some very real differences between the two studies in traits such as disk diameter (DISKDIA), leaf shape (LFSHAP), and leaf CI. These differences are probably best accounted for by intraspecific variation in these traits because different populations of each species were employed in the two studies and/or by environment-dependent trait expression. The previous study was conducted in the fall with lower light and temperature conditions as compared to this study, which was conducted in the summer.

Ecological Interpretation of Trait Differences

A long-term goal of this work is to determine whether the trait differences observed among the species represent adaptations to the habitat in which they occur. However, the comparative approach we are employing cannot discriminate ad-

aptations from neutral traits or from nonadaptive traits that are by-products of developmental processes (Gould and Lewontin 1979; Arntz and Delph 2001). Nonetheless, we can search for correlations between traits and habitats and develop hypotheses that can be tested experimentally.

Helianthus anomalus. This sunflower is a true sand dune endemic, often extending further into sand dune habitat than any other species occurring in the Great Basin Desert. Dune habitats are relatively mesic compared to open sandy desert, and water is usually not the limiting factor for growth (Pavlik 1980). The environmental factors that are most limiting to plant growth and existence on dunes are moving sand and low nutrient levels, particularly inorganic nitrogen (Pavlik 1980; Bowers 1982; Moreno-Casasola 1986; Danin 1996; Maun 1998). This creates a dilemma; plants need to grow fast enough to avoid burial by shifting sand, but their growth rate is limited by low nutrient levels.

The emerging literature on sand dunes has identified a suite of traits associated with the dune habitat specifically and with desert annuals more generally. Large seeds and rapid seedling growth characterize many dune plants and are thought to represent adaptations for avoiding burial by moving sand (Danin 1991; Bowers 1996). Substantial internal nutrient storage also enables rapid early root growth for tapping water reservoirs. Once plants become established, however, there may be little advantage to continued rapid stem growth because light generally is not limiting in the dune habitat. *Helianthus anomalus* may employ this strategy because it has significantly larger seeds than either parent (Schwarzbach et al. 2001; table 2), and seed weight is tightly correlated with initial seedling growth (HYPOLG) in this species (fig. 3). However, growth rates of seedlings and established plants of *H. anomalus* did not differ significantly from its parental species (table 2).

Less is known about how plants tolerate the low nutrient conditions of the dune habitat. Some dune species can fix nitrogen, whereas others appear to increase nutrient uptake by forming associations with vesicular-arbuscular mycorrhizae (Perumal and Maun 1999). While *H. anomalus* growing in dunes likely forms mycorrhizal associations that were absent in this greenhouse study, it is not a nitrogen-fixing species. Photosynthetic nitrogen use efficiency (PNUE) in *H. anomalus* is high when compared to other desert annuals (Field and Mooney 1986), but it cannot be differentiated from its parental species for the trait (table 2). *Helianthus anomalus* does have higher leaf nitrogen than either parental species when measured on a leaf-area basis (LFNAREA).

Photosynthetic rate, stomatal conductance, and various measures of WUE in *H. anomalus* and its parental species are discussed in detail by Schwarzbach et al. (2001), so our comments are brief. Like many other dune plants (Pavlik 1980; Jiang and He 1999), the photosynthetic rate and stomatal conductance exhibited by *H. anomalus* are high but within the range of its parental species (table 2). Data on WUE are mixed. Schwarzbach et al. (2001) found *H. anomalus* to have a less conservative water use strategy than *H. annuus* whether measured in terms of instantaneous or leaf-lifetime integrated WUE. In this study, however, no differences were detected for either measure of WUE.

Helianthus deserticola. This hybrid sunflower species is native to desert regions of Nevada, Utah, and Arizona. Unlike

Table 2

Kruskal-Wallis Comparisons of 41 Morphological and Ecophysiological Traits in Five Wild Sunflower (*Helianthus*) Species

Trait	χ^2	<i>P</i>	<i>H. annuus</i> (mean [SD])	<i>H. petiolaris</i> (mean [SD])	•ANN	<i>H. anomalus</i> (mean [SD])	•ANN •PET	<i>H. deserticola</i> (mean [SD])	•ANN •PET	<i>H. paradoxus</i> (mean [SD])	•ANN •PET	
Morphological traits:												
BRHGT	44.41	0.0000	58.31 (14.75)	24.67 (10.62)	S	43.73 (Intermediate)	S S	23.44 (PET-like)	S	36.67 (ANN-like)	S S	
BRNUM	16.39	0.0025	19.28 (5.43)	13.86 (5.05)	S	16.00 (Intermediate)		13.92 (PET-like)	S	22.08 (ANN-like)	S S	
BUDDAY	50.68	0.0000	37.85 (11.11)	34.35 (6.91)		45.31 (+Transgressive)	S S	29.50 (–Transgressive)	S S	100.06 (+Transgressive)	S S	
DISKDIA	57.72	0.0000	27.48 (5.14)	16.24 (2.40)	S	13.19 (Intermediate)	S S	12.04 (Intermediate)	S S	15.24 (PET-like)	S S	
FLODAY	37.15	0.0000	59.35 (13.94)	48.71 (7.24)	S	60.19 (ANN-like)	S	42.50 (–Transgressive)	S S	106.00 (+Transgressive)	S S	
FLRNUM	64.07	0.0000	0.50 (0.76)	9.06 (4.68)	S	1.63 (Intermediate)	S S	10.94 (PET-like)	S	0.00 (–Transgressive)	S S	
HARHGT	30.01	0.0000	286.51 (34.21)	203.55 (39.17)	S	248.34 (ANN-like)	S S	209.85 (PET-like)	S	288.56 (ANN-like)	S S	
HGT1	64.65	0.0000	41.49 (7.97)	14.61 (3.62)	S	29.56 (Intermediate)	S S	18.25 (Intermediate)	S S	20.62 (Intermediate)	S S	
HYPOLG	7.37	0.1177	11.85 (4.28)	8.86 (3.54)		11.48 (ANN/PET-like)		10.36 (ANN/PET Like)		11.83 (ANN/PET-like)		
LFAREA	66.79	0.0000	63.30 (36.79)	24.97 (9.48)	S	19.25 (–Transgressive)	S S	20.21 (–Transgressive)	S S	92.18 (+Transgressive)	S S	
LFSHAP	69.20	0.0000	1.71 (0.27)	2.35 (0.58)	S	4.47 (+Transgressive)	S S	3.63 (+Transgressive)	S S	2.79 (+Transgressive)	S S	
LFTOUG	53.43	0.0000	172.08 (28.00)	121.47 (19.52)	S	182.08 (ANN-like)	S S	121.98 (PET-like)	S	205.28 (+Transgressive)	S S	
LFSUC	41.84	0.0000	23.95 (4.47)	23.75 (3.77)		32.16 (+Transgressive)	S S	24.09 (ANN/PET-like)		32.16 (+Transgressive)	S S	
LIGNUM	51.36	0.0000	18.32 (3.06)	11.59 (2.15)	S	10.63 (–Transgressive)	S S	10.25 (–Transgressive)	S S	14.70 (Intermediate)	S S	
LIGLGTH	28.89	0.0000	45.47 (48.49)	28.28 (4.30)	S	29.65 (PET-like)	S	25.68 (PET-like)	S	26.25 (PET-like)	S S	
LIGWDTH	18.40	0.0010	13.42 (2.10)	11.70 (2.17)	S	12.35 (Intermediate)		11.51 (PET-like)	S	9.77 (–Transgressive)	S S	
PETLEN	23.38	0.0001	54.40 (29.79)	22.09 (7.63)	S	32.75 (PET-like)	S	28.61 (PET-like)	S	33.47 (PET-like)	S S	
PHYNUM	40.88	0.0000	27.00 (4.61)	18.82 (2.40)	S	17.19 (PET-like)	S	16.88 (–Transgressive)	S S	18.60 (PET-like)	S S	
PHYSHAP	62.04	0.0000	2.68 (0.61)	4.73 (1.17)	S	15.32 (+Transgressive)	S S	7.59 (+Transgressive)	S S	4.01 (Intermediate)	S S	
RGR	30.01	0.0000	2.56 (0.40)	2.37 (0.50)		2.36 (PET-like)		2.34 (PET-like)		1.19 (–Transgressive)	S S	
SEEDW	79.79	0.0000	5.64 (0.37)	1.71 (0.32)	S	7.39 (+Transgressive)	S S	3.11 (Intermediate)	S S	4.24 (Intermediate)	S S	
SLA	28.91	0.0000	207.88 (50.55)	320.17 (72.87)	S	240.53 (Intermediate)	S S	268.67 (Intermediate)	S S	241.85 (Intermediate)	S S	
STEMDIA	60.83	0.0000	14.54 (1.63)	7.55 (1.16)	S	6.02 (–Transgressive)	S S	7.74 (PET-like)	S	11.15 (Intermediate)	S S	
Ecophysiological traits:												
B	47.51	0.0000	66.04 (19.18)	66.54 (16.93)		61.86 (ANN/PET-like)		32.10 (–Transgressive)	S S	39.81 (–Transgressive)	S S	
Ca	10.37	0.0347	16,927.34 (7064.30)	19,685.13 (7358.02)		13,436.70 (ANN/PET-like)	S	16,843.16 (ANN/PET-like)		22,338.20 (ANN/PET-like)	S S	
CI	26.93	0.0000	305.71 (15.26)	300.20 (8.65)	S	293.86 (PET-like)	S	298.43 (PET-like)	S	314.86 (ANN-like)	S S	
COND	11.69	0.0198	2.59 (0.88)	2.15 (0.47)		1.78 (PET-like)	S	2.08 (ANN/PET-like)		2.38 (ANN/PET-like)		
CNRAT	23.01	0.0001	11.39 (2.42)	7.26 (0.70)	S	7.45 (PET-like)	S	8.21 (Intermediate)	S S	7.93 (PET-like)	S S	
DEL13C	10.97	0.0269	–30.29 (0.64)	–31.36 (0.49)	S	–30.56 (Intermediate)		–31.02 (Intermediate)		–30.70 (Intermediate)	S S	
DEL15N	15.30	0.0041	–0.09 (0.58)	–0.97 (0.93)	S	–0.33 (Intermediate)		–1.98 (PET-like)	S	–1.32 (PET-like)	S S	
K	32.24	0.0000	49,226.90 (6831.37)	61,708.53 (5885.27)	S	44,451.29 (–Transgressive)	S S	49,298.71 (ANN-like)	S	38,310.09 (–Transgressive)	S S	
LEAFC	13.92	0.0076	0.43 (0.02)	0.41 (0.02)	S	0.41 (PET-like)	S	0.42 (Intermediate)		0.39 (PET-like)	S S	
LEAFNG	21.01	0.0003	0.04 (0.01)	0.06 (0.01)	S	0.05 (PET-like)	S	0.05 (PET-like)	S	0.05 (Intermediate)	S S	
LEAFNMOL	21.01	0.0003	2.79 (0.45)	4.04 (0.49)	S	3.91 (PET-like)	S	3.70 (PET-like)	S	3.59 (Intermediate)	S S	
LFNAREA	10.51	0.0326	146.47 (22.45)	136.65 (26.26)		174.87 (+Transgressive)	S S	147.75 (ANN/PET-like)		139.50 (ANN/PET-like)		
Mg	49.76	0.0000	3283.18 (582.83)	4212.85 (542.28)	S	3455.07 (Intermediate)	S	4271.48 (PET-like)	S	5698.12 (+Transgressive)	S S	
Mn	34.04	0.0000	196.69 (32.79)	216.42 (61.76)		215.35 (ANN/PET-like)		189.62 (ANN/PET-like)		354.12 (+Transgressive)	S S	
Na	39.09	0.0000	697.41 (279.39)	781.05 (420.63)		689.74 (ANN/PET-like)		987.81 (ANN/PET-like)		2194.64 (+Transgressive)	S S	
P	16.33	0.0026	2371.77 (832.76)	3228.64 (738.02)	S	3113.98 (PET-like)	S	3110.93 (PET-like)	S	3282.03 (PET-like)	S S	
PHOTO	36.49	0.0000	41.99 (4.03)	45.99 (2.56)	S	45.40 (PET-like)	S	45.18 (PET-like)	S	34.81 (–Transgressive)	S S	
PNUE	18.47	0.0010	289.80 (62.60)	332.62 (61.60)		268.17 (ANN-like)	S	314.63 (ANN/PET-like)		221.97 (–Transgressive)	S S	

Note. ANN = *H. annuus*; PET = *H. petiolaris*. χ^2 and *P* values derive from Kruskal-Wallis tests of species differences for each trait. Least significant difference methods (Sokal and Rohlf 1995) were employed in the context of the Kruskal-Wallis test to identify significant pairwise combinations (*P* < 0.05), which are indicated by the letter *S*. Significance levels were corrected using the sequential Bonferroni method (Rice 1989). Trait abbreviations are from table 1. Twenty individuals of *H. annuus*, 11 of *H. anomalus*, 16 of *H. deserticola*, 18 of *H. paradoxus*, and 17 of *H. petiolaris* were analyzed.

H. anomalus, *H. deserticola* grows in stabilized sandy soils on the desert floor. Desert annuals typically germinate only after heavy seasonal rain and then complete their reproductive cycle very quickly (Aronson et al. 1993; Clauss and Venable 2000). Traits associated with this strategy include rapid growth rate, rapid flowering, reduced height at maturity, and high rates of photosynthesis and stomatal conductance (Wullschlegel 1993; Smith 1997). In addition, desert plants often have narrower, smaller leaves to reduce water loss and avoid fatal overheating (Chapin et al. 1987; Gibson 1998).

Our measurements of *H. deserticola* are largely consistent with these predictions. It grows rapidly (RGR) like its parental species, but it buds (BUDDAY) and flowers (FLODAY) significantly earlier than either of them (table 2). Surprisingly, glasshouse plants of *H. deserticola* are as tall at harvest (HARHGT) as one of its parental species, *H. petiolaris*. However, in nature, its final height typically is less than half that of either parental species (Heiser et al. 1969). As with other annual sunflowers (Schwarzbach et al. 2001), *H. deserticola* displays high rates of photosynthesis and stomatal conductance, but both values are in the range of the parental species. Physiological experiments indicate that *H. deserticola* is no more conservative in WUE than its parental species under well-watered conditions, but it does display the predicted reduction in the size and shape of leaves, phyllaries, and ligules (table 2).

Perhaps the most interesting finding in relation to *H. deserticola* is its reduction in boron uptake. Boron is the most common micronutrient deficiency in field-grown sunflower crops (Blamey et al. 1997), but it also can cause toxicity in low-rainfall regions that require irrigation (Blamey et al. 1997; Jefferies et al. 1999) and may limit sunflower cultivation in these areas. *Helianthus deserticola* germplasm may be useful for breeding increased boron toxicity tolerance in the cultivated sunflower.

***Helianthus paradoxus*.** This species is a rare sunflower known only from brackish marshes in five counties of New Mexico and Texas (van Auken and Bush 1998). Soil sodium concentrations in these marshes range from 80–240 mM (Rogers et al. 1982; Welch and Rieseberg 2002), making *H. paradoxus* a true halophyte (Flowers et al. 1986). In contrast, its parental species occur in habitats with very low soil sodium concentrations: 1.1 ± 0.42 mM SE for soil from five *H. annuus* populations and 5.2 ± 1.2 mM SE for soil from six *H. petiolaris* populations (Welch and Rieseberg 2002).

Halophytes are typically characterized by a suite of traits that reduce the toxic effects sodium may have on the function of cytoplasmic enzymes (Yeo 1998; Hasegawa et al. 2000). This may be achieved by actively excluding sodium from plant tissues (Flowers et al. 1977, 1986; Ashraf and O'Leary 1995; Francois 1996) or by sequestering sodium internally in vacuoles by means of sodium pumps (Hasegawa et al. 2000). In addition to exclusion and/or sequestration, salt tolerance can be enhanced through greater WUE and increased succulence (Yeo 1998). Decreased transpiration can reduce Na uptake, and succulence may both increase WUE (because of a thicker palisade layer in the mesophyll) and reduce cytoplasmic ion concentrations.

Helianthus paradoxus displays many of the predicted traits for a halophyte. Corroborating a prior study (Welch and Rie-

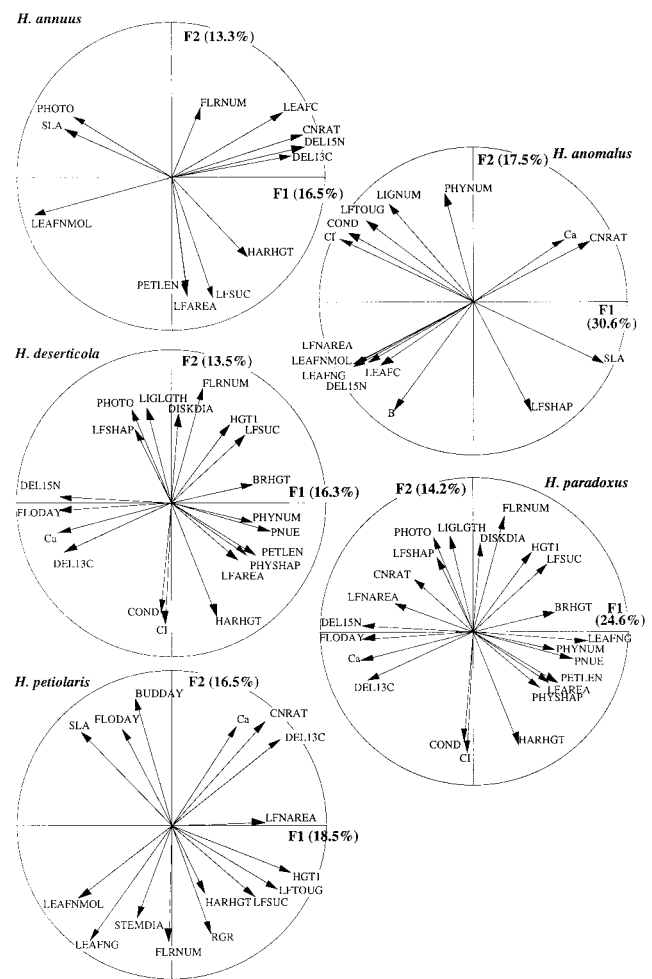


Fig. 3 Graphic representation of intraspecific character correlations of each of the five wild sunflower species. Traits with arrows in the same direction and close to the edge of the circle are highly correlated. However, note that only a subset of intraspecific correlations are shown (only characters with high loadings in factor 1 \times factor 2 of a principal components analysis of each species).

seberg 2002), the leaves of *H. paradoxus* are significantly more succulent and contain more than six times the sodium found in leaves of either parental species (table 2). The latter observation indicates that *H. paradoxus* has some means of sequestering sodium internally. Consistent with these data, growth chamber experiments revealed that *H. paradoxus* had a five- to 14-fold fitness advantage relative to its parental species when all three were exposed to Na levels similar to those reported from natural habitat of *H. paradoxus* (Welch and Rieseberg 2002).

The interpretation of other traits is less clear. *Helianthus paradoxus* does grow significantly slower (RGR) than its glycophytic parental species (table 2), which is consistent with expectations for a halophyte (Flowers et al. 1986; Ishikawa and Kachi 2000). Presumably, the slow relative growth results from the reduced photosynthetic rates that characterize this species (table 2). However, it is least conservative with respect to instantaneous WUE (though not significantly different from

Table 3
Distribution of Trait Values in Three Hybrid Sunflower Species

Trait classification	<i>Helianthus anomalus</i>		<i>Helianthus deserticola</i>		<i>Helianthus paradoxus</i>	
	Traits (no.)	Traits (%)	Traits (no.)	Traits (%)	Traits (no.)	Traits (%)
-Transgressive	4	9.8	6	14.6	7	17.1
+Transgressive	6	14.6	2	4.9	9	22.0
ANN-like ^a	4	9.8	1	2.4	4	9.8
PET-like ^b	12	29.3	17	41.5	8	19.5
ANN/PET-like ^{a,b}	5	12.2	8	19.5	4	9.8
Intermediate	10	24.4	7	17.1	9	22.0
Totals:						
Transgressive	10	24.4	8	19.5	16	39.0
Parental	21	51.2	26	63.4	16	39.0
Intermediate	10	24.4	7	17.1	9	22.0

^a ANN = *Helianthus annuus*.

^b PET = *Helianthus petiolaris*.

H. annuus) and well within the range of the parental species for lifetime integrated WUE.

With respect to nutrients, *H. paradoxus* is significantly less efficient than its parental species in PNUE, potentially resulting from the use of N in compatible solutes and Na tolerance. It also displays a reduction in boron uptake similar to that found in *H. deserticola*. Levels of other leaf micronutrients appear to be highly correlated with Na accumulation, with Mn and Mg exhibiting a positive correlation and K a negative one. Uptake of these elements is known to be affected by Na and Cl (Glenn and O'Leary 1984; Donovan et al. 1997; Silberbush and Ben-Asher 2001), and salt tolerance sometimes is achieved by selectivity of K over Na (Bartal et al. 1991). However, this does not appear to be the case for *H. paradoxus*, in which K concentrations actually are significantly reduced relative to its parental species (table 2).

Conclusions and Future Directions

Results from this comparative study indicate that the three hybrid sunflower species display numerous traits that are ex-

treme relative to their parental species. Comparison to literature for species in similar habitats indicates that most of these trait differences likely represent adaptations to the environments the species occur in, but this has not been proven, nor is it clear that the extreme traits actually arose via hybridization. However, for hybrid speciation to be successful, at least some ecological divergence is required, and this divergence must occur early in the speciation process. This implies that hybridization likely contributed to the extreme phenotypes observed in these ancient hybrid taxa, but further experimentation is needed confirm these predictions.

Acknowledgments

We thank Jennifer Lance for her assistance in all phases of the phenotyping and Tracy Burton, Scott Cornman, Javier Espeleta, Steve Franks, Jill Johnston, Christina Richards, Jason West, and others who helped during the harvest. This research was supported by National Institutes of Health award GM59065 to L. H. Rieseberg.

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