Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*

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Summary

- Adaptive trait introgression is increasingly recognized as common. However, it is unclear whether adaptive genetic exchanges typically affect only a single trait, or instead affect multiple aspects of the phenotype. Here, we examine introgression of abiotic tolerance traits between two hybridizing North American sunflower species, *Helianthus annuus* and *Helianthus debilis.*
- In two common gardens in the hybrid range, we measured 10 ecophysiological, phenological, and architectural traits for parents and their natural and artificial hybrids, and examined how fitness covaried with trait values.
- Eight of the 10 traits showed patterns consistent with introgression from *H. debilis* into *H. annuus*, and suggested that *H. debilis*-like traits allowing rapid growth and reproduction before summer heat and drought have been favored in the hybrid range. Natural selection currently favors BC_1 hybrids with *H. debilis*-like branching traits.
- We demonstrate that introgression has altered multiple aspects of the *H. annuus* phenotype in an adaptive manner, has affected traits relevant to both biotic and abiotic environments, and may have aided expansion of the *H. annuus* range into central Texas, USA.

Introduction

Adaptive trait introgression, the movement of fitness-increasing alleles and their associated phenotypes from one differentiated population or species to another (Anderson, 1949; Rieseberg & Wendel, 1993), is increasingly recognized as an important evolutionary phenomenon (Arnold, 2004). Such introgression has the potential to allow adaptation at rates that considerably exceed those possible for non-hybridizing populations that are dependent on mutation for genetic novelty (Barton, 2001). Numerous hypothesized examples of adaptive introgression have been proposed (for some recent work, see Friedman *et al.*, 2008; Kim *et al.*, 2008; Gagnaire *et al.*, 2009; Trucco *et al.*, 2009), but well-documented cases identifying the key traits and confirming their adaptive value are relatively scarce. Two excellent examples are Louisiana irises, in which flooding tolerance has introgressed from *Iris fulva* into *Iris brevicaulis* and has increased survival in hybrids (Martin *et al.*, 2005, 2006), and Darwin's finches, in which alleles influencing beak

morphology have introgressed bidirectionally between *Geospiza fortis* and *Geospiza scandens* and have increased fitness via improved feeding performance (Grant & Grant, 1996, 2008).

Despite the growing number of examples of adaptive trait introgression, many outstanding issues remain. One issue concerns the extent of the process: do adaptive genetic exchanges typically affect only a single trait, or does adaptive introgression simultaneously affect multiple aspects of the phenotype? Extant studies of adaptive trait introgression have typically focused on only a single trait or a group of functionally related traits (e.g. the *Iris* and *Geospiza* examples in the preceding paragraph; plumage coloration in *Manacus*, Uy & Stein, 2007; the presence of ray floret petals in *Senecio*, Kim *et al.*, 2008). In fact, to our knowledge, systems in which multiple introgressed traits have been conclusively identified are limited to a single crop-wild system (*Sorghum*, Paterson *et al.*, 1995). However, this pattern may simply reflect investigators' focus on a single hypothesis per system, and/or the large investment of study effort

required to build a case for adaptive introgression of multiple traits. A second issue concerns the types of traits that introgress: we need a better understanding of the relative importance of introgression in adaptation to abiotic (e.g. temperature, water availability) vs biotic (e.g. predation, competition) environments. Progress on this issue could be more rapid if investigators examined both biotic and abiotic traits in the same system.

Here, we examine two North American sunflower species to ask whether introgression simultaneously affects multiple aspects of the phenotype in an adaptive manner. Heiser (1951a) first proposed that *Helianthus annuus* has captured advantageous genetic material from *Helianthus debilis* ssp. *cucumerifolius*, a sunflower of central Texas, and by doing so has expanded its range southward (Fig. 1). Subsequent work has indicated that there are few barriers to the movement of morphological quantitative trait loci (QTL) alleles between the species (Kim & Rieseberg, 1999, 2001), and has confirmed via molecular markers that the two species have formed a stabilized hybrid, *Helianthus annuus* ssp. *texanus* (*H. a. texanus*, Rieseberg *et al.*, 1990, 2007; Scascitelli *et al.*, 2010). In a companion study (Whitney *et al.*, 2006), we demonstrated that *H. a. texanus* has higher fitness than either parent in the field, and examined 11 herbivore resistance traits for evidence of adaptive introgression. We concluded that traits conferring resistance to two types of herbivore (seed-feeding midges and seed- and receptacle-feeding caterpillars) have introgressed from *H. debilis* to *H. annuus* and have resulted in increased fitness (Whitney *et al.*, 2006).

In the present study, we focus on potential introgression of 10 ecophysiological, phenological, and architectural traits hypothesized to influence adaptation to the abiotic environment. Given that the hybrid range is more southern than that of the *H. annuus* (recipient) parent, and has higher

temperatures but similar rainfall, we predict that traits increasing performance in warmer, more water-limited conditions will be favored. We ask:

• Are trait means consistent with the adaptive trait introgression hypothesis; namely, do abiotic tolerance traits exist for which the mean *H. a. texanus* phenotype differs significantly from *H. annuus* ssp. *annuus* (*H. a. annuus*) in the direction of *H. debilis*?
• Is the direction of selection on these traits consistent with the adaptive trait introgression hypothesis; namely, do hybrids with *H. debilis*-like trait values have relatively high fitness in the novel/hybrid environment?

Materials and Methods

Study system

Helianthus annuus L. is a weedy, self-incompatible diploid annual with a wide distribution in North America. Heiser (1951b, 1954) proposed that *H. annuus* has been able to capture advantageous genetic material from *Helianthus debilis* Nutt. var. *cucumerifolius* (Torr. & A. Gray) Heiser, a small sunflower endemic to central Texas and some dispersed areas along the eastern seaboard. According to Heiser's hypothesis, this introgression allowed a southward range expansion of *H. annuus* into central and southern Texas and Mexico (Fig. 1). In contrast to the northern taxon *H. a. annuus* from which it is derived, the putative introgressant was named *H. a. texanus* (Heiser, 1954). Morphologically, *H. a. texanus* is intermediate between the parents (Heiser, 1951b; Kim & Rieseberg, 1999; Whitney *et al.*, 2006), and appears to occupy a novel ecological niche combining the edaphic preferences of the *H. annuus* parent (clay rather than sandy soil) with the southerly latitudinal range of *H. debilis* (Heiser, 1951b). Abiotic conditions in the hybrid range differ from those in the southern edge of the *H. a. annuus* range (northern Texas and southern Oklahoma). While annual rainfall is similar in the two regions (rainfall isoclines run roughly north–south in this area; Spatial Climate Analysis Service, 2000), mean annual temperatures are 1.6–5.6°C warmer in the hybrid range (Griffiths & Orton, 1968), leading to higher evapotranspiration rates and greater drought stress.

To date, molecular data support Heiser's view on the origin of *H. a. texanus*. Using both chloroplast DNA and ribosomal DNA markers, Rieseberg *et al.* (1990) confirmed that *H. a. texanus* populations contain introgressed *H. debilis* material (and northern populations of *H. annuus* do not). Regions of genetic differentiation between the taxa appear to be small (< 1 cM) and estimates of recent immigration/admixture are in the < 2–14% range (Scascitelli *et al.*, 2010). Certain *H. debilis* markers reach high frequencies in natural *H. a. texanus* populations, suggestive of positive selection (Rieseberg *et al.*, 2007).

Fig. 1 Geographic distributions of parents and hybrids in the United States: light gray, the hypothesized range of *Helianthus annuus* before the colonization of North America by humans; black, the current Texas range of *Helianthus debilis* ssp. *cucumerifolius* (Rogers *et al.*, 1982); dark gray, the current range of *H. annuus* ssp. *texanus* (Heiser, 1951b).

Progress has been made in connecting parental alleles with hybrid morphology and in the search for key traits driving this case of putative adaptive introgression. Kim & Rieseberg (1999, 2001) mapped QTL for 15 morphological traits differentiating the parental species, as well as QTL influencing pollen sterility. Loose linkage between the two types of loci suggested few barriers to the introgression of the morphological QTL, and it was estimated that the movement of three small chromosomal regions from *H. debilis* would be sufficient to recover the *H. a. texanus* phenotype, at least with respect to the traits examined. A subset of the high-frequency *H. debilis* markers in natural *H. a. texanus* populations identified by Rieseberg *et al.* (2007) were associated with QTL underlying morphological traits (e.g. flower and inflorescence size) that vary in the direction of *H. debilis*. While the fitness consequences of these traits/QTL have not yet been tested, an additional study identified specific herbivore resistance traits that differ between the parents and for which *H. debilis*-like values are favored in hybrids in the field (Whitney *et al.*, 2006).

Plant material

Four classes of plant material were compared in field plantings: *H. debilis* ssp. *cucumerifolius, H. a. annuus*, backcrosses (BC$_1$) between them, and *H. a. texanus*. Achenes (seeds hereafter) of the three wild taxa were collected from three to four Texas populations and nine to 10 families per species (see table 1 of Whitney *et al.* (2006) for locality details). To sample the range of variability within each taxon, these collections spanned a wide area within Texas and, in the case of *H. a. annuus*, also included a northerly population from Nebraska. In experiments, an equal number of individuals from each population/family was used. The BC$_1$ generation was obtained by first mating *H. debilis* ssp. *cucumerifolius* from Texas to wild *H. a. annuus* from Oklahoma to produce F$_1$ progeny in the glasshouse.

In order to produce enough BC$_1$ seed for replicate field populations, a single progeny from the F$_1$ generation was selected and propagated vegetatively to produce 14 F$_1$ clones. A single *H. a. annuus* pollen donor was then mated to the F$_1$ clones to produce 3758 BC$_1$ seeds. This limited-parent design was necessary to allow QTL mapping of the relevant traits in a parallel study. Seeds were nicked, germinated on filter paper, and on day 6 transplanted into peat pots (6 × 10 cm, Jiffy A/S, Ryomgaard, Denmark) containing field soil. Seedlings were grown in a glasshouse for *c.* 4 wk before transplanting to the field.

Study sites and experimental design

Plant performance was examined at two sites in central Texas (a novel habitat with respect to *H. a. annuus*): the Brackenridge Field Laboratory of the University of Texas,

Austin (hereafter BFL), and the Lady Bird Johnson Wildflower Center (hereafter LBJ; Austin, TX, USA). The BFL site is characterized by sandy riverbottom soil, while LBJ is characterized by clay soil in an oak savanna. Further site details are available in Whitney *et al.* (2006).

Common gardens To examine traits and fitness in the hybrid derivative *H. a. texanus* relative to the parental species, common garden plots were planted at the two sites. Plots were divided into two and five blocks, respectively, at BFL and LBJ. In late March 2003, individuals of four taxa (*H. debilis* ssp. *cucumerifolius, H. a. annuus, H. a. texanus*, and BC$_1$) were planted at random in a grid pattern within each block (90 cm spacing). Twenty and 45 individuals per taxon were planted at BFL and LBJ, respectively. Final sample sizes per site for most taxa were also 20 and 45, but because of early transplant mortality and missing data, final sample sizes for *H. a. annuus* and *H. debilis* were both $n = 19$ at BFL, and were $n = 44$ and 37, respectively, for *H. a. annuus* and *H. debilis* at LBJ. Seedlings were kept moist via hand-watering for 9 d. Plant traits and fitness were measured as described later.

Selection plots Natural selection on BC$_1$ hybrids was examined in a separate plot at each of the two sites (500 BC$_1$ seedlings per site; 90 cm spacing). Because these hybrid populations were planned for use in a long-term selection experiment which would be disrupted by extensive gene flow, they were each established at 0.5 km from the four-taxa common gardens. Seedlings were transplanted to the field in late March and kept moist via hand-watering for 9 d. A frost at the LBJ plot killed 300 seedlings on 29 March; these were replaced with new transplants on 3 April. Plant traits and fitness were measured as described in the following sections. At analysis, 29 plants from BFL and 37 from LBJ were excluded because of early transplant mortality or missing trait data. In total, 1223 plants were analyzed from the selection plots and common gardens.

Ecophysiological traits

Water-use efficiency (WUE) is the rate of carbon gained via photosynthesis per unit of water lost via transpiration. We measured integrated WUE via carbon stable isotope ratios; the more negative the $\delta^{13}C$ value, the poorer the WUE (Pate, 2001). The youngest fully expanded leaf on each plant was collected and dried at 37°C to constant mass in mid-May. Samples of dried leaf tissue (2.5 mg) were then analyzed for carbon and nitrogen using an isotope ratio mass spectrometer (PDZ Europa Integra, Cheshire, UK) at the UC Davis Stable Isotope Facility (CA, USA). Owing to cost considerations, only a subset of the plants ($n = 17$ per taxon) were sampled in the LBJ common garden; all plants in the three remaining plots were sampled for this trait.

Specific leaf area (SLA) is the ratio of leaf area (cm^2) to dry mass (g), and thus plants with high SLA values have relatively thin leaves and a relatively high degree of light interception per unit biomass. Leaf succulence is calculated as (leaf wet mass − dry mass)/wet mass. One fully expanded leaf per plant was collected in the period before first flowering. Leaf area was assessed with a Li-Cor leaf area meter Li-Cor, (Lincoln, NE, USA). Wet mass was measured on a microbalance, the leaf was dried in a drying oven until constant mass was reached, and dry mass was then measured.

We hypothesized that traits allowing rapid spring growth in these annual plants would contribute to drought escape (Ludlow, 1989) and therefore would be favored in the hybrid region. Given a recognized tradeoff between growth rate and efficient conservation of nutrients in plants generally (Poorter & Garnier, 1999), we thus hypothesized that low WUE and high SLA would be favored in the hybrid region. Additionally, we hypothesized that high leaf succulence would also be favored.

Phenological traits

Phenological status of all plants was recorded every third day from transplanting until all plants had died. Bud initiation time is the number of days between transplanting and the first appearance of the immature apical flowering head. Seed maturation time is the number of days between the end of stigma receptivity and achene maturity (measured for the apical flowering head). Plant longevity is the number of days between transplanting and mortality.

Under the hypothesis that introgression aided expansion of *H. annuus* into more southern areas experiencing warmer temperatures and greater drought stress, we hypothesized that early flowering and rapid maturation would contribute to a drought escape strategy (Ludlow, 1989) and thus would be favored in the hybrid region.

Architectural traits

Disk diameter is the diameter (mm) of the central disk of the apical flowering head, measured during stigma receptivity. Height of lowest branch is the height (cm) above ground of the lowest branching point on the main stem. Bushiness describes the degree of higher-order branching and is estimated as the mean branch position of all flowering heads on a plant (Pilson & Decker, 2002). Heads originating from a meristem on the main stem have a branch position of 1, heads originating from a primary branch have a branch position of 2, heads originating from a secondary branch have a branch position of 3, and so forth. Relative branch diameter reflects the degree of investment in branches relative to the main stem and is the average primary branch diameter divided by the diameter of the main stem.

We hypothesized that smaller disks would reduce water loss and thus be favored in the hybrid region. We had no particular hypotheses for height of lowest branch and relative branch diameter, but we hypothesized that greater bushiness would be favored in the hybrid region. Since each flowering head is necessarily subtended by a branch, and higher-order branches are less costly to produce than lower-order branches, plants capable of assuming a bushier phenotype might be able to add more inflorescences than less bushy plants in response to unpredictable rainfall events.

Statistical analysis

Comparison of trait means in parental and hybrid taxa
Trait means were compared using MANOVA and ANOVA (proc GLM and proc MIXED; SAS Institute, 2003). Response variables were square-root- or log-transformed to improve the normality of residuals and homoscedasticity. Preliminary MANOVAs on the three sets of plant traits (ecophysiological, phenology, and plant architecture) examined the effects of taxon, site, their interaction, and block (nested within site). All effects were considered fixed except for block, which was considered a random effect; therefore site was tested over variation among blocks. While site could arguably be considered a random effect, the fact that only two sites were used (and were chosen for their known environmental differences) led us to consider it fixed (Underwood, 1997). MANOVAs were evaluated using the Pillai's trace test criterion (Scheiner, 2001). Following a significant taxon effect or taxon × site interaction in the MANOVA for a trait set, protected ANOVAs (Scheiner, 2001) were carried out for each trait, again examining the factors taxon, site, taxon × site, and block (nested within site). Following a significant ANOVA, trait means for *H. debilis*, *H. a. texanus*, and BC_1 were each compared with the *H. a. annuus* mean (the 'control') using Dunnett's adjustment for multiple comparisons.

Selection analyses Phenotypic selection analysis (Lande & Arnold, 1983) was used to examine natural selection on traits in the two BC_1 hybrid populations. Because these analyses become misleading when relevant traits are omitted from the regression model, a single global selection analysis was performed for each population, including the 10 putative abiotic tolerance traits discussed here as well as 11 herbivore resistance-related traits discussed in Whitney *et al.* (2006). Viable seed production was chosen as the measure of fitness in these annual plants, and was estimated by multiplying the total number of heads by the average number of viable seeds per head in a pooled sample of bagged heads (see Whitney *et al.*, 2006 for further details). Relative fitness was calculated as individual viable seed production divided by the mean viable seed production for the population.

Predictor variables were transformed as necessary to improve normality, and standardized to a mean of 0 and a standard deviation of 1. Collinearity between predictor variables was assessed with Proc Reg (SAS Institute, 2003). All variance inflation factors (VIFs) were < 6.5 and all condition indices were < 6.9; therefore multicollinearity is unlikely to compromise the results (Myers, 1990; Freund & Littell, 2000). Correlations between variables are reported in Supporting Information, Table S1.

Linear selection differentials (s') are given by the covariance between the trait and relative fitness, and linear selection gradients (β) are the partial regression coefficients simultaneously fitted to all traits in a multiple regression on relative fitness (Proc Reg). Using untransformed relative fitness, the assumption of normality of residuals was violated as indicated by significant Shapiro–Wilks tests (Proc Univariate), invalidating traditional significance tests. Consequently, 95% confidence intervals for selection gradients were estimated via resampling techniques (Mitchell-Olds & Shaw, 1987) using a bias-corrected accelerated bootstrap with 10 000 resamples.

Results

Are at least some abiotic tolerance trait means in hybrid *H. a. texanus* shifted towards *H. debilis*?

Overall, traits differed strongly between taxa for ecophysiological, phenological, and architectural traits (MANOVA, Table 1). In all cases, there were significant site and/or taxon × site interactions, indicating that the direction and/or magnitude by which the taxa differed depended on the site context.

The *H. debilis* parent differed significantly from the *H. a. annuus* parent in eight of the 10 traits examined (Table 2). Relative to *H. a. annuus*, *H. debilis* was characterized by

lower WUE, higher SLA, more rapid bud initiation and seed maturation, smaller inflorescence disk diameter, and greater allocation to branching (branching started lower on the stem and branches were relatively thicker). One trait showed site-dependent patterns: relative to *H. a. annuus*, *H. debilis* had significantly greater longevity at LBJ but no differences were apparent at BFL.

Helianthus annuus ssp. *texanus* showed multiple instances of phenotypic intermediacy between its parental taxa. In particular, *H. a. texanus* phenotypes for WUE, SLA, seed maturation time, disk diameter, height of lowest branch, and relative branch diameter differed significantly from *H. a. annuus*, and in each case were shifted towards *H. debilis* (Table 2). In addition, relative branch diameter and longevity of *H. a. texanus* were shifted towards *H. debilis* at BFL and LBJ, respectively, although differences between the hybrid and the *H. a. annuus* parent for these two traits were not apparent at the alternate site. Finally, *H. a. texanus* exhibited significantly greater bushiness than either parent (Table 2), potentially indicating the presence of transgressive segregation.

Does selection favor resynthesized hybrids with abiotic tolerance trait values shifted towards those of *H. debilis*?

Selection differentials and gradients for the two BC_1 populations are given in Table 3. The 21 abiotic and biotic traits examined collectively explained a fairly high proportion of the variance in relative fitness (R^2 = 0.48 and 0.59 for BFL and LBJ, respectively). In the BFL population, selection favored phenotypes with high WUE, low SLA, longer seed maturation time, larger disk diameters, branching from a lower height, greater bushiness and lower relative branch diameter. However, examination of selection gradients indicated that many of these patterns were caused by selection on correlated characters; direct selection was found only for larger disk diameters and bushier plants. With reference to the mean trait values at BFL (Table 2), direct selection on these two traits did not favor hybrids with *H. debilis*-like traits. However, selection on both traits was in the direction of the *H. a. texanus* phenotype.

In the LBJ population, total selection favored changes in all measured aspects of the phenotype (Table 3). The direction of total selection on each trait was generally very similar to that at BFL, with the exception of SLA (s' was negative at BFL and positive at LBJ). Again, many of these patterns were driven by indirect selection via correlated characters, as direct selection was found only for longer bud initiation time, larger disk diameters, branching from a lower height, greater bushiness, and higher relative branch diameters. Direct selection favored hybrids with *H. debilis*-like values for relative branch diameter and height of the lowest branch. Furthermore, selection on both relative branch

Table 1 MANOVAs for three sets of traits measured on four *Helianthus* taxa in common gardens in two central Texas sites (USA)

Trait set	Effect	df	Pillai's trace	F	P
Ecophysiological	Taxon	9, 399	0.471	8.25	< 0.0001
	Site	3, 3	0.986	72.99	0.0027
	Taxon × site	9, 399	0.040	0.59	0.8092
	Block (site)	15, 399	0.401	4.11	< 0.0001
Phenological	Taxon	9, 663	0.848	29.02	< 0.0001
	Site	3, 3	0.876	7.03	0.0717
	Taxon × site	9, 663	0.136	3.50	0.0003
	Block (site)	15, 663	0.138	2.14	0.0072
Architectural	Taxon	12, 687	0.924	25.49	< 0.0001
	Site	4, 2	0.980	24.45	0.0397
	Taxon × site	12, 687	0.147	2.95	0.0005
	Block (site)	20, 920	0.289	3.58	< 0.0001

The traits contained within each set are listed in Table 2.

Table 2 Trait values (mean ± SE) for four *Helianthus* taxa in common gardens in two central Texas sites (USA)

Trait/Site	H. a. annuus		H. a. texanus		H. debilis		BC$_1$	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Ecophysiological traits								
Water-use efficiency (δ^{13}C)								
BFL	−28.0 ± 0.2	−29.4 to −26.8	**−28.6 ± 0.2**	−29.8 to −27.4	**−29.0 ± 0.1**	−30.1 to −28.3	−28.0 ± 0.1	−29.2 to −27.1
LBJ	−28.6 ± 0.1	−29.9 to −27.8	**−29.1 ± 0.1**	−30.2 to −28.2	**−29.3 ± 0.1**	−30.7 to −27.6	−28.6 ± 0.0	−29.2 to −28.2
Specific leaf area (cm^2 g^{-1})								
BFL	224.1 ± 6.3	177.2 to 277.4	**245.0 ± 7.1**	186.3 to 310.6	**270.8 ± 9.5**	201.4 to 364.8	216.2 ± 6.0	174.1 to 278.6
LBJ	139.8 ± 3.1	74.6 to 178.4	**154.1 ± 2.8**	115.2 to 204.0	**164.8 ± 4.0**	102.4 to 227.1	137.8 ± 2.9	105.9 to 221.2
Leaf succulence								
BFL	0.870 ± 0.002	0.851 to 0.889	0.874 ± 0.002	0.858 to 0.889	0.872 ± 0.002	0.852 to 0.885	0.876 ± 0.002	0.864 to 0.892
LBJ	0.776 ± 0.004	0.690 to 0.821	0.780 ± 0.005	0.696 to 0.917	0.776 ± 0.006	0.661 to 0.830	0.792 ± 0.003	0.738 to 0.833
Phenological traits								
Bud initiation time (d)								
BFL	54.8 ± 3.1	37 to 91	50.7 ± 1.4	35 to 58	**42.2 ± 2.0**	24 to 61	54.5 ± 1.9	32 to 68
LBJ	61.5 ± 2.8	28 to 97	63.9 ± 1.5	40 to 82	**43.3 ± 1.9**	29 to 74	**70.4 ± 1.3**	52 to 88
Seed maturation time (d)								
BFL	29.2 ± 1.0	22 to 40	**26.3 ± 0.8**	18 to 30	**16.9 ± 0.5**	12 to 21	**24.0 ± 0.6**	19 to 29
LBJ	28.6 ± 0.6	18 to 38	**27.2 ± 0.4**	17 to 33	**19.3 ± 0.4**	15 to 27	**23.8 ± 0.3**	17 to 27
Plant longevity (d)								
BFL	141.9 ± 7.0	105 to 187	162.1 ± 6.1	109 to 211	140.7 ± 6.3	92 to 211	155.4 ± 4.5	128 to 187
LBJ	158.7 ± 4.7	90 to 208	182.6 ± 3.3	129 to 223	**187.7 ± 5.8**	122 to 235	**183.1 ± 1.7**	148 to 205
Plant architectural traits								
Disk diameter (cm)								
BFL	33.5 ± 1.2	24.2 to 42.3	29.5 ± 0.8	23.0 to 39.1	**17.8 ± 0.5**	14.4 to 21.2	**28.3 ± 0.8**	21.5 to 35.4
LBJ	37.7 ± 1.0	22.5 to 48.0	31.4 ± 0.5	23.8 to 39.5	**17.6 ± 0.4**	13.0 to 22.0	**32.6 ± 0.7**	24.8 to 43.0
Height of lowest branch (cm)								
BFL	51.5 ± 6.6	12.0 to 102.0	35.5 ± 4.5	11.0 to 103.0	**10.5 ± 1.3**	3.0 to 22.0	**31.4 ± 4.5**	1.0 to 65.0
LBJ	33.3 ± 3.9	6.0 to 106.0	28.2 ± 2.4	4.0 to 72.0	**8.6 ± 0.7**	0.0 to 21.0	**25.1 ± 2.6**	0.0 to 64.0
Bushiness								
BFL	2.2 ± 0.1	1.8 to 2.8	2.4 ± 0.1	1.9 to 2.9	2.2 ± 0.1	1.0 to 2.8	2.2 ± 0.1	1.5 to 2.8
LBJ	2.5 ± 0.0	1.8 to 2.9	2.7 ± 0.0	2.3 to 2.9	2.5 ± 0.1	1.0 to 2.9	2.6 ± 0.0	2.0 to 2.9
Relative branch diameter								
BFL	0.29 ± 0.02	0.19 to 0.40	**0.36 ± 0.02**	0.28 to 0.57	**0.48 ± 0.02**	0.33 to 0.62	0.32 ± 0.01	0.21 to 0.38
LBJ	0.33 ± 0.01	0.21 to 0.56	0.33 ± 0.01	0.23 to 0.44	**0.53 ± 0.01**	0.34 to 0.77	0.30 ± 0.01	0.23 to 0.43
Fitness traits								
Viable seed no.								
BFL	1703.4 ± 467.4	177.4 to 7663.2	**2371.6 ± 621.5**	251.5 to 12238.2	932.3 ± 161.6	65.0 to 3033.8	**570.1 ± 130.0**	11.0 to 2520.2
LBJ	2566.1 ± 492.9	133.0 to 17990.3	**4370.3 ± 375.8**	625.5 to 12344.0	1604.3 ± 300.2	44.0 to 10091.1	**1201.9 ± 184.3**	38.0 to 5031.0

H. a. annuus, *Helianthus annuus* ssp. *annuus*; *H. a. texanus*, *Helianthus annuus* ssp. *texanus*; BFL, Brackenridge Field Laboratory of the University of Texas, Austin; LBJ, Lady Bird Johnson Wildflower Center, Austin, Texas.

Trait values for *H. a. texanus*, *H. debilis*, and BC$_1$ are in bold if they differ significantly ($P < 0.05$) from *H. a. annuus* using Dunnett's adjustment for multiple comparisons in a mixed-model ANOVA. Initial MANOVAs (Table 1) controlled for inflated type I error resulting from tests of multiple traits. Sample sizes per taxon are $n = 20$ for BFL and $n = 45$ for LBJ, except for *H. a. annuus* and *H. debilis* at LBJ ($n = 44$ and 36, respectively) and carbon isotope ratio for all taxa at LBJ ($n = 17$ per taxon).

Table 3 Selection differentials (s′) and selection gradients (β) for putative abiotic tolerance traits in two *Helianthus annuus* ssp. *annuus* × *Helianthus debilis* BC$_1$ populations in central Texas (USA)

Population/Trait	s′	P	β	95% CI	
				Lower	Upper
Brackenridge Field Laboratory (BFL)					
Water-use efficiency (δ^{13}C)	**0.27**	< 0.0001	0.02	−0.08	0.12
Specific leaf area (cm^2 g^{-1})	−**0.09**	0.0215	0.03	−0.11	0.20
Succulence	−0.02	0.6958	−0.03	−0.20	0.12
Bud initiation time (d)	−0.01	0.8138	−0.01	−0.10	0.08
Seed maturation time (d)	**0.15**	0.0003	0.04	−0.03	0.11
Longevity (d)	−0.04	0.2803	0.04	−0.03	0.11
Disk diameter (cm)	**0.38**	< 0.0001	**0.23**	0.14	0.31
Height of lowest branch (cm)	−**0.08**	0.0452	0.03	−0.04	0.10
Bushiness	**0.24**	< 0.0001	**0.20**	0.11	0.28
Relative branch diameter	−**0.11**	0.0106	0.05	−0.02	0.13
Lady Bird Johnson Wildflower Center (LBJ)					
Water-use efficiency (δ^{13}C)	**0.35**	< 0.0001	0.05	−0.03	0.14
Specific leaf area (cm^2 g^{-1})	**0.12**	0.0363	0.09	−0.03	0.22
Succulence	**0.31**	< 0.0001	−0.03	−0.15	0.11
Bud initiation time (d)	−**0.12**	0.0323	**0.21**	0.09	0.35
Seed maturation time (d)	**0.27**	< 0.0001	0.07	−0.01	0.14
Longevity (d)	−**0.15**	0.0090	−0.02	−0.09	0.05
Disk diameter (cm)	**0.45**	< 0.0001	**0.10**	0.00	0.20
Height of lowest branch (cm)	−**0.25**	< 0.0001	−**0.15**	−0.29	−0.03
Bushiness	**0.38**	< 0.0001	**0.18**	0.07	0.30
Relative branch diameter	−**0.41**	< 0.0001	**0.13**	0.00	0.27

Sample sizes are $n = 471$ for BFL and $n = 463$ for LBJ. For differentials/gradients that are significantly different from zero ($P < 0.05$), values are in bold.

diameter and bushiness was in the direction of the *H. a. texanus* phenotype.

Discussion

Our results imply that traits influencing adaptation to the abiotic environment have introgressed from *H. debilis* into the *H. annuus* background. Seven of 10 traits measured in the *H. a. texanus* hybrid lineage differ significantly from the *H. a. annuus* parent and are shifted in the direction of the *H. debilis* parent in one or both of the sites. In addition, an eighth trait, bushiness, reaches its maximum mean values in the hybrid lineage, suggesting that transgressive segregation has occurred. Of these eight traits, four are associated with significant selection gradients in resynthesized hybrids (BC$_1$s) at one or both sites. Although data from a single year may not be sufficient to generalize about adaptation, the selection gradients suggest that these four traits are likely important in current-day adaptation to the environment of central and south Texas.

Ecophysiological traits

We did not detect current selection on any of the three eco-physiological traits. However, relative to the *H. a. annuus* parent, both *H. debilis* and the *H. a. texanus* hybrid lineage exhibited low WUE and high SLA. Such traits are charac-

teristic of plant species that capture resources quickly in order to maximize growth rates at the expense of efficient conservation of nutrients (Poorter & Garnier, 1999), and low WUE has been associated with an early-flowering, drought-escape strategy in other wild species (Geber & Dawson, 1997; McKay *et al.*, 2003). Thus, we hypothesize that the central Texas environment favors a drought-escape strategy in these annual sunflowers, expressed as rapid spring growth and flowering before the intense heat and dry conditions of summer.

Phenological traits

Bud initiation time is very rapid in *H. debilis*, consistent with an environment in the hybrid range that favors rapid spring growth and flowering before the heat of summer. However, selection on hybrids at LBJ appears to favor longer bud initiation times than currently exist in any of the extant taxa. While we have no good explanation for this pattern, it could be that the direction of selection in this particular year and site is opposite to that of its long-term average direction. While seed maturation time was not under current direct selection, seed maturation times are consistent with adaptive introgression in an environment favoring rapid growth, as the *H. debilis* and *H. a. texanus* phenotypes were accelerated by 1.5–13 d relative to *H. a. annuus*.

Architectural traits

For branching-related traits – relative branch diameter, height of the lowest branch, and bushiness – the beneficial effect of putative *H. debilis* alleles seems clear. Hybrid BC$_1$ plants that showed a *H. debilis*-like allocation to branches (thick branches relative to stem diameter and branches that start low on the plant) were more fit than sparsely branched, *annuus*-type plants. In addition, the environment favors bushy plants with large numbers of higher-order branches. While the bushiness of the two parents does not differ, it is likely that the extreme values of bushiness observed in *H. a. texanus* are the result of combinations of alleles from both parents, again suggesting that introgression of *H. debilis* alleles has been adaptive. We speculate that the environment favors a branchier, bushier phenotype for two reasons. First, self-shading changes with increasing allocation to branches (Valladares, 1999) and could alter light reception and/or water relations to better match the relatively warm hybrid environment. Second, bushiness could influence the size of the potential flowering response to water pulses. Since each flowering head is necessarily subtended by a branch, and higher-order branches are smaller and less costly to produce than lower-order branches, plants capable of assuming a bushier phenotype might be able to add more inflorescences than less bushy plants in response to isolated rainfall events.

As with the previous three traits, floral disk diameter is also under current direct selection. Disk diameters are small in *H. debilis* and intermediate in *H. a. texanus*, but selection on BC$_1$s apparently favors larger disks in both sites. Note, however, that disk size in the BC$_1$ hybrids is smaller than that in *H. a. texanus*, so the patterns are still consistent with introgression of *H. debilis* alleles followed by natural selection to approach a more optimal disk size from below. We hypothesize that relatively small floral disks might reduce water losses and thus be advantageous in the central Texas environment, but direct experiments are needed.

Fitness traits

It is expected that early-generation (but post-F$_1$) hybrids will have lower mean fitness than parentals because of Dobzhansky–Muller incompatibilities and/or chromosomal rearrangements and because trait combinations have not yet been filtered by natural selection (Arnold *et al.*, 1999; Barton, 2001). If introgression has been adaptive, it will be expressed in a relatively small number of fit individuals, which in some cases may 'escape from the mass of unfit recombinants' (Barton, 2001, p. 562) and lead to a stabilized hybrid lineage. As predicted, mean fitness of early-generation BC$_1$ hybrids in the *H. a. texanus* system is quite low (33 and 47% of the *H. a. annuus* parental fitness at BFL and LBJ, respectively). Also as predicted, fit individuals are present: seed productions by the best-performing BC$_1$ plant was 148 and 196% of the *H. a. annuus* mean at BFL and LBJ, respectively.

Relative importance of introgression in adaptation to abiotic vs biotic environments

Organismal traits are subject to a high degree of integration, and in practice an individual trait might shape interactions with both the biotic and abiotic environments. However, it may still be valuable to ask whether traits that are more associated with one or the other type of adaptation introgress differentially. In the *H. annuus* system, we have documented intermediate or transgressive patterns for three biotic traits of 11 tested (Whitney *et al.*, 2006) and eight of 10 putative abiotic traits tested (the current study). Two and four of the biotic and abiotic traits (biotic: resistance to seed midges, resistance to receptacle-feeding Lepidoptera; abiotic: disk diameter, height of lowest branch, bushiness, relative branch diameter) were under contemporary direct natural selection in the wild, indicating their importance to adaptation. These counts could indicate that introgression of the latter class of tolerance traits is more important to adaptation in this system. However, more informative tests would directly compare the fitness benefits of 'biotic' vs 'abiotic' introgressed alleles in artificial hybrids, and/or compare the frequencies of such alleles in natural populations of the hybrid lineage. We are currently pursuing the former approach using QTL mapping in field populations of *H. annuus* × *H. debilis* BC$_1$ hybrids, including estimation of selection coefficients for *H. debilis* QTL alleles associated with abiotic vs biotic traits (K.D. Whitney *et al.*, unpublished). The latter approach was taken in a study finding high frequencies of an *H. debilis* marker associated with floral disk diameter in wild populations of *H. a. texanus* (Rieseberg *et al.*, 2007). This approach could be extended to compare the frequencies of introgressed markers associated with a variety of abiotic vs biotic traits, thereby allowing inferences about the strength of past selection on these trait types.

Conclusions

Our evidence strongly suggests that abiotic tolerance traits have introgressed across a species boundary, and that the introgression was adaptive for the recipient species *H. annuus*. More generally, our studies of the hybrid lineage *H. a. texanus* and its parents have found evidence for the likely introgression of alleles influencing 23 traits affecting gross morphology, herbivore resistance, and interaction with the abiotic environment (Kim & Rieseberg, 1999; Whitney *et al.*, 2006; this study). Clearly, introgression can influence multiple aspects of a phenotype and can shape interactions with the biotic and abiotic environment in complex ways.

This pattern has several implications. First, it suggests that studies focusing solely on introgression of alleles underlying a single focal trait – the vast majority of studies to date – may be missing introgression of traits of equal, or perhaps greater, adaptive significance. Second, it highlights the importance of examining correlations between traits via such methods as phenotypic selection analysis (Lande & Arnold, 1983) or via mapping of colocalized QTL alleles. Because an association between a single trait and fitness could reflect indirect selection via correlated traits, single trait studies are at greater risk of misinterpreting the forces behind the movement of alleles between species. Finally, the patterns described here give some guidance for interpreting the many cases of widespread marker-based molecular introgression currently being described (Morrell *et al.*, 2005; Gagnaire *et al.*, 2009; Gaskin & Kazmer, 2009). Simply, widespread marker-based introgression may reflect multiple adaptive consequences.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Correlation matrices: Pearson correlation coefficients for traits in two *Helianthus annuus* ssp. *annuus* × *Helianthus debilis* BC$_1$ populations

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