

Adaptive Introgression of Herbivore Resistance Traits in the Weedy Sunflower *Helianthus annuus*

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ABSTRACT: The role of hybridization in adaptive evolution is contentious. While many cases of adaptive trait introgression have been proposed, the relevant traits have rarely been identified, resulting in a lack of clear examples of this process. Here, we examine a purported case of adaptive introgression in which the annual sunflower *Helianthus annuus annuus* has captured alleles from a congener (*Helianthus debilis*) to form a stabilized hybrid, *Helianthus annuus texanus*. We tested the hypotheses that herbivore resistance traits have introgressed from *H. debilis* to *H. annuus* and have increased adaptation in the latter. In two common gardens, fitness (estimated by seed production) was on average 55% higher in *H. a. texanus* than in *H. a. annuus*. For *H. a. texanus*, three damage traits (of seven tested) differed significantly from the *H. a. annuus* parent in one or both sites and were shifted in the direction of the more resistant *H. debilis*. Natural selection favored *H. a. annuus* × *H. debilis* BC₁ hybrids (synthesized to mimic the ancestors of *H. a. texanus*) with *H. debilis*-like resistance to seed midges *Neolasioptera helianthis* and to receptacle/seed feeding Lepidoptera at one or both sites. Assuming similar herbivore pressures in the past, these results suggest that introgression of biotic resistance traits was important in the adaptation of *H. annuus* to central and southern Texas.

Keywords: adaptive trait introgression, herbivory, hybridization, natural selection, range expansion, seed predation.

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The role of hybridization in adaptive evolution has been the subject of a long debate (Rieseberg and Wendel 1993; Arnold et al. 1999; Barton 2001). On one hand, it has been argued that hybridization produces only transient phenomena that are incidental to the major patterns of adaptation and diversification (Wagner 1970; Mayr 1992; Schemske 2000). However, the rate of adaptation can be mutation limited, as evidenced in selection experiments by the stepped pattern of fitness increases that corresponds to the fixation of newly arising mutations (e.g., Lenski et al. 1991; Burch and Chao 1999). Given this limitation, it is plausible that hybridization could result in the acquisition of beneficial alleles that speed adaptation. Anderson (1949), Stebbins (1959), Grant (1971), and Arnold (1997, 2004) have argued that despite constraints imposed by fertility/inviability problems in early-generation hybrids, hybridization has made a substantial contribution to adaptation and to evolutionary diversification.

Hybridization potentially can contribute to adaptive evolution by triggering phenotypic change along two routes. First, hybridization can result in novel or extreme traits, as has been suggested for the tephritid fly *Bactrocera tyroni* (Lewontin and Birch 1966) and shown for the hybrid sunflowers *Helianthus paradoxus*, *Helianthus deserticola*, and *Helianthus anomalus* (Lexer et al. 2003; Gross et al. 2004; Ludwig et al. 2004). Such traits are often the product of transgressive segregation, in which alleles from both parents combine to produce extreme phenotypes (Rieseberg et al. 1999). Second, hybridization can, in theory, result in adaptive trait introgression, in which fitness-increasing alleles (and their associated phenotypic effects/trait values) are transferred from one population to another (Rieseberg and Wendel 1993). Many potential instances of adaptive introgression have been proposed for both plants (Heiser 1951*b*; Klier et al. 1991; Arnold and Bennett 1993; Paterson et al. 1995; Wang et al. 1997; Neuffer et al. 1999; Milne and Abbot 2000; Abbott et al. 2003; Bleeker 2003; Pysek et al. 2003) and animals (Grant and Grant 1996; Doiron et al. 2002; Pfennig and Simovich 2002; Fonseca et al. 2004).

Ideally, adaptive trait introgression would be demon-

strated via evidence that particular alleles or chromosomal segments (with known phenotypic effects) from a donor parent are under positive selection in a hybrid lineage. However, the genetic bases for most traits in wild species are unknown. Also, advantageous alleles may spread rapidly to fixation in the recipient species, making adaptive introgression difficult to “catch in the act” (Barton 2001). In the absence of such information, documentation of adaptive trait introgression should reasonably comprise five criteria: (1) evidence of genetic introgression; (2) evidence that the introgression is not ephemeral, that is, that a stabilized hybrid lineage has formed; (3) evidence that later-generation hybrid genotypes are more fit than parentals (notably, because heterosis is transitory, high fitness in early-generation [particularly F_1] hybrids does not guarantee criterion 2; Rieseberg and Carney 1998; Barton 2001); (4) evidence that mean values of one or more fitness-related traits in the hybrids are shifted toward those of the donor parent; and (5) evidence that natural selection has favored hybrids with trait values shifted toward those of the donor parent. While historical patterns of selection are ultimately unknowable, insight may be gained by evaluating current selective regimes and assessing whether past regimes may have been similar.

It is rare to have evidence bearing on these five criteria for adaptive trait introgression in a single system (Abbott et al. 2003). However, good evidence for some of the criteria has been found. In Darwin’s finches (Grant and Grant 1996), hybridization frequencies (determined via pedigree analysis) dramatically increased following a severe El Niño event. Hybrid fitness increased as well, so that F_1 ’s and early backcrosses had equal and sometimes greater fitness than parental species. High fitness in hybrids was related to beak traits: selection favored beak shape values characteristic of the hybrids, which were observed to more efficiently exploit an assemblage of small-seeded plant species that became common after the El Niño event. These data suggest the occurrence of the early stages of adaptive introgression, but it is not known whether this introgression is ephemeral. In a second example, hybrid Louisiana irises have demonstrably higher fitness than parentals in an intermediate habitat, where light levels fall between those typically experienced by the parental species, *Iris fulva* and *Iris hexagona* (Bennett and Grace 1990; Arnold and Bennett 1993; Emms and Arnold 1997). Shade tolerance derived from *I. fulva* is the proposed introgressive trait. However, evidence that this trait is responsible for the elevated fitness is lacking: in a shade manipulation experiment, hybrids did not outperform the supposedly shade-intolerant parent, *I. hexagona* (Bennett and Grace 1990), suggesting that the key traits remain to be determined. Finally, two *Senecio* species have apparently hybridized to produce a taxon (*Senecio vulgaris* var. *hiber-*

nicus) that has spread over much of the British Isles (Abbott et al. 2003). Some evidence (an allozyme locus; Abbott et al. 1992) suggests introgression while other evidence (random amplified polymorphic DNA and simple sequence repeat markers; Abbott et al. 2003) does not, as is expected when small amounts of genetic material may have been transferred. While this system is an excellent candidate for adaptive trait introgression, a complex pattern of differences in outcrossing rates, seed production, and seed germination behavior in parents versus hybrids means that further work is needed to demonstrate that introgression has resulted in a net fitness benefit (Abbott et al. 2003).

Here, we use a classic system in *Helianthus* to examine whether herbivore resistance traits have crossed a species boundary and provide adaptive benefits. By attempting to link evidence of introgression, high fitness in a stabilized hybrid lineage, and natural selection on particular traits, we hope to make the most complete assessment yet of adaptive trait introgression. Heiser (1951*b*) 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. Subsequent work has confirmed via molecular markers that the two species have indeed formed a stabilized hybrid, *Helianthus annuus texanus* (criteria 1, 2; Rieseberg et al. 1990, forthcoming), and that there are few barriers to the movement of morphological quantitative trait loci (QTL) alleles between them (Kim and Rieseberg 1999, 2001). *Helianthus annuus texanus* occupies an environment that combines characteristics of the parental habitats, and there is suggestive evidence that herbivore resistance may be higher in both *H. a. texanus* and *H. debilis* than in the *H. a. annuus* parent (see “Study System,” below). However, fitness comparisons have not been made, nor have particular adaptive traits been identified. We asked these questions: Does the hybrid taxon have higher fitness than the *H. a. annuus* parent in the novel environment of central Texas (criterion 3)? Are trait means consistent with the adaptive trait introgression hypothesis; namely, do fitness-related traits exist for which the mean *H. a. texanus* phenotype differs significantly from *H. a. annuus* in the direction of *H. debilis* (criterion 4)? Can traits important in adaptation to the biotic environment be identified, and is the direction of selection on them 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 (criterion 5)?

In a companion study currently underway (R. A. Randell, K. D. Whitney, and L. H. Rieseberg, unpublished manuscript), we examine a second set of traits (including drought tolerance and avoidance) relating to adaptation

of *H. a. texanus* to the abiotic environment. Together, these two studies provide a comprehensive assessment of the adaptive value of traits differing between parental species and the potential for their introgression to influence fitness in hybrid lineages.

Methods

Study System

Helianthus annuus is a weedy, self-incompatible diploid annual with a wide distribution in North America. It is hypothesized to have achieved this wide distribution in part via introgression with congeneric species adapted to different areas (Heiser 1949, 1951a, 1951b, 1954, 1961, 1965). Although introgression has been discounted in some of these cases, it has been confirmed in several others (Rieseberg et al., forthcoming). In particular, Heiser (1951b, 1954) proposed that *H. annuus* has been able to capture advantageous genetic material from *H. debilis* ssp. *cucumerifolius*, 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. 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 and Rieseberg 1999) 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). Heiser (1951b) suggested that amalgamation of *H. a. texanus* and *H. debilis* is not occurring, despite extensive sympatry; rather, *H. a. texanus* appears to be a stabilized hybrid lineage.

To date, molecular data supports 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). Subsequently, Kim and 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. Finally, a recent survey (Rieseberg et al., forthcoming) has demonstrated that certain *H. debilis* markers reach high frequencies in natural *H. a. texanus* populations, which is suggestive of positive

selection. Furthermore, a subset of these high-frequency markers was associated with QTL underlying morphological traits (e.g., flower and inflorescence size) that vary in the direction of *H. debilis*, as is expected if the morphological intermediacy of *H. a. texanus* is the result of introgression (Rieseberg et al., forthcoming). However, the fitness consequences of these traits/QTL are unknown, so the key traits driving this case of putative adaptive introgression have not yet been identified.

In this study, we focus on herbivore and predator resistance traits for three reasons. Herbivory and seed predation are known to have strong impacts on fitness in annual sunflowers (Cummings et al. 1999; Pilson 2000; Snow et al. 2003). In addition, in studies comparing wild *H. annuus* to *H. debilis*, the latter species frequently exhibits greater resistance to a variety of insect taxa (e.g., Rogers 1981; Rogers and Seiler 1985). Finally, F₁ hybrids of *H. annuus* and *H. debilis* possess a unique sesquiterpene lactone defensive chemistry, including novel compounds in addition to a mixture of parental types (Buschmann and Spring 1995). These patterns suggest that introgression from *H. debilis* could have a substantial impact on herbivore resistance and fitness in *H. annuus*.

Plant Material

Four classes of plant material were compared in field plantings: *H. debilis*, *H. a. annuus*, F₁ × *H. a. annuus* backcrosses (BC₁), and *H. a. texanus*. Achenes (seeds hereafter) of the three wild taxa were collected from three to four populations and nine to 10 families per species (table 1). To sample the range of variability within each taxon, these collections spanned a wide area within the Texas range of each taxon 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 were used. The BC₁ generation was obtained by first mating *H. debilis* from Texas to wild *H. annuus* ssp. *annuus* from Oklahoma (for locality information, see table 1) to produce F₁ progeny in the greenhouse. In order to produce enough BC₁ seed for replicate field populations, a single progeny from the F₁ generation was selected and propagated vegetatively to produce 14 F₁ clones. A single *H. a. annuus* pollen donor was then mated to the F₁ clones to produce 3,758 BC₁ seeds. This limited-parent design was necessary to allow QTL mapping of the relevant traits in a parallel study.

To obtain seedlings for the field experiments, seeds were surface-sterilized in a 5% bleach solution, nicked with a razor blade, and germinated on damp filter paper in late February 2003. Germinating seeds were kept in the dark at room temperature; seedlings were moved into the light after they produced fine root hairs. Approxi-

Table 1: Source populations for *Helianthus* plant material used in the common gardens and in production of the experimental backcross (BC₁) populations

Taxon/population no.	State	Collection locality	Latitude, longitude	Use
<i>Helianthus annuus annuus</i> :				
RAR 58	Texas	At exit to County Rd. 551 off of Hwy. 30/65N	32.932°N, 96.382°W	CG
RAR 59/SCK 21	Texas	North of Trenton on Hwy. 69	33.524°N, 96.403°W	CG, RA
LHR 1238	Nebraska	West of white gate, Cedar Point Biological Research Station, Keith County	41.125°N, 101.39°W	CG
LHR 1223	Oklahoma	18 mi NE of Lawton on I-44, Caddo County	34.90°N, 98.10°W	A
<i>Helianthus annuus texanus</i> :				
RAR 18	Texas	Next to entrance of Chant Ranch; railroad tracks	29.102°N, 98.940°W	CG
RAR 47	Texas	Hwy. 97 and County Rd. 135 (dirt road alongside)	29.077°N, 98.223°W	CG
RAR 51	Texas	Olmos Rd. pull-out and Hwy. 476	29.440°N, 98.039°W	CG
RAR 52	Texas	Hwy. 466 east, west of Cantu Creek	29.528°N, 97.909°W	CG
<i>Helianthus debilis cucumerifolius</i> :				
RAR 46	Texas	Hwy. 97 east, before Floresville	29.056°N, 98.278°W	CG
RAR 50	Texas	Hwy. 467 north, between 475 and 5390; 2 mi south of Blue Creek	29.425°N, 98.100°W	CG
RAR 53	Texas	Hwy. 466 east, mile marker 536; 0.4 mi west of Salt Creek	29.514°N, 97.838°W	CG
RN	Texas	Galveston	...	D

Note: CG = used in common garden; A = used as *H. annuus annuus* parent in cross; D = used as *H. debilis* parent in cross; RA = used as recurrent *H. annuus annuus* parent in backcross.

mately 6-day-old seedlings were transplanted into peat pots (6 cm × 10 cm; Jiffy, Denmark) containing field soil and grown in a University of Texas at Austin greenhouse for approximately 4 weeks before transplanting to the field.

Study Sites and Experimental Design

Plant performance was examined at two sites in central Texas (novel habitats with respect to *H. a. annuus*): the Brackenridge Field Laboratory of the University of Texas at Austin (hereafter BFL; 30°16.890'N, 97°46.815'W), and the Lady Bird Johnson Wildflower Center (hereafter LBJ; 30°10.886'N, 97°52.580'W). These locations are separated by approximately 14.5 km. The BFL site is characterized by sandy riverbottom soil, while LBJ is characterized by clay soil in an oak savanna. Plots were fenced with plastic deer fencing (height 2.1 m) to reduce disturbance by deer and rabbits. Before planting, plots were tilled to remove standing vegetation. After planting, local vegetation was allowed to colonize the plots unhindered.

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*, *H. a. annuus*, *H. a. texanus*, and BC₁) 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; space limitations at BFL dictated smaller sample sizes there. Final sample sizes per site for most taxa were also 20 and 45, but because of early transplant mortality, missing data, and the exclusion of a single plant that grew abnormally large due to a nearby leaking water pipe, final sample sizes for *H. a. annuus* and *H. debilis* were both $n = 19$ at BFL and $n = 44$ and 37, respectively, for *H. a. annuus* and *H. debilis* at LBJ. Seedlings were kept moist via hand watering for 9 days following transplanting. Plant traits and fitness were measured as described below.

Selection plots. Natural selection on BC₁ hybrids was examined in a separate plot at each of the two sites (500 BC₁ seedlings per site; 90-cm spacing). Because these hybrid populations were planned for use in a long-term selection experiment that would be disrupted by extensive gene flow, they were 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 days. 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 below. At analysis, 29 plants from BFL and 37 from LBJ were excluded because of early transplant mortality or missing trait data. In total, 1,223 plants were analyzed from the selection plots and common gardens.

Putative Resistance/Palatability Traits

Trichome density. Densities of glandular trichomes and nonglandular trichomes were measured on dried leaf material. In mid-May 2003, a 13.2-mm-diameter leaf sample was taken with a no. 7 cork borer from the youngest fully expanded leaf on each plant and dried at 37°C. Trichome counts were made in a 3.8-mm² area on the leaf underside using a $\times 64$ dissecting scope fitted with a gridded eyepiece; counts were then converted to densities (trichomes mm⁻²). Glandular trichome density in many species of *Helianthus* is linearly related to leaf sesquiterpene lactone concentration (K. D. Whitney and C. Orians, unpublished data).

Leaf C : N ratio. The ratio of carbon to nitrogen may influence plant palatability to herbivores because many herbivores are nitrogen limited (Mattson 1980). The youngest fully expanded leaf on each plant was collected and dried at 37°C to constant mass in mid-May 2003. The 2.5-mg samples of dried leaf tissue were then analyzed for carbon and nitrogen using an isotope ratio mass spectrometer (PDZ Europa Integra, Cheshire, U.K.) at the University of California, Davis, Stable Isotope Facility. This method estimates total elemental concentrations, including both structural and nonstructural forms. Because of cost considerations, only a subset of the plants ($n = 17$ per taxon) was sampled in the LBJ common garden; all plants in the three remaining plots were sampled for this trait.

Plant volume. Plant size may influence herbivore attack through several mechanisms (Marquis 1992). Because destructive harvest to determine plant biomass would have interfered with the final stages of seed set, aboveground plant volume (cm³) was estimated by modeling the main stem as a cylinder with volume $\pi r^2 l$. In late August (near plant senescence), basal diameter of the main stem was measured with digital calipers, and length of the stem was measured to the nearest centimeter. In a sample of wild *H. a. texanus* growing near the study sites, plant volume as estimated by this method was highly correlated with dry biomass ($r^2 = 0.86$, $P < .0001$, $n = 18$ plants).

Damage Traits

Leaf damage. Insect damage to leaves was scored twice for each plant, once in mid-June 2003 and once in late July 2003. On each date, the three oldest nonsenesced leaves were examined. The mid-June scoring corresponded approximately with the fourth through sixth true leaves. By late July, the previously scored leaves had senesced, so newer leaves were chosen for a total of six leaves per plant across the two dates. Damage scored (and the agents) included holes and pits (Orthoptera), scrapes (Lepidoptera), leaf mines (Diptera), leafhopper burn (Cicadellidae: Homoptera), and infestations of aphids (Aphididae: Homoptera) and lacebugs (*Corythucha morrilli*, Tingidae: Hemiptera). The percentage of total leaf area affected by each type of damage was estimated with the aid of a transparent grid, and assigned a score from 0 to 4, where 0 = no damage, 1 = 0.1%–5% damage, 2 = 5.1%–25% damage, 3 = 25.1%–50% damage, and 4 = 50.1%–100% damage. Scores were used to calculate a damage index for each damage type and each plant:

$$D = \sum_{i=1}^4 \frac{n_i(C_i)}{N},$$

where i is the damage category, n_i is the number of leaves in the i th category of damage, C_i is the midpoint of each category (i.e., $C_3 = 37.5\%$), and N is the total number of leaves scored per plant (six). Units of D are percentage leaf area. Such indices have been used extensively in assessing herbivore and pathogen damage (see Benitez-Malvido and Kossmann-Ferraz 1999 and references therein). For simplicity of analysis, composite damage indices for leaf-vascular-tissue feeders (Hemiptera, Homoptera) and for leaf chewers (Orthoptera, Lepidoptera, Diptera) were constructed by summing D scores for each of the component taxa.

Stems/petiole damage. Damage by *Rhodoabaenus* weevils (Coleoptera: Curculionidae) was quantified by summing the number of lesions on all stems and petioles of each plant in late May, after the height of weevil attack. Damage caused by stem-boring larvae (Coleoptera, Lepidoptera) was quantified by summing the number of entry and exit holes per plant in either late June (BFL) or mid-July (LBJ).

Receptacle damage. Receptacles (the structures subtending the inflorescences) were subject to attack by head-feeding Lepidoptera (*Isophrictis* sp., Lepidoptera: Gelechiidae). Damage was measured by counting the number of larval holes in a sample of two to eight ($x = 6.1$) mature receptacles per plant. The average number of holes per receptacle was used as the measure of damage.

Seed damage. Because seed heads in *H. annuus* are progressively smaller in diameter and contain fewer seeds as

Table 2: Incidence of herbivore damage in the four experimental populations

Damage type	Incidence (% plants affected)			
	BFL site		LBJ site	
	BC ₁ plot	Common garden	BC ₁ plot	Common garden
Leaf-vascular-tissue damage	97.0	88.5	53.5	83.0
Leaf-chewing damage	99.8	96.2	95.4	100.0
Receptacle damage	29.5	24.4	62.3	43.9
Stem borer damage	38.8	6.4	4.0	3.5
<i>Rhodoabaenus</i> damage	66.9	42.3	.0	.0
Seed midge damage	89.6	70.5	99.6	84.8
Hole damage	16.9	2.6	54.3	35.7
Gray seed weevil damage	1.8	.0	4.0	5.3

Note: For the common gardens, incidence is averaged across the four taxa present (*Helianthus annuus annuus*, *Helianthus annuus texanus*, *Helianthus debilis*, BC₁). If a particular type of damage affected fewer than 10% of the plants at both plots within a site, it was excluded from the data analysis for that site. BFL = Brackenridge Field Laboratory; LBJ = Lady Bird Johnson Wildflower Center.

the season progresses (K. D. Whitney, personal observation; Pilson and Decker 2002), heads were bagged throughout the season (June to September) to obtain a representative sample. Bags (8 cm × 8 cm) made from plastic mesh (DelStar Technologies, Delaware) were secured with twist ties to prevent seed loss in the field. Bags were attached after pollination but before shattering (seed drop) to allow seed predators ample time to attack the heads. At plant death (August to October), a minimum of eight bagged heads per plant were collected unless the plant produced fewer heads. Heads were divided into relative size classes (large, medium, and small) according to perceived breaks in the size distribution; we recognize that this criterion was arbitrary, but it did result in identification of heads with low seed production (the small category; see below). Damage to seeds was scored at ×10 using a dissecting microscope. At least 50 ($x = 82.7$) seeds per head-size class per plant were sorted into categories including viable, midge damaged (the sunflower seed midge *Neolasioptera helianthis*; Diptera: Cecidomyiidae), hole damaged (*Isophrictis* sp.), and weevil damaged (the gray seed weevil *Smicronyx sordidus*; Coleoptera: Curculionidae). In total, 202,709 seeds were scored. For each plant, total numbers of seeds in each category were extrapolated based on counts of large- and medium-head-size classes, as was done for viable seeds (see next section). Damage scores were calculated as fractions (number of seeds in a given category/total seeds per plant).

Fitness

Viable seed production was chosen as the measure of fitness in these annual plants. For each head-size class, seed production was estimated by multiplying the total number of heads (bagged + unbagged) by the average number of viable seeds per head in a pooled sample of the bagged

heads. Preliminary analysis indicated that heads in the small-size class contained so few seeds that total seed production could be accurately estimated by the large- and medium-size classes alone ($r^2 = 0.95$, $P < .0001$, $n = 86$ BC₁ plants). Therefore, data collection was restricted to the two largest head-size classes, and fitness for each plant was estimated by the sum of seeds in these classes.

Statistical Analysis

All analyses were carried out in SAS (ver. 9.1; SAS Institute 2003). Before statistical analysis, the incidence of different types of damage was examined (table 2), and infrequent types were dropped from further consideration on a site-by-site basis. Damage types were retained for analysis if they affected more than 10% of the plants at one or both of the plots within a site (table 2).

Comparison of trait means and fitness 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, log, or arcsine-square root transformed to improve the normality of residuals and homoscedasticity. Preliminary MANOVAs on the two sets of plant traits (resistance and damage) examined the effects of taxon, site, taxon × site 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). 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 BC₁ were each compared with the *H. a. annuus* mean (the “control”) using Dunnett’s adjustment for multiple comparisons. An identical ANOVA and trait means comparison were used to identify differences in fitness (viable seed set) among the taxa and sites.

Selection analyses. Phenotypic selection analysis (Lande and Arnold 1983) was used to examine natural selection on traits in the two BC₁ 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 and included the 11 herbivore resistance-related traits discussed here as well as 10 abiotic-tolerance traits discussed in a companion article (Randell et al., unpublished manuscript). Predictor variables were transformed as necessary to improve normality and standardized to a mean of 0 and an SD of 1. Collinearity between predictor variables was assessed with Proc Reg (SAS Institute 2003). All variance inflation factors were <4.8, and all condition indices were <5.3; therefore, multicollinearity is unlikely to compromise the results (Myers 1990; Freund and Littell 2000). Correlations between variables are reported in table A1 in the online edition of the *American Naturalist*. Relative fitness was calculated as individual viable seed production divided by the mean viable seed production for the population. 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 and Shaw 1987), using a bias-corrected accelerated bootstrap with 10,000 resamples. Nonlinear selection was not examined for two reasons: first, we hypothesized directional (and not stabilizing or disruptive) selection toward the *H. debilis* phenotype; and second, the very large number of traits examined would require a tremendously complicated model (i.e., one with 231 quadratic and cross-product terms as predictor variables).

Results

Does Hybrid Helianthus annuus texanus Have Higher Fitness than the Helianthus annuus annuus Parent (Criterion 3)?

Comparisons of means indicated that *H. a. texanus* had high fitness; viable seed production was 139% and 170%

that of the *H. a. annuus* parents at BFL and LBJ, respectively, and the two taxa differed significantly (table 3). Some of this difference was the result of poor performance by the *H. a. annuus* accession from Nebraska. However, removal of this population from the analysis (i.e., comparison of only Texas accessions of the two subspecies) still demonstrated high relative fitness for *H. a. texanus* (seed production 110% and 125% that of *H. a. annuus* at BFL and LBJ, respectively), although the reduced sample sizes resulted in a loss of significance.

Fitness in the *H. debilis* parent tended to be lower than the *H. a. annuus* parent, but differences were not significant (table 3). As expected for an early generation cross segregating for sterility factors, the BC₁ hybrids had low mean fitness relative to the other three taxa, although particular individuals had quite high fitness. For example, seed production in the best-performing BC₁ plant was 148% and 196% of the *H. a. annuus* mean at BFL and LBJ, respectively (table 3). Overall, there were large fitness differences between taxa but not between sites (taxa: $F = 22.8$, $P < .0001$; site: $F = 4.73$, $P = .0842$; taxon \times site: $F = 1.17$, $P = .3223$). Block effect explained a significant portion of the variation ($F = 2.65$, $P = .0235$).

Are at Least Some Trait Means in Hybrid H. a. texanus Shifted toward Helianthus debilis (Criterion 4)?

Overall, traits differed strongly between taxa for both “resistance” and “damage” types of traits (MANOVA; table 4). There were significant site and taxon \times site interactions, indicating that the direction and/or magnitude by which the taxa differ depend on the site context. The block effect explained a significant portion of the variation for both sets of traits (table 4).

The *H. debilis* parent differed significantly from the *H. a. annuus* parent in seven of the 11 resistance and damage traits (table 3). Relative to *H. a. annuus*, *H. debilis* was characterized by few trichomes (both glandular and non-glandular), smaller plant volume, and reduced amounts of damage from *Rhodobaenus* weevils and seed midges. Two traits showed site-dependent patterns: relative to *H. a. annuus*, *H. debilis* had a significantly higher leaf C : N ratio at BFL and significantly lower leaf-vascular-tissue damage at LBJ, but no differences were apparent at the alternate site.

Helianthus annuus texanus also differed substantially from *H. a. annuus* for many herbivory-related traits. In particular, *H. a. texanus* phenotypes for leaf-vascular-tissue damage, receptacle damage, and seed midge damage differed significantly from *H. a. annuus* and in each case were shifted toward the more resistant *H. debilis* (table 3). For all of these traits, shifts in *H. a. texanus* phenotypes were consistent in direction across both sites, although for leaf-

Table 3: Trait values (mean \pm SE) for four *Helianthus* taxa in common gardens in two central Texas sites

Trait/site	<i>H. annuus annuus</i>		<i>H. annuus texanus</i>		<i>H. debilis</i>		BC ₁	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Putative resistance/palatability traits:								
Glandular trichome density (mm ⁻²):								
BFL	14.1 \pm 1.0	6.0–2.6	14.2 \pm 1.4	1.8–30.2	.3 \pm .2	.0–3.1	5.8 \pm 1.0	.0–13.3
LBJ	28.2 \pm 1.7	7.6–59.6	29.2 \pm 1.4	13.8–50.0	2.4 \pm .6	.0–15.6	15.8 \pm 1.1	4.2–33.7
Nonglandular trichome density (mm ⁻²):								
BFL	11.5 \pm 1.0	4.2–20.6	11.5 \pm .7	7.0–18.0	5.8 \pm .6	2.9–15.1	13.6 \pm .9	6.5–22.9
LBJ	17.5 \pm 1.1	7.8–43.7	16.7 \pm .6	10.4–24.7	12.7 \pm .8	.3–25.2	17.4 \pm .7	9.9–26.8
Leaf C : N:								
BFL	7.0 \pm .2	6.0–8.2	7.3 \pm .2	6.3–8.8	8.4 \pm .3	6.7–11.0	7.4 \pm .2	6.5–9.3
LBJ	9.7 \pm .3	7.9–11.7	9.8 \pm .3	8.6–11.8	9.5 \pm .5	7.8–16.8	9.9 \pm .2	7.8–11.3
Plant volume (cm ³):								
BFL	300.3 \pm 99.7	14.3–1,595.7	244.8 \pm 56.2	10.1–958.6	28.8 \pm 5.0	4.7–88.6	204.7 \pm 35.7	27.4–642.7
LBJ	441.3 \pm 64.2	4.4–1,866.9	393.2 \pm 39.5	52.9–1,316.5	39.9 \pm 6.2	1.5–173.7	378.8 \pm 24.0	106.0–786.7
Damage traits:								
Leaf-vascular-tissue damage (% leaf area)								
Hemiptera, Homoptera:								
BFL	12.6 \pm 2.4	.0–33.3	10.3 \pm 2.2	.0–36.7	7.2 \pm 1.7	.0–26.7	20.3 \pm 2.8	.0–42.5
LBJ	24.9 \pm 3.6	.0–117.9	17.7 \pm 2.7	.0–75.8	9.1 \pm 2.1	.0–40.8	18.2 \pm 3.3	.0–122.5
Leaf-chewing damage (% leaf area)								
Orthoptera, Lepidoptera, Diptera:								
BFL	3.0 \pm .5	.0–6.7	2.3 \pm .3	.4–5.4	2.8 \pm .8	.0–15.4	3.2 \pm .6	.4–10.4
LBJ	9.4 \pm 1.2	1.7–42.5	7.2 \pm .6	.4–18.3	13.7 \pm 1.7	.8–38.3	9.6 \pm 1.3	.8–32.1
Receptacle damage (no. holes/receptacle)								
<i>Isophrictis</i> sp.:								
BFL	.15 \pm .07	.00–1.00	.04 \pm .02	.00–.33	.10 \pm .05	.00–.80	.09 \pm .05	.00–.80
LBJ	.30 \pm .07	.00–2.40	.08 \pm .02	.00–.63	.17 \pm .04	.00–.70	.20 \pm .04	.00–.83
Stem borer damage (no. holes/plant):								
BFL	.21 \pm .16	.00–3.00	.00 \pm .00	.00–.00	.00 \pm .00	.00–.00	.80 \pm .55	.00–10.00
LBJ
<i>Rhodoabaenus</i> damage (no. lesions/plant):								
BFL	1.9 \pm .7	.0–10.0	1.1 \pm .6	.0–11.0	.0 \pm .0	.0–.0	1.3 \pm .3	.0–4.0
LBJ
Midge damage (fraction seeds killed)								
<i>Neolasioptera helianthis</i> :								
BFL	.18 \pm .04	.00–.49	.14 \pm .04	.00–.59	.00 \pm .00	.00–.01	.49 \pm .06	.04–.96
LBJ	.42 \pm .04	.00–.89	.18 \pm .03	.00–.69	.01 \pm .00	.00–.13	.81 \pm .02	.40–.98
Hole damage (fraction of seeds killed)								
<i>Isophrictis</i> sp.:								
BFL	.000 \pm .000	.000–.000	.002 \pm .002	.000–.033	.000 \pm .000	.000–.000	.001 \pm .001	.000–.026
LBJ	.011 \pm .003	.000–.110	.003 \pm .001	.000–.036	.008 \pm .002	.000–.039	.008 \pm .003	.000–.144
Fitness traits:								
No. viable seeds:								
BFL	1,703.4 \pm 467.4	177.4–7,663.2	2,371.6 \pm 621.5	251.5–12,238.2	932.3 \pm 161.6	65–3,033.8	570.1 \pm 130.0	11.0–2,520.2
LBJ	2,566.1 \pm 492.9	133.0–17,990.3	4,370.3 \pm 375.8	625.5–12,344.0	1,604.3 \pm 300.2	44.0–10,091.1	1,201.9 \pm 184.3	38.0–5,031.0

Note: BFL = Brackenridge Field Laboratory; LBJ = Lady Bird Johnson Wildflower Center. Trait values for *H. a. texanus*, *H. debilis*, and BC₁ are in bold if they differ significantly ($P < .05$) from *H. a. annuus* using Dunnett's adjustment for multiple comparisons in a mixed-model ANOVA. Initial MANOVAs (table 4) 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 BFL ($n = 19$ for each), *H. a. annuus* and *H. debilis* at LBJ ($n = 44$ and 37, respectively) and leaf C : N ratio for all taxa at LBJ ($n = 17$ per taxon). Ellipses indicate that damage was absent for the site.

Table 4: MANOVAs for two sets of traits measured on four *Helianthus* taxa in common gardens in two central Texas sites

Trait set/effect	df	Pillai's	F	P
Resistance:				
Taxon	12, 702	1.278	43.39	<.0001
Site	4, 2	.975	19.56	.0492
Taxon × site	12, 702	.174	3.59	<.0001
Block (site)	20, 940	.197	2.43	.0004
Damage:				
Taxon	15, 702	.694	14.08	<.0001
Site	5, 1	.991	21.31	.1629
Taxon × site	15, 702	.151	2.48	.0015
Block (site)	25, 1,180	.399	4.10	<.0001

Note: Traits contained within each set are listed in table 3.

vascular-tissue damage and midge damage, differences reached statistical significance only at LBJ, a site with both higher herbivore attack rates and larger sample sizes.

*Does Selection Favor Resynthesized Hybrids with
Trait Values Shifted toward Those of
H. debilis (Criterion 5)?*

Selection differentials and gradients for the two BC₁ populations are given in table 5. The traits examined collectively explained a fairly high proportion of the variance in relative fitness ($R^2 = 0.48$ and 0.59 in BFL and LBJ, respectively). In the BFL population, total selection favored phenotypes with high densities of glandular trichomes, reduced leaf C : N ratios, increased plant volume, increased levels of damage by stem borers and *Rhodobaenus* weevils, and decreased levels of damage by leaf-vascular-tissue feeders and seed midges. However, examination of selection gradients indicated that many of these patterns were caused by selection on correlated characters; direct selection was found only for increased plant volume and decreased levels of damage by receptacle-feeding insects and seed midges. With reference to the mean trait values (table 3), direct selection on all of these traits was in the direction of the *H. a. texanus* phenotype.

In the LBJ population, total selection favored phenotypes with reduced leaf C : N ratios, increased volume, increased levels of damage by feeders on leaf vascular tissue, and decreased levels of several types of damage (receptacle, seed midge, and hole damage). Again, many of these patterns were driven by indirect selection via correlated characters because direct selection was found only for increased leaf C : N ratio, increased plant volume, decreased seed midge damage, and decreased hole damage (*Isophrictis* sp.) to seeds. Direct selection on three of the four traits (all but leaf C : N ratio) was in the direction of the *H. a. texanus* phenotype.

Discussion

Here, we have compiled evidence for adaptive trait introgression from Texas populations of *H. debilis* into *H. annuus*. Previous work (Rieseberg et al. 1990, forthcoming) has confirmed the introgression of chromosomal blocks and the formation of a stabilized hybrid lineage (criteria 1 and 2 in the introduction to this article). New evidence presented here consists of three major observations on fitness effects and on the adaptive value of specific traits, corresponding to criteria 3, 4, and 5.

First, hybridization may have contributed to increased adaptation in *H. annuus*. The stabilized hybrid *H. a. texanus* had consistently higher fitness than the *H. a. annuus* parent when both were grown in the novel environment of central Texas (mean seed production increase of 55% across two sites).

Second, traits fulfilling the expectations of introgression do exist. These are traits for which the mean hybrid phenotype differs significantly from the recipient parent in the direction of the donor parent. In addition to several intermediate morphological traits documented in Kim and Rieseberg (1999), at LBJ, three damage traits (leaf-vascular-tissue damage, receptacle damage, and seed midge damage) in *H. a. texanus* differed significantly from the *H. a. annuus* parent and were shifted in the direction of the more resistant *H. debilis*. At BFL, these shifts were in the same direction but reached statistical significance only for receptacle damage, probably reflecting lower herbivore attack rates at that site. In the schema of Fritz (1999), damage patterns are consistent with additive inheritance of resistance alleles for leaf-vascular-tissue damage and seed midge damage and also with the "hybrid resistance" pattern (a type of transgressive segregation model) for receptacle damage.

Third, of these three traits, two are demonstrably important in present-day adaptation to the environment of central south Texas. As evidenced by selection gradients on the resynthesized hybrids (BC₁s), plants with reduced levels of receptacle damage had higher fitness (at the BFL site), as did plants with reduced levels of seed midge predation (at both sites). These patterns suggest that the introgression of resistance to these insect attackers was adaptive during the formation of *H. a. texanus*, although the specific resistance mechanisms remain to be elucidated. Overall, the selective patterns on the resynthesized hybrids (BC₁s) suggest that current selective regimes resemble past regimes during the evolution of the *H. a. texanus* phenotype. Considering all measured traits, direct selection in most cases was in the direction of the *H. a. texanus* phenotype (86% of the seven significant gradients). Total selection was also in the direction of *H. a. texanus* (77% of the 13 significant differentials), indicating that the current

Table 5: Selection differentials (s') and selection gradients (β) for resistance and damage traits in two *Helianthus annuus annuus* × *Helianthus debilis* BC₁ populations in central Texas

Population/trait	s'	P	β	95% CI	
				Lower	Upper
BFL:					
Glandular trichome density (mm ⁻²)	.16^T	.0001	.04	-.033	.115
Nonglandular trichome density (mm ⁻²)	.05	.2432	.03	-.028	.094
Leaf C : N	-.14^T	.0008	-.01	-.093	.064
Plant volume (cm ³)	.46^T	<.0001	.26^T	.179	.342
Leaf-vascular-tissue damage (fraction of leaf area)	-.14^T	.0006	-.02	-.093	.048
Leaf-chewing damage (fraction of leaf area)	-.01	.8691	.03	-.034	.093
Receptacle damage (no. holes/head)	-.05	.2403	-.07^T	-.124	-.018
Stem borer damage (no. holes/plant)	.08^A	.0397	.05	-.018	.138
<i>Rhodoabaenus</i> damage (no. petioles/plant)	.11^A	.0105	.02	-.033	.084
Midge damage (fraction seeds killed)	-.36^T	<.0001	-.31^T	-.387	-.244
Hole damage (fraction seeds killed)	-.02	.5814	.01	-.037	.058
LBJ:					
Glandular trichome density (mm ⁻²)	-.06	.2461	.03	-.048	.128
Nonglandular trichome density (mm ⁻²)	.06	.3184	.06	-.024	.139
Leaf C : N	-.37^T	<.0001	.14^A	.024	.262
Plant volume (cm ³)	.64^T	<.0001	.51^T	.358	.695
Leaf-vascular-tissue damage (fraction of leaf area)	.18^A	.0011	.07	-.007	.185
Leaf-chewing damage (fraction of leaf area)	-.06	.246	-.04	-.110	.030
Receptacle damage (no. holes/head)	-.14^T	.0154	.03	-.051	.110
Midge damage (fraction seeds killed)	-.63^T	<.0001	-.55^T	-.693	-.419
Hole damage (fraction seeds killed)	-.13^T	.0192	-.18^T	-.248	-.102

Note: BFL = Brackenridge Field Lab; LBJ = Lady Bird Johnson Wildflower Center. Sample sizes are $n = 471$ for BFL and $n = 463$ for LBJ. For differentials/gradients that are significantly different from 0 ($P < .05$), values are in boldface, and superscripts indicate whether selection is predicted to move the trait toward or away from the *H. a. texanus* phenotype (based on table 3). Superscript T = toward; superscript A = away; CI = confidence interval.

genetic correlation structure may approximate its past structure as well. These observations give us greater confidence that the identification of traits that are currently adaptive is relevant to understanding past adaptation, that is, in the period following the original introgression.

In at least one way, the results presented here represent a conservative test of the adaptive trait introgression hypothesis for this system. Because a limited-parent design was necessary a parallel QTL mapping study, only three individuals of the parental species contributed genes to the BC₁ generation. Since this is probably far fewer individuals than would have been involved in the ancient hybridization event, our chances of recovering fit hybrid genotypes were correspondingly reduced.

It is worthwhile to contrast introgressive race formation with hybrid speciation in the context of the *H. a. texanus* system. Hybrid lineages may or may not be reproductively isolated from their parental species and are typically referred to as species and introgressive races, respectively. Crossing studies indicate that *H. a. texanus* is interfertile with other subspecies of *H. annuus* (Heiser 1954), so it is best classified as an introgressive race. Although we do not know why hybridization has resulted in introgressive race

formation rather than speciation in this instance, conditions favoring the latter are stringent and include the presence of a novel habitat that is not easily colonized by either parental species, as well as substantial spatial isolation (Buerkle et al. 2000). Neither of these conditions is fulfilled in the *H. a. texanus* system.

Adaptive Introgression of Resistance to Seed Predators

Resistance to seed midges (*Neolasioptera helianthis*) may be a key trait in these annual sunflowers, which rely on seeds for reproduction. In the *H. a. annuus* populations at the two sites, seed midges destroyed an average of 18% and 42% of the seed crop, respectively, and individual plants had up to 89% seed loss (table 3). In contrast, *H. a. texanus* experienced average losses of 14% and 18%, with the worst performance of an individual plant at 69% seed loss. *Helianthus debilis* experienced average losses of <1% across sites. This trait was under strong selection in both resynthesized hybrid populations ($\beta = -0.31$ and $\beta = -0.55$).

It is not clear what plant traits may underlie this resistance to seed midges; possibilities include both physical

and chemical traits. Physical traits such as ovary or achene wall thickness may affect oviposition success and/or larval development of the midge. A layer of phytomelanin in *Helianthus* seeds has been shown to reduce seed predation by moth larvae (see Rogers et al. 1987) and conceivably could affect seed midge attack. Another class of compounds, sesquiterpene lactones, is present in glands on the anthers in many *Helianthus* species (Seaman 1982; Rossiter et al. 1986; Rogers et al. 1987), and as mentioned previously, unique sesquiterpene lactone defensive chemistry may be present in *H. annuus* × *H. debilis* hybrid lineages (Buschmann and Spring 1995). A future avenue of research will be examination of sesquiterpene lactone presence and composition in flowers of *H. a. texanus* and the effects of these compounds on oviposition success and damage by the seed midge.

Interestingly, *N. helianthis* is not restricted to Texas, and in fact, it attacks sunflowers at least as far north as Nebraska (D. Pilson, personal communication). This begs the question of why *H. debilis*-derived resistance has not introgressed further northward into "pure" *H. a. annuus* populations. One general possibility is that resistance alleles have pleiotropic effects that are maladaptive in northern climes. Another possibility is that seed midge attack rates are geographically variable, with lower attack rates and reduced selective pressure for resistance at higher latitudes. While this idea is speculative, there are known clines in factors that could affect attack rates. For example, there is a well-documented cline in *Helianthus* seed oil composition, in which the ratio of saturated : unsaturated oils decreases with latitude (Linder 2000). Conceivably, this cline could have impacts on palatability and/or larval development and therefore oviposition rates of *N. helianthis*.

In addition to the seed midge, resistance to another seed predator (*Isophrictis* sp.) appears to be important in this system. These caterpillars tunnel through the receptacle, interfering with normal seed development, and also chew holes into maturing seeds. As *H. a. texanus* shows reduced receptacle damage relative to both parent species, this enhanced resistance may result from transgressive segregation. Given that selection sometimes favors reductions in damage by this predator in central Texas (receptacle damage at BFL, $\beta = -0.07$; hole damage at LBJ, $\beta = -0.18$), introgression of alleles affecting resistance to *Isophrictis* could also have played a role in the adaptation of *H. annuus* to more southern areas.

Resistance to Leaf-Feeding Insects

Perhaps surprisingly, damage by leaf-feeding insects did not appear to affect plant fitness at either site; selection gradients were nonsignificant for damage by both leaf

chewers and vascular-tissue feeders (table 5). These patterns are consistent with a concurrent field experiment at LBJ in which weekly treatment with the pesticide Sevin (carbaryl) reduced herbivore leaf damage but did not increase seed production relative to plants treated with a water control ($n = 45$ plants per treatment per taxon, unpublished data). One possibility is that *H. annuus* may exhibit a high degree of tolerance to folivory, as it does to herbivory by head-clipping weevils (Pilson and Decker 2002). Alternately, levels of leaf damage (averaging 16% and 28% of leaf area at BFL and LBJ, respectively) may simply have been insufficient to produce a fitness effect in this particular year. In many systems, herbivory affects fitness components in some years but not others (e.g., Horvitz and Schemske 2002).

Patterns of selection on putative resistance traits in leaves (glandular trichome density, nonglandular trichome density, and leaf C : N ratio) were generally consistent with these negligible fitness effects of leaf herbivory. Selection gradients were significant only for leaf C : N ratio and only at a single site. Selection differentials were consistently much larger in magnitude than selection gradients, indicating that correlations with other traits were more important than direct impacts on phenotypes in determining selection on these leaf traits.

Time Course of Adaptive Trait Introgression

A major unresolved issue in the study of adaptive trait introgression is the time course of adaptation. 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. The timing of this transition from low to high mean hybrid fitness (relative to parentals) is unknown in any system. As predicted, in the *H. a. texanus* system, mean fitness of early generation BC_1 hybrids 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 production by the best-performing BC_1 plants was 148% and 196% of the *H. a. annuus* mean at BFL and LBJ, respectively. Given higher mean fitness of the stabilized introgressant *H. a. texanus* than the *H. a. annuus* parent as shown here, we predict that mean fitness of hybrids should increase over time. Currently, the BFL and LBJ backcross populations are being maintained for long-term data collection, along with

a population of *H. a. annuus* to control for local adaptation. Annual fitness measurements should help resolve the question of whether mean fitness of hybrids can trump the fitness of parentals within five to 10 generations of the hybridization event.

Conclusions

Here, we have collected evidence strongly suggesting that herbivore resistance traits have introgressed across a species boundary and the introgression was adaptive for the recipient species *H. annuus*. In an ongoing QTL mapping project, we are bringing the question full circle by asking whether QTL derived from the donor species *H. debilis* (linked to the traits identified here) are under positive selection in hybrids. This work should provide the first definitive evidence bearing on the adaptive significance of introgressed chromosomal blocks in a natural system.

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