Published in final edited form as:

Mol Ecol. 2015 May ; 24(9): 2194–2211. doi:10.1111/mec.13044.

QTL mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower

Kenneth D. Whitney¹, Karl W. Broman², Nolan C. Kane³, Stephen M. Hovick⁴, Rebecca A. Randell⁵, and Loren H. Rieseberg^{5,6}

Kenneth D. Whitney: whitneyk@unm.edu

¹Department of Biology, MSC03 2020, University of New Mexico, Albuquerque, NM 87131-0001 USA, phone 505 277-4408; fax 505 277-0304

²Department of Biostatistics and Medical Informatics, University of Wisconsin–Madison, 2126 Genetics-Biotechnology Center, 425 Henry Mall, Madison, Wisconsin 53706 USA

³EBIO Department, UCB 334, University of Colorado, Boulder, CO 80309, USA

⁴Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43210, USA

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

⁶Department of Botany, University of British Columbia, 3529-6270 University Blvd., Vancouver, B.C. Canada V6T 1Z4

Abstract

The wild North American sunflowers *Helianthus annuus* and *H. debilis* are participants in one of the earliest identified examples of adaptive trait introgression, and the exchange is hypothesized to have triggered a range expansion in *H. annuus*. However, the genetic basis of the adaptive exchange has not been examined. Here, we combine quantitative trait locus (QTL) mapping with field measurements of fitness to identify candidate *H. debilis* QTL alleles likely to have introgressed into *H. annuus* to form the natural hybrid lineage *H. a. texanus*. Two 500-individual BC₁ mapping populations were grown in central Texas, genotyped for 384 SNP markers, and then phenotyped in the field for two fitness and 22 herbivore resistance, ecophysiological, phenological, and architectural traits. We identified a total of 110 QTL, including at least one QTL for 22 of the 24 traits. Over 75% of traits exhibited at least one *H. debilis* QTL allele that would shift the trait in the direction of the wild hybrid *H. a. texanus*. We identified three chromosomal regions where *H. debilis* alleles increased both female and male components of fitness; these regions are expected to be strongly favored in the wild. QTL for a number of other ecophysiological, phenological, and architectural traits co-localized with these three regions and are candidates for the actual traits driving adaptive shifts. G × E interactions played a modest role,

AUTHOR CONTRIBUTIONS

Designed the study: KDW LHR RAR. Fieldwork and phenotyping: KDW RAR. SNP development, DNA extraction and genotyping: NCK KDW RAR. QTL mapping and data analysis: KWB KDW. Wrote the manuscript: KDW KWB SMH LHR.

DATA ACCESSIBILITY

Phenotype and SNP genotype data for the mapping populations, as well as the estimated linkage map, are available from the Dryad data repository DOI:10.5061/dryad.5dh5f.

with 17% of the QTL showing potentially divergent phenotypic effects between the two field sites. The candidate adaptive chromosomal regions identified here serve as explicit hypotheses for how the genetic architecture of the hybrid lineage came into existence.

Keywords

hybridization; adaptive trait introgression; quantitative trait locus; SNP; herbivory; seed predation; phenology; plant architecture; range expansion; invasion

INTRODUCTION

Adaptive trait introgression is the transfer of fitness-increasing traits between species via hybridization and backcrossing (Rieseberg and Wendel 1993). Early potential examples were described by Anderson (1949), Heiser (1951) and Stebbins (1959). Their view that adaptive introgression is a frequent and important evolutionary process has received increasing support (Abbott et al. 2013, Arnold 2006, Hedrick 2013). By sidestepping the waiting time for new mutation, introgression has the potential to allow adaptation at rates that exceed those possible for populations dependent solely on mutation for genetic novelty (Abbott et al. 2013, Hedrick 2013; but see Barton 2013). Introgression has been suggested as a trigger of macroevolutionary patterns such as adaptive radiation, via the generation of large amounts of genetic and phenotypic novelty (Seehausen 2004, 2013), and can also have cascading effects on fundamental ecological processes such as colonization and species invasion (Baker & Stebbins 1965, Hovick et al. 2012, Hovick & Whitney 2014; see also Whitney et al 2009).

Until recently, adaptive trait introgression was most often inferred from patterns in phenotypic traits, with infrequent determination of the underlying genetics (Chapman & Abbott 2010). Identification of the actual genomic regions/alleles involved in adaptive trait introgression is key to both demonstrating the existence of this phenomenon and understanding its role in adaptive evolution. In the past few years, there has been a burst of progress in this area: specific genes or quantitative trait loci (QTL) have been identified that control introgressing traits such as self-incompatibility in wild *Arabidopsis* (Castric et al. 2008), inflorescence morphology affecting pollination in *Senecio* (Kim et al. 2008; Chapman & Abbott 2010), rodenticide resistance in house mice (Song et al. 2011), and mimetic wing coloration affecting predation in *Heliconius* butterflies (Pardo-Diaz et al. 2012, Heliconius Genome Consortium 2012). In addition, other studies have identified introgressing traits and their genetic bases, but have not yet experimentally confirmed that they are indeed adaptive in the wild (e.g. melanism in wolves, Anderson et al. 2009, see also Hedrick 2013; pigment and leaf traits in maize, Hufford et al. 2013). Other studies have identified QTL alleles that appear to have high adaptive value across species boundaries, even though introgression for these alleles has not been documented in nature (e.g. alleles for flood tolerance in wetland *Iris* species; Martin et al. 2005, 2006). Still other studies have used molecular signatures of selection to identify introgressing genomic regions without identification of the phenotypic traits affected (e.g., Fitzpatrick et al. 2009, Gagnaire et al. 2009, Roux et al. 2013).

The North American sunflower subspecies *Helianthus annuus texanus* represents one of the earliest identified (Heiser 1951, 1954) and most prominent (Grant 1971; Arnold 2004, 2006) examples of adaptive trait introgression, but to date, the genetic basis of the adaptive introgression event(s) have not been elucidated. Heiser (1951) 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. *H. a. texanus* 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 1951). Subsequent work has confirmed via molecular markers that the two species have indeed formed a stabilized hybrid, *H. a. texanus* (Rieseberg et al. 1990, Scascitelli et al. 2010), that there are few barriers to the movement of morphological quantitative trait loci (QTL) alleles between them (Kim and Rieseberg 1999, 2001), and that some *H. debilis*-derived markers reach high frequencies and thus appear to be linked to *H. debilis* alleles under positive selection in natural populations of the hybrid lineage (Rieseberg et al. 2007).

Potential traits that may have been influenced by adaptive introgression of *H. debilis* alleles have been identified in two ways (Whitney et al. 2006, 2010). First, comparison of the phenotypes of the parents and naturally occurring individuals from the hybrid lineage have suggested several traits where a) the hybrid phenotype differs significantly from the *H. annuus* parent in the direction of *H. debilis*, potentially indicating past transfer of *H. debilis* alleles; or b) the hybrid has extreme trait values, indicating that past transfer of *H. debilis* alleles plus transgressive segregation (wherein extreme phenotypes can arise from particular combinations of parental alleles, Rieseberg et al. 1999) could explain the hybrid phenotype. Second, phenotypic selection analysis (Lande & Arnold 1983) of resynthesized hybrid populations grown in nature has been used to identify traits that may have been under strong selection during the formation of the natural hybrid lineage. In cases where the *H. debilis* phenotype is in the direction of greater fitness, adaptive transfer of *H. debilis* alleles has been hypothesized. Candidate introgressed traits identified by these methods include increased resistance to insect seed predators and herbivores (Whitney et al. 2006), as well as lower water use efficiency, higher specific leaf area, more rapid phenology, and a bushier plant architecture with increased allocation to branches (Whitney et al. 2010). Thus, while the vast majority of documented cases of adaptive trait introgression identify a single introgressing trait per species (e.g. Martin et al., 2005, 2006; Grant & Grant, 1996, 2008; Uy & Stein, 2007; Kim et al., 2008), introgression in the case of *H. a. texanus* has apparently affected multiple aspects of the phenotype, making the genetic architecture of introgression in this system of particular interest.

The above methods identify candidate traits involved in adaptive introgression between these sunflower species, but do not identify candidate genomic regions/alleles. Here, we combine quantitative trait locus (QTL) mapping approaches with field measurements of fitness to identify *H. debilis* QTL alleles that increase fitness in the hybrid background. These candidate alleles serve as explicit hypotheses for how the genetic architecture of the hybrid lineage came into existence. We first created 1,000 *H. annuus* x *H. debilis* hybrid seedlings to mimic the early ancestors of the natural hybrid lineage *H. a. texanus*. We then

split these seedlings among replicate mapping populations and planted them into two field locations in the hybrid's natural range in central Texas, USA. This area represents a southerly range extension with respect to the *H. annuus* parent. We measured 22 traits (comprising ecophysiological, phenological, architectural, and herbivore/predator resistance traits) and fitness. We genotyped the plants at 384 single-nucleotide polymorphism (SNP) loci and constructed QTL maps for the two mapping populations. We asked:

1) Could introgression of *H. debilis* alleles explain the *H. a. texanus* phenotype?

In other words, are there *debilis*-derived alleles capable of pushing the phenotype towards *texanus*? Previous studies have confirmed this hypothesis for alleles affecting gross morphological traits such as height and leaf serration (Kim & Rieseberg 1999), but here we focus on alleles affecting potential adaptive traits known to interact with the biotic and abiotic environment.

2) Are there *H. debilis*-derived alleles that increase fitness in hybrids?

These are candidate QTL alleles for the adaptive introgression event(s). We evaluate the effects of these alleles in two different habitats to obtain a preliminary assessment of how consistently favored they might have been across environments.

METHODS

Study species

H. a. annuus, *H. debilis*, and their stabilized hybrid lineage *H. a. texanus* are all annual outcrossing taxa (Heiser 1951, 1954). *H. a. annuus* is a widespread species in North America; *H. debilis* has a very restricted distribution in central Texas and a few small, widely dispersed locations on the Eastern seaboard; and *H. a. texanus* is distributed in central and southern Texas (Rogers et al. 1982). In central Texas, germination occurs during the winter, flowering commences in May, seeds are set in mid- to late summer, and plants senesce from mid-August through September or October.

Backcross (BC₁) mapping populations and field sites

We synthesized Backcross 1 (BC₁) mapping populations with an eye towards mimicking the ancestral early-generation hybrids that gave rise to *H. a. texanus*. An F₁ generation was first obtained by mating wild *H. debilis* ssp. *cucumerifolius* from Texas to wild *H. annuus* ssp. *annuus* from Oklahoma in the greenhouse. In order to produce enough BC₁ seed for replicate mapping populations, a single progeny from the F₁ generation was selected and propagated vegetatively to produce 14 F₁ clones. A single wild *H. a. annuus* pollen donor from Texas was then mated to the F₁ clones to produce 3,758 BC₁ seeds. Locality information for the three individuals used in crosses is presented in Table 1 of Whitney et al. (2006).

To extend the generality of our results and to look for QTL × Site interactions, replicate mapping populations (500 BC₁ seedlings per site; 90 cm spacing) were planted at the Brackenridge Field Laboratory of the University of Texas, Austin (hereafter BFL) and the Lady Bird Johnson Wildflower Center (hereafter LBJ). The BFL site is characterized by

sandy river bottom soil, while LBJ is characterized by clay soil in an oak savanna; the sites are separated by roughly 14.5km. Both sites are near wild populations of *H. a. texanus* and thus represent appropriate habitat for the resynthesized hybrids. Further site details are given in Whitney et al. (2006). Seeds were nicked, germinated on filter paper, and on day six transplanted into peat pots (6 × 6 × 10 cm, Jiffy A/S, Denmark) containing field soil. Seedlings were grown in a greenhouse for approximately 4 weeks before transplanting to the field in late March 2003. Seedlings were then 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 from the original germination cohort on 3 April.

Phenotyping and genotyping

BC₁ plants were measured in the field for 11 herbivore/predator resistance traits and 10 ecophysiological, phenological, and architectural traits related to tolerance of abiotic conditions (Table 1; Appendix S1, Supporting Information). Further details on these two groups of traits are found in Whitney et al. (2006) and Whitney et al. (2010), respectively. In addition, individual seed (achene) weight (mg) was estimated by weighing a batch of 50 seeds per plant and dividing by 50. Finally, two fitness traits were measured, inflorescence number and the number of seeds per inflorescence (averaged across several inflorescences per plant; see Appendix S1, Supporting Information). The former is expected to be a good estimate of lifetime male fitness, since pollinator visitation (and thus expected pollen removal) is highly correlated with inflorescence number in these species (KDW and SMH, unpubl. data). The product of the two (inflorescence number × number of seeds per inflorescence) is expected to be a good estimate of lifetime female fitness for these annual plants.

Based on expressed sequence tag (EST) libraries for 12 *H. a. annuus* individuals and 7 *H. debilis* individuals, 1649 single nucleotide polymorphisms (SNPs) were identified as fixed for alternate alleles in the two species. From this initial list, 384 SNPs were chosen such that a) only one SNP locus per gene was allowed; b) primer design criteria were met; and c) there was evidence at 100 of the 384 loci that *H. debilis* alleles were present in two sequenced individuals of the natural hybrid *H. a. texanus*. The BC₁ mapping populations were then genotyped at these 384 loci. DNA was extracted from 40mg samples of fresh leaf tissue using Qiagen DNeasy 96 plant kits in conjunction with a Qiagen Mixer Mill MM 300. SNP genotyping was accomplished via the Illumina Goldengate platform at the Texas Children's Hospital Genomics and Proteomics Core Laboratory, Houston, TX.

Linkage map construction

Genetic map construction was performed with R/qtl (Broman et al. 2003), an add-on package for the general statistical software, R (R Core Team 2013). Full details on data cleaning and linkage map construction are given in Appendix S2 (Supporting Information). Briefly, we began with genotype data at 384 markers on 975 BC₁ individuals; of the original 1000 individuals planted, 25 did not survive long enough for trait measurements to be completed. We then omitted 41 individuals with greater than 10% missing genotypes, and we omitted 32 markers with more than 200 missing genotypes. We further omitted 95 markers that appeared to be monomorphic.

Markers were expected to segregate AA:AD, where “A” and “D” represent alleles from the *H. annuus* and *H. debilis* parents, respectively. However, several markers appeared to segregate as AD:DD, and in some cases there were differences among genotyping plates in the segregation patterns, indicating problems with genotyping calls. These issues were handled as follows. First, we initially focused on 109 markers that had consistent genotype patterns across all plates. We then formed initial linkage groups by first considering all possible pairs of markers and, for each pair, estimating the recombination fraction (rf) and calculating a LOD score for the test of $rf = 0.5$. Two markers were placed in the same linkage group if they had $rf < 0.35$ and $LOD > 25$. This gave 15 linkage groups. We then ordered the markers within linkage groups by a “greedy” algorithm: We started with two random markers from the linkage group and then added one marker at a time, keeping the order of previously-mapped markers fixed, considering all possible locations for the new marker, and placing the new marker in the position that minimized the total number of obligate crossovers. This was followed by the exploration of alternate orders by considering all possible permutations in a sliding window of up to 7 markers.

The majority of the 148 remaining unmapped markers were clearly linked to some of the mapped markers, but there were a number of plate-specific problems in the genotype calls. These were corrected by inspection of plate-specific two-locus genotype tables for each unmapped marker with the most closely linked mapped marker, to infer the correct genotype pattern in the unmapped marker. We omitted markers with complex genotype patterns that could not be resolved. The unmapped markers, with corrected genotype calls, were then placed on the initial linkage map in their best-estimated position, followed by another round of alternate orders by considering all possible permutations in a sliding window of up to 7 markers.

Linkage group nomenclature follows the standard for *H. annuus* (Bowers et al. 2012), and was determined by locating 244 of our 384 SNP markers on an ultra-high density map of *H. annuus* (Renaut et al. 2013). The final map included 190 markers in 15 linkage groups, with genotype data on 906 individuals (449 from BFL and 457 from LBJ).

QTL mapping

QTL analysis was performed in R/qtl (Broman et al. 2003) using Haley-Knott regression (Haley and Knott 1992). We considered the mapping populations at the BFL and LBJ sites separately as well as jointly. In the joint analysis, an additive covariate for Site was included. That is, we allowed for a shift in the average phenotype between the two sites. We further evaluated the possibility of $QTL \times Site$ interactions by including Site as an interactive covariate (that is, allowing the effect of the QTL to be different in the two sites), comparing this to the model in which Site was strictly additive, and calculating a LOD score for the interaction (i.e., the $\log_{10}$ likelihood ratio comparing the two models). Statistical significance of the QTL mapping results, accounting for the genome scans, were established by permutation tests (Churchill and Doerge 1994), using 1000 permutation replicates.

Multiple-QTL analyses were then performed using the method of Broman and Speed (2002). This approach uses a stepwise model selection approach for additive QTL models (i.e., no epistatic interactions), with a penalized LOD score criterion to select a QTL model, with the

penalty on QTL being to the 5% significance threshold from the single-QTL analysis. A common model for the multiple traits was selected by extending the Broman and Speed (2002) approach to use the multivariate QTL mapping method of Knott and Haley (2000).

Some traits were square-root transformed prior to QTL analysis (see Table 1) in order to reduce the skewness of the distributions. For these traits, QTL effect sizes and their 95% confidence limits reported in the Results are back-transformed to the original units (following Sokal & Rohlf 1981, Table 13.3); however note that back-transformed values are often substantially smaller than raw values.

Analyses and interpretations

Could introgression of *H. debilis* alleles explain the *H. a. texanus* phenotype?

—For each QTL found, we compared the direction of the allelic effect of the *H. debilis* allele with the mean phenotypes of *H. a. annuus* and *H. a. texanus* (grown in common gardens) as reported in Whitney et al. (2006, 2010). We interpret cases where the *H. debilis* allelic effect is in the direction of *H. a. texanus* as evidence that introgression of *H. debilis* alleles could have led to the phenotype of the natural hybrid lineage.

Are there *H. debilis*-derived alleles that increase fitness in hybrids?—Candidate alleles involved in adaptive introgression event(s) in the history of *H. a. texanus* were identified as beneficial *H. debilis* alleles at QTL that influenced fitness directly (via effects on inflorescence number or number of seeds per inflorescence), or at QTL for other traits that co-localized with fitness QTL. We used overlapping 1-LOD support intervals as the criterion for co-localization (Lexer et al. 2003, Wessinger et al 2014). To make a preliminary assessment of which alleles are likely to have been widely favored across sites and which may have provided patchier selective advantages, we tested for the presence of G × E (that is, QTL × Site) interactions. However, we note that QTL detection is plagued by problems of repeatability across environments (Mauricio 2005), and thus even statistically significant QTL × Site interactions in our study should be treated as hypotheses of differential phenotypic effects rather than as proof of such.

RESULTS

QTL map

A condensed map showing QTL for the two fitness traits (and QTL for other traits that co-localize with them) is presented as Fig 1. The full QTL map is presented as Figure S1 (Supporting Information). LOD support intervals for each QTL (used in assessing co-localization) are presented in Table S1 (Supporting Information). Our linkage map recovered 15 linkage groups, relative to the expected chromosome number $n=17$ for these *Helianthus* species. The reduced number of linkage groups may reflect reciprocal translocations that are known to distinguish the parental species (Chandler et al. 1986) which would tend to join markers from two chromosomes in a single linkage group (e.g., chromosomes 12 and 17, designated here as linkage group 12/17).

Could introgression of *H. debilis* alleles explain the *H. a. texanus* phenotype?

Excluding QTL for the two fitness traits (treated in the next section), we found a total of 98 QTL influencing traits in our field-grown BC₁ hybrids (Table 1, Figure S1). Eighty-one of these QTL had consistent effects across sites, while 17 of them showed potential site-specific effects (i.e., the QTL $\times$ Site interaction term was significant). Of 22 traits examined, 20 were associated with at least one significant QTL (all but stem borer damage and *Rhodobaenus* damage).

Sixteen of the 20 traits for which at least one QTL was found showed evidence of at least one *H. debilis* allele that would shift the trait in the predicted direction (Table 1). Of the 81 QTL found with consistent effects across sites, the *debilis* allele is in the direction of the *H. a. texanus* phenotype in 54 cases (67%) and in the opposite direction in the remaining 27 cases (33%; Table 1). For the 17 QTL found with site-specific effects, there are 34 possibilities (17 QTL $\times$ 2 sites): the *debilis* allele was in the direction of the *H. a. texanus* phenotype in 14 cases (41%), in the opposite direction in 8 cases (24%), and had no significant effect in 12 cases (35%; Table 1).

The 11 traits suggested previously to have been strong candidates for showing adaptive introgression from *H. debilis* (Whitney et al. 2006, 2010) are indicated via footnotes in Table 1. Of these traits, nine (82%) are associated with at least one QTL, and seven (64%) are associated with three or more QTL, where the *debilis* alleles would shift the phenotype both a) in the direction of the *H. a. texanus* phenotype and b) in the direction of increased adaptation.

Are there *H. debilis*-derived alleles that increase fitness in hybrids?

We found seven QTL influencing inflorescence number and five QTL influencing the number of seeds per inflorescence (Table 1, Fig. 1). *H. debilis* alleles were associated with fitness increases at four of the inflorescence number loci (linkage groups 7, 9, 12/17@0 cM, and 12/17@58.6 cM). At an additional inflorescence number locus on linkage group 15, we found evidence of a QTL $\times$ Site interaction where the *H. debilis* allele was associated with increased fitness at the BFL site but did not affect fitness at the LBJ site. For the seeds per inflorescence loci, *H. debilis* alleles were never associated with increases in fitness (Table 1, Fig. 1).

While QTL for these two types of fitness traits often did not co-localize (Fig. 1), in other regions they did form clusters: two clusters on linkage group 12/17 and one on linkage group 15 (Fig. 1). Each cluster could represent either single pleiotropic QTL or multiple closely linked QTL. Given that *H. debilis* genetic material acted antagonistically in these clusters (i.e., an increase in inflorescence number was associated with a decrease in the number of seeds per inflorescence, and vice versa), we estimated the net effect on female fitness of an *H. debilis* chromosomal segment containing each set of co-localizing QTL (Table 2). Overall, we estimate that *H. debilis* genetic material is highly favored in three regions of the genome: LG7@4.7 cM, LG9@25.1 cM, and the distal end of LG12/17@58.6–60 cM (Table 2). Inheritance of *H. debilis* genetic material at all three locations simultaneously would increase female fitness (total seeds) by an estimated 136.5

and 49.5 seeds at BFL and LBJ, respectively (Table 2), and would also likely increase male fitness through increased pollen export. We note that an additional *H. debilis* allele (in the cluster on LG15@4.0–8.9 cM) could also be favored, if the beneficial effect on male fitness of producing more inflorescences (seen at the BFL site only) outweighs the net negative effect on female fitness (seen at both sites).

Hypotheses for how these three key *H. debilis* chromosomal regions actually shape the plant phenotype to increase fitness can be generated by examining trait QTL that co-localize with them. A total of 8, 6 and 13 trait QTL were found with 1-LOD support intervals that overlap the three regions on LG7, LG9 and LG12/17, respectively (see Figure 1 and Table S1).

Evidence for G × E interactions

Among fitness traits (inflorescence number, number of seeds per inflorescence) we found evidence of two loci showing G × E (i.e., QTL × Site) interactions. As mentioned above, LG15 contains a locus where the *H. debilis* allele was associated with increased inflorescence number at the BFL site but had no significant effect on that trait at the LBJ site (Table 1). LG13 contains a locus where the *H. debilis* allele decreases seeds per inflorescence at the BFL site but had no significant effect on that trait at the LBJ site (Table 1). In contrast, the 10 additional loci affecting these two fitness traits showed consistent effects across sites (no evidence for G × E).

There was also evidence of G × E interactions at a number of loci affecting the 22 other measured traits (Table 1, Figure S1). In several cases, a given allele had opposite effects on the phenotype in alternate sites. For example, at a locus on LG4, the *H. debilis* allele increased plant size by 2.5 cm³ on average at one site but decreased it by 8.42 cm³ at the other.

In total, 17% of the 110 QTL identified showed evidence of G × E interactions while the remaining 83% showed consistent effects across the two habitats examined.

DISCUSSION

Heiser (1951) first proposed that *H. a. texanus* was a stabilized introgressive lineage of *H. annuus* that had received genetic input from *H. debilis*, allowing it to colonize novel habitats in central and south Texas, USA. More recent phenotypic analyses of wild material grown in common gardens (Whitney et al. 2006, 2010) have demonstrated that the hybrid lineage has higher fitness than the *H. annuus* parent in this novel geographic range, and have also identified several adaptive traits in the hybrid lineage that appear to be derived from *H. debilis*. Here, we have shown that the majority of the phenotypic shifts in adaptive traits seen in the hybrid lineage could be explained by the action of particular *H. debilis* QTL alleles (see also Kim & Rieseberg 1999, 2001 for identification of QTL controlling general morphological traits not necessarily linked to fitness in this system). More to the point, we identified three *H. debilis* chromosomal regions which had positive impacts on both female and male fitness in resynthesized BC₁ hybrids grown in the field. To the extent that these resynthesized hybrids are representative of the spontaneous early-generation hybrids that gave rise to *H. a. texanus*, these three regions are strong candidates for the historic regions

of introgression driving both phenotypic change and increased adaptation in *H. a. texanus*. Numerous QTL for ecophysiological, phenological, and architectural traits co-localized with these three fitness-enhancing regions (see discussion below). This pattern suggests that introgression of the three regions could be responsible for much of the phenotypic shift from *H. a. annuus* to *H. a. texanus*, while not excluding the possibility that other regions of the genome might also have been subject to introgression.

Identification of QTL for adaptive (and non-adaptive) trait shifts

Hypotheses for how the three key *H. debilis* chromosomal regions shape the plant phenotype to increase fitness can be generated by examining trait QTL that co-localize with them. Potential key phenotypic effects of *H. debilis* alleles in these three regions include reductions in seed maturation time (2 QTL), floral disk diameter (3 QTL), the height of the lowest branch (2 QTL), and relative branch diameter (1 QTL); as well as increases in bushiness (2 QTL) and specific leaf area (1 QTL; increase seen at the LBJ site only).

All of the above-listed traits have been previously identified as strong candidates for adaptive introgression from *H. debilis* (Whitney et al. 2006, 2010; see Table 1), and the direction of the *H. debilis* allelic effects are as predicted from the relative rankings of the phenotypes of *H. annuus*, the hybrid *H. a. texanus*, and *H. debilis*. For example, seed maturation time in wild *H. a. texanus* hybrids is shifted towards the *H. debilis* phenotype (by -2.2 days; Table 1); and *H. debilis* alleles at QTL in two of the three regions under discussion reduce seed maturation time (by -1.1 and -1.9 days on LG7 and LG12/17, respectively; Table 1 and Fig. 1). Similarly, increased bushiness (the degree of higher-order branching) is adaptive in BC₁s in the field (selection gradients $\beta = 0.18$ to 0.20 ; Whitney et al. 2010); bushiness in wild *H. a. texanus* hybrids is shifted towards the *H. debilis* phenotype (by 0.2 units or 9%; Table 1); and *H. debilis* alleles at QTL in two of the three regions under discussion increase bushiness (by 0.05 and 0.64 units on LG7 and LG12/17, respectively; Table 1 and Fig. 1). Therefore we hypothesize that adaptive introgression of *H. debilis* chromosomal segments in these three regions may have occurred, and furthermore may have been driven in part by natural selection for more rapid seed maturation time and increased bushiness, along with possible selection on other traits exhibiting QTL in these regions.

This focus on potentially adaptive alleles derived from *H. debilis* should not be allowed to obscure the fact that a large fraction of *H. debilis* QTL alleles clearly have negative phenotypic consequences in the *H. annuus* background. These include reductions in the number of seeds per inflorescence on LG4, LG13, the proximal end of LG12/17, and LG15. These alleles would be expected to be disfavored in hybrids and thus are not expected to have been involved in historical adaptive introgression events.

A surprising result is that we found very few *H. debilis* alleles that improve herbivore resistance in the *H. annuus* background. Phenotypic analyses showed that *H. debilis* and *H. a. texanus* are more resistant than *H. annuus* to many types of damage (Whitney et al. 2006). Furthermore, resistance in resynthesized hybrids is under relatively strong positive selection, especially with respect to the midge seed predator *Neolasioptera helianthis* (selection gradients β of 0.31 to 0.55) and hole damage by *Isophrictis* sp. ($\beta = 0.18$ at LBJ). Despite the expectation that *H. debilis* would harbor resistance alleles, we found that *H. debilis* alleles

increased damage at all QTL that affected either midge or hole damage. We did find a QTL where the *H. debilis* allele increased resistance to leaf vascular tissue damage (typically inflicted by Hemiptera and Homoptera), but this QTL was in a region (the proximal end of LG12/17) where *H. debilis* genetic material had a negative effect on fitness components and thus would not be expected to be favored. Potential reasons for this mismatch between expectations and reality are several. First, QTL where *H. debilis* alleles increase herbivore resistance could exist in regions of the genome not well covered by markers, and thus may not have been located; such resistance QTL could be of small effect and thus hard to detect; and/or the particular *H. debilis* individual used in crosses may not have contained typical *H. debilis* resistance alleles. Alternately, it could be that higher herbivore resistance in the hybrid lineage is simply not derived from *H. debilis* and instead represents other processes, e.g. *de novo* mutation or introgression from species other than *H. debilis*.

Putative G × E interactions could affect patterns of adaptive introgression

For 17% of the QTL discovered here, the *H. debilis* allele had a significant phenotypic effect in one site and the opposite (or no) effect in the other site. Given that QTL detection is often incompletely repeatable across environments (Mauricio 2005), it is possible that some fraction of these G × E interactions are artifactual: repeated mapping experiments could find more consistent allelic effects. However, if some portion of these putative G × E interactions were real, they might indicate alleles with lower absolute magnitudes of the selection coefficient *s* (measured across environments) relative to alleles not exhibiting G × E. Low *s* in turn would mean that the rate of allelic introgression (or rate of loss) would be slower and/or more governed by stochastic events as predicted by basic population genetic theory (Gillespie 1998).

To our knowledge, this is the first investigation of site-specific effects of candidate alleles in an adaptive trait introgression scenario. The closest relevant study is Martin et al.'s (2007) investigation of the genetic architecture of reproductive barriers between the naturally hybridizing Louisiana irises *Iris fulva* and *I. brevicaulis*, which differ in flowering phenology. In that study, QTL for flowering time were mapped in four environments: the greenhouse in two different years and two different field plots separated by approx. 1 km. Seventeen QTL were found that affected flowering time in one or a few environments, but no single locus had effects in all four environments. In some cases, QTL on the same linkage group had opposite effects in different environments, though it was unclear if these QTL represented a single locus or multiple loci. While Martin et al. (2007) caution that low power to detect QTL may underlie some of their results, like the current study their findings suggest that G × E interactions could be important in understanding the fates of alleles exchanged by hybridizing species.

Introgression of single vs. multiple traits

We have previously pointed out (Whitney et al. 2010) that the vast majority of examples of adaptive trait introgression have identified introgression of a single key trait or a group of functionally related traits (e.g. Martin et al., 2005, 2006; Grant & Grant, 1996, 2008; Uy & Stein, 2007; Kim et al., 2008). In contrast, introgression in the *H. a. texanus* system seems to have involved multiple phenotypic axes, including herbivore resistance, ecophysiological,

phenological, and architectural traits. We hypothesize that this pattern may reflect the high degree of clustering of trait QTL around fitness loci, and/or the fact that southerly range expansion by *H. annuus* required modification of multiple aspects of the phenotype. Alternately, it may be that introgression involves multiple traits in most or all systems, and the typical pattern mentioned above (introgression of a single key trait or a group of functionally related traits) 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.

Future directions

The candidate *H. debilis* alleles identified here serve as explicit hypotheses for how the genetic architecture of the wild hybrid lineage *H. a. texanus* came into existence. These hypotheses can then be tested via a) surveys of allele frequencies in wild populations of *H. a. texanus*, and b) examination of allele frequency changes in experimental populations of resynthesized hybrids allowed to evolve over time in the field (i.e. by “replaying the evolutionary clock”). Both of these tests are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Many thanks to the Brackenridge Field Laboratory and the Lady Bird Johnson Wildflower Center of the University of Texas, Austin, for space and support during fieldwork. Particular thanks to John Abbott, John Crutchfield, Larry Gilbert, Randy Linder, Tom Juenger and Damon Waitt. Sincere thanks to Serena Barnes, Lauren Blume, Amanda Hill, Laurel Klein, Samantha Morgan, and Robin Reister for field assistance, and to Serena Barnes, Phuong Nguyen, Scott Johns, Jason Harper, Erin Miller, and Mike Green for assistance in the lab. Special thanks to Jennifer Durphy, John Randell, Jennifer Rudgers, and Mark Stoutemeyer for help with fencing, plowing, and planting, as well as to Chris Grassa for locating our SNP markers on the high density genetic linkage map for sunflower. This work was supported by USDA NRI 2003-35320 to KDW, NSF DEB 0716868 and DEB 1257965 to KDW and LHR, and NIH R01 GM074244 to KWB.

References

- Abbott R, Albach D, Ansell S, et al. Hybridization and speciation. *Journal of Evolutionary Biology*. 2013; 26:229–246. [PubMed: 23323997]
- Anderson, E. Introgressive hybridization. Chapman & Hall; London: 1949.
- Anderson TM, vonHoldt BM, Candille SI, et al. Molecular and evolutionary history of melanism in North American gray wolves. *Science*. 2009; 323:1339–1343. [PubMed: 19197024]
- Arnold ML. Transfer and origin of adaptations through natural hybridization: were Anderson and Stebbins right? *Plant Cell*. 2004; 16:562–570. [PubMed: 15004269]
- Arnold, ML. *Evolution through genetic exchange*. Oxford University Press; Oxford, UK: 2006.
- Baker, HG.; Stebbins, GL. *The genetics of colonizing species*. Academic Press; New York: 1965.
- Barton NH. Does hybridization influence speciation? *Journal of Evolutionary Biology*. 2013; 26:267–269. [PubMed: 23324003]
- Bowers JE, Bachlava E, Brunick RL, et al. Development of a 10,000 locus genetic map of the sunflower genome based on multiple crosses. *G3: Genes | Genomes | Genetics*. 2012; 2:721–729.
- Broman KW, Speed TP. A model selection approach for the identification of quantitative trait loci in experimental crosses. *Journal of the Royal Statistical Society B*. 2002; 64:641–656.

- Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics*. 2003; 19:889–890. [PubMed: 12724300]
- Castric V, Bechsgaard J, Schierup MH, Vekemans X. Repeated adaptive introgression at a gene under multiallelic balancing selection. *PLoS Genetics*. 2008; 4
- Chandler JM, Jan CC, Beard BH. Chromosomal differentiation among the annual *Helianthus* species. *Systematic Botany*. 1986; 11:354–371.
- Chapman MA, Abbott RJ. Introgression of fitness genes across a ploidy barrier. *New Phytologist*. 2010; 186:63–71. [PubMed: 19912548]
- Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics*. 1994; 138:963–971. [PubMed: 7851788]
- Fitzpatrick BM, Johnson JR, Kump DK, et al. Rapid fixation of non-native alleles revealed by genome-wide SNP analysis of hybrid tiger salamanders. *BMC Evolutionary Biology*. 2009; 9
- Gagnaire PA, Albert V, Jonsson B, Bernatchez L. Natural selection influences AFLP intraspecific genetic variability and introgression patterns in Atlantic eels. *Molecular Ecology*. 2009; 18:1678–1691. [PubMed: 19302349]
- Gillespie, JH. Population genetics: a concise guide. Johns Hopkins University Press; Baltimore, Maryland, USA: 1998.
- Grant BR, Grant PR. High survival of Darwin's finch hybrids: Effects of beak morphology and diets. *Ecology*. 1996; 77:500–509.
- Grant BR, Grant PR. Fission and fusion of Darwin's finches populations. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*. 2008; 363:2821–2829.
- Grant, V. Plant speciation. Columbia University Press; New York, New York, USA: 1971.
- Haley CS, Knott SA. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*. 1992; 69:315–324. [PubMed: 16718932]
- Hedrick PW. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology*. 2013; 22:4606–4618. [PubMed: 23906376]
- Heiser CB. Hybridization in the annual sunflowers: *Helianthus annuus* X *H. debilis* var. *cucumerifolius*. *Evolution*. 1951; 5:42–51.
- Heiser CB. Variation and Subspeciation in the Common Sunflower, *Helianthus annuus*. *American Midland Naturalist*. 1954; 51:287–305.
- Heliconius Genome Consortium. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*. 2012; 487:94–98. [PubMed: 22722851]
- Hovick SM, Campbell LG, Snow AA, Whitney KD. Hybridization alters early life-history traits and increases plant colonization success in a novel region. *American Naturalist*. 2012; 179:192–203.
- Hovick SM, Whitney KD. Hybridization is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridization-invasion hypothesis. *Ecology Letters*. 2014; 17:1464–1477. [PubMed: 25234578]
- Hufford MB, Lubinsky P, Pyhajarvi T, et al. The genomic signature of crop-wild introgression in maize. *PLoS Genetics*. 2013; 9
- Kim M, Cui ML, Cubas P, et al. Regulatory genes control a key morphological and ecological trait transferred between species. *Science*. 2008; 322:1116–1119. [PubMed: 19008450]
- Kim S-C, Rieseberg LH. Genetic architecture of species differences in annual sunflowers: Implications for adaptive trait introgression. *Genetics*. 1999; 153:965–977. [PubMed: 10511571]
- Kim S-C, Rieseberg LH. The contribution of epistasis to species differences in annual sunflowers. *Molecular Ecology*. 2001; 10:683–690. [PubMed: 11298979]
- Knott SA, Haley CS. Multitrait least squares for quantitative trait loci detection. *Genetics*. 2000; 156:899–911. [PubMed: 11014835]
- Lande R, Arnold SJ. The measurement of selection on correlated characters. *Evolution*. 1983; 37:1210–1226.
- Lexer C, Welch ME, Durphy JL, Rieseberg LH. Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. *Molecular Ecology*. 2003; 12:1225–1235. [PubMed: 12694286]

- Martin NH, Bouck AC, Arnold ML. Loci affecting long-term hybrid survivorship in Louisiana irises: Implications for reproductive isolation and introgression. *Evolution*. 2005; 59:2116–2124. [PubMed: 16405157]
- Martin NH, Bouck AC, Arnold ML. Detecting adaptive trait introgression between *Iris fulva* and *I. brevicaulis* in highly selective field conditions. *Genetics*. 2006; 172:2481–2489. [PubMed: 16415358]
- Martin NH, Bouck AC, Arnold ML. The genetic architecture of reproductive isolation in Louisiana irises: Flowering phenology. *Genetics*. 2007; 175:1803–1812. [PubMed: 17237511]
- Mauricio R. Ontogenetics of QTL: the genetic architecture of trichome density over time in *Arabidopsis thaliana*. *Genetica*. 2005; 123:75–85. [PubMed: 15881682]
- Pardo-Diaz C, Salazar C, Baxter SW, et al. Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genetics*. 2012; 8
- R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; Vienna, Austria: 2013.
- Renaut S, Grassa CJ, Yeaman S, et al. Genomic islands of divergence are not affected by geography of speciation in sunflowers. *Nature Communications*. 2013; 4:article 1827.
- Rieseberg LH, Archer MA, Wayne RK. Transgressive segregation, adaptation and speciation. *Heredity*. 1999; 83:363–372. [PubMed: 10583537]
- Rieseberg LH, Beckstrom-Sternberg S, Doan K. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:593–597. [PubMed: 11607056]
- Rieseberg LH, Kim SC, Randell RA, et al. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica*. 2007; 129:149–165. [PubMed: 16955330]
- Rieseberg, LH.; Wendel, JF. Introgression and its consequences in plants. In: Harrison, RG., editor. *Hybrid zones and the evolutionary process*. Oxford University Press; Oxford: 1993. p. 70–114.
- Roux C, Tsagkogeorga G, Bierne N, Galtier N. Crossing the species barrier: genomic hotspots of introgression between two highly divergent *Ciona intestinalis* species. *Molecular Biology and Evolution*. 2013; 30:1574–1587. [PubMed: 23564941]
- Scascitelli M, Whitney KD, Randell RA, et al. Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H. debilis*) reveals asymmetric patterns of introgression and small islands of genomic differentiation. *Molecular Ecology*. 2010; 19:521–541. [PubMed: 20355258]
- Seehausen O. Hybridization and adaptive radiation. *Trends in Ecology & Evolution*. 2004; 19:198–207. [PubMed: 16701254]
- Seehausen O. Conditions when hybridization might predispose populations for adaptive radiation. *Journal of Evolutionary Biology*. 2013; 26:279–281. [PubMed: 23324007]
- Sokal, RR.; Rohlf, FJ. *Biometry*. 2. W. H. Freeman and Company; New York: 1981.
- Song Y, Endepols S, Klemann N, et al. Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. *Current Biology*. 2011; 21:1296–1301. [PubMed: 21782438]
- Stebbins GL. The role of hybridization in evolution. *Proceedings of the American Philosophical Society*. 1959; 103:231–251.
- Uy JAC, Stein AC. Variable visual habitats may influence the spread of colourful plumage across an avian hybrid zone. *Journal of Evolutionary Biology*. 2007; 20:1847–1858. [PubMed: 17714302]
- Wessinger C, Hileman L, Rausher M. Identification of major quantitative trait loci underlying floral pollination syndrome divergence in *Penstemon*. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2014; 369:20130349.
- Whitney KD, Ahern JR, Campbell LG. Hybridization-prone plant families do not generate more invasive species. *Biological Invasions*. 2009; 11:1205–1215.
- Whitney KD, Randell RA, Rieseberg LH. Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. *American Naturalist*. 2006; 167:794–807.
- Whitney KD, Randell RA, Rieseberg LH. Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. *New Phytologist*. 2010; 187:230–239. [PubMed: 20345635]

Fig. 1. Partial Quantitative Trait Locus (QTL) map for *H. annuus* x *H. debilis* BC₁ hybrids grown in the field in central Texas. Linkage groups lacking QTL that affect fitness traits are not shown (see Fig. S1 for full map). Loci affecting fitness traits are shown in **Red** and co-localizing trait QTL are shown in **Black**. Marker names are shown to the right of the linkage group; marker positions (cM) are shown to the left. Solid bars indicate the QTL position +/- 0.5 cM, while thin capped lines indicate the +/- 1-LOD support interval. Additive effects of the *H. debilis* allele are shown in parentheses following the trait name; effect sizes have been back-transformed for traits subject to square-root transformation (see Table 1). Separate additive effects (for the BFL and LBJ sites, respectively) are displayed for loci showing significant QTL x Site interactions.

Table 1

Quantitative trait loci (QTL) for 22 traits from two *H. a. annuus* x *H. debilis* BC₁ mapping populations planted in two field sites in Texas. When the QTL x Site interaction is significant ($P < 0.02$), separate additive effects are presented for each site (BFL = Brackenridge Field Laboratory; LBJ = Lady Bird Johnson Wildflower Center); otherwise the effects are pooled across sites. *H. a. annuus* and *H. a. texanus* trait values are means across common gardens at the BFL and LBJ sites (derived from data presented in Whitney et al. 2006, 2010). **Boldface** values of the *H. a. texanus* phenotypic mean indicate that it is significantly different from the *H. a. annuus* phenotypic mean. *Italicized* values indicate traits that were square-root transformed prior to analysis; values for additive effects and confidence limits have been back-transformed. *Asterisks* indicate QTL where *H. debilis* allelic effects are significantly different from zero and are in the direction of the phenotype of the natural hybrid *H. a. texanus*, based on traits measured in field common gardens.

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
Putative resistance/palatability traits											
Glandular trichome density (mm ⁻²)	GlandDens	21.15	21.70	4	6.0	1.9	9.9	-0.11	-0.19	-0.05	
				9	34.9	1.5	7.7	-0.07	-0.13	-0.03	
				10A	17.0	1.9	9.9	-0.10	-0.18	-0.05	
				12/17	0.0	4.0	20.1	0.33*	0.20	0.49	
				12/17	32.0	1.1	5.5	-0.20	-0.41	-0.07	
				12/17	58.0	4.5	22.5	-0.58	-0.84	-0.37	
				13	5.0	27.5	108.1	-1.29	-1.52	-1.09	
Non-glandular trichome density (mm ⁻²)	HairDens	14.50	14.10	16	0.0	0.5	2.7	-0.03	-0.07	0.00	
				8	26.0	2.4	5.7	0.03	0.01	0.06	
				12/17	17.0	1.3	1.5	-0.20	-0.14	0.00	interaction
								0.04	0.01	0.10	BFL
								0.00	-0.01	0.01	LBJ
Leaf Carbon:Nitrogen	CNratio	8.35	8.55	15	9.4	7.4	16.7	-0.08*	-0.13	-0.05	
				12/17	21.0	0.9	4.5	0.78*	0.41	1.14	
				12/17	58.0	0.8	4.1	-0.68	-1.02	-0.35	
				15	12.0	0.5	2.7	0.35*	0.14	0.56	
Plant volume (cm ³)	Volume	370.80	319.00	4	1.9	1.9	4.4	4.48	5.58	43.43	interaction
								-8.42*	-20.10	-1.74	BFL
								2.48	0.03	8.88	LBJ

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
<u>Damage traits</u>											
§ Leaf-vascular-tissue damage (%)	SuckDam	18.75	14.00	12/17	21.0	7.4	20.7	-66.29*	-97.79	-40.90	
				12/17	57.0	4.0	11.4	31.26	15.61	52.29	
				13	24.0	0.9	2.5	-2.82*	-7.47	-0.39	
				15	12.0	2.5	7.1	-6.83*	-12.88	-2.68	
Leaf-chewing damage (%)	ChewDam	6.20	4.75	7	3.3	0.8	2.4	-0.83	-1.86	-0.08	interaction
				10B	1.5	1.7	3.9	0.32	0.03	0.93	BFL
								-0.07	-0.38	0.01	LBJ
								-1.06	-2.54	-0.27	interaction
								0.83	0.27	1.70	BFL
				11	0.0	1.3	3.5	-0.02	-0.26	0.05	LBJ
								-1.01	-2.37	-0.22	interaction
								0.58	0.14	1.35	BFL
								-0.06	-0.36	0.01	LBJ
								-2.69*	-5.76	-0.78	
								1.78	0.34	4.32	
§ Receptacle damage (no. holes/receptacle): <i>Isophrictis</i> sp.	RecepDam	0.23	0.06	12/17	19.0	1.3	4.6	0.340	0.007	0.358	interaction
				12/17	46.0	0.9	3.2	-0.002	-0.048	0.018	BFL
				2	4.4	1.3	1.7	0.088	0.012	0.236	LBJ
				9	41.0	1.6	3.7	0.006	0.001	0.014	
§ Midge damage (fraction seeds killed): <i>Neolastoptera helianthis</i>	MidgeDam	0.30	0.16	12/17	55.7	1.8	1.3	0.096	0.000	0.032	interaction
				15	7.0	1.0	2.3	0.000	-0.001	0.006	BFL
				12/17	10.0	3.8	14.3	0.013	0.003	0.031	LBJ
				15	18.0	1.9	7.1	0.004	0.000	0.010	
Hole damage (fraction seeds killed): <i>Isophrictis</i> sp.	HoleDam	0.006	0.003	15	41.0	1.9	4.3	0.14	0.10	0.18	
				9	41.0	1.9	4.3	0.09	0.06	0.12	
								0.001	0.000	0.001	

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
<u>Ecophysiological traits</u>											
§ Water-use efficiency (δ 13C)	WUE	-28.30	-28.85	12/17	55.7	2.6	5.8	0.001	0.000	0.002	
				4	20.0	0.8	2.3	-0.16*	-0.27	-0.05	
				10A	2.0	1.2	3.4	-0.18*	-0.27	-0.08	
				15	8.9	3.5	9.7	-0.29*	-0.39	-0.20	
§ Specific leaf area (cm²/g)	SLA	181.95	199.55	8	0.0	0.6	3.3	-6.00	-9.32	-2.69	
				9	36.0	0.4	2.1	9.92	3.11	16.72	interaction
								-4.44	-9.98	1.09	BFL
								5.48*	1.48	9.47	LBJ
Leaf succulence	Succ	0.82	0.83	12/17	14.0	0.8	4.4	-7.99	-11.78	-4.20	
				15	5.6	2.2	2.3	-10.09	-16.73	-3.46	interaction
								15.24*	9.99	20.50	BFL
				7	2.3	0.8	2.5	0.005*	0.002	9.20	LBJ
<u>Phenological traits</u>											
Bud initiation time (days)	DaysToBud	58.15	57.30	12/17	61.6	0.8	2.5	-0.005	-0.008	0.008	
				5	13.0	1.4	4.1	-2.66*	-3.96	-1.35	
				7	5.0	1.2	3.5	-2.31*	-3.56	-1.07	
				9	23.6	7.0	1.5	3.48	0.65	6.31	interaction
§ Seed maturation time (days)	SMT	28.90	26.75					-7.06*	-9.24	-4.88	BFL
								-3.58*	-5.39	-1.76	LBJ
				12/17	58.6	7.5	21.2	-5.83*	-7.07	-4.59	
				13	3.4	3.4	1.8	3.90	1.01	6.80	interaction
								-5.32*	-7.55	-3.08	BFL
								-1.41	-3.26	0.43	LBJ
				14	1.5	2.1	6.1	-3.05*	-4.29	-1.82	
				15	5.6	9.0	25.0	-6.41*	-7.66	-5.16	
				16	0.0	0.9	2.5	-1.99*	-3.25	-0.74	
				2	2.0	0.8	1.7	-1.23	-2.18	-0.27	interaction

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
§ Plant longevity (days)	Longevity	150.30	172.35					0.61*	-0.05	1.27	BFL
				3	0.0	2.2	5.7	-0.62*	-1.30	0.07	LBJ
				4	3.3	2.9	7.5	-0.99*	-1.40	-0.57	
				5	23.0	4.3	11.1	-1.20*	-1.64	-0.76	
				7	2.3	2.7	7.1	-1.43*	-1.86	-1.01	
				11	7.0	1.0	2.6	-1.11*	-1.53	-0.70	
				12/17	16.0	2.7	7.1	-0.68*	-1.10	-0.25	
				12/17	36.0	1.9	4.9	-3.12*	-4.28	-1.95	
				12/17	52.0	1.3	3.4	2.89	1.58	4.19	
				15	10.1	4.3	11.1	-1.87*	-2.89	-0.85	
§ Disk diameter (mm)	DiskDiam	35.60	30.45	9	34.9	5.8	16.0	-1.40*	-1.81	-0.98	
				12/17	0.0	3.6	1.5	6.36*	4.80	7.92	interaction
								-3.98	-7.23	-0.73	
								6.61*	4.42	8.81	BFL
								2.64*	0.25	5.03	LBJ
				2	4.4	1.7	6.5		0.61	1.40	
				4	5.0	2.5	9.4	-1.30*	-1.72	-0.88	
				7	4.0	3.3	12.7	-1.41*	-1.80	-1.02	
				8	13.0	1.8	7.1	-1.11*	-1.53	-0.70	
				9	23.6	2.2	8.6	-1.13*	-1.52	-0.75	
§ Height of lowest branch (cm)	HtLow	42.40	31.85	12/17	17.0	1.3	5.0	-2.47*	-3.58	-1.37	
				12/17	44.0	0.8	3.1	-1.86*	-2.91	-0.81	
				13	6.1	2.7	10.4	-1.25*	-1.64	-0.87	
				15	1.0	8.4	30.5	-2.21*	-2.59	-1.82	
				7	4.0	3.0	6.5	-9.04*	-12.57	-5.51	
				10A	9.1	1.2	2.6	-5.66*	-9.17	-2.15	
				12/17	37.2	3.0	6.4	-9.50*	-13.22	-5.77	
				15	9.3	4.3	9.3	-10.79*	-14.29	-7.28	

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
§ Bushiness	Bushy	2.35	2.55	7	4.7	1.0	2.2	0.05*	0.02	0.08	
				10A	13.0	1.9	4.4	-0.07	-0.11	-0.04	
				12/17	0.0	2.0	4.6	0.09*	0.05	0.13	
				12/17	52.0	2.9	6.6	-0.61	-0.85	-0.37	
				12/17	56.0	3.9	8.8	0.64*	0.43	0.86	
				13	0.0	2.1	2.5	-0.11	-0.18	-0.04	interaction
								0.10*	0.05	0.15	BFL
								-0.01	-0.05	0.04	LBJ
				15	9.4	1.6	3.7	0.06*	0.03	0.09	
				4	1.9	1.4	3.0	-0.03	-0.04	-0.01	interaction
§ Relative branch diameter	RelBrDiam	0.31	0.35					0.01	-0.005	0.015	BFL
								-0.02	-0.04	-0.01	LBJ
				7	7.0	0.9	3.0	-0.01	-0.02	-0.01	
				12/17	1.0	1.8	5.8	0.02*	0.01	0.03	
				13	0.0	1.8	5.6	0.02*	0.01	0.03	
				14	2.3	1.7	5.3	0.02*	0.01	0.03	
				3	0.2	2.0	8.1	-0.39*	-0.53	-0.25	
				4	10.0	2.9	11.6	-0.54*	-0.70	-0.38	
				5	26.1	0.9	3.6	-0.26*	-0.40	-0.12	
				8	19.6	1.5	6.2	-0.34*	-0.48	-0.20	
Seed mass (mg)	SeedWt	8.34	7.29	9	33.5	1.2	4.9	-0.30*	-0.44	-0.16	
				10A	6.0	1.1	4.4	-0.29*	-0.43	-0.15	
				10B	0.0	1.6	1.6	0.49	0.10	0.88	interaction
								-0.50*	-0.77	-0.22	BFL
								-0.01	-0.28	0.27	LBJ
				12/17	0.0	3.6	14.4	0.74	0.55	0.93	
				12/17	22.0	0.7	2.7	-0.76*	-1.21	-0.30	
				12/17	44.0	2.8	11.4	-1.42*	-1.84	-1.00	
				13	26.0	2.4	1.9	0.57	0.16	0.98	interaction

Trait	Trait Abbreviation	<i>H. a. annuus</i> mean	<i>H. a. texanus</i> mean	Linkage Group	Position (cM)	PVE	LOD	Additive Effect of <i>H. debilis</i> allele	95% CI lower	95% CI upper	Site
Fitness traits	InfNum	34.06	54.62	15	5.6	3.0	12.0	0.49	0.35	0.63	LBJ
Seeds per inflorescence	SeedsPerInfl	64.13	66.30	4	10.0	4.6	16.4	-0.78	-1.20	-0.45	LBJ
Seeds per inflorescence	SeedsPerInfl	64.13	66.30	13	30.0	1.6	2.9	0.85	0.12	1.81	interaction BFL
Seeds per inflorescence	SeedsPerInfl	64.13	66.30	15	4.0	16.8	54.6	-2.21	-2.80	-1.69	LBJ

[§] Traits previously suggested to have been strong candidates for adaptive introgression from *H. debilis* (Whitney et al. 2006, 2010)

PVE = Percent Variance Explained

95% CI = 95% Confidence Interval (associated with the additive effect of the *H. debilis* allele)

Table 2

Effects of *H. debilis* alleles at QTL for two fitness traits, inflorescence number (InflNum) and the number of seeds per inflorescence (SeedsPerInfl). Total seed number (InflNum $\times$ SeedsPerInfl) is our measure of female fitness; net allelic effects on female fitness (rightmost column) are calculated with respect to a hypothetical BC₁ plant with average trait values at each field site. InflNum is our measure of male fitness. Effects for three QTL which consistently had positive effects on both female and male fitness are highlighted in bold text.

Field Site	Linkage Group	QTL positions (cM)			Additive effects of <i>H. debilis</i> alleles on			Total seed number [§]		Net effect of <i>H. debilis</i> alleles (seeds)
		InflNum	SeedsPerInfl	InflNum	SeedsPerInfl	Average BC ₁ phenotype	<i>H. debilis</i> genotype			
BFL	4		10.0		-0.78	862.0	836.4	-25.6		
	7	4.7		0.69		862.0	880.2	18.2		
	9	25.1		0.47		862.0	874.3	12.3		
	10A	27.1		-0.38		862.0	852.0	-9.9		
	12/17	0.0, 29.0	15.0	-2.15*	-1.92	862.0	746.6	-115.4		
	12/17	58.6	60.0	4.78	-0.51	862.0	968.0	106.0		
	13		30.0		-0.34	862.0	850.9	-11.1		
LBJ	15	8.9	4.0	1.65	-2.21	862.0	828.9	-33.1		
	4		10.0		-0.78	1355.2	1289.4	-65.8		
	7	4.7		0.69		1355.2	1366.3	11.1		
	9	25.1		0.47		1355.2	1362.7	7.5		
	10A	27.1		-0.38		1355.2	1349.1	-6.1		
	12/17	0.0, 29.0	15.0	-2.15*	-1.92	1355.2	1162.3	-192.9		
	12/17	58.6	60.0	4.78	-0.51	1355.2	1386.0	30.8		
	13		30.0		0.07 [†]	1355.2	1361.1	5.9 [†]		
	15	8.9	4.0	-0.03 [†]	-2.21	1355.2	1167.6	-187.6		

[§]Total seed number = InflNum $\times$ SeedsPerInfl. Average trait values for BFL and LBJ BC₁ plants were: InflNum: 32.9, 84.7; SeedsPerInfl: 26.2, 16.0.

* Sum of additive effects of the two co-localizing QTL for InflNum

[†]Not significantly different from zero (see Table 1)