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INVASION GENETICS: THE BAKER AND STEBBINS LEGACY

Quantitative trait locus mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower

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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 BC_1 mapping populations were grown in central Texas, genotyped for 384 single nucleotide polymorphism (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 favoured in the wild. QTL for a number of other ecophysiological, phenological and architectural traits colocalized with these three regions and are candidates for the actual traits driving adaptive shifts. G × E interactions played a modest role, 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: adaptive trait introgression, herbivory, hybridization, invasion, phenology, plant architecture, quantitative trait locus, range expansion, seed predation, single nucleotide polymorphism

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Introduction

Adaptive trait introgression is the transfer of fitness-increasing traits between species via hybridization and backcrossing (Rieseberg & Wendel 1993). Early potential examples were described by Anderson (1949), Heiser

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(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) has 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 & 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 has suggested several traits where (i) 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 (ii) 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 1000 *H. annuus* × *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:

- 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.
- 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 favoured they might have been across environments.

Methods

Study species

Helianthus annuus annuus, H. debilis and their stabilized hybrid derivative *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 (BC_1) mapping populations and field sites

We synthesized Backcross 1 (BC_1) mapping populations with an eye towards mimicking the ancestral early-generation hybrids that gave rise to *H. a. texanus*. An F_1 generation was first obtained by mating wild *H. debilis* ssp. *cucumerifolius* from Texas to wild *H. annuus* ssp. *annuus* from Oklahoma in the greenhouse. To produce enough BC_1 seed for replicate mapping populations, a single progeny from the F_1 generation was selected and propagated vegetatively to produce 14 F_1 clones. A single wild *H. a. annuus* pollen donor from Texas was then mated to the F_1 clones to produce 3758 BC_1 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 BC_1 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 approximately 14.5 km. 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

BC_1 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, 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

Table 1 Quantitative trait loci (QTL) for 22 traits from two *Helianthus annuus annuus* × *H. debilis* BC_1 mapping populations planted in two field sites in Texas. When the QTL × 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	*H. a. annuus* mean	*H. a. texanus* mean	Linkage group	Position (cM)	PVE	LOD	Additive effect of *H. debilis* 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*	
				16	0.0	0.5	2.7	*−0.03*		*−0.07*	*0.00*	
Nonglandular trichome density (mm^{-2})	HairDens	14.50	14.10	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
				15	9.4	7.4	16.7	*−0.08*	*	*−0.13*	*−0.05*	
Leaf Carbon:Nitrogen	CNratio	8.35	8.55	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
				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*	

Table 1 *Continued*

Trait	Trait abbreviation	*H. a. annuus* mean	*H. a. texanus* mean	Linkage group	Position (cM)	PVE	LOD	Additive effect of *H. debilis* allele		95% CI lower	95% CI upper	Site
Damage traits												
Leaf−vascular−tissue damage (%)†	SuckDam	18.75	14.00	7	3.3	0.8	2.4	*−0.83*		*−1.86*	*−0.08*	Interaction
								0.32		*0.03*	*0.93*	BFL
								−0.07		*−0.38*	*0.01*	LBJ
				10B	1.5	1.7	3.9	*−1.06*		*−2.54*	*−0.27*	Interaction
								0.83		*0.27*	*1.70*	BFL
								−0.02		*−0.26*	*0.05*	LBJ
				11	0.0	1.3	3.5	*−1.01*		*−2.37*	*−0.22*	Interaction
								0.58		*0.14*	*1.35*	BFL
								−0.06		*−0.36*	*0.01*	LBJ
				12/17	19.0	1.3	4.6	*−2.69*	*	*−5.76*	*−0.78*	
				12/17	46.0	0.9	3.2	*1.78*		*0.34*	*4.32*	
Leaf−chewing damage (%)	ChewDam	6.20	4.75	2	4.4	1.3	1.7	*0.340*		*0.007*	*0.358*	Interaction
								−0.002		*−0.048*	*0.018*	BFL
								0.088		*0.012*	*0.236*	LBJ
Receptacle damage (no. holes/ receptacle): *Isophrictis* sp.†	RecepDam	0.23	**0.06**	9	41.0	1.6	3.7	*0.006*		*0.001*	*0.014*	
				12/17	55.7	1.8	1.3	*0.096*		*0.000*	*0.032*	Interaction
								0.000		*−0.001*	*0.006*	BFL
								0.013		*0.003*	*0.031*	LBJ
				15	7.0	1.0	2.3	*0.004*		*0.000*	*0.010*	
Midge damage (fraction seeds killed): *Neolasioptera helianthis*†	MidgeDam	0.30	**0.16**	12/17	10.0	3.8	14.3	0.14		0.10	0.18	
				15	18.0	1.9	7.1	0.09		0.06	0.12	
Hole damage (fraction seeds killed): *Isophrictis* sp.	HoleDam	0.006	0.003	9	41.0	1.9	4.3	*0.001*		*0.000*	*0.001*	
				12/17	55.7	2.6	5.8	*0.001*		*0.000*	*0.002*	
Ecophysiological traits												
Water−use efficiency (δ 13C)†	WUE	−28.30	**−28.85**	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 (cm^2/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
				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
								5.15	*	1.10	9.20	LBJ

Table 1 *Continued*

Trait	Trait abbreviation	*H. a. annuus* mean	*H. a. texanus* mean	Linkage group	Position (cM)	PVE	LOD	Additive effect of *H. debilis* allele		95% CI lower	95% CI upper	Site
Leaf succulence	Succ	0.82	0.83	7	2.3	0.8	2.5	0.005	*	0.002	0.008	
				12/17	61.6	0.8	2.5	−0.005		−0.008	−0.002	
Phenological traits												
Bud initiation time (days)	DaysToBud	58.15	57.30	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
								−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	
Seed maturation time (days)†	SMT	28.90	**26.75**	2	2.0	0.8	1.7	−1.23		−2.18	−0.27	Interaction
								0.61	*	−0.05	1.27	BFL
								−0.62	*	−1.30	0.07	LBJ
				3	0.0	2.2	5.7	−0.99	*	−1.40	−0.57	
				4	3.3	2.9	7.5	−1.20	*	−1.64	−0.76	
				5	23.0	4.3	11.1	−1.43	*	−1.86	−1.01	
				7	2.3	2.7	7.1	−1.11	*	−1.53	−0.70	
				11	7.0	1.0	2.6	−0.68	*	−1.10	−0.25	
				12/17	16.0	2.7	7.1	−3.12	*	−4.28	−1.95	
				12/17	36.0	1.9	4.9	2.89		1.58	4.19	
				12/17	52.0	1.3	3.4	−1.87	*	−2.89	−0.85	
				15	10.1	4.3	11.1	−1.40	*	−1.81	−0.98	
Plant longevity (days)†	Longevity	150.30	**172.35**	9	34.9	5.8	16.0	6.36	*	4.80	7.92	
				12/17	0.0	3.6	1.5	−3.98		−7.23	−0.73	Interaction
								6.61	*	4.42	8.81	BFL
								2.64	*	0.25	5.03	LBJ

Table 1 *Continued*

Trait	Trait abbreviation	*H. a. annuus* mean	*H. a. texanus* mean	Linkage group	Position (cM)	PVE	LOD	Additive effect of *H. debilis* allele		95% CI lower	95% CI upper	Site
Plant architectural traits												
Disk diameter (mm)†	DiskDiam	35.60	**30.45**	2	4.4	1.7	6.5	1.01		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	
				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	
Height of lowest branch (cm)†	HtLow	42.40	**31.85**	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	
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	
Relative branch diameter†	RelBrDiam	0.31	**0.35**	4	1.9	1.4	3.0	−0.03		−0.04	−0.01	Interaction
								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	
Seed mass (mg)	SeedWt	8.34	**7.29**	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	
				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	

Table 1 *Continued*

Trait	Trait abbreviation	*H. a. annuus* mean	*H. a. texanus* mean	Linkage group	Position (cM)	PVE	LOD	Additive effect of *H. debilis* allele		95% CI lower	95% CI upper	Site
				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
								−0.64	*	−0.94	−0.35	BFL
								−0.07		−0.36	0.21	LBJ
				15	5.6	3.0	12.0	0.49		0.35	0.63	
Fitness traits												
Inflorescence number	InflNum	34.06	**54.62**	7	4.7	1.9	4.3	*0.69*	*	*0.19*	*1.52*	
				9	25.1	1.3	3.0	*0.47*	*	*0.08*	*1.18*	
				10A	27.1	1.0	2.3	*−0.38*		*−1.05*	*−0.04*	
				12/17	0.0	1.0	2.3	*0.62*	*	*0.07*	*1.72*	
				12/17	29.0	2.0	4.5	*−2.77*		*−6.02*	*−0.77*	
				12/17	58.6	5.1	11.1	*4.78*	*	*2.36*	*8.04*	
				15	8.9	2.4	3.1	*−1.46*		*−5.27*	*−0.40*	Interaction
								1.65	*	*0.51*	*3.44*	BFL
								−0.03		*−0.62*	*0.18*	LBJ
Seeds per inflorescence	SeedsPerInfl	64.13	66.30	4	10.0	4.6	16.4	*−0.78*		*−1.20*	*−0.45*	
				12/17	15.0	5.8	20.5	*−1.92*		*−2.83*	*−1.18*	
				12/17	60.0	1.9	7.2	*−0.51*		*−0.95*	*−0.20*	
				13	30.0	1.6	2.9	*0.85*		*0.12*	*1.81*	Interaction
								−0.34		*−0.92*	*−0.04*	BFL
								0.07		*0.00*	*0.35*	LBJ
				15	4.0	16.8	54.6	*−2.21*		*−2.80*	*−1.69*	

PVE, per cent variance explained. 95% CI, 95% confidence interval (associated with the additive effect of the *H. debilis* allele).
[†]Traits previously suggested to have been strong candidates for adaptive introgression from *H. debilis* (Whitney *et al.* 2006, 2010).

inflorescences per plant; see Appendix S1, Supporting information). The former is expected to be a good estimate of lifetime male fitness, as pollinator visitation (and thus expected pollen removal) is highly correlated with inflorescence number in these species (K. Whitney & S. Hovick, 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 seven *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 (i) only one SNP locus per gene was allowed; (ii) primer design criteria were met; and (iii) 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 BC_1 mapping populations were then genotyped at these 384 loci. DNA was extracted from 40 mg 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 BC_1 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 >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 seven 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 & 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 × Site interactions by including Site as an interactive covariate (i.e. 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, was established by permutation tests (Churchill & Doerge 1994), using 1000 permutation replicates.

Multiple-QTL analyses were then performed using the method of Broman & 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 & Speed (2002) approach to use the multivariate QTL mapping method of Knott & Haley (2000).

Some traits were square-root transformed prior to QTL analysis (see Table 1) 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 colocalized with fitness QTL. We used overlapping 1-LOD support intervals as the criterion for colocalization (Lexer *et al.* 2003; Wessinger *et al.* 2014). To make a preliminary assessment of which alleles are likely to have been widely favoured across sites vs. those which may have provided patchier selective advantages, we tested for the presence of G × E (i.e. 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

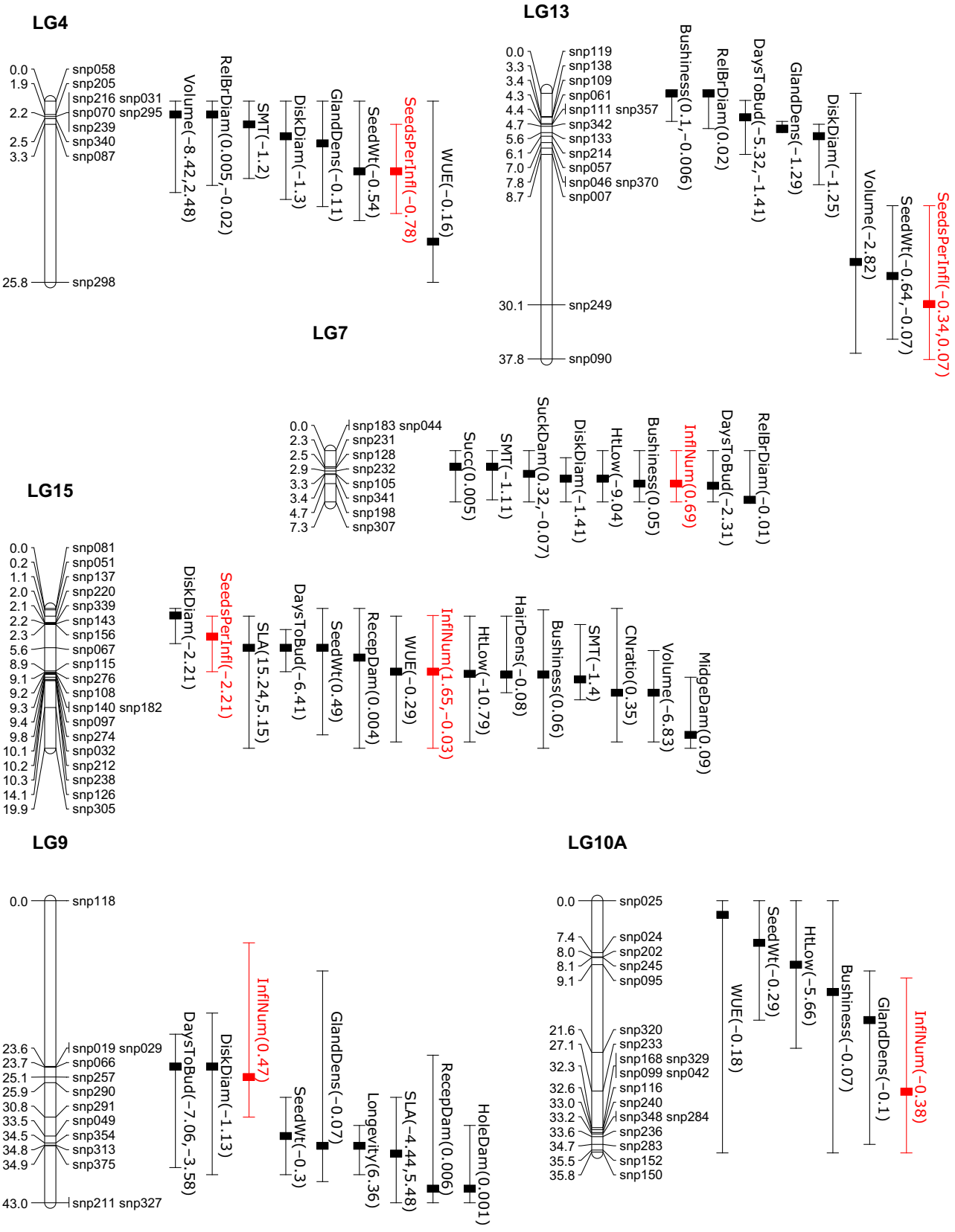
A condensed map showing QTL for the two fitness traits (and QTL for other traits that colocalize with them) is presented as Fig 1. The full QTL map is presented as Fig. S1 (Supporting information). LOD support intervals for each QTL (used in assessing colocalization) are presented in Table S1 (Supporting 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 BC_1 hybrids (Table 1, Fig. S1, Supporting information). Eighty-one of these QTL had consistent effects across sites, while 17 of them showed potential site-specific effects (i.e. the QTL × 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

Fig. 1 Partial quantitative trait locus (QTL) map for *Helianthus annuus* × *Helianthus debilis* BC_1 hybrids grown in the field in central Texas. Linkage groups lacking QTL that affect fitness traits are not shown (see Fig. S1, Supporting information for full map). Loci affecting fitness traits are shown in Red and colocalizing 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 × Site interactions.



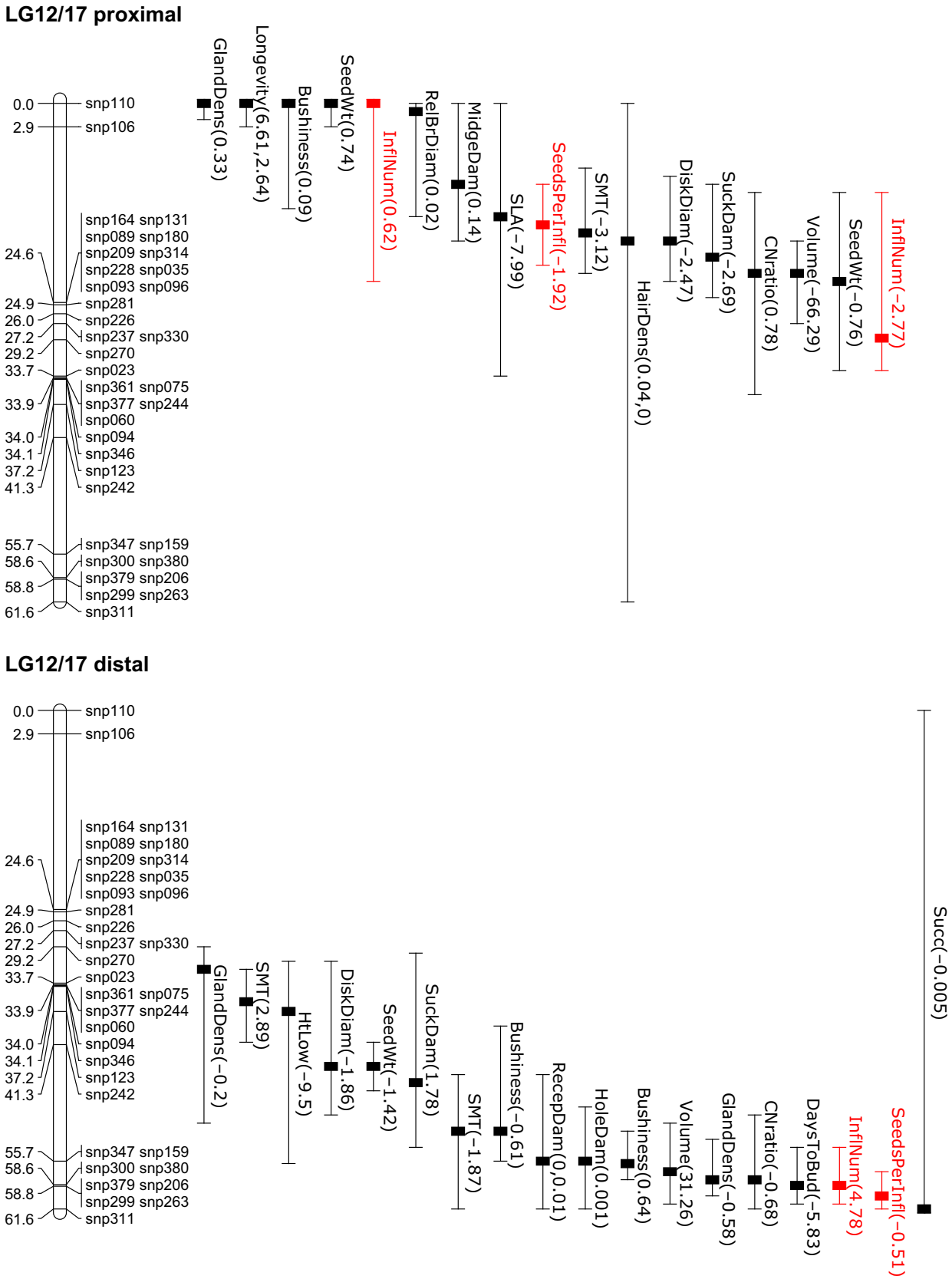


Fig. 1 Continued

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 × 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 (i) in the direction of the *H. a. texanus* phenotype and (ii) 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 × 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 colocalize (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 colocalizing QTL (Table 2). Overall, we estimate that *H. debilis* genetic material is highly favoured 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

Table 2 Effects of *Helianthus debilis* alleles at quantitative trait loci (QTL) for two fitness traits, inflorescence number (InflNum) and the number of seeds per inflorescence (SeedsPerInfl). Total seed number (InflNum × SeedsPerInfl) is our measure of female fitness; net allelic effects on female fitness (rightmost column) are calculated with respect to a hypothetical BC_1 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

		QTL positions (cM)		Additive effects of *H. debilis* alleles on		Total seed number‡		Net effect of *H. debilis* alleles
Field site	Linkage group	InflNum	SeedsPerInfl	InflNum	SeedsPerInfl	Average BC_1 phenotype	*H. debilis* genotype	(seeds)
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
	15	8.9	4.0	1.65	−2.21	862.0	828.9	−33.1
LBJ	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

*Sum of additive effects of the two colocalizing QTL for InflNum.
†Not significantly different from zero (see Table 1).
‡Total seed number = InflNum × SeedsPerInfl. Average trait values for BFL and LBJ BC_1 plants were as follows: InflNum: 32.9, 84.7; SeedsPerInfl: 26.2, 16.0.

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 favoured, 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 colocalize 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 Fig. 1 and Table S1, Supporting information).

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, Fig. S1, Supporting information). 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 cm^3 on average at one site but decreased it by 8.42 cm^3 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 BC_1 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 historical regions of introgression driving both phenotypic change and increased adaptation in *H. a. texanus*. Numerous QTL for ecophysiological, phenological and architectural traits colocalized with these three fitness-enhancing regions (see further 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 nonadaptive) 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 colocalize 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 is 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 BC_1s in the field (selection gradients β = 0.18–0.20; 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 disfavoured 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. (β = 0.18 at LBJ). Despite the expectation that *H. debilis* would harbour 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 favoured. 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. Alternatively, it could be that higher herbivore resistance in the hybrid lineage is simply not derived from *H. debilis* and instead represents other processes, for example 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 artefactual: 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, although 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. Alternatively, 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.

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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.

Supporting information

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

Fig. S1 Quantitative Trait Locus (QTL) map for *H. annuus* x *H. debilis* BC1 hybrids showing locations of 22 phenotypic traits as determined by Multiple-QTL analysis.

Table S1 LOD support intervals for QTL positions.

Appendix S1 Trait descriptions and trait measurement methods.

Appendix S2 Sunflower genetic map.