Genetic Architecture of Species Differences in Annual Sunflowers: Implications for Adaptive Trait Introgression

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ABSTRACT

Genetic architecture may profoundly influence the ability of adaptive traits to spread between species via introgressive hybridization. Here, we examine the genomic location of quantitative trait loci (QTL) associated with pollen sterility and morphological traits distinguishing two annual sunflowers, *Helianthus annuus* and *H. debilis* ssp. *cucumerifolius*. These species are of particular interest since they hybridize naturally, and the form of *H. annuus* in Texas (called ssp. *texanus*) is thought to have arisen through introgression. Analysis of 226 BC_1 progeny from a cross between *H. annuus* and *H. debilis* revealed 56 QTL for 15 morphological traits and 2 QTL for pollen sterility. Four morphological QTL are tightly linked (<10 cM) to one or more sterility factors and 7 are closely allied with underrepresented and presumably negatively selected chromosomal blocks. Although these 11 QTL seem unlikely to move between the species, no barrier to introgression was detected for the remaining 45 morphological QTL. In fact, due to widespread pleiotropy (or tight linkage), the introgression of just three small chromosomal blocks appears sufficient to largely recover the phenotype of ssp. *texanus*. Subsequent work will test for the occurrence and fitness consequences of the identified QTL in natural populations of ssp. *texanus*.

IN his monograph on introgressive hybridization, Anderson (1949) reasoned that hybrids might serve as a bridge for the exchange of genetic adaptations between otherwise isolated species. Given the large number of plant and animal species that at least occasionally hybridize (Ellstrand *et al.* 1996; Dowling and Secor 1997), this process could, under Anderson's scenario, potentially contribute to adaptive evolution in many organismal groups. However, there are theoretical difficulties associated with the transfer of adaptations across species barriers in nature. First, alleles contributing to an adaptation must integrate into a new genetic background before the chromosomal block they are associated with is eliminated by selection (Barton and Hewitt 1985). If many genes contribute to reduced hybrid fitness, then much of the genome may be resistant to introgression due to linkage. Second, if the adaptation is polygenic, quantitative trait loci (QTL) contributing to the trait will become disassociated by recombination. Therefore, only alleles that are individually favorable are likely to cross the hybrid zone.

Despite these difficulties, numerous natural examples of adaptive trait introgression have been postulated (e.g., Heiser 1951, 1979; Harlan and De Wet 1963; Stutz and Thomas 1964; Monaghan and Hall 1976; Parsons *et al.* 1993). However, in most of these examples, it was not possible to distinguish between introgression and alternative explanations for the observed patterns such as convergent evolution or the joint retention of ancestral character states (symplesiomorphy). In other studies, neutral molecular markers have been observed to cross hybrid zones (*e.g.*, Rieseberg *et al.* 1990; Arnold *et al.* 1991; Abbott *et al.* 1992; Parsons *et al.* 1993). Neutral introgression might be viewed as synonymous with adaptive trait introgression since advantageous alleles will cross hybrid zones more readily than neutral markers. However, Rieseberg and Wendel (1993) argued that this conclusion was premature due to the likely preponderance of neutral alleles relative to favorable ones in hybrid zones.

Perhaps the best evidence to date for the movement of adaptations across species barriers in nature comes from studies in which the introgression of both neutral molecular markers and presumably advantageous traits have been examined (*e.g.*, Rieseberg *et al.* 1990; Klier *et al.* 1991; Abbott *et al.* 1992; Parsons *et al.* 1993). One such study (Heiser 1951) involves hybridization between the common sunflower, *Helianthus annuus*, which is abundant throughout the western U.S., and a closely related congener, *H. debilis* ssp. *cucumerifolius*, which is native to eastern Texas. Because *H. annuus* is not found in natural sites in eastern Texas, Heiser (1951) suggested that it was recently introduced by Native Americans. The east Texan form of *H. annuus* (now called ssp. *texanus*) approaches *H. debilis* ssp. *cucumerifolius* in several morphological features. Hybrid swarms

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are common in this region, and fertile plants can be recovered after several generations of backcrossing. These observations led Heiser (1951) to speculate that the successful colonization of eastern Texas by *H. annuus* was facilitated by the acquisition of advantageous alleles from *H. debilis* ssp. *cucumerifolius*, which was already "well adapted to this area" (pp. 44–45). Rieseberg *et al.* (1990) demonstrated the introgression of molecular markers between these species. However, because presumably neutral molecular markers rather than genes encoding phenotypic traits were assayed, Rieseberg *et al.* (1990) were cautious in their interpretation of the data, noting that molecular evidence for introgression does not necessarily account for morphological variation of ssp. *texanus* in the direction of *H. debilis* ssp. *cucumerifolius* or prove that the observed introgression was in any way adaptive.

Here we assess the plausibility of Heiser's (1951) hypothesis by mapping QTL that contribute to the sterility barrier that isolates *H. annuus* and *H. debilis* ssp. *cucumerifolius* and those that contribute to morphological traits that differentiate the two species. The object of this study is to ask whether the genetic architecture of the sterility barrier is conducive to adaptive trait introgression. A large number of sterility factors scattered throughout the genome would represent a much more formidable barrier to introgression than would a small number of loci or loci that are clustered. Likewise, we ask whether morphological traits (some likely adaptive) that distinguish the species have architectures that facilitate introgression. For example, if morphological QTL are linked to sterility factors, the likelihood of successful introgression is greatly reduced. Finally, we ask if there are differences between genetic architecture of morphological traits that are thought to have successfully transversed the sterility barrier and those that have not.

The data generated by this study also provide an opportunity to investigate the genetic basis of morphological adaptations that differentiate species. In the conventional view, derived from the synthetic theory of evolution (Charlesworth *et al.* 1982; Maynard Smith 1983), very small mutations are thought to drive evolution because mutations of large effect are unlikely to be favorable (Fisher 1930). However, others have argued, on the basis of classical crossing studies, that mutations with large morphological effects often contribute to adaptive evolution (Grant 1975; Templeton 1981; Turner 1981; Gottlieb 1984). This latter viewpoint has received increased support in recent years because factors with large phenotypic effects are often detected in QTL studies (*e.g.*, Doebley and Stec 1991, 1993; Vlot *et al.* 1992; Dorweiler *et al.* 1993; Tanksley 1993; Van Houten *et al.* 1994; Bradshaw *et al.* 1995, 1998; Mitchell-Olds 1995; Kuittinen *et al.* 1997) and because of the recent recognition that larger mutations, while less likely to be favorable, are more likely to be fixed in the population when advantageous (Kimura 1983; Orr and Coyne 1992). Orr (1998) has modeled the approach of a population to a phenotypic optimum and has shown that the distribution of factors during adaptation is roughly exponential and that the first factor fixed often can be fairly large. Thus, we ask whether QTL of large magnitude contribute to the morphological differences between *H. annuus* and *H. debilis* ssp. *cucumerifolius*.

Study species: *H. annuus* and *H. debilis* ssp. *cucumerifolius* are self-incompatible annuals with haploid chromosome numbers of 17. They are easily distinguished by numerous morphological features and have different ecological requirements. *H. annuus* occurs fairly commonly in the western U.S. and prefers heavier, clay-based soil. In contrast, *H. debilis* ssp. *cucumerifolius* occurs primarily in east Texas and is restricted to exposed, sandy soils (Heiser 1951). Meiotic analyses of interspecific hybrids indicates that *H. annuus* is differentiated from *H. debilis* by at least one paracentric inversion and several (possibly three) reciprocal translocations. These chromosomal differences may at least partially explain the sterility of F_1 hybrids between the species, which average 6.7% pollen viability (Heiser 1951). Nonetheless, successful backcrosses toward *H. annuus* are possible and pollen viability increases rapidly in the backcross generations (BC_1: 3–67%; BC_2: 20–98%; Heiser 1951), suggesting that introgression can occur despite the highly reduced fertility of the F_1 plants.

H. annuus ssp. *texanus* is fully fertile with other subspecies of *H. annuus* (ssp. *annuus* from the central and eastern U.S. and ssp. *lenticularis* from western North America; Heiser 1954) and exhibits a similar preference for heavy soils. Mean pollen and seed viabilities in F_1 hybrids between *H. annuus* ssp. *texanus* and *H. debilis* ssp. *cucumerifolius* (Heiser 1951) are similar to those described above for crosses between other subspecies of *H. annuus* and *H. debilis* ssp. *cucumerifolius*. However, *H. annuus* ssp. *texanus* appears to be a morphological mosaic, resembling typical *H. annuus* for some species-typical morphological characters such as phyllary (bracts that surround each flowering head) length and achene (one-seeded fruits) shape, but approaching *H. debilis* ssp. *cucumerifolius* for other traits, including stem speckling, ligule (fused petal of ray flowers) size and shape, phyllary width and shape, and achene size (Heiser 1951).

MATERIALS AND METHODS

Plant materials: We employed a BC_1 mapping strategy, using self-compatible, inbred lines of the cultivated *H. annuus* as the recurrent parent. This approach was used to counter several difficulties such as high levels of intraspecific polymorphism for molecular markers, sporophytic self-incompatibility, and low F_1 fertility. Cultivars of *H. annuus* are fully fertile with all wild forms of the species and represent an extreme form of the wild phenotype for most of the morphological traits that distinguish *H. debilis* ssp. *cucumerifolius* and *H. annuus*. The

specific details of the crossing program are as follows. A cytoplasmic male-sterile line of *H. annuus* (CMS89) was pollinated with a wild individual of *H. debilis* ssp. *cucumerifolius* collected near Austin, TX. Although *H. debilis* populations are polymorphic for restorer alleles for CMS, attempts to cross F_1's back to CMS89 failed due to low F_1 pollen viability, forcing us to use a closely related cultivar of *H. annuus*, USDA894, as the paternal parent, and a single male-sterile F_1 as the maternal parent, of the BC_1 mapping population. A total of 226 achenes were obtained from this crossing program and grown in greenhouses at Indiana University. Measurements of quantitative traits were taken after plants were fully grown (see below).

Phenotypic trait measurements: Fifteen morphological traits that differentiate *H. annuus* from *H. debilis* ssp. *cucumerifolius* were measured in the 226 BC_1 plants (Table 1). *H. annuus* ssp. *texanus* is intermediate between *H. debilis* ssp. *cucumerifolius* and other forms of *H. annuus* for all of these traits, with the exception of phyllary length and achene shape. Stem speckling was measured by counting the number of internodes that have at least some purple pigmentation. Leaf serration was measured as the average tooth height (cm) of the first three major leaves below the first head (at least five measurements from each leaf). Head number was a simple count, except that false heads (heads with short peduncles that did not develop fully) were excluded. The number of ray flowers was averaged over five heads when possible (some plants produced fewer than five heads). The length and width of ligules (cm) were measured from at least five ray flowers and averaged over multiple heads when possible. Ligule shape was estimated as a ratio of ligule length to width. Disk size (cm) was estimated as the internal diameter of fully opened heads and averaged over five heads when possible. The length and width of five of the outermost phyllaries (cm) were measured from the first head. Phyllary shape was estimated as the ratio of phyllary length to width. Phyllary pubescence was estimated by visually ranking the degree of pubescence on the outer phyllary on a scale of one to five, with one being glabrous and five being pubescent. Ten achenes from the first head were measured for the length and width in cm and achene shape was estimated as the ratio of length to width. Lastly, the pollen viability of backcross progenies was estimated by staining with 30% sucrose and 0.1% 3-(4.5-dimethylthiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Chandler *et al.* 1986), and counting at least 600 pollen grains per plant.

Means and variance were measured for each character in the BC_1 population, and deviations from normality were tested by calculating skewness and kurtosis. Traits with non-normal distributions were log-transformed using Mapmaker/QTL 1.1 (Lincoln *et al.* 1992). Transgressive segregation was detected by comparing the numbers of BC_1 progeny that exceed the high or low means of the parental traits by two, three, or four standard deviations, with the numbers expected by chance based on a population size of 226. All phenotypic trait measurements are available upon request from the corresponding author.

DNA isolations and genotyping: Total genomic DNA was isolated from 100 mg of fresh leaf tissue using DNeasy plant mini kits (QIAGEN, Chatsworth, CA) and then quantified on a TKO-100 fluorometer (Hoefer Scientific Instruments, San Francisco). All 226 BC_1 progeny were genotyped using amplified fragment length polymorphisms (AFLPs) following a slightly modified standard protocol (Vos *et al.* 1995; Travis *et al.* 1996). Briefly, 300 ng genomic DNA was digested for 1 hr at 37° with 3 units *Eco*RI, 3 units *Mse*I, 4 mg bovine serum albumin (BSA), 4 μl 10× buffer 2 (New England Biolabs, Beverly, MA), and ddH_2O to a final volume of 40 μl. Adapters were ligated to the digested fragments by adding 15 pmol *Eco*RI adapters, 150 pmol *Mse*I adapters, 0.5 μl T4 polynucleotide DNA ligase, 1 μl 10 mm ATP, 1 mg BSA, 2.0 μl 10× buffer 2, and ddH_2O, to a total volume of 50 μl and incubated for 3 hr at 37°.

Preamplification reactions were performed with two AFLP primers having a single selective nucleotide (A). The preamplification reactions contained 1 μl of template DNA from the ligation reaction, 187.5 ng *Eco*RI+A and 187.5 ng *Mse*I+A primers, 0.4 μl dNTPs (each at 25 mm), 2.5 μl PCR buffer (500 mm KCl, 15 mm $MgCl_2$, and 200 mm Tris HCl), 0.4 units Taq polymerase (Biology Department, Indiana University, Bloomington, IN), and ddH_2O in a total volume of 25 μl. The reactions were placed in a thermal cycler (MJ Research, Watertown, MA) programmed for 20 cycles, each consisting of 30 sec at 94°, 30 sec at 60°, and 1 min at 72°.

Once the preamplifications were complete, selective amplifications were performed using 2.5 μl of 1:20 diluted preamplification reaction as a template, 5 ng of the *Eco*RI+3 nucleotide selective primer (Vos *et al.* 1995), 15 ng of the *Mse*I+3 nucleotide selective primer (Vos *et al.* 1995), 0.16 μl dNTPs, 1 μl PCR buffer, Taq Polymerase, and ddH_2O to a final volume of 10 μl. Amplifications were conducted in a MJ Research thermal cycler programmed for 36 cycles, each consisting of 30 sec at 94°, 1 min 30 sec at 65° (see below), and 1 min 30 sec at 72°. The 65° annealing temperature of the first cycle was subsequently reduced by 1° for the next 10 cycles and then continued at 54° for the remaining 26 cycles. Likewise, the extension time of 1 min 30 sec was reduced to 1 min for the last 26 cycles.

All enzymes and buffers were purchased from New England Biolabs. Adapter and primer sequences are the same as those described in Vos *et al.* (1995). The *Eco*RI+3 primers were labeled using a 5′ oligonucleotide Texas Red labeling kit (Amersham and Life Science, Buckinghamshire, England) or a FluoReporter Oregon Green Oligonucleotide Amine labeling kit (Molecular Probes Inc., Eugene, OR). Oligonucleotides labeled with Texas Red were purified with TE Select-D, G-25 Spin Columns (5 Prime→3 Prime, Inc., Boulder, CO), whereas those labeled with Oregon Green were purified with the FluoReporter labeled oligonucleotide purification kit (Molecular Probes).

Following amplification, reaction products were mixed with an equal volume (10 μl) of formamide dye (98% formamide, 10 mm EDTA pH 8.0, and bromphenol blue). The resulting mixtures were heated for 3 min at 90°, and then quickly cooled on ice. Each sample (5.5 μl) was loaded on a 5% denaturing polyacrylamide gel and run at constant power, 50 W, for 1 hr 30 min. After electrophoresis, gels were scanned and visualized using an FMBIO II scanner and Image Analysis software (Hitachi Software Engineering Co., Ltd., Tokyo, Japan) with wavelengths of 625 nm for Texas Red-labeled and 505 nm for Oregon Green-labeled reactions, respectively. The Prism Genescan-500 ROX ladder (PE Applied Biosystems, Warrington, Great Britain) and MapMarker LOW 70-400BP fluorescein ladder (Bioventures Inc., Murfreesboro, TN) were run with the samples to estimate the size of Texas Red- and Oregon Green-labeled fragments, respectively. AFLP markers were named using the last two selective nucleotides in the *Eco*RI and *Mse*I primers followed by estimated fragment sizes: for example, GCCC.209 denotes reaction from primer combination of *Eco*RI+AGC and *Mse*I+ACC and the fragment size of 209 bp.

Linkage analysis: For each segregating locus, segregation distortion from an expected 1:1 ratio was determined by chi-square analysis. The effective number of independent markers per chromosome (M_{eff}), was calculated using equation 22 in Cheverud (1999). M_{eff} is determined using the variance of the observed eigenvalues of an intermarker correlation matrix relative to the maximum variance of the eigenvalues. For each

linkage group, the intended significance level ($\alpha = 0.05$) was divided by the effective number of markers (*i.e.*, the Bonferroni test) to provide an estimate of chromosome-wide significance levels. The effects of markers with heavily distorted ratios on linkage relationships were monitored throughout the mapping process to guard against the formation of pseudolinkages that join physically independent blocks or highly biased map distances (Cloutier *et al.* 1997).

MAPMAKER 3.0 (Lander *et al.* 1987) was used to infer linkage relationships among markers. Markers were first divided into linkage groups using the group command (parameters LOD > 3.5; $\theta < 0.25$). For linkage groups with 9 or fewer markers, an exhaustive analysis (*i.e.*, the compare command) was used to order markers. For linkage groups with 10 or more markers, three-point analysis (parameters LOD > 3.0; $\theta < 0.25$) was first performed, followed by the order command. The ordered marker sequences were confirmed using the ripple command. Haldane's (1919) mapping function was then used to transform the recombination frequency between linked loci into centimorgan (cM) distances.

QTL analysis: For traits with normal distributions (all but three), untransformed phenotypic data were analyzed with QTL Cartographer version 1.13 (Basten *et al.* 1997), whereas for the remaining three traits (number of speckled internodes, head number, and phyllary pubescence), log-transformed data were analyzed. Interval mapping (IM; model 3; Lander and Botstein 1989) as implemented by the program Zmapqtl in QTL Cartographer was used as a preliminary step to identify single QTL, and then composite interval mapping (CIM; model 6; Jansen and Stam 1994; Zeng 1994), also implemented by Zmapqtl, was used to identify multiple QTL associated with the same trait. CIM was run with a 10-cM window size and the number of markers for the background control was set to five. This means that the five most significant markers detected through stepwise regression (forward regression with the backward elimination option in the SRmapqtl program) that were outside the window were fitted to the model. Empirical experimentwise threshold values ($\alpha = 0.05$) for declaring the presence of QTL were estimated using the permutation resampling tests described by Churchill and Doerge (1994) and Doerge and Churchill (1996) as implemented by Zmapqtl. For both IM and CIM, threshold values were estimated from 1000 permutations of each phenotypic trait (*ca.* 4.5 hr/trait/model on a Power Macintosh G3). We recognize that the permutation thresholds do not take cofactors into account and thus may not be accurate for CIM. Nonetheless, we feel that the the permutation thresholds presented and based upon the standard Churchill and Doerge (1994) method are a good preliminary guideline and are more specific to the experiment than the standards set by Kruglyak and Lander (1995). A likelihood ratio decline of ≥ 9.212 (equivalent to a LOD score decline of two) between adjacent peaks on a single linkage group was considered evidence for multiple QTL. The program Eqtl in QTL Cartographer was used to summarize the positions and effects of the QTL using significance threshold values from the permutation tests. However, Eqtl appeared to have overestimated number and magnitude of QTL for several traits, and for these traits more conservative estimates were derived from Zmapqtl. One-LOD support limits for each QTL were calculated for QTL positions. Only the results of CIM are reported herein.

RESULTS

Phenotypic traits: *H. annuus* and *H. debilis* ssp. *cucumerifolius* differ dramatically for the phenotypic traits assayed (Table 2). For several characters (head number, disk diameter, and phyllary and achene size) these differences are exaggerated by the use of a cultivated line to represent *H. annuus* (Table 2). Generally, floral parts of *H. debilis* are much smaller than those of either wild or domesticated *H. annuus* (Tables 1 and 2).

Analysis of the BC_1 population revealed segregation for all traits, indicating the presence of *H. debilis* alleles with dominant or additive effects on each trait. None of the traits had a bimodal phenotypic distribution as

TABLE 1

Comparison of morphological traits among wild *H. annuus* ssp. *annuus*, *H. annuus* ssp. *texanus*, and *H. debilis* ssp. *cucumerifolius*

Trait	*H. annuus* ssp. *annuus*	*H. annuus* ssp. *texanus*	*H. debilis* ssp. *cucumerifolius*
Stem speckling	None	Intermediate	Heavy
Leaf serration	Serrate	Deeply irregular	Jagged
Head no.	Few	Intermediate	Many
Ray flower no.	21–35	19–25	13–18
Ligule length (l)	3.0–5.0 cm	2.4–3.2 cm	1.2–2.3 cm
Ligule width (w)	1.0–1.5 cm	0.8-1.2 cm	0.5–1.2 cm
Ligule shape (l/w)	3.0–3.8	2.7–3.1	1.9–2.1
Disk diameter	3.0–5.0 cm	2.1–3.0 cm	1.0–2.2 cm
Phyllary length (l)	1.0–1.5 cm	1.0–1.5 cm	1.0–1.1 cm
Phyllary width (w)	0.4–0.7 cm	0.35–0.6 cm	0.15–0.3 cm
Phyllary shape (l/w)	2.0–3.0	2.5–4.0	5.0–8.3
Phyllary pubescence	Hirsute	Sparingly hispid	Glabrous or sparingly hispid
Achene length (l)	0.4–0.7 cm	0.33–0.5 cm	0.32–0.4 cm
Achene width (w)	0.25–0.3 cm	0.2–0.25 cm	0.12–0.18 cm
Achene shape (l/w)	1.7–2.4	1.6–2.3	1.7–3.0

Data were obtained from Heiser (1951, 1954), Heiser *et al.* (1969), and from personal observations of greenhouse-grown plants.

TABLE 2

Morphological trait variation in the *H. annuus* and *H. debilis* ssp. *cucumerifolius* parental individuals used for mapping and their BC_1 progeny

Trait	*H. annuus* (CMS89)	*H. debilis* ssp. *cucumerifolius*	BC_1
No. speckled internodes	0	12	2.17 ± 4.75
Tooth height (cm)[a]	0.16 ± 0.04	0.27 ± 0.04	0.38 ± 0.14
Head no.	1	31	3.58 ± 2.71
Ray flower no.	33.8 ± 6.3	14.3 ± 2.5	28.6 ± 7.4
Ligule length (cm)[a]	4.94 ± 0.21	2.28 ± 0.17	4.52 ± 0.83
Ligule width (cm)[a]	1.56 ± 0.05	1.13 ± 0.06	1.54 ± 0.41
Ligule shape (l/w)[a]	3.17 ± 0.16	2.03 ± 0.08	3.03 ± 0.45
Disk diameter (cm)	7.0	1.54 ± 0.09	4.49 ± 1.33
Phyllary length (cm)[a]	2.80 ± 0.29	1.03 ± 0.05	2.94 ± 0.05
Phyllary width (cm)[a]	1.46 ± 0.07	0.16 ± 0.02	1.27 ± 0.32
Phyllary shape (l/w)[a]	1.92 ± 0.18	6.65 ± 0.95	2.45 ± 0.59
Phyllary pubescence	5	1	1.92 ± 1.00
Achene length (cm)	1.04 ± 0.05	0.34 ± 0.03	0.71 ± 1.00
Achene width (cm)	0.56 ± 0.06	0.15 ± 0.02	0.36 ± 0.11
Achene shape (l/w)	1.84 ± 0.16	2.32 ± 0.39	2.02 ± 0.27
Pollen viability	NA	>90%	24.4%

Values are means ± SD. NA, not applicable.
[a] Traits that exhibit significant transgressive segregation.

would be expected if the traits were controlled by a single Mendelian locus, although for head number and the number of speckled nodes, a large number of BC_1 plants recovered the *H. annuus* phenotype. The BC_1 population also segregated for male sterility due to heterozygosity at a fertility restorer locus in the backcross parent (USDA894). Thus, it was only possible to assay pollen viability in the 110 individuals that produced pollen. Pollen viability in these plants ranged from 1–83.7% with an average of 24.4%.

For 9 of the 15 traits, the phenotypic values of some individuals from the BC_1 population actually exceeded that of both parental individuals. However, for only 7 of these traits (tooth height, ligule length, ligule width, ligule shape, phyllary length, phyllary width, and phyllary shape; Table 2) were the number of progeny with extreme or transgressive phenotypes in excess of the numbers expected by chance. Six traits were transgressive in the direction of the recurrent parent, *H. annuus*, and 1 trait (tooth height), was transgressive in the direction of *H. debilis* ssp. *cucumerifolius*.

Marker segregation and linkage analysis: From a survey of over 100 AFLP primer combinations, 27 primer pairs were used to generate an initial 261-marker map for 84 progeny. Upon completion of this preliminary map, which consisted of 15 linkage groups, the remaining 142 progeny were genotyped for 133 markers (15 primer pairs) that provided fairly complete genomic coverage. Of these 133 markers, 40 (30%) exhibit significant segregation distortion. Of the 40 distorted markers, 26 were significantly overrepresented (*i.e.*, 26 *H. debilis* markers are significantly overrepresented in an *H. annuus* genetic background), whereas 14 markers were significantly underrepresented.

Linkage analysis of the 133 marker × 226 progeny data set revealed the same 15 linkage groups detected in the initial analysis (Figure 1). Because this set of AFLP markers has not been placed on other genetic maps for sunflower, linkage group designations were assigned on the basis of the results of the group output of MAPMAKER 3.0. There are several loose linkages on this map (on linkage groups 1, 4, 7, 8, and 11), each of which is associated with a heavily distorted marker. It is possible that one or more of these represents a pseudolinkage, and may explain the difference between the number of linkage groups mapped here (15) and the haploid chromosome number of these species (n = 17; Heiser 1951). Pseudolinkage also can be caused by translocations, which are known to differentiate these species. With respect to this latter point, it is noteworthy that a genetic factor affecting pollen viability is also associated with heavily distorted markers on linkage groups 2 and 8.

The map distance covered by the 15 linkage groups is 954.9 cM, with an average distance between markers of 7.18 cM. The map length reported here is considerably less than the estimated length of the *H. annuus* genome of 1650 cM (Gentzbittel *et al.* 1995). This difference may reflect incomplete genomic coverage in the current study or reduced recombination, which is a common feature of interspecific mapping populations.

Numbers and magnitudes of QTL: Composite interval mapping detected 56 QTL for the 15 morphological traits and 2 QTL for pollen viability (Table 3; Figure

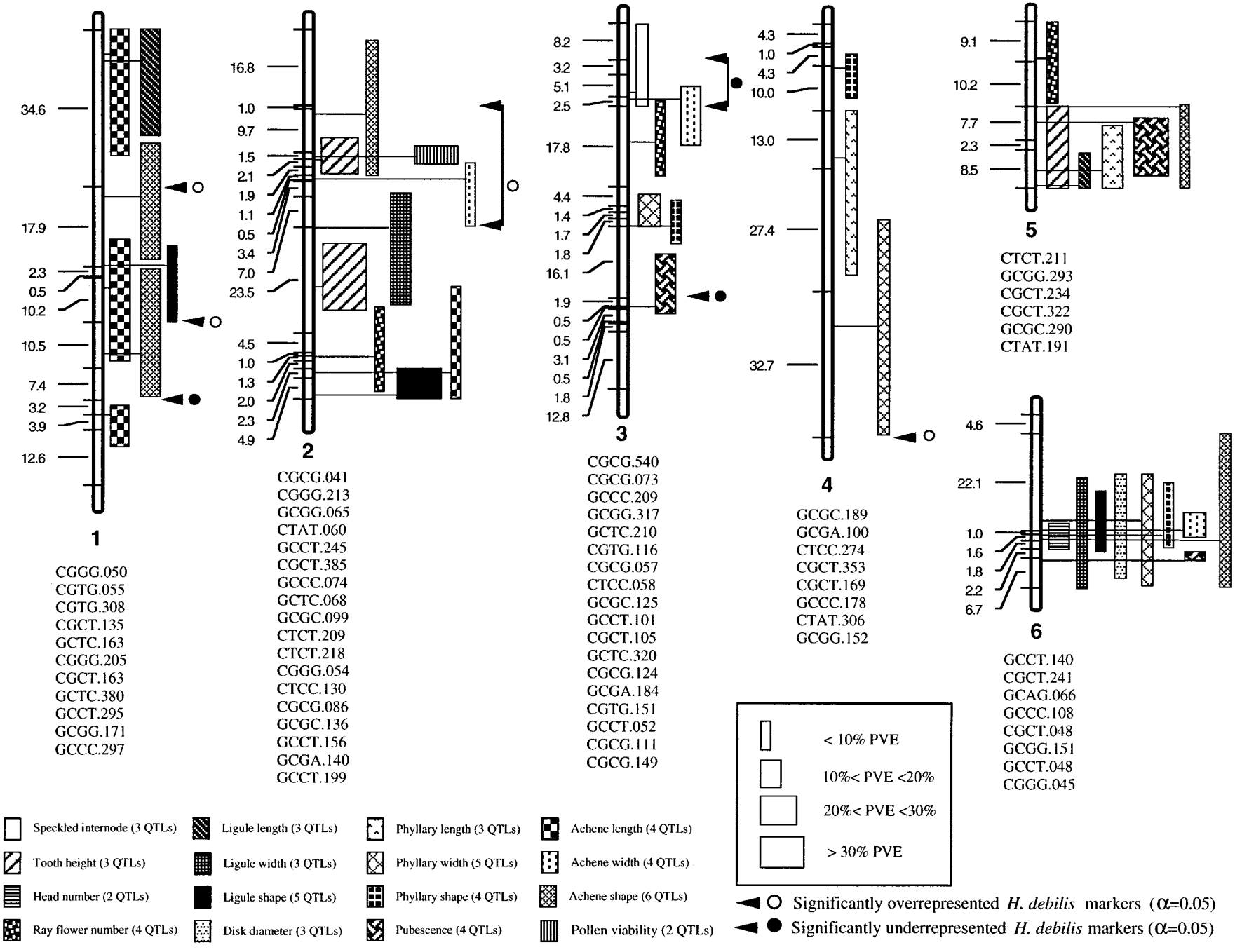
34.6
17.9
2.3
0.5
10.2
10.5
7.4
3.2
3.9
12.6
1
CGGG.050
CGTG.055
CGTG.308
CGCT.135
GCTC.163
CGGG.205
CGCT.163
GCTC.380
GCCT.295
GCGG.171
GCCC.297
16.8
1.0
9.7
1.5
2.1
1.9
1.1
0.5
3.4
7.0
23.5
4.5
1.0
1.3
2.0
2.3
4.9
2
CGCG.041
CGGG.213
GCGG.065
CTAT.060
GCCT.245
CGCT.385
GCCC.074
GCTC.068
GCGC.099
CTCT.209
CTCT.218
CGGG.054
CTCC.130
CGCG.086
GCGC.136
GCCT.156
GCGA.140
GCCT.199
8.2
3.2
5.1
2.5
17.8
4.4
1.4
1.7
1.8
16.1
1.9
0.5
0.5
3.1
0.5
1.8
12.8
3
CGCG.540
CGCG.073
GCCC.209
GCGG.317
GCTC.210
CGTG.116
CGCG.057
CTCC.058
GCGC.125
GCCT.101
CGCT.105
GCTC.320
CGCG.124
GCGA.184
CGTG.151
GCCT.052
CGCG.111
CGCG.149
4.3
1.0
4.3
10.0
13.0
27.4
32.7
4
GCGC.189
GCGA.100
CTCC.274
CGCT.353
CGCT.169
GCCC.178
CTAT.306
GCGG.152
9.1
10.2
7.7
2.3
8.5
5
CTCT.211
GCGG.293
CGCT.234
CGCT.322
GCGC.290
CTAT.191
4.6
22.1
1.0
1.6
1.8
2.2
6.7
6
GCCT.140
CGCT.241
GCAG.066
GCCC.108
CGCT.048
GCGG.151
GCCT.048
CGGG.045
< 10% PVE
10%< PVE <20%
20%< PVE <30%
> 30% PVE
Speckled internode (3 QTLs)
Tooth height (3 QTLs)
Head number (2 QTLs)
Ray flower number (4 QTLs)
Ligule length (3 QTLs)
Ligule width (3 QTLs)
Ligule shape (5 QTLs)
Disk diameter (3 QTLs)
Phyllary length (3 QTLs)
Phyllary width (5 QTLs)
Phyllary shape (4 QTLs)
Pubescence (4 QTLs)
Achene length (4 QTLs)
Achene width (4 QTLs)
Achene shape (6 QTLs)
Pollen viability (2 QTLs)
Significantly overrepresented H. debilis markers (α=0.05)
Significantly underrepresented H. debilis markers (α=0.05)

Figure 1.—Linkage map derived from the *H. annuus* (CMS89) × *H. debilis* ssp. *cucumerifolius* BC_1 population and QTL positions of 13 putatively introgressed and two non-introgressed vegetative and floral traits, as well as pollen viability. Marker positions are shown by horizontal lines, map distances between markers by numerals to the left, and QTL positions (with one-LOD support limits) and their magnitudes by vertical bars to the right of each linkage group. Marker names are listed according to order below each linkage group.

1). For each trait, between 2 and 6 QTL were found, and for 4 traits there was evidence for more than one QTL on a single linkage group (Figure 1).

Many of the phenotypic differences between *H. annuus* and *H. debilis* ssp. *cucumerifolius* are likely to have resulted from disruptive selection, creating the expectation that the effects of most *H. debilis* alleles will be in the direction of the *H. debilis* phenotype (Tables 2 and 3). This expectation was confirmed for 7 of the 15 phenotypic traits. However, single factors for ray number, ligule length, ligule width, ligule shape, phyllary length, phyllary shape, and achene width had effects in the direction of the *H. annuus* phenotype. For achene shape, three of six factors have effects in the direction of *H. annuus.*

Individual QTL explained 5.4–36.5% of the corresponding phenotypic variance for the 15 morphological traits (Table 3). If a threshold of ≥25% of phenotypic variance explained (PVE) is employed to declare a major QTL (Bradshaw *et al.* 1995, 1998), only three traits (speckled internodes, tooth height, and ligule shape) appear to be affected by one or more major QTL. Thus, most of the morphological traits differentiating *H. annuus* and *H. debilis* ssp. *cucumerifolius* appear to be controlled by QTL with intermediate or small effects. By contrast, pollen viability is affected by two genomic regions with large effects. However, the number and magnitude of QTL contributing to this trait should be interpreted cautiously given the reduced number of plants that could be assayed for pollen viability.

There are no apparent differences in the numbers and magnitudes of effects of QTL that are thought to have transversed the species barrier and those that have not. The two traits for which ssp. *texanus* does not differ from other forms of *H. annuus* (*i.e.*, nonintrogressed traits), phyllary length and achene shape, are controlled by three and six QTL, respectively, and this is in the range of the number of QTL for putatively introgressed traits (Table 3). Likewise, magnitudes of effects of QTL for nonintrogressed traits broadly overlap those of putatively introgressed traits (Table 3).

Genomic location of QTL: The detected QTL occur on 12 of the 15 mapped linkage groups. Nonetheless, some clustering of QTL is evident: eight QTL overlap in range of position (*i.e.*, one-LOD support limits) on linkage group 6, five QTL on linkage group 5, and four QTL on linkage group 7 and on linkage group 8 (Figure 1). At least some of the clustering is an artifact of scoring both shape and size characters for a given trait since shape is calculated as a ratio of trait length to width. It is not clear whether the remaining clustering is due to the nonrandom distribution of genes that affect the phenotypic differences between these species or whether it is due to pleiotropy. Some support for the pleiotropy hypothesis comes from observations that size-affecting QTL across several traits were clustered as follows: linkage group 1 (achene length/ligule length), linkage group 2 (achene width/ligule width), linkage group 5 (ligule length/phyllary length), linkage group 6 (ligule width/phyllary width/disk size), and linkage group 7 (achene width/phyllary width). Likewise, QTL for head number (linkage groups 6 and 7) are grouped with QTL that control size characters. Again, this relationship is predictable since head number is negatively correlated with head size.

Comparison of the location of QTL affecting phenotype with the location of sterility factors (linkage groups 2 and 8) indicates that only 4 QTL (out of 56) are tightly linked (<10 cM) to sterility factors. These include 1 QTL that accounts for 21.9% PVE for tooth height, 2 QTL accounting for 27.6% PVE for achene shape, and a single QTL for achene width (7.5% PVE).

Sterility is likely to be only one of several postmating barriers that isolate these species, so we also searched for possible linkages between QTL and chromosomal blocks that were underrepresented (and presumably negatively selected) in the BC_1 progeny. Seven QTL were within 10 cM of a significantly underrepresented marker: achene length (linkage group 1, 16.4% PVE), achene shape (linkage group 1, 13.2% PVE), speckled internode (linkage group 3, 6.3% PVE), achene width (linkage group 3, 11.2% PVE), ray flower number (linkage group 3, 9.1% PVE), phyllary pubescence (linkage group 3, 10.4% PVE), and phyllary width (1 QTL, linkage group 12, 6.6%). Surprisingly, the sterility QTL discussed in the previous paragraph occurred on overrepresented rather than underrepresented linkage blocks. Thus, a total of 11 morphological QTL appear to be linked to postmating isolating barriers. These QTL contribute to six traits for which *H. annuus* ssp. *texanus* approaches *H. debilis* ssp. *cucumerifolius* (tooth height, achene length and width, ray flower number, speckled internode, and phyllary width and pubescence), and one trait (achene shape) for which *H. annuus* ssp. *texanus* resembles other forms of the species.

DISCUSSION

Genetic architecture and introgression: The permeability of hybrid zones to gene flow or introgression depends in large part on the number and genomic distribution of factors that contribute to reproductive isolation (Barton and Hewitt 1985; Harrison 1990). If a large number of evenly dispersed factors contribute to isolation, the likelihood of successful introgression is low due to linkage. On the other hand, if isolation is due to a small number of factors and/or these factors are clustered, the possibility of introgression is considerably enhanced and will depend both on genomic location of the locus in question and on the fitness effects of the donor allele in the genetic background of the recurrent parent.

In this study, only two factors were detected that contribute to the sterility barrier between *H. annuus* and

TABLE 3

Putative QTL positions, LOD thresholds, LOD scores, and magnitude of effects for 15 morphological traits and pollen viability in a backcross population of *H. annuus* (CMS89) × *H. debilis* ssp. *cucumerifolius*

Trait[a]	LOD threshold	Linkage group	Position[b]	Interval markers[c]	LOD score	PVE (%)[d]
Speckled internode	2.68	3	13.41	GCCC.209-GCGG.317	3.09	6.3
		8	107.67	CGGG.110-GCCT.164	14.82	27.1
		11	38.34	CGCG.380-CGTG.097	22.26	36.5
Tooth height	2.68	2	28.92	GCCT.245-CGCT.385	11.65	21.9
		2	56.89	CTCT.218-CGGG.054	9.05	32.1
		5	33.28	GCGC.290-CTAT.191	6.41	13.8
Head no.	2.80	6	27.70	GCCC.108-CGCT.048	7.06	13.4
		7	131.78	GCCC.175-GCTC.150	5.11	9.9
Ray no.	2.69	2	73.89	CGCG.086-GCGC.136	4.21	8.7
		3	24.99	GCTC.210-CGTG.116	3.46	*9.1*
		5	8.01	CTCT.211-GCGG.293	4.13	8.8
		8	115.96	CGGG.235-GCGG.110	5.79	11.8
Ligule length	2.72	1	10.01	CGGG.050-CGTG.055	6.08	16.2
		5	37.28	GCGC.290-CTAT.191	3.91	*8.9*
		7	131.78	GCCC.175-GCTC.150	3.70	7.6
Ligule width	2.72	2	43.84	CTCT.209-CTCT.218	6.10	12.9
		6	27.70	GCCC.108-CGCT.048	2.81	*5.8*
		9	0.01	CGCG.360-CGTG.226	4.41	9.0
Ligule shape	2.75	1	52.49	CGTG.308-CGCT.135	3.36	6.9
		2	81.49	GCGA.140-GCCT.199	17.83	32.7
		6	26.72	GCAG.066-GCCC.108	3.87	*7.9*
		9	0.01	CGCG.360-CGTG.226	3.86	7.9
		13	0.01	CTCT.063-CTCT.133	3.25	6.7
Disk diameter	2.63	6	29.26	CGCT.048-GCGG.151	3.59	7.1
		8	105.67	CGGG.110-GCCT.164	4.93	10.2
		8	117.96	CGGG.235-GCGG.110	5.04	10.5
Phyllary length	2.53	4	29.60	CGCT.169-GCCC.178	2.88	6.8
		5	33.28	GCGC.290-CTAT.191	4.14	*10.8*
		11	49.88	GCCC.050-GCCC.071	5.40	12.1

(*continued*)

H. debilis ssp. *cucumerifolius*. This would suggest that much of the genome between these species is permeable to introgression. In fact, of the 56 QTL detected that contribute to the morphological differences between these two species, only 4 are likely to be seriously impeded by linkage to these sterility factors.

Although sterility is thought to be the primary reproductive barrier between *H. annuus* and *H. debilis*, we also detected five chromosomal blocks that were significantly underrepresented in the BC_1 population. If these blocks are negatively selected in hybrids as our data suggest, the 7 QTL within these blocks (or tightly linked to them) are also unlikely to introgress. Thus, a total of 11 QTL seem less likely to introgress due to linkage to sterility factors or other negatively selected loci.

Our inability to detect additional QTL affecting isolation in this study does not mean they do not exist. Sample sizes were small for the sterility analysis, and we did not analyze possible prezygotic barriers such as pollen competition or habitat differentiation. Nonetheless, the present investigation contrasts sharply with a similar study for *H. annuus* and *H. petiolaris*, in which 21 factors were detected that contribute to pollen sterility, and 26 blocks were significantly underrepresented in hybrids (Rieseberg *et al.* 1999b). Not surprisingly, little introgression is observed between *H. annuus* and *H. petiolaris* in nature (Heiser 1947; Rieseberg *et al.* 1999a,b).

Although 11 QTL are unlikely to move across the species barrier, could introgression of the remaining 45 QTL account for the phenotypic intermediacy of *H. annuus* ssp. *texanus*? The answer to this question appears to be yes. Because *H. annuus* ssp. *texanus* is intermediate between *H. debilis* and other forms of *H. annuus*, the recovery of its phenotype should not require all *H. debilis* QTL for a given trait. Moreover, some of the *H. debilis* QTL detected here probably differentiate it from the cultivated sunflower rather than wild *H. annuus*. In fact, given the clustering of QTL, introgression of small blocks from linkage groups 5, 6, and 8 might be sufficient to recover all aspects of the *H. annuus* ssp. *texanus* phenotype with the possible exception of achene

TABLE 3

(Continued)

Trait[a]	LOD threshold	Linkage group	Position[b]	Interval markers[c]	LOD score	PVE (%)[d]
Phyllary width	2.66	3	44.29	GCGC.125-GCCT.101	5.20	10.7
		4	72.01	CTAT.306-GCGG.152	3.20	9.4
		6	24.59	CGCT.241-GCAG.066	2.85	6.5
		7	131.78	GCCC.175-GCTC.150	3.72	7.7
		12	15.69	CTCT.127-CTCT.116	3.12	6.6
Phyllary shape	2.65	3	44.29	GCGC.125-GCCT.101	4.42	9.3
		4	9.64	CGCT.353-CGCT.169	2.79	*5.9*
		6	27.70	GCCC.108-CGCT.048	4.08	8.4
		8	103.67	CGGG.110-GCCT.164	3.57	7.4
Phyllary pubescence	2.66	3	64.63	CGCG.124-GCGA.184	5.40	10.4
		5	23.25	CGCT.234-CGCT.322	10.18	20.6
		6	33.24	GCCT.048-CGGG.045	8.78	16.3
		8	108.92	GCCT.164-GCAG.094	4.01	7.8
Achene length	2.25	1	8.01	CGGG.050-CGTG.055	6.98	16.4
		1	63.34	GCTC.163-CGGG.205	8.79	18.1
		1	86.59	GCCT.295-GCGG.171	7.54	15.2
		2	77.22	GCCT.156-GCGA.140	2.56	5.4
Achene width	2.65	2	33.99	GCTC.068-GCGC.099	3.53	7.5
		3	16.53	GCGG.317-GCTC.210	5.46	*11.2*
		6	26.72	GCAG.066-GCCC.108	8.56	17.1
		7	131.78	GCCC.175-GCTC.150	2.78	5.9
Achene shape	2.66	1	38.59	CGTG.055-CGTG.308	5.47	*13.2*
		1	71.51	CGGG.205-CGCT.163	4.90	*11.2*
		2	19.76	GCGG.065-CTAT.060	3.54	8.1
		5	19.25	CGCT.234-CGCT.322	3.56	7.6
		6	27.70	GCCC.108-CGCT.048	2.68	5.7
		8	22.01	GCCC.044-GCTC.083	5.86	*19.5*
Pollen viability	3.98	2	27.44	GCGG.065-CTAT.060	7.47	38.2
		8	38.00	GCTC.083-GCGA.295	4.57	38.8

[a] Nonintrogressed traits are in italics.
[b] Absolute position from left telomere in cM.
[c] Marker nearest to QTL is underlined.
[d] Proportion of phenotypic variation explained by QTL using CIM. QTL effects in the direction of the *H. annuus* phenotypes are in italic type.

length. This prediction can of course be tested via crossing experiments, which are currently underway.

Although the mapping data indicate that Heiser's (1951) introgression hypothesis is plausible, they do not provide definite proof of it. Rather, they do set the stage for further experimentation (currently underway) that may provide unambiguous verification or rejection. Briefly, we are testing natural populations of *H. annuus* ssp. *texanus* for *H. debilis* markers that flank the QTL thought to be responsible for the morphological intermediacy of ssp. *texanus*. Detection of flanking markers in ssp. *texanus* will provide strong evidence for the introgression of the QTL linked to them.

If evidence for the introgression of morphological QTL can be found, it would be crucial to determine whether putatively introgressed QTL do in fact provide some selective advantage to *H. annuus* in eastern Texas. Some traits such as head number and achene size seem likely to be adaptive because of their important effects on fecundity, whereas the adaptive value of other traits, such as phyllary shape, are less clear. Nonetheless, it should be feasible to generate backcross plants that differ by single QTL, which would enable field studies of the fitness effects of these loci.

Sterility QTL: As noted in the previous section, two major QTL were detected that contribute to reduced pollen viability. Although our data cannot discriminate between chromosomal rearrangements and genic factors as possible causes for the reduced fertility observed when these QTL are present, several lines of evidence point to a role for chromosomal rearrangements. These include observations that (1) the two species are karyotypically divergent, differing by a single paracentric inversion and 2–3 translocations; (2) F_1 pollen sterility is roughly correlated with the number of translocations that differentiate different Helianthus species (Whelan

1978; Chandler *et al.* 1986); and (3) much of the variation in pollen viability observed in segregating hybrids maps to translocation breakpoints (Quillet *et al.* 1995; Rieseberg *et al.* 1999b). However, meiotic study of BC_1 progeny that possess or lack the sterility QTL will be required to determine whether chromosomal rearrangements account for the pollen sterility of hybrids between *H. annuus* and *H. debilis.*

Another puzzle is that the pollen viability QTL are significantly overrepresented in the BC_1 mapping family. If these QTL correspond to chromosomal rearrangements, then we may have an example of meiotic drive. Although it is feasible for drive to move chromosomal rearrangements through hybrid zones, this does not appear to have taken place in the *H. annuus* × *H. debilis* hybrid zone since *H. annuus* ssp. *texanus* has the karyotype of other forms of *H. annuus* rather than the karyotype of *H. debilis.* Typically, exposure to a meiotically driven chromosomal rearrangement leads to strong selection for the accumulation of unlinked suppressors which reduce drive (Lyttle 1989).

Segregation distortion: Segregation distortion appears to be correlated with increasing genetic divergence between parental lines (Zamir and Tadmor 1986; Paterson *et al.* 1991; Quillet *et al.* 1995; Grandillo and Tanksley 1996) and was expected for the interspecific backcross performed here. However, the observation that >50% of distorted markers (26 out of 40) were distorted in the direction of the donor parent, *H. debilis*, was not expected. In fact, a ratio in the opposite direction was predicted since segregation distortion is often interpreted as evidence for the presence of postmating barriers (Burke *et al.* 1998; Whitkus 1998). Our results contrast with reports from introgression experiments involving other interspecific combinations in Helianthus (*e.g.*, Quillet *et al.* 1995; Rieseberg *et al.* 1996) and other groups of plants (*e.g.*, Liu *et al.* 1996; Fulton *et al.* 1997), in which most donor parent alleles are underrepresented. Possibly, many of the overrepresented *H. debilis* alleles confer some type of fitness advantage to hybrids. However, it seems equally likely that they represent segregation distorters that enhance the success of the gametes they inhabit even if they pose a significant fitness cost to the diploid phase (Haldane 1932). If the latter explanation is correct, they are likely to have only a short-term favorable impact on the introgression of linked QTL, because (as with meiotic drive) unlinked suppressors are likely to evolve rapidly.

Transgressive segregation: Most studies of morphological variation in segregating plant hybrids report the presence of extreme or transgressive phenotypes (Rieseberg and Ellstrand 1993), and transgressive segregation is the primary mechanism by which the extreme or novel adaptations observed in new hybrid ecotypes or species are thought to arise. However, only with the recent application of marker-assisted QTL methods has the genetic basis of transgressive trait expression become understood (*e.g.*, deVicente and Tanksley 1993; Xu *et al.* 1998). These studies point to the complementary action of genes from the two parental lines as the major cause of transgression. That is, transgression occurs when crosses are made between parental lines that are fixed for sets of alleles with opposing effects and when some QTL have effects that are in the opposite direction of the species differences for those traits. Alleles reducing a trait value are sometimes derived from the species that has the highest value for that trait and vice versa.

The results presented here are largely consistent with complementary gene action as an explanation for transgression. Five of six traits that were significantly transgressive toward *H. annuus* appear to be so because of *H. debilis* alleles with effects in that direction. However, observations of transgressive segregation for the remaining trait, phyllary width, cannot be explained by this mechanism. Possibly, QTL with effects in the direction of *H. annuus* exist for this character, but we failed to detect them. Alternatively, the transgression observed may be due to a different mechanism such as heterosis. A seventh trait, tooth height, also was significantly transgressive, but in the direction of *H. debilis.* Because our backcrosses were in the direction of *H. annuus*, we were unable to assess the complementary gene hypothesis for this trait.

Magnitude of QTL controlling species differences: As alluded to in the introduction, there has been considerable disagreement in the past regarding the genetic basis of adaptation, with some authors advocating an infinitesimal mode and others a role for major genes. However, this issue is now much less contentious because of recent theoretical work suggesting that there is an exponential distribution of factors during adaptation (Orr 1998) and that the first factor fixed can be fairly large. Although the present experiment does not provide sufficient resolution to assess whether the distribution of QTL magnitudes is exponential, we did have sufficient power to detect QTL with large magnitudes. Surprisingly, only 3 (2 vegetative and 1 floral) of the 15 phenotypic traits were affected by major QTL (≥25% PVE). This contrasts with (1) studies of other wild species in the sunflower family in which both vegetative and floral traits typically have major QTL (Vlot *et al.* 1992; Battjes *et al.* 1994; Van Houten *et al.* 1994; Hombergen and Bachmann 1994; Comes and Kadereit 1996); (2) the results of Bradshaw *et al.* (1995, 1998) who detected major genes at 9/12 floral traits that differentiate two Mimulus species; and (3) most studies involving crop species in which major QTL are common (reviewed in Tanksley 1993).

We have no ready explanation for the paucity of major QTL in this study. However, *H. annuus* and *H. debilis* occur in two fairly divergent clades within Helianthus section Helianthus, and are probably more divergent genetically than most lines or species employed in the

studies cited above. Perhaps with increasing genetic divergence, phenotypic diversity is partitioned among a larger number of QTL, reducing the likelihood of detecting major QTL. However, we are unaware of any studies that demonstrate a link between genetic divergence and the number or magnitude of detected QTL.

Limitations of this study: There are several limitations to the current study. The first of these involves the use of a cultivated form of *H. annuus* in the backcross mating design. Although this facilitated linkage and QTL mapping, we were unable to distinguish between QTL that were involved in the domestication of *H. annuus* and those that differentiate the wild species. This does not detract from our overall conclusion that the simple genetic architecture of hybrid sterility poses only a small impediment to the introgression of QTL that differentiate these species. However, it does detract from the usefulness of these data for future experiments (below) to study these QTL in wild populations. Another problem associated with the use of cultivated lines is that major QTL with negative pleiotropic effects are more likely to be fixed by artificial selection (*i.e.*, domestication) than by natural selection. Thus, even the very small number of major QTL detected in this study must be evaluated cautiously, since they may result from artificial rather than natural selection. Finally, the backcross QTL mapping design employed here was unable to detect QTL that have a recessive *H. debilis* allele.

Conclusions and future directions: The purpose of this study was to examine the relationship between sterility and morphological QTL using *H. debilis* ssp. *cucumerifolius* × *H. annuus* as an example. The simple architecture of the sterility barrier and the lack of linkage between most phenotypic QTL and sterility factors is consistent with the proposed origin of *H. annuus* ssp. *texanus* through introgression (Heiser 1951). We plan to further test this hypothesis by assaying individuals from natural populations of *H. annuus* ssp. *texanus* for the *H. debilis* markers that flank critical QTL and by assessing the adaptive value of these QTL in nature. Of course, the proposed field experiments will employ backcrossed progeny between populations of *H. annuus* from Oklahoma (that presumably gave rise to ssp. *texanus*) and *H. debilis*. The proposed experiments will not only provide the final chapter of a classic tale of introgression, but will also represent a rigorous test of Edgar Anderson's (1949) assertions regarding the role of introgression in adaptive evolution.

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