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Quantitative Trait Locus Analysis of the Early Domestication of Sunflower

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ABSTRACT

Genetic analyses of the domestication syndrome have revealed that domestication-related traits typically have a very similar genetic architecture across most crops, being conditioned by a small number of quantitative trait loci (QTL), each with a relatively large effect on the phenotype. To date, the domestication of sunflower (*Helianthus annuus* L.) stands as the only counterexample to this pattern. In previous work involving a cross between wild sunflower (also *H. annuus*) and a highly improved oilseed cultivar, we found that domestication-related traits in sunflower are controlled by numerous QTL, typically of small effect. To provide insight into the minimum genetic changes required to transform the weedy common sunflower into a useful crop plant, we mapped QTL underlying domestication-related traits in a cross between a wild sunflower and a primitive Native American landrace that has not been the target of modern breeding programs. Consistent with the results of the previous study, our data indicate that the domestication of sunflower was driven by selection on a large number of loci, most of which had small to moderate phenotypic effects. Unlike the results of the previous study, however, nearly all of the QTL identified herein had phenotypic effects in the expected direction, with the domesticated allele producing a more crop-like phenotype and the wild allele producing a more wild-like phenotype. Taken together, these results are consistent with the hypothesis that selection during the post-domestication era has resulted in the introduction of apparently maladaptive alleles into the modern sunflower gene pool.

PLANT domestication typically involves intense directional selection, which produces large changes in quantitative traits, often accompanied by some degree of reproductive isolation between wild and domesticated taxa. Crop evolution thus allows for the investigation of basic evolutionary phenomena such as the phenotypic response of populations to long-term directional selection, the genetic consequences of recent selective sweeps, and the limitations imposed on selection response by genetic architecture (*e.g.*, Stuber *et al.* 1980; Wang *et al.* 1999; Bost *et al.* 2001). Unlike researchers studying most wild systems, students of domestication often enjoy historical insights into the likely timing of selection, as well as the types of traits that have been subjected to selection. Common domestication traits include: increased seed or fruit size, more determinate growth and flowering, suppression of natural seed dispersal, and loss of self-incompatibility. Termed the "domestication syndrome" (Harlan 1992), these traits make crop plants easier to cultivate and result in more valuable products for human use.

Genetic analyses of the domestication syndrome have revealed that these traits have a similar genetic architecture across most crops (*e.g.*, Doebley *et al.* 1990; Doebley and Stec 1991, 1993; Paterson *et al.* 1991; Koinange *et al.* 1996; Xiong *et al.* 1999). More specifically, crop-related traits are typically conditioned by a small number of quantitative trait loci (QTL), each with a relatively large effect on the phenotype (reviewed in Ross-Ibarra 2005). Perhaps the most well-known example of this is maize, wherein just five genomic regions account for the majority of the phenotypic differentiation between teosinte and maize (Doebley and Stec 1991, 1993). Doebley and Stec (1991, p. 294) argued that if "evolution is opportunistic, one would predict that major shifts in the morphological traits of plants could be controlled by the full range of genetic mechanisms from few genes with large effects to many genes with small effects." They further argued that "The relative importance in plant evolution of these contrasting modes of inheritance remains to be determined."

Although wild populations have been shown to respond to selection in a variety of ways (*e.g.*, Bradshaw *et al.* 1998; Fishman *et al.* 2002), the pattern of few QTL of large effect is nearly universal in crop plants. In fact, there is just one counterexample—the evolution of domesticated sunflower, *Helianthus annuus* L. (Burke *et al.* 2002). In terms of the phenotypic response to cultivation, sunflower is a very typical crop. Human-mediated selection has resulted in a dramatic increase in apical dominance relative to its wild progenitor (common sunflower, also *H. annuus*), an increase in seed size, and the loss of natural seed dispersal, seed dormancy, and self-incompatibility. However, when these traits were

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investigated at a genetic level in a cross between common sunflower and an elite oilseed cultivar, they were found to be under the control of a large number of QTL of predominantly minor effect, with only 5% of all QTL detected accounting for ≥25% of the segregating phenotypic variation. Traits of obvious importance for domestication, such as seed weight, branching, and shattering, all lacked QTL of major effect, and seed dormancy was later shown to be under similarly complex genetic control in a different crop × wild mapping population (Gandhi *et al.* 2005).

The foregoing results suggest that sunflower may indeed be an exception to the rule, thereby supporting the notion that evolution under domestication is an opportunistic process, making use of whatever genetic variation happens to be available (Doebley and Stec 1991). However, a subsequent study of seed oil content and composition revealed that these original findings may have been influenced by the complex postdomestication breeding history of sunflower (Burke *et al.* 2005). In fact, it now seems clear that certain portions of the cultivated sunflower genome experienced postdomestication selective sweeps, meaning that the use of a modern inbred line as the cultivar parent in the original study likely confounded the effects of selection during domestication with the effects of selection during the subsequent period of breeding and improvement.

Here, we report the results of an investigation of the genetic architecture of sunflower domestication utilizing a cross between common sunflower and a primitive Native American domesticate. This work is thus designed to provide insight into the genetic changes that were necessary for the initial transformation of the weedy common sunflower into a useful crop plant. We have focused primarily on a suite of domestication-related traits that have previously been analyzed and are thus able to make a direct comparison to the results of earlier research.

MATERIALS AND METHODS

Mapping population: The mapping population described in this study was derived from a common × domesticated sunflower cross. The wild parent used in this cross was drawn from the same population (Ann1238) in Keith County, Nebraska, that served as the source of the wild parent in the previous QTL analysis of sunflower domestication (Burke *et al.* 2002). This population is located within the same general range of the common sunflower that is thought to have given rise to domesticated sunflower (Harter *et al.* 2004). The domesticated parent was the Hopi sunflower (USDA PI 432504), which was selected for analysis because it represents one of the two most primitive extant cultivated sunflower lineages (Tang and Knapp 2003; Harter *et al.* 2004; Wills and Burke 2006). A single, self-compatible F_1 individual from the initial wild × domesticated cross was self-pollinated to produce the F_2 generation. F_2 seeds were nicked with a razor blade and allowed to germinate on moist filter paper prior to being sown in flats. Seedlings were then transplanted into pots and grown under 16-hr days in the greenhouse. The final mapping population consisted of 378 F_2 individuals. Fifteen individuals each from the Hopi landrace and the Ann1238 population were grown along with the mapping population to estimate the phenotypic means of the parental lines when grown under these conditions.

TABLE 1

Comparison of 14 traits between a primitive sunflower domesticate (the Hopi landrace) and its wild progenitor (*Helianthus annuus* var. *annuus*)

Trait	Hopi landrace	Common sunflower
Days to flower	100.0 ± 4.4	84.8 ± 4.4
Stem diameter (mm)	21.7 ± 0.7	10.8 ± 0.7
Height (cm)	358 ± 14.0	171 ± 16.9
No. main stem leaves	47.5 ± 1.0	21.7 ± 1.9
Leaf size (cm^2)	687 ± 23.8	335 ± 27.7
No. branches	0.4 ± 0.2	9.4 ± 1.6
No. heads	1 ± 0.0	4.2 ± 0.6
Disk diameter (mm)	75.3 ± 4.3	23.3 ± 1.9
No. ray flowers	42.5 ± 3.8	23.7 ± 1.6
Self-compatibility	Yes	No
Achene weight (g/100)	2.9 ± 1.1	0.6 ± 0.1
Shattering	No	Yes
Seed dormancy	No	Yes

All values are expressed as mean ± standard error

Phenotypic trait measurements: Thirteen domestication-related traits that have been shown to differ between wild and domesticated sunflower were measured in all 378 F_2 plants as well as in the 15 individuals from each parental line (or their selfed progeny in the case of seed traits; Table 1). The number of days to flowering was recorded for each individual. At flowering, the number of rays, disc diameter of the primary head, stem length, length and width of largest leaf, and stem diameter 3 cm above the soil were recorded for each individual. Leaf size was calculated as length × width. The primary head on each individual was bagged to prevent pollination from neighboring plants and rubbed to ensure self-pollination until florets ceased to emerge (~9 days). Plants were maintained in the greenhouse until their seeds were mature, at which time the primary head was harvested, and the number of heads and branches were recorded for each individual. Primary heads were then dried for 3 days at 40°. To quantify shattering of the capitulum, the dried heads were dropped three times from a height of 12 cm. The total number of seeds released from the capitulum was then recorded, the heads were threshed, and the total seed output was recorded. Shattering was scored as the percentage of seeds released and 100-seed weight was estimated for each line. Seeds were then stored at 4° for 3 months. To quantify seed dormancy, 20 seeds from each selfed F_2 individual with sufficient seed output were sown in pots at a soil depth of 2 cm and allowed to germinate in a growth chamber under 16-hr days with constant bottom watering. The number of germinated seeds was recorded each day, and pots were monitored for 100 days. Seeds that failed to germinate during the course of this trial were scored as having germinated on the 100th day, and the mean number of days until germination was calculated for each F_2 line.

Genotyping: Total genomic DNA was extracted from a sample of leaf tissue from each F_2 individual using the Qiagen DNeasy plant mini kit (Qiagen, Valencia, CA). Genotyping for the genetic map was then carried out for 111 variable codominant loci, including 108 simple-sequence repeats

(SSRs) that were previously mapped in sunflower (Tang *et al.* 2002, 2003; Yu *et al.* 2003; Lai *et al.* 2005). The SSRs were fluorescently labeled with 6FAM, TET, HEX, or VIC either by direct labeling of the 5′ end of the forward primer or using a modification of the three-primer PCR methodology presented by Schuelke (2000), previously adapted for sunflower by Wills *et al.* (2005). This technique involves incorporation of an arbitrarily selected sequence (the M13 Forward [−29] sequencing primer, 5′-CAC GAC GTT GTA AAA CGA C-3′) to the 5′ end of the forward primer. PCR products are then labeled by including a fluorescently tagged (6FAM, TET, or HEX) M13 forward (−29) primer in the reaction mixture. All reactions were performed in 10 μl total volume containing 10 ng of template DNA, 30 mm Tricine pH 8.4-KOH, 50 mm KCl, 2mm $MgCl_2$, 100 μm of each dNTP, 0.02 μm forward primer, 0.1 μm of both the reverse primer and the fluorescently labeled M13F primer, and 2 units of *Taq* polymerase. When PCR was carried out with directly labeled primer pairs, the M13F primer was left out, and the forward primer was increased to 0.1 μm. Cycling conditions were as follows: initial denaturation at 95° for 3 min, followed by 10 cycles of 30 sec at 94°, 30 sec at 58° (annealing temperature was reduced by one degree per cycle), 45 sec at 72°, followed by 30 cycles of 30 sec at 94°, 30 sec at 48°, 45 sec at 72°, and a final extension time of 20 min at 72°.

Amplification products were visualized on either an MJ Research BaseStation automated DNA sequencer (South San Francisco, CA) or an Applied Biosystems 3730xl DNA analyzer (Foster City, CA). MapMarker 1000 ROX size standard (Bio-Ventures, Murfreesboro, TN) was included in each lane to allow for accurate determination of fragment size. Alleles were called using the software package Cartographer (MJ Research) for the BaseStation runs or GeneMarker (SoftGenetics, State College, PA) for the 3730 data. The final map included three additional, previously unpublished markers: HT39, HT135, and HT1490 (S. Tang and S. J. Knapp, unpublished data). HT39 amplicons were visualized via SSCP gel electrophoresis using 0.5× MDE gels that were run for 14 hr at 4 W (Slabaugh *et al.* 1997) followed by silver staining (Sanguinetti *et al.* 1994). HT135 exhibited a length polymorphism in this cross and was scored in the same manner as the SSRs described above. HT1490 was not length polymorphic and could not be reliably scored via SSCP analysis. Thus, this locus was sequenced, and a restriction polymorphism corresponding to an *Fnu*4HI restriction site was found to be segregating within the mapping population. The forward primer was therefore 5′ end labeled with 6FAM, and each individual was amplified as described above. All PCR amplicons were then digested at 37° overnight with 1 unit of *Fnu*4HI (New England Biolabs, Ipswich, MA). The PCR–RFLP products were then run on an Applied Biosystems 3730xl and scored using GeneMarker.

Map construction: The linkage map was constructed using MAPMAKER 3.0/EXP (Lander *et al.* 1987; Lincoln and Lander 1992). Initial linkage groups were identified using the "group" command with LOD > 5.0 and $\theta < 0.2$. Preliminary map orders within groups were then set based on the results from previous sunflower mapping studies (Burke *et al.* 2002; Tang *et al.* 2002, 2003; Yu *et al.* 2003; Lai *et al.* 2005). Final map orders were then confirmed using the "ripple" and "compare" commands, such that the map orders presented herein reflect the statistically most likely order on the basis of the data at hand.

QTL analysis: The initial QTL analysis followed the same general approach as outlined by Burke *et al.* (2002). Because shattering was scored as a proportion, the data for this trait were arcsine-square root transformed prior to analysis using JMP 4 (SAS Institute, Cary, NC). Composite interval mapping (CIM) (Zeng 1993, 1994) was then performed as implemented by the program Zmapqtl (model 6) of the software package QTL Cartographer version 1.17 (Basten *et al.* 1994, 2004). CIM was run with a 10-cM window and five background cofactors. Tests were performed at 2-cM intervals, and cofactors were selected via forward–backward stepwise regression using the program SRmapqtl. Genomewide threshold values ($\alpha = 0.05$) for declaring the presence of QTL were estimated from 1000 permutations for each trait (Churchill and Doerge 1994; Doerge and Churchill 1996). A likelihood-ratio decline of 9.21 (equivalent to a LOD decline of 2.0) between adjacent peaks on a linkage group was taken as evidence of multiple linked QTL and one-LOD support limits for the position of each QTL were calculated from the CIM results. The degree of dominance of the Hopi allele at each locus was calculated as the dominance effect divided by the additive effect (d/a), and the following arbitrary thresholds were used to classify the mode of gene action for each QTL: underdominant $\leq -1.25 <$ recessive $\leq -0.75 <$ partially recessive $\leq -0.25 <$ additive $\leq 0.25 <$ partially dominant $\leq 0.75 <$ dominant $< 1.25 \leq$ overdominant. Finally, to allow a direct comparison to the results of Burke *et al.* (2002), we used arbitrary percentage of variance explained (PVE) thresholds of 10 and 25% to classify QTL as having "minor," "intermediate," or "major" effects.

Multiple interval mapping (MIM) (Kao and Zeng 1997; Kao *et al.* 1999) was then used to search for epistatic interactions amongst the QTL identified via CIM. The CIM results were used as the initial model for the MImapqtl module in QTL Cartographer (Basten *et al.* 1994, 2004), and the maximum number of allowable pairwise interactions was set to 19. Only those interactions that significantly improved the fit of the model were retained. As recommended by the authors, significance was determined on the basis of the information criterion $IC(k) = -2(\log(L) - k\,c(n)/2)$, where $c(n) = \log(n)$ as the penalty function and a threshold of 0.0.

RESULTS

Linkage analysis: The map coalesced into the expected 17 linkage groups and covered a total of 906.4 cM with an average intermarker distance of 8.2 cM. As has been previously observed for common × cultivated sunflower crosses (*e.g.*, Burke *et al.* 2002), this map showed evidence of suppressed recombination, with common marker intervals exhibiting nearly 20% compression when compared against the sunflower reference map, which is based on a cross between two elite inbred lines (RHA280 × RHA801) (Tang *et al.* 2002; S. Tang and S. J. Knapp, unpublished data). On the basis of a comparison of shared markers, coverage of the map described herein is equal to or exceeds that of the map constructed by Burke *et al.* (2002) for 16 of the 17 linkage groups (LGs). The one exception was a portion of the top of LG17, which we were unable to cover due to a lack of polymorphism. However, no QTL have been detected previously in this region, so this small gap in coverage is unlikely to influence our overall findings.

QTL analysis: For the 13 domestication-related traits that we analyzed, CIM detected 61 QTL (Table 2; Figure 1). The number of QTL per trait ranged from 2 to 8 (mean = 4.7) (Figure 2A) with shattering, and the number of heads produced being the only traits with multiple QTL on a single linkage group. QTL were found on all linkage groups with the exception of LG11, and the one-LOD support intervals, which provide an

TABLE 2

Putative QTL positions, effect magnitudes, and modes of gene action for 13 traits using composite interval mapping in an F_2 population derived from a cross between a primitive sunflower domesticate (the Hopi landrace) and its wild progenitor (*H. annuus* var. *annuus*)

Trait	Linkage group[a]	Position[b]	Nearest marker	One-LOD interval[c]	Additive effect[d]	Dominance ratio[e]	PVE[f]	Previously identified?[g]
Days to flower	6	57.6	ORS483	53.6–57.7	4.6	−0.23	7.6	Yes
	7	1.0	ORS1041	0–5.3	−2.6	−0.11	2.5	Yes
	15	57.1	ORS687	57–58.2	10.4	−0.49	46.9	No
Stem diameter	1	7.0	HT1018	4.6–10.4	1.2	0.72	10.0	Yes
	2	1.7	ORS925	0–15.0	0.7	−0.03	3.0	No
	3	3.4	ORS665	0–9.9	1.1	−0.33	6.5	Yes
	8	43.8	HT668	37.8–46.8	1.3	0.84	8.0	No
	15	56.4	ORS1141	52.4–58.2	1.7	0.16	15.7	No
Height	1	8.0	ORS716	4.6–10.0	29.6	0.45	11.9	No
	6	57.6	ORS483	47.6–57.7	23.1	0.01	6.4	Yes
	9	10.0	ORS1265	2.0–19.0	15.7	0.56	3.0	No
	14	16.1	HT319	10.1–18.0	11.5	1.19	3.0	No
	15	57.1	ORS687	57.0–58.2	53.2	−0.31	39.4	No
No. main stem leaves	6	57.6	ORS483	55.6–57.7	2.7	0.09	4.9	Yes
	7	1.0	ORS1041	0–7.3	−1.8	0.31	2.7	Yes
	9	19.0	HT294	13.0–39.8	2.6	−0.23	5.3	Yes
	15	57.1	ORS687	57.1–58.2	8.0	−0.49	57.0	No
Leaf size	5	31.6	ORS852	21.6–44.5	64.8	−0.71	9.1	Unknown
	8	35.6	ORS1161	32.4–35.8	56.4	−0.14	5.6	No
	10	15.8	ORS437	7.8–18.9	46.7	0.64	4.4	No
	14	10.1	ORS307	3.1–18.0	36.4	1.34	4.9	No
	15	57.0	ORS1141	50.5–58.2	42.2	0.97	3.7	No
	16	45.4	ORS407	37.4–60.1	−31.6	−1.96	5.1	No
No. branches	10	17.1	ORS437	9.8–24.8	−1.3	0.26	4.6	No
	13	0	HT848	0–17.6	−1.4	−0.1	5.2	No
	16	30.1	ORS899	22.0–36.1	−1.3	−0.87	7.0	No
	17	22.0	ORS735	16.0–30.1	−0.2	11.47	8.4	No
No. heads	6	41.1	ORS1229	22.8–53.6	0.8	−0.43	3.1	No
	8	29.5	ORS147	19.5–35.2	−1.3	−0.06	5.9	No
	10	15.8	ORS437	11.8–18.9	−2.4	0.59	28.1	No
	13a	0	HT848	0–2.0	−1.2	−0.2	5.3	No
	13b	15.6	ORS317	5.6–27.6	−1.3	0.03	6.5	No
	16	34.1	ORS993	28.1–45.4	−0.5	−2.19	3.4	No
	17	24.8	ORS735	14.0–32.8	−0.4	2.31	3.4	Yes
Disc diameter	1	14.4	HT39	0–18.3	2.7	0.67	4.4	No
	6	53.6	ORS381	45.6–57.7	2.3	−1.09	4.9	No
	8	35.2	ORS456	32.4–45.8	4.1	0.23	9.0	No
	9	17.0	ORS1265	8.0–22.5	3.8	0.16	7.7	No
	10	13.8	ORS437	7.8–17.1	4.4	0.74	13	No
	14	12.1	ORS307	0–18.0	2.8	0.7	5.6	No
	15	50.5	ORS7	35.1–57.1	2.0	1.61	4.3	No
	17	4.0	ORS565	0–10.0	3.8	−0.14	5.5	No
No. ray flowers	5	19.6	ORS505	8.5–29.6	1.9	−0.2	6.7	No
	8	32.4	ORS147	23.5–41.8	0.8	1.77	3.1	No
	10	13.8	ORS534	4.0–22.9	1.4	0.04	3.6	No
	12	72.3	HT466	65.7–72.8	1.9	0.28	4.3	No
	15	57.1	ORS687	48.5–58.2	2.8	0.23	13.1	No
	17	26.8	ORS735	10.0–33.7	1.2	0.92	4.6	No
No. selfed seeds	1	14.4	HT39	8.0–30.3	48.7	−0.5	6.6	No
	8	15.5	ZVG34	6.2–29.5	44.2	0.69	5.4	No
	12	72.3	HT466	65.7–72.8	60.4	0.06	6.8	No
	17	18.0	ORS735	10.0–33.7	37.3	1.08	7.2	Yes
Achene weight	1	6.6	HT1018	2.0–18.3	1.6	0.70	8.6	No
	8	35.2	ORS456	19.5–35.8	1.7	0	7.6	No
	9	19.0	HT294	6.0–35.8	1.3	0.37	4.2	Yes

(*continued*)

TABLE 2

(Continued)

Trait	Linkage group[a]	Position[b]	Nearest marker	One-LOD interval[c]	Additive effect[d]	Dominance ratio[e]	PVE[f]	Previously identified?[g]
	10	15.8	ORS437	9.8–18.9	2.6	0.36	19.0	Yes
Shattering[g]	4a	0	HT298	0–4.0	−0.1	0.26	10.7	n/a
	4b	33.4	ORS674	32.6–41.4	0.1	0.04	6.4	n/a
	10	15.8	ORS437	7.8–20.1	−0.1	0.38	9.0	n/a
Seed germination	12	72.3	HT466	71.4–72.8	−10.0	−1.31	17.3	n/a
	15	57.1	ORS687	48.5–58.2	−20.3	0.16	17.8	n/a

[a] When multiple QTL for a single trait occurred on the same linkage group, a letter was used to uniquely identify them.
[b] Absolute position from the top of the linkage group (in centimorgans).
[c] Refers to the region flanking each QTL peak in which the LOD score declines by one.
[d] Refers to the additive effect (a) of the Hopi allele. Underlined values indicate instances in which the allelic effects are in the wrong direction. See text for details.
[e] Refers to the dominance ratio (d/a) of the Hopi allele.
[f] Percentage of phenotypic variation explained by each QTL using CIM. PVE values for QTL with effects in the direction of the wild phenotype are underlined.
[g] Indicates whether or not a given QTL was detected in the previous cultivated × wild sunflower QTL analysis (Burke *et al.* 2002). The determination of overlap between studies was based on the one-LOD confidence intervals. Note that the previous analysis of shattering was based on a different (indirect) measure and that seed germination has not been previously analyzed.

approximate confidence interval for the true location of a given QTL, ranged from 1.1 to 30.8 cM (mean = 13.0 cM). Multiple overlapping QTL were observed on most linkage groups; the exceptions were LG2, LG3, and LG4, as well as LG11, which (as noted above) was devoid of QTL.

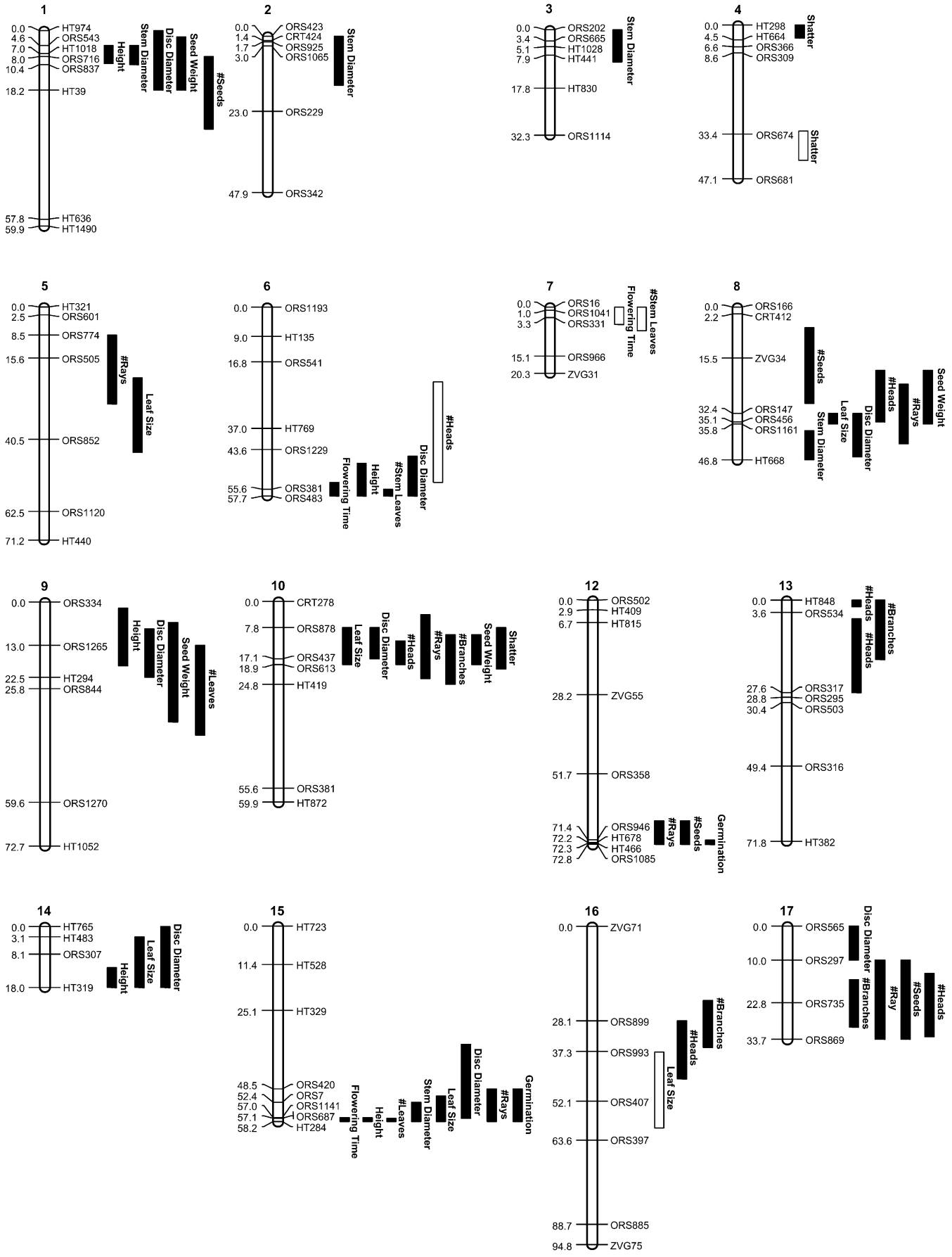
As previously documented, there was a paucity of QTL of major effect. Individual QTL explained 2.5–59.0% of the observed phenotypic variance for a particular trait, but only four QTL had PVE ≥25% (Figure 2B). Three of these major-effect QTL co-occur on the bottom of LG15 and influence days to flower, plant height, and number of leaves along the main stem. The only other QTL of major effect is located on LG10 and influences the number of heads produced. Contrary to previous findings, the majority of domestication-related QTL identified here (56 of 61) had effects in the expected direction (Figure 2C). That is, the Hopi allele produced a more crop-like phenotype, and the wild allele produced a more wild-like phenotype. The exceptions were QTL for shattering on LG4, number of heads produced on LG6, days to flower and number of leaves along the main stem on LG7, and leaf size on LG16. All five QTL with effects in "wrong" direction were minor, explaining from 2.5 to 6.4% of the phenotypic variance. In terms of the mode of gene action, the Hopi allele at each locus exhibited a dominance ratio (d/a) ranging from −2.19 to 11.47 with a mean of 0.38 (Table 2; Figure 2D).

Results of the search for epistasis amongst significant QTL using MIM are presented in Table 3. There were 30 significant pairwise interactions across the 13 traits. For the most part, these interactions were minor, and their phenotypic effects were mixed. Indeed, 28 of 30 interactions had an effect of <5%, and half were in the expected direction, whereas the other half were in the wrong direction. The two exceptions were interactions for branching and seed germination. In the former case, there was a dominant × additive interaction with an effect of 9.3% between QTL on LG13 and LG17, whereas the latter case had an additive × additive interaction with an effect of 11.5%. In both cases, the interactions acted in the expected direction, with Hopi/Hopi genotypes producing an even more Hopi-like phenotype.

DISCUSSION

QTL numbers and magnitudes of effect: In general terms, the results of this study confirm that sunflower is an exception to the rule in that its domestication involved changes at a large number of loci, each of relatively small effect (Figure 2, A and B). Indeed, we analyzed 13 traits and identified a total of 61 QTL, only 4 of which had PVE > 25%. In contrast, the domestication of crops such as maize (Doebley and Stec 1991, 1993), rice (Xiao *et al.* 1998; Xiong *et al.* 1999), and beans (Koinange *et al.* 1996) were all driven by relatively major changes at a much smaller number of loci. This observed lack of major QTL suggests that the transition from wild to domesticated sunflower was relatively smooth with few major phenotypic leaps.

Gene action and interaction: In terms of the predominant mode of gene action, our results mirror those of Burke *et al.* (2002) and stand in stark contrast to the view that domestication is generally driven by recessive genetic changes (*e.g.*, Ladizinsky 1985; Lester 1989). In fact, inspection of Figure 2D reveals a preponderance of nonrecessive QTL, suggesting that selection during domestication likely resulted in a rapid phenotypic response, as most of these QTL would have been at least


1
0.0 HT974
4.6 ORS543
7.0 HT1018
8.0 ORS716
10.4 ORS837
18.2 HT39
57.8 HT636
59.9 HT1490
Height
Stem Diameter
Disc Diameter
Seed Weight
#Seeds
2
0.0 ORS423
1.4 CRT424
1.7 ORS925
3.0 ORS1065
23.0 ORS229
47.9 ORS342
Stem Diameter
3
0.0 ORS202
3.4 ORS665
5.1 HT1028
7.9 HT441
17.8 HT830
32.3 ORS1114
Stem Diameter
4
0.0 HT298
4.5 HT664
6.6 ORS366
8.6 ORS309
33.4 ORS674
47.1 ORS681
Shatter
Shatter
5
0.0 HT321
2.5 ORS601
8.5 ORS774
15.6 ORS505
40.5 ORS852
62.5 ORS1120
71.2 HT440
#Rays
Leaf Size
6
0.0 ORS1193
9.0 HT135
16.8 ORS541
37.0 HT769
43.6 ORS1229
55.6 ORS381
57.7 ORS483
Flowering Time
Height
#Stem Leaves
Disc Diameter
#Heads
7
0.0 ORS16
1.0 ORS1041
3.3 ORS331
15.1 ORS966
20.3 ZVG31
Flowering Time
#Stem Leaves
8
0.0 ORS166
2.2 CRT412
15.5 ZVG34
32.4 ORS147
35.1 ORS456
35.8 ORS1161
46.8 HT668
#Seeds
Stem Diameter
Leaf Size
Disc Diameter
#Heads
#Rays
Seed Weight
9
0.0 ORS334
13.0 ORS1265
22.5 HT294
25.8 ORS844
59.6 ORS1270
72.7 HT1052
Height
Disc Diameter
Seed Weight
#Leaves
10
0.0 CRT278
7.8 ORS878
17.1 ORS437
18.9 ORS613
24.8 HT419
55.6 ORS381
59.9 HT872
Leaf Size
Disc Diameter
#Heads
#Rays
#Branches
Seed Weight
Shatter
12
0.0 ORS502
2.9 HT409
6.7 HT815
28.2 ZVG55
51.7 ORS358
71.4 ORS946
72.2 HT678
72.3 HT466
72.8 ORS1085
#Rays
#Seeds
Germination
13
0.0 HT848
3.6 ORS534
27.6 ORS317
28.8 ORS295
30.4 ORS503
49.4 ORS316
71.8 HT382
#Heads
#Heads
#Branches
14
0.0 HT765
3.1 HT483
8.1 ORS307
18.0 HT319
Height
Leaf Size
Disc Diameter
15
0.0 HT723
11.4 HT528
25.1 HT329
48.5 ORS420
52.4 ORS7
57.0 ORS1141
57.1 ORS687
58.2 HT284
Flowering Time
Height
#Leaves
Stem Diameter
Leaf Size
Disc Diameter
#Rays
Germination
16
0.0 ZVG71
28.1 ORS899
37.3 ORS993
52.1 ORS407
63.6 ORS397
88.7 ORS885
94.8 ZVG75
#Branches
#Heads
Leaf Size
17
0.0 ORS565
10.0 ORS297
22.8 ORS735
33.7 ORS869
Disc Diameter
#Branches
#Ray
#Seeds
#Heads

partially visible to selection, even when rare. These findings are in accord with previous QTL results from other taxa, including tomato (Paterson *et al.* 1991) and maize (Doebley *et al.* 1994).

With regard to the role of gene interaction in domestication, our results provide somewhat limited evidence of epistasis. When combined with the overall lack of epistasis documented by Burke *et al.* (2002), these results suggest that neither the initial domestication of sunflower nor its subsequent improvement relied heavily upon the fixation of favorably interacting gene complexes. While MIM detected significant QTL × QTL interactions for 10 of the 13 traits, the vast majority of these interactions had effects of ≤5% (Table 3). The exceptions to this were an interaction between two branching QTL located on LG13 and LG17 and an interaction between the two seed dormancy QTL on LG12 and LG15. This latter case, which involves a synergistic additive × additive interaction between two QTL of intermediate effect (PVE = 17.3 and 17.8%, respectively), is particularly noteworthy because seed dormancy was not previously analyzed by Burke *et al.* (2002). Gandhi *et al.* (2005) did, however, map QTL related to seed dormancy in a different elite × wild cross and identified a QTL of intermediate effect in the same region of LG15, as well as two other QTL that were not recovered here; no significant epistatic interactions were detected among those QTL.

QTL concordance: Despite the foregoing similarities between our results and those of Burke *et al.* (2002), a direct comparison of QTL locations reveals a relatively low level of concordance. Indeed, comparing the 59 QTL identified in this study (ignoring the two seed dormancy QTL because this trait was not previously analyzed) to the 56 QTL previously identified for this same suite of traits reveals only 15 cases in which QTL for the same trait mapped to the same linkage group in both studies. Twelve of these cases involved QTL with overlapping one-LOD support intervals, suggesting that the same QTL was detected in both crosses, 2 showed clear evidence of nonoverlap, and in one case the degree of overlap could not be determined because of a paucity of shared markers (LG5). This relatively low rate of correspondence between studies is likely due to a combination of factors, including differences between the parents used in each cross, QTL × environment interactions, and difficulties associated with reliably detecting QTL of small effect (Beavis 1994).

Because sunflower is an annual plant, it was impossible to use the same wild individual in both the present and previous crosses. Moreover, wild sunflower is an obligate outcrosser that exhibits high levels of heterozygosity (*e.g.*, Ivanov 1975; Fernandez-Martinez and Knowles 1978; Tang and Knapp 2003; Harter *et al.* 2004). Thus, in an attempt to minimize problems with intra-taxon polymorphism and maintain continuity with previous work, the wild parent for the present cross was drawn from the same population that Burke *et al.* (2002) utilized. Although variation is evident in the wild for all of the traits in question, the phenotypic differences between wild and cultivated sunflower are largely consistent across environments. Despite this, it is still possible that some of the differences between the two studies resulted from allelic variation between the wild parents used in the two studies.

A more likely explanation is that a sizable fraction of the differences result from the cultivar parents used in the two studies having very different evolutionary histories; in fact, this was the primary motivation of the present study. The cultivar parent utilized by Burke *et al.* (2002) was a highly improved, elite oilseed line that has subsequently been found to bear the signature of post-domestication selective sweeps, presumably due to selection on oil-related characters (Burke *et al.* 2005). In contrast, the cultivar parent used in the present study is a primitive Native American landrace. The results presented herein should, therefore, provide a much more accurate picture of the genetic changes necessary to transform wild into domesticated sunflower, as they are largely free from the confounding effects of improvement subsequent to the initial domestication event. In this context, it is worth noting that LG6 has previously been shown to a harbor a large cluster of QTL that mostly have effects in the wrong direction (Burke *et al.* 2002). Subsequent work has suggested that at least some of these QTL arose as a byproduct of selection during sunflower improvement (Burke *et al.* 2005), and our results are fully consistent with this hypothesis. Indeed, only a subset of the QTL that were initially identified were recovered in the present analysis, and all but one of the QTL on this linkage group now have effects in the expected direction.

Another key difference between the cultivars utilized in these two studies is that they are adapted to relatively different habitats. As such, some of the QTL that have been identified in just one population are likely to reflect differences in local adaptation. Most notable in this context is flowering time (and associated traits such as height and number of stem leaves) in the Hopi × wild cross analyzed here. The Hopi landrace exhibits late flowering, presumably as an adaptation to the extremely long growing season of the desert southwest. Our results

Figure 1.—Results of the CIM analysis for the 16 linkage groups on which QTL were detected. QTL positions are indicated by bars alongside each linkage group. The length of each bar is equal to the one-LOD support interval for that QTL. Loci at which the crop allele had the expected effect are indicated by a filled bar, whereas those at which the crop allele conferred a wild-like phenotype are represented by unfilled bars.

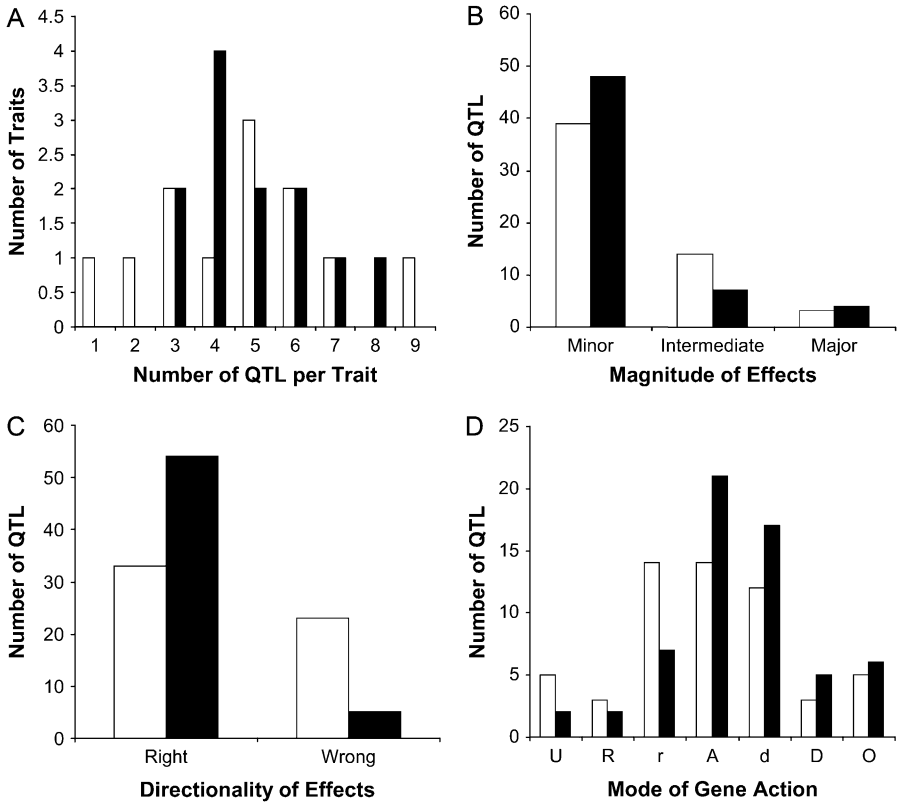


FIGURE 2.—Comparison of the number of QTL (A) per trait, (B) magnitude of effects, (C) directionality of effects, and (D) mode of gene action between a previous study based on an elite × wild sunflower mapping population (open bars) (BURKE *et al.* 2002) and the present study, which was based on a primitive × wild mapping population (closed bars). The following thresholds were used to classify the mode of gene action for each QTL: underdominant ≤ -1.25 < recessive ≤ -0.75 < partially recessive ≤ -0.25 < additive ≤ 0.25 < partially dominant ≤ 0.75 < dominant < $1.25 \leq$ overdominant.

indicate that this flowering time difference is conditioned by a major QTL at the bottom of LG15, which, as one might expect, was not present in the previous cross.

As noted above, other factors that could account for the relatively low level of QTL concordance are QTL × environment interactions and the difficulties associated with reliably detecting QTL of small effect. With regard to QTL × environment interactions, it has previously been shown that individual QTL can vary in their degree of environmental sensitivity, with some QTL being robust across environments, while others can be detected only under certain conditions (*e.g.*, PATERSON *et al.* 1991, 2003). Thus, even though both populations were greenhouse grown, and the traits of interest are reasonably robust across environments, it is conceivable that some fraction of the QTL were detected in one study but not the other because of differences in growing conditions.

Regarding the issue of detectability, it is well known that QTL of minor effect suffer a higher false-negative rate as compared to QTL of major effect (BEAVIS 1994). Indeed, DOEBLEY and STEC (1993) found much higher agreement in QTL locations in a comparison between two teosinte × maize populations for QTL of major effect (81% concordance for QTL with $r^2 \geq 20\%$) as compared to QTL of intermediate or minor effect (55 and 28% concordance for QTL with $10\% \leq r^2 \leq 20\%$ and QTL with $r^2 < 10\%$, respectively). Given the typically small effect sizes associated with QTL identified in both the present and previous analyses, the relatively low QTL concordance is therefore not surprising. Consistent with this idea is the fact that the handful of major QTL identified by BURKE *et al.* (2002), including QTL for flowering time and the number of stem leaves on LG6 and the number of selfed seeds on LG17, were all recovered in the present study. The key difference is that the estimated effect sizes for all of these QTL were much lower in the present study. In the case of flowering time and the number of stem leaves, the reduced PVE in the Hopi × wild cross is likely due to an overall increase in phenotypic variance for these traits within the mapping population (our unpublished data) due to the adaptation of the Hopi sunflower to the long growing seasons of the desert southwest (see above). In the case of selfed seed production, the prior identification of two QTL at the bottom of LG17 (BURKE *et al.* 2002) has subsequently been shown to be an artifact of inconsistent locus ordering; this region is now believed to harbor the *S* locus (GANDHI *et al.* 2005). Values reported in Figure 2 from the earlier study have been adjusted to account for the reordering of these markers. The low PVE associated with this locus in the present analysis is potentially an artifact of extreme segregation distortion in this region (all four markers on this linkage group deviate significantly from the expected segregation ratios, with all $P < 0.001$).

Conversely, the four QTL of major effect identified in the present study had not been previously identified. This result is not surprising for the QTL related to flowering time that are located near the bottom of LG15, as they are likely a byproduct of adaptation of the

TABLE 3

Summary of significant interactions amongst individually significant QTL

Trait	Linkage groups	Type of interaction[a]	Phenotypic effect[b]	Effect (%)
Height	6 × 15	A × A	12.5	2.2
	9 × 15	D × A	17.8	1.3
	9 × 15	D × D	−22.9	1.0
No. main stem leaves	6 × 15	A × A	2.4	3.8
	7 × 9	A × A	−1.2	1.3
Leaf size	5 × 8	A × A	−46.8	1.6
	5 × 14	D × D	100.2	2.2
	10 × 14	D × A	70.5	1.2
No. branches	10 × 17	D × A	−1.5	0.8
	13 × 17	D × A	−5.0	9.3
	13 × 17	D × D	3.0	−1.1
	16 × 17	A × A	1.4	3.7
No. heads	8 × 16	A × A	0.8	0.8
	8 × 17	D × A	−1.4	2.2
	13a × 17	D × A	−1.2	1.1
Disk diameter	1 × 8	A × A	−1.9	0.4
	1 × 14	A × A	−2.3	1.0
	1 × 17	A × A	−2.9	2.1
	6 × 14	D × A	2.7	1.1
	6 × 15	A × A	2.3	1.8
	6 × 17	A × A	2.3	1.3
	8 × 14	A × A	−2.7	1.8
	15 × 17	A × A	−2.2	0.3
No. ray flowers	8 × 15	D × A	2.1	1.8
	10 × 12	D × A	−2.0	1.9
	10 × 15	D × A	−1.9	1.5
No. selfed seeds	1 × 17	A × A	38.5	0.3
	8 × 17	A × A	42.2	1.0
Achene weight	1 × 8	A × A	−2.3	2.0
Seed germination	12 × 15	A × A	−14.3	11.5

[a] A × A = additive × additive; A × D = additive × dominant; D × A = dominant × additive; D × D = dominant × dominant.

[b] Underlined values indicate an interaction with effects in the wrong direction.

Hopi landrace to local growing conditions. However, in the one remaining case (number of heads produced; LG10), this is somewhat unexpected. Indeed, this QTL explains 28% of the segregating phenotypic variance and colocalizes with the *B* locus, which is known to influence apical branching (Tang *et al.* 2006). In fact, the region harboring the *B* locus is known to have manifold effects, influencing not only plant architecture but also achene/seed morphology. In the present analysis, this QTL is embedded within a larger cluster of loci that influence apical dominance as well as leaf and disc morphology, seed weight, and shattering. In fact, Burke *et al.* (2002) also found QTL related to seed size in this vicinity, but none related to branching. One possible explanation for this is that, despite their simple inheritance in crosses between cultivars, branching-related traits in wild × cultivar crosses are thought to be genetically complex (Burke *et al.* 2002) and may well be influenced by genetic background.

QTL directionality and evidence of selection: Perhaps the greatest departure between our results and those of Burke *et al.* (2002) relates to the directionality of QTL effects (Figure 2C). As previously noted, nearly one-third of all QTL identified by Burke *et al.* (2002) had effects in the wrong direction, with the wild allele producing a more crop-like phenotype and vice versa. As noted above, however, a subsequent analysis of improvement-related traits (*i.e.*, seed oil content and composition) combined with a population genetic scan for selection suggested that this result was due to post-domestication selection and breeding (Burke *et al.* 2005). The low frequency of wrong-way QTL in the present study (only 5 of 61 QTL had such effects) is fully consistent with this hypothesis. While data on QTL directionality can be used to statistically test for past directional selection (Orr 1998), the power of this approach is limited by QTL numbers. Thus, following the methods of Rieseberg *et al.* (2002), we pooled our data across traits and tested for selection on the domestication syndrome as a whole. In this case, the results were highly significant ($P < 0.001$), providing clear evidence that sunflower domestication was driven by consistent directional selection on a wide variety of traits.

Conclusions: Our results confirm that the domestication of sunflower was driven by selection on a large number of loci, most of which had small to moderate phenotypic effects. However, the underlying cause of this departure from the typical genetic architecture of domestication remains a mystery. For example, while sunflower is an ancient polyploid (Adams and Wendel 2005; Sossey-Alaoui *et al.* 1998), and thus potentially exhibits high levels of genetic redundancy across the genome, this sort of redundancy alone cannot be the explanation. Indeed, virtually all major crops have experienced large-scale genome duplication at some point in their evolutionary history. Another possibility is that the population bottleneck leading to domesticated sunflower was less severe than that which occurred during the evolution of other crop lineages, resulting in a relatively large effective population size during domestication. This, in turn, would allow for selection to target mutations of minor effect more efficiently in sunflower than in other crop lineages. While the available data indicate that sunflower suffered a similar loss in genetic diversity during domestication as compared to other crop plants (*e.g.*, Liu and Burke 2006)—a fact that argues against the idea that sunflower experienced a relatively mild bottleneck—a better understanding of the dynamics of the sunflower domestication bottleneck awaits more rigorous analysis. Ultimately, it may be that Doebley and Stec (1991) had it right; evolution under domestication may simply be an opportunistic process that makes use of whatever genetic variation happens to be available. Whether or not this lack of suitable

mutations of major effect in sunflower reflects some sort of fundamental genomic constraint remains an open question.

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