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Genetic Architecture of Novel Traits in the Hopi Sunflower

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Abstract

Following domestication, crop lineages typically undergo diversification either to adapt to disparate habitats or to fill novel agricultural roles. This process has produced the numerous varieties found in modern-day crop germplasm collections. Here, we mapped quantitative trait loci (QTLs) underlying unique traits in the Hopi sunflower, a primitive, Native American domesticate. These traits included a variety of achene (i.e., single-seeded fruit) characters as well as the extremely late flowering time of the Hopi sunflower. Composite interval mapping identified 42 QTLs underlying the 12 traits of interest. Although these QTLs were found on 10 of the 17 sunflower linkage groups, strong genetic correlations were evidenced by the clustering of QTLs across traits in certain genomic regions. The number of QTLs per trait ranged from 2 to 6, and the average QTL explained 14.7% of the variance (range: 2.5–46.9%). The apparent contribution of epistasis was minor, as has previously been observed for domestication-related traits. Unlike typical domestication-related traits in sunflower, the traits under consideration here exhibited a relatively simple genetic basis, with 2 QTL clusters being largely responsible for the unique characteristics of the Hopi sunflower. Based on the rarity of these traits in domesticated sunflower, it would appear that they evolved within the Hopi lineage following domestication. The simple genetic architecture of these traits may be a by-product of genetic constraints imposed by the genetically complex nature of domestication-related traits in sunflower, with the large number of domestication-related QTLs limiting the fraction of the genome that is available for subsequent diversification.

Key words: *crop evolution, flowering time,* Helianthus annuus, *Hopi sunflower, QTL analysis, seed morphology*

The evolution of crop plants can be viewed as occurring in somewhat distinct phases (e.g., Burke et al. 2005; Yamasaki et al. 2005; Burke et al. 2007). During the initial phase of domestication from its wild progenitor, the proto-domesticate typically experiences changes in a suite of traits collectively known as the "domestication syndrome" (Hammer 1984; Harlan 1992). Following this initial period of domestication, crop lineages often experience selection for adaptation to disparate environments and/or to fill unique agricultural roles. Beyond this, crop plants are generally subjected to selection on traits such as yield, quality, and disease resistance. This complex evolutionary history is often preserved, at least in part, in germplasm collections.

Sunflower (*Helianthus annuus* L.) is thought to have been initially domesticated as a source of edible seeds and was ultimately developed into an important oilseed crop (Heiser 1945, 1954; Putt 1997). Previous genetic analyses of phenotypic evolution under domestication have revealed that domestication-related traits often have a relatively simple genetic architecture, in that they are typically influenced by a small number of quantitative trait loci (QTLs), each of relatively large effect (e.g., Doebley et al. 1990; Doebley and Stec 1991; Paterson et al. 1991; Doebley and Stec 1993; Koinange et al. 1996; Xiong et al. 1999; Liu et al. 2007). The domestication of sunflower is perhaps the clearest counterexample to this pattern. Sunflower domestication appears to have been driven by selection on a large number of loci, most of which had small to moderate phenotypic effects (Burke et al. 2002; Wills and Burke 2007). In addition to domestication traits common to all domesticated sunflowers, certain lineages have also become adapted for growth in novel environments and/or for new agricultural uses. For example, the Hopi sunflower, which is native to the desert southwest of the United States, exhibits exceptionally late flowering and ultimately came to be used as an important source of dye for basketry, textiles, and body paint in the Hopi culture (Whiting 1939; Heiser 1950, 1951). The pigments used for these purposes were water-soluble anthocyanins extracted from the achene

(i.e., single-seeded fruit) pericarps—that is, the "shell" surrounding the sunflower kernel.

In addition to late flowering and high anthocyanin concentration, the Hopi sunflower possesses achenes with a unique overall morphology, including an elevated kernel-to-pericarp weight ratio as well as a high achene length-to-width ratio, which results in a cylindrical shape. Furthermore, seed oil content in the Hopi sunflower is somewhat elevated relative to that of wild sunflower, though there is no evidence that this trait was directly selected by Native Americans. In this article, we report on the genetic architecture of the unique postdomestication traits observed in the Hopi sunflower, including delayed flowering, increased pericarp anthocyanin and seed oil content, and elongated seed shape. We analyzed these traits in a Hopi × wild sunflower mapping population that was previously used to map QTLs underlying domestication-related traits; as such, we are able to directly compare the genetic basis of these changes to the types of changes that occurred during the initial domestication of sunflower.

Materials and Methods

Mapping Population and Genotyping

The mapping population used for this study was previously described by Wills and Burke (2007). This population consists of 378 F_2 individuals derived from a cross between a wild sunflower (*H. annuus* var. *annuus*) from a population in Keith County, Nebraska (Ann1238) and the Hopi sunflower (USDA PI 432504; *H. annuus* var. *macrocarpus*). The resulting population was grown in the greenhouse in a completely randomized fashion. In order to minimize environmental variation, plant were rotated amongst locations within the greenhouse until they were too big to move (ca. 6 weeks). In order to prevent outcrossing, heads were bagged prior to flowering. The linkage map, which consists of 111 co-dominant loci, was constructed using MapMaker 3.0/EXP (Lander et al. 1987; Lincoln and Lander 1992). The initial linkage groups (LGs) were identified using the "group" command with a logarithm of odds (LOD) score >5.0 and $\theta < 0.2$, and the final orders were then confirmed using the "ripple" and "compare" commands such that the map orders presented herein reflect the statistically most likely order based on the available data.

Phenotypic Analysis

The phenotypes examined in this study include 9 aspects of seed morphology, pericarp anthocyanin concentration, percent seed oil content, and flowering time for a total of 12 traits. Because self-incompatibility was segregating in this population, only 300 of the 378 F_2 individuals set selfed seeds and were thus available for analysis. Parental phenotypic values are based on averages from 8 wild (Ann1238) and 7 Hopi individuals which were grown concurrently with the mapping population in the greenhouse, and only those individuals that produced enough seeds for analysis were included in this study.

The flowering time and achene weight data are the same values that were reported previously (Wills and Burke 2007). The number of days from germination to flowering was recorded for all 378 individuals and 100 achene weight is based on the 300 F_2 individuals that set selfed seeds. Pericarp weights were only available for the 150 families assayed for anthocyanin concentration (see below). For those 150 lines, kernel weight was estimated as average achene weight minus average pericarp weight, and kernel-to-pericarp weight ratio was estimated from those measures. The remaining seed size and shape phenotypes were measured using Tomato Analyzer 2.1.0.0 (Brewer et al. 2006), which is a software package capable of measuring a variety of morphological characteristics of fruits or seeds from a scanned image. Fifteen achenes from all lines with sufficient seed, 230 in total, were scanned at 1200 dpi and saved as JPEG files. The achene images were rotated to a common orientation, and the perimeter, area, length, width, and achene shape were estimated for each. Using the standard nomenclature of Brewer et al. (2006), these measures correspond to perimeter, area, height at midpoint, width at midpoint, and fruit shape index (fs II, which is essentially a length-to-width ratio), respectively. These values were then averaged across seeds for each line.

Due to the destructive nature of the anthocyanin assay, the 150 F_2 plants with the largest number of seeds were selected for analysis. To quantify pericarp anthocyanin content, we extracted anthocyanin from achenes of each line using an adaptation of the protocol described by Bullard et al. (1989). The pericarps from 5 achenes from each of 7 Hopi individuals were removed, weighed, and placed in a 2-ml microcentrifuge tube containing 250 mg of sand, one 4.83 mm steel bead, and 1.2 ml of 0.5% HCl in methanol. Seeds were ground for 2 min at 30 Hz in a Qiagen TissueLyser (Qiagen, Valencia, CA). Samples were then centrifuged for 10 min at 16 100 × g and 1 ml of supernatant was removed. A SmartSpec 3000 spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA) was used to find the absorbance of the supernatant from 400 to 800 nm in 1-nm intervals. Peak heights were divided by the mass of pericarp material extracted, and the value at each wavelength was averaged across all 7 individuals. The highest average peak was 525 nm, which corresponds closely to the expected sunflower anthocyanin value reported by Mazza and Gao (1994). Phenotyping of the mapping population involved the characterization of pericarps from 5 selfed seeds from each of 150 F_2 individuals. Extractions were performed as above, and absorbance at 525 nm was measured. Trait values were recorded as OD525/gram pericarp weight and Box-Cox transformed in JMP 4.0 (SAS Institute, Cary, NC) prior to QTL analysis.

Seed oil content was estimated following the methods outlined by Burke et al. (2005) and Tang et al. (2006). Briefly, a single, pooled achene sample weighing between

0.5 and 5.0 g was drawn from the selfed offspring of each of 150 selfed F_2 individuals and placed in flat-bottomed sample tubes. Percentage oil content was then measured by pulsed nuclear magnetic resonance (NMR) analysis on a Bruker MQ20 Minispec NMR Analyzer (The Woodlands, TX). The data were proportions of total mass, so trait values were arcsine square-root transformed for data analysis (Sokal and Rohlf 1995).

QTL Analysis

The QTL analysis followed the same general approach outlined by Wills and Burke (2007) and Burke et al. (2002) using QTL Cartographer version 1.17 (Basten et al. 1994, 2004). Composite interval mapping (CIM) (Zeng 1993, 1994) was performed with a 10-cM window and a maximum of 5 background cofactors identified using both forward and backward regression, with tests performed at 2-cM intervals. One thousand permutations for each trait were performed to set threshold values ($\alpha = 0.05$) to identify the presence of QTL (Churchill and Doerge 1994; Doerge and Churchill 1996). A LOD decline of 2.0 between adjacent peaks was taken as evidence of multiple, linked QTL on a LG. To search for epistatic interactions between the identified QTL, multiple interval mapping was employed (Kao and Zeng 1997; Kao et al. 1999). The maximum number of allowable pairwise interactions was set to 19, and significance was determined based on the information criterion $IC(k) = -2(\log(L) - kc(n)/2)$ with $c(n) = \log(n)$ as the penalty function and a threshold of 0.0, as recommended by the authors. The degree of dominance of the Hopi allele at each locus was calculated as the dominance effect divided by the additive effect (d/a) such that a value of -1 reflects recessive gene action, a value of 0 reflects additivity, and a value of $+1$ reflects dominance. Arbitrary percentage of variance explained (PVE) thresholds of 10% and 25% were used to classify QTL as having "minor," "intermediate," and "major" effects. These were the same thresholds used to describe previously identified QTL in sunflower (Burke et al. 2002; Wills and Burke 2007).

RESULTS

The linkage map used for the present study has been described elsewhere (Wills and Burke 2007). This map, which was derived from a mapping population comprised 378 F_2 individuals from a cross between wild sunflower and the Hopi landrace, was 906.4 cM long, with the expected 17 LGs, and had an average intermarker distance of 8.4 cM. This map is somewhat compressed relative to prior maps in sunflower (but see Burke et al. 2002), with common marker intervals in this map spanning approximately 80% of the genetic distance covered in maps derived from crosses between 2 cultivated lines (e.g., Tang et al. 2002). This map compression is likely due to somewhat suppressed recombination in this slightly wider wild × cultivated cross, resulting in relatively even compression across LGs.

CIM identified 42 QTLs for the 12 traits under consideration (including flowering time and achene weight from the previous analysis; Table 1). QTLs were mapped to 10 of the 17 LGs (Figure 1 and Table 2). One-LOD support intervals for QTL locations ranged from 0.1 to 29.8 cM (mean = 9.3 cM). The average PVE was 14.7% (range: 2.5–46.9%), and the number of QTLs per trait ranged from 2 to 6. Achene shape, pericarp weight, and kernel-to-pericarp weight ratio were the only traits with multiple QTLs on the same LG. Each of these traits had 2 nonoverlapping QTLs on LG 16 (Figure 1). The QTL for achene perimeter on LG 4 was the only QTL for which the one-LOD interval did not overlap with previously identified domestication-related QTLs. Although we cannot strictly rule out the possibility that inbreeding depression may have influenced our QTL results—wild sunflower populations are highly heterozygous, and some fraction of the observed phenotypic variation could be due to inbreeding depression when deleterious recessives were made homozygous in the F_2 generation—all plants developed normally, and none showed obvious signs of inbreeding depression. Segregation distortion was observed for 15 of 111 markers based on χ^2 tests against the Mendelian expectation (Bonferroni-adjusted $P < 0.05$). The observed instances of segregation distortion typically occurred near the ends of LGs, primarily on LGs 9, 12, 15, and 17.

Table 1 Comparison of parental and F_2 population means for the 12 traits under consideration

Trait	Hopi landrace[a]	Common sunflower[a]	F_2 population[a]
Days to flower	100.0 ± 4.4	84.4 ± 4.4	64.8 ± 0.61
Achene weight (g/100)	2.9 ± 1.1	0.6 ± 0.10	2.4 ± 0.05
Pericarp weight (g/100)	1.3 ± 0.31	0.3 ± 0.04	0.9 ± 0.03
Kernel weight (g/100)	4.0 ± 0.47	0.4 ± 0.04	1.6 ± 0.04
Kernel-to-pericarp weight ratio	3.9 ± 0.48	1.2 ± 0.12	2.1 ± 0.06
Achene length (mm)	10.8 ± 0.62	4.8 ± 0.09	6.8 ± 0.05
Achene width (mm)	3.9 ± 0.39	2.2 ± 0.07	3.4 ± 0.04
Achene area (mm^2)	35.0 ± 4.82	8.7 ± 0.40	19.7 ± 0.31
Achene perimeter (mm)	28.8 ± 1.67	14.3 ± 0.97	19.5 ± 0.16
Achene shape	2.9 ± 0.17	2.2 ± 0.06	2.1 ± 0.02
Oil content	29.6 ± 1.17	24.5 ± 0.80	25.9 ± 0.27
Anthocyanin concentration (OD525/g of pericarp)	252.6 ± 59.16	5.0 ± 0.56	8.1 ± 0.71

[a] All trait values are expressed as mean ± standard error.

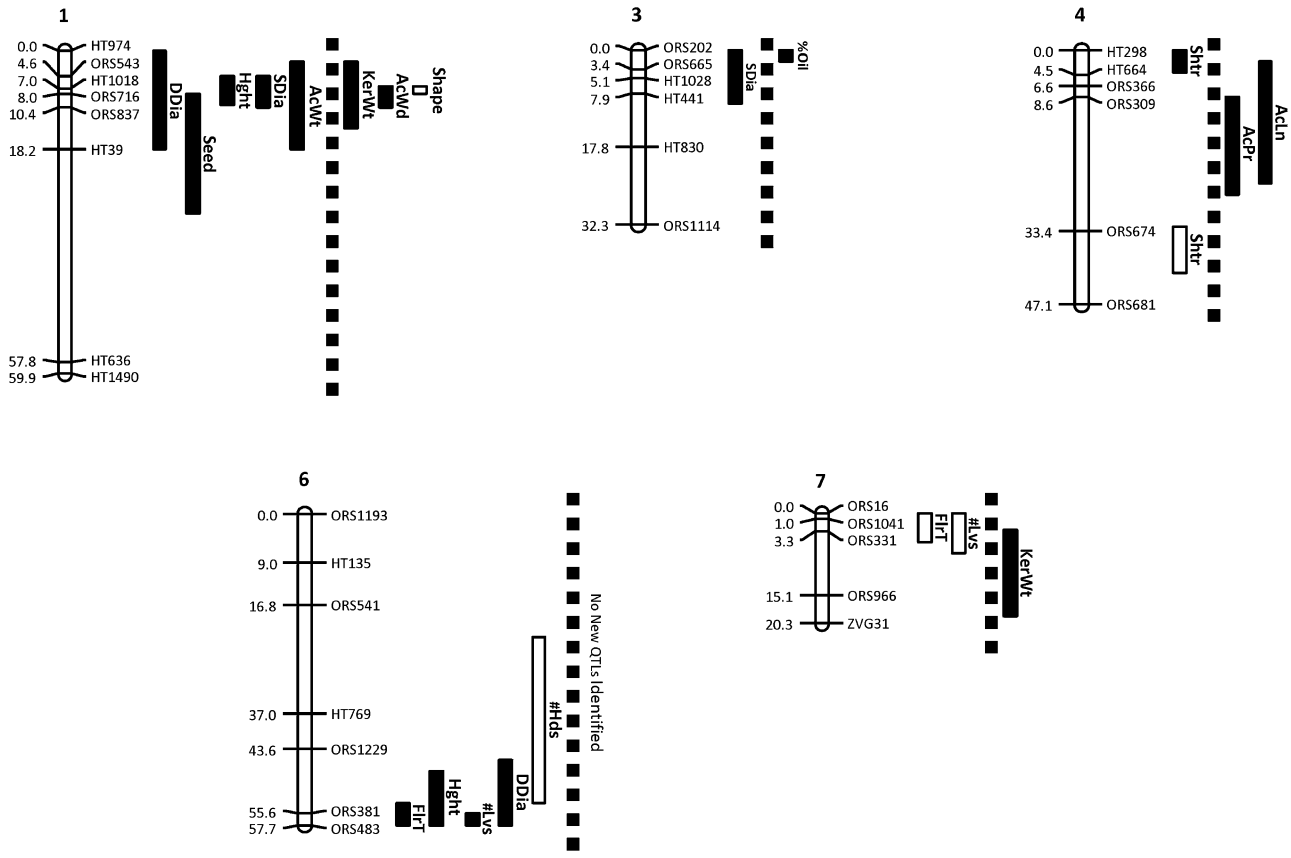
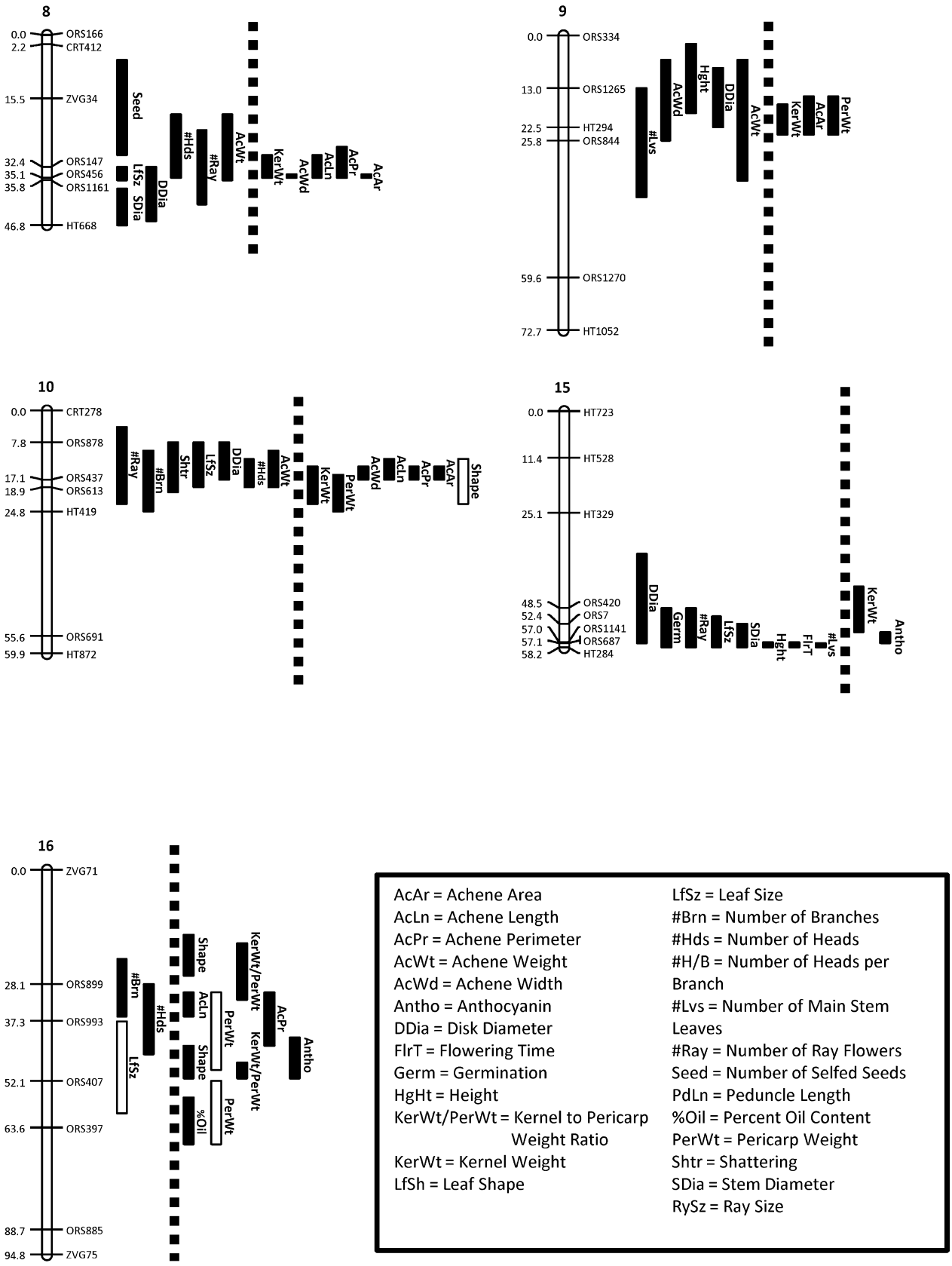


Figure 1. Results of the CIM analysis. For the 10 LGs carrying QTLs for the traits of interest, QTL positions are indicated by bars alongside each LG. The length of each bar is equal to the one-LOD support interval for that QTL. Loci at which the crop allele had the expected effect are indicated by a filled bar, whereas those at which the crop allele conferred a wild-like phenotype are represented by unfilled bars. To the left of the dashed lines are QTLs identified by Wills and Burke (2007). QTLs to the right of the dashed line influence the postdomestication traits phenotyped in this study. Note that LG 6 is included because it harbors a flowering time QTL, though no new QTLs were identified on this LG.

In our previous investigation of sunflower domestication (Wills and Burke 2007), 3 QTLs were identified for flowering time on LGs 6, 7, and 15 and 4 QTLs for achene weight were identified on LGs 1, 8, 9, and 10. Each of the achene weight QTLs colocalized with a QTL for kernel weight or pericarp weight in the present analysis but not always both. Furthermore, QTLs for kernel and pericarp weight were identified on LGs 7, 15, and 16 without a significant effect on total seed weight. Above that location on LG 16 is a second QTL for pericarp weight that colocalizes with a QTL of large effect for kernel-to-pericarp weight ratio (40.8% PVE). A final QTL for kernel-to-pericarp ratio, which mapped to a third genomic region on LG 16, above the other 2, explains 11.6% of the variance and once again had no significant effect on total seed weight. Most of the QTLs (17 of 19) for achene area, perimeter, length, width, and achene shape colocalized with QTLs for achene, kernel, or pericarp weight identified in the present or previous analyses of this cross (Wills and Burke 2007). The only 2 exceptions were QTLs for achene length and perimeter on LG 4. Only 2 QTLs were detected for seed oil content in the Hopi × wild population, with the QTL on LG 16 controlling 39.5% of the phenotypic variance. Similarly, the concentration of anthocyanin in the pericarp was influenced by 2 QTLs in this cross, one each on LGs 15 and 16. These QTLs explained 28.7% and 17.7% of the trait variance, respectively. Multiple significant correlations between traits were observed (Table 3), which was expected based on the observed pattern of QTL colocalization.

Multiple interval mapping revealed 10 significant 2-way interactions accounting for 0.1–8.1% of the phenotypic variability for 13 traits (Table 4). The contribution of epistasis to the observed phenotypes was thus minor, as has previously been observed for domestication-related traits in sunflower (Burke et al. 2002; Wills and Burke 2007). However, the power to detect epistasis in QTL analyses is relatively low (Mackay 2001), and the potentially important role of epistasis is often only discovered following more detailed analyses (Doebley et al. 1995). Only a single



AcAr = Achene Area	LfSz = Leaf Size
AcLn = Achene Length	#Brn = Number of Branches
AcPr = Achene Perimeter	#Hds = Number of Heads
AcWt = Achene Weight	#H/B = Number of Heads per Branch
AcWd = Achene Width	#Lvs = Number of Main Stem Leaves
Antho = Anthocyanin	#Ray = Number of Ray Flowers
DDia = Disk Diameter	Seed = Number of Selfed Seeds
FlrT = Flowering Time	PdLn = Peduncle Length
Germ = Germination	%Oil = Percent Oil Content
HgHt = Height	PerWt = Pericarp Weight
KerWt/PerWt = Kernel to Pericarp Weight Ratio	Shtr = Shattering
KerWt = Kernel Weight	SDia = Stem Diameter
LfSh = Leaf Shape	RySz = Ray Size

Figure 1. Continued

Table 2 Putative QTL positions, effect magnitudes, and modes of gene action for the 12 traits of interest using CIM in an F_2 population derived from a cross between the Hopi landrace and common sunflower

Trait	LG[a]	Position[b]	LOD score	Nearest marker	One-LOD interval[c]	Additive effect[d]	Dominance ratio[e]	PVE[f]
Days to flower	6	57.6	13.7	ORS483	53.6–57.7	4.6	−0.23	7.6
	7	1.0	4.8	ORS1041	0–5.3	−2.6	−0.11	2.5
	15	57.1	66.1	ORS687	57–58.2	10.4	−0.49	46.9
Achene weight	1	6.6	8.1	HT1018	2.0–18.3	1.6	0.70	8.6
	8	35.2	7.0	ORS456	19.5–35.8	1.7	0	7.6
	9	19.0	3.5	HT294	6.0–35.8	1.3	0.37	4.2
	10	15.8	15.9	ORS437	9.8–18.9	2.6	0.36	19.0
Pericarp weight	9	21.0	3.7	HT294	15.0–24.5	0.74	0.15	7.7
	10	18.9	7.5	ORS613	15.8–24.8	0.21	0.41	16.1
	16a	43.4	3.8	ORS399	30.1–49.4	−0.03	−8.43	10.6
	16b	60.1	6.8	ORS397	52.1–67.6	−0.16	−1.09	14.8
Kernel weight	1	7.0	4.1	HT1018	2.0–14.4	0.18	0.92	8.0
	7	9.3	3.6	ORS966	3.0–19.1	0.19	−0.4	8.0
	8	34.4	3.7	ORS456	29.5–35.2	0.17	−0.42	6.7
	9	22.5	4.4	HT294	17.0–24.5	0.21	0.16	9.0
	10	20.9	9.6	ORS613	13.8–22.9	0.34	−0.08	22.2
	15	48.5	4.6	ORS420	43.1–54.4	0.11	2.46	8.3
Kernel weight/ pericarp weight	16a	26.0	4.3	ORS899	18.0–32.1	0.17	−2.57	11.6
	16b	49.4	11.3	ORS407	47.4–51.4	0.56	−0.93	41.1
Achene length	4	4.5	5.0	HT664	2.0–24.6	0.19	0.64	5.0
	8	35.2	8.4	ORS456	29.5–35.2	0.30	0.48	8.7
	10	13.8	21.4	ORS437	11.8–17.1	0.44	0.56	25.9
	16	32.1	15.0	ORS899	30.1–36.1	0.41	−0.21	18.3
Achene width	1	7.0	4.9	HT1018	6.6–10.4	0.20	0.50	5.8
	8	35.2	4.1	ORS456	34.4–35.2	0.18	−0.03	4.9
	9	17.0	4.4	HT294	6.0–24.5	0.20	0.23	5.7
	10	15.8	23.3	ORS437	13.8–17.1	0.43	0.41	33.3
Achene area	8	35.2	6.2	ORS456	34.4–35.2	1.94	−0.04	7.8
	9	22.5	5.8	HT294	15.0–24.5	1.82	0.57	6.4
	10	15.8	23.4	ORS437	13.8–17.1	3.78	0.36	35.4
Achene perimeter	4	14.6	4.0	ORS309	8.6–26.7	0.73	0.73	6.0
	8	35.2	4.3	ORS456	27.5–35.2	0.85	0.45	5.5
	10	15.8	17.9	ORS437	13.8–17.1	1.62	0.45	26.0
	16	37.4	5.3	ORS399	30.1–43.4	0.79	0.78	6.7
Achene shape	1	7.0	8.5	HT1018	6.6–8.0	−0.15	0.47	11.5
	10	15.8	7.3	ORS437	11.8–22.9	−0.13	0.48	10.3
	16a	22.0	7.4	ORS899	16.0–26.0	0.11	−1.84	16.8
	16b	47.4	11.5	ORS407	43.4–51.4	0.16	−0.95	21.3
% Oil content	3	0.0	4.8	ORS202	0.0–2.0	1.49	−0.69	9.9
	16	58.1	16.2	ORS397	56.1–67.6	2.87	−0.82	39.5
Anthocyanin	15	58.2	12.1	HT284	54.4–58.2	3.52	−0.26	28.7
	16	47.4	6.9	ORS407	43.4–49.4	1.89	−1.24	17.7

[a] When multiple QTL for a single trait occurred on the same LG, a letter was used to uniquely identify them.

[b] Absolute position from the top of the LG (in cM).

[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 were in the wrong direction. See text for details. Units are the same as Table 1 except for oil content and anthocyanin content which were arcsin square-root and Box-Cox transformed, respectively.

[e] Refers to the dominance ratio (*d*/*a*) of the Hopi allele.

[f] Percentage of phenotypic variation explained by each QTL using CIM.

dominant × additive interaction for achene shape had an effect greater than 5%. Pericarp weight was the only other trait for which the total effect for all epistatic interactions summed to greater than 5%. No significant epistatic interactions were detected for oil or anthocyanin content, though our power to detect an interaction for those traits was reduced by the sample size for which these trait data were available (150 individuals). Nonetheless, 150

Table 3 Pairwise correlation coefficients of achene phenotypes in the F_2 mapping population

	Achene weight	Pericarp weight	Kernel weight	Kernel weight/ pericarp weight	Achene length	Achene width	Achene area	Achene perimeter	Achene shape	% Oil content	Anthocyanin
Days to flower	−0.15	−0.12	−0.05	0.14	−0.11	−0.16	−0.14	−0.11	0.15	0.11	0.4***
Achene weight		0.86***	0.92***	−0.37***	0.58***	0.88***	0.86***	0.71***	−0.64***	0.01	−0.07
Pericarp weight			0.6***	−0.77***	0.37***	0.86***	0.77***	0.58***	−0.75***	−0.29*	−0.23
Kernel weight				0	0.57***	0.79***	0.84***	0.68***	−0.53***	0.23	0.03
Kernel weight/ pericarp weight					0.05	−0.48***	−0.3*	−0.17	0.58***	0.51***	0.31*
Achene length						0.62***	0.79***	0.85***	−0.03	0.23	0.15
Achene width							0.95***	0.82***	−0.78***	−0.1	−0.17
Achene area								0.91***	−0.58***	0.02	−0.07
Achene perimeter									−0.39***	0.11	0.02
Achene shape										0.26	0.31**
% Oil content											0.43***

*, **, and *** reflect significance at the 0.05, 0.01, and ≥ 0.001, respectively, probability level after the Bonferroni correction for multiple tests.

observations were sufficient to identify 2 minor epistatic interactions for pericarp weight.

DISCUSSION

As one of the most basal domesticated sunflower lineages, the Hopi landrace has provided important insights into the genetic architecture of the early stages of the evolution of cultivated sunflower (Wills and Burke 2007). Interestingly, the Hopi sunflower exhibits a number of unique traits that distinguish it from other primitive domesticates. Some traits appear to be the by-product of postdomestication selection for adaptation to local growing conditions, or for cultural purposes, whereas others may be the result hitchhiking or genetic drift. As noted above, these traits include a suite of unique seed phenotypes, as well as exceptionally late flowering time.

Table 4 Summary of significant interactions amongst individually significant QTLs

Trait	LGs	Type of interaction[a]	Phenotypic effect[b]	effect (%)
Days to flower	None			
Achene weight	1 × 8	A × A	−0.2	2.0
Pericarp weight	10 × 16a	D × D	0.3	4.9
	9 × 16b	A × A	−0.1	2.7
Kernel weight	7 × 10	D × A	−0.2	1.5
	7 × 10	A × A	0.2	0.1
Kernel weight/ pericarp weight	16a × 16b	D × D	0.6	3.9
Achene length	None			
Achene width	1 × 12	D × A	−0.2	0.7
Achene area	9 × 10	A × A	1.3	2.7
Achene perimeter	None			
Achene shape	16a × 16b	A × A	−0.3	−2.6
	16a × 16b	D × A	−0.3	8.1
% Oil content	None			
Anthocyanin	None			

[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.

Achene Pigmentation

Anthocyanin pigmentation in sunflower has previously been shown to be under multilocus control, and pleiotropic effects have been observed for some of the pigment loci. The presence of anthocyanin in any tissue is controlled by *T*, a locus with epistatic effects (Stoenescu 1974; Joshi et al. 1994), which has been mapped in close proximity to a nuclear male sterility locus on LG 11 (Burke et al. 2002; Perez-Vich et al. 2005). Prior to our work, this was the only locus controlling anthocyanin pigmentation that had been mapped in sunflower. Although the interval containing *T* was well covered in our map, QTLs for anthocyanin concentration were not found on LG 11. This locus was not segregating in our mapping population as all offspring produced anthocyanin in their disc florets, indicating that the wild and Hopi parents both contributed functional alleles at this locus. Mosjidis (1982) found evidence for 3 loci controlling the presence of anthocyanin in sunflower. A dominant allele at one locus (*C*, which likely corresponds to *T*) was necessary, but not sufficient, for anthocyanin production. The *P* locus causes anthocyanin pigmentation in the hypodermis of the pericarp as well as the corolla (note that the *P* locus in this instance is distinct from the *P* locus controlling phytomelanin pigmentation; Stoenescu 1974; Joshi et al. 1994). Independent of *P*, the *Y* locus produces a "diluted" purple phenotype in the hypodermis. The anthocyanin pigment QTLs detected in the present study may correspond to *Y* and *P*, though we did not observe a diluted purple phenotype in any individuals. It is conceivable that the phenotype observed by Mosjidis (1982) was the result of the joint effects of the QTL that we mapped to LG 16 and the *Hyp* locus (a phenotypically defined locus causing a white pigment to be deposited in the hypodermis of the pericarp; Leon et al. 1996), whose location falls in an adjacent, nonoverlapping interval. Together, these loci might control the deposition of both anthocyanin and the

white pigment in the pericarp, resulting in a diluted purple phenotype.

Seed Morphology

Any investigation of the unique achene morphology exhibited by the Hopi landrace must first account for the tremendous increase in seed size that likely drove sunflower domestication (Burke et al. 2002). As noted above, 17 of 19 QTLs conditioning increases in achene length, width, perimeter, and area, or altering achene shape, colocalized with previously mapped QTLs controlling some aspect of achene mass on LGs 1, 8, 9, and 10 (Burke et al. 2002; Tang et al. 2006; Wills and Burke 2007), and are thus likely a by-product of prior selection for increased seed size during domestication. When the change in seed morphology was expressed in terms of achene shape, 2 QTLs were detected on LG 16 which account for 16.8% and 21.3% of the observed phenotypic variance. These 2 QTLs colocalized with 2 QTLs underlying kernel-to-pericarp weight ratio on that same LG (Figure 1). Thus, LG 16 appears to be a key determinant of the unique achene morphology of the Hopi sunflower, independent of the increase in seed mass during domestication. The one QTL of major effect (PVE = 39.5%) for seed oil content is also present on this LG. This QTL has been detected in multiple crosses and environments and has been found in other studies to colocalize with the aforementioned *Hyp* locus (Mosjidis 1982; Leon et al. 1996; Tang et al. 2006).

Flowering Time

On average, the Hopi individuals included in our study initiated flowering after 100 days, which was over 2 weeks (15.2 days) later than individuals from the wild parental population (Table 1). This result accords well with the previous findings of Heiser (1951), who observed a mean flowering time in the field of 102 days postgermination. This finding led him to note that "Such late maturing forms obviously can only be grown where there is an extremely long growing season" (Heiser 1951). Our results indicate that this phenological shift is largely due to a single QTL on LG 15 with an additive effect of 10.4 days, with 2 other QTLs playing a lesser role (Table 2; Figure 1). This delay in flowering time results in significantly larger, taller plants with a larger primary inflorescence. It is unclear if these late flowering forms were intentionally selected for their increased height and/or disk diameter or if the late flowering is the by-product of selection for increased anthocyanin pigment in the achene pericarp acting on LG 15.

Insights into the Origin of the Hopi Sunflower

Two genomic regions harbor clusters of QTLs influencing seed morphology: the interval containing the *B* locus (which is known to influence branching) on LG 10 and the middle portion of LG 16. QTLs on LG 10 are predominantly related to seed size and are thus likely a by-product of domestication. In contrast, QTLs on LG 16 influence most of the achene characters that are unique to the Hopi landrace, including achene shape, increased kernel-to-pericarp weight ratio, and pericarp anthocyanin pigmentation. Thus, the distinguishing characteristics of the Hopi landrace—that is, the unique achene shape and anthocyanin pigmentation, as well as the exceptionally late flowering and resulting tremendous plant size—are largely controlled by QTLs on LGs 15 and 16.

The foregoing results suggest that the unique traits of the Hopi sunflower have a relatively simple genetic basis; yet, the source of the necessary alleles remains unknown. In principle, there are 3 possible sources of such allelic variation: mutation, introgression, or standing variation within the domesticated sunflower gene pool. Although novel mutations are a possibility, this explanation would require the occurrence of major mutations at 2 loci shortly after domestication, and therefore seems improbable. If the necessary variation arose via introgression, one might expect to see a pattern similar to that documented by Burke et al. (2005) in an analysis of oilseed sunflower evolution, wherein a putatively introgressed region harbored QTLs conditioning desirable traits, as well as multiple QTLs with maladaptive (i.e., wild-like) effects. However, all the QTLs found at the bottom of LG 15 acted in the expected direction, and just one QTL underlying a domestication-related trait on LG 16 had an effect in the wrong direction. In the case of standing variation, the genetic bottleneck imposed by domestication will determine the amount of residual variation present for selection. For domestication-related traits, very little (if any) genetic variation is expected to make it through the bottleneck (Hanson et al. 1996; Tenaillon et al. 2004), but for other traits, the severity of the bottleneck will ultimately determine the amount of available variation (Eyre-Walker et al. 1998). In the case of sunflower, a substantial amount of molecular variation is thought to have passed through the domestication bottleneck (Liu and Burke 2006; Kolkman et al. 2007), suggesting that there may have been a large amount of functional variation segregating in the early domesticates. For the flowering time and anthocyanin QTLs on LGs 15 and 16, the Hopi alleles were all partially or completely recessive. Thus, even if these alleles were initially deleterious, they would have been able to persist at low frequencies until they became desirable. Given that sunflower was domesticated just once (Harter et al. 2004; Wills and Burke 2006) and yet achene pigmentation patterns/levels, flowering time, and seed oil content vary across domesticated lineages, the available evidence suggests that the unique characteristics of the Hopi landrace were selected from standing variation following domestication.

Whatever the source of the variation necessary for producing the specialized traits that distinguish the Hopi lineage, these traits appear to be largely conditioned by the QTL-rich regions on LGs 15 and 16. This relatively simple genetic architecture may be a by-product of constraints due to the genetically complex nature of domestication-related traits in sunflower. That is, the large number of domestication-related QTLs may limit the fraction of the genome that is available for subsequent diversification. The strong genetic correlation between late flowering time and high anthocyanin

pigmentation on the bottom of LG 15 suggests 2 possible scenarios. First, local adaptation to the long growing season of the desert southwest could have initially brought the "Hopi" allele in this genomic region to higher frequency, at which time culturally mediated selection for high anthocyanin content took over. Alternatively, the dissemination of domesticated sunflower to a locale with an exceptionally long growing season, such as the desert southwest, may have allowed for the evolution of highly pigmented seeds in the absence of natural selection against late flowering. Whether the simple genetic control of these traits is a result of the complex genetic architecture of domestication or an inherent quality of the underlying developmental pathways of the traits themselves remains an open question.

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