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Fitness effects and genetic architecture of plant–herbivore interactions in sunflower crop–wild hybrids

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Summary

- Introgression of cultivar alleles into wild plant populations via crop–wild hybridization is primarily governed by their fitness effects as well as those of linked loci. The fitness of crop–wild hybrids is often dependent on environmental factors, but less is understood about how aspects of the environment affect individual cultivar alleles.
- This study investigated the effects of naturally occurring herbivory on patterns of phenotypic selection and the genetic architecture of plant–herbivore interactions in an experimental sunflower crop–wild hybrid population in two locales.
- Phenotypic selection analyses suggested that cultivar alleles conferring increased size were generally favored, but at one site cultivar-like flowering time was favored only if three types of herbivory were included in the selection model. Quantitative trait locus (QTL) mapping identified three regions in which the cultivar allele conferred a selective advantage for a number of co-localized traits. Quantitative trait loci for several measures of insect herbivory were detected and, although the cultivar allele increased herbivory damage at the majority of these QTLs, they rarely colocalized with advantageous cultivar alleles for morphological traits.
- These results suggest that a subset of cultivar traits/alleles are advantageous in natural environments but that herbivory may mitigate the selective advantage of some cultivar alleles.

Introduction

Since the dawn of agriculture, wild plant populations have experienced gene flow from their cultivated relatives. It was not until recently, however, that concerns regarding the economic, ecological, and evolutionary consequences of crop-to-wild gene flow began to mount. These concerns have been largely driven by the introduction of genetically modified (GM) genotypes (Colwell *et al.*, 1985; Goodman & Newell, 1985; Ellstrand *et al.*, 1999; Chapman & Burke, 2006), though concerns have also been expressed about nontransgenic gene flow (De Wet & Harlan, 1975; Ellstrand, 2003). Much of this attention has focused on the possible introduction of adaptive cultivar alleles into wild populations, which could promote the expansion of wild species ranges or result in the evolution of increasingly weedy or invasive species (Ellstrand, 2003). Range expansion following the introgression of cultivar alleles into wild populations has been demonstrated for the invasive species *Sorghum halapense* (Johnson grass) (De Wet & Harlan, 1975) and *Rhododendron ponticum* (Milne & Abbott, 2000), although no direct link between cultivar alleles and invasiveness has been proven.

The dominant parameter governing the spread of crop alleles into wild populations is the selective advantage of the allele (Rieseberg & Burke, 2001; Morjan & Rieseberg, 2004; Chapman & Burke, 2006). Several other factors, such as low transmission rates or poor F_1 viability, also influence the rate of allelic introgression, but an allele that maintains a moderate selective advantage (e.g. $s = 0.1$) will eventually overcome these barriers and spread across the species' range (Pialek & Barton, 1997; Haygood *et al.*, 2004). While increased fitness does not necessarily translate into increased invasiveness, fitness remains the best predictor of allelic establishment and spread.

In order to better predict the types of cultivar traits that may be expected to persist in wild populations, several studies have examined the fitness effects of crop-related traits in early generations of crop–wild hybrids (Cummings *et al.*, 1999;

Alexander *et al.*, 2001; Snow *et al.*, 2003; Mercer *et al.*, 2006, 2007; Campbell & Snow, 2007). Some crop-related traits, such as transgenic herbivore resistance, appear to confer a selective advantage in crop–wild hybrids (Snow *et al.*, 2003; Vacher *et al.*, 2004). Upon further examination, however, the results are more complex. For example, introgression of the *Bt* transgene into wild *Brassica rapa* and *H. annuus* increased the fitness of crop–wild hybrids in the presence of herbivores. In the absence of herbivores, however, the *Bt* gene resulted in a fitness cost in *B. rapa* but not *H. annuus* (Snow *et al.*, 2003; Vacher *et al.*, 2004). These results and others demonstrate that selection on cultivar alleles can vary greatly across environments; therefore, their fitness effects should be assessed under a range of natural conditions in order to better predict the persistence and spread of cultivar alleles in wild populations.

Herbivore pressure has also been shown to influence the selective advantage of nontransgenic cultivar-like phenotypic traits. For example, large seed size is often considered to be adaptive (in the absence of dispersal limitation or seed size-seed number trade-offs), as large seeds generally predict larger, more vigorous seedlings (Roach & Wulff, 1987). However, large seeds are also often preferred by seed predators (Kelrick *et al.*, 1986; Hulme, 1994; Moegenburg, 1996). Consistent with this hypothesis, first-generation sunflower crop–wild hybrids typically produce larger seeds than their wild counterparts and are more susceptible to post-dispersal seed predation than wild individuals (Alexander *et al.*, 2001).

Herbivory also imposes selection on flowering phenology (English-Loeb & Karban, 1992; Cummings *et al.*, 1999; Pilson, 2000). In a study of wild *H. annuus*, selection favored late-flowering individuals if herbivory was not considered, but when moth damage to inflorescences was taken into account, intermediate flowering dates were favored (Pilson, 2000). Flowering phenology has also been shown to partly explain increased pre-dispersal seed herbivory in sunflower crop–wild hybrids relative to wild plants, in that more hybrid plants flowered at peak damage times (Cummings *et al.*, 1999). Although some of the effects of predispersal and postdispersal herbivory on crop–wild hybrids could be explained by other measured phenotypic traits, the relationship between herbivory and cross type (hybrid and nonhybrid) remained after the influence of flowering date, head size, number of heads, and seed size were considered (Cummings *et al.*, 1999; Alexander *et al.*, 2001). Clearly, the effects of plant–herbivore interactions on the fitness of crop–wild hybrids are complex and warrant further investigation.

Most studies to date have focused on the phenotypic effects of plant–herbivore interactions on fitness in early-generation crop–wild hybrids; thus, the underlying genetic architecture of these effects is largely unknown. In F_1 hybrids, it is impossible to separate the effects of individual cultivar loci from general heterosis (Hooftman *et al.*, 2007) or linkage disequilibrium between favorable and unfavorable cultivar alleles (Warren & James, 2006; Baack *et al.*, 2008). The novel trait variation and allelic combinations produced in segregating progenies (e.g. F_2 or recombinant inbred lines; RILs) make it possible to both assess selection on many traits as well as identify the quantitative trait loci (QTL) associated with selectively advantageous traits in the wild (Mauricio, 2001). Quantitative trait locus studies in crop–wild hybrids can discern the direction of allelic effects for the cultivar (and wild) alleles, as well as reveal pleiotropic effects or tight linkage relationships that may constrain the evolution of correlated traits (Baack *et al.*, 2008). In addition, QTL-mapping may aid in the identification of candidate genes that are responsible for fitness differences and which could conceivably be applied in the mitigation of transgene escape (Gressel, 1999).

In this study, we examine the fitness effects and genetic architecture of plant–herbivore interactions in sunflower crop–wild hybrid RILs. Specifically, we investigate: (1) the relationships between naturally occurring herbivory and plant morphology in two noncrop field environments; (2) which (if any) cultivar-like traits are favored in these environments; (3) the effects of herbivory on selection on cultivar-like traits; and (4) the QTL architecture of these effects.

Materials and Methods

Study system

Sunflower (*Helianthus annuus* L., Asteraceae) is a globally-important oilseed crop and a common source of confectionery seeds. Cultivated sunflower is derived from the common sunflower (*H. annuus* var. *annuus*), and the two groups are completely interfertile (Burke *et al.*, 2002a). In the USA and Canada, the majority of cultivated sunflower occurs within the range of common sunflower, flowering seasons overlap considerably (Burke *et al.*, 2002a), and hybridization in the field occurs at distances up to 1000 m (Arias & Rieseberg, 1994). Cultivated *H. annuus* alleles commonly introgress into wild populations (Linder *et al.*, 1998) and can persist for at least five generations (Whitton *et al.*, 1997). Cultivated and wild sunflowers exhibit a number of morphological differences related to domestication (Burke *et al.*, 2002b). Cultivated plants are characterized by earlier flowering, reduced branching, and a single flower head (inflorescence) with many large seeds. Wild sunflower commonly displays extensive branching with many smaller flower heads. Resistance to herbivory has been associated with several of the phenological and morphological differences between cultivated and wild sunflower (Cummings *et al.*, 1999; Alexander *et al.*, 2001).

Mapping population

Development of the mapping population has been described in detail previously (Burke *et al.*, 2002b; Baack *et al.*, 2008). Briefly, RILs were derived from a cross between an *H. annuus* cultivar, cmsHA89 (USDA Ames 3693) and a wild *H. annuus* var. *annuus* individual (ANN1238) that was grown from seed

collected from a wild plant in Keith County, NE, USA. A single self-compatible F_1 individual was selected from this cross, self-pollinated, and the resulting F_2 generation was field grown in isolation from other *H. annuus* in Mexico. The F_3 and F_4–F_6 generations were glasshouse-grown at the University of Indiana, Bloomington, Indiana, USA and Oregon State University, Corvallis, Oregon, USA, respectively. Plants were self-pollinated and advanced via single-seed descent every generation. This resulted in 184 RILs from which the linkage map was produced. One caveat of this approach is that wild *H. annuus* is a naturally outcrossing species. As such, inbreeding depression is likely to be present in RILs of this species. Heterogeneity in inbreeding depression may increase among-trait correlations and subsequently result in the detection of QTL that indirectly affect several traits via inbreeding. The extent of inbreeding depression in these RILs has not been determined, but preliminary observations and measurements of the RILs in the field and wild *H. annuus* growing nearby suggest, while inbreeding depression may be present, its effects are relatively minor (Baack *et al.*, 2008; J. Dechaine, unpublished data).

Map construction

The RIL population was previously mapped using 109 codominant simple sequence repeat (SSR), single-strand confirmation polymorphism (SSCP) or restricted fragment length polymorphism (RFLP) PCR-generated markers (Baack *et al.*, 2008). In addition, we mapped several marker loci that were recently shown to be under strong directional selection during sunflower domestication and/or improvement (Chapman *et al.*, 2008b). These are excellent candidate genes for traits that differ between cultivated and wild sunflowers. Using the data and methods described in Chapman *et al.* (2008b), 28 'selected' loci were genotyped on the RIL population. These loci were added to the previously published linkage map (Baack *et al.*, 2008) using MAPMAKER 3.0/EXP. The 'near' command was used to identify previously mapped markers that were most closely associated with the new markers. Marker order was determined using the 'compare' command. Recombination distances were converted into cM following Kosambi (Kosambi, 1944). Only one marker at each cM location was retained in the final linkage map, which was produced from 131 markers and all 184 RILs.

Study sites

Recombinant inbred lines were planted at two Midwestern sites, Nebraska (NE) and North Dakota (ND), USA; both sites are within the range of both cultivated sunflower production and the distribution of wild common sunflower. The Nebraska study site was located at Cedar Point Biological Station (41°12.4′ N, 101°40.2′ W), near Ogallala, Keith County (NE). The wild parent was also collected in Keith County; therefore, the wild alleles may be better adapted to the NE site than the ND site, although there is no evidence to suggest that wild alleles were maladapted to ND. The ND study site was established on the North Dakota Agricultural Experiment Station land at North Dakota State University in Fargo (46°89.2′ N, 096°88.0′ W). Each site was prepared by plowing in early spring 2007 and installing a perimeter fence to prevent wildlife and cattle (in NE) from grazing on study plants. With the exception of native and nonnative volunteer sunflowers, weeds were allowed to grow in and around the experimental plants. Both sites were weeded as necessary by hand every 1–3 wk to maintain an even competitive density across all plots within a site. Green foxtail (*Setaria viridis* (L.) P. Beauv.) represented > 90% of the competition in ND and Venice mallow (*Hibiscus trionum* L.) accounted for most of the remaining 10%; these two species were very evenly distributed. A diverse assemblage of species, including cocklebur (*Xanthium strumarium* L.), sandbur (*Cenchrus longispinus* (Hack.) Fernald), western ragweed (*Ambrosia psilostachya* DC.), and Russian thistle (*Salsola tragus* L.), occurred in NE.

Planting design

A total of 171 RILs and their progenitors (accession HA89 and individuals of NE wild population ANN1238) were planted on April 18–21, 2007 in NE and May 15–17, 2007 in ND. Thirteen RILs (of 184) were not included because of seed limitation (11 RILs) or 0% germination (2 RILs). Lines were planted at 0.5-m intervals once in each of 10 blocks in a fully randomized complete-block design at each site. Blocks measured 7 × 8 m, consisted of 12 rows, and were spaced at a minimum distance of 2 m from one another. Because of space limitations, two blocks were located approximately 2 km south of the remaining six in NE.

Four seeds of each line were planted at marked locations in 12-cm diameter paper coffee filters to ensure the identity of emerging seedlings within each block. All seeds were covered with 2–3 cm of soil. Rainfall occurred in NE immediately following planting, whereas seeds were hand-watered after planting in ND. Seedling emergence was first observed on May 1 in NE and May 31 in ND. Seedlings were thinned to one individual per planting location, and extra individuals were transplanted as needed at the four-leaf stage. Field and glasshouse-grown transplants replaced one to seven individuals of 127 RILs in NE (323 total) and one to three individuals of 58 RILs in ND (89 total). Supplemental watering was provided as needed throughout the seedling stage. Carbaryl insecticide (Sevin; GardenTech, Lexington, KY, USA) mixed to the manufacturer's specifications was applied twice in early May at the NE site to reduce seedling death from cutworm (Noctuidae) larvae.

Phenotypic measurements

Flowering began on June 26 in NE and July 17 in ND, after which plots were visited at 3-d intervals until all plants had

flowered. The date of first flower was recorded at anthesis of the outer ring of disk flowers. Several other characteristics were also recorded at flowering, including disk diameter across the widest part of the head, length from tip to base of a representative ray flower and the plant's stem diameter directly above the first true leaves. If the primary (apical) head did not flower (e.g. owing to herbivore damage), flowering date and floral characteristics were recorded for the next head that opened. Heads were covered with a mesh bag (Delnet; Delstar Technologies, Inc., Middleton, DE, USA) 2–3 wk after anthesis to prevent seed loss. About 1 month after first flower, several additional plant traits were surveyed. These included stem height from soil level to the apical inflorescence, total number of leaves on the plant, and the length and width at the longest/widest point of a randomly chosen, fully-expanded leaf on the primary stem. Incidence of fungal leaf damage (caused by Septoria leaf spot (*Septoria helianthi* Ell & Kell.) or *Alternaria* spp.) was scored as 0/1, presence/absence of yellow or brown necrotic leaf spots. General leaf damage by insect herbivores was also assessed as an estimated ranking of the percentage of leaf tissue missing: 0, no visible leaf damage; 1, 5–10%, of total leaf area damaged; 2, 5–25%; 3, 25–50%.

In late August, several measures of herbivore damage were assessed on the primary (apical) flowering head and the first three secondary heads. At both sites, incidence of moth damage was recorded by the presence of insect frass on each head: 0, no visible frass; 1, light frass; 2, half to entire head covered by frass. Moth damage was caused by at least four species in NE, including *Homoeosoma electellum* (Pyralidae), *Cochylis hospes* and *Suleima helianthana* (Tortricidae) and *Plagiomimicus spumosum* (Noctuidae); the most abundant and damaging of these was *S. helianthana.* The two most abundant moths in ND were *H. electellum* and *C. hospes*, although other moth species may have caused some head damage. The incidence of the sunflower head-clipping weevil (*Haplorhynchites aeneus*, Curculionidae) was also recorded for each head: 0, head intact; 1, head removed by clipping. In ND, each seed head was rated for sunflower midge (*Contarinia schulzi*, Cecidomyiidae) damage according to the scale proposed by Bracken (1990): 0, no visible damage; 1, light bract damage; 2, bract damage evident; 3, heavy bract damage and parts of head are seedless; 4, large damage and seedless area; 5, complete seed loss. No midge damage was observed in NE.

Plants began senescing in September in NE and October in ND. At senescence, all primary branches and flower heads > 1 cm diameter were counted. The head count included immature and mature heads, as well as heads that flowered but failed to produce seed because of damage by herbivores (including the head-clipping weevil) and/or pathogens. All heads that had flowered were collected, dried, and processed. General head damage by herbivory was assessed in the laboratory by counting the number of heads that were severely damaged (> 75% seedless, including heads that were aborted because of insect damage). Seeds were removed by crushing the heads over a series of sieves; mature, viable seeds were further cleaned from chaff and debris by passing samples through a seed blower (Seedblower table, 757; Seedburo, Des Plaines, IL, USA). All mature, viable seeds were pooled by plant and weighed. A random sample of up to 50 mature seeds was also counted and weighed for each plant. Individual plant seed totals were calculated by dividing total seed weight by the average weight of a single seed, as estimated from 50 seeds from the plant in question.

Statistical analyses

Descriptive statistics Leaf and head herbivory damage likely resulted from several types of insects including: Lepidopteron larvae (see above), grasshopper species (Orthoptera: Acrididae), beetles (Coleoptera: Cerambycidae, Chrysomelidae, Curculionidae), and flies (Diptera: Cecidomyiidae). However, unique feeding patterns, such as head clipping (*H. aeneus*), extensive frass production by moths (*S. helianthana*, and, to a lesser degree, *H. electellum*), and head inflation by the sunflower midge, allowed damage by each of these herbivores to be analysed separately. General head damage and damage by the head-clipping weevil were calculated as the per cent of measured heads (up to four) per plant that were affected by each type of damage. Head-clipping was observed for just one head on each of three plants in ND and was therefore analysed only for NE. The severity of midge damage and moth damage, using the ratings scales described above, were averaged over the measured heads for an individual. Midge damage was only analysed in ND.

A restricted maximum likelihood (REML) procedure was used to test the fixed effects of site and the random effects of block, RIL, and site × RIL on plant characters, as well as to generate least-square means and 95% confidence intervals for each trait (PROC MIXED, SAS, 2001). Degrees of freedom were determined by Satterthwaite. The data were then split by site, and RIL best linear unbiased predictors (BLUPs) were generated for use in QTL mapping. Ray length and stem height displayed constant residual variance. All other traits were power transformed using the Box–Cox method (Box & Cox, 1964). Transformations greatly improved homoscedasticity for all traits except damage by the head-clipping weevil and moth damage, which did not reach a normal distribution.

To examine among-trait relationships, Pearson correlation coefficients (r^2) were generated by site for all trait combinations (PROC CORR). The significance of correlations were tested using *t*-tests, and multiple comparisons were corrected for using the Bonferroni method based on the number of comparisons within each site. Correlations resulting from transformed or untransformed values were essentially the same, so untransformed trait correlations are presented here for ease of interpretation. Individuals that survived to the start of flowering at each site (i.e. individuals that successfully emerged or

survived transplanting) were included in all analyses. Sample sizes ranged from 922 (ray length) to 1263 (most traits) in ND and from 658 (head-clipping weevil and moth damage) to 867 (several traits) in NE.

Phenotypic selection analyses Separate phenotypic selection analyses were performed for each site using the ASTER program (Geyer *et al.*, 2007; Shaw *et al.*, 2008) in R (R Development Core Team, 2008). ASTER employs a maximum-likelihood approach to examine the relationships between traits and lifetime fitness. ASTER improves on earlier methods for estimating selection by incorporating multiple dependent components of fitness (e.g. survival to reproduction and fecundity) into an overall fitness measure and modeling each fitness component with a different statistical distribution (Shaw *et al.*, 2008). In this study, two dependent fitness components – survival to reproduction and seed total – were used to model overall fitness. Survival to reproduction was modeled following a Bernoulli distribution (0, did not produce any seed; 1, produced seed) and seed total was modeled following a Poisson distribution. Multiple nested models were fitted to each data set, and likelihood-ratio tests were used to compare each set of models. All models included block and RIL. Selection was not significant for fungal leaf damage at either site or moth damage in ND, and the best fit models did not include these traits. The best fit models included all other traits, and these results are shown below, but we also fit models that excluded the various herbivory traits (leaf and head damage by herbivory at both sites, midge damage in ND and damage by the head-clipping weevil and moths in NE) in order to examine how herbivory influences selection on flowering day and morphological traits in sunflower crop–wild hybrids (Pilson, 2000).

QTL-mapping Mapping was performed using BLUPs (as described earlier). The QTL were mapped using the composite interval mapping (CIM) procedure in Windows QTL Cartographer (Wang *et al.*, 2007). Quantitative trait loci were initially analysed using forward and backward stepwise regressions ($P = 0.05$) and a walk speed of 2 cM. Up to 10 control markers were selected and the CIM procedure was performed using the default model (model 6) and a window size of 5 cM. Significance thresholds were determined via permutation tests with 1000 permutations per trait (Churchill & Doerge, 1994). The additive effect of the cultivar allele (cmsHA89) and the per cent variance explained for each QTL were estimated in QTL Cartographer. Additive effects were standardized to the standard deviation (α/SD) of each trait.

Results

Descriptive statistics

All traits exhibited highly significant genetic variation (RIL effect, $P < 0.01$), except for moth damage in ND. Site effects were also significant ($P < 0.05$) for all traits, indicating substantial environmental variation between sites. Plants flowered earlier and produced larger flowers in NE than ND (see the Supporting Information, Table S1). Plants were generally larger in NE and produced more heads and seeds in NE; however, heavier seeds were produced in ND. Leaf and head herbivory were slightly more severe in NE, but fungal leaf damage was more severe in ND. Incidence of stem cankers and seedling death resulting from the fungal pathogens, *Phomopsis helianthi* Munt.-Cvet *et al.* and downy mildew (*Plasmopara halstedii* Farl.), respectively, were also observed in ND but not NE (J. Burger, pers. obs). Moth damage was much less severe in ND; frass was only found on 20 individuals at that site (vs 337 individuals in NE).

Despite highly significant site effects, among-trait correlations for morphological characters were similar between sites (Table 1). Several size traits were positively correlated: plants that were taller and had wider stems also produced more leaves and branches, larger flower heads and more heads. In general, larger plants also flowered earlier and had leaves that were wider in relation to their length. Several plant size characters were also positively correlated with seed mass and seed total, suggesting that larger plants had higher fitness.

Herbivory was less consistent between sites. Although leaf and head herbivory were generally less severe on larger plants in NE, leaf herbivory was more severe on plants that produced more leaves, branches and heads in ND. In addition, leaf herbivory and seed total were negatively correlated in NE but uncorrelated in ND. In NE, moth damage was negatively correlated with several size characters and had a detrimental effect on seed total; these patterns were not observed in ND. Interestingly, several herbivory characters were correlated with flowering day. Flowering day was negatively correlated with leaf herbivory at both sites and moth damage at ND, indicating that early-flowering plants received more damage by these herbivores. Although damage by the most damaging herbivore at each site (midge in ND and head-clipping weevil in NE) was positively correlated with flowering day, this is probably because plants that received more damage by these herbivores flowered later. If only the flowering day of the primary head was considered, midge damage was significantly negatively correlated with flowering day ($r^2 = -0.26$, $P < 0.0001$), indicating that midge damage was more severe early in the flowering season. No clear relationship between head-clipping and flowering day was detected, but head-clipping may have dissipated later in the flowering season, as it was only recorded on the first four flowers of a plant.

Phenotypic selection analysis

Selection analyses showed significant linear selection gradients for all but one trait (leaf size in NE) included in the best fit models (Table 2). Early flowering and large plant and flower head size (includes disk diameter, ray length, stem height,

Table 1 Among-trait phenotypic correlations in North Dakota (above-diagonal) and Nebraska (below-diagonal)

	Flowering day	Disk diameter	Ray length	Stem diameter	Stem height	Leaf number	Leaf size	Leaf shape	Branch number	Head total	Seed mass	Seed total	Leaf herbivory	Head herbivory	Leaf fungal damage	HC weevil	Midge damage	Moth damage
Flowering day		(0.39) <0.0001	(0.12) 0.0004	(0.10) 0.0013	(0.29) <0.0001	0.05 0.1076	(0.20) <0.0001	0.32 <0.0001	(0.07) 0.0276	(0.09) 0.0037	(0.16) <0.0001	(0.11) 0.0002	(0.25) <0.0001	(0.08) 0.0082	(0.21) <0.0001	–	0.41 <0.0001	(0.13) 0.0001
Disk diameter	(0.56) <0.0001		0.36 <0.0001	0.72 <0.0001	0.68 <0.0001	0.46 <0.0001	0.70 <0.0001	(0.59) <0.0001	0.53 <0.0001	0.45 <0.0001	0.61 <0.0001	0.50 <0.0001	0.21 <0.0001	(0.32) <0.0001	0.01 0.6459	–	(0.29) <0.0001	0.08 0.018
Ray length	(0.52) <0.0001	0.75 <0.0001		0.26 <0.0001	0.22 <0.0001	0.18 <0.0001	0.25 <0.0001	(0.20) <0.0001	0.20 <0.0001	0.17 <0.0001	0.28 <0.0001	0.18 <0.0001	0.10 0.0017	(0.09) 0.0069	(0.03) 0.4405	–	(0.16) <0.0001	0.08 0.024
Stem diameter	(0.43) <0.0001	0.71 <0.0001	0.57 <0.0001		0.60 <0.0001	0.66 <0.0001	0.83 <0.0001	(0.56) <0.0001	0.68 <0.0001	0.64 <0.0001	0.58 <0.0001	0.56 <0.0001	0.17 <0.0001	(0.34) <0.0001	(0.05) 0.0736	–	(0.13) <0.0001	0.04 0.2619
Stem height	(0.31) <0.0001	0.56 <0.0001	0.49 <0.0001	0.66 <0.0001		0.41 <0.0001	0.64 <0.0001	(0.46) <0.0001	0.55 <0.0001	0.49 <0.0001	0.58 <0.0001	0.52 <0.0001	0.09 0.0008	(0.30) <0.0001	0.07 0.0172	–	(0.26) <0.0001	0.04 0.1535
Leaf number	(0.06) 0.119	0.20 <0.0001	0.16 <0.0001	0.54 <0.0001	0.41 <0.0001		0.66 <0.0001	(0.35) <0.0001	0.76 <0.0001	0.76 <0.0001	0.48 <0.0001	0.58 <0.0001	0.10 0.0004	(0.30) <0.0001	(0.10) 0.0007	–	(0.01) 0.7978	(0.01) 0.7726
Leaf size (l × w)	(0.33) <0.0001	0.63 <0.0001	0.51 <0.0001	0.84 <0.0001	0.62 <0.0001	0.53 <0.0001		(0.46) <0.0001	0.69 <0.0001	0.70 <0.0001	0.62 <0.0001	0.72 <0.0001	0.18 <0.0001	(0.30) <0.0001	(0.02) 0.4097	–	(0.24) <0.0001	0.03 0.3672
Leaf shape (l/w)	0.27 <0.0001	(0.42) <0.0001	(0.29) <0.0001	(0.46) <0.0001	(0.33) <0.0001	(0.22) <0.0001	(0.43) <0.0001		(0.42) <0.0001	(0.36) <0.0001	(0.37) <0.0001	(0.26) <0.0001	(0.22) <0.0001	0.15 <0.0001	(0.10) 0.0004	–	0.10 0.0016	(0.05) 0.0909
Branch number	(0.19) <0.0001	0.39 <0.0001	0.35 <0.0001	0.69 <0.0001	0.54 <0.0001	0.69 <0.0001	0.69 <0.0001	(0.33) <0.0001		0.82 <0.0001	0.56 <0.0001	0.53 <0.0001	0.11 <0.0001	(0.30) <0.0001	(0.09) 0.0012	–	(0.06) 0.0473	0.00 0.9302
Head total	(0.10) 0.0043	0.23 <0.0001	0.20 <0.0001	0.52 <0.0001	0.46 <0.0001	0.84 <0.0001	0.56 <0.0001	(0.22) <0.0001	0.74 <0.0001		0.55 <0.0001	0.62 <0.0001	0.12 <0.0001	(0.26) <0.0001	(0.08) 0.006	–	(0.05) 0.1046	0.00 0.9388
Seed mass	(0.25) <0.0001	0.29 <0.0001	0.32 <0.0001	0.28 <0.0001	0.26 <0.0001	0.07 0.0401	0.30 <0.0001	(0.16) <0.0001	0.20 <0.0001	0.13 0.0001		0.60 <0.0001	0.11 0.0002	(0.47) <0.0001	(0.05) 0.0992	–	(0.27) <0.0001	(0.02) 0.6245
Seed total	(0.11) 0.0029	0.18 <0.0001	0.15 <0.0001	0.37 <0.0001	0.34 <0.0001	0.64 <0.0001	0.39 <0.0001	(0.14) <0.0001	0.46 <0.0001	0.65 <0.0001	0.13 <0.0001		0.09 0.0021	(0.31) <0.0001	(0.04) 0.1411	–	(0.24) <0.0001	(0.03) 0.4164
Leaf herbivory	(0.21) <0.0001	0.00 0.8882	(0.02) 0.5691	(0.06) 0.0664	(0.18) <0.0001	(0.16) <0.0001	(0.11) 0.0017	(0.04) 0.2612	(0.14) <0.0001	(0.14) <0.0001	(0.01) 0.858	(0.13) 0.0002		0.05 0.1161	0.11 <0.0001	–	0.03 0.3538	0.06 0.0666
Head herbivory	0.07 0.0779	(0.11) 0.0033	(0.14) 0.0004	(0.17) <0.0001	(0.14) 0.0003	(0.14) 0.0003	(0.15) 0.0001	0.10 0.0106	(0.12) 0.0025	(0.15) <0.0001	(0.21) <0.0001	(0.21) <0.0001	0.01 0.8464		0.02 0.6115	–	0.29 <0.0001	0.01 0.6534
Leaf fungal damage	(0.08) 0.0248	0.10 0.0044	0.11 0.0026	0.02 0.5594	(0.08) 0.0235	(0.01) 0.7331	0.10 0.003	(0.07) 0.0286	0.08 0.0143	0.02 0.4839	0.07 0.035	0.01 0.7421	0.05 0.13	0.17 <0.0001		–	0.09 0.0023	0.07 0.0231
HC weevil	0.34 <0.0001	(0.13) 0.0007	(0.10) 0.0105	(0.08) 0.0304	(0.05) 0.1791	0.00 0.9271	(0.01) 0.7193	(0.06) 0.1095	0.12 0.0014	0.12 0.0019	(0.12) 0.003	(0.09) 0.0159	0.00 0.9053	(0.10) 0.0139	0.08 0.0312		–	–
Midge damage	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		(0.03) 0.2744
Moth damage	0.00 0.9498	(0.10) 0.0114	(0.10) 0.013	(0.19) <0.0001	(0.09) 0.0184	(0.13) 0.0006	(0.22) <0.0001	0.12 0.002	(0.21) <0.0001	(0.20) <0.0001	(0.08) 0.031	(0.16) <0.0001	(0.02) 0.6522	0.39 <0.0001	(0.01) 0.8749	(0.53) <0.0001	–	

Pearson correlation coefficients and *P*-values are shown. HC Weevil (head-clipping weevil). Positive (dark gray) and negative (light gray) correlations significant after Bonferroni correction ($P < 0.0003$) are highlighted. Correlations that reverse across sites or are significant in one site but nonsignificant ($P > 0.05$) in the other site are boxed in black.

Table 2 Phenotypic selection analyses

	North Dakota			Nebraska		
	Estimate	SE	z-value	Estimate	SE	z-value
Flowering day	**–8.73E–04**	**3.04E–04**	**–2.87****	–1.98E–03	1.75E–04	–11.34***
Disk diameter	7.91E–02	6.27E–03	12.61***	1.59E–01	3.64E–03	43.77***
Ray length	2.84E–02	3.29E–03	8.64***	2.54E–01	4.16E–03	61.03***
Stem height	1.26E–02	2.82E–04	44.59***	5.03E–03	1.55E–04	32.37***
Stem diameter	–2.33E–01	1.86E–02	–12.55***	**6.26E–02**	**1.08E–02**	**5.78*****
Leaf number	1.09E–02	3.48E–04	31.18***	2.87E–03	1.57E–04	18.22***
Leaf size	1.92E–03	6.60E–05	29.10***	**–2.17E–05**	**4.09E–05**	**–0.53**
Leaf shape	**–4.69E–02**	**1.82E–02**	**–2.58****	–1.60E–01	1.01E–02	–15.84***
Branch number	4.59E–02	1.91E–03	24.01***	8.43E–02	7.25E–04	116.18***
Head total	2.22E–02	7.28E–04	30.48***	5.25E–03	1.99E–04	26.37***
Seed mass	1.20E+00	1.18E–02	101.13***	1.47E+01	1.80E–01	81.74***
Leaf herbivory	–6.04E–02	6.95E–03	–8.68***	–2.79E–01	4.48E–03	–62.17***
Head herbivory	–4.68E–01	1.64E–02	–28.45***	–8.77E–01	9.99E–03	–87.84***
Midge damage	–3.37E–02	2.78E–03	–12.10***	–		
Head-clipping weevil	–			–9.41E–01	9.40E–03	–100.04***
Moth damage	–			–2.98E–01	6.87E–03	–43.40***

Estimates of linear selection, standard errors (SE), and z-values are shown for each trait. Selection analyses were performed in the program ASTER, which uses maximum-likelihood methods to estimate selection on overall fitness. Overall fitness is modeled by survival to reproduction and seed total. Estimates of selection that differed in direction or significance when herbivory traits were removed from the analyses are in bold type. ***, $P < 0.0001$; **, $P < 0.001$.

total leaf number, branch number, head total and seed size) were favored at both sites, although narrower stems were favored in ND. Cultivar-like leaf shape (decreased length-width ratio) was also favored at both sites.

Susceptibility to herbivores was universally selected against. In ND, the removal of any one herbivory term (leaf herbivory, head herbivory or midge damage) from the selection model resulted in less significant or nonsignificant selection on flowering day and leaf shape. If all three herbivory terms were removed, selection on flowering day and leaf shape became significantly positive (flowering day, estimate = 9.14E–4, $P < 0.01$; leaf shape, estimate = 2.21E–3, $P < 0.001$). In NE, selection on stem diameter became nonsignificant when leaf herbivory was removed from the model ($P = 0.27$) and selection favored larger leaves ($P < 0.0001$) if any herbivory term other than head herbivory was removed from the analyses.

QTL-analysis

We mapped a total of 61 QTL affecting 18 traits and were successful in detecting QTL for all types of herbivory, as well as leaf fungal damage (Table 3). The QTL were located on all linkage groups (LGs) except 2 and 15, but QTL clustered on several LGs (Fig. 1) as reported previously in the same population (Baack *et al.*, 2008). Individual QTL explained 5.96–22.67% of the phenotypic variance for a trait, and additive effects of the cultivar allele ranged over 0.25–0.59 standard deviation units. The majority of morphological traits for which we mapped two or more QTL displayed at least one QTL with an additive effect in each direction. The exception was that the cultivar allele increased the trait value for all stem height and seed mass QTL. Of the 14 QTL detected for the five herbivory traits, 11 displayed positive additive effects of the cultivar allele (i.e. damage was increased in RILs with the cultivar allele at these loci). By contrast, the cultivar allele led to decreased susceptibility for all leaf fungal damage QTL. Twenty-six QTL mapped to intervals flanked by one or more candidate loci (Table S2, Fig. 1).

We identified several chromosomal regions in which one allele (wild or cultivar) conferred a selective advantage for several colocalized (overlap of 1-LOD scores) traits (Fig. 1). The wild allele was favored for the majority of morphological and herbivory damage QTL on the top-center of LG 7, center of LG 12, and top of LG 13. By contrast, the cultivar allele was generally favored on the top of LGs 3 and 4 and bottom of LG 9. On LG 3, the cultivar allele increased plant size for several QTL in ND, including cultivar-like traits (stem diameter and seed mass) and wild-like traits (stem height, branch number; same effect in NE), and head total. Leaf fungal damage was also reduced by the cultivar allele in this region at both sites. The cultivar allele resulted in an increase in seed total in ND on LG 4, as well as larger leaves and stems at the same experimental site. On LG 9, QTL colocalized for seed mass and leaf herbivory in ND, as well as ray length and branch number in NE, and the cultivar allele increased the trait values for these QTL.

There were only five examples of QTL for the same trait that colocalized across experimental sites (ND and NE): leaf fungal damage and branch number on LG 3 and leaf herbivory,

Table 3 Quantitative trait locus (QTL) mapping results

			North Dakota			Nebraska		
Trait	LG	Flanking markers	2-LOD	α	PVE	2-LOD	α	PVE
Flowering day	1	HT1018, c1774	(0.01–21.01)	0.32	10.79			
	6	HT913, **c2603**				(69.71–71.71)	0.51	22.67
	7	ZVG29, **c1533**	(0.01–9.01)	–0.33	10.29			
	8	HT71, ORS70	(26.81–50.71)	0.26	6.56			
	14	**c1666, G13K16**				(9.81–37.41)	–0.35	8.21
	17	ORS561, ORS735	(35.51–43.31)	–0.41	7.24			
Disk diameter	14	**c0211**, HT528	(13.11–47.41)	0.37	10.17	None		
Ray length	9	**CYC5B**, ORS176	None			(23.51–55.61)	0.33	9.10
Stem height	3	**c1144**, HT1031	(0.01–17.01)	0.29	7.86	None		
	13	HT568, CRT504	(0.01–20.81)	–0.28	7.13			
	14	**c2693, c5666**	(0.01–12.21)	0.59	10.47			
Stem diameter	1	ORS371, CRT391	(23.01–39.11)	0.39	12.74			
	3	HT1031, ORS949	(4.01–36.41)	0.28	7.49			
	4	ORS963, HT298	(0.01–10.01)	0.28	6.53			
	10	ORS878, HT347	(0.01–24.21)	0.32	7.06			
	12	**c0019, c3115**	(36.01–49.91)	–0.32	8.87			
	13	HT568, CRT504	(0.01–24.81)	–0.34	10.55	(0.01–28.81)	–0.32	9.86
	13	ORS511, ORS578	(36.51–50.51)	0.38	9.83			
Leaf number	4	ORS674, HT221				(73.61–84.51)	–0.37	13.18
	7	ORS331, **c1921**	(0.01–23.21)	–0.27	6.07			
	12	**c0019, c3115**	(36.01–49.91)	–0.36	12.27			
	16	HT208, ORS172				(100.21–110.31)	–0.34	11.38
Leaf area	3	HT1031, ORS949	(5.31–42.41)	0.26	6.64			
	4	ORS963, HT298	(0.01–10.01)	0.27	6.14			
	5	ORS852, ORS1120				(19.11–51.11)	–0.30	8.57
	5	ORS1120, HT440	(43.11–67.01)	–0.27	7.14			
	10	**c1700**, ORS878	(2.01–20.21)	0.38	9.96			
	13	HT568, CRT504	(0.01–29.71)	–0.31	9.05			
Leaf shape	4	**c1258**, HT989	None			(22.11–34.11)	0.36	9.87
	4	HT339, ORS674				(44.11–61.61)	–0.33	7.70
	12	**c3115**, HT490				(40.01–60.11)	–0.33	9.88
Branch number	3	**c1144**, HT1031				(5.31–23.01)	0.38	13.71
	3	HT1031, ORS949	(4.01–42.41)	0.28	7.87			
	9	**CYC5B**, ORS176				(25.51–55.61)	0.30	8.31
	12	**c3115**, HT490	(32.01–58.11)	–0.34	11.26			
	16	HT208, ZVG75b	(86.21–110.31)	0.32	10.13			
Head total	3	HT1031, ORS949	(11.31–45.31)	0.32	7.02	None		
	12	c5456, **c0019**	(30.51–61.11)	–0.34	10.82			
Seed mass	3	**c1144**, ORS949	(5.31–40.41)	0.30	8.91	None		
	9	**CYCB5**, ORS176	(49.61–55.61)	0.31	9.32			
Seed total	4	ORS963, HT298	(0.01–6.51)	0.35	10.44			
	6	HT913, **c2603**				(60.11–71.71)	–0.39	14.21
Leaf herbivory	1	c1774, ORS371	(4.01–31.01)	–0.25	5.96			
	6	HT913, **c2603**				(52.11–71.71)	–0.27	6.21
	9	**CYC5B**, ORS176	(39.51–55.61)	0.30	7.52			
	11	HT821, HT390				(54.91–66.91)	0.30	7.70
	13	HT568, CRT504	(0.01–16.81)	0.38	12.61	(0.01–22.81)	0.32	9.42
Head herbivory	7	**c1921**, ORS966	(5.01–27.21)	0.35	11.72			
	13	HT568, CRT504	(0.01–6.81)	0.40	15.48	(0.01–22.81)	0.36	12.78
Leaf fungal damage	3	ORS555, **c1144**				(0.01–15.31)	–0.31	8.64
	3	**c1144**, HT1031	(4.01–23.01)	–0.41	15.84			
	6	HT769, ORS57	(52.11–71.71)	–0.30	8.12			
	11	**c1649**, HT821	(31.51–38.91)	–0.33	8.96			
Head-clipping weevil	6	ORS57, HT913	NA			(52.11–71.71)	0.31	9.01
	11	ORS62, c5763				(0.01–10.71)	–0.29	7.86
	12	ORS358, c5456				(8.01–32.01)	0.35	11.44
Midge damage	10	ORS613, HT419	(10.41–32.71)	0.35	10.06	NA		
Moth damage	4	HT664, ORS366	None			(4.01–36.11)	0.36	12.99

Columns 1 and 2 present the linkage group (LG) and flanking markers for each QTL. Candidate (selected) loci are highlighted in bold. Columns 4–6 and 7–9 show the range of the 2-LOD support limits in cM, the standardized additive effect of the cultivar allele (α), and per cent variance explained (PVE) for each QTL in North Dakota and Nebraska, respectively.

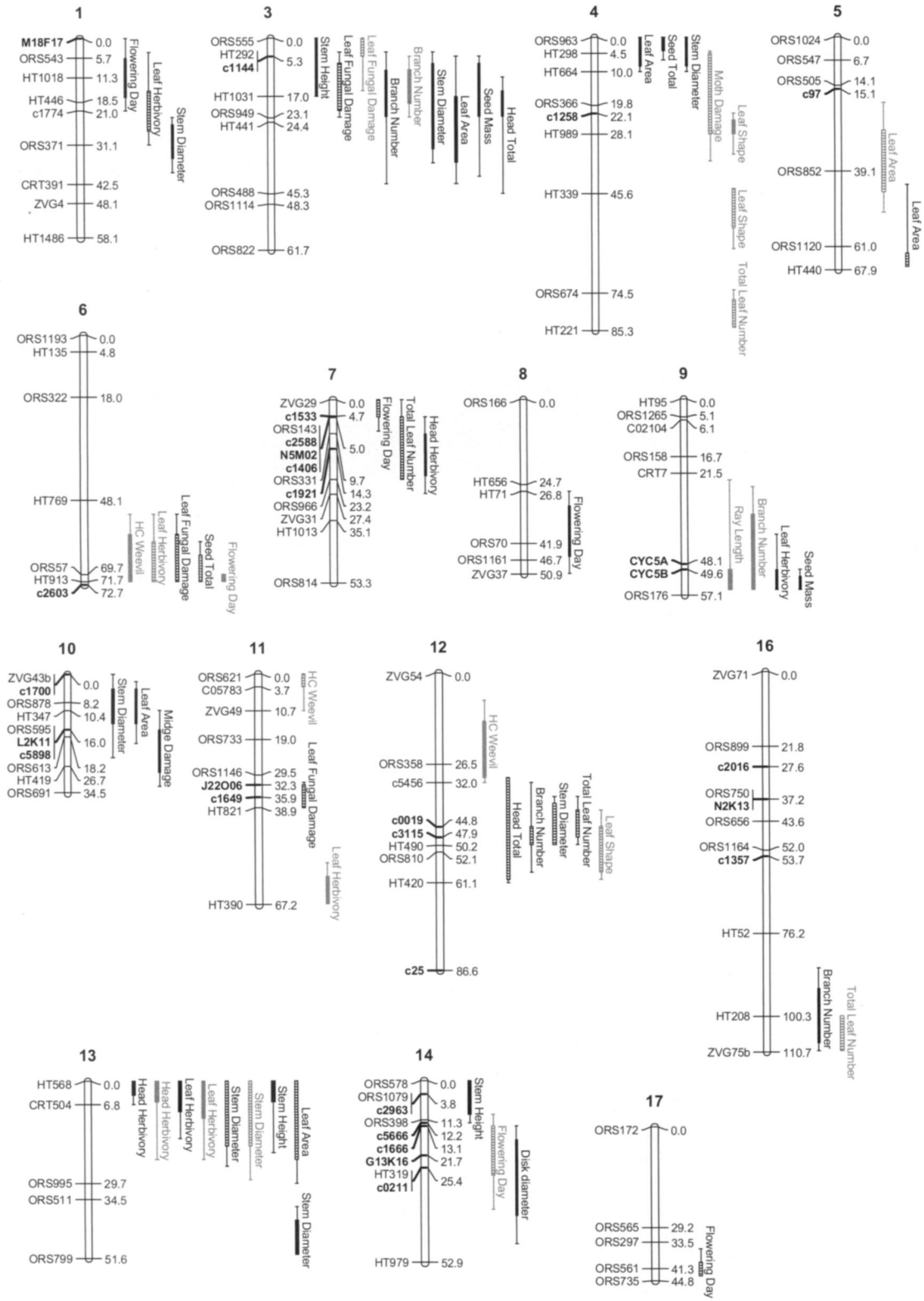

Fig. 1 Graphical representation of quantitative trait locus (QTL)-mapping results. Quantitative trait loci for flowering phenology, herbivory (and one pathogen) susceptibility, and morphological traits were mapped in recombinant inbred lines (RILs) of sunflower crop–wild hybrids using composite interval mapping (CIM) in QTL-CARTOGRAPHER. HC weevil (head-clipping weevil). Site, North Dakota (black) or Nebraska (gray); additive effects, positive (solid) or negative (hatched); and 1-LOD (thick bars) and 2-LOD (tails) support limits are indicated for each QTL. All markers that mapped to any one cM location are listed. Candidate (selected) loci are highlighted in bold.

head herbivory, and stem diameter on LG 13 (Fig. 1). The additive effect of the cultivar allele was always in the same direction for both colocalized QTL of the same trait. The remaining 51 QTL were detected only in ND or NE, indicating possible QTL × environment interactions at these loci.

Discussion

Selection on phenotypic traits

We detected significant directional selection for almost every trait at both sites. Larger plant and inflorescence size were generally favored whether the traits were cultivar-like (e.g. larger leaves, disks, rays and seeds) or wild-like (e.g. greater branch, leaf and head numbers). The one exception was that thinner stems (wild-like) were favored in ND. This result is perplexing because stem diameter was positively correlated with several other traits that were favored by selection in both sites. A likely explanation for the negative selection on stem diameter is that this trait was correlated with an unanalysed trait that was selected against in ND. There was some evidence in ND that stem diameter was positively correlated ($r^2 = 0.17$) with the presence of stem cankers formed by the fungal pathogen, *P. helianthi*. The *P. helianthi* survey was not complete enough to test for selection on this trait, but previous studies have shown a negative effect of *P. helianthi* on seed production in sunflower (Gulya *et al.*, 1997). Stem cankers were not observed in NE (J. Burger, pers. obs.), which may partly explain the differential selection on stem diameter between sites.

Patterns of selection on flowering phenology and three morphological traits were altered depending on which herbivory characters were included in the selection analyses. Selection favored earlier flowering (cultivar-like) and cultivar-like leaves (smaller value for leaf shape) at both sites if all herbivory terms were included in the selection models. However, if any one type of herbivory was excluded from the ND analyses, selection on leaf shape and flowering day became nonsignificant. If all three herbivory terms were removed from the model in ND, estimates of selection for leaf shape and flowering day were reversed (i.e. wild-like leaves and later flowering – also wild-like) were favored. Comparison of selection estimates with and without herbivory terms in ND suggests that, in the absence of herbivory, crop-like phenotypes for flowering time and leaf shape would be favored. In addition, selection favoring wild-like later flowering can be explained by the effects of herbivory, probably because all types of herbivory examined in ND were more severe early in the flowering season. Plants that flowered later (more wild-like) would have avoided some damage by these herbivores. Our results are consistent with those of a previous study in which increased predispersal seed herbivory in sunflower crop–wild hybrids vs wild plants was partly attributable to earlier hybrid flowering (Cummings *et al.*, 1999). In addition, these results suggest that naturally-occurring herbivory may mitigate the selective advantage of some cultivar alleles.

Herbivory was also found to affect selection on flowering time in a previous study of wild sunflower (Pilson, 2000). Consistent with our results, Pilson (2000) found that later flowering was only favored when herbivory by two moth species was not accounted for in the selection model, and these results were largely attributed to greater seed predation on early-flowering plants (Pilson, 2000). The Pilson (2000) study was conducted in NE at Cedar Biological Station (close to our NE site) on moth species, *S. helianthana* and *H. electellum*, which we also observed in NE. However, we found no effects of herbivory on selection for flowering time in NE. Instead, cultivar-like early flowering was favored in all NE selection models. These results suggest that selection on flowering time may be highly environmentally dependent. Although we also found that moth damage was more severe early in the flowering season in NE, damage by the head-clipping weevil showed no clear phenological pattern, which may explain why we found no effect of herbivory on selection for flowering time at the NE site.

In addition, we found significant selection against head-clipping in NE. Pilson & Decker (2002) found no effect of head-clipping on total plant fitness, largely because wild sunflower compensated for simulated head-clipping by producing more inflorescences, more filled seeds, and larger seeds (Pilson, 2000; Pilson & Decker, 2002). One possible explanation for the different effects of head-clipping on plant fitness between studies is that head-clipping may be more detrimental to a hybrid than a wild sunflower population, because many hybrid individuals produce a single head (similar to the crop parent) or fewer heads than a typical wild plant and therefore cannot compensate for head-clipping.

Patterns of selection observed in our study suggest that cultivar-like traits that increase plant size are generally favored in natural environments. Consistent with this finding, larger plant or inflorescence size has been shown to increase the fitness of sunflower crop–wild hybrids in several studies (Campbell & Snow, 2007; Mercer *et al.*, 2007; Baack *et al.*, 2008). Based on these results, cultivar alleles that increase plant or inflorescence size without a detrimental effect on wild-like size traits, such as increased branching or head number, would be expected to introgress into wild populations. However, our results also indicate that naturally occurring herbivory may reduce the selective advantage of other cultivar alleles in wild populations. Consequently, if herbivore populations were to decrease or if crop–wild hybrids were isolated from natural herbivores, cultivar alleles could become more advantageous. Patterns of selection on some traits, such as flowering day, are highly dependent on environmental conditions. For example, our results may have been different if damage by late-season herbivores, such as seed weevils (Curculionidae), had been investigated. Nevertheless, this is one of few studies to identify environmental factors that could reduce the likelihood that crop alleles will introgress into wild populations.

Selective advantage of cultivar alleles

In order to predict the spread of cultivar alleles into wild populations, it is most interesting to identify chromosomal regions in which the cultivar allele confers a selective advantage for one or more co-localized QTL. In addition, cultivar alleles are more likely to spread if they are not pleiotropic for, or tightly linked to, selectively disadvantageous traits, such as increased susceptibility to herbivory. In this study, the cultivar allele was favored for all, or nearly all, QTL at the top of LGs 3 and 4 and bottom of LG 9. Only one of these regions (and only in ND) was associated with a direct effect on fitness; the cultivar alleles on LG 4 led to an increase in seed total, leaf area and stem diameter. No seed total QTL were detected on LGs 3 and 9, but the cultivar allele conferred an increase in size for several traits in both of these regions. In the three regions in which the cultivar allele was generally favored, a maladaptive effect of the cultivar allele was only detected for one QTL, increased leaf herbivory on LG 9. A previous study in crop–wild sunflowers also demonstrated that crop alleles were favored for several traits on the bottom of LG 9 (Baack *et al.*, 2008). Cultivar alleles in this region (and those on the top of LGs 3 and 4) are the most likely to spread into wild populations should selection pressures remain the same.

Several additional QTL for which the cultivar allele was favored were found throughout the genome. It is difficult to predict the likelihood that many of these QTL will spread into wild populations, because they colocalize with QTL for traits in which the cultivar allele was disadvantageous. For example, the QTL for stem height on LG 13 seems unlikely to spread into the wild, because cultivar alleles in this region conferred an adaptive increase in stem height, as well as a maladaptive increase in leaf and head herbivory and decrease in leaf size. In addition to the top of LG 13, the wild allele was favored for the majority of QTL on the top of LGs 4 (only in NE) and 7, and center of LG 12. A direct increase in fitness was associated with the wild allele on LG 6, as was earlier flowering and decreased susceptibility to the head-clipping weevil. The wild allele also increased leaf herbivory and leaf fungal damage at this region. Very similar effects were found for flowering day and fitness QTL on the bottom of LG 6 in a previous study of crop–wild sunflower hybrids (Baack *et al.*, 2008).

Interestingly, allelic effects on co-localized herbivory and morphological traits were, for the most part, similarly advantageous or disadvantageous. There were only two examples (seed mass with leaf herbivory on LG 9 and stem height with leaf and head herbivory on LG 13) of a selectively advantageous morphological QTL that co-localized with a maladaptive herbivory trait QTL at the same experimental site. These results suggest that only in rare cases will maladaptive herbivory alleles hinder the spread of advantageous cultivar alleles into wild populations. One caveat to these results is that QTL architecture can vary significantly with experimental conditions and/or the parental lines used to generate the mapping population (Mauricio, 2001; Burke *et al.*, 2002b; Baack *et al.*, 2008). In this study, QTL co-localized across sites for only five traits (10 of 61 QTL detected). A portion of this QTL × environmental variation is likely caused by differing sample sizes between sites (i.e. we detected more QTL in ND, for which sample sizes were 20–40% greater than in NE). An unknown number of these QTL are environment-specific and would not necessary be detected at another site or under different herbivore pressures. In addition, QTL-mapping is limited to the allelic variation between the two parental lines, and an initial cross with different parents, particularly a different wild parent, could alter our results. Nevertheless, for the five traits with QTL that colocalized between sites, additive effects for QTL pairs were always in the same direction and generally in the expected direction (e.g. the cultivar allele increased susceptibility to insect herbivores at most QTL). Therefore, we expect that many of our results would be observed in additional environments or crop–wild parental crosses, though further studies are necessary to confirm these results.

The cultivar allele conferred opposite effects for damage by insect herbivores vs damage by fungal pathogens. Susceptibility to insect herbivory was increased by the cultivar allele for all herbivory QTL except one (of three) QTL for damage by the head-clipping weevil and two (of six) QTL for general leaf herbivory; whereas, all cultivar alleles decreased susceptibility to leaf fungal damage. These results are partly explained by the breeding history of crop sunflowers. Resistance to insect herbivores has not been actively bred into crop sunflowers; instead, insecticides are the primary control strategy (Gulya *et al.*, 1997). By contrast, sunflower cultivars have been selectively bred for resistance to several fungal pathogens such as *P. halstedii* (downy mildew), *Sclerotinia sclerotiorum*, *Diaporthe helianthis*, and *Phoma macdonadii* (Vear *et al.*, 2008). Although resistance to *S. helianthi* or *Alternaria* spp. (the species that caused leaf fungal damage in our study) has not been actively selected in cmsHA89, resistance to one pathogen often negatively affects others (Gulya *et al.*, 1997).

Candidate genes

Twenty-six QTL mapped to intervals bordered by possible candidate genes, and several of these genes exhibited significant sequence similarity to proteins with putative functions in other plant species (Table S2, adapted from Chapman *et al.*, 2008b). Although their function in sunflower is unknown, a number of candidates were associated with particularly interesting QTL. For example, a flowering day QTL mapped to a region on LG 14 linked to the candidate gene, G13K16. This region was also associated with flowering day in a previous study of crop–wild sunflower hybrids (Baack *et al.*, 2008). Locus G13K16 shows sequence homology to an *Arabidopsis* putative fructose-2,6-biphosphatase protein (Chapman *et al.*, 2008b), which is involved in general carbohydrate metabolism (Okar & Lange, 1999). Carbon metabolism has been previously

linked to environmental effects on flowering time in *Arabidopsis* (Calenge *et al.*, 2006), and it is possible that altered carbon metabolism in sunflower played a similar role during domestication.

A QTL for fungal leaf damage mapped to a region on LG 3 bordered by the candidate locus, c1144, which shows sequence similarity to an *Arabidopsis* calmodulin-binding protein (Chapman *et al.*, 2008b). Calmodulin-binding transcription factors have been linked to plant stress response (Yang & Poovaiah, 2002), and at least one calmodulin-binding gene is involved in the regulation of salicylic acid-mediated resistance to plant pathogens (Du *et al.*, 2009). If c1144 plays a similar role in sunflower, it may be of interest for plant breeding.

The most promising candidate gene result is that QTL for four traits mapped to the region bordering the *CYCLOIDEA*-like gene, *CYC2b* on LG 9. *CYC* genes are a family of transcription factors involved in flower symmetry in *Antirrhinum majus* L. and *Lotus japonica*, as well as members of the Asteraceae (Luo *et al.*, 1996; Cubas *et al.*, 1999; Feng *et al.*, 2006; Broholm *et al.*, 2008; Kim *et al.*, 2008). Other members of the gene family are involved in the loss of branching in maize (Doebley *et al.*, 1997) and rice (Takeda *et al.*, 2003). Expression patterns of *CYC*-like genes suggest that *CYC2c* may play a role in the development of ray florets in sunflower (Chapman *et al.*, 2008a). *CYC2c* maps to the same region as *CYC2b* (as does a third *CYC*-like gene, *CYC2e*; Chapman *et al.*, 2008a) well within the 1-LOD interval of QTL for ray length, branch number, seed total and leaf damage. It is possible that sunflower crop and wild *CYC2* alleles differentially affect ray length and/or branch number.

Conclusions

In this study, several cultivar-like size traits were favored, in combination with certain size-related wild traits, consistently across two different natural sites. Although most cultivar alleles at herbivory-related QTL conferred an increase in susceptibility to herbivores, these QTL rarely colocalized with selectively advantageous cultivar alleles for other phenotypic traits. The results of this study combined with previous work demonstrating the selective advantage of cultivar-like plant and inflorescence size traits (Mercer *et al.*, 2007; Baack *et al.*, 2008) suggest that cultivar alleles for several traits could spread into wild sunflower populations should selection pressures remain constant. However, our data suggest that naturally occurring herbivory might hinder the spread of otherwise advantageous cultivar alleles into wild populations, perhaps explaining why wild sunflower populations still retain many wild-like traits even in the face of extensive reproductive contact (Baack *et al.*, 2008). Nonetheless, cultivar alleles are often retained in natural populations (Arias & Rieseberg, 1994), and if wild species are able to combine favorable cultivar and wild alleles, the evolution of increased weediness or invasiveness could easily follow.

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Supporting Information

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

Table S1 Least-squared means (LSmeans) and 95% confidence intervals for measured traits at both sites

Table S2 Homology search results for candidate genes

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