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Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*

TAKESHI KAWAKAMI,* THEODORE J. MORGAN,* JESSE B. NIPPERT,* TROY W. OCHELTREE,* ROSE KEITH,† PREETI DHAKAL* and MARK C. UNGERER*

**Division of Biology, Kansas State University, Manhattan, KS 66506, USA, †Mount Holyoke College, 50 College Street, South Hadley, MA 01075, USA*

Abstract

In plants, ecologically important life history traits often display clinal patterns of population divergence. Such patterns can provide strong evidence for spatially varying selection across environmental gradients but also may result from nonselective processes, such as genetic drift, population bottlenecks and spatially restricted gene flow. Comparison of population differentiation in quantitative traits (measured as Q_{ST}) with neutral molecular markers (measured as F_{ST}) provides a useful tool for understanding the relative importance of adaptive and nonadaptive processes in the formation and maintenance of clinal variation. Here, we demonstrate the existence of geographic variation in key life history traits in the diploid perennial sunflower species *Helianthus maximiliani* across a broad latitudinal transect in North America. Strong population differentiation was found for days to flowering, growth rate and multiple size-related traits. Differentiation in these traits greatly exceeds neutral predictions, as determined both by partial Mantel tests and by comparisons of global Q_{ST} values with theoretical F_{ST} distributions. These findings indicate that clinal variation in these life history traits likely results from local adaptation driven by spatially heterogeneous environments.

Keywords: clinal variation, F_{ST} vs. Q_{ST}, life history, local adaptation, microsatellite, population genetics

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Introduction

Widely distributed species often exhibit considerable variation in developmental, physiological and other fitness-related traits. When examined across latitudinal or altitudinal transects, these traits often display clinal patterns of variation (e.g., Olsson & Agren 2002; Storz 2002; Hall *et al.* 2007). Natural selection represents a plausible driver of clinal phenotypic differentiation because of gradients in climatic conditions and/or local selection pressures (Slatkin 1973; Endler 1977). Latitude is closely correlated with temperature, solar radiation, precipitation and moisture availability. For plants, these environmental variables are especially critical determinants of developmental timing events and life history characteristics that optimize fitness while avoiding unfavourable environmental conditions such as heat, frost and drought (Hoffmann & Parsons 1991).

Clinal patterns of phenotypic variation also can be produced by neutral or nonadaptive processes. For example, repeated large-scale displacement of flora and fauna in North America during glacial maxima was followed by recolonization from refugia after climate amelioration (Avise 2000). It is possible that concordant clinal variation in phenotypic traits and allelic frequencies at genes underlying these traits can result from range expansion, drift or admixture of multiple genetically distinct populations during recolonization (Vasemagi 2006). Therefore, determining the relative importance of adaptive and nonadaptive processes in the formation and maintenance of clinal phenotypic variation represents an essential first step towards

Correspondence: Mark C. Ungerer, Fax: +1 785 532 5845;
E-mail: mcungere@ksu.edu

understanding the genetic basis of local adaptation and the mechanistic nature of spatially varying selection.

An effective means of discriminating between adaptive and nonadaptive processes acting in populations involves comparing relative levels of between-population differentiation using neutral molecular markers (as measured by F_{ST}) and quantitative traits that may be targets of selection (as measured by Q_{ST}) (Spitze 1993). F_{ST} values are calculated from putatively neutral molecular markers and provide a standardized measure of genetic differentiation between populations through neutral or nonadaptive processes. Q_{ST} is an F_{ST} analogue for quantitative traits and measures the amount of between-population additive genetic variance relative to the total genetic variance (Wright 1951). Q_{ST} values significantly larger than F_{ST} values are taken as evidence of spatially varying selection favouring different genotypes along environmental gradients. Conversely, Q_{ST} values significantly smaller than F_{ST} values are taken as evidence of spatially uniform selection where the same genotypes are favoured in different environments. Finally, Q_{ST} values not significantly different from F_{ST} values are taken as evidence that between-population differentiation in a quantitative trait is not different from that produced by neutral or nonadaptive processes. The F_{ST} vs. Q_{ST} approach has been applied in a variety of organisms where populations are differentiated along gradients of latitude (Storz 2002; Palo *et al.* 2003), altitude (Ramirez-Valiente *et al.* 2009) and longitude (Jaramillo-Correa *et al.* 2001) (see Leinonen *et al.* 2008 for a comprehensive review).

Helianthus maximiliani is a native perennial sunflower species that occurs throughout much of the USA and southern Canada. This species has a broad latitudinal distribution, ranging from 28°N to 55°N (Global Biodiversity Information Facility, http://www.gbif.org/; University of Manitoba Herbarium, http://www.umanitoba.ca/botany/research.html) with the greatest concentration of populations found in central North America between Texas, USA and Manitoba, CA. In his 1969 monograph on *Helianthus*, Heiser (1969) noted that when grown under common garden conditions, *H. maximiliani* plants collected from northern and southern margins of the species range showed striking variation in height, flowering time and other size-related morphological traits. He predicted that 'Detailed studies might show the existence of north–south clines in the species for some characters' (p. 157).

In the current study, we test and confirm this prediction of clinal variation by characterizing differences in several life history traits among latitudinally diverse *H. maximiliani* populations in a common garden greenhouse experiment. In addition, variation at seven microsatellite loci was surveyed to characterize between-population differentiation (F_{ST}) at putatively neutral markers and estimate the degree of isolation by distance for these populations. Comparisons of between-population differentiation in quantitative traits (measured by Q_{ST}) and neutral differentiation at molecular markers (measured by F_{ST}) suggest that clinal variation in key life history traits in *H. maximiliani* is likely maintained by a balance between gene flow and recurrent spatially varying selection.

Methods

Sunflower populations

Seeds from natural *H. maximiliani* populations were wild-collected in the field or obtained from the USDA National Plant Germplasm System (Fig. 1, Table 1). USDA seeds also represent original wild-collected material. An F_2 population was generated that segregates for alleles from Manitoba and TX populations. These F_2 seeds were generated by crossing F_1 plants derived from two different crosses of Man-5 × TX-2 individuals. Man-5 was used as maternal parent in initial crosses, and thus, all F_2 individuals possess a Man-5 cytoplasm. Crosses to generate F_2 seeds were performed between 2006 and 2008. For the experiments described herein, all seeds were germinated in the dark on moist filter paper in Petri dishes on 11 May 2009. Germinated seedlings were transferred to four-inch pots and grown in a Kansas State University greenhouse under ambient light and temperature conditions. Plants were transferred to eight-inch pots on 1 June 2009. Plant positions were randomized across three 1.2 × 2.4 m benches. All plants were watered daily or as needed and provided with a weak nutrient solution (N:P:K = 15:30:15) once per week.

Life history traits

A total of 15 quantitative life history traits related to aspects of vegetative size and morphology, capitulum size and structure, growth and physiology were measured from May to October 2009 (Table 2).

Vegetative size and morphology. Plant height and stem diameter 2 cm above soil level were measured on the day of flowering. Above-ground dry biomass (DB) was measured at the end of the flowering period. The number of capitula (#Cap) was counted for each plant and standardized by biomass (#Cap/DB). On 31 July 2009, a fully expanded mature leaf was harvested from each plant and the leaf area was measured using a LiCOR 3100 leaf area metre. Leaves were subsequently dried, weighed and ground with a mortar and pestle in

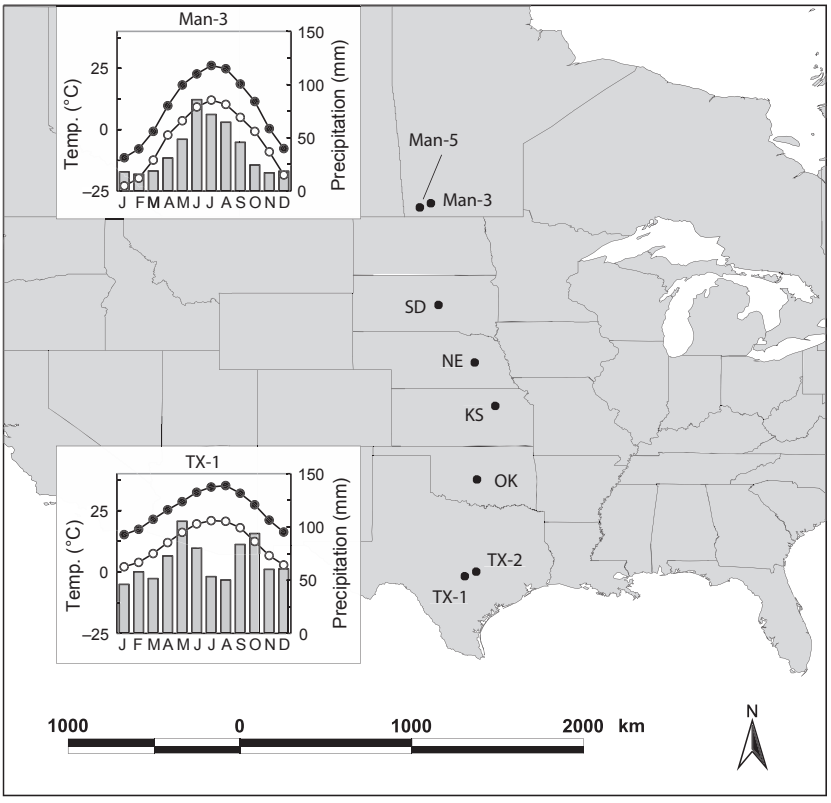

Fig. 1 Map of sampling localities across North America. Insets show mean highest and lowest monthly temperatures (filled and open circles, respectively) and mean monthly precipitation (shaded bars) from 1901 to 2002 for the Man-3 (top) and TX-1 (bottom) collection locations. Meteorological data from Integrated Database Information System.

Table 1 Source of plant material and summary statistics of seven microsatellite loci genotyped in *Helianthus maximiliani*

					Microsatellite data (7 loci)			
Population	State/Province	Lat. (N), Long. (W)	Accession*	*N*	R_t	H_E	H_O	F_{IS}
Man-3	MB, Canada	49.709, 99.962	PI 592333	17	4.57	0.63	0.56	0.11
Man-5	MB, Canada	49.486, 100.533	PI 592335	18	4.07	0.55	0.56	-0.02
SD	SD, USA	44.383, 99.550	PI 586900	18	5.01	0.64	0.60	0.06
NE	NE, USA	41.367, 97.667	PI 586895	18	4.26	0.67	0.73	-0.09
KS	KS, USA	39.100, 96.580	MCU-KS	17	4.85	0.66	0.63	0.05
OK	OK, USA	35.247, 97.539	MCU-OK	17	4.27	0.60	0.56	0.08
TX-2	TX, USA	30.422, 97.592	RT-TX	18	4.84	0.67	0.74	-0.10
TX-1	TX, USA	30.199, 98.180	MCU-TX	16	5.15	0.64	0.60	0.07
F2	(Man5 × TX-2)	—	—	37	—	—	—	—

N, number of individuals analyzed; R_t, mean allelic richness corrected for sample size; H_E, expected heterozygosity (unbiased gene diversity); H_O, observed heterozygosity; F_{IS}, inbreeding coefficient.
*USDA-National Plant Germplasm System accession ID (http://www.ars-grin.gov/npgs/) or collected in the field by MCU or Ruth Timme (RT).

preparation for mass spectrometry analyses (see section below on Physiological traits).

Capitulum size and structure. Disk diameter, ligule width, ligule length and number of ligules per capitulum were measured for two capitula per plant and averaged. Measurements were made 2–3 weeks after the day of flowering when fully opened flowers were most abundant.

Growth. Plant height was measured once a week, and these measurements were fit to the logistic growth model to estimate growth rate (Freund *et al.* 2003).

Table 2 Differentiation across eight *Helianthus maximiliani* populations for 15 quantitative traits (Global Q_{ST}) and the correlation between pairwise Q_{ST} and geographic distance between populations analysed by Mantel tests and partial Mantel tests

		Mantel test		Partial Mantel test	
Traits*	Global Q_{ST}†	*r*	*P*‡	*r*	*P*‡
Vegetative size/morphology					
Height	**0.748**	0.662	**0.003**	0.658	**0.001**
Stem diameter	**0.731**	0.710	**0.002**	0.670	**0.001**
Dry biomass	**0.698**	0.709	**0.002**	0.644	**0.002**
No. of capitula/biomass	**0.607**	0.545	0.011	0.656	**0.003**
Leaf mass	**0.395**	0.523	0.018	0.475	0.020
Capitulum size/structure					
Disk diameter	**0.518**	0.674	**0.002**	0.683	**0.002**
Ligule width	**0.589**	0.571	0.009	0.430	0.024
Ligule length	**0.537**	0.694	**0.002**	0.620	**0.003**
Ligule no.	0.342	0.282	0.095	0.294	0.078
Growth					
Growth rate	**0.799**	0.730	**<0.001**	0.760	**<0.001**
Days to flowering	**0.946**	0.545	**<0.001**	0.369	0.035
Physiological traits					
$\delta^{15}N$	0.152	0.587	0.012	0.562	0.011
% Leaf N	0.109	0.213	0.115	0.110	0.293
$\delta^{13}C$	0.306	0.536	0.011	0.428	0.042
% Leaf C	0.013	0.435	0.026	0.414	0.031

*Underline indicates traits with significant partial Mantel tests and with global Q_{ST} estimates exceeding the 97.5% value of the theoretical F_{ST} distribution (see text).
†Bold indicates global Q_{ST} values falling outside the tail of simulated F_{ST} distribution (97.5% percentile, see text).
‡Bold indicates significant after sequential Bonferroni corrections ($\alpha = 0.05$).

Days to flowering was recorded as the number of days before the opening of the first flower.

Physiological traits. $\delta^{13}C$ of leaf tissue is commonly used as an index of intrinsic water use efficiency, while $\delta^{15}N$ reflects the net effect of a range of ecophysiological processes, such as the assimilation, transport and loss of nitrogen (Dawson *et al.* 2002). %C is an approximate assessment of carbon allocation to structural molecules in leaves, such as cellulose (Nagel *et al.* 2002), and changes in leaf %N reflect differences in nitrogen-rich photosynthetic enzymes, which influence photosynthetic capacity (Field & Mooney 1983) and are often correlated with latitude and temperature differences between species (Reich & Oleksyn 2004). The stable carbon and nitrogen isotopic signature and percent C and N content of dried leaf tissue was measured using an EA and Conflo II interface connected to a continuous-flow Thermo-Finnigan Delta Plus isotope ratio mass spectrometer. Results are reported using standard delta notation:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000 \quad (1)$$

where R_{sample} and R_{standard} are the molar abundance ratios of the sample and standard, respectively. Data are expressed in per mil (‰).

Population genetic analyses

One mature leaf per plant was harvested on July 13 and immediately frozen in liquid nitrogen. DNA was extracted from these samples using a DNeasy 96 Plant Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. One hundred and thirty-nine plants from eight populations along the latitudinal transect (Table 1) were genotyped at seven microsatellite loci (Table S1, Supporting information). The PCR conditions and primer sequences for these loci are given in Aurelle *et al.* (2010) and associated supplemental data. In addition, 37 F_2s, two F_1s and the parental plants from which they were derived (two Man-5 and two TX-2) were genotyped to confirm that these microsatellite loci follow a standard pattern of Mendelian segregation. PCR-amplified fragments were analysed on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and fragment sizes were scored using GENEMARKER (SoftGenetics, State College, PA, USA).

Standard population genetic parameters were calculated using FSTAT 2.9.3 (Goudet 1995) or Microsatellite Toolkit 3.1.1 (Park 2001). Neutral divergence between populations was evaluated by estimating F_{ST} (Weir & Cockerham 1984) using the program FSTAT 2.9.3. A permutation test (5000 runs of randomization of alleles

among individuals within populations) was used to determine whether observed inbreeding coefficient (F_{IS}) values were significantly different from zero for each locus and over all loci using FSTAT 2.9.3. For each population (Table 1) and for all loci, tests for pairwise linkage disequilibrium and departure from Hardy–Weinberg equilibrium were conducted with Genepop 4.0 (Raymond & Rousset 1995; Rousset 2008). It has been suggested that F_{ST}-type statistics tend to underestimate actual population differentiation when highly polymorphic molecular markers with high within-population diversity (such as microsatellites) are utilized (Hedrick 2005; Jost 2008; Heller & Siegismund 2009; Ryman & Leimar 2009). Consequently, we also estimated D_{est} (Jost 2008) using the program SMOGD 1.2.5 (Crawford 2010) (Fig. S2, Supporting information).

Analysis of quantitative trait differentiation

Tests for normality and unequal variance of trait data were conducted with Shapiro–Wilk's and Bartlett's tests. The existence of clinal variation was assessed by least-squares linear regression analyses in JMP version 7.0.1. To estimate differentiation in quantitative traits among populations, we used Wright's (1951) approach, which shows that the total additive genetic variance for a quantitative character in a population under Hardy–Weinberg equilibrium can be partitioned into within- and between-population components of variation based on his fixation indices (*F*-statistics) as shown below.

$$\begin{aligned}\sigma_b^2 &= 2F_{ST}\sigma_0^2 \\ \sigma_w^2 &= (1-F_{IS})(1-F_{ST})\sigma_0^2\end{aligned} \tag{2}$$

where σ_0^2 is the total additive genetic variance under Hardy–Weinberg equilibrium and σ_b^2 and σ_w^2 are the between- and within-genetic variances, respectively. If we assume that local populations are in Hardy–Weinberg equilibrium (F_{IS} = 0; Table 1) and then solve for σ_0^2, the result is a measure for differentiation in quantitative traits that is similar to F_{ST} for neutral molecular markers, referred to as Q_{ST} by Spitze (1993).

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2} \tag{3}$$

For each trait, we estimated Q_{ST} for all pairs of populations (pairwise Q_{ST}) and across all populations (global Q_{ST}). We determined the within- and among-population variance components by equating the observed mean squares with the expected mean squares (Lynch & Walsh 1998) from a one-way ANOVA for each of the pairwise data sets. Variance components were computed using REML in PROC VARCOMP in SAS version 9.2 (SAS-Institute 2010).

It should be noted that studies of Q_{ST} traditionally utilize a quantitative genetic breeding design for each population measured under common environmental conditions to minimize environmental variance in the experimental design and to estimate the within-population additive genetic variance. In such a design, the within-population variance is an estimate of the additive genetic variance in each population being compared, while the between-population variance is phenotypic variance between the populations measured under common environmental conditions and thus is taken as a surrogate for the between-population genetic variance. In the current study, plants were grown from seed in a greenhouse under uniform condition to minimize environmental effects; however, we did not employ a quantitative genetic breeding design within each population. Therefore, our estimates of the within-population variance (σ_w^2) are actually estimates of the within-population phenotypic variance in a common garden. This experimental design modification is expected to result in conservative estimates of Q_{ST}, because the between-population variance estimated in a common garden experiment should be nearly identical to that estimated in a common garden with a breeding design, while the within-population phenotypic variance will be larger than the additive variance (Falconer & Mackay 1996) and thus decrease the magnitude of the Q_{ST} estimate, thereby resulting in a conservative test between neutral and quantitative trait differentiation.

Comparison of neutral genetic and quantitative trait divergence

Assuming that migration decreases as a function of geographical distance, genetic differentiation (measured by pairwise F_{ST} for neutral markers) is expected to increase as geographical distances increase between populations (Wright 1943). To test this neutral 'isolation-by-distance' expectation, the association between F_{ST} and geographical distance was evaluated with a Mantel test. Similarly, pairwise Q_{ST} estimates for each quantitative trait were compared with geographic distance by Mantel tests. To test whether clinal variation in quantitative traits may be attributable to spatially varying selection, each pairwise Q_{ST} distance matrix was compared with the geographic distance matrix while controlling for the effect of neutral genetic divergence measured by pairwise F_{ST} (partial Mantel tests). If partial Mantel correlations (r) remain significant after controlling for the effect of neutral genetic divergence, this would suggest that clinal variation in quantitative traits cannot be explained by nonadaptive (i.e., isolation-by-distance) mechanisms

alone. All Mantel and partial Mantel tests were calculated using ZT version 1.1 (Bonnet & Van de Peer 2002) with 100 000 permutations.

We also evaluated neutral genetic and quantitative trait divergence by comparing global Q_{ST} values against a distribution of F_{ST} values as suggested by Whitlock (2008). Lewontin & Krakauer (1973) found that the distribution of F_{ST} among loci can be approximated by a chi-square distribution with $(n - 1)$ degrees of freedom:

$$\frac{(n-1)F_{ST}}{\bar{F}_{ST}} \quad (4)$$

where n is the number of populations and $\bar{F}_{ST}$ is the mean F_{ST} value. The variance of the distribution is given by $2\ F_{ST}^2/(n-1)$. The values of global Q_{ST} for each trait that fall in the extreme tail (>97.5% percentile value) of the simulated distribution of F_{ST} may be regarded as traits likely to be under spatially varying selection (Whitlock 2008). We used the $\bar{F}_{ST}$ value estimated by FSTAT 2.9.3 to simulate the distribution of F_{ST} expected by the Lewontin–Krakauer prediction.

Results

Clinal variation in life history traits

Fourteen of fifteen quantitative traits displayed significant clinal variation across latitude, as determined by regression analyses (Fig. 2 and Fig. S1, Supporting information). Days to flowering exhibited the most striking clinal pattern ($R^2 = 0.974$; $P < 0.0001$), with individuals from northern populations flowering earliest and individuals from southern populations flowering latest (Fig. 2c). Strong north–south trends also were observed for plant height at flowering (Fig. 2a), disk diameter (i.e., capitulum size) (Fig. 2b) and multiple additional morphological traits (Fig. S1, Supporting information), indicating that at reproductive maturity, plants from southern latitudes are larger overall when compared with their northern counterparts. The ratio of capitula number to above-ground biomass increased with increasing latitude (Fig. S1C, Supporting information), suggesting that plants in northern populations may have a proportionally higher reproductive allocation.

Growth rate also increased with increasing latitude (Fig. 2d). This pattern was pronounced; plants from Manitoba were approximately twice as tall as plants from Texas on day 67 of the experiment (mean plant height = 43.1 cm vs. 22.6 cm for Manitoba and Texas plants, respectively), despite reaching less than half the final height of their Texas counterparts at reproductive maturity (Fig. 2a). Three of four physiological traits

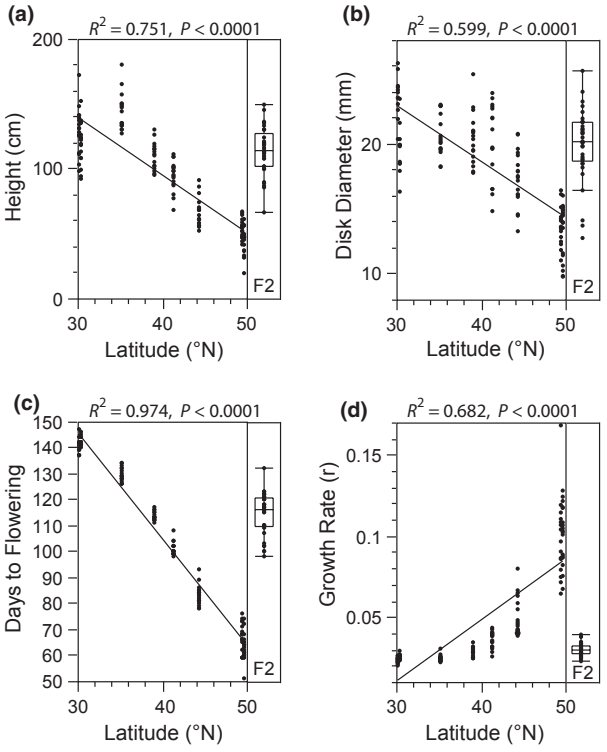

Fig. 2 Latitudinal clines for plant height (a), disk diameter (b), days to flowering (c) and growth rate (d). Values of these traits in F_2 hybrid plants (Man-5 × TX-2) are indicated with box plots. Least-squares linear regressions against latitude are shown as solid lines. Results of the remaining 11 quantitative traits are found in Fig. S1 (Supporting information).

showed significant clinal variation along the latitudinal transect (Fig. S1H–K, Supporting information), although R^2 values for these traits were considerably lower than those observed for developmental timing and morphological traits.

The population of F_2 plants derived from a Man-5 × TX-2 cross generally exhibited intermediate values relative to the parental lines and larger variance for most traits when compared to the natural populations, suggesting abundant segregating variation (Fig. 2 and Fig. S1, Supporting information). Exceptions to these general patterns include growth rate (Fig. 2d) and the number of capitula per above-ground biomass (Fig. S1C, Supporting information), where F_2s more closely resembled populations from southern latitudes. These observations suggest that the TX-2 population may harbour multiple dominant alleles influencing these traits.

Genetic variation at microsatellite loci

A total of seven microsatellite loci were scored for 139 *H. maximiliani* plants in eight populations utilized in this experiment (Table 1). Allelic richness (R_t) and

unbiased gene diversity (H_E) of these microsatellite loci were relatively uniform across the eight populations, and the inbreeding coefficient (F_{IS}) was not significantly different from zero in any population. Across all populations, the mean number of alleles per locus was 10.9, and mean observed and expected heterozygosities (H_O and H_E) were 0.62 and 0.63, respectively (Table S1, Supporting information). High within-population diversity in these markers is consistent with the obligate outcrossing mode of reproduction resulting from a functional self-incompatibility system in this species.

Exact tests failed to find significant departures from Hardy–Weinberg equilibrium for any of these loci across all populations with the exception of one locus (*Hmax*19) in one populations (Oklahoma, $P = 0.0004$). Because estimates of population differentiation with and without this locus were virtually identical, we present results using all loci. Pairwise linkage disequilibrium was not detected for any pair of loci in any population after sequential Bonferroni corrections (Rice 1989).

Comparison of neutral genetic and quantitative trait divergence

Multilocus measures of genetic differentiation (F_{ST}) in pairwise comparisons among the eight populations show a typical pattern of 'isolation by distance' (Fig. 3, open squares; Mantel test, $r = 0.501$, $P = 0.001$). A similar pattern is observed with D_{est} as an estimate of differentiation (Fig. S2, Supporting information; Mantel test, $r = 0.553$, $P = 0.002$). These results suggest geographic structure along the latitudinal gradient with restricted gene flow. Pairwise Q_{ST} for 13 of 15 traits also exhibits a significant increase as a positive function of distance (Fig. 3 and Fig. S3, Supporting information, Table 2), with seven traits remaining significant after sequential Bonferroni correction (Table 2).

To test whether divergence in quantitative traits is significantly greater than that caused by neutral processes, we compared neutral divergence (pairwise F_{ST}) and quantitative trait divergence (pairwise Q_{ST}) as a function of distance using partial Mantel tests. The tests revealed significant correlations between pairwise Q_{ST} and geographic distance even after controlling for neutral divergence for 13 of 15 traits, with seven traits remaining significant after sequential Bonferroni correction (Table 2).

We also evaluated global Q_{ST} values for each trait in relation to the predicted theoretical distribution of F_{ST} (Lewontin & Krakauer 1973) to test whether there were traits with exceedingly large Q_{ST} values relative to the neutral expectation (Whitlock 2008). Global population divergence (Q_{ST}) for 15 quantitative traits ranged from 0.013 to 0.946 (Table 2). The predicted F_{ST} distribution had a global mean of 0.160 with a 97.5 percentile value equal to 0.360. Ten traits had Q_{ST} values exceeding this 97.5 percentile value (Table 2) and, hence, are unlikely to have evolved under neutral expectations. There is strong agreement between the results of this latter analysis and those from the partial Mantel tests (Table 2).

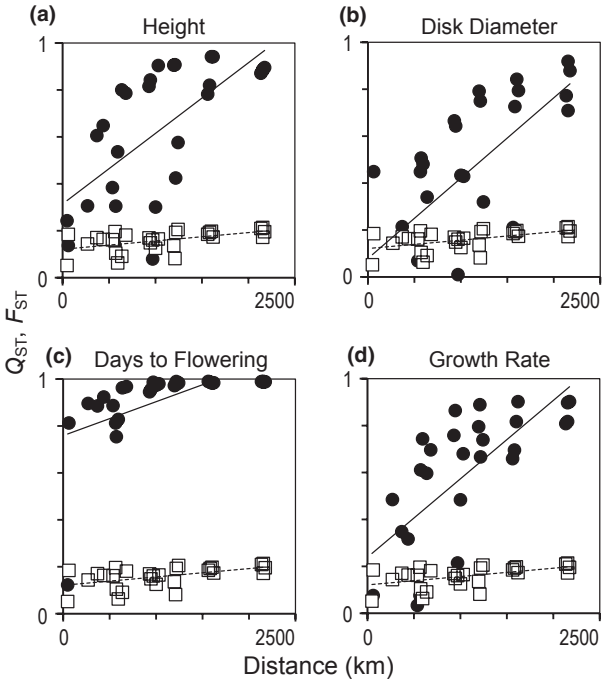

Fig. 3 Correlations of F_{ST} and Q_{ST} values with geographic distances for all pairwise combinations of populations. Q_{ST} values for plant height (a), disk diameter (b), days to flowering (c) and growth rate (d) are indicated as filled circles. F_{ST} values for neutral divergence are indicated as open squares. Least-squares linear regressions for F_{ST} and Q_{ST} against geographic distance are shown as dashed and solid lines, respectively. Results of the remaining 11 quantitative traits are found in Fig. S3 (Supporting information).

Discussion

Clinal variation in morphological, physiological and other quantitative traits is common within species and is often invoked as strong support for spatially varying selection pressures that ultimately facilitate local adaptation (Slatkin 1973; Endler 1977). Populations along latitudinal gradients typically experience different climatic conditions, including temperature, precipitation, moisture availability and solar radiation, which for plants influence the length of the growing season and, consequently, strategies for growth and reproduction. However, latitudinal variation in quantitative traits also can be the result of various nonadaptive evolutionary

processes, such as vicariance events followed by secondary contact, founder events during population expansion and spatially structured populations with restricted gene flow (Vasemagi 2006). Therefore, evaluating the relative importance of neutral and adaptive processes as determinants of differentiation in quantitative traits among populations is a central theme of evolutionary biology (McKay & Latta 2002; Whitlock 2008).

We observed a highly significant linear relationship between latitude of origin of *H. maximiliani* populations and measures of several morphological and developmental timing traits (Fig. 2 and Fig. S1, Supporting information). This relationship was less strong, but still apparent, for several additional traits related to physiological performance. For the majority of traits measured, segregating F_2s derived from a Man-5 × TX-2 cross showed intermediate values with large variance relative to the parental lines used to construct them, indicating abundant segregating genetic variation.

F_{ST} *vs.* Q_{ST}

While pairwise population differentiation based both on neutral molecular markers (F_{ST}) and on quantitative traits (Q_{ST}) increased with increasing geographic distance, differentiation was far more pronounced for quantitative traits. These observations support a hypothesis of adaptive population divergence in the face of gene flow and suggest that these clinal patterns are driven by natural selection. While this conclusion seems warranted in this system, two issues relating to our analyses deserve further mention: (i) the reliability of estimates of neutral population differentiation as an unbiased baseline measure of divergence using microsatellite markers and (ii) the validity of Q_{ST} estimates to accurately represent additive genetic variance in the quantitative traits under study.

Wright's F_{ST} (1951) and its relatives (e.g., G_{ST} for the finite island model with multiple alleles, Nei 1975) have been traditionally used as measures of population differentiation. However, it has been suggested that F_{ST}-type statistics do not provide accurate estimates of differentiation between subpopulations when microsatellites are employed (Hedrick 1999, 2005; Jost 2008). Properties of microsatellite markers, including large numbers of alleles per locus, elevated within-population heterozygosity and increased mutation rates, result in a downward bias in estimates of population differentiation in F_{ST}-type measurements because of their dependence on within-population heterozygosity (H_S) (Jost 2008; Heller & Siegismund 2009). Such a downward bias in neutral population differentiation can potentially overestimate the difference between Q_{ST} and F_{ST} and thus result in falsely invoking adaptive processes. Another method for measuring population differentiation, D_{est}, takes into account the effective number of alleles while reducing dependence on H_S and may reflect a more accurate level of genetic differentiation when utilizing this class of molecular marker (Jost 2008). Nonetheless, Mantel tests showed comparable levels of isolation by distance for both F_{ST} and D_{est} (Fig. S2, Supporting information). In addition, partial Mantel tests revealed that the same traits showed significant correlation with distance after controlling for the effect of neutral genetic divergence by either F_{ST} (Table 2) or D_{est} (data not shown). Therefore, despite the potential underestimate of neutral population differentiation in F_{ST} for microsatellite markers, the conclusion that clinal variation in quantitative traits cannot be explained by isolation by distance alone appears reasonably robust.

While an F_{ST} vs. Q_{ST} comparison appears theoretically straightforward and provides a practical framework for identifying natural selection acting within populations, obtaining an accurate estimate of Q_{ST} is challenging. For the F_{ST} vs. Q_{ST} comparison to be completely valid, quantitative traits should have an additive genetic basis with minimal confounding effects of variation resulting from dominance effects, maternal environment and phenotypic plasticity (Lynch & Walsh 1998; Merila & Crnokrak 2001). In the current study, a common garden design was employed to minimize environmental variance, although maternal effects cannot be ruled out completely given that plants representing the eight populations analysed in this study were grown from wild-collected seeds. Several lines of evidence, however, indicate that F_{ST} vs. Q_{ST} comparisons are robust to the factors discussed earlier. For example, sensitivity analyses of different simulated heritability values demonstrated that effects of nonadditive genetic variation are negligible in estimates of Q_{ST} relative to F_{ST} (Saether *et al.* 2007; Lehtonen *et al.* 2009). Moreover, meta-analysis of 62 published empirical studies showed that the inference of spatially variable selection drawn from the pattern of $Q_{ST} > F_{ST}$ is robust to effects of maternal environment (Leinonen *et al.* 2008). Furthermore, another simulation study suggested that dominance generally deflates Q_{ST} values, and thus, the existence of dominance variance would have a net effect of biasing against obtaining a significant result (i.e., that $Q_{ST} \neq F_{ST}$) (Goudet & Buchi 2006).

Our Q_{ST} estimates are thus likely to be conservative if there are nonadditive genetic effects on the quantitative traits under investigation. This may be particularly true for growth rate and the number of capitula per biomass, given the observed mean and variance of these

traits in the F_2 population (Fig. 2d and Fig. S1C, Supporting information).

Spatially varying selection and targets of selection

Comparison of F_{ST} and Q_{ST} is a useful exploratory tool to identify candidate life history traits that are likely to be targets of spatially varying selection (Leinonen *et al.* 2008). If traits are correlated, however, it may be difficult to distinguish true target(s) of selection from correlated responses as both are expected to display significantly large Q_{ST} values compared to F_{ST}. Therefore, we regard traits with the most extreme differentiation between populations (i.e., the highest Q_{ST} values or strongest relationships between geographic distance and pairwise Q_{ST}) as primary candidate traits under direct selection.

Days to flowering displayed the steepest latitudinal variation with the largest global Q_{ST} (Fig. 2c, Table 2), indicating that this trait is likely to be under strong selection across the latitudinal transect. Days to flowering decreased sharply with increasing latitude (e.g., mean days to flowering is 142 days in Texas vs. 65 days in Manitoba). A similar level of strong population differentiation was reported in the timing of bud set in Scots pine *Pinus sylvestris* along a latitudinal gradient in Finland (Q_{ST} = 0.82, Hurme 1999). Population differentiation in reproductive timing represents a classic example of clinal variation in plants and is considered an adaptive response to different photoperiod and temperature conditions that maximize reproductive outputs while avoiding unfavourable environmental conditions, such as frost and heat (Engelmann & Purugganan 2006; Zhang *et al.* 2008). Genes responsible for regulating flowering time are well characterized in the model plant species, *Arabidopsis thaliana* (Simpson & Dean 2002; Caicedo *et al.* 2004; Stinchcombe *et al.* 2004), and are beginning to be described in sunflower (Blackman *et al.* 2010), thus providing a useful list of candidates for further study in the *H. maximiliani* system.

Growth rate also showed striking clinal variation and interpopulational differentiation (Fig. 2d), with populations from higher latitudes exhibiting accelerated rates of growth (but smaller overall size at flowering) as compared with populations from lower latitudes. These patterns are again consistent with differences in the length of the growing season along the latitudinal transect. Genotypes with rapid growth coupled with early flowering are likely to be favoured at high latitudes that experience shorter growing seasons, whereas genotypes that grow more slowly but achieve larger sizes at flowering may be favoured at lower latitudes where the growing season is much longer. These patterns likely represent the classic life history trade-off between growth and timing of reproduction (Obeso 2002).

Patterns of variation in capitulum size and the number of capitula as a function of biomass may represent differential reproductive strategies to achieve fitness optimum in a given local environment. The significant positive relationship between latitude and number of capitula/biomass suggests that plants in northern populations allocate more resources to capitula production per biomass than plants in southern populations (Fig. S1C, Supporting information). In contrast, the strong negative relationship between latitude and traits related to capitulum size (Fig. 2b and Fig. S1E–G, Supporting information) indicate that while fewer capitula per unit DB are produced by plants from lower latitudes, these capitula are larger and likely harbour more and/or larger seeds. Evaluation of net seed production and seed size/viability will provide more definitive evidence concerning differential reproductive strategies and fitness outcomes between southern and northern populations as related to seed size/number variation.

In conclusion, we have shown strong population-level divergence in *H. maximiliani* for several quantitative life history traits across a latitudinal gradient. Divergence in these traits is significantly higher than neutral divergence as determined by seven microsatellite loci, providing preliminary evidence for local adaptation. While these patterns of life history variation are consistent with predictions based on abiotic variables (temperature, light, length of the growing season) along the gradient, reciprocal transplant experiments will be necessary to confirm that these patterns of variation are truly adaptive. Transcriptome sequencing for *H. maximiliani* currently is underway in our laboratory and will offer a valuable genomic resource for further investigation into the clinal variation described herein.

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T.K.'s research focuses on on speciation, hybridisation and local adaptation. T.J.M.'s research focuses on the evolution of complex ecologically important traits in animals and plants. J.B.N.'s research focuses on physiological responses of plants to environmental variation and resource availability. T.W.O. is a senior technician with expertise in plant physiological analyses. R.K. is interested in population genetics of animals and plants. P.D. is a technician with expertise in molecular genetic analyses. M.C.U.'s research focuses on hybridisation, genome evolution, and genetics of adaptation.

Data accessibility

Microsatellite genotypes and values for 15 quantitative traits are available in the Supporting information (see Tables S2 and S3).

Supporting information

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

Fig. S1 Latitudinal clines for 11 quantitative traits.

Fig. S2 Correlations between genetic differentiation (F_{ST} or D_{est}) and geographic distance for all pairwise combinations of populations.

Fig. S3 Correlations between F_{ST} or Q_{ST} values and geographic distances for all pairwise combinations of populations.

Table S1 Per locus and global summary statistics of seven microsatellite loci used in the population genetic analyses of *Helianthus maximiliani*.

Table S2 Microsatellite genotypes at seven loci for eight populations of *Helianthus maximiliani*.

Table S3 Values for 15 quantitative traits for eight populations of *Helianthus maximiliani*.

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