

Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient

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Received: 13 May 2013

Accepted: 21 October 2013

New Phytologist (2014) **201**: 1316–1327

doi: 10.1111/nph.12628

Key words: adaptation, drought, *Helianthus*, selection, soil fertility, sunflower, water limitation.

Summary

- Plant resource-use traits are generally hypothesized to be adaptively differentiated for populations distributed along resource gradients. Although nutrient limitations are expected to select for resource-conservative strategies, water limitations may select for either resource-conservative or -acquisitive strategies. We test whether population differentiation reflects local adaptation for traits associated with resource-use strategies in a desert annual (*Helianthus anomalous*) distributed along a gradient of positively covarying water and nutrient availability.
- We compared quantitative trait variation (Q_{ST}) with neutral genetic differentiation (F_{ST}), in a common garden glasshouse study, for leaf economics spectrum (LES) and related traits: photosynthesis (A_{mass} , A_{area}), leaf nitrogen (N_{mass} , N_{area}), leaf lifetime (LL), leaf mass per area (LMA), leaf water content (LWC), water-use efficiency (WUE, estimated as $\delta^{13}C$) and days to first flower (DFF).
- $Q_{ST} - F_{ST}$ differences support adaptive differentiation for A_{mass} , N_{mass} , N_{area} , LWC and DFF. The trait combinations associated with drier and lower fertility sites represent correlated trait evolution consistent with the more resource-acquisitive end of the LES. There was no evidence for adaptive differentiation for A_{area} , LMA and WUE.
- These results demonstrate that hot dry environments can selectively favor correlated evolution of traits contributing to a resource-acquisitive and earlier reproduction 'escape' strategy, despite lower fertility.

Introduction

Plant traits related to resource use are expected to be adaptively differentiated in habitats differing in resource availability. Low nutrient availability is generally hypothesized to select for resource-conservative strategies associated with slower growth rate (Chapin, 1980; Grime, 1988; Chapin *et al.*, 1993; Arendt, 1997; Wright & Westoby, 2002; Wright *et al.*, 2002; Reich *et al.*, 2003; but see Stanton *et al.*, 2000). Water limitation may also select for resource-conservative strategies and slow growth rate for many life forms, particularly woody perennials and evergreen life forms that tolerate low water potentials (Ludlow, 1989; Dudley, 1996; Etterson, 2004; Wright *et al.*, 2005; Knight *et al.*, 2006). However, annual plants and perennials that go dormant may escape water limitation with a resource-acquisitive strategy and/or accelerated phenology that permits completion of reproduction before water limitation occurs (Ludlow, 1989; Arendt, 1997; Geber & Dawson, 1997; Ackerly *et al.*, 2000; Stanton *et al.*, 2000; McKay *et al.*, 2003; Heschel & Reginos, 2005; Franks *et al.*, 2007; Franks & Weis, 2008; Kigel *et al.*, 2011; Brachi *et al.*, 2012; Ivey & Carr, 2012). We test whether population differentiation in a desert annual (*Helianthus anomalous*) reflects local adaptation for traits associated more with a resource-acquisitive or resource-conservative

strategy in response to a gradient of decreasing water and nutrient availability.

The leaf economics spectrum (LES) is a well-documented pattern of leaf trait correlations that is generally explained in resource economic terms of quantifying leaf income as carbon fixation via photosynthesis and leaf expenditures as metabolic and construction costs (Orians & Solbrig, 1977; Bloom *et al.*, 1985; Reich *et al.*, 1997; Wright *et al.*, 2004). Trait combinations on one end of the spectrum are thought to represent fast-growing species that have the potential for quick returns on investment, with leaves that have resource-acquisitive trait combinations: high photosynthetic rate (A_{mass} , A_{area}) to support faster growth, high nitrogen content (N_{mass} , N_{area}) to support high photosynthesis, low leaf mass per unit area (LMA) that permits high metabolic rates, and short leaf lifetime (LL) as a result of low LMA (Reich *et al.*, 1997; Wright *et al.*, 2004). The trait combinations at the other end of the spectrum are thought to represent slow-growing species that yield slower returns on investment, with leaves that have more conservative resource traits: low A_{mass} , A_{area} , N_{mass} , N_{area} , high LMA and long LL (Reich *et al.*, 1997; Wright *et al.*, 2004). While there is some discussion about whether mass- or area-based traits are more important, both are biologically relevant and should be included when possible (Lloyd *et al.*, 2013; Osnas *et al.*, 2013; Westoby *et al.*, 2013). Additional traits such

as higher leaf water content (LWC), lower water-use efficiency (WUE), and fewer days to first flower (DFF) are often discussed as traits associated with the resource-acquisitive end of the LES traits spectrum (Cohen, 1970; Geber & Dawson, 1997; Stanton *et al.*, 2000; McKay *et al.*, 2003; Knight *et al.*, 2006; Shipley *et al.*, 2006).

Several different approaches can be used to support the interpretation of different combinations of LES and associated traits as adaptive plant growth strategies (Reich *et al.*, 1997, 1999; Wright *et al.*, 2004; Westoby & Wright, 2006). At the macroevolutionary level, phylogenetically explicit contrasts of species that are largely perennials have demonstrated correlated evolution of a few LES and related trait combinations, providing strong inference for selection on at least some traits and the association of more conservative traits with lower fertility and rainfall sites (Cunningham *et al.*, 1999; Wright & Westoby, 1999; Mediavilla *et al.*, 2008). However, these studies do not identify the relative strength of adaptive differentiation for resource-use traits across habitats, or whether resource limitations select for more resource-conservative or -acquisitive strategies in annuals. At the microevolutionary level, a few within-population studies of predominately annuals have demonstrated phenotypic selection on individual LES and related traits (Stanton *et al.*, 2000; Etterson, 2004; Scheepens *et al.*, 2010; Donovan *et al.*, 2011 and references therein; Galloway & Burgess, 2012; Ivey & Carr, 2012). However, these studies do not identify any consistent direction of phenotypic selection in different resource treatments. Additionally, because response to selection is dependent on heritable variation in the traits targeted by selection (Lande & Arnold, 1983), measurements of phenotypic selection cannot be used to infer adaptive trait evolution. Another microevolutionary approach, performing reciprocal transplants, is the gold standard for demonstrating local adaptation of populations (Clausen *et al.*, 1940; Conner & Hartl, 2004), but trait differentiation in each transplant garden provides only indirect evidence for which traits are most important for local adaptation unless phenotypic selection analyses are additionally included. Common garden studies, including reciprocal transplants, can be used to demonstrate correlations between plant traits and population source site characteristics (e.g. altitude, latitude, climate or resource availability) that infer a response of individual traits to hypothesized selective agents (Billings, 1985; Arntz & Delph, 2001; Christman *et al.*, 2008; Kawakami *et al.*, 2011; Scheepens & Stocklin, 2011; but see Hubner *et al.*, 2013). Correlational analyses (between common garden plant traits and source site characteristics) can be complemented with Q_{ST} - F_{ST} analyses to provide a powerful approach for investigating adaptive evolution of traits in response to gradients (Kawakami *et al.*, 2011; Keller *et al.*, 2011; Dutkowski & Potts, 2012; Frei *et al.*, 2012; Hubner *et al.*, 2013).

Q_{ST} - F_{ST} analyses test for patterns of population differentiation that are indicative of past selection and thus provide evidence of local adaptation. The extent of population differentiation in neutral genetic variation (F_{ST}) is calculated as the proportion of total genetic variation that is partitioned among populations (Wright, 1951). F_{ST} measured on genetic markers that are selectively neutral provides an estimate of the

partitioning of genetic variation that is the result of neutral processes such as drift, mutation and migration. This provides a baseline measurement of population differentiation that can be compared to an analogous parameter, Q_{ST} , calculated for phenotypic traits (Spitze, 1993; Yang *et al.*, 1996). If Q_{ST} for a trait is not significantly different from F_{ST} , then trait differentiation among populations is not distinguishable from neutral processes and there is no evidence that diversifying selection is causing divergence among populations. If Q_{ST} is greater than F_{ST} , then the trait has diversified more than would be expected by neutral processes alone, providing evidence that populations have responded to diversifying selection and are probably locally adapted. If Q_{ST} is less than F_{ST} , the trait is more similar across populations than expected, suggesting stabilizing selection across the range. Although some aspects of the methodology are currently being debated (Hedrick, 2005; Jost, 2008; Pujol *et al.*, 2008; Edelaar & Bjorklund, 2011; Whitlock, 2011), this is still a powerful method for identifying the signature of past selection on phenotypic traits, allowing tests of hypotheses about the selective pressures driving adaptation. Several Q_{ST} - F_{ST} studies that included a subset of LES and related traits found evidence for selection on some traits but not others (Steane *et al.*, 2006; Ramirez-Valiente *et al.*, 2009; Scheepens *et al.*, 2010; Kawakami *et al.*, 2011; Keller *et al.*, 2011; Frei *et al.*, 2012; Hubner *et al.*, 2013). However, to our knowledge none have focused on the hypothesized response to selection for the suite of LES and related traits across a resource gradient where local adaptation to resource limitations is expected. If different combinations of LES and related traits represent locally adaptive plant growth strategies along a resource gradient, then Q_{ST} should be greater than F_{ST} for multiple LES and related traits, and the correlated trait evolution should favor trait combinations along the multi-dimensional resource-acquisitive to resource-conservative axis of LES.

Helianthus anomalus Blake, the study species, is an annual sunflower endemic to nutrient-poor desert sand dunes in the southwestern United States. It is a stable homoploid hybrid species derived from *Helianthus annuus* L. and *Helianthus petiolaris* Nutt. (Rieseberg, 1991; Schwarzbach & Reiseberg, 2002; Rieseberg *et al.*, 2003). Reciprocal transplant experiments have demonstrated that *H. anomalus* is adapted to its actively moving sand dune habitat when compared to its ancestral parents grown in that habitat (Donovan *et al.*, 2010). Because *H. anomalus* grows on active sand dunes, it was originally hypothesized that water limitation was an important selective force driving the evolution of the species (Thompson *et al.*, 1981; Rieseberg, 1991). However, nutrient limitations seem to be as important as or more important than water limitations for *H. anomalus* productivity in some populations, potentially acting as an additional selective agent (Rosenthal *et al.*, 2005; Ludwig *et al.*, 2006). *H. anomalus* also appears to be more tolerant of nutrient stress than its ancestral parents based on a lower relative growth rate and higher nutrient-use efficiency, although experiments at the microevolutionary level have demonstrated phenotypic selection for increased N_{mass} in *H. anomalus* habitats (Brouillette *et al.*, 2006; Donovan *et al.*, 2007, 2009; Brouillette & Donovan, 2011).

Thus, *H. anomalus* provides a good system for testing adaptive differentiation of resource-related traits.

In this study, we investigate the expectation of adaptive differentiation of *H. anomalus* along a resource gradient for LES (A_{mass} , A_{area} , N_{mass} , N_{area} , LMA and LL) and related traits (LWC, WUE and DFF) in a common garden study. Specifically, we test the following hypotheses: population differentiation for trait variation (Q_{ST}) will be greater than differentiation for neutral genetic variation (F_{ST}), providing evidence for diversifying selection; for traits demonstrating evidence of diversifying selection, correlated trait evolution will follow the axis of the LES towards a more resource-acquisitive or resource-conservative strategy; combinations of putatively adaptive traits will be correlated with source site climate and soil fertility characteristics, providing support for these abiotic factors as selective agents driving adaptive differentiation. These results will allow us to determine whether a gradient of positively covarying water and nutrient availability has selected for correlated trait evolution of a more resource-conservative or -acquisitive resource strategy in this desert annual. If a more resource-conservative strategy is associated with drier low fertility sites, then it will not be possible to distinguish between the relative importance of water and nutrient limitations as selective agents. However, if a more resource-acquisitive escape strategy is associated with drier low fertility sites, this would suggest that water limitation has been a more important selective agent than nutrient limitation along this resource gradient.

Materials and Methods

The glasshouse experiment was conducted at the University of Georgia Plant Biology Greenhouses in Athens, GA, USA. Achenes (hereafter 'seeds') for the study were collected as maternal half-sibling families from eight natural populations of *Helianthus anomalus* S.F. Blake in August 2007 (Fig. 1, Supporting Information, Table S1). For germination, seeds were scarified (blunt end removed) on 10 January 2008, soaked overnight in 0.005% solution of fusicoccin to break dormancy, and then germinated on filter paper moistened with deionized water. Seedlings were transplanted into azalea pots, 25 cm in diameter and 19 cm deep, filled with a 3:1 sand:Surface mixture (Profile Products, Buffalo Grove, IL, USA) in the glasshouse on 13–14 January 2008. The planned experimental design was a randomized complete block design with a target of 12 families from each of eight populations replicated once in each of three blocks ($N=288$). However, mortality and poor germination of seeds from White Sands (WHS) and Jericho (JER) populations resulted in an unbalanced design of eight to 14 families and 15–45 individuals per population (Table S1). Plants were watered daily using an automated drip irrigation system and fertilized three times each wk with half-strength Hoagland's solution (Epstein & Bloom, 2005) applied after the automatic irrigation, resulting in high-fertility common garden conditions that were probably higher in nutrient availability than native population sites. Supplemental metal halide lighting was used to extend day length to 14 h and photosynthetically active radiation (PAR) in the glasshouse averaged $16.5 \text{ mol m}^{-2} \text{ d}^{-1}$. The average daytime temperature was

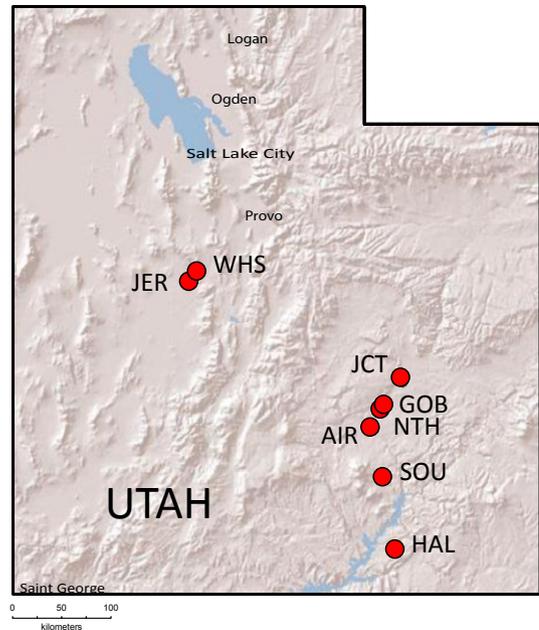


Fig. 1 Locations in the USA of eight populations of *Helianthus anomalus* that served as the seed sources for this study (see Table S1 for GPS locations): AIR, Hanksville airport; GOB, Goblin Valley; HAL, Hall's Crossing; JCT, Junction; JER, Jericho; NTH, North of Hanksville; SOU, South of Hanksville; and WHS, White Sands.

27.3°C and the average daytime humidity was 54.9%. The average night-time temperature was 22.9°C .

Plant traits

Photosynthesis on a leaf area basis (A_{area}) was measured on the most recently fully expanded leaf on 18–20 February, which were sunny days before flowering. One experimental block was measured each day using a portable photosynthesis system (Li-Cor 6400, Li-Cor Biosciences, Lincoln, NE, USA). Gas exchange cuvette conditions were set to 380 ppm CO_2 , 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR, 30°C block temperature, and relative humidity slightly above ambient in the glasshouse (*c.* 50–55%). The morning following photosynthesis measurements, when leaves were maximally hydrated for the day, the gas exchange leaf was excised, weighed, and digitally scanned. The leaf area that had been inside the Li-Cor 6400 chamber and the area of the entire leaf were determined with ImageJ freeware (National Institutes of Health, Bethesda, MD, USA). Gas exchange leaves were then dried to constant weight at 60°C , weighed, and ball mill ground to estimate leaf nitrogen (N_{mass} ; NA1500, Carlo Erba Strumentazione, Milan, Italy) and leaf carbon isotope ratio $\delta^{13}\text{C}$ (Finnegan, continuous-flow mass spectrometer, Bremen, Germany). Leaf $\delta^{13}\text{C}$ provides an integrated measure of leaf intercellular CO_2 concentration (C_i) over the lifetime of the leaf. Integrated C_i is, in turn, a relative measure of integrated instantaneous WUE, provided leaf temperatures are similar (Farquhar *et al.*, 1989; Ehleringer *et al.*, 1992; Ehleringer, 1993). A higher (less negative) value of leaf $\delta^{13}\text{C}$ reflects greater WUE. LMA was calculated as the dry mass of the gas exchange leaf divided by leaf area. Leaf photosynthetic rate on a mass basis (A_{mass}) was

calculated as A_{area} divided by LMA. Leaf nitrogen on an area basis (N_{area}) was calculated as N_{mass} multiplied by LMA. LWC was calculated as the difference between fresh and dry leaf mass divided by leaf dry mass (Shipley *et al.*, 2006). The most recently fully expanded leaf opposite the gas exchange leaf was tagged *c.* 1 month after transplant and tracked for LL, estimated as the time (d) between tagging and leaf color change to 25% of leaf area turned yellow (Brouillette & Donovan, 2011). Tagging of the leaf was done during the vegetative stage of plant growth when leaves were actively being produced so that leaves were of comparable age. The DFF was recorded when at least one ligule was fully extended, making the disk of the inflorescence visible. In addition, 38 other morphological and ecophysiological traits were measured, although they are not the focus of this manuscript. The additional trait list, methods and results are included in the supporting materials (Methods S1, Table S2, Fig. S1).

Source site characterization

Each population source site was characterized for climate and soil fertility. Site climate characteristics (mean annual temperature (MAT) and mean annual precipitation (MAP)) were determined using WorldClim, which interpolates a 50 yr average based on observations from 1950 to 2000 and has resolution to *c.* 1 km² (Hijmans *et al.*, 2005). Five soil cores (0–10 cm depth) collected at each site spanned the area in which seeds were collected. Soils were dried at 60°C and analyzed for soil C by Dumas combustion with a CHN analyzer (NA1500, Carlo Erba). For soil N, the CHN analyzer was subsequently modified to remove the CO₂ produced via combustion and allow detection of low soil N amounts below the normal detection limit. Soil P and K were analyzed with an inductively coupled plasma optical emission spectrometer (Thermo Jarrell-Ash Enviro 36, Franklin, MA, USA) after double acid extraction (Mehlich, 1953).

ANOVA, bivariate correlations and principal component analyses (PCAs)

Populations were compared for plant traits and soil fertility characteristics with ANOVA (SAS proc mixed; SAS Institute Inc., Cary, NC, USA). Across all populations, bivariate correlations among traits were determined with Pearson correlations (SAS proc corr). Although the trait data in the original global GLOPNET dataset describing the LES required log transformation in order to meet the assumptions of parametric statistics (Wright *et al.*, 2004), log transformation was not necessary for our data. Five different PCAs (SAS proc princomp) were used to provide population values that summarize: source site climate (MAT, MAP); source site fertility characteristics that differed by population; source site climate and fertility combined (see the Results section); plant traits for which $Q_{ST} > F_{ST}$; and all nine plant traits regardless of the relationship of Q_{ST} to F_{ST} . Only the first axis of each analysis was used as it captured the bulk of variation in each case. Correlations were then used to assess the relationships among the primary principal components summarizing source site characteristics and plant traits.

Simple sequence repeat (SSR) genotyping and analysis

Leaf samples from each plant were collected and placed in polypropylene tubes containing silica gel desiccant and stored at room temperature until use. DNA was extracted from one individual from each of the 97 maternal families using DNeasy Plant Mini kits (Qiagen). A 12-locus genotype was obtained for each sample using SSR markers located in intergenic regions of the nuclear genome from the Compositae Genome Database (CGP, <http://cgpdb.ucdavis.edu>). Markers were chosen to be largely unlinked in the parental species *H. annuus* (Tang *et al.*, 2003) and polymorphic in a test panel of *H. anomalus* samples: (ORS229, ORS297, ORS511, ORS588, ORS618, ORS844, ORS896-A, ORS896-B, ORS1008-A, ORS1008-B, ORS1017, ORS1141). Fluorescently labeled primers (FAM, NED, HEX, TET) were used to amplify SSRs with a touchdown 58 protocol (Don *et al.*, 1991). Products from the reaction were diluted 1 : 20 and analyzed using capillary gel electrophoresis (ABI 3730xl; Applied Biosystems, Valencia, CA, USA). The size of fragments was determined by comparison with a fluorescently labeled size standard (GS500 LIZ, Applied Biosystems) using Genescan software (Applied Biosystems). Observed and expected heterozygosities were calculated in FSTAT (Goudet, 1995).

Q_{ST} - F_{ST} comparison

Genetic variation in quantitative traits, when partitioned into between-population and within-population components, can be used to calculate population divergence (Q_{ST}) in a manner analogous to F_{ST} calculated from neutral genetic markers:

$$Q_{ST} = \frac{\sigma_b^2}{2\sigma_w^2 + \sigma_b^2} \quad \text{Eqn 1}$$

where σ_b^2 is the genetic variance among populations and σ_w^2 is the genetic variance within populations. When traits are purely additive and neutral, $Q_{ST} = F_{ST}$ (Spitze, 1993). Divergence from this expectation can be used to test for adaptive response to selection.

Variance for all traits was partitioned into population, family nested within population, and block and error components using a completely random model with restricted maximum likelihood estimation (SAS 9.3 proc mixed). The variance component for populations was used as the estimate of among-population variance (σ_b^2). The within-population genetic variance (σ_w^2) was estimated as four times the family within-population variance component because half-siblings were used (Lynch & Walsh, 1998). Owing to high mortality in some populations, our data were unbalanced and thus unsuitable for standard parametric bootstrap methods (which use a χ^2 distribution to simulate the distribution around variance components) for estimating confidence intervals around Q_{ST} (O'Hara & Merila, 2005; Whitlock, 2008; Whitlock & Guillaume, 2009). Additionally, estimation of confidence intervals using Bayesian methods, which do not require balanced data (O'Hara & Merila, 2005), were highly dependent on initial conditions and thus not suitable. Thus,

standard error for the mean Q_{ST} value of each trait was estimated as the standard deviation of Q_{ST} estimates from 1000 samples bootstrapped over all observations and used to construct confidence intervals using the standard formula (± 1.96 SE) (O'Hara & Merila, 2005; but see Leinonen *et al.*, 2008, p. 11).

F_{ST} was calculated using GDA (Genetic Data Analysis software; Weir & Cockerham, 1984; Lewis & Zaykin, 2001), with bootstrapped confidence intervals estimated over 1000 iterations. There remain concerns that markers with high mutation rates, including SSRs, which were used in this study, may increase the rate of type I error when testing for divergent selection. Within-population heterozygosities are increased with highly polymorphic markers, which deflates F_{ST} (Hedrick, 1999) and makes it easier to achieve a statistically significant $Q_{ST} > F_{ST}$. While alternative metrics that are not dependent on within-population heterozygosity have been proposed (e.g. D , Jost, 2008; and G'_{ST} , Hedrick, 2005), only F_{ST} is appropriate for comparisons with Q_{ST} (Edelaar & Bjorklund, 2011). Thus, we acknowledge that some of the traits identified in this study as being under divergent selection may be artifacts of the genetic markers used. However, several traits in this study had Q_{ST} values much greater than F_{ST} , and for these it is likely that $Q_{ST} > F_{ST}$ would remain statistically significant regardless of marker choice. We must also point out that our plants were grown from field-collected seeds. Our analysis may overestimate population divergence in individual traits because variation in the maternal environments could have exaggerated differences between the populations. Additionally, because paternity is unknown, it is likely that some of the families contain full and half siblings. If this is the case, our estimate of σ_w^2 will be inflated, artificially decreasing Q_{ST} . The inclusion of some full siblings would affect Q_{ST} similarly across all traits, but differences caused by maternal effects would vary from trait to trait.

Results

Characterization of source site environment

Across all eight of the population sites, the MAP ranged from 161 to 292 mm, and the MAT ranged from 9.6 to 12.5°C. PCA analysis of MAP and MAT ($n = 8$ populations) resulted in a composite variable designated as climate PC1 that captured 87.6% of the variation (loadings of 0.71 and -0.71 , respectively). Among the population source sites, the more northern populations, for example (JER, WHS), had higher annual precipitation and lower temperatures (Table S1). The populations differ for precipitation predominately during the late fall and winter months when plants are overwintering as dormant seeds (Fig. S2).

Across all eight of the population sites, the desert sand dune soils had low soil fertility. Soil C and N were extremely low ($0.591 \pm 0.200\%$ and $0.003 \pm 0.001\%$ by mass, respectively). Soil P was 12.3 ± 1.2 ppm and soil K was 16.4 ± 1.6 ppm. There were significant population differences for soil C, P and K (see Table S1 for population means; ANOVA, $F > 8.47$, $P < 0.001$, df 7,32 for all) but not for soil N ($F = 1.57$, $P = 0.18$, df 7,29). PCA analysis ($n = 8$ populations) for the three soil

fertility measures that differed by population (C, P and K) resulted in a composite variable designated as 'soil PC1' that captured 72.2% of the variation. Higher values of soil PC1 generally represented higher C, P and K, with loadings of 0.65, 0.47 and 0.59, respectively. The more northern populations generally had higher fertility.

When 'climate PC1' was plotted against soil PC1, there was a positive correlation ($r^2 = 0.82$, $P = 0.002$). Thus, the climate and soil data (MAP, MAT, soil C, soil P and soil K) were combined in an additional PCA analysis that resulted in a composite variable that captured 74.7% of the variation (loadings of 0.47, -0.47 , 0.50, 0.34 and 0.43, respectively), designated as 'environment PC1' (Fig. 2, x -axis).

Genetic variation in SSR markers

F_{ST} was 0.18, demonstrating moderate population genetic differentiation for an outcrossing herbaceous annual. Total expected heterozygosity (H_e) across all individuals was 0.621. Populations were moderately diverse, with population-level H_e ranging from 0.300 (JER) to 0.655 (HAL) (Table S3).

Comparison of neutral genetic and quantitative trait divergence

Comparisons of neutral genetic variation (F_{ST}) to quantitative trait variation (Q_{ST}) indicated that Q_{ST} was greater than F_{ST} for A_{mass} , N_{mass} , N_{area} , LWC and DFF, consistent with population differentiation for these traits being the result, in part, of a strong response to direct or indirect divergent selection (Fig. 3). For

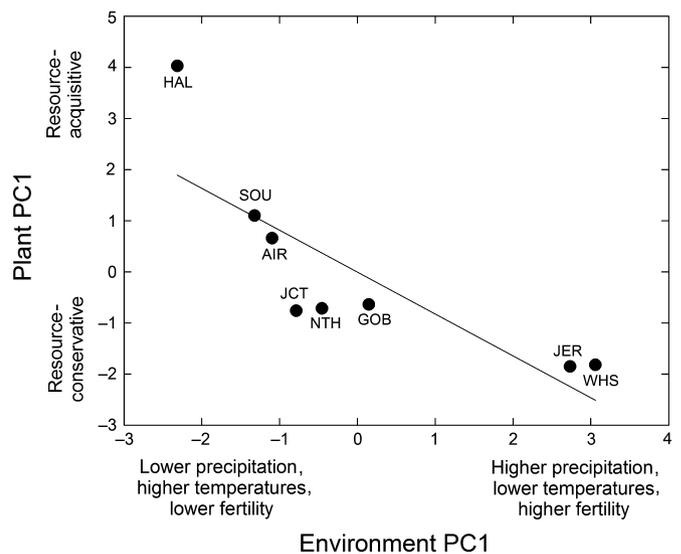


Fig. 2 Relationship between *Helianthus anomalous* population source site climate and fertility characteristics (environment PC1, first axis from climate and soil fertility principal components analysis (PCA)) and population plant traits from the common garden glasshouse study (plant PC1, first axis from plant PCA for traits with $Q_{ST} > F_{ST}$): AIR, Hanksville airport; GOB, Goblin Valley; HAL, Hall's Crossing; JCT, Junction; JER, Jericho; NTH, North of Hanksville; SOU, South of Hanksville; and WHS, White Sands.

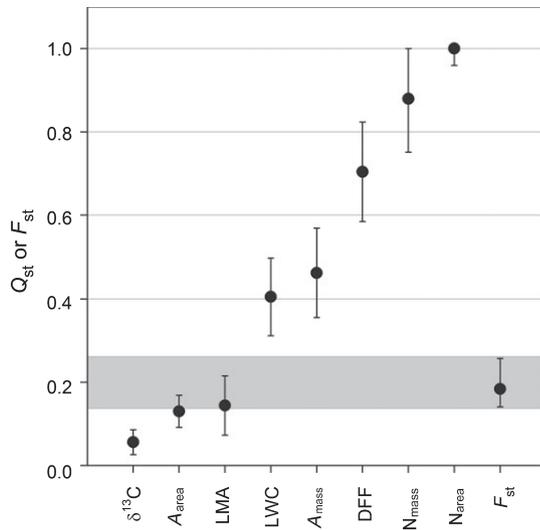


Fig. 3 Q_{ST} and F_{ST} (point estimates and bootstrapped 95% confidence intervals) for *Helianthus anomalus* carbon isotope ratio ($\delta^{13}\text{C}$), leaf photosynthesis on an area basis (A_{area}), leaf mass per area (LMA), leaf water content (LWC), leaf photosynthesis on a mass basis (A_{mass}), days to first flower (DFF), and leaf nitrogen on a mass and an area basis (N_{mass} and N_{area} , respectively). Values represent the proportion of total heritable variance that is partitioned among populations.

A_{area} and LMA, Q_{ST} was not significantly different from F_{ST} , providing no evidence for adaptive differentiation in response to selection. For leaf WUE estimated from $\delta^{13}\text{C}$, Q_{ST} was less than F_{ST} , suggesting stabilizing selection. For LL, the Q_{ST} could not be estimated because there were essentially no significant differences among families within populations or among populations. For the eight morphological, physiological and life-history traits in this study with estimable Q_{ST} values, the mean was 0.473, with Q_{ST} often greater than F_{ST} .

Quantitative trait patterns

For *H. anomalus* the glasshouse-grown plant traits occur at the resource-acquisitive end of the cross-species LES spectrum initially described for field-grown plants (Wright *et al.*, 2004), with high A_{mass} , N_{mass} and LMA (Fig. 4a). The species-level trait means (\pm SE) for *H. anomalus* were as follows: A_{mass} , $897.0 \pm 16.24 \text{ nmol g}^{-1} \text{ s}^{-1}$; A_{area} , $35.60 \pm 0.44 \mu\text{mol m}^{-2} \text{ s}^{-1}$; N_{mass} , $5.95 \pm 0.11\%$; N_{area} , $169.3 \pm 1.8 \text{ mmol m}^{-2}$; LMA, $40.01 \pm 0.43 \text{ g m}^{-2}$; LL, 1.09 ± 0.02 months; LWC, $11.30 \pm 0.22 \text{ g g}^{-1}$; $\delta^{13}\text{C}$, $-31.62 \pm 0.06 \text{ ‰}$; and DFF, $55.5 \pm 1.5 \text{ d}$.

There were significant population differences for A_{mass} , A_{area} , N_{mass} , LMA, LWC, $\delta^{13}\text{C}$ and DFF (ANOVA $P < 0.001$, df 7,181–182 for all), but only a trend for N_{area} ($P = 0.07$, df 7,182) and no difference for LL ($P = 0.62$, df 7,182). Across populations, correlated trait evolution generally followed the main axis of the LES for A_{mass} , N_{mass} and LMA (Fig. 4b, Table 1; population means are presented in Table S1). A_{mass} was positively correlated with A_{area} , N_{mass} and N_{area} , as expected, and tended to be negatively correlated with LMA ($P = 0.06$). N_{mass} was additionally positively correlated with N_{area} , and negatively correlated

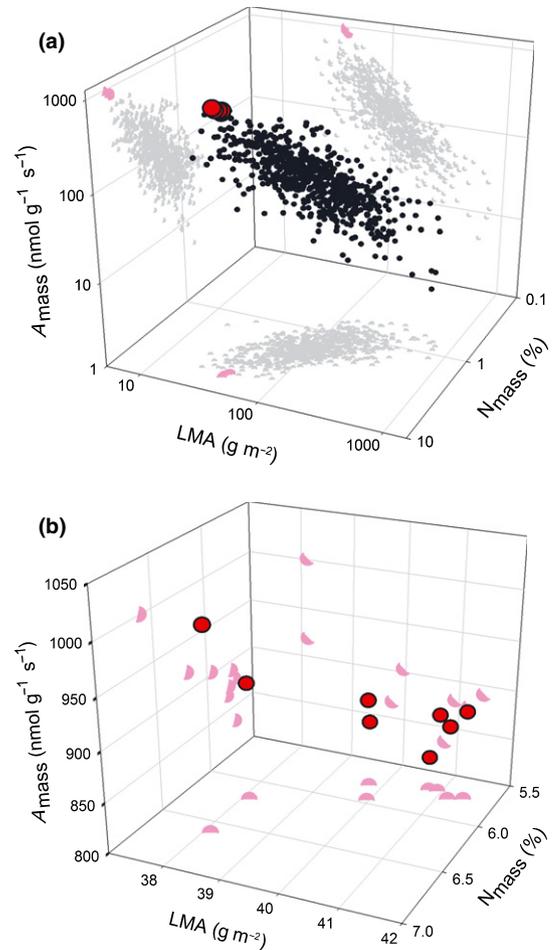


Fig. 4 *Helianthus anomalus* population means of traits for glasshouse-grown plants (in red) plotted at two different scales: (a) in relation to GLOPNET data (Wright *et al.*, 2004, in black and gray): leaf photosynthetic rate (A_{mass}), leaf nitrogen, (N_{mass}), and leaf mass per area (LMA); and (b) at a finer resolution which clarifies relationships among populations (see Table 1 for correlation coefficients and significance).

with LMA. Contrary to expectation, LL and LWC were not correlated with any other LES traits. Also contrary to expectation, less negative leaf $\delta^{13}\text{C}$ (i.e. higher WUE) was associated with higher A_{mass} , A_{area} , N_{mass} , N_{area} and LWC. Later flowering (higher DFF) was associated with lower N_{mass} and higher LMA but was not correlated with A_{mass} , A_{area} , N_{area} or WUE estimated from leaf $\delta^{13}\text{C}$.

Principal component analysis ($n = 8$ populations) for traits that have evidence of population differentiation in response to selection (i.e. $Q_{\text{ST}} > F_{\text{ST}}$ for A_{mass} , N_{mass} , N_{area} , LWC and DFF) resulted in a composite variable designated as ‘plant PC1’ that captures 74.6% of the variation among traits. Higher values represented higher A_{mass} , N_{mass} , N_{area} and LWC, and lower DFF, with loadings of 0.51, 0.49, 0.48, 0.36 and -0.37 , respectively (Fig. 2, y -axis). PCA ($n = 8$ populations) for all nine plant traits (instead of just those for which $Q_{\text{ST}} > F_{\text{ST}}$) resulted in a composite variable that captures 61.1% of the variation among traits. Higher values represented higher A_{mass} , N_{mass} , N_{area} and LWC, lower DFF, higher A_{area} and leaf $\delta^{13}\text{C}$, and lower LMA and LL,

Table 1 Trait correlation coefficients (r , bold indicates $P < 0.05$, df 6) among population means for *Helianthus anomalus* grown under common garden glasshouse conditions

	A_{area}	N_{mass}	N_{area}	LMA	LL	LWC	$\delta^{13}\text{C}$	DFF
A_{mass}	0.7280	0.9372	0.9258	-0.6911	-0.1924	0.67508	0.9161	-0.6087
A_{area}	-	0.4640	0.8500	-0.0183	-0.2534	0.69974	0.7364	-0.1397
N_{mass}		-	0.8225	-0.8628	-0.0587	0.53492	0.8003	-0.7619
N_{area}			-	-0.4277	-0.1637	0.64541	0.8210	-0.5325
LMA				-	0.0032	-0.31113	-0.5863	0.7080
LL					-	0.13682	-0.3666	-0.3912
LWC						-	0.7343	-0.2185
$\delta^{13}\text{C}$							-	-0.2685

A_{mass} and A_{area} , leaf photosynthetic rate on a mass and an area basis, respectively; N_{mass} and N_{area} , leaf nitrogen on a mass and an area basis, respectively; LMA, leaf mass per area; LL, leaf lifetime; LWC, leaf water content; $\delta^{13}\text{C}$, leaf carbon isotope ratio; DFF, days to first flower.

with loadings of 0.42, 0.40, 0.40, 0.31, -0.26, 0.31, 0.39, -0.29 and -0.05, respectively.

Relationship between plant traits and source site environment

There was a significant correlation between plant PC1 and the source site characteristics assessed as either climate PC1 ($n = 8$, $r = -0.746$, $P = 0.03$), soil PC1 ($n = 8$, $r = -0.842$, $P = 0.01$), or the combined climate and soil variable, environment PC1 ($n = 8$, $r = -0.817$, $P = 0.01$, Fig. 2). The more resource-acquisitive LES and related traits were associated with drier (lower precipitation, higher temperatures) and lower fertility sites. If all nine plant traits are included in the plant PCA then the r and P values remain similar ($r = -0.778$, $P = 0.02$; $r = -0.847$, $P = 0.01$; $r = -0.834$, $P = 0.01$, respectively).

Discussion

We compared neutral and genetic variation in the annual *H. anomalus* to test for adaptive divergence in LES and related traits along a water and nutrient gradient. The F_{ST} estimate of 0.18, which was the neutral measure for comparison with Q_{ST} , suggested a moderate degree of genetic differentiation among populations similar to other wild *Helianthus* spp. (Ellis *et al.*, 2006; Ellis & Burke, 2007; Gevaert *et al.*, 2013; Mandel *et al.*, 2013). For individual traits, we found evidence for population differentiation consistent with response to diversifying selection (i.e. $Q_{\text{ST}} > F_{\text{ST}}$) for N_{mass} , N_{area} , DFF, A_{mass} and LWC. Our support for adaptive differentiation of leaf N_{mass} and N_{area} is consistent with that found for *Quercus suber* along a climate gradient (N_{mass} , Ramirez-Valiente *et al.*, 2009), and among populations and subpopulations for *Populus balsamifera* (N_{area} ; Keller *et al.*, 2011), but not for *Helianthus maximiliani* along a latitudinal gradient (N_{mass} ; Kawakami *et al.*, 2011). Our support for adaptive differentiation of DFF is consistent with reports for some species (*H. maximiliani*, Kawakami *et al.*, 2011; *Arabidopsis thaliana* using bolting as a proxy, Le Corre, 2005), but not for others (*Lythrum salicaria*, Chun *et al.*, 2009; *Hordeum spontaneum*, Hubner *et al.*, 2013). To our knowledge, A_{mass} and LWC have not been previously investigated in $Q_{\text{ST}}-F_{\text{ST}}$ analyses. For LMA,

A_{area} and leaf $\delta^{13}\text{C}$, our lack of support for adaptive differentiation is also consistent with some but not all reports for other species (Ramirez-Valiente *et al.*, 2009; Kawakami *et al.*, 2011; Keller *et al.*, 2011; Frei *et al.*, 2012). Thus, for each trait examined, there is substantial variation among studies as to whether $Q_{\text{ST}}-F_{\text{ST}}$ analyses support adaptive differentiation. This suggests either that individual traits are not as important as hypothesized for adaptation to the variety of gradients and landscape heterogeneity examined, or that genetic constraints may prevent them from responding to selection for some species and populations (Donovan *et al.*, 2011). However, multiple traits need to be considered to assess whether adaptive trait divergence contributes more to a resource-acquisitive or a resource-conservative strategy in response to greater resource limitation.

For trait combinations, *H. anomalus* populations with high A_{mass} , N_{mass} and low LMA clustered with other annuals and short-lived herbaceous perennials at the resource-acquisitive end of the cross-species LES based on field data (Wright *et al.*, 2004; Fig. 4a). The *H. anomalus* trait population means covered a relatively small portion of the entire LES range, as expected for closely related populations in a common garden glasshouse study that minimized environmentally induced variation. Despite that small range, correlated trait evolution represented by population differentiation for A_{mass} , N_{mass} and LMA was consistent with the primary axis of the LES (Fig. 4b, Table 1). Higher N_{mass} was also associated with the earlier flowering time (lower DFF), indicating a resource-acquisitive earlier flowering strategy. The lack of correlation of LWC with other traits (only nonsignificant positive trends with A_{mass} , A_{area} and N_{area}) does not support the hypothesis that LWC is an important biophysical driver of LES traits in this system (Shipley *et al.*, 2006).

The correlation between *H. anomalus* traits with a signature of adaptive divergence (plant PC1 for traits with $Q_{\text{ST}} > F_{\text{ST}}$) and source site characteristics (environment PC1) supports our hypothesis that climate and/or soil fertility are selective agents acting on plant traits and driving population differentiation (Fig. 2). The more southerly sites have plants with inherently higher N_{mass} , N_{area} , DFF and A_{mass} . These sites have lower fertility and a hotter drier climate, with less soil moisture to sustain the growing season as a result of less winter precipitation. Thus, for *H. anomalus*, the hotter, drier environment of the more

southerly sites selectively favored correlated evolution of traits contributing to a resource-acquisitive and earlier reproductive strategy to escape soil moisture depletion predictably occurring late in the growing season, despite lower fertility. This is consistent with the growing literature documenting earlier onset of reproduction as an evolutionary response of annual species to many stresses, including drought, although earlier onset of reproduction is not always accompanied by the greater pre-reproductive growth rates expected for a 'live fast, die young' strategy (Arendt, 1997; Geber & Dawson, 1997; Stanton *et al.*, 2000; McKay *et al.*, 2003; Griffith & Watson, 2005; Heschel & Reginos, 2005; Franks *et al.*, 2007; Franks & Weis, 2008; Kigel *et al.*, 2011; Ivey & Carr, 2012).

Although multitrait LES studies on annuals are generally lacking, we can compare the adaptive differentiation of multiple traits for this desert annual to Q_{ST} – F_{ST} studies for several woody species. For the evergreen *Quercus suber* (Ramirez-Valiente *et al.*, 2009), populations from cooler, drier sites had leaves with lower N and higher LMA, suggesting that water limitation selected for a more resource-conservative strategy. For the deciduous *Populus balsamifera*, populations from more northerly sites with a shorter, drier growing season had leaves with higher A_{area} and N_{area} , but also a higher LMA, suggesting that water limitation selected for a more resource-acquisitive strategy except for LMA (Soolanayakanahally *et al.*, 2009; Keller *et al.*, 2011). Thus, there is growing evidence at the microevolutionary level of adaptive trait differentiation of multiple traits consistent with the primary axis of the LES in response to diversifying selection, with the direction of the response (more resource-acquisitive or resource-conservative) probably influenced by life history and associated differences in LL, for example, annuals and deciduous perennials vs evergreen perennials. This is consistent with observed macroevolutionary patterns (Wright *et al.*, 2005). However, more studies that provide evidence of adaptive differentiation along environmental gradients will be needed before we can say that microevolutionary studies support this generalization. Additionally, other approaches will be needed to distinguish whether correlated trait evolution is a result of direct selection on individual traits, correlational selection, and/or indirect selection mediated through pleiotropy or linkage that may be reflected as genetic correlations (Chen & Lubberstedt, 2010; Donovan *et al.*, 2011).

In *H. anomalus*, the difference between Q_{ST} and F_{ST} was greatest for N_{mass} , N_{area} and DFF, indicating that these traits have the strongest support for adaptive differentiation. These results suggest several interpretations. First, direct selection may be stronger on leaf N_{mass} , N_{area} and DFF than on A_{mass} , LWC and LMA, but that would require the assumption that there was similar heritable variation for all of the traits and that there were no constraints as a result of indirect selection mediated through genetic correlations. Alternatively, the stronger support for adaptive differentiation for these traits might be the result of equally strong or diversifying selection on other traits that did not result in similar population differentiation because of genetic constraints in the form of limited heritable variation or genetic correlations. Quantitative genetic approaches and phenotypic selection analyses would be needed to test these alternative

hypotheses. Phenotypic selection analyses carried out in the *H. anomalus* JER population have demonstrated that leaf N_{mass} was under direct selection within the context of leaf traits measured in that study (N_{mass} , $\delta^{13}C$, area, succulence) (Donovan *et al.*, 2009). However, that analysis did not include other LES traits, so there was no power to determine whether N_{mass} was under direct or indirect selection within the context of other LES traits.

The results for WUE estimated from leaf $\delta^{13}C$ were surprising for *H. anomalus*. First, Q_{ST} was less than F_{ST} , suggesting stabilizing or uniform selection, although this pattern ($Q_{ST} < F_{ST}$) is not frequently reported in the literature and is difficult to interpret (Scheepens *et al.*, 2010; Frei *et al.*, 2012; Lamy *et al.*, 2012). The analysis of intrinsic WUE, estimated as C_i (approx. A_{area} /stomatal conductance), yielded the same result (Fig. S1). Secondly, higher leaf-level WUE (less negative $\delta^{13}C$) was associated with higher A_{mass} , A_{area} , N_{mass} and N_{area} , which is contrary to the expectation of an association of lower WUE with faster growth. Thirdly, leaf $\delta^{13}C$ was not correlated with DFF. Thus, the resource-acquisitive and earlier-flowering strategy associated with drier lower fertility sites did not have lower WUE, as might be expected from theory and empirical studies (Cohen, 1970; Geber & Dawson, 1997; McKay *et al.*, 2003). The trait patterns suggest that despite the lower fertility at the drier sites, these populations achieve a higher leaf N (on mass and area bases) which permits a higher photosynthetic capacity and, thus, both higher photosynthesis and leaf-level WUE.

The population differences for LMA, coupled with the correlation of LMA with N_{mass} , seem to be at odds with the lack of evidence for adaptive differentiation for LMA. Mathematically, we can consider that F_{ST} and Q_{ST} represent the proportion of total variance attributable to population differences, such that the high within-population variance for LMA decreased Q_{ST} (Table 2). Taken alone, the Q_{ST} – F_{ST} results for LMA suggest that population differentiation in this trait is indistinguishable from divergence resulting from neutral, rather than adaptive evolutionary processes. However, the correlations observed between LMA and

Table 2 Components of phenotypic variance of traits within and among eight populations of *Helianthus anomalus* grown under common garden glasshouse conditions, with total phenotypic variance partitioned using restricted maximum likelihood into variance among populations (V_{pop}), within-population genetic variance (V_{fam} ; four times the variance among families within populations) and residual variance (V_{res})

Trait	V_{pop}	V_{fam}	V_{res}
A_{mass}	2174.14	316.40	9451.62
A_{area}	1.0014	0.8373	9.7197
N_{mass}	0.09618	0.00165	0.184
N_{area}	6.6649	0	416.08
LMA	1.6115	1.1977	14.1349
LL	0	0	57.5191
LWC	0.3779	0.0694	1.1828
$\delta^{13}C$	0.02167	0.0155	0.2724
DFF	18.8144	0.9899	21.3786

A_{mass} and A_{area} , leaf photosynthetic rate on a mass and an area basis, respectively; N_{mass} and N_{area} , leaf nitrogen on a mass and an area basis, respectively; LMA, leaf mass per area; LL, leaf lifetime; LWC, leaf water content; $\delta^{13}C$, leaf carbon isotope ratio; DFF, days to first flower.

two of the traits with support for adaptive differentiation (DFF and N_{mass}) suggest that LMA values among and within populations are not random, as would be predicted by genetic drift. An alternative explanation may be that weak direct or indirect selection drove population differentiation for LMA, but the response to selection was too weak to be detected in this study. Studies with low numbers (<20) of populations have generally had low power to detect selection (O'Hara & Merila, 2005; Goudet & Buchi, 2006; Whitlock, 2008). Robust estimates of heritabilities and the structure of genetic covariance matrices would further our understanding of the role of selection and constraints in the correlated response to selection for LES and related traits (Chapuis *et al.*, 2008; Chenoweth & Blows, 2008; Martin *et al.*, 2008).

We were unable to make an $F_{\text{ST}}-Q_{\text{ST}}$ comparison for LL in this study, because both within- and among-population variance components were estimated as zero. There may be several explanations for the lack of detectable variance for LL. First, LL is a difficult trait to measure and error may have obscured genetically based variation, although we have used this same methodology to determine that *H. anomalus* LL is longer than that of its ancestral parental species (Brouillette & Donovan, 2011). DFF might serve as a proxy for LL in some annuals (Luquez *et al.*, 2006; Vasseur *et al.*, 2012), but we found no correlation between DFF and LL. Secondly, there may be minimal heritable genetic variation in *H. anomalus*. Longer LL could be favored by selection because it increases nitrogen-use efficiency by increasing the length of time nitrogen remains in the plant (Aerts & Chapin, 2000; Brouillette & Donovan, 2011), and traits under extremely strong selection are expected to show low degrees of genetic variance (Geber & Griffen, 2003 and references therein). Low soil fertility might be a strong selective force reducing heritable variation for LL in the *H. anomalus* populations. However, population differentiation and heritable variation have been documented for LL in *A. thaliana* (Luquez *et al.*, 2006). Additionally, sexual selection for flower size has been reported to result in a correlated trait response of higher LMA and shorter LL (Delph *et al.*, 2005). Thus, LL deserves further investigation as a putative adaptive trait.

In summary, we found that combining common garden correlational approaches with $Q_{\text{ST}}-F_{\text{ST}}$ approaches supports the adaptive differentiation of some LES and related traits. A more resource-acquisitive and yet more water-use efficient strategy associated with a higher N_{mass} and N_{area} is found in hotter, drier sites, despite lower fertility. A comparison of our results with the literature suggests that while adaptive divergence of traits along stress gradients may generally follow the primary axis of the LES, variation in trait combinations favored by selection is likely to occur as a result of variation in life-history characteristics of the species, the dominance of particular abiotic and biotic components in the stress gradient, and potential genetic constraints within populations.

Acknowledgements

This research was funded by National Science Foundation grants 0614739 and 1122842 to L.A.D. and a Garden Club of America

Award in Desert Studies to L.C.B. We thank C. Darragh for help with the glasshouse work and the laboratory of S.J. Knapp for genotyping support.

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Supporting Information

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

Fig. S1 F_{ST} and Q_{ST} (point estimates and bootstrapped 95% confidence intervals) for *Helianthus anomalus* traits in Table S2.

Fig. S2 Mean monthly precipitation and temperature of *Helianthus anomalus* population sites determined with WorldClim.

Table S1 *Helianthus anomalus* population information for the glasshouse common garden study

Table S2 Species means and SE for *Helianthus anomalus* plant traits ($n = 8$ populations) that appear in Fig. S1

Table S3 *Helianthus anomalus* population sample size for number of maternal families sampled for SSR markers (one plant per maternal family) (N), expected heterozygosity (H_e), and observed heterozygosity (H_o) based on SSR markers

Methods S1 Supporting methods for additional plant traits not in the main text.

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