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Importance of whole-plant biomass allocation and reproductive timing to habitat differentiation across the North American sunflowers

Chase M. Mason[1,2,*], **Eric W. Goolsby**[2,3,4], **Kaleigh E. Davis**[1,2], **Devon V. Bullock**[2] and **Lisa A. Donovan**[2]

[1]*University of Central Florida, Orlando, FL 32816, USA,* [2]*Department of Plant Biology, University of Georgia, Athens, GA 30602, USA,* [3]*Interdisciplinary Toxicology Program, University of Georgia, Athens, GA 30602, USA and* [4]*Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA*
For correspondence. E-mail chasemason.evolution@gmail.com

● **Background and Aims** Trait-based plant ecology attempts to use small numbers of functional traits to predict plant ecological strategies. However, a major gap exists between our understanding of organ-level ecophysiological traits and our understanding of whole-plant fitness and environmental adaptation. In this gap lie whole-plant organizational traits, including those that describe how plant biomass is allocated among organs and the timing of plant reproduction. This study explores the role of whole-plant organizational traits in adaptation to diverse environments in the context of life history, growth form and leaf economic strategy in a well-studied herbaceous system.
● **Methods** A phylogenetic comparative approach was used in conjunction with common garden phenotyping to assess the evolution of biomass allocation and reproductive timing across 83 populations of 27 species of the diverse genus *Helianthus* (the sunflowers).
● **Key Results** Broad diversity exists among species in both relative biomass allocation and reproductive timing. Early reproduction is strongly associated with resource-acquisitive leaf economic strategy, while biomass allocation is less integrated with either reproductive timing or leaf economics. Both biomass allocation and reproductive timing are strongly related to source site environmental characteristics, including length of the growing season, temperature, precipitation and soil fertility.
● **Conclusions** Herbaceous taxa can adapt to diverse environments in many ways, including modulation of phenology, plant architecture and organ-level ecophysiology. Although leaf economic strategy captures one key aspect of plant physiology, on their own leaf traits are not particularly predictive of ecological strategies in *Helianthus* outside of the context of growth form, life history and whole-plant organization. These results highlight the importance of including data on whole-plant organization alongside organ-level ecophysiological traits when attempting to bridge the gap between functional traits and plant fitness and environmental adaptation.

Key words: Biomass, bud, climate, daylength, flower, growing season, *Helianthus*, soil fertility, life history, leaf, root, stem.

INTRODUCTION

The past few decades have seen major strides in the development of 'trait-based' plant ecology. Such approaches focus on functional traits, often ecophysiological traits, individually and in small suites as proxies for whole-plant ecological strategies when attempting to understand adaptation (e.g. Westoby *et al.*, 2002; Reich *et al.*, 2003; Westoby and Wright, 2006; Ackerly and Cornwell, 2007). This approach is exemplified by the leaf economics spectrum (Wright *et al.*, 2004), which summarized major aspects of ecophysiological variation among diverse species with a handful of key traits, and the broader plant economics spectrum (Reich *et al.*, 2014), which attempts to unite functional trait axes from leaves, stems and roots into a coherent description of whole-plant ecological strategy. However, the link between easily measured functional traits and plant fitness is recognized to be rather tenuous (Shipley *et al.*, 2016), as is the implicit assumption that ecophysiological traits contribute similarly to plant function and overall fitness across diverse

species regardless of other aspects of whole-plant biology (Shipley *et al.*, 2016). This is problematic given that the same classic literature that ties key ecophysiological traits to adaptation emphasizes almost equally the integration of such traits into whole-plant phenotypes, in particular the relative allocation of resources among the major plant organs (Mooney, 1972; Chapin, 1980; Bloom *et al.*, 1985). Emerging empirical evidence has highlighted the importance of biomass allocation and whole-plant organization alongside key ecophysiological traits (Edwards *et al.*, 2014; Freschet *et al.*, 2015). Essentially, organ physiology ('quality') coupled with its relative abundance ('quantity') together determine the overall amount of organ function achieved by a plant. For example, from a carbon-capture perspective, whole-plant relative growth rate can be expressed as the product of three main factors – leaf construction cost, net photosynthetic return and leaf mass fraction (Poorter *et al.*, 2012). Likewise, from a nutrient perspective, relative growth rate can be expressed as the product of net root uptake rate, average plant nutrient concentration and root mass fraction

(Poorter *et al.*, 2012). The relative allocation of biomass among organs is therefore theoretically on an equal footing with organ ecophysiology in driving plant resource acquisition and growth. Because of this, selection on organ ecophysiological traits is directly dependent on the larger context of biomass allocation, which determines the total whole-plant function achieved by a given set of ecophysiological traits. This interaction probably plays an important role in shaping plant adaptation to and persistence under disparate environmental conditions.

At a broad scale, the balance in allocation among organs is influenced by a variety of factors, including growth form and the relative availability of different key resources such as light, water and nutrients (Poorter *et al.*, 2012). Modulation of allocation among organs is hypothesized to vary in response to resource supply, until the resources obtained by each organ limit growth equally (Chapin, 1980; Bloom *et al.*, 1985). For instance, low water or nutrient availability should favour the evolution of increased allocation to roots, while insufficient carbon acquisition should favour increased allocation to leaves, or stems if the proximate cause is low light availability (Chapin, 1980; Bloom *et al.*, 1985). Life history is also thought to influence biomass allocation, in particular the relative allocation to reproduction and storage functions between annuals and perennials (Bazzaz *et al.*, 1987). While thorough meta-analyses have been conducted to examine a wide variety of factors affecting broad differences in biomass allocation among species (Poorter *et al.*, 2012, 2015), there have been very few phylogenetically explicit studies of the role of biomass allocation in adaptation across diverse environments.

In addition to biomass allocation, reproductive timing is another whole-plant organizational trait that is key to determining plant fitness. Among habitats, a variety of factors may influence the optimal timing of reproduction, including growing season length and resource availability (Bazzaz *et al.*, 1987; Guilbaud *et al.*, 2015; Matthews and Mazer, 2016). If a plant begins reproduction too early, it sacrifices the potentially higher reproductive output that might be gained by delaying reproduction and growing to a larger vegetative size. If a plant begins reproduction too late, it may not complete seed production before the end of the growing season, whether terminated by frost, drought or other factors. Given the strong selection the environment places on optimal reproductive timing, this trait is known to be readily and rapidly differentiated among differing habitats (e.g. Hall and Willis, 2006; Franks *et al.*, 2007; Kawakami *et al.*, 2011; Brouillette *et al.*, 2014; Matthews and Mazer, 2016). Plants possess multiple mechanisms for sensing environmental conditions, such that reproduction can be evolutionarily programmed to occur at the proper time, using cues in the form of temperature (Song *et al.*, 2013; Kazan and Lyons, 2016), water availability (Fox, 1990; Kazan and Lyons, 2016), nutrient uptake (Guilbaud *et al.*, 2015) and, perhaps most commonly, photoperiod (Blackman *et al.*, 2011; Song *et al.*, 2013). Reproductive timing is also expected to interact with biomass allocation, because allocation to productive vegetative structures has a compound interest effect on plant growth such that delaying allocation to reproduction in suitable environments can improve fitness (Bazzaz *et al.*, 1987).

Through the dual lenses of biomass allocation and reproductive timing, this study examines the importance of whole-plant organization in the diversification of the herbaceous genus

Helianthus. Wild sunflowers are an excellent system in which to examine trait evolution, as species vary widely in morphology and physiology, and occupy diverse habitats across North America, including deserts, wetlands, grasslands, forests, rock outcrops and coastal dunes (Heiser *et al.*, 1969). Here we address two main questions using a phylogenetic comparative approach. First, how integrated are whole-plant organizational traits with one another and with organ-level ecophysiology? If biomass allocation and reproductive timing are tightly tied to one another and to leaf economic traits, then organ-level functional traits such as those of the leaf economics spectrum may be reasonable proxies for whole-plant ecological strategies. If not, then whole-plant organizational traits may be needed to describe plant function adequately. Secondly, what is the relative importance of whole-plant organizational traits to habitat differentiation as compared with organ-level functional traits? If biomass allocation or reproductive timing are more strongly tied to species diversification across environmental gradients than leaf economic traits, this calls into question the utility of using organ-level functional traits alone as proxies for whole-plant ecological strategies. Similarly, if the same leaf economic strategy allows for the occupancy of dramatically different habitats through interactions with plant architecture, phenology, growth form or life history, this undermines the assumption that organ-level functional traits contribute in similar ways to plant function and fitness across diverse species and environments (Shipley *et al.*, 2016). By examining the relative predictive power of biomass allocation and reproductive timing in this system, we aim to gain an understanding of whether the current weak points in 'trait-based' ecology can be strengthened through the explicit consideration of plant architecture and phenology, and how these whole-plant organizational traits interact with organ-level ecophysiology.

MATERIALS AND METHODS

Study system

To assess the evolution of biomass allocation and reproductive timing across wild sunflowers, a diverse group of 27 diploid non-hybrid species of *Helianthus* (Asteraceae, Helantheae) were selected for inclusion in this study, along with one of the two members of the sister genus *Phoebanthus* as an outgroup. This includes over half of the approx. 50 species described within *Helianthus* as well as four-fifths of diploid non-hybrid taxa (Heiser *et al.*, 1969; Timme *et al.*, 2007). Furthermore, these species were all included in the most recent and well-resolved phylogeny of the diploid backbone of *Helianthus* (Stephens *et al.*, 2015), allowing for the use of robust phylogenetic comparative approaches. In order to incorporate natural intraspecific variation into assessments of trait evolution, multiple populations were included from across the geographic range of each species (2–4 each, 83 in total). Seed from these populations was either directly wild-collected or obtained from accessions established with the USDA National Genetic Resources Program (Supplementary Data Dataset S1).

Experimental design and plant growth

Because biomass allocation and reproductive timing are environmentally labile, plants were grown and traits assessed under controlled conditions in a high-resource greenhouse common garden experiment. By minimizing environmentally driven variation in this way, underlying genetic trait differentiation can be assessed for potential adaptive significance. Given the large number of populations under study, it was not feasible to grow all species simultaneously. Accordingly, the 28 species were divided into two common gardens grown in 2012 and 2013 (hereafter CG-1 and CG-2, respectively) at the University of Georgia Plant Biology greenhouses in Athens, GA, USA. To minimize differences between the two common gardens, the same experimental timing was used in both years, and environmental conditions were kept as similar as possible, including the use of identical pots, soil mixture, fertilization, watering regime and greenhouse temperature controls. To assess and correct for any remaining uncontrollable differences in conditions between years, three species reflecting a cross-section of growth form, life history and overall morphology (*H. annuus*, *H. radula* and *H. silphioides*) were replicated in both common gardens to serve as phytometers. Given the suitability of this design for addressing a variety of research questions, these common gardens were also used for the study of several other classes of physiological traits, including leaf economics (Mason and Donovan, 2015), leaf defences and secondary chemistry (Mason *et al*., 2016), and floral trait diversity (Mason *et al*., unpubl. res.). Experimental conditions are therefore described in detail in Mason and Donovan (2015). In short, seeds were scarified to induce uniform germination on moist filter paper in Petri dishes (Julian days 129–131), and transferred to seedling trays under a 12 h photoperiod once cotedylons had turned green (within 2–4 d). Eight replicate plants per population were then transplanted upon the emergence of true leaves (Julian days 142–154) into 6 L pots filled with a 3:1 mixture of sand and calcined clay. Pots were arranged in a randomized complete block design to account for any spatial environmental variation in the greenhouse. To provide high-nutrient conditions for plant growth, pots received 20 g of 9 month slow-release fertilizer with micronutrients (Osmocote Plus 15-9-12, Scotts, Marysville, OH, USA) mixed into the soil, as well as an initial liquid fertilization with supplemental Ca, Fe and Mg to promote seedling establishment. To ensure ample water availability, pots received daily drip irrigation to field capacity. Once transplanted into pots in the greenhouse, plants received ambient photoperiod for the remainder of the experiment, ranging from a maximum daylength of 14·4 h in mid-June (Julian days 168–174) to a minimum daylength of 9·9 h in mid-December (Julian days 350–360). Greenhouse temperatures were controlled by an automated system of heaters and evaporative coolers, set to 18 °C at night and 27 °C during the day (though daytime temperatures on especially hot summer days varied a few degrees above the daytime set point due to limitations of evaporative cooling).

Trait measurement

All plants were evaluated three times per week for the development of the first visible bud, and thereafter for the opening of the first flower (anthesis). These two dates were recorded for each plant. Almost all plants survived and successfully flowered (a mean of 7·52 ± 1·13 s.d. per population), and only a handful of individual plants remained vegetative and did not flower during the experiment. Once plants reached reproductive maturity, standardized harvests were performed. All members of a species (across all populations) were harvested together over a short period of time (Supplementary Data Table S1), with two criteria – reproduction and leaf senescence – used to define maturity. Timing harvests by the application of these two criteria captures both plant size and biomass allocation on reproductively mature plants at the end of a simulated growing season, in a way relevant to the biology of each species. First, to meet the reproduction criterion, all plants of a given species had to be past peak flowering, with the exception of any plants that remained vegetative and were not approaching reproduction (e.g. no buds). A small number of plants remained vegetative in a handful of species, mostly perennials. Secondly, to meet the leaf senescence criterion, all plants of a given species had to have senesced their most juvenile leaves. As these common gardens were also used to assess leaf economics traits, leaves were tagged on juvenile plants to estimate leaf life span (Mason and Donovan, 2015), and plants were not harvested until these leaves had senesced. In practice, the senescence of these juvenile leaves typically corresponded to the period between bud production and the onset of seed filling (and associated whole-plant senescence) in annual species, the onset of programmed end of growing season shoot senescence in the erect perennials (which are deciduous) and either the shift to winter dormancy or the production of new leaves after breaking dormancy in the basal rosette perennials. By combining these two criteria, we avoid issues that might arise from using either criterion in isolation – for instance, using only reproduction would not adequately capture the mature size or allocation of biomass in most basal rosette species, as these species often reproduce well before the end of their growing season and produce substantial additional vegetative growth before entering winter dormancy. Similarly, using only leaf senescence would result in barely capturing flowering in many annual species. Together, these two criteria allow for estimation of plant biomass traits across diverse taxa in a way that determines end of growing season maturity relative to the biology of each species.

At harvest, shoots were detached at the soil surface and divided into three categories: stems, leaves and reproductive parts (buds and flower heads). Pots were then emptied over a fine mesh screen, and below-ground biomass was gently washed to remove the soil mixture. This biomass consisted of coarse and fine roots, as well as rhizomes in many of the perennial species. Across *Helianthus* species, there is a wide continuum in the degree of rhizome formation, and in fact many perennial species lack rhizomes and instead root tissue or crown buds give rise to new shoots each year (Heiser *et al*., 1969). In many cases, rhizomes are not readily distinguishable from coarse roots, so rhizomes could not be consistently separated from roots with confidence and were thus included with roots as a single category of below-ground biomass. Separated biomass was dried at 60 °C in a forced-air drying oven for at least 72 h and then weighed for dry mass. Total plant biomass was calculated as the sum of below-ground, stem, leaf and reproductive biomass, and mass fractions were calculated as the most preferable

presentation of biomass allocation patterns following the recommendations of Poorter and Sack (2012). Mass fractions were favoured over ratios (e.g. root:shoot) given the inherent problems with ratio-based metrics, including unboundedness, asymmetry, strong non-normality and loss of biological information (Poorter and Sack, 2012).

Data assessment and standardization

To test for differences between the two common gardens (CG-1 and CG-2) in total plant biomass and mass fractions, all replicates of the three phytometer species (nine populations) were assessed by a single analysis of variance (ANOVA; including year, population, year × population and block effects). Block was not found to have a significant effect for total biomass or any mass fractions ($\alpha = 0.05$ was used as the significance threshold for all analyses unless otherwise stated). Total plant biomass was on average 36 % larger in CG-2 than in CG-1 across the phytometers, with no significant year × population interaction. While plant size differed substantially, mass fractions were found to vary negligibly between years (1–5 % of total dry mass). These phytometer results were used to inform standardization of data between the two common gardens. Population means were calculated over replicate plants without incorporating block effects. Population means for total biomass were then corrected upward by 36 % for all populations grown in CG-1, while mass fractions were not corrected between years. The summer of 2012 was hot and sunny, while the summer of 2013 was unusually rainy with milder peak temperatures. While precipitation itself could not affect plants in the greenhouse, differences in peak temperatures and cumulative insolation between years probably drove the differences seen in total plant size. It is important to point out that this phytometer-based correction is by no means a perfect solution to the problem of inter-year variation in total biomass, as the differences between years probably had differential impacts on different taxa and the phytometers cannot completely account for all variation. However, phytometer correction should put species grown in different years on a roughly equal footing, and in any case the shortcomings of phytometer correction for inter-year variation should only affect the trait of total biomass, not biomass allocation patterns (mass fractions).

With respect to reproductive timing, all but two plants of the KYL population of *H. maximiliani* did not flower in the greenhouse, either not producing buds or stalling after bud production, so this population was removed from analyses of reproductive timing. Additionally, plants of all three populations of *H. radula* grown in CG-2 behaved similarly, with most stalling after producing a few buds and only around a third of plants flowering after abnormally long delays. This slow-growing species does not always flower in the first year in the wild, and differences in conditions in CG-2 may have inhibited flowering in favour of vegetative growth. As such, populations of *H. radula* grown in CG-2 were excluded from further consideration for reproductive analyses and not used as a phytometer species for assessment of reproductive timing. The other two phytometer species were used to test for differences in the Julian dates of first bud and first flower between years in the same manner as for biomass traits. Block was not found to have

a significant effect for either first bud or first flower. Date of first bud was on average 5·0 % later in CG-2, with a significant year × population interaction (varying between 1·4 and 7·4 % later among populations). Date of first flower was on average 9·7 % later in CG-2, also with a significant year × population interaction (varying between 4·4 and 13·9 % among populations). It seems that the same difference in conditions that resulted in overall larger plants in CG-2 also delayed reproduction. As such, population means were calculated over replicate plants without incorporating block effects, and first bud and first flower dates were corrected upward by 5·0 and 9·7 %, respectively, for all populations grown in CG-1. Again, this correction by phytometer is far from a perfect solution, but should ameliorate some of the effect of inter-year variation and put species on a roughly equal footing.

After correction, population means of first bud and first flower dates were used to calculate the length of time between bud initiation and anthesis (hereafter referred to as 'interim period'). Daylengths were also calculated for each observed first bud and first flower date using the US Naval Observatory sunrise and sunset tables for Athens, GA.

Environmental data

Climate and soil characteristics of each population source site were obtained as reported previously in Mason and Donovan (2015). In brief, climate data were aggregated from a variety of sources. Altitude, mean annual temperature (MAT), mean diurnal temperature range, temperature seasonality, mean annual precipitation (MAP) and precipitation seasonality were obtained from the WorldClim database (Hijmans *et al*., 2005). Potential evapotranspiration (PET) and aridity index (temperature-adjusted water availability, the ratio of MAP to PET) were taken from the CGIAR Global Aridity and PET database (Zomer *et al*., 2008). Additionally, length of the frost-free period was derived from the USDA NRCS Soil Survey Geographic Database (Soil Survey Staff, NRCS), and moisture-based growing season length was retrieved from the UN FAO Global Agro-Ecological Zones version 3·0 Module I (IIFAS/FAO, 2012).

To characterize soil characteristics, five approx. 20 cm deep soil cores were taken spread throughout the population at each source site, dried at 60 °C and homogenized prior to analysis. Soil carbon content, nitrogen content and C:N ratio were assessed with Micro-Dumas combustion (NA1500, Carlo Erba Strumentazione, Milan, Italy) at the University of Georgia Analytical Chemistry Laboratory. Soil samples were also submitted for standard bulk soil analysis by A&L Eastern Laboratories (Richmond, VA, USA). This analysis provided estimates of soil pH, organic matter content, cation exchange capacity (CEC), available phosphorus and exchangeable calcium, potassium and magnesium. All soil characteristics were averaged across the five soil cores to yield population site means (Dataset 1). Covariation among environmental characteristics is given in Supplementary Data Table S3.

Phylogenetic analysis

All phylogenetic analyses were performed using the phylogeny of Stephens *et al*. (2015), the most robust and well-

resolved phylogeny of the diploid backbone of the genus *Helianthus*. Phylogenetic signal was assessed as Pagel's λ for all plant traits using the 'phylosig' function in the R package 'phytools' (Pagel, 1999; Revell, 2012). All but one trait was found to exhibit significant phylogenetic signal (Supplementary Data Table S2), confirming the need for phylogenetically explicit analyses. Macroevolutionary correlations among plant traits and between plant traits and environmental characteristics were assessed using phylogenetic mixed models on population means (Housworth *et al.*, 2004; Felsenstein, 2008). These models were implemented using the 'phylopars' function in the R package 'Rphylopars' to estimate evolutionary covariance while accounting for within-species variation and missing data (Bruggeman *et al.*, 2009; Goolsby *et al.*, 2016). Additionally, macroevolutionary correlations were assessed between the plant traits assessed in this study and leaf economic traits assessed on the common gardens in a previous study (Mason and Donovan, 2015). Furthermore, to test for trait differences between annuals, erect perennials and basal rosette perennials, species means were calculated on population means, and phylogenetic ANOVA was performed using the 'phylANOVA' function in the R package 'phytools' (Garland *et al.*, 1993; Revell, 2012), which tests for differences among groups while accounting for expected residual autocorrelation due to evolutionary relatedness.

RESULTS

Variation in biomass allocation and reproductive timing

Across the genus, there is broad diversity in plant size and organization (Fig. 1). Total plant biomass varies approx. 50-fold, from the very small *H. cusickii* to the very large *H. floridanus*. Below-ground mass fraction varies from <5 % of total plant biomass in several annual species to >55 % in *H. divaricatus*. Stem mass fraction is similarly variable, ranging from 28 % in *H. occidentalis* ssp. *occidentalis* to nearly 78 % in *H. agrestis*. Leaf mass fraction varies somewhat less, from <12 % in *H. porteri* to >37 % in both *H. heterophyllus* and *H. radula*. Reproductive mass fraction varies the least though still substantially, from about 1 % in *H. salicifolius* and *H. niveus* ssp. *tephrodes* to >18 % in *H. annuus*. Among species, the evolution of larger overall plant size at maturity is associated with the evolution of higher stem mass fraction and lower leaf mass fraction (Table 1). Stem mass fraction trades off strongly with below-ground mass fraction, and to a lesser extent with leaf mass fraction, though no other significant pairwise trade-offs exist among mass fractions (Table 1). With respect to growth form and life history, there are no significant differences in overall plant size, though there are large differences in vegetative mass fractions (Fig. 2). Annual species have significantly higher allocation to stems than perennial species, while basal rosette species have significantly higher allocation to leaves than erect species (Fig. 2). Erect perennials also have significantly higher allocation to roots and rhizomes than annual species (Fig. 2). Reproductive allocation, while on average higher in annuals than in perennials, does not vary significantly among groups (Fig. 2).

Helianthus species vary substantially in reproductive timing, here with first bud varying from as little as under 2 months post-germination in several annual species to well over 4 months in multiple south-eastern species. Date of first flower varies even more, from a little over 2 months to well over 6 months post-germination. This corresponds to variation in the interim period between bud and flower from just over 2 weeks to over 2 months. Dates of first bud and first flower are strongly positively correlated among species, as expected, and later reproducing species also have longer interim periods (Table 1). The evolution of later reproduction is positively correlated with the evolution of both higher total plant biomass and higher stem mass fraction at maturity, but later reproduction is also associated with lower reproductive mass fraction (Table 1). Dates of first bud and first flower do not appear to be significantly associated with life history or growth form (Fig. 2).

Reproductive timing, biomass allocation among vegetative organs, and plant size at maturity are all evolutionarily correlated with previously reported leaf economic traits. The evolution of more resource-acquisitive leaf traits (higher photosynthetic rate and leaf nutrient contents) is correlated with the evolution of earlier reproduction (Table 2). The evolution of higher leaf mass per area (LMA), indicating higher leaf construction cost, is correlated with the evolution of higher leaf mass fraction, while the evolution of higher leaf phosphorus and longer leaf life span is correlated with increased allocation to roots and rhizomes over stems (Table 2). Overall, the evolution of higher total plant biomass at maturity is associated with more resource-acquisitive traits such as lower LMA and higher leaf phosphorus (Table 2). Allocation to reproductive organs, however, appears unrelated to leaf economic traits (Table 2).

Diversification across environmental gradients

Both total plant biomass at maturity and relative mass fractions are significantly evolutionarily correlated with a wide variety of environmental factors. Higher total plant biomass at maturity is correlated with evolutionary shifts into habitats with longer frost-free periods and moisture-based growing periods as well as higher MAT and MAP and lower temperature seasonality, all of which predominate at lower latitudes and altitudes (Table 3). Soil characteristics appear unrelated to the evolution of total plant biomass at maturity (Table 3).

Given their strong trade-off among species, below-ground mass fraction and stem mass fraction are inversely related to many of the same climate factors. Habitats with shorter frost-free periods, lower MAT, higher temperature seasonality and lower precipitation seasonality (all conditions typical of higher latitudes) favour the evolution of increased allocation to roots and rhizomes at the expense of stems (Table 3). Unlike below-ground and stem allocation, climate factors do not appear to influence the evolution of leaf mass fraction or reproductive mass fraction, with the single exception that a higher reproductive mass fraction evolves repeatedly at higher altitudes (Table 2). Underground, some soil fertility metrics appear to mediate relative allocation between below-ground biomass and stems, while others appear to drive relative allocation between stems and leaves. Shifts onto soils with higher soil calcium, CEC and C:N ratio are evolutionarily correlated with lower below-ground allocation and higher stem allocation (Table 2). Shifts onto soils with higher nitrogen, carbon and organic matter content are all
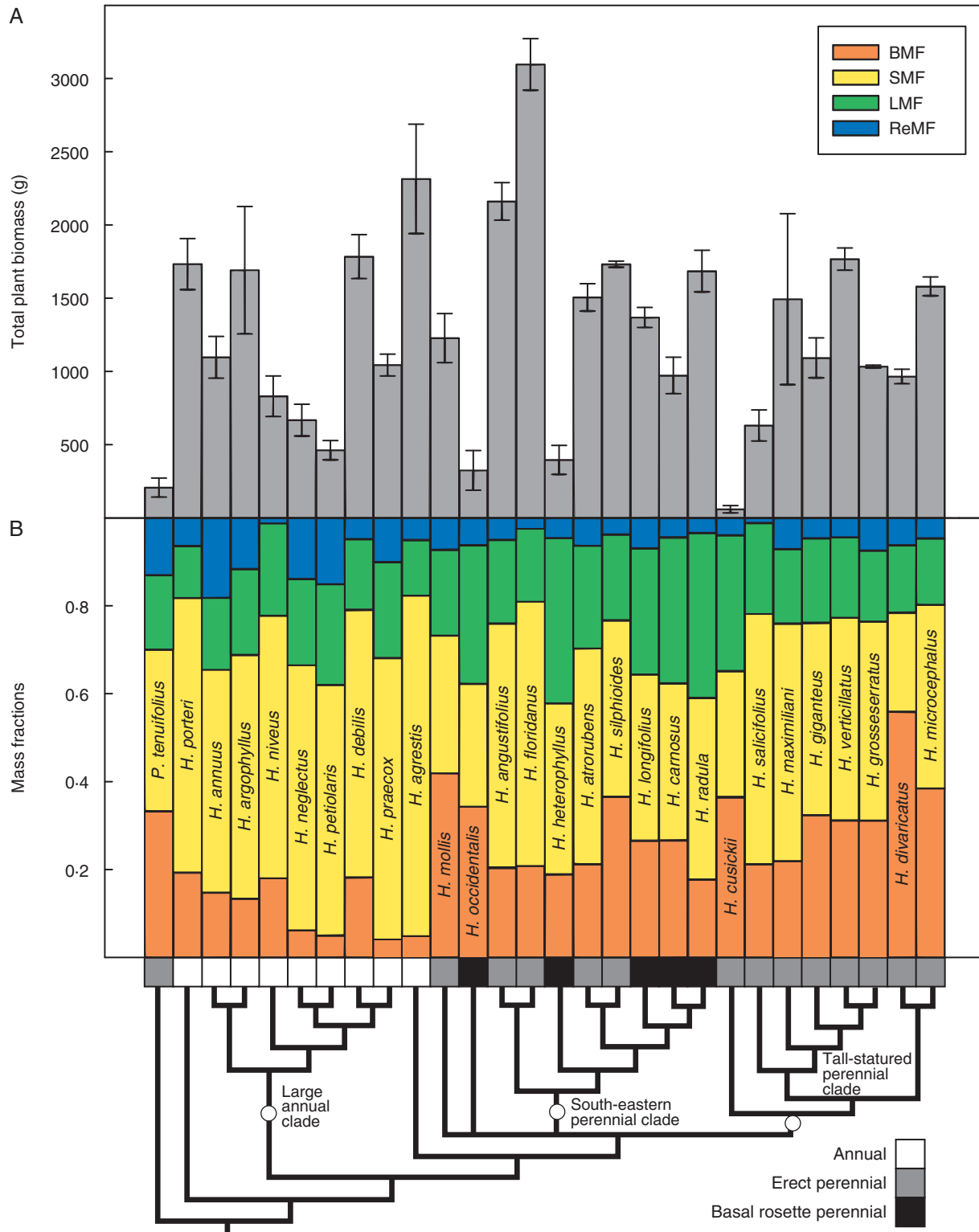
FIG. 1. Variation in biomass allocation and total plant size across *Helianthus*. (A) Species mean total plant biomass at harvest. Error bars reflect standard errors of species means calculated from population means. (B) Relative biomass allocation among plant organs represented as species mean below-ground, stem, leaf and reproductive mass fractions. Note that infraspecific epithets are omitted for space.

TABLE 1. *Macroevolutionary correlations among plant traits as assessed by a phylogenetic mixed model incorporating intraspecific variation using population means (*Housworth* et al., 2004; Felsenstein, 2008)*

	BMF	SMF	LMF	ReMF	First bud	First flower	Interim period
Total biomass	–	(+) 0·32	(–) 0·34	–	(+) 0·38	(+) 0·44	(+) 0·42
BMF		(–) 0·66	–				
SMF			(–) 0·20	–	(+) 0·20	(+) 0·18	–
LMF				–	–	–	–
ReMF					(–) 0·33	(–) 0·28	–
First bud						(+) 0·93	(+) 0·56
First flower							(+) 0·80

R^2 and directionality of significant correlations are presented.

Abbreviations: BMF, below-ground mass fraction; SMF, stem mass fraction; LMF, leaf mass fraction; ReMF, reproductive mass fraction.

First bud and first flower dates are considered in Julian days.

evolutionarily correlated with lower leaf allocation and higher stem allocation (Table 2). In all cases shifts into habitats with less fertile soils appear to favour decreased allocation to stems. Additionally, the occupation of soils with higher phosphorus content is evolutionarily correlated with increased allocation to reproduction.

Much like total plant biomass at maturity, the evolution of later reproductive timing is strongly correlated with evolutionary shifts into habitats with longer growing seasons and associated environmental factors (Table 2). In addition to those factors correlated with the evolution of total plant biomass at maturity, a high potential evapotranspiration and aridity index also favour the evolution of later reproduction (Table 2). Geographically, it is easy to see that earlier reproduction is associated with populations distributed in the cooler northern and more arid western USA, while later reproduction is largely found in the warm and mesic south-east (Fig. 3). With respect to soil fertility, earlier reproduction appears to be favoured by shifts onto more acidic soils, as well as those with higher levels of phosphorus, potassium and magnesium (Table 2).

DISCUSSION

Integration of traits into whole-plant ecological strategies

The most current sunflower phylogeny (Stephens *et al*., 2015) indicates that the diploid backbone of *Helianthus* is comprised of three main clades – a large annual clade, a clade of tall-statured perennials and a south-eastern perennial clade made up of both erect and basal rosette perennials. With respect to whole-plant organization, members of the large annual clade typically have low below-ground allocation (not built for multi-year persistence) coupled with high stem allocation allowing for the shading of competitors in the open habitats they typically occupy. This architecture is accompanied by a resource-acquisitive leaf economic strategy supporting rapid growth (Mason and Donovan, 2015), as well as a generally high reproductive allocation. These species also have fairly early reproductive timing regardless of geography, a characteristic that is probably favoured in the habitats this clade occupies: more arid

environments such as deserts and grasslands, or edaphically stressful habitats such as coastal dunes and sand prairies in less arid regions (Heiser *et al*., 1969). Members of the tall-statured perennial clade typically have high allocation to roots and rhizomes, supporting their multiyear persistence, as well as a typically resource-acquisitive leaf economic strategy capable of generating large deciduous shoots each growing season (Mason and Donovan, 2015). Reproductive timing in this clade is highly variable by geographic region – quite early in northern latitudes vs. later in southern Appalachia and the middle Great Plains. The south-eastern perennial clade contains an even mix of basal rosette and erect perennial species, united by a more resource-conservative leaf economic strategy supporting slower growth and late reproduction over the long growing seasons characteristic of the warm and moist south-eastern USA (Mason and Donovan, 2015). The growth forms, however, differ in allocation, with the erect perennials having similar allocation patterns to those in the large-statured perennial clade, while the basal rosette species maintain above-ground rosettes year-round and have high leaf allocation. Across the genus as a whole, biomass allocation is not particularly strongly integrated with reproductive timing. Later reproducing species do have higher average stem allocation and lower reproductive allocation, but both early and late reproducing species possess a diversity of allocation patterns. Surprisingly, life history is only associated with significant differences in vegetative allocation, and not with significant differences in reproductive timing, overall plant size or reproductive mass fraction. Annual plants are typically expected to invest more in reproduction than perennial close relatives, especially for annuals that occupy ruderal environments (Gaines *et al*., 1974; Bazzaz *et al*., 1987). While some annual *Helianthus* species often occupy human-disturbed habitats (e.g. *Helianthus annuus* and *Helianthus petiolaris*) and several more are native to habitats with natural forms of disturbance (e.g. unstable sand dunes), others are found in habitats with little disturbance (e.g. *H. porteri* and *H. agrestis*) which might obscure a general pattern. For rhizomatous perennials, estimates of below-ground mass fraction may also contain some clonal reproductive function, especially in the handful of members of the tall-statured perennial clade known to achieve substantial vegetative spread and senesce connections to parent plants (Heiser *et al*., 1969). However, given that this study only assessed a single year of growth from seed, the rhizomatous allocation that could be considered 'reproductive' rather than simply storage for resprouting is negligible.

Whole-plant traits and environmental differentiation

Across North America, biomass allocation in *Helianthus* varies strongly across environmental gradients. Meta-analysis of biomass allocation in herbs suggests that several of the macroevolutionary patterns seen here mirror typical plastic plant responses to environmental conditions (Poorter *et al*., 2012). For instance, lower nutrient availability favours increased root allocation, lower temperatures favour increased root and decreased stem allocation, and larger herbs on average have lower leaf mass fraction and higher stem mass fraction (Poorter *et al*., 2012). Water availability is an apparent exception, as plastically herbs typically increase root allocation in
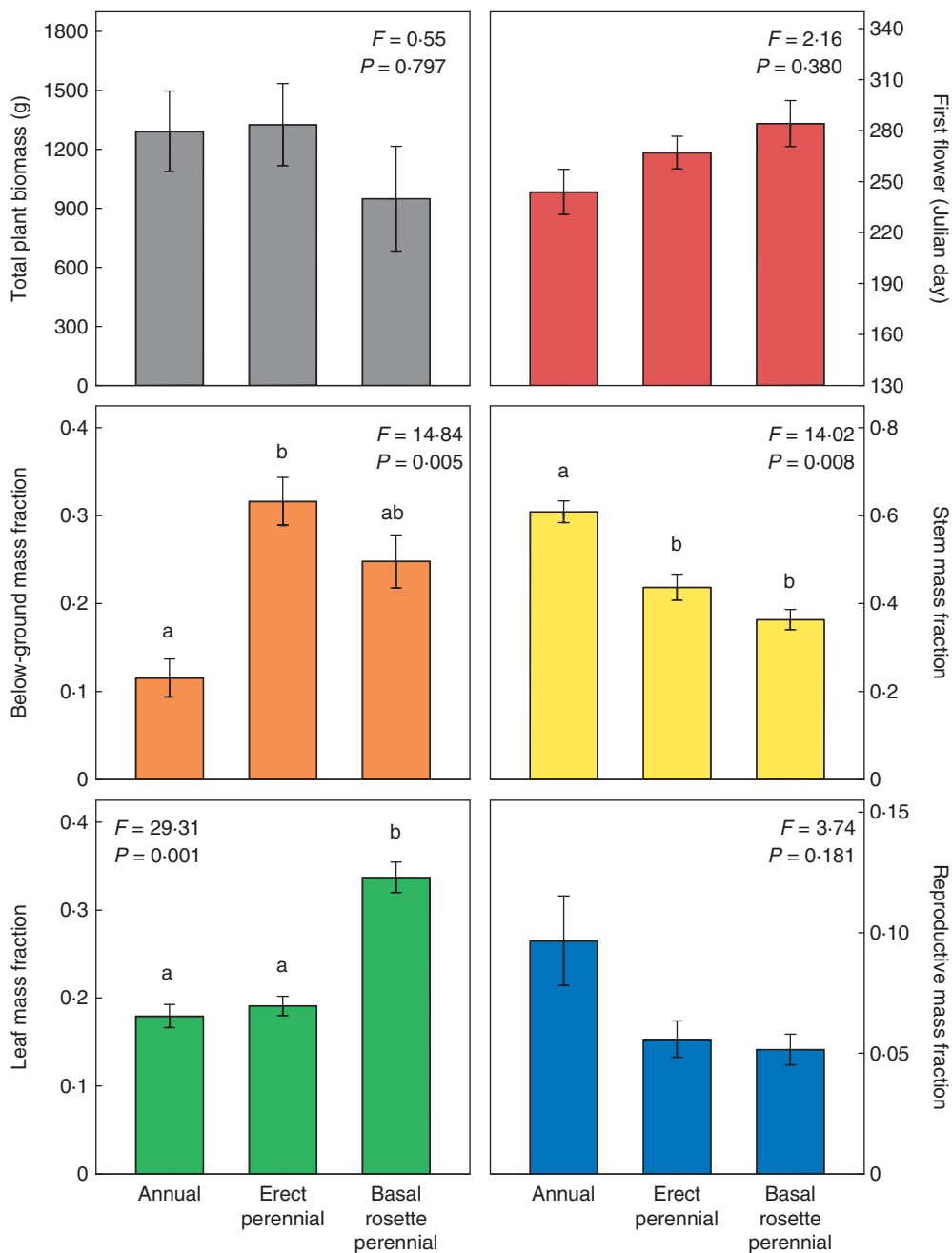
FIG. 2. Differences in plant traits among species based on growth form and life history using phylogenetic ANOVA (Garland *et al*., 1993) in the R package 'phytools' (Revell, 2012). Bars that do not share letters are significantly different by the Holm post-hoc test.

response to drought (Poorter *et al*., 2012), though among *Helianthus* there are no significant relationships between below-ground mass fraction and mean annual precipitation or aridity index. Additionally, a lower below-ground mass fraction is associated with higher precipitation seasonality and higher potential evapotranspiration, indicating if anything lower root allocation in environments more prone to low water availability. This is probably due to contrasting architectural mechanisms for achieving drought escape in annuals vs. deciduous perennials – in annuals, growing quickly and completing reproduction during favourable periods, in deciduous perennials,

investing heavily in below-ground storage and becoming dormant during droughts (Kigel *et al*., 2011; Brouillette *et al*., 2014). Unlike water availability, macroevolutionary differentiation with respect to soil fertility appears to be unified across *Helianthus*, with low soil fertility favouring higher allocation to roots to increase plant acquisition of growth-limiting nutrients (Chapin, 1980; Bloom *et al*., 1985). Additionally, high soil fertility favours reduced allocation to leaves, probably because more resource-acquisitive leaf economic strategies are found on more fertile soils, and more productive leaves allow for the

maintenance of total plant carbon capture with lower relative leaf allocation (Mason and Donovan, 2015).

Reproductive timing across *Helianthus* is even more strongly correlated with environmental gradients than biomass allocation. Reproduction across *Helianthus* is known to be strongly photoperiod controlled, with most species native to lower latitudes possessing an obligate short-day requirement for flowering (Henry *et al.*, 2014). Previous work in three species shows adaptation for earlier flowering in populations at higher latitudes as well as in drier habitats (Blackman *et al.*, 2011; Kawakami *et al.*, 2011; Brouillette *et al.*, 2014), identical to the

patterns seen here among species. Overall *Helianthus* species appear to tailor reproduction strongly to growing season length, whether defined by temperature or water availability. Additionally, the relationship between soil nutrient availability and reproductive timing is consistent with the 'peak-nutrient' hypothesis of flowering time, where selection favours the trigger for flowering occurring just before the whole-plant nutrient uptake rate is maximized, a point that is reached earlier in faster-growing species and in more fertile soils (Guilbaud *et al.*, 2015). This phenomenon is probably folded on top of selection in response to growing season length across the genus.

TABLE 2. *Macroevolutionary correlations between biomass allocation and reproductive timing traits in this study vs. leaf economic traits reported previously (Mason and Donovan, 2015)*

	A_{mass}	R_{mass}	N_{mass}	P_{mass}	LMA	LL
Total biomass	–	–	–	(–) 0·21	(–) 0·22	–
BMF	–	–	–	(+) 0·16	–	(+) 0·21
SMF	–	–	–	(–) 0·16	–	(–) 0·21
LMF	–	–	–	–	(+) 0·17	–
ReMF	–	–	–	–	–	–
First bud	(–) 0·26	–	(–) 0·23	–	–	–
First flower	(–) 0·36	–	(–) 0·26	(–) 0·18	–	–
Interim period	(–) 0·43	–	(–) 0·23	(–) 0·19	–	(+) 0·19

Correlations were assessed by a phylogenetic mixed model.
R^2 and directionality of significant correlations ($P < 0.05$) are presented.
Abbreviations: A_{mass}, leaf photosynthetic rate; BMF, below-ground mass fraction; LL, leaf life span; LMA, leaf mass per area; LMF, leaf mass fraction; N_{mass}, leaf nitrogen concentration; P_{mass}, leaf phosphorus concentration; R_{mass}, leaf respiration rate; ReMF, reproductive mass fraction; SMF, stem mass fraction.
First bud and first flower dates are considered in Julian days.

On the relative predictive power of leaf economic traits

In general, biomass allocation and reproductive timing are as or more strongly linked to differentiation across environments as leaf economics are in *Helianthus* (Mason and Donovan, 2015). Leaf economic strategy and reproductive timing appear tightly integrated across the genus, but both are less strongly associated with biomass allocation. On the whole, it seems that all three classes of traits are shaped by selection from the environment in the context of plant growth form and life history, with biomass allocation and leaf economic strategy evolving together to support the rate of whole plant growth necessary given optimal reproductive timing. Our results underscore the importance of whole-plant strategies in plant adaptation and the interaction of multiple classes of traits, in agreement with emerging evidence in other systems, both herbaceous and woody (Edwards *et al.*, 2014; Freschet *et al.*, 2015). While different growth forms may differ strongly in typical allocation across organs (e.g. forbs vs. trees), species within these forms (and

TABLE 3. *Macroevolutionary correlations between plant traits and source site environmental characteristics as assessed by a phylogenetic mixed model incorporating intraspecific variation using population means (Housworth* et al., *2004; Felsenstein, 2008)*

	Total biomass	BMF	SMF	LMF	ReMF	First bud	First flower	Interim period
Latitude	**(–) 0·25**	**(+) 0·25**	**(–) 0·31**	–	–	**(–) 0·26**	**(–) 0·29**	**(–) 0·24**
Altitude	**(–) 0·28**	–	–	–	**(+) 0·30**	**(–) 0·42**	**(–) 0·44**	**(–) 0·37**
Frost-free period	**(+) 0·23**	(–) 0·18	**(+) 0·31**	–	–	**(+) 0·28**	**(+) 0·32**	**(+) 0·28**
Moisture-based growing period	(+) 0·19	–	–	–	–	**(+) 0·34**	**(+) 0·38**	**(+) 0·35**
Mean annual temperature	(+) 0·21	(–) 0·20	**(+) 0·31**	–	–	**(+) 0·27**	**(+) 0·30**	**(+) 0·25**
Mean diurnal range	–	–	–	–	–	–	–	–
Temperature seasonality	(–) 0·18	(+) 0·15	(–) 0·16	–	–	(–) 0·19	(–) 0·22	(–) 0·18
Mean annual precipitation	(+) 0·21	–	–	–	–	**(+) 0·34**	**(+) 0·38**	**(+) 0·33**
Precipitation seasonality	–	**(–) 0·39**	(+) 0·19	–	–	–	–	–
Potential evapotranspiration	–	(–) 0·14	**(+) 0·24**	–	–	**(+) 0·25**	**(+) 0·27**	(+) 0·22
Aridity index	–	–	–	–	–	(+) 0·19	(+) 0·21	(+) 0·20
Soil N	–	–	(+) 0·19	(–) 0·19	–	–	–	–
Soil C	–	–	(+) 0·20	(–) 0·20	–	–	–	–
Soil C:N	–	**(–) 0·29**	**(+) 0·38**	–	–	–	–	–
Soil P	–	–	–	–	**(+) 0·23**	(–) 0·15	(–) 0·22	**(–) 0·29**
Soil K	–	–	–	–	–	(–) 0·22	(–) 0·20	–
Soil Mg	–	–	–	–	–	**(–) 0·27**	**(–) 0·28**	(–) 0·21
Soil Ca	–	(–) 0·18	**(+) 0·23**	–	–	–	–	–
Soil pH	–	–	–	–	–	(–) 0·14	(–) 0·17	(–) 0·17
Soil organic matter content	–	–	(+) 0·15	(–) 0·18	–	–	–	–
Soil cation exchange capacity	–	(–) 0·15	(+) 0·17	–	–	–	–	–

R^2 and directionality of significant correlations ($P < 0.05$) are presented, with correlations significant after implementation of a false discovery rate correction at $q = 0.05$ presented in bold (Benjamini and Hochberg, 1995).
Abbreviations: BMF, below-ground mass fraction; SMF, stem mass fraction; LMF, leaf mass fraction; ReMF, reproductive mass fraction.
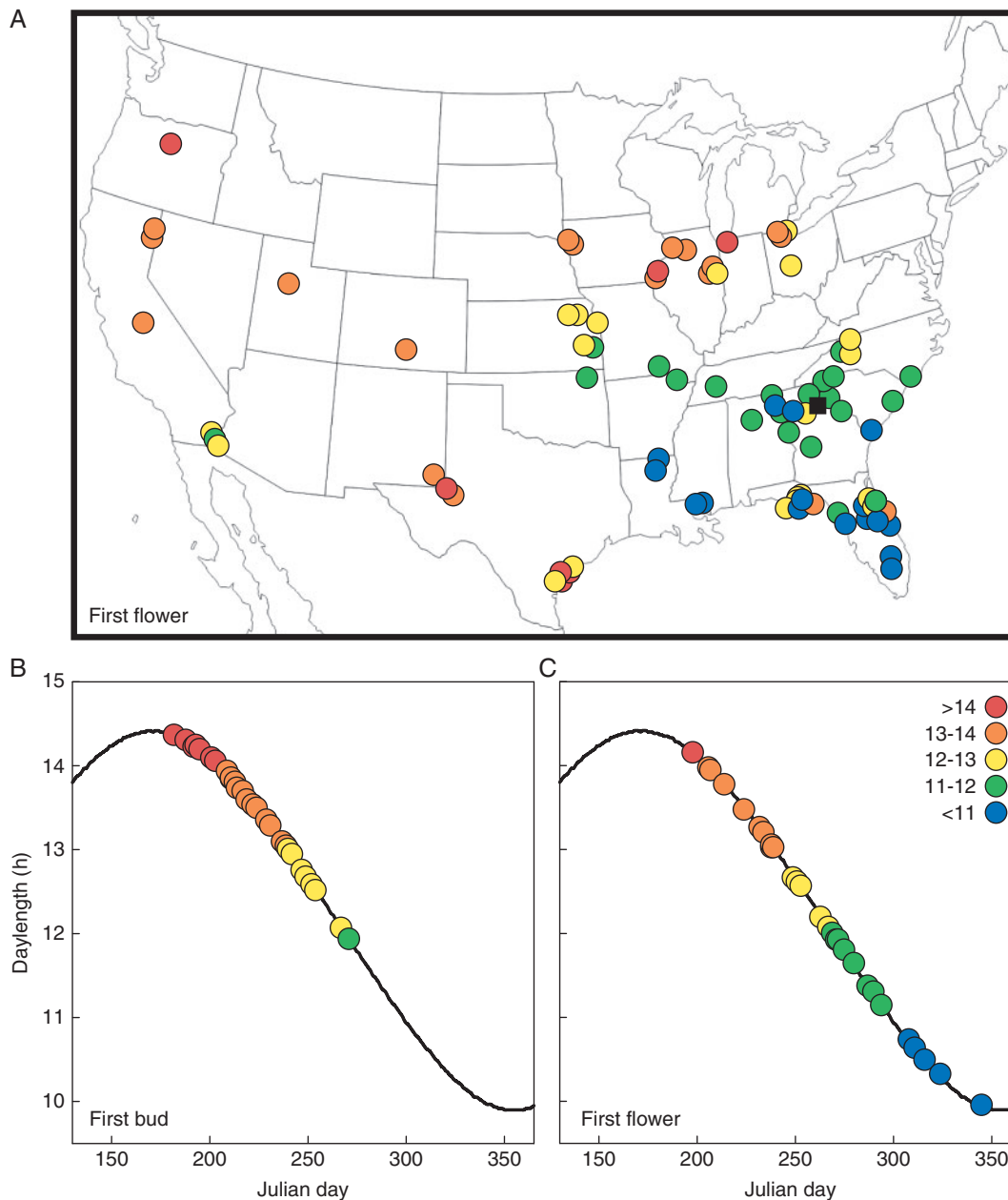First bud and first flower dates are considered in Julian days.

FIG. 3. Variation in reproductive timing across *Helianthus*. (A) Population mean daylength at first flower in Athens, Georgia common gardens (black square) plotted on population source sites across North America. (B) Species mean daylength at first bud and (C) first flower in Athens, Georgia common gardens.

often even within closely related groups such as genera) vary so much in size, architecture, longevity and seasonal phenology that they very probably experience similar interactions between organ functional traits and whole-plant allocation and phenology. Single classes of traits such as those that are usually considered to define the leaf economic spectrum are often poor proxies for whole-plant strategies without the context of whole-plant ecology and economics, as seen in *Helianthus* where species can have similar leaf economic traits despite differing dramatically in habitat occupancy, flowering time and whole-plant architecture. For example, using the first principal component of the standard six mass-based leaf economic traits, leaf

economic strategy is nearly identical between the desert annual *H. neglectus* and the tallgrass prairie perennial *H. grosseserratus*, between the granite outcrop annual *H. porteri* and the forest gap perennial *H. verticillatus*, and between the wetland annual *H. agrestis* and the pine savanna basal rosette perennial *H. radula* (Mason and Donovan, 2015). Clearly more information than leaf functional traits alone is needed to understand the role of leaf functional traits in species diversification, especially predicting how trait values determine individual fitness and how trait values are shaped by environment (Shipley *et al.*, 2016). Both of these current weak points in 'trait-based' ecology described by Shipley *et al.* (2016) seem largely

unaddressable outside of the context of whole-plant organization. Furthermore, the idea of building broad generalizations about functional traits in plant ecology without at least a rough understanding of how general plant architecture and phenology interact with key ecophysiological traits in roots, stems and leaves seems insurmountable (Godoy and Levine, 2014; Klimešová and Herben, 2016). The current lack of data on whole-plant organization in functional trait databases would therefore appear to be a major impediment to progress in 'trait-based' ecology, and expanded consideration of these key aspects of plant physiology in light of growth form and life history is needed to achieve meaningful synthesis.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: harvest dates for all species in both common garden years. Table S2: phylogenetic signal in plant traits across *Helianthus*. Table S3: pairwise correlations among source site environmental characteristics. Dataset S1: source information, trait data and environmental metrics used in this study.

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Data accessibility: Data used in this study are available in the Supplementary Data, and also available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.v3824.

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