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Genetic variation in reproductive timing in a long-lived herbaceous perennial

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Abstract

Premise: Reproductive fitness of individual plants depends on the timing of flowering, especially in mate-limited populations, such as those in fragmented habitats. When flowering time traits are associated with differential reproductive success, the narrow-sense heritability (h^2) of traits will determine how rapidly trait means evolve in response to selection. Heritability of flowering time is documented in many annual plants. However, estimating h^2 of flowering time in perennials presents additional methodological challenges, often including paternity assignment and trait expression over multiple years.

Methods: We evaluated the h^2 of onset and duration of flowering using offspring–midparent regressions and restricted maximum likelihood methods in an experimental population of an iterocarpic, perennial, herbaceous plant, *Echinacea angustifolia*, growing in natural conditions. We assessed the flowering time of the parental cohort in 2005 and 2006; the offspring in 2014 through 2017. We also examined the effects of the paternity assignment from Cervus and MasterBayes on estimates of h^2.

Results: We found substantial h^2 for onset and duration of flowering. We also observed variation in estimates among years. The most reliable estimates for both traits fell in the range of 0.1–0.17. We found evidence of a genotype by year interaction for onset of flowering and strong evidence that genotypes are consistent in their duration of flowering across years.

Conclusions: Substantial heritabilities in this population imply the capacity for a response to natural selection, while also suggesting the potential for differential contributions to adaptive evolution among seasons.

KEYWORDS

assortative mating, Asteraceae, *Echinacea angustifolia*, flowering phenology, heritability, offspring–midparent regression, paternity analysis, restricted maximum likelihood

The expression of flowering time arises from joint genetic and environmental influences on individuals and has a multitude of consequences for their reproductive success. For example, an individual's flowering time determines its access to potential mates (Galen and Stanton, 1991; Hatchwell, 1991; Ollerton and Lack, 1998), the resulting extent of assortative mating, and the conditions under which seeds develop, mature, and disperse. The specific outcomes for an individual's reproductive success depend on the magnitude and interaction of genetic and environmental effects on flowering time. Additionally, the degree to which variation in flowering time is attributable to genetic variation, or is heritable, determines the potential for adaptive evolution across generations. The consequences of flowering phenology for individuals' reproductive success and the long-term evolution of populations make it important to study the heritability of the timing of flowering.

Previous investigations have revealed genetic (Mora et al., 2009; Leinonen et al., 2013) and environmental (Dahlgren et al., 2007) contributions to among- and within-population variation in flowering phenology. Variation in reproductive phenology is widely observed, and many studies have found a genetic component to flowering time

(e.g., Best and McIntyre, 1972; Mazer, 1987; Andersson, 1996; O'Neil, 1997; Weis and Kossler, 2004; Franks et al., 2007). In a study of the annual plant species, *Plectritis congesta*, heritability of flowering time estimated in selfed and outcrossed natural populations ranged from 0.42 to 0.72 (Carey, 1983), while for open-pollinated experimental plots of the annual species *Brassica rapa* heritability estimates ranged from 0.51 to 0.67 (Austen and Weis, 2016; Ison and Weis, 2017). Studies of inbred accessions of annual plant species have demonstrated that numerous genes influence the date of flowering onset (maize, Buckler et al., 2009; *Arabidopsis thaliana*, Wilczek et al., 2010). Previous research has focused on annual plants, often in laboratory or greenhouse conditions. Far fewer studies have estimated genetic contributions to reproductive phenology of a perennial in a natural environment (Sedlacek et al., 2016; Fogelström and Ehrlén, 2019).

In many species, both onset and duration of flowering contribute to an individual's fitness. Both the onset of flowering and the duration will determine an individual's pool of potential mates. For example, an individual with early onset of flowering relative to other individuals and a short duration of flowering will have far fewer potential mates than an early-flowering individual with a long duration of flowering. Further, both the peak flowering date of a population and its degree of flowering synchrony, the overlap among individuals in a population (Augspurger, 1983), depend on the distribution of onset and duration (or cessation) of individuals' flowering (Elzinga et al., 2007). Thus, both onset and duration of flowering influence mating success and patterns of mating and are likely to be subject to both genetic and environmental influences (Sherry et al., 2007; Miller-Rushing and Primack, 2008; Wadgymar et al., 2018). However, the reproductive phenology of plants is often only characterized by the onset of pollen production or stigma receptivity (e.g., Buckler et al., 2009; Salomé et al., 2011), but characterizing both the onset and the duration of flowering will help to clarify reproductive consequences of variation in flowering phenology.

One result of variation among individuals in timing of flowering is potential for assortative mating, which can lead to temporal isolation of individuals within a population and complicates the estimation of heritability. Flowering time often varies sufficiently among members of a population (e.g., Whittet et al., 2017) to prevent mating between individuals whose flowering periods do not overlap, which reduces the number of potential mates and results in mating between individuals with similar phenology (phenological assortative mating). Over extended periods, assortative mating influences the evolution of genetic variance in populations (Godineau et al., 2022 and references therein) and can result in reproductive isolation of subpopulations by time. This isolation can create or exacerbate temporal genetic structure via limited gene transfer among subpopulations differing in reproductive phenology (Hendry and Day, 2005; Daïnou et al., 2012; Ison and Weis, 2017). The severity of isolation by time and temporal genetic structure within a population will depend heavily on the degree to which variation in parental phenotypes is heritable. Assortative mating also has immediate effects on the resemblances between pairs of relatives in that they are expected to be more similar than in the absence of assortative mating (Nagylaki, 1978, Falconer and Mackay, 1996). Few empirical studies address how assortative mating complicates the estimation of heritability.

As with most quantitative traits, environmental variation contributes to overall variation in flowering phenology. Phenotypic plasticity, defined as the difference in average trait value in response to a particular environmental factor, may be detected because environmental influences such as soil microbes (Wagner et al., 2014) act on individuals early in development but affect phenotypes throughout an individual's lifespan. In contrast, conditions that vary over an organism's lifespan, such as snow-melt date (Inouye et al., 2002), may influence phenotypic expression in each season. The relative contributions of environmental and genetic sources to variation in flowering time may change across an individual's lifespan, especially if gene expression is age- or stage-dependent or if effects of differences in the environment compound over time. Changes in these contributions are likely each year for traits like onset and duration of flowering, which are expressed multiple times per individual and subject to different environments. There may also be significant genetic variation for the expression of a trait in response to environmental conditions or cues, i.e., genotype by environment interactions (G × E), allowing for the evolution of plasticity in the trait. One consequence of G × E is that heritability estimates depend on the context in which they are estimated. Additionally, the subset of individuals expressing the trait varies each year affecting heritability estimates (Mazer and Schick, 1991). The context dependency of the expression of genetic variation means that the trajectory and rate of selection for a trait with substantial genotype–environment interaction can differ from year to year as environments vary (Allard and Bradshaw, 1964; Sultan, 2021). Because environments differ among years, evolutionary potential of reproductive timing depends not only on the genetic variance in traits, but also on the contributions of environmental sources of variation in these traits, the accumulation of environmental variation over individuals' lifetimes, and the dependence of genetic effects on environmental conditions in nature.

To clarify the capacity for adaptive evolution of flowering phenology, we here present a study of an experimental population of a widespread and common perennial native to the North American plains and prairie, *Echinacea angustifolia* (Asteraceae). In this study, we considered the onset and duration across multiple years and the challenges presented by assortative mating by accounting for trait correlations between mates. Finally, we quantified both the additive genetic variance and environmental variance in flowering time and their interaction across multiple years. We specifically (1) estimated the

heritability of two key flowering time traits, onset, and duration of flowering, across multiple years; and (2) assessed the genetic and environmental variation within years for these traits and quantified the interaction between genotype and year.

MATERIALS AND METHODS

Study site and species

This study focuses on the perennial plant *Echinacea angustifolia* DC, commonly known as the narrow-leaved purple coneflower, which is native to the Great Plains of North America. *Echinacea angustifolia* is found in remnants of prairie west of the Mississippi River, from Texas to Canada. It is a long-lived herbaceous species (Hurlburt, 1991), for which the time between germination and the first reproductive bout is considerable, generally from 3 to 8 years, but can extend to over 21 (Echinacea Project records). Once an individual flowers for the first time, it may not flower again for several years. Individuals flowering in a given year typically produce a single inflorescence (hereafter, "head"), but it is not unusual for an individual to produce multiple heads, especially after a spring fire (Wagenius et al., 2020). In west-central Minnesota where we conducted this study, *E. angustifolia* usually begins flowering in late June to early July and ends in early to mid-August.

The systematic progression of flowering within the elongated heads of *E. angustifolia* allows precise determination of the first and last days of flowering without requiring daily observations. We identify the onset of flowering as the day that the row of florets along the basal circumference of the head presents anthers shedding pollen. Then, 24 h later, styles emerge from those florets, and anthers emerge from the florets one row up the head. While the number of anthers and styles presented each day varies, this general pattern continues for the remainder of flowering (Wagenius, 2004). In this self-incompatible species, whose habitat is now severely reduced and extremely fragmented, flowering phenology plays a crucial role in pollination biology and evolutionary dynamics (Wagenius et al., 2020). Access to mates hinges on its flowering phenology, and phenology strongly influences reproductive fitness and assortative mating (Ison et al., 2014; Waananen et al., 2018).

This study focuses on an open-pollinated parental cohort and a cohort of its offspring from a single year (2005) growing in restored prairie habitat among species common to remnant sites where natural *E. angustifolia* populations are found. We quantified the date of flowering onset and duration of flowering of the offspring cohort for four seasons, 2014–2017, and parents for two, 2005–2006. During 1996–2000, almost all parental individuals were collected as seed from prairie remnants of varying sizes and quality located within 5 km of the planting site. A small proportion of the parental individuals resulted from a cross between the initial set collected from remnants; these individuals made up less than 4% of the parental cohort in our study. Seeds were then germinated and planted as juveniles into existing vegetation of an experimental field (see Ison and Wagenius, 2014: 2005 panels in Figures 1 and 2). This experimental restoration is called P1. Individual *E. angustifolia* plants were planted into P1 as seedlings in a grid formation with 0.33 to 1 m between plants. Within a planting-year cohort, individual plants were randomized, thus obviating spatial genetic structure that has been observed in nearby remnant *E. angustifolia* populations (Wagenius et al., 2007). During this study, the nearest flowering *E. angustifolia* individuals outside of P1 were in a much smaller experimental plot about 250 m away, while the nearest remnant *E. angustifolia* populations were over 400 m away. Previous research has found limited successful immigration of pollen, around 2%, from the experiment plot 250 m away (Ison et al., 2014). For more information regarding the spatial layout, establishment, and management of P1, please refer to Geyer et al. (2007), Wagenius et al. (2010), and Ison and Wagenius (2014).

The offspring originated from open pollination of the 224 of these plants that flowered in P1 during summer 2005. We randomly sampled 123 of these plants as the maternal plants for the offspring cohort. To span the range of a maternal plant's flowering, we aimed to sample the earliest 10 fertilized achenes (achenes in the basal rows of the earliest flowering head) and the latest 10 fertilized achenes (achenes in the top rows of the latest flowering head) per maternal plant (see Ison et al., 2014 for additional details). In May 2006, we planted a total of 3916 offspring into a second experimental field, P2, at the Hegg Lake Wildlife Management Area, ~8 km from P1. These plants were also planted into a grid (1×1 m spacing) in randomized locations, thus preventing spatial genetic structure within this cohort. A mean of 31.8 offspring were planted per maternal parent (SD ± 17.1). In 2014, 55% of these offspring were still alive.

Both P1 and P2 support diverse vegetation common in old fields, restorations, and prairie remnants in western Minnesota. They have access to native pollinator communities, and they are managed with prescribed burns. P1 is burned almost every 2 years, including spring 2005; P2 is burned infrequently, but was burned before summer 2015. In a 20-year study at a nearby prairie remnant, Wagenius et al. (2020) found that dormant-season fires usually increase population flowering synchrony of *E. angustifolia* in the season immediately following a burn.

Data collection

For the 224 flowering individuals in the parental cohort, flowering phenology was recorded in 2005 and for the 86 plants that flowered again in 2006. The offspring cohort's flowering phenology was recorded from 2014 to 2017. We observed the flowering of 209 individuals for the first time in 2014 and for 646 in 2015, 570 in 2016, and 661 in 2017.

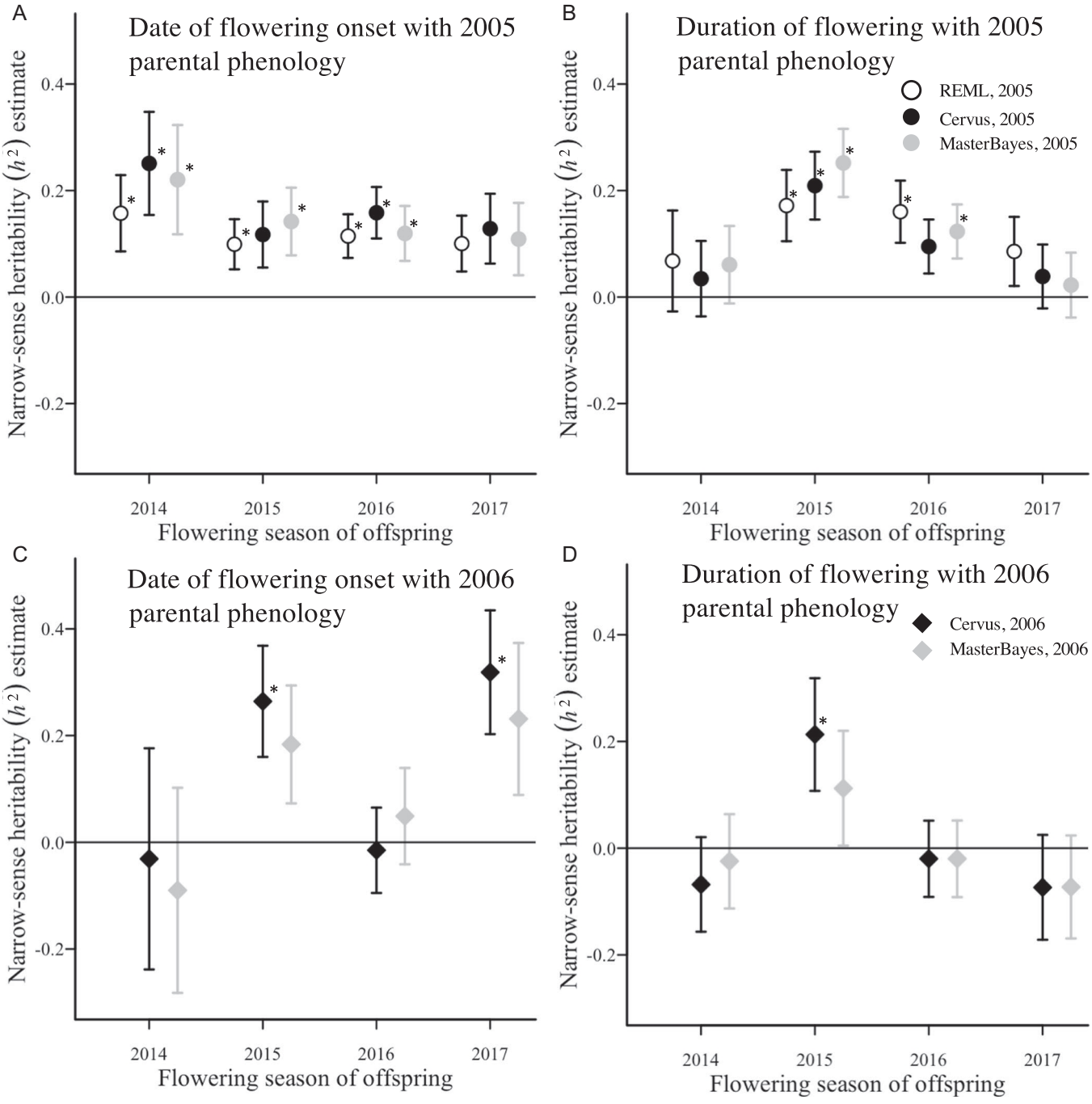

FIGURE 1 Estimates of narrow-sense heritability (h^2) from multiple paternity and estimation methods. (A) Estimates of h^2 for the date of flowering onset and (B) duration of flowering. Estimates in A and B are based on parental traits from 2005 and offspring traits in 2014–2017 using offspring–midparent (O-MP) regressions with Cervus and MasterBayes derived pedigrees and restricted maximum likelihood methods with Cervus pedigrees. (C) Estimates of h^2 for the date of flowering onset and (D) duration of flowering again but based on parental phenology from 2006 and offspring phenology from 2014 to 2017, including O-MP regression with Cervus and MasterBayes pedigrees. (*$P < 0.05$)

During 2005, we visited each plant every day between 24 June and 8 August (Appendix S1). For all plants, we recorded the first day of flowering, defined as the first day that pollen is produced by one or more anthers on an individual, and the last day of flowering, defined as the last day that pollen is produced by an individual. In 2006, we observed phenology between 24 June and 10 August. During the 2014–2017 observations, we visited each individual every 2 to 3 days when flowering began 2 July in 2014, 4 July in 2015, and 22 June in 2016 and 2017. Observations of the offspring population concluded on 10 August in 2014, 16 August in 2015, 24 July in 2016, and 8 August in 2017. We determined the date of flowering onset and the last day of flowering exactly for observations

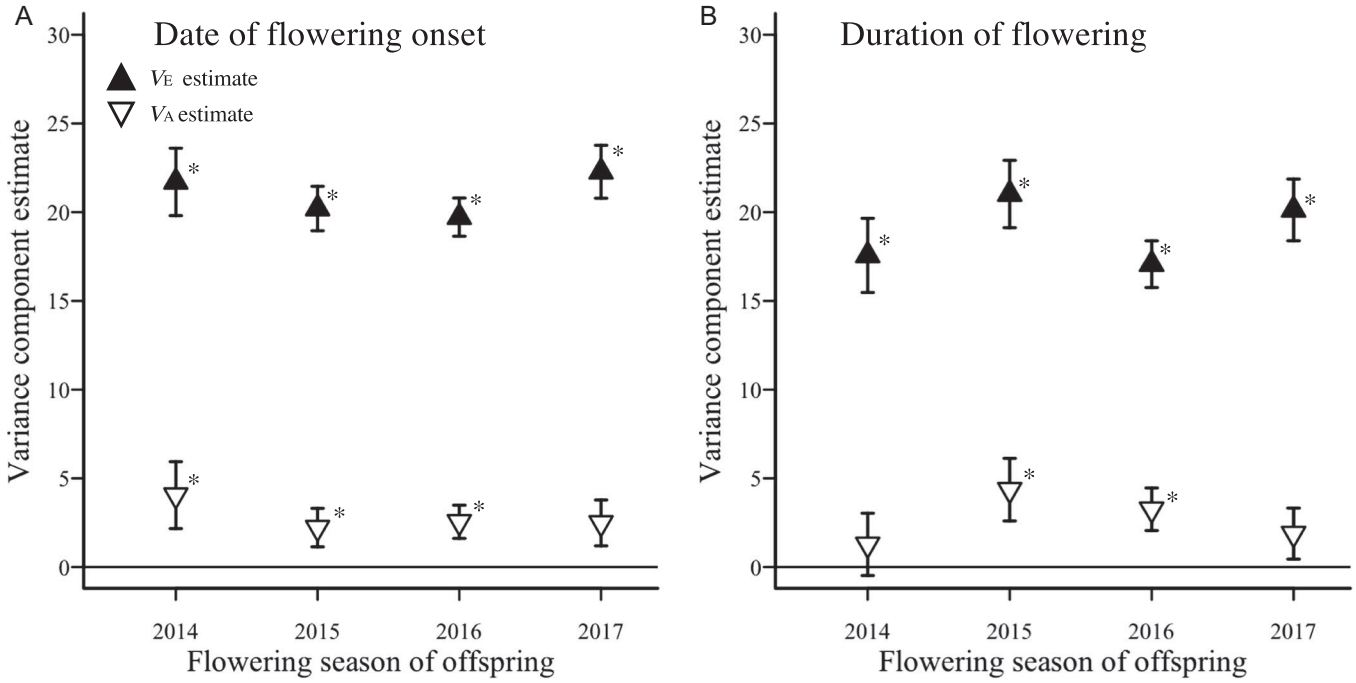

FIGURE 2 Estimates of additive (V_A) and environmental (V_E) variance components for date of flowering onset and duration of flowering in our study population of *E. angustifolia*. (A) V_A and V_E estimates for flowering onset. (B) V_A and V_E estimates for the duration of flowering. All results were produced using restricted maximum likelihood estimation methods. Phenotypic comparisons were made between offspring observed in 2014–2017 and parents observed in 2005. (*$P < 0.05$)

made at 2-day intervals; we interpolated these dates for observations at 3-day intervals. These records have an error of no more than 1 day.

Determining parent–offspring relationships

Estimation of h^2 requires information on the genetic relatedness between observed individuals. By noting the maternal plant of each offspring when the seeds were collected in 2005, we had direct knowledge of relationships with and through mothers, but not fathers. To infer the fathers, we collected leaf tissue from each flowering plant in P1 cohort and from the nearest flowering *E. angustifolia* in the small experimental plot ~250 m away. Finally, we collected samples of leaf tissue from individuals in the offspring cohort, either at planting or when the individuals first flowered. We extracted DNA from each tissue sample and amplified 11 microsatellite loci primarily using the protocols of Ison et al. (2013). All flowering plants and 182 offspring were genotyped for the Ison et al. (2014) study. An additional 157 offspring were genotyped for the Page et al. (2019) study. We genotyped the remaining 448 offspring at 10 microsatellite loci (dropping *Ech07*) using the Ison et al. (2013) procedure with the following adjustments. For these samples, fragments were analyzed using an ABI 3730 DNA Analyzer (Applied Biosystems, Waltham, MA, USA) and dye set G5. Each forward primer was labeled with a fluorescent dye (Ech03 dye NED, Ech05 dye VIC, Ech11 dye NED, Ech13/Ech13Z dye VIC, Ech15 dye PET, Ech28 dye 6-FAM, Ech36 dye PET, Ech37 dye 6-FAM, Ech47 dye VIC) (Thermo Fisher Scientific, Waltham, MA) and the size standard used dye LIZ. To ensure consistent allele scoring between the two procedures, we genotyped a haphazard selection of 96 samples, including 16 parental plant samples, a second time using the updated procedure.

We used two methods of parentage analysis to infer the father of each individual in the offspring cohort: categorical allocation and a full probability parentage analysis (reviewed by Jones et al., 2010). Each method is widely used but has potential advantages and disadvantages in ease of use, data requirements, and paternity assignment accuracy and number (Walling et al., 2010). We used Cervus 3.03 to assign paternity to offspring using a categorical allocation approach that applies maximum likelihood (Kalinowski et al., 2007). Parentage analyses typically allow a small number of genotypic mismatches between candidate fathers and offspring to allow for genotyping errors; when multiple individuals are non-excluded, categorical allocation approaches assign paternity to the candidate father with the highest likelihood or posterior probability of being the true parent, based on information such as allele frequencies, genotyping error rates, and the heterozygosity of the candidate fathers (Jones et al., 2010). In Cervus, a likelihood ratio is calculated for each potential sire, and

Delta (the difference between the natural log of the likelihood ratios of the two sires with the highest likelihood ratios) is calculated for the most likely sire. A user-parameterized simulation determines a critical Delta threshold for a given level of confidence in the assignment. For comparison, we also performed a full probability paternity analysis using the R package MasterBayes, which accommodates not only genotypic information but also other attributes, such as the proximity of candidate fathers to the maternal plant or other phenotypic information, in making assignments (Hadfield et al., 2006). MasterBayes uses a Bayesian framework to infer posterior distributions of parameters representing these attributes, including the parent–offspring pedigree, that maximize the model's overall posterior probability. The candidate father most frequently assigned to an individual offspring is then reported as its most likely father. In our model, we included parameters for the location, and thus proximities, of the maternal plant and candidate fathers and added a term to exclude self-pollination as a potential paternity assignment.

We assigned paternity to 787 offspring that flowered between 2014 and 2017. For the majority of these offspring (472 of 787), Cervus and MasterBayes assigned the same father. For two offspring, Cervus assigned paternity, but MasterBayes did not; for 54 offspring, MasterBayes assigned paternity, but Cervus did not. A small number of offspring (3 of 791) received paternity assignments that did not reach our confidence thresholds for either method. The remaining 189 offspring were assigned different fathers under the different methods. In these cases, the flowering traits of the different fathers assigned by each program flowering time and duration of the fathers assigned by each program were not substantially correlated (start date in 2005: Pearson's r [186] = 0.06; $P = 0.43$; duration in 2005: r [186] = –0.06; P = 0.38). Accordingly, we do not expect erroneous assignments to bias heritability estimates; rather, they may reduce estimates and increase their uncertainty to a modest degree.

For our heritability analyses, we included offspring that flowered at least once between 2014 and 2017 and to which we were able to assign paternal plants using Cervus or MasterBayes. Using Cervus, we assigned paternity to 90% of the offspring that flowered between 2014 and 2017 and exceeded the commonly used relaxed (80% confidence) critical value threshold of Delta scores (708 of 787 genotyped offspring). Using MasterBayes, we assigned paternity to 93 percent of these offspring (738 of 787 genotyped offspring) based on pedigrees that maximized the model posterior probability. We restricted the pedigree to offspring assignments in which the same candidate father was assigned in at least 20% of the MCMC iterations. These pedigrees represent over half of the offspring that flowered between 2014 and 2017 (1178 individuals). Because Cervus and MasterBayes differ both in their statistical properties and in the data used for inference, the two methods did not always make the same paternity assignments. Thus, we separately analyzed the data based on pedigrees estimated by each program.

Heritability estimation

We estimated narrow-sense heritabilities (h^2), the proportion of variance in a trait (V_P) that is due to additive genetic variance (V_A), for flowering onset and duration of flowering in two ways. First, for each trait, we used regressions of offspring trait value on the average of the two parental trait values, with separate analyses for the traits of offspring expressed in each year and for the traits expressed by parents in 2005 and 2006 (i.e., altogether eight analyses). Note that only 38% of parental plants flowered in 2006. To assess the stability of heritability estimates among years and as plants age, we estimated heritability in each of a series of years for a subset of the offspring, those that first flowered in 2014, hereafter referred to as the 2014 flowering cohort. Estimates for this 2014 flowering cohort were based on *any* individual that first flowered in 2014. For this analysis, we used parental traits as expressed in 2005. In these regressions of offspring on mid-parent traits, the estimate for narrow-sense heritability (h^2) is given by the estimate of the slope in each of the regressions and is not influenced by assortative mating (Falconer and Mackay, 1996). We used base R version 4.1.2 (R Core Team, 2021) for these regressions.

Estimation of heritability via offspring–parent regression is appealing for its simplicity and has been widely used. However, this approach assumes that all offspring–parent pairs are independent and does not account for the resemblance between individuals in other relationships, such as full and half-sibs, including reciprocals, which are prevalent in both cohorts (Table 1). Thus, this approach does not make use of all the available information, nor does it validly represent the sampling variance. To take full advantage of the data using general, rigorous methods, we also analyzed these data sets via restricted maximum likelihood (REML) using Quercus quantitative genetic software (Shaw and Shaw, 1994). We estimated the additive genetic variance (V_A) and the environmental variance (V_E) using a model that constrained the dominance and maternal variances to zero. Because only a small proportion of the relationships were full sibs, there is little information about

TABLE 1 Maternal family size summaries for offspring cohort in each year including overall family size.

Statistic	2014	2015	2016	2017	Overall
Mean	2.4	3.86	4.89	3.59	13.08
Median	2	3	4	3	10
Minimum	1	1	1	1	1
Maximum	9	14	18	13	53

dominance variance. We expected maternal variance to be small because no traits were measured on juveniles, in which maternal effects tend to be strongly expressed (Roach and Wulff, 1987), and preliminary analyses estimated maternal variance to be very close to 0. To account for assortative mating with respect to flowering onset, we incorporated the observed correlation between mates into the expressions of the expected resemblances between relatives (Nagylaki, 1978; Lynch and Walsh, 1998) by modifying a version of Quercus to include the enhanced correlations in the phenotypic covariance matrix and its derivatives (as also done by Shaw et al., 1995). The phenotypic correlation between mates with respect to flowering onset was included for all pairs of plants that produced offspring included in this study. For flowering duration, the correlation between mates was very near zero and was not included in the analysis of this trait.

Our REML models included year as a categorical fixed factor to account for the effect on phenology of differences in environments between the years that parent and offspring cohorts were observed. To estimate the standard error for the heritability estimates obtained via REML, we implemented parametric bootstrapping using the R package MASS (Venables and Ripley, 2002) and R version 4.1.2 (R Core Team, 2021) using the estimates of V_A and V_E, together with their sampling variances and covariances.

To investigate interaction between genotypes and annual environmental conditions, we analyzed each trait expressed in different years as distinct character states (Falconer, 1952), obtaining estimates of V_A and V_E for trait states in different years and the genetic and environmental correlations between them. This approach has the benefit of distinguishing the two contributors to G × E interaction: a difference in genetic variance between environments, which would suggest a difference in the amount of genetic variation available to support response to selection, and a difference in genotypic ranking between environments, which is reflected in a genetic correlation between conditions substantially less than one (Falconer, 1952). We present this correlation as an estimate of the extent to which genotypic expression is consistent between environmental contexts. We note that, for correlations between estimated breeding values, in contrast to standard Pearson product-moment correlations, there is no straightforward significance test of the null hypothesis that the genetic correlation equals one, corresponding to the case of fully consistent genotypic expression in different environments. We provide these correlations only as a rough guide to the degree of genetic association. We also estimated the environmental correlations as the measure of the effects of individuals' local conditions on their trait expression in the 2 years. As noted above, burn incidence differed between years in both cohorts; though we recognize that many other environmental conditions also likely differed among years, the trait states that we designated align with this distinction. For the parents, trait values from 2005 (the year the offspring were produced) were expressed in a burn year and those from 2006 as expression in a year without a burn. Mirroring this structure, for offspring, phenotype expressed in a burn year comprised trait values observed in 2015 and the non-burn phenotype expressed in a non-burn year comprised values recorded in 2016.

All phenology and microsatellite data are archived on the Echinacea Project website (http://echinaceaproject.org/datasets/h2-fl-phenology-2022/); all scripts used in analyses are archived on the bitbucket code repository (https://bitbucket.org/wjreed2/heritability_2). Quercus REML software (and modified versions used in these analyses) is available at https://cbs.umn.edu/academics/departments/eeb/quercus.

RESULTS

Qualitative results do not differ between results based on pedigrees from assignments by MasterBayes or Cervus. Here we present quantitative results based on assignments made in Cervus. Quantitative results based on MasterBayes assignments and comparison of paternity assignment methods are available in Appendices S2 and S3.

Mid-parent regressions

Both date of flowering onset and duration of flowering exhibited substantial heritability based on regressions of offspring values observed in single years in 2014–2017 on mid-parent values from 2005, the year in which the offspring were produced (Figure 1). For flowering onset, all estimates of h^2 exceeded 0.1 and were statistically significant or marginally so. Estimates of heritability for duration of flowering were sizable in 2015 and 2016, but not 2014 or 2017) (Table 2).

To further evaluate the context-dependence of heritability estimates, we conducted a set of offspring–midparent regressions using offspring values observed in 2014–2017 with midparent values observed in 2006, the year following offspring production (Figure 1C, D), when only about one third of parental individuals flowered. Thus, sample sizes were considerably smaller for these analyses, which yielded estimates of heritability that differ strikingly from those based on trait values of parents obtained in 2005. In these analyses, we found little support for heritability in flowering duration except in 2015. To assess the possible impact of plant size on the heritability estimates, we included results that consider the number of flowering heads produced by an individual as a covariate in regressions in Appendix S4. When including the number of flowering heads, patterns of h^2 across years remained the same when considering the number of flowering heads. However, the magnitude of estimates varied some, particularly for the duration of flowering.

TABLE 2 Estimates of narrow-sense heritability based on different paternity assignment methods and for multiple years of parental and offspring phenology observed. All estimates are based on offspring–midparent regressions.

Trait	Paternity assign. method	Parental	Offspring	h^2	SE	*P*	df	*N*
Onset	Cervus	2005	2014	0.25	0.10	0.010	200	202
			2015	0.12	0.06	0.059	408	410
			2016	0.16	0.05	0.001	551	553
			2017	0.13	0.07	0.051	390	392
		2006	2014	−0.03	0.21	0.881	76	78
			2015	0.26	0.10	0.012	155	157
			2016	−0.01	0.08	0.852	223	225
			2017	0.32	0.12	0.007	137	139
	MasterBayes	2005	2014	0.22	0.10	0.033	190	192
			2015	0.14	0.06	0.026	386	388
			2016	0.12	0.05	0.021	522	524
			2017	0.11	0.07	0.109	369	371
		2006	2014	−0.09	0.19	0.641	75	77
			2015	0.18	0.11	0.099	157	159
			2016	0.05	0.09	0.588	224	226
			2017	0.23	0.14	0.107	141	143
Duration	Cervus	2005	2014	0.03	0.07	0.627	200	202
			2015	0.21	0.06	0.001	408	410
			2016	0.09	0.05	0.062	551	553
			2017	0.04	0.06	0.518	390	392
		2006	2014	−0.07	0.09	0.446	76	78
			2015	0.21	0.11	0.045	155	157
			2016	−0.02	0.07	0.782	223	225
			2017	−0.07	0.10	0.457	137	139
	MasterBayes	2005	2014	0.06	0.07	0.404	190	192
			2015	0.25	0.06	0.001	386	388
			2016	0.12	0.05	0.016	522	524
			2017	0.02	0.06	0.712	369	371
		2006	2014	−0.02	0.09	0.782	75	77
			2015	0.11	0.11	0.298	157	159
			2016	−0.02	0.07	0.782	224	226
			2017	−0.07	0.10	0.453	141	143

Restricted maximum likelihood

REML analysis yielded estimates of additive genetic variance (V_A) and environmental variance (V_E); from these estimates, we derived narrow-sense heritability estimates by $V_A/(V_A + V_E)$ (Figure 1A, B). As with the heritability estimated by offspring–midparent regression, the REML estimates also differed somewhat among the single flowering years, though generally within the standard error of the estimate. The estimated components of variance offered insight into the variation in estimates of heritability. For both traits, the year with the highest heritability estimate

was also the year with the greatest estimate of V_A (2014 for flowering onset; 2015 for duration), despite the relatively large, estimated V_E in each case (Figure 2, Table 3).

Heritability in different years

Considering the 2014 flowering cohort in the years 2015–2017, we observed a decline in heritability estimates for flowering onset (Figure 3A), though the estimates were within sampling error. This trend was reflected in both offspring–midparent regressions and REML analysis. Results for heritability estimates were mirrored by a large decrease in the estimates of V_A, particularly for 2016 and 2017. V_E estimates for 2014, 2016, and 2017 were similar and exceeded those for 2015 (Appendix S5). Results for duration were similar overall to our estimates for the entire population (Figure 3B). Estimates of V_A for duration of flowering largely followed the same pattern as h^2 estimates for this trait. V_E decreased marginally during this study (Appendix S5). Heritability from offspring–midparent regressions and REML analyses for the 2014 flowering cohort are summarized in Table 4. We also included heritability estimates for onset and duration of flowering for the cohort that first flowered in 2015 in Appendix S6.

TABLE 3 Estimates for additive (V_A) and environmental (V_E) variance components of phenotypic variation in date of flowering onset and duration of flowering in a single year estimated via restricted maximum likelihood. In each case, the likelihood ratio test of the null hypothesis that $V_A = 0$ has 1 df (*$P < 0.05$).

Trait	Offspring	V_A	SE	V_E	SE	h^2	SE	N
Onset	2014	4.05*	1.88	21.71*	1.90	0.16*	0.07	202
	2015	2.22*	1.09	20.21*	1.25	0.10*	0.05	410
	2016	2.55*	0.94	19.72*	1.07	0.11*	0.04	553
	2017	2.49	1.29	22.28*	1.49	0.10	0.05	392
Duration	2014	1.28	1.76	17.56*	2.09	0.07	0.09	202
	2015	4.36*	1.76	21.02*	1.90	0.17*	0.07	410
	2016	3.26*	1.20	17.07*	1.32	0.16*	0.06	553
	2017	1.89	1.44	20.13*	1.74	0.09	0.06	392

Genotype × environment interaction

The genetic correlation for the date of flowering between burn and non-burn years was 0.34 (CovA, not significantly different from 0), indicating weak consistency of genetic effects between years and suggesting a genetic contribution to differences among years in the order of flowering onset. For the duration of flowering, by contrast, we detected a strong genetic correlation of 0.84 between the two seasons. We also estimated the environmental correlation between burn and non-burn environments, and we found small, nonsignificant correlations for both the date of flowering

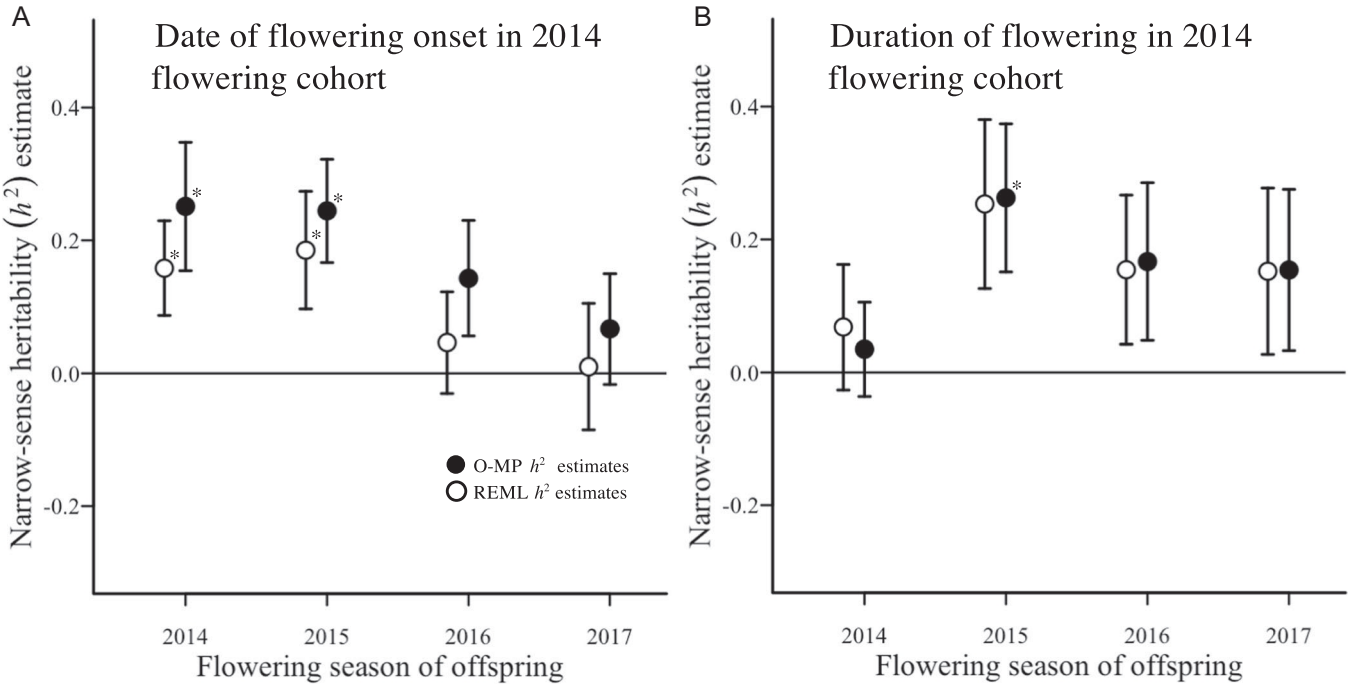

FIGURE 3 Estimates for the narrow-sense heritability (h^2) of the 2014 flowering cohort based on offspring–midparent (O-MP) and restricted maximum likelihood methods (REML). (A) Estimates for the date of flowering onset. (B) Estimates for duration of flowering. All estimates are derived from the subset of the offspring that flowered for the first time in 2014 and then flowered again in subsequent years. Not all individuals that first flowered in 2014 flowered again from 2015 to 2017. (*$P < 0.05$)

TABLE 4 Estimates of narrow-sense heritability (h^2) for the cohort of individuals that first flowered in 2014. All values in this table compare offspring traits from 2014–2017 and to parent trait values from 2005. Heritabilities estimated using regression of offspring on parent ($h^2_{O\text{-}MP}$) and by REML, taking into account all pedigree information (h^2_{REML}). Reported *P*-values show significance of $h^2_{O\text{-}MP}$ while an asterisk indicates h^2_{REML} estimates that are significant at a 0.05 level or lower. N reports the number of offspring included in each estimate.

Trait	Offspring	$h^2_{O\text{-}MP}$	$SE_{O\text{-}MP}$	*P*	h^2_{REML}	SE_{REML}	$df_{O\text{-}MP}$	*N*
Onset	2014	0.25	0.10	0.010	0.16*	0.07	200	202
	2015	0.24	0.08	0.002	0.19*	0.09	129	131
	2016	0.14	0.09	0.103	0.05	0.08	144	146
	2017	0.07	0.08	0.426	0.01	0.10	121	123
Duration	2014	0.03	0.07	0.627	0.07	0.09	200	202
	2015	0.26	0.11	0.020	0.25	0.13	129	131
	2016	0.17	0.12	0.163	0.15	0.11	144	146
	2017	0.15	0.12	0.207	0.15	0.13	121	123

TABLE 5 Estimates of additive genetic variance (V_A) and environmental variance (V_E) from analysis to estimate genotype × environment interactions. Estimates here are derived from both offspring (2015, 2016) and parental cohorts (2005, 2006) in consecutive years. In each case, the likelihood ratio test of the null hypothesis that $V_A = 0$ has 1 df (*$P < 0.05$).

Trait	Year	V_A	SE_{va}	V_E	SE_{ve}
Onset	2015	6.48*	2.12	17.28*	2.03
	2016	3.73*	1.16	17.39*	1.34
Duration	2015	3.08	2.09	24.25*	2.40
	2016	2.96*	1.14	17.21*	1.43

onset and duration of flowering. The environmental correlation between burn and non-burn conditions for flowering onset was −0.03. Duration of flowering had an environmental correlation of 0.12. All estimates of variance components for the G × E analysis are summarized in Table 5. These very low values imply that individuals' local conditions, such as factors specific to their position in the garden, do not induce strong similarity in flowering phenology between years. Visualization of offspring traits across the 2015 and 2016 annual environments is available in Appendix S7.

DISCUSSION

We detected substantial additive genetic contributions to intraseasonal variation in flowering phenology in a Minnesota population of the herbaceous perennial, *E. angustifolia*, represented by an 8- to 11-year-old cohort of offspring from open pollination, along with their parents. Our estimates elucidate this population's capacity for genetic response to selection on both the onset date and duration of flowering, the former trait generally exhibiting stronger heritability (h^2). In both traits, our estimates varied in magnitude among seasons. For flowering onset, we found substantial evidence of interaction between genotype and year of flowering.

Consequences for heritability of flowering time traits

Our estimates of heritability indicate this population of *E. angustifolia* could respond to selection on onset of flowering, which is prevalent in our population and many others. Specifically, in a 3-year study focusing on the parental cohort of this study, individual seed set (a measure of female fitness) decreased with later onset dates and the effect was consistent each year (Ison and Wagenius, 2014). A meta-analysis of 71 studies reporting onset and measures of fitness (predominantly fruit or seed set) found prevalent phenotypic selection for earlier flowering time (Munguía-Rosas et al., 2011). Given this evidence of selection, we might expect to see a shift toward earlier onset dates, though the strength of the response to selection each year would fluctuate with the magnitude of heritability and changes in selective pressures, which vary among years in some systems due to effects of antagonists (Ehrlén, Münzbergová, 2009) and other environmental conditions (Ehrlén and Valdés, 2020).

We found heritable variation in duration of flowering, a prerequisite for response to selection to occur. In other species, duration of flowering, measured by the duration of stigma receptivity, was linked to higher seed set (Liu et al., 2021). However, the relationship between reproductive success and duration of flowering in our system is less clear. In this population's parental cohort, seed set was not strongly or consistently associated with flowering duration (Ison and Wagenius, 2014). However, in the same populations (Ison et al., 2014) showed

that siring success, a component of male reproductive fitness, increased with flowering synchrony, which is influenced by the duration over which plants flower (Augspurger, 1983). Similarly, when flowering duration in *E. angustifolia* was studied over 20 years in a nearby preserve (Wagenius et al., 2020), a longer flowering duration was associated with increased flowering synchrony and higher seed set, though not consistently over years. Thus, a longer duration of flowering allows for more overlap between individuals in the population, increasing synchrony and allowing for selection on duration of flowering. Selection for longer duration of flowering may particularly enhance fitness of individuals who flower early (Austen et al., 2017) given that onset and duration of flowering are often correlated (Hendry and Day, 2005).

The years an individual reproduces also influences its access to mates and potential reproductive success (Waananen et al., 2018). Additionally, in this study, we observed the potential for different contributions to evolutionary change among seasons for this population of *E. angustifolia*. This result suggests that the subset of individuals flowering in a year with high additive genetic will have the potential to contribute more to the evolutionary trajectory of the population than individuals that flower in years with low V_A. If selection on flowering time in our population varies from year to year, higher heritability could offset the effects of weak selection or amplify strong selection. Differential genetic contributions among years could also have important ramifications if there are genetic determinants for which years an individual flowers, especially if they are correlated with certain phenotypes for the onset and duration of flowering. We emphasize that in this study we considered only genetic variation in the timing of reproduction within years. To fully understand how selection on flowering time may proceed within populations, it is likely important to also consider genetic variation in the timing of reproduction among years, including age at first flowering and intervals between flowering bouts.

Our declining estimates of h^2 for 2014–2017 derived from the cohort of offspring that flowered for the first time in 2014 (Figure 3) suggest that resemblance between offspring and parental phenotypes for flowering onset decreased as individuals aged. Estimates of V_A and V_E indicate that this decline is related to a decrease in V_A rather than an increase in V_E. The scale of these contributions could also change (possibly directionally) over an individual's lifespan. It is important to note that we cannot separate the effects of age from year effects, but this finding does suggest that there could be larger contributions to evolutionary change earlier in an individual's lifetime, a potentially important consideration for iteroparous organisms, whether it is due to accumulated environmental effects or effects of age.

Genotype × environment interaction

The analyses of traits in pairs of successive years address the questions: How consistent are the genetic effects on flowering phenology in different years? If they are not consistent, in what ways do they differ between years? Two aspects of genetic expression can contribute to G × E interactions: imperfect correlation (i.e., deviation from 1) between genetic effects on trait values expressed in different environments (here, years) and difference in the additive genetic variance of traits expressed in different years. In our study, we found substantial evidence for both contributors to G × E interaction.

The genetic and environmental correlations for the onset of flowering imply a genetic basis for variation in response to annual environmental conditions and the potential for the evolution of plasticity in this trait. Thus, there is a capacity for the evolution of onset of flowering in our study population through selection on this trait in environmentally distinctive years, such that plasticity of this trait also evolves. The conditional expression of genetic variation for onset of flowering is consistent with patterns in our estimates of heritability in different years and indicates that the rate of evolutionary change will vary from year to year (Allard and Bradshaw, 1964; Sultan, 2021). For example, substantially greater V_A (and h^2) in 2015 implies that selection under these conditions would more greatly contribute to evolutionary change in this trait than in 2016. While there were many differences between the 2015 and 2016 seasons, one large difference was a spring burn in 2015. Further study will be needed to assess the generality of burn effects on these genetic properties. In addition to the consequences for selection, the low genetic correlation between years for flowering onset indicates that the genetic tendency of a plant to flower early or late is not preserved across annual environments, a phenomenon observed in other studies of responses to environmental cues (Blackman, 2017), and an important adaptation to varying environments (Anderson et al., 2012). Shifts between years in the genotypic rank order of flowering onset likely increases the genotypic variability of the pool of potential mates for individuals across their lifetimes. Thus, while assortative mating is reported in our study system (Ison et al., 2014), shifts in rank order of flowering time by genotypes may alleviate its potentially detrimental consequences, hindering the formation of temporal genetic structure.

By contrast, for the duration of flowering between two consecutive seasons, we found little evidence of interaction between genotype and year of flowering. The duration of flowering for genotypes in this population was extremely consistent; the genetic correlation between years for this trait exceeded 0.8, and the additive genetic variances were similar between years. Moreover, our estimate for the environmental correlation of duration of flowering suggests that there is no predictable effect of an individual's local environment between years. Our results imply that

regardless of the environmental variation experienced, including a spring burn, the genetic contribution to the duration of flowering remains consistent.

Genetic inferences and methods: paternity assignments and heritability estimates

We estimated the h^2 of two components of reproductive phenology in *E. angustifolia* in eight distinct flowering combinations of seasons, two parental and four offspring, using two methods for paternity assignment and two for estimation (Figure 1; Tables 2 and 3). Among all methods, there is qualitative agreement in their detection of a substantial quantitative genetic basis for flowering time in our study population. Despite differences in the pedigrees assigned independently by Cervus and MasterBayes, for both traits, h^2 estimates and interannual patterns based on each differed only modestly. Likewise, our annual estimates from offspring–midparent regressions were mostly similar to their REML counterparts (Figure 1A, B). Nevertheless, we emphasize that the REML analyses provide a more nuanced estimate of h^2 by accounting for the phenotypic correlation between mates (assortative mating) and by considering sibling and half-sibling relationships in this data set. Further, the REML analysis directly estimates both additive and environmental variance, allowing for a more robust examination of contributions from genetics and the environment (Figure 3).

Heritability and its implications depend on the context in which it is estimated

The abundance of studies in the literature about selection on flowering onset reflect a long-standing interest in the evolutionary dynamics of flowering phenology (Widén, 1991; Ollerton and Lack, 1992; O'Neil, 1997). In our study, heritability estimates for the date of flowering onset varied across the distinct annual environments in which offspring cohorts were observed (Figure 1A) and varied even more dramatically when observing the parental cohort in different seasons (Figure 1C). We found larger differences in h^2 estimates for duration of flowering among years (Figure 1B). This variation across years is an important consideration when interpreting how populations will respond to selection over time, especially in iteroparous species. One potential contributor to the observed variation, could be that a different subset of individuals flowers each year. Which individuals are measured could be as important in estimating heritability as when and where they are measured. Our results imply substantial context-dependence of heritability of flowering time traits. The differences in h^2 estimates we observed across environments and among cohorts indicates the potential perils of making inferences about the capacity for evolutionary response from short-term studies. These results also highlight the value of estimating selection and h^2 in the same system and at the same time.

AUTHOR CONTRIBUTIONS

J.L.I. and S.W. designed and established experimental populations and began fieldwork. A.W., J.L.I., S.W., and W.J.R. collected the data. A.W., J.L.I., F.H.S., R.G.S., and W.J.R. conducted all statistical analyses. W.J.R. prepared the first draft of the manuscript. All authors contributed to manuscript revisions.

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DATA AVAILABILITY STATEMENT

Data and analysis scripts are available for this study and can be accessed at: http://echinaceaproject.org/datasets/h2-fl-phenology-2022.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Flowering schedules of *Echinacea angustifolia* for years included in this study.

Appendix S2. Further comparisons of paternity inference software.

Appendix S3. Estimates for heritability (h^2) when using pedigrees in which the same candidate father was assigned by both Cervus and MasterBayes.

Appendix S4. Estimation of heritability including measures of plant size.

Appendix S5. Estimates of additive (V_A) and environmental (V_E) variance components for the cohort of individuals that first flowered in 2014.

Appendix S6. Heritability estimates for cohort that first flowered in 2015 and in subsequent years (2016 and 2017).

Appendix S7. Offspring trait values across annual environments in 2015 and 2016.

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