The strength of assortative mating for flowering date and its basis in individual variation in flowering schedule

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Abstract

Although it has been widely asserted that plants mate assortatively by flowering time, there is virtually no published information on the strength or causes of phenological assortment in natural populations. When strong, assortative mating can accelerate the evolution of plant reproductive phenology through its inflationary effect on genetic variance. We estimated potential assortative mating for flowering date in 31 old-field species in Ontario, Canada. For each species, we constructed a matrix of pairwise mating probabilities from the individual flowering schedules, that is the number of flower deployed on successive dates. The matrix was used to estimate the phenotypic correlation between mates, ρ, for flowering date. We also developed a measure of flowering synchrony within species, S, based upon the eigenstructure of the mating matrix. The mean correlation between pollen recipients and potential donors for flowering date was $\bar{\rho} = 0.31$ (range: 0.05–0.63). A strong potential for assortative mating was found among species with high variance in flowering date, flowering schedules of short duration and skew towards early flower deployment. Flowering synchrony, S, was negatively correlated with potential assortment ($r = -0.49$), but we go on to show that although low synchrony is a necessary condition for phenological assortative mating, it may not be sufficient to induce assortment for a given phenological trait. The potential correlation between mates showed no seasonal trend; thus, as climate change imposes selection on phenology through longer growing seasons, spring-flowering species are no more likely to experience an accelerated evolutionary response than summer species.

Introduction

Assortative mating can accelerate evolution by amplifying additive genetic variance. Fisher's foundational paper on quantitative genetics (Fisher, 1918) dealt explicitly with heritable effects of assortative mating, which he quantified as the phenotypic correlation between mates for the assorting trait, denoted here as ρ. When the assorting trait is heritable, there will be a genetic correlation between mates equal to the product ρh^2. Because of this correlation, offspring receive alleles with similar trait effects from both parents. This increases the frequency of extreme genotypes, causing departures from Hardy–Weinberg and gametic equilibrium, all without any change in underlying allele frequencies (Fisher, 1918; Wright, 1921; Breese, 1956; Felsenstein, 1981; Lynch & Walsh, 1998).

Wright (1921) quantified the potent inflationary effect of assortative mating on genetic variance. Assume an infinite, randomly mating, ancestral population with additive genetic variance G_0 in a selectively neutral focal trait and no mutation. Subjecting this population to many generations of assortative mating for the focal trait at level ρ, inflates the genetic variance to an

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equilibrium level

$$\hat{G} = \frac{G_0}{1 - \rho \hat{h}^2 \left(1 - \left[\frac{1}{2n_e}\right]\right)},$$

where $\hat{h}^2$ is the trait's heritability at equilibrium and n_e is the effective number of segregating loci (see Felsenstein (1981) for a detailed account of this relationship). Perfect assortative mating ($\rho = 1$) inflates genetic variance to nearly $2n_e G_0$ (Devaux & Lande, 2008). This inflation makes the descendent generation more responsive to selection than the ancestral.

Additionally, assortative mating can contribute to population differentiation by diminishing gene flow (Dieckmann & Doebeli, 1999; Vines & Schluter, 2006; Bolnick & Kirkpatrick, 2012) and can reduce effective population size (Devaux & Lande, 2008).

These potential effects on trait evolution invites the question of how strong assortative mating might be for particular types of traits. Jiang *et al.* (2013) noted that although many examples of assortative mating had been documented for traits involved in animal mate choice, such as size, colour and condition, it was unclear if the average correlation between mates was weak or strong. Their meta-analysis of published estimates of ρ revealed a mean correlation of $\bar{\rho} = 0.28$. In light of Wright's formulation, these levels of assortative mating could have consequential effects on selection response and population divergence.

Are there plant traits subject to comparable levels of assortative mating? Many have suggested *a priori* that plants mate assortatively by flowering date (Breese, 1956; Jain, 1979; Hartl & Clark, 1989; Lynch & Walsh, 1998; Hedrick, 2000; Kirkpatrick, 2000; Fox, 2003). The logic is clear: early bloomers tend to exchange pollen with other early bloomers, and late with late. Given the extensive evidence for genetic variation in flowering time (e.g. Pors & Werner, 1989; Fox, 1990; Dorn & Mitchell-Olds, 1991; Mazer & Schick, 1991; Conner & Via, 1993; O'Neil, 1997; Quinn & Wetherington, 2002; Gerber & Griffin, 2003), Fox (2003) remarked that assortative mating by flowering time should be ubiquitous.

Knowing the general intensity of phenological assortative mating is important for several reasons. A strong correlation between mates for flowering date would facilitate evolutionary responses to climate change. Evidence is growing that the earlier arrival of spring and later end of summer is imposing selection on the timing of life history transitions (Bradshaw & Holzapfel, 2008; Forkner *et al.*, 2008; Hoffmann & Sgro, 2011). In plants, selection should favour flowering times that take better advantage of the new growing season length (Franks *et al.*, 2007). Phenological mismatch between plants and their pollinators or seed predators, caused by differential plastic responses to warming, may also generate selection on flowering date.

Assessing the strength of phenological assortment can also inform our understanding of local adaptation and speciation. Flowering time frequently diverges between adjacent populations or sister species (e.g. Antonovics & Bradshaw, 1970; Ellis *et al.*, 2006; Savolainen *et al.*, 2006; Martin *et al.*, 2007; Lowry *et al.*, 2008; Devaux & Lande, 2009; Franks & Weis, 2009; Levin, 2009; Colautti *et al.*, 2010) as the timing of transition from vegetative growth to reproduction responds to local selection. Thus, flowering time is what has been called a 'magic trait' (Servedio *et al.*, 2011); in the course of adapting to local conditions, it constricts gene flow. Finally, when coupled with seasonal shifts in selection, phenological assortative mating could lead to the formation of adaptive temporal clines (Hendry & Day, 2005).

Despite the potential importance of phenological assortative mating, there have been very few attempts to verify its occurrence (Gutierrez & Sprague, 1959; Ennos & Dodson, 1987; Lyons & Mully, 1992; Gérard *et al.*, 2006). We know of only one estimate of ρ to be published for flowering time and that was for a greenhouse experiment (Weis & Kossler, 2004). This paucity of knowledge reflects the challenges to estimating the correlation between pollen donor and recipient in large natural populations. Plants mate cryptically, copiously and promiscuously. Although genetic paternity analysis could be applied to the problem (Ison *et al.*, 2014), genotyping all potential partners and their offspring remains a daunting task for all but the smallest of natural populations.

This study examines the *potential* strength of assortative mating for flowering date and its basis in individual variation in the flowering schedule, that is the number of flowers deployed on successive dates. We compiled an unprecedented data set that includes complete flowering schedules for 25–50 individuals in each of 31 co-occurring old-field species. We used Weis' (2005) method to estimate ρ and develop a new index for flowering synchrony. The results show that the level of assortative mating for a given species depends on its pattern of among-individual variation in flowering schedule.

Conceptual framework

Building on work by Fox (2003) and Weis (2005) showed that the pairwise mating probabilities for a random sample of n plants from a large population can be estimated from differences in their individual flowering schedules, that is, in the number of flowers they display on each day of the flowering season (e.g. Michalski & Durka, 2007; Grogan & Loveless, 2013; Austen *et al.*, 2014). The number of mating opportunities between any two sampled individuals on a given day is assumed to be proportional to the number of open flowers each has on that day. Their total number of shared mating opportunities is obtained by summing across days. Although mating is assumed to be random on each day (with respect to phenological traits), it is assortative

across the season due to differences in the temporal position and shape of the flowering schedules (Fig. 1). The pairwise mating probabilities are assembled into an $n \times n$ matrix, $\mathbf{\Phi}$. The supplemental material presents code to construct the mating matrix.

Specifically, each element ϕ_{ij} of matrix $\mathbf{\Phi}$ is the proportion of all opportunities for pollen transfer in the entire population, across the entire season, that are to recipient i from donor j. The matrix elements thus sum to 1.0. Assuming that the sample is drawn from a very large population, all matings are equally fertile and mating is random within days, ϕ_{ij} estimates the probability of pollen transfer to all individuals sharing the same flowering schedule as individual i from those sharing the schedule of j. This point is important when interpreting the diagonal elements of $\mathbf{\Phi}$, that is when $i = j$. These diagonal elements are not strictly the frequency of self-pollination under random pollen movement, but rather, that between all individuals sharing the same particular flowering schedule (Weis, 2005). Issues of self-pollination and self-incompatibility are covered in the Discussion.

The mating matrix encodes information on flowering synchrony. Under complete synchrony, all individuals have the same probability of exchanging pollen and so each $\phi_{ij} = \{1/n\}^2$. In this case, the population forms single panmictic mating pool. At the other extreme, when the flowering schedule for each individual fails to overlap with that of any other plant, the diagonal elements are all $1/n$ and the nondiagonal elements are zero. Complete asynchrony thus leads to a highly structured matrix, indicating n mating pools. Intermediate levels of matrix structure arise when the sample is drawn from a population comprised of several overlapping pools. The degree of synchrony can be quantified through the eigenstructure of $\mathbf{\Phi}$. Specifically,

$$S = \frac{\lambda_1}{\sum_{k=1}^{n} \lambda_k},$$

that is, the ratio of the first eigenvalue, λ_1, to the sum of all n eigenvalues for $\mathbf{\Phi}$. With complete synchrony and equal flower production among all plants (all matrix elements equal), the first eigenvalue will be $1/n$ whereas all remaining are zero. Thus, $S = 1$ when all plants have the same flowering schedule; all plants are included in a single mating pool. In contrast, if the plants are completely asynchronous, then *all* of the eigenvalues for $\mathbf{\Phi}$ equal $1/n$; and as there are n of them, they sum to 1. So, with complete asynchrony, $S = 1/n$, which is the relative size of all n mating pools. As sample size increases, the minimum possible value of S declines to zero. Values of S between the two extremes indicate that the population is divided into several overlapping mating pools.

Other measures of flowering synchrony exist (Augspurger, 1983; Freitas & Bolmgren, 2008; see review by Elzinga *et al.*, 2007), but because it is an attribute of the mating matrix, $\mathbf{S}$ informs our understanding of the correlation between mates. Weis (2005) showed that

$$\rho = \mathbf{z}^T \mathbf{\Phi} \mathbf{z}$$

where $\mathbf{\Phi}$ is the mating matrix and $\mathbf{z}$ is the vector of the observed phenotypic values for a trait of interest, such as
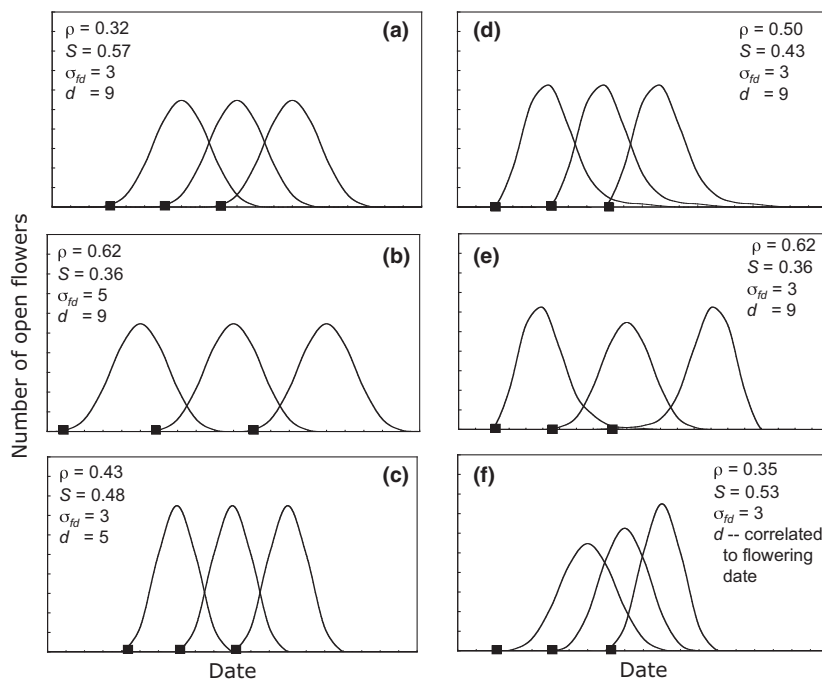
Fig. 1 The effect of variation in flower schedule components on the intensity of assortative mating for flowering date, ρ and flowering synchrony, S. Each curve indicates the number of open flowers per day over the course of the season for a given flowering schedule phenotype. The flowering date is indicated by the square symbol at the foot of each flowering schedule curve. The symbol σ_{fd} denotes the standard deviation in flowering date among the three schedules, whereas d is the duration of flowering, in days. Panels: (a) the benchmark example; (b) standard deviation in flowering date increased, relative to *a*; (c) the duration of flowering increased relative to *a*; (d) the asymmetry of the flowering schedule increased, relative to *a*; (e) schedule asymmetry correlated to flowering date, relative to *a* and *d*; (f) duration correlated with flowering date, relative to *a* and *c*.

flowering date, in the n sampled plants (standardized to zero mean and unit variance). When variance in trait z contributes to asynchrony, ρ will be positive. A ρ of zero indicates either that flowering is synchronous, or that trait z is unassociated with mating synchrony. Thus, mating asynchrony (low S) is a necessary but not sufficient cause for phenological assortative mating in a given trait. Note that populations can simultaneously mate assortatively for multiple traits. The correlation between mates for each trait, and the cross-correlations among traits (e.g. the correlation between flowering date of pollen recipients and height of potential donors), is obtained from an extension of this formula (see Weis, 2005).

Individual flowering schedules, synchrony and the potential for assortative mating

Reproductive asynchrony and potential assortment arise when the individuals in a population differ in their flowering schedules (Fox, 2003; Weis & Kossler, 2004). This section shows how the mean, variance and covariance among flowering date, the duration of individual flowering periods and the symmetry of flower production influences S and ρ.

Consider a series of hypothetical, very large, hermaphroditic populations, each with a different array of three equally abundant flowering schedule phenotypes (Fig. 1). How would these populations differ in assortative mating for flowering date? Fig. 1a illustrates a population with limited temporal overlap in flower production. The mating matrix for this case,

$$\Phi_a = \begin{vmatrix} 0.22 & 0.10 & 0.01 \\ 0.10 & 0.14 & 0.10 \\ 0.01 & 0.10 & 0.22 \end{vmatrix},$$

yields a synchrony index of $S = 0.57$. Although mating opportunities tend to occur within phenotypic classes (high mating probabilities along the diagonal), 42% of all matings are among classes. The resulting phenotypic correlation between mates for flowering date is $\rho = 0.32$.

Temporal overlap could be decreased in several ways. Increasing the variance in flowering date, as in Fig. 1b, increases the proportion of within-type mating,

$$\Phi_b = \begin{vmatrix} 0.31 & 0.02 & 0.00 \\ 0.02 & 0.30 & 0.02 \\ 0.00 & 0.02 & 0.31 \end{vmatrix},$$

which reduces among-type mating to 8%. Here, the synchrony index falls $S = 0.36$, and correlation between mates for the flowering date increases to $\rho = 0.62$.

Changing other schedule components also changes assortment. Shortening the mean schedule duration increases ρ (Fig. 1a vs. c). Keeping the variance in starting dates and mean schedule duration the same as in Fig. 1a, a decrease in the symmetry (increased skew) of the flowering schedule reduces temporal overlap (Fig. 1d) and increases assortment.

The covariance among schedule components also affects temporal overlap. In Fig. 1e, we have used the same parameters as in Fig. 1a, but we have added a negative correlation between flowering date and symmetry. This greatly increases the potential for assortment. A positive correlation between these schedule attributes would decrease the potential for assortment (see Austen *et al.*, 2014). The correlation between flowering date and schedule duration in Fig. 1f slightly decreased the percentage of among-type matings to 38%, causing a modest increase in ρ, relative to Fig. 1a.

Study goals

In the following empirical study, we assembled mating matrices for 31 species to explore patterns of variation in flowering synchrony and assortative mating by flowering date. Specifically, we ask: (1) What is the average correlation between pollen donor and recipient for flowering date?; (2) Do species with more synchronous flowering have a weaker potential for assortative mating?; (3) How do species-level differences in flowering schedule components (mean and variance in the start date, flowering duration, flowering schedule symmetry and the covariance among these) contribute to the among-species variation in the correlation between mates?; (4) Does the correlation between mates for flowering date vary with other species attributes?; and (5) Is there a seasonal trend with respect to the strength of assortment? We focus on flowering date (i.e. Julian date on which the first flower bud opens) for several reasons. For one, there has been much progress in uncovering the genetic basis for this trait (Jung & Muller, 2009; Amasino, 2010). Further, flowering date is a 'first-order' trait; it determines which interval of the seasonally shifting environment the plant occupies during reproduction, and thereby affects the environmental variance in the other schedule components. Finally, flowering date can be measured independently of the other flowering schedule components.

Materials and methods

Data collection

Beginning in May of 2008, we recorded the flowering schedules for 31 species of old-field flowering plants (Table S1) at the Koffler Scientific Reserve (KSR) (44°01′N, 79°31′W). The Reserve sits atop the Oak Ridges Moraine, where soils range from sand to sandy loam. The study species occurred in one of several old-fields that were deforested by the 1880s. Between the 1940s and 1998, the fields were either cut for hay or fenced as horse paddocks. Most of the study species were herbaceous perennials, although we also included two woody species growing near the field margins.

Given the number of species and the diversity of their growth habits, a universal sampling scheme was not feasible other than that all individuals were identified and marked before any had come into flower. For each species, we marked 25 to 50 plants. To increase the likelihood that plants within a species were from a single breeding population, marked individuals within a species were within a ~50 m radius, and in most cases less. To the extent possible, we chose plants so as to avoid marking multiple individuals within tight clusters of immediate neighbours. For abundant species, we used evenly spaced points along transects to select 50. For clonal species, care was taken to ensure that each marked individual was from a different clone, as could be established from proximity and differences in vegetative phenotypes. For *Aquilegia canadensis* and *Rudbeckia hirsute*, we counted all flowering units on all stalks. For *Solidago altissima* and *Vicia cracca*, we counted flower units on three ramets per clone, and for the two woody species, *Cornus alternifolia* and *Prunus serotina*, we counted flowers on all inflorescences from three representative branches; no adjustments for number of ramets/branches were made. For members of the Asteraceae and for *Daucus carrota*, counting total flowers was not practical, and so, the number of inflorescences was recorded at each counting date. In recognition of the differences between flowers and inflorescences, we refer to counts of flowering units. In total, over 400 000 flowering unit observations were tallied.

Each day, we inspected every marked plant and recorded the flowering date. We then censused the number of open flowers on each marked plant in each species at 3-day intervals until no more viable flower buds were visible. Data collection continued to late October of 2008, when all study plants had stopped flowering. Duration of the flowering period was the number of elapsed days between first and last census dates for which flowers were recorded. Schedule symmetry was calculated as $sym = (m - \frac{1}{2}d)/d$, where m is the median flower day (number of days to produce the first 50% of all flowering units) and d is duration; negative values indicate the median occurred before the midpoint of the flowering period, and positive values indicate later. The total number of flowering units counted was the sum of counts across all census dates; it is likely to be strongly correlated with total flowers produced.

Analysis

The synchrony index, S, was obtained from the mating matrix, Φ, which was based on flower counts on the census dates. Because the matrix is typically not symmetrical, the algorithm calculated the imaginary components of the eigenvalues; these were zero or vanishingly small ($< 10^{-8}$). Values of ρ, the phenotypic correlation between pollen donors and recipients, were obtained for flowering date (see above). Confidence intervals for S and ρ were obtained by bootstrapping 1000 random samples. The online supplemental material contains R code (R Development Core Team, 2008) for estimating S and ρ and their confidence intervals.

Following arguments presented in Fig. 1, we explored the effect of variation in flowerings schedule components on S and on ρ for flowering date. Both simple correlation and multiple regression were used. Predictor variables included the species-level means of the four schedule components: flowering date, duration of the flowering schedule, schedule symmetry and number of flowering units counted. The values of ρ and S are expected to vary not only with species trait means, but also with their within-species variances and covariances (Fig. 1). To evaluate the impact of the variance and covariance among schedule components on ρ, our set of predictor variables was expanded to use the standard deviations of each component trait plus the within-species correlations among them. Standardized regression coefficients were calculated to assess the relative impact of the retained variables.

Results of these multiple regression analyses are descriptive. With the exception of flowering date, the values used for the individual flowering schedule components were derived from the same data used to calculate the mating matrix, Φ. As Φ is included in the calculation of both S and ρ, the predictor and predicted variables are not independent, so the calculated regression coefficients describe the contribution of variance in the part to the variance in the whole. We report standard errors and t-tests for the coefficient calculated for each predictor; however, these should be interpreted as indicators of the 'noisiness' around the signal from each predictor. We used backward selection to simplify the model to that which yielded the lowest AIC. When models were < 2 AIC units apart, we reported the one with the fewest coefficients. We also compared the magnitude of ρ among groups of species with contrasting attributes, such as self-compatibility and origin (native vs. introduced). All statistical analyses were performed in R (R Development Core Team, 2008).

Results

The 31 study species at KSR varied widely in their potential for assortative mating by flowering date. Figure 2 plots flowering schedules for three species, illustrating cases with low, medium and high potential. The mean correlation between mates for flowering date was $\bar{\rho} = 0.31$ (Fig. 3) with an approximate 95% confidence interval of 0.25–0.38. *Leonurus cardiaca* showed the weakest potential for assortment for flowering date ($\rho = 0.046$), and *Verbascum thapsus* showed the strongest ($\rho = 0.624$). The synchrony of flower deployment varied strongly among the study species (Fig. 3); *Leonurus cardiaca* was the most synchronous ($S = 0.905$),
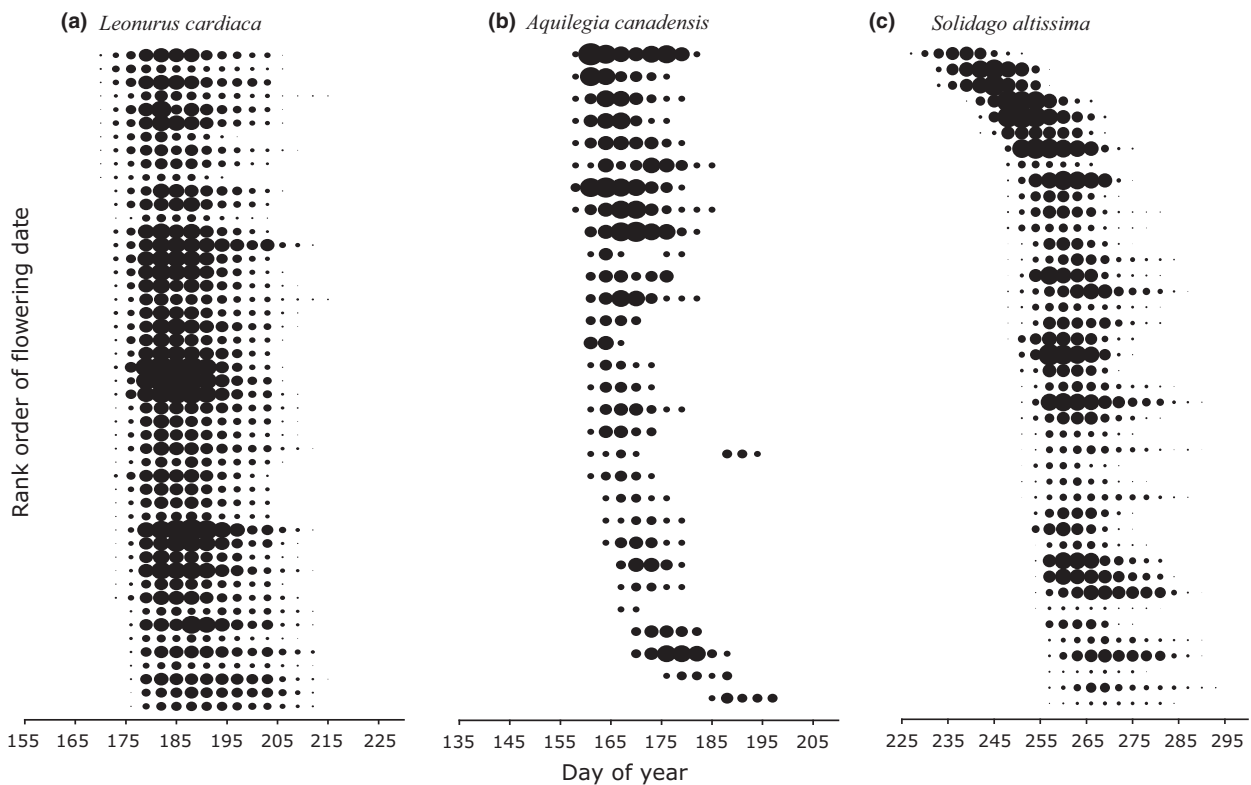
Fig. 2 Individual flowering schedules for three of the 31 study species. Each row of bubbles represents a single individual. Bubble area is proportional to the number of open flowering units on the given census date. (a) *Leonurus cardiaca,* weak assortative mating for flowering date and high flowering synchrony. (b) *Aquilegia canadensis*, moderate assortative mating and moderate synchrony. (c) *Solidago altissima*, strong assortative mating and low synchrony.

whereas *Vicia cracca* was the least ($S = 0.364$). Note that the minimum possible value for S for any given species in this data set ranged from 0.04 down to 0.02, depending on its sample size. As expected, flowering synchrony was negatively correlated with potential for assortative mating by flowering date ($r = -0.49$, $P < 0.003$; Fig. S1).

The potential for assortative mating is also expected to depend upon the mean and variance in the flowering schedule components (Fig. 1), and the 31 study species showed considerable differences in these, as seen in the supplementary material (Table S1). There was a six-fold difference in the standard deviation for flowering date among the species; *Leonurus cardiaca* was the least variable with respect to start date (s.d. = 1.87), whereas *Cirsium vulgare* was the most (s.d. = 11.86). Mean flowering duration also varied over a six-fold range, from 13.2 *d* for *Galium aparine* up to 80.5 *d* for *Erigeron philadelphicus* (mean across species = 32.3 *d*). The standard deviations of duration also varied six-fold. These differences in duration variance were not exclusively due to scaling with the mean; a four-fold difference in coefficient of variation for duration was observed between the most uniform species (*Arctium

minus, $CV = 12\%$) and the least uniform (*Geranium robertianum*, $CV = 58\%$). With regard to flowering schedule symmetry, most species were skewed left (the first 50% of flowers produced before the duration midpoint, $sym < 0$). In absolute value, the most symmetrical species was *Cirsium vulgare* ($sym = 0.005$) and the least was *Ranunculus acris* ($sym = 0.441$). As with other schedule components, species varied nearly six-fold in their standard deviation for schedule symmetry.

As argued above, the within-species correlations among schedule components will also influence flowering synchrony and thus affect the potential for assortative mating (see Fig. 1a vs. e and f). Table S2 in the supplementary material reveals three inter-related tendencies among the 31 study species. First, the individuals within a species that flowered late also flowered for fewer days (Table S2); across the study species, the mean correlation between flowering date and duration of flowering was $\bar{r} = -0.36$ (H_o, mean correlation is zero: $t = 3.33$, $P = 0.002$). Secondly, later individuals tended to produce fewer flowers ($\bar{\rho} = -0.34$, $t = 3.48$, $P = 0.001$). Thirdly, individuals that flowered longer produced more flowering units ($\bar{r} = 0.65$, $t = 9.56$, $P < 0.0001$). In summary, early-flowering individuals

(a)

ρ, Correlation between mates for first flower date

(b)

S, Flowering synchrony
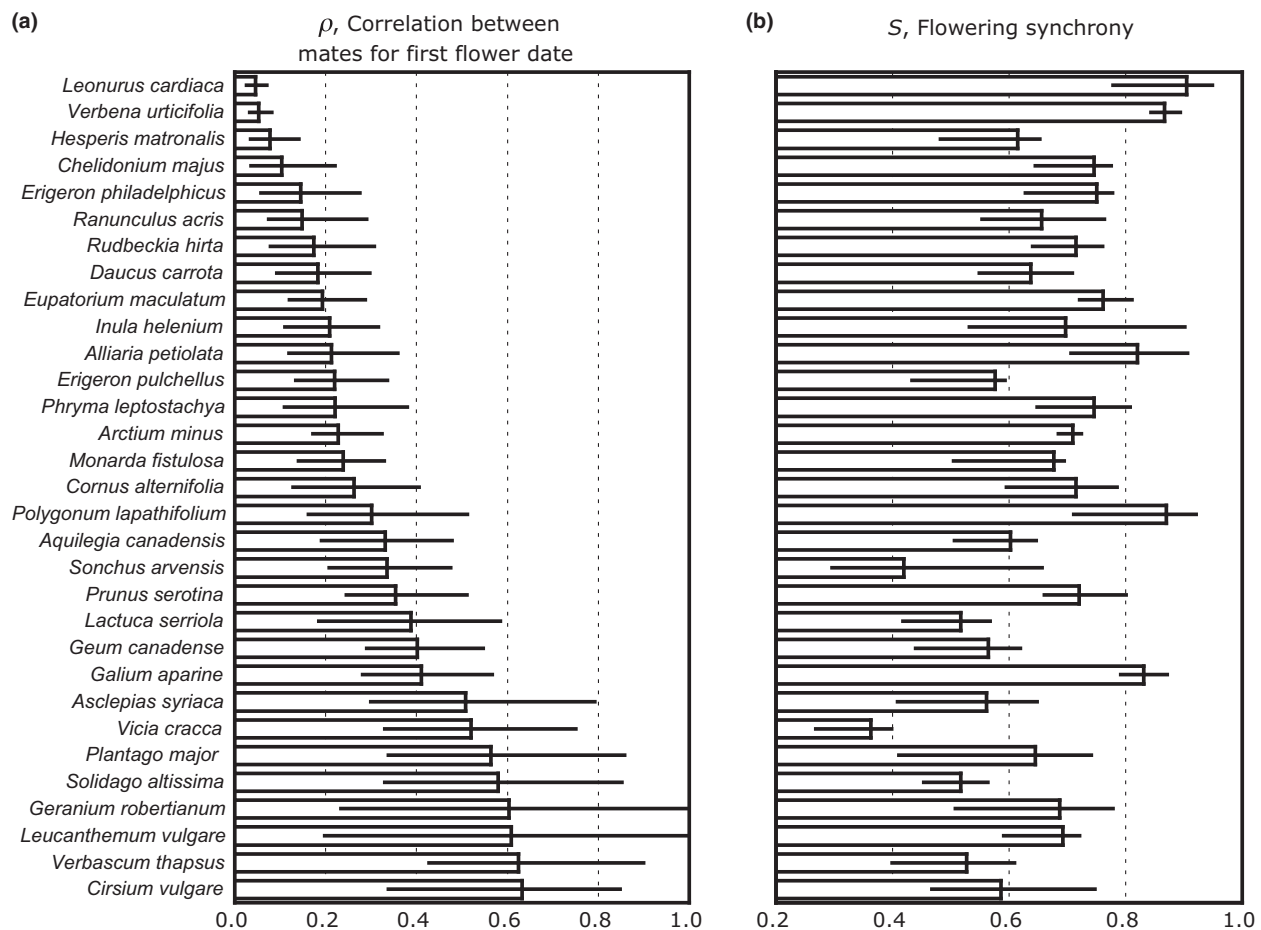
Fig. 3 The potential for (a) assortative mating by flowering date, ρ, and (b) flowering synchrony, S, based on flowering schedules for the 31 study species in. Error bars indicate 90% confidence interval based on 1000 bootstrapped samples.

flowered longer and produced more flowers. The remaining correlations among flowering schedule components were neither more nor less variable among species than the three already noted, but their across-species means did not differ significantly from zero (Table S2).

As predicted from first principles (Fig. 1), flowering synchrony and potential assortative mating for a given species depended on the mean, variance and covariance among its flowering schedule components. Taking species as the experimental unit, we calculated the correlations among S, ρ and schedule component statistics. Correlation coefficients are presented in the supplementary material (Table S3). Given the large number of coefficients calculated, their values should be interpreted as descriptive. Here, we mention univariate correlations of schedule components to ρ and S that were nominally significant (Table S3).

Early-blooming species were more synchronous in flowering, as evidenced by the negative correlation between S and mean flowering date ($r = -0.41$, $P < 0.02$). This did not translate into weaker assortative mating for early bloomers, as the correlation between ρ and mean flowering date ($r = 0.20$ $P = 0.28$) was not significant.

As expected (see Fig. 1a vs. b), the within-species standard deviation in flowering date correlated positively with ρ, but negatively with S ($r = 0.51$, $P = 0.003$ and $r = -0.63$, $P = 0.001$, respectively). Species in which individuals deploy flowers over a long time (see Fig. 1a vs. c) tend to be more synchronous ($r = 0.44$, $P = 0.013$) and have a lower potential for assortment by flowering date ($r = -0.39$, $P = 0.03$). Species with greater variation in schedule symmetry (Fig. 1e) were modestly more prone to assortment ($r = 0.35$, $P = 0.053$). A strong positive correlation between duration and symmetry tended to further increase synchrony ($r = 0.45$, $P = 0.011$) and weaken assortment ($r = -0.40$, $P = 0.025$); see the explanation of symmetry in the multiple regression analysis below. We also

found that species with stronger positive correlations between total flower production and schedule symmetry tended to also have lower levels of assortative mating and greater synchrony (Table S3).

Many of the flowering schedule components were intercorrelated (Table S3), and so, a linear model approach was taken to identify those with strong direct effects on ρ and S. The among-species variation in potential assortative mating can be most efficiently predicted from four underlying schedule components (Table 1a). Based on the standardized partial regression coefficients, the strongest predictor of ρ for flowering date was the species-level standard deviation in flowering date; that is, species with a greater difference among individuals in flowering start date predictably showed a stronger correlation between mates (see Fig. 1a vs. b). Similarly, the shorter the mean schedule duration, the stronger was the assortment (see Fig. 1a vs. c). With respect to mean schedule symmetry, the 31 species trended towards left-skewed flowering schedules, yielding negative estimates of *sym* (Table S1). Thus, the negative relationship between symmetry and ρ suggests that potential assortment decreased as mean schedules became more symmetrical (see Fig. 1a vs. d). The final predictor of ρ was the within-species correlation between flowering date and duration; the more positive (less negative) this correlation, the weaker was the assortment for a species. This confirms the prediction that assortment is intensified when the earliest plants in the population have the longest flowering duration (see Fig. 1a vs. f).

There is partial overlap between the set of schedule components that best predict synchrony with the set predicting assortative mating (Table 1b). If the two were perfectly coupled, the coefficients predicting ρ and S would be of the same magnitude but opposite sign. The standard deviation in flowering date, the strongest predictor of ρ, was a weaker predictor of S. Mean flowering duration strongly predicted S: when individuals within a species produced flowers over a longer period, they were more synchronous. Holding the effect of mean duration statistically constant, species with a small standard deviation in duration were even more synchronous. When duration and symmetry were more positively correlated, synchrony increased. The strongest predictor of synchrony was the correlation between flowering date and duration, which had a negative coefficient. The date-duration correlations were mostly negative in the 31 speceis (late bloomers flower for fewer days; Table S2). The negative regression coefficient thus indicates higher synchrony in species where flowering date and duration were uncorrelated and lower synchrony where they were negatively correlated (see Fig. 1f vs. a).

A phylogenetic analysis was not made, but variation between families suggests weak trends associated with ancestry. Ten species were from the Asteraceae, and these showed nearly the full range of assortative mating for flowering date. The three Brassicaceae and the two Verbenaceae showed low values of ρ. The ranges of values for ρ from the families Rosacae, Ranunculaceae and Lamiaceae (two species each) all broadly overlapped.

As for other attributes, the potential for assortative mating in species known to be self-compatible was on average $\bar{\rho} = 0.34$ (SE = 0.06), which is not different from the average for self-incompatible species, $\bar{\rho} = 0.26$ (SE = 0.04) ($t_{28} = 0.98$, $P = 0.83$). Assortative mating in the context of self-pollination is discussed below. Native and introduced species did not differ (native, $\bar{\rho} = 0.31$ {SE = 0.02}; non-native, $\bar{\rho} = 0.33$ {SE = 0.04}; $t_{28} = -0.326$, $P = 0.75$).

Discussion

We examined the potential for phenological assortative mating in 31 co-occurring plant species, using an

Table 1 Results from stepwise multiple regression predicting potential for assortative mating from species-level statistics on flowering schedules. Traits identified by same letter as in Table S3.

	Coefficient (SE)	Standardized coefficient	*t* Value
(a) Phenotypic correlation between mates for flowering date, ρ			
B: Mean schedule duration	−0.003 (0.001)	−0.30	−2.64
C: Mean schedule symmetry	−0.718 (0.202)	−0.43	−3.54***
E: SD of flowering date	0.039 (0.008)	0.58	4.50***
I: COR of Flr date, duration	0.257 (0.088)	0.26	2.91*
$R^2 = 0.88$			
(b) Flowering synchrony, S			
B: Mean schedule duration	0.006 (0.0013)	0.63	4.00***
E: SD of flowering date	−0.018 (0.0058)	−0.34	−3.04***
F: SD of duration	−0.012 (0.0043)	−0.45	−2.84
H: SD of Total flower units	−0.00004 (0.00003)	−0.15	−1.47
I: COR of Flr date, duration	−0.336 (0.0561)	−0.73	−5.98***
M: COR of Dur, symmetry	0.237 (0.0667)	0.53	3.56***
$R^2 = 0.77$			

*P < 0.05; ***P < 0.001.

unprecedented data set containing individual-level flowering schedules for 25–50 plants per species. The correlation between pollen donors and potential recipients for flowering date ranged from weak (0.046) to strong (0.624). In general, species with greater synchrony across their entire flowering schedules showed a weaker potential for assortment. The potential for assortment was highest in species with a high variance in flowering date and that deployed more of their flowers towards the beginning of a short flowering period. Factors such as self-compatibility and non-native status had no detectable effect on the potential for assortment. Spring-flowering species showed greater synchrony than summer species, but there was no detectable seasonal trend in the correlation between mates for flowering date.

This study clarifies the distinction between mating asynchrony and phenological assortative mating – not made by Fox (2003) but first noted by Weis (2005). The important point is that the former is a necessary but not sufficient condition for the latter. Figures 3 and S1 show that variation in flowering date leads to asynchrony, but variation in other phenological traits also diminish *S*.

The strength of phenological assortative mating

Given these results, is assortative mating by flowering date strong or weak? There are several perspectives on this question. One is to compare assortment for this single plant trait to the multiple animal traits considered by Jiang *et al.* (2013). Their meta-analysis encompassed 360 published estimates of ρ, each for a unique species–trait combination; like ours, all were single population estimates. They found an average value of $\bar{\rho} = 0.28$ (95% confidence interval, 0.25–0.31) for traits such as body size, colour and condition. Our results on flowering date yielded a mean of $\bar{\rho} = 0.31$ (95% confidence interval, 0.25–0.38). Neither study suffers publication bias against statistically nonsignificant results (see fig. 5 in Jiang *et al.*, 2013; all estimates are reported from our data set). There could be, however, investigation bias; some of the animal studies may have been prompted by preliminary observations suggesting assortment. Thus, the true mean strength of assortative mating by a trait – say by body size – across all animals might factor in a higher proportion of zero values than Jiang *et al.* (2013) found in the literature. Against this, assortative mating by body size is predicted *a priori* in some groups due to body size compatibility (e.g. Brown, 1993; Arnqvist *et al.*, 1996). The situation is similar with flowering date, where it is expected from first principles that like-by-like matings occur more often than by chance (Breese, 1956; Hartl & Clark, 1989; Lynch & Walsh, 1998; Kirkpatrick, 2000; Fox, 2003; Devaux & Lande, 2008). All in all, it is safe to say that assortative mating for flowering date is as strong as for animal traits such as body size, colour and condition, in cases where there is reason to suspect assortment in the first place.

A more salient perspective for judging the strength of phenological assortative mating is to ask if the observed values of ρ are sufficient to accelerate adaptation. For the sake of argument, suppose that the 31 study species fit Wright's scenario, as laid out in the Introduction. Using our observed mean of 0.31 for ρ, and 0.4 as a reasonable level of heritability for flowering date (Geber & Griffen, 2003) and assuming many contributing loci ($1/2n_e \approx 0$), one would surmise that the 'average species' would show a ~15% increase in genetic variance over that expected in the absence of assortative mating. A population with the lowest observed ρ would show just a 1.6% increase, whereas that with the highest ρ would show a 33% increase. Although real species do not fit Wright's restrictive assumptions (infinite population size, no mutation or selection), these calculations suggest that the larger values of ρ can have nontrivial impact.

The shape and size of the flowering schedule determine the potential for assortative mating

The contributions of variance in flowering schedule components to assortment and synchrony closely followed those predicted from first principles (Fig. 1, Table 1). Importantly, as the standard deviation of flowering date increased for a species, so did ρ. Similarly, species with more asymmetric flowering schedules tended to have a larger ρ; this was because, all else being equal, when flower production was concentrated into fewer days, there was a decline in the number of shared mating opportunities between individuals of different start dates (Fig. 1).

Returning to the idea that mating asynchrony is a necessary but not sufficient condition for phenological assortative mating, not all factors predicting *S* likewise predicted ρ, and *vice versa*. Variation in flowering date predicted both, as expected. An increase in the mean duration of the flowering period increased ρ and *S*, but its effect on the latter also included negative contributions through its standard deviation and its correlation to flowering date. Taken together, species with long, uniform, overlapping flowering periods were more synchronous. A strong negative correlation between flowering date and duration also tended to increase ρ.

The contribution of multiple flowering schedule components to flowering asynchrony bears upon a situation that Fox (2003) called 'cryptic assortment'. He applied this term to the asynchrony caused by variation in the skew, kurtosis or modality of flower production schedules, independent of variation in the flowering date. For example, all individuals could start and stop flowering simultaneously, yet mating would still be nonrandom because they reach flowering peak(s) on different

dates. This situation is one of many in which mating is asynchronous and therefore nonrandom, but may not be assortative for the traits of interest. Examination of Φ, the matrix summarizing co-occurrence in the mating pool, enables a broader range of inferences than examination of ρ, which pertains to a particular trait.

Individual flowering schedules also highlight the mechanistic differences between phenological assortative mating and that caused by mating preferences (Weis *et al.*, 2005). Behavioural assortment is commonly viewed as involving two traits – the preference trait and the criterion trait, such that assortment is by the criterion, but caused by the preference. With mating phenology, only one trait causes assortment. In the two-trait context, Rubenstein (1984) suggested that assortment can be 'true' or 'apparent' (see also Crespi, 1989 and Arnqvist *et al.*, 1996). With apparent assortment, mates are correlated because individuals at one phenotypic extreme have access to all mates, whereas those at the other extreme obtain mates like themselves. For instance, sexually antagonistic behaviours may prevent small males from mating with large females, whereas large males can mate with females of all sizes. Large males will have larger mates on average than small males, but only the small males mate within type.

Something similar to 'apparent assortative mating' appears in the flowering schedule data set. There is a tendency for stronger assortment for flowering date in species where late-flowering plants also flower for fewer days (Table 1a, see Fig. 1f). At the extreme, one would see this negative correlation if late bloomers come up against end-of-season events at the same time as early bloomers – the flowering period for the last plant is nested within the flowering period for the first. Late bloomers would have mating opportunities with plants of all flowering dates during every day of their flowering period. Early bloomers, however, have days when the only available mates are other early bloomers. This extreme case would be similar to what Rubenstein meant by 'apparent assortment' – not all individuals are equally restricted in mate access. Does this mean that assortment by flowering date is not real? We would argue that the distinction between 'real' and 'apparent' is important only when one is asking if a particular behavioural mechanism generates the correlation between mates.

Interestingly, however, a negative correlation between start date and duration could induce a heteroscedastic association between the flowering dates of pollen recipients and donors. The impact of this heteroscedasticity on genetic variance merits further consideration. Does it skew the distribution of breeding values? If so, does it then lead to asymmetric response to upward and downward selection on the assorting trait (e.g. Burgess *et al.*, 2007)?

Potential vs. realized phenological assortative mating

Flower counts estimate mating opportunities and thus the *potential* for phenological assortative mating. Weis & Kossler (2004) performed a greenhouse quantitative genetics experiment on flowering time where they imposed random mating on a daily basis, as assumed in the estimates for ρ. They found strong agreement between the ρ obtained by flower counts and that based on the inflation of the maternal–offspring regression (see Lynch & Walsh, 1998).

In natural populations, however, the realized levels of assortment may deviate from their potential when pollen exchange is influenced by factors over and above phenology. For instance, pollinator response to the wax and wane of floral abundance can lead to a nonlinear relationship between flower count and pollen transfer. Some pollinators preferentially visit plants with large floral displays, although per-flower visitation rates can decline with display size (Brys & Jacquemyn, 2010; Dudash *et al.*, 2011; Karron & Mitchell, 2012). Flower counts could either over- or underestimate assortment for flowering date, depending on how these opposing trends balance. If the attraction to large displays predominates, the effect of flowering date on asynchrony would decrease, but the effect of peak flower number would increase. Realized levels of assortative mating for flowering date would be overestimated by flower counts, whereas assortment by date of peak flowering would be underestimated. Plant density and dispersion can also bias pollinator movement. Pollen generally moves from donors to nearby recipients (Thomson & Thomson, 1989; Fenster, 1991), although near neighbours can get bypassed in dense aggregations (Cresswell, 2000). Recently, Ison *et al.* (2014) found through genetic paternity analysis that mating between *Echinacea angustifolia* individuals increases with both their flowering synchrony and spatial proximity.

The flower count method assumes that self-pollination occurs no more frequently than expected from random pollen movement. Moderate to high selfing rates will increase the true ϕ_{ii} and thereby increase the phenotypic correlation between mates. It is important to note, however, that selfing inflates genetic variance for flowering date solely by increasing homozygosity and *not* by increasing gametic disequilibrium among contributing loci (Felsenstein, 1981; Lynch & Walsh, 1998). Gametic disequilibrium inflates genetic variance far more than homozygosity when multiple loci contribute to the assorting trait (Felsenstein, 1981). Thus, although highly selfing species have larger phenotypic correlations between mates than indicated by flower counts, the genetic consequence of assortment will be weaker than in outcrossers.

Final considerations

Climate change is imposing selection on phenology by changing the length of the growing season (van Asch *et al.*, 2007; Bradshaw & Holzapfel, 2008; Forkner *et al.*, 2008; Hoffmann & Sgro, 2011). This brings an urgency to understanding the potential for evolution of flowering time, including the role of assortative mating. No doubt, observed shifts to earlier flowering over recent decades (e.g. Fitter & Fitter, 2002; Inouye, 2008) are largely plastic responses to warmer temperatures. However, phenotypic plasticity in flowering date does not preclude evolutionary (genetic) change. Indeed, the rate of evolutionary response may increase as populations reach the limits of their plasticity (Anderson *et al.*, 2012). At least one case of recent, rapid evolution in flowering time has been detected. Using a resurrection experiment, Franks *et al.* (2007) documented a 2–8 day evolutionary acceleration in flowering time for California *Brassica rapa* populations. This shift towards earlier reproduction was attributed to natural selection induced by an extended drought, which contracted the growing season in that Mediterranean climate. In view of all this, what are the interesting and important questions to ask about phenological assortative mating in this era of changing climate?

One question is how the diversity of flowering schedule shape varies among climatic zones and how this affects mating synchrony and assortment. Our 31 study plants were predominantly herbaceous, animal-pollinated eudicots from a short-seasoned, northern temperate climate. These species showed unimodal, slightly skewed schedules, with a moderate correlation between start date and duration. This schedule shape may be common, but multimodal schedules are also known. Michalski & Durka (2007) found strong multimodal individual schedules in the wind-pollinated grass genus *Juncus* in Europe. In the climatically more extreme Arizona desert, some *Erigonium abertianum* individuals flower during the spring rainy season, others during the summer monsoon and still others that flower during both (Fox, 1990). Multimodality is common in Costa Rican tropical forests, with multiple flowering pulses across the year, each triggered by rain events (Opler *et al.*, 1976). A few species in this region flower continuously through the year (Frankie *et al.*, 1974), although not necessarily with uniform intensity across time. With polymodal flowering schedules, assortment for date of first flowering will depend on variance in start date and mean duration of flowering, just as with the unimodal case (Fig. 1). However, when flowering is pulsed, there is potentially strong assortative mating for the developmental traits controlling the response to environmental flowering cues: plants with similar response rates will reach flowering peaks simultaneously, but out of synch with those responding at faster or slower rates. Investigating the association

between flowering schedule shape and climatic regime will sharpen predictions on which types of species might show accelerated selection responses.

A second question relates to the effect of life history on phenological assortment. The response to selection in long-lived species is slowed by long generation times. Does assortative mating ameliorate the generation time effect in the case of phenological adaptation? Monocarpic perennials present an extreme case. When all individuals from each annual seed cohort mature after a fixed number of years, each cohort constitutes a separate mating pool, but because there is no variation in age a reproduction, there is no assortment for that trait (ρ is undefined). With variation within cohorts (seeds produced in a given year mature over a span of years), assortative mating for age at reproduction depends on the relative contributions of the different cohorts to the mating pool; increased variance in age of reproduction increases assortment. In this vein, Loveless *et al.* (1998) determined that isolation by distance could not explain the spatial genetic structure of the tropical monocarpic tree *Tachigali versicolor*, but suggested that the observed structure would arise if patches of trees were comprised of different mixtures of multiple seed cohorts. In iteroparous species, ontogenetic shifts in flowering behaviour can affect the potential for phenological assortative mating. For instance, Bustamante & Búrquez (2008) showed that organ pipe cactus (*Stenocereus thurberi*) shifts the beginning or ending date of its flowering period as they grow larger. Grogan & Loveless (2013) found that mature, large-sized individuals of big-leaf mahogany (*Swietenia macrophylla*) flower at predictable times every year, whereas younger, smaller trees frequently skip years. These examples indicate that in long-lived species, the opportunities for pollen exchange between individuals during any one year may not reflect total opportunities over the lifetime; strong phenotypic correlations between mates within years may or may not result in strong genetic correlations between mates across years.

Finally, from a community ecology perspective, one could ask if systematic differences among plant species in the evolutionary response of flowering phenology to climate change could have consequences for higher trophic levels, including pollinators and predispersal seed predators. Aldridge *et al.* (2011) showed a greater plastic advancement of flowering date by spring-blooming mountain wildflower species, compared with summer bloomers. This created a mid-season gap in floral resources for pollinators. Seasonal trends among species in phenological assortative mating could facilitate or constrain the development of such gaps through evolutionary responses, depending on assortment's concordance/discordance with seasonal trends in selection. We did not detect a seasonal trend in the correlation between mates for flowering date, but we did detect such a trend in flowering asynchrony, which in turn

may lead to systematic differences in assortment for other flowering schedule components, such as date of peak flower production or duration of the flowering period. Changes in these traits could also impact resource availability for consumers. Are seasonal trends in assortment wide-spread?

Phenological assortative mating, caused by individual differences in flowering schedule, is in the mix of factors that will determine the range of adaptive responses in flowering phenology in the coming decades of climate change. Efforts, such as Project Baseline (Franks *et al.*, 2008), are poised to detect such evolutionary shifts: seeds from current generations of plant populations are being systematically collected and preserved (frozen). In the future, these can be resurrected, grown side-by-side with descendant generations from the same populations. Comparing the phenotypes of ancestral and descendant generations (and their hybrids) in a common environment will provide a strong test for evolutionary change. It will be interesting to see if species with flowering schedule variation that promotes assortative mating are measurably more responsive to selection than those that lack it.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Conceptual background.

Table S1 Study species, with mean (standard deviation) of individual flowering date (Julian), duration of their flowering schedule (days), flowering schedule symmetry and total number of flowering units. Arranged by order of flowering.

Table S2 Within-species correlations among flowering schedule components.

Table S3 Among-species correlations of flowering schedule components to flowering synchrony, S, and the potential phenotypic correlation between mates.

Figure S1 The correlation between the potential for assortative mating by flowering date, r, and flowering synchrony, S, based on individual-level flowering schedules for the 31 study species.