RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

Temporal population genetic structure in the pollen pool for flowering time: A field experiment with *Brassica rapa* (Brassicaceae)[1]

Jennifer L. Ison[2,3,5] and Arthur E. Weis[3,4]

PREMISE OF THE STUDY: Assortative mating by flowering time can cause temporal genetic structure in species with heritable flowering times. A strong temporal structure, when coupled with a seasonal shift in selection, may lead to adaptive temporal clines. We implemented a prospective and retrospective method to estimate the temporal genetic structure in the pollen pool of *Brassica rapa*.

METHODS: The prospective method uses flowering schedules to estimate the seasonal shift in the pollen donors' phenotype. By examining the offspring generation, we can get a direct estimate of temporal genetic structure, i.e., a retrospective estimate. However, this estimate is problematic because of the phenotypic correlation of the trait of interest, flowering time, between dam and sire. We developed a novel retrospective method that isolates flowering time by holding the maternal contribution constant and sampled the pollen pool in eight open-pollinated field plots throughout the flowering season.

KEY RESULTS: We found temporal genetic structure for flowering time in seven of the eight field plots. Interestingly, the direct (retrospective) temporal structure estimate was 35% larger than the prospective estimate based on flowering schedules. Spatial clumping of pollen donors did not affect temporal structure, but structure intensified when heritability was experimentally enhanced.

CONCLUSIONS: Temporal genetic structure, especially for flowering time, likely occurs in many plant populations and may be underestimated using a prospective method. We discuss the genome-wide consequences of temporal genetic structure and the potential for adaptive temporal clines in plant populations.

KEY WORDS assortative mating; *Brassica rapa*; flowering phenology; gamete pool; gene traps; isolation by time; temporal cline

Flowering date is a key plant life history trait that determines synchrony with pollinators, avoidance of florivores, and exposure to seasonal shifts in climate (e.g., Elzinga et al., 2007; Franks, 2015). The date and duration of an individual's flowering period can determine its opportunities for pollen exchange with the other plants in the population (e.g., Schmitt, 1983; Ison et al., 2014; Weis et al., 2014). Even though flowering phenology is affected by environmental conditions (reviewed by Rathcke and Lacey, 1985; Elzinga et al., 2007), there is often a strong genetic component of flowering time (reviewed by Geber and Griffen, 2003; Hendry and Day, 2005). It has been noted that genetic variation in flowering time should be prone to temporal population structure (Weis and Kossler, 2004; Hendry and Day, 2005). When individual plants enter and exit the mating pool asynchronously, "early" flowering alleles predominate in the gamete pool as the season begins, while "late" flowering alleles predominate at the end. As a result, the mean breeding value for mating phenology shifts directionally over the mating season (Hendry and Day, 2005), which in turn can lead to assortative mating for flowering time, hereafter, phenological assortative mating (Hartl and Clark, 1989; Fox, 2003).

Temporal population genetic structure of flowering time, and the assortative mating it induces can have several notable consequences. Because offspring inherit similarly acting alleles from both parents, there will be more individuals with an excess of early

[1] Manuscript received 31 May 2017; revision accepted 11 September 2017.

[2] Department of Biology, The College of Wooster, 1189 Beall Avenue, Wooster, Ohio 44691 USA;

[3] Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario, M5S 3B2, Canada; and

[4] Koffler Scientific Reserve at Jokers Hill, University of Toronto 17000 Dufferin Street, King City, Ontario, L7B 1K5, Canada

[5] Author for correspondence (e-mail: jison@wooster.edu)

https://doi.org/10.3732/ajb.1700210

(late) alleles than expected under random mating. Therefore, assortative mating inflates the expressed additive genetic variance in the assorting trait (Wright, 1921; Felsenstein, 1981) and can thereby accelerate its response to selection. However, in the absence of selection, genetic drift at phenology loci can theoretically lead to the formation of temporally isolated, sympatric "quasi-species" (Devaux and Lande, 2008). Hendry and Day (2005) argued that genetically based temporal isolation acts like isolation by distance (Wright, 1943), at least qualitatively, and when coupled with season shifts in directional selection, the temporal structure can catalyze the evolution of temporal clines. At the extreme, strong disruptive selection paired with strong temporal assortative mating contributes to speciation (Kirkpatrick, 2000; Savolainen et al., 2006).

The amount of temporal assortative mating between any two individuals will be in part delimited by the overlap in their flowering schedules, i.e., the number of active flowers one plant has on display on each day across the flowering season. For instance, Weis et al. (2014) used the overlap in flowering schedules (i.e., counts of open flowers on each individual, repeated over successive intervals) to estimate pairwise mating opportunities among individuals for each of 31 old-field plant species. The phenotypic correlation for flowering onset date between pollen recipients and their possible donors ranged from 00.05 to 0.62. In the lowest correlation species, most individuals started and stopped flowering on the same days, leading to high overlap in schedules. In the highest correlation species, the latest individuals did not start flowering until the earliest were finishing, leading to generally low schedule overlap. The genetic correlation between mates can be estimated by multiplying this phenotypic correlation by heritability of flowering onset (Weis and Kossler, 2004). Such estimates are prospective, in that they are based on the opportunities for pollen exchange. These calculations make the explicit assumption that each open flower receives a random sample of pollen in circulation during the days it is receptive. Even if mating is random within days, it will be nonrandom across time as different plants go into and out of flowering as the season progresses.

Both flowering time and the spatial distribution of plants affect mating patterns (e.g., Gérard et al., 2006; Kitamoto et al., 2006; Ison et al., 2014). Yet the spatial distribution of flowering plants has been largely ignored in prospective phenological assortative mating estimates (Weis and Kossler, 2004). Research has demonstrated that pollinators, thus pollen grains, move from donors to nearby recipients (e.g., Thomson and Thomson, 1989; Fenster, 1991). However, pollinators may skip over the nearest neighbors in dense aggregations (Cresswell, 2000). It seems probable that the strength of phenological assortative mating would vary based on the spatial distribution of plants in a population. Since areas of high conspecific density have many nearby potential mates, we would predict that the strength of phenological assortative mating to be greater in these areas compared to areas of low conspecific density where the most synchronous potential mates maybe quite distant.

Many other ecological factors could also cause realized pairwise mating frequencies to differ from this prospective expectation. For instance, pollinator preference for large inflorescences could disproportionately increase the contribution of plants at peak flower to the daily pollen pool unless there is a compensatory decline in the per-flower visitation rate with display size (e.g., Brys and Jacquemyn, 2010; Dudash et al., 2011; Karron and Mitchell, 2012). Finally, flowers produced early in a plant's flowering schedule are more likely to set fruit than those produced near the end (Ison and Wagenius, 2014) and pollen donated late in the season is less likely to sire offspring (Austen et al., 2015). Thus, the pool of successful gametes on any day may deviate genetically from the overall gamete pool at flowering time loci. If so, the prospective estimate of temporal population structure, based on flowering schedule overlap, will be an over-estimate.

Direct estimates of temporal genetic structure in mating phenology comes only with a retrospective analysis, i.e., one that infers the genetic makeup of the successful gametes by examining the offspring generation. This estimate can be straightforward, if tedious, for the ovule pool: record the number of flowers produced on each individual, on each day, and monitor each flower's success in seed set (Austen et al., 2015). Pollen is more problematic. Genetic markers can indicate the siring success of phenologically divergent pollen donors (Gutierrez and Sprague, 1959; Ennos and Dodson, 1987). For instance, Ison et al. (2014) used microsatellite markers to demonstrate significant phenological assortative mating in an open-pollinated population of the generalist-insect-pollinated *Echinacea angustifolia*. However, this method indirectly measures the parameter of interest, i.e., the change in the breeding value for flowering date in the pollen pool over the season.

In this study, we compare prospective and retrospective estimates for seasonal shift in flowering date breeding value of pollen pools. We used a manipulative field approach, in which plants were open-pollinated but we were able to isolate the trait of interest, flowering date, by controlling the maternal contribution to the trait. This design is an improvement over previous studies because we are able to compare the prospective and retrospective estimates, allowing us to quantify the effect of ecological dynamics (i.e., pollinator foraging behavior, plant density) in natural populations on temporal genetic structure.

MATERIALS AND METHODS

Conceptual background—The temporal genetic structure in the pollen pool for a quantitative trait is revealed by a shift in mean breeding value of pollen donors across time (Hendry and Day, 2005). In this section, we first explain how asynchronous flowering leads to temporal genetic structure in flowering time, i.e., the number of days from sowing to first flower. We then describe how temporal structure can be estimated prospectively and retrospectively. Finally, we explain our "gene trap" method, which isolates the temporal genetic structure among pollen donors from that of pollen recipients.

For heritable focal trait z, define the phenotypic mean of plants contributing to the pollen pool on any given day as the weighted mean of all individuals in the population, with the weighting factor for each plant being the number of open flowers it has on that day (Fig. 1). Assume no variation in pollen export per flower (i.e., no selection through pollen fertility or gametic competition). The weighted mean phenotype of plants contributing to the pollen pool on day t is

$$\overline{z}_t^{\mathrm{P}} = \frac{\sum_{i=1}^{N} z_i F_{it}}{\sum_{i=1}^{N} F_{it}}, \tag{1}$$

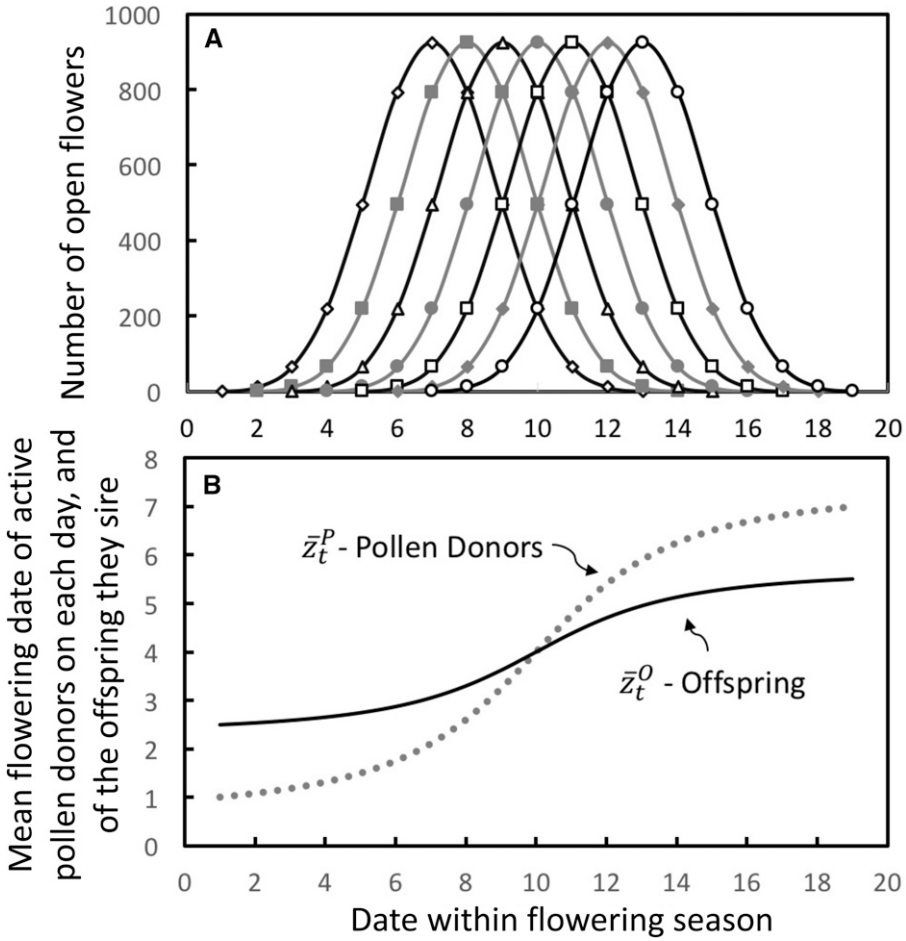

FIGURE 1 The basis for temporal genetic structure in date of first flowering. Panel A is the flowering schedules for seven plants. A single individual starts to flower on each of seven consecutive days; each reaches peak production on their seventh day and makes its final flower on the 13th day. Each day of the flowering season, every open flower has an equal chance of exchanging pollen with every other open flower. Panel B is the flowering date of the pollen donors (plants that contribute to the pollen pool) across the flowering season. The dotted curve connects the daily mean of active pollen donors, weighted by the number of open flowers. The solid line connects the expected flowering date of the offspring produced on each day (i.e., the breeding value). The difference between the linear regressions of the two types of daily means of date is the trait's heritability.

where superscript *P* denotes pollen donor, F_{it} is the number of flowers dispensing pollen on plant *i* on day *t*, and *N* is the population size. In essence, if a pollen grain is randomly sampled from the pool on day *t*, $\bar{z}_t^{\mathrm{P}}$ is the expected phenotype of the plant that produced it (Fig. 1B). Taking flowering time as the focal trait, Fig. 1B shows a shift in the trait mean value for contributors to the pollen pool over the course of the mating season, given the flowering schedules in Fig. 1A. Because donor and recipeint phenotypes shift concordantly through time, mating is assortative with respect to *z*. The slope of the regression of $\bar{z}_t^{\mathrm{P}}$ over *t* is the number of days that the mean pollen donor phenotype changes per calendar day (days per day). The slope is steep when the standard deviation in flowering time is large and the duration of individual flowering is brief (see Weis et al., 2014). A zero slope indicates that all donors contribute to the pollen pool in equal proportions on every day of the mating season, i.e., the population is composed of a single, unstructured mating pool.

This regression provides a phenotypic measure for temporal structure in the mating pool; the impact of pool structure on genetic structure depends on the trait's heritability. If the variation in flowering time is heritable, its mean breeding value shifts over the course of the season. Under panmixia, the breeding value of an individual sire, A_t, is twice the deviation of the expected (mean) phenotype of his offspring (sired across a random array of dams) from the population grand mean. The deviation is multiplied by two because the offspring get only half their genes from the sire. A sire's breeding value for a focal trait can be estimated as his phenotypic value multiplied by trait heritability (Falconer and MacKay, 1996). Thus, with negligible variance due to dominance and epistasis, the mean breeding value for the sires contributing to the pollen pool during time interval *t* will be

$$\bar{A}_t = 2\left(\bar{z}_t^{\mathrm{O}} - \hat{z}^{\mathrm{O}}\right) = \left(\bar{z}_t^{\mathrm{P}} - \hat{z}^{\mathrm{P}}\right)h^2, \qquad (2)$$

where $\hat{z}^{\mathrm{O}}$ and $\hat{z}^{\mathrm{P}}$ are the grand means of the offspring and parental generations, respectively, and h^2 is flowering time heritability. If mean donor phenotype shifts over time, so does breeding value (Fig. 1B). When the regression of $\bar{A}_t$ over *t* has zero slope, there is no temporal population genetic structure. A slope of 1.0 indicates two things: (1) There is complete turnover in the mating pool each successive day (all plants flower for a single day), dividing the population into a series of reproductively isolated mating pools, and (2) heritability of *z* is 1.0. Under these extreme conditions, not only is the mating pool of the current generation temporally structured, the offspring from each parental pool will form a distinct mating pool, dividing the population into a series of temporally reproductively isolated "quasi-species' (Devaux and Lande, 2008).

Equation 2 suggests that a two-generation experiment can determine whether the temporal population genetic structure for flowering time differs from that predicted by flowering schedule overlap. Flowering schedules of the parental generation are used to derive $\bar{z}_t^{\mathrm{P}}$ (Eq. 1), which is regressed over *t*. The slope of this regression, when multiplied by an independently derived estimate of h^2, is then the prospective estimate. The actual structure is then estimated retrospectively through the regression of the regression of $\bar{z}_t^{\mathrm{O}}$ over *t*. However, there is a problem; Eq. 2 estimated temporal structure among pollen donors and is valid only so long as their breeding values are uncorrelated to the pollen recipients. Assortative mating would normally induce such a correlation. We devised the "gene trap" method to isolate the genetic contribution of the pollen pool.

Gene trap methodology background—We broke the donor-recipient correlation by exposing successive batches of genetically uniform pollen recipients (dams) to "trap" genes from the natural pollen pool. Since the dam mean genotype is constant across the season,

shift in the mean offspring phenotype from the successive gene trap batches reflects genetic structure in the pollen pool exclusively.

A complication in this design, when applied to normal hermaphrodites, is that pollen recipients are also pollen producers that genetically contaminate the pollen pool. Our experiment avoided contamination by using male-sterile plants from a tester line as gene traps. We sowed successive batches of this line in the greenhouse, and upon flowering, set male-sterile individuals among the natural pollen donors for a limited interval. We then returned the gene traps to the greenhouse to mature their seed. Subsequently, we reared and phenotyped their progeny. This procedure minimized the genetic, developmental, and environmental variance among maternal plants to reveal the temporal genetic structure in the pollen pool.

The advantages of using male-sterile plants from the tester line as gene traps could be countered by strong dominance and epistatic interactions among phenology alleles from the wild and tester lines. If alleles interact strongly, the relationship of offspring phenotype to donor phenotype could have nonlinear components (Falconer and MacKay, 1996; Hallauer et al., 2010), biasing or reducing precision in the estimates of the donor breeding value. Recognizing this possibility, the deviation of offspring batch mean from the overall offspring mean is more precisely termed the mean combining ability of the pollen producers with the gene trap line. We constructed the gene traps from source materials that were unlikely to cause strong nonlinearities. Weis (2015) used the same source material (see below) and found that when these plants were pollinated by plants from a California wild population, he was able to account for the entire sire component of additive genetic variance for flowering time that was known to exist in the wild population. No variation in genetic contributions of the source material parents to offspring flower time was detected. These findings support our assumption of a linear relationship of breeding value to the combining ability of wild pollen donors to the gene trap line and that our method greatly restricted genetic variation for flowering time among the gene traps.

Constructing the gene trap line—Starting in the autumn of 2010, we produced the gene traps in the greenhouse by crossing male-sterile individuals from the *Brassica rapa* (Brassicaceae) Wisconsin FastPlants line 1-107 (Mendelian recessive male sterile gene) to five very-early-flowering Quebec pollen donors. We crossed the F_1 offspring within full-sibling groups and planted the resulting F_2 seeds in successive batches during the summer of 2011. The tester line plants were grown in ConeTainers (SC10-U; Stuewe & Sons, Tangent, Oregon, USA) filled with a mixture of 67% soil-less mix and 33% coarse sand in the greenhouse. We fertilized these plants with 20:20:20 soluble fertilizer on days 7 and 14 after planting. Throughout the summer, we placed the resulting male-sterile segregants (i.e., gene traps) into experimental plots to trap genes from pollen donors.

Pollen donors and spatial arrangement of plots—For the pollen donors, we used seed stock that came from a naturalized *B. rapa* Quebec population (46°39′N, 071°16′W). Heritability for flowering time for this population was previously estimated at 0.506 to 0.610, using a multigeneration animal model (Austen and Weis, 2016).

We conducted the two open-pollinated field experiments using *B. rapa* at the Koffler Scientific Reserve (44°1′N, 079°32′W), King Township, Ontario. The site is within the geographic range of *B. rapa*, but pollen contamination from the wild was highly unlikely: searches across several years found no natural populations within a 3-km radius. Bees in the Apidae and Halictidae and flies in the Syrphidae were the most frequent floral visitors (Parker et al., 2015).

In the first experiment (hereafter experiment one), we planted six plots of pollen donors, each with either 217 or 222 flowering plants, depending on spatial arrangement. We set up the pollen donor plants in a hexagonal plot where each side was 4 m long (Fig. 2). Each plot was at least 300 m from its closest plot. Pollinators may forage differently in a patchy (i.e., clumped) plant distribution compared a uniform (i.e., even) plant distribution (e.g., Cresswell, 2000; Geslin et al., 2014). Therefore, in three plots, we planted a pollen donor plant at 0.5 m spacing (Fig. 2A; hereafter, even plots). Within this matrix, six evenly spaced positions were reserved for the gene trap plants (Fig. 2A). In the other three plots, we grouped pollen donor plants into each corner of the hexagon (Fig. 2B; hereafter, clumped plots) at 0.17-m spacing and a minimum of 2.7 m between clumps. We reserved the position in the center of each clump for a gene trap (Fig. 2B). Cresswell (2000) found significant differences in the foraging behavior of *Bombus lapidaries* using similar interplant distances (0.4 m in uniform arrays and 0.2 m in patchy arrays). On each plant, we recorded the first flowering date the number of newly open flowers every 3–4 days (Fig. 3A, B). We predicted that the clumped plots would have a retrospective estimate that more closely matches the prospective estimate because of the high local conspecific density (see introduction).

Amplified heritability experiment—To fully confirm the relationship between flowering schedules and temporal population genetic structure, we performed an additional experiment (hereafter, the amplified heritability experiment) in which we artificially elevated the heritability of flowering time to see if that increased the temporal shift

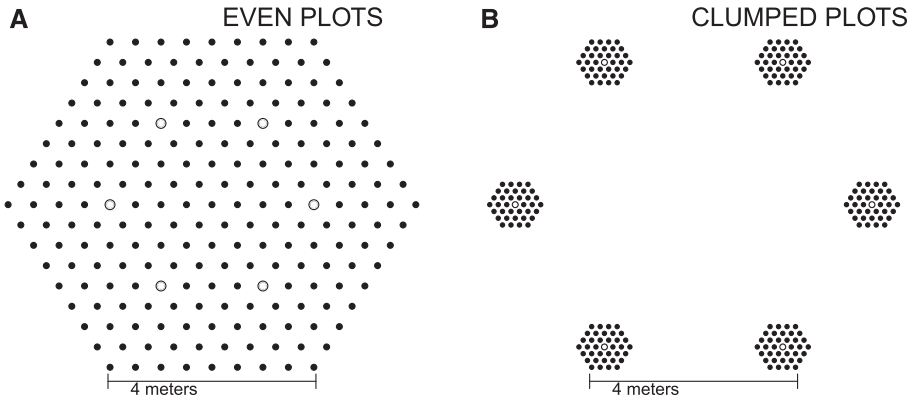

FIGURE 2 Diagrams of the experimental plot spatial arrangements. All plots were hexagon-shaped with each side measuring 4 m. In the even plots (A), plants were evenly spaced (0.5 m. apart; 217 total plants). In the clumped plots (B), plants were arranged into smaller hexagons in the corners of each larger hexagon. Within each clump, plants were spaced 0.17 m apart, and the shortest distance between clumps was 2.7 m (222 total plants). The black points represent a flowering plant that was producing pollen and contributing to the pollen pool. The six open circles represent the "gene trap" positions where male sterile FastPlant individuals were placed in regular intervals throughout the flowering season.

in flowering time breeding value, as estimated from slope of the regression $\bar{z}_t^{\mathrm{O}}$ over t (see Eq. 2). In two additional plots (one even and one clumped), we used pollen donor plants that were offspring from a greenhouse generation of *B. rapa* Quebec plants grown in the spring of 2011. We recorded flowering times, assortatively pollinated all parental plants by flowering time, then grew the progeny (i.e., pollen donor plants) in the greenhouse during the summer of 2011 before setting them into the field plots. We enhanced the heritability of flowering time among donors in two ways. First, we applied within-family stabilizing selection: specifically, we planted in the field plots only those individual progenies that had identical flowering time to their seed parent, discarding those that bloomed earlier or later. This approach would tend to eliminate individuals with extreme contributions of both genetic segregation and environment to flowering time variance, thereby increasing the genetic covariance between parent and offspring. Second, we limited variance in the developmental environment of pollen donors by growing them for their entire pre-flowering period under greenhouse conditions. This experiment ran from mid-August until early October in 2011. We recorded the date of first flowering for each plant, and the number of open flowers every 4 days (Fig. 3C, D).

Flowering time of gene trap offspring—To estimate the change in the combining ability for flowering time over the season, we planted the seeds matured from the gene trap plants and recorded the days to first flowering. The offspring were grown in the University of Toronto-St. George greenhouse during the winter of 2011–2012 with supplemental artificial light. We grew seven randomly chosen seeds from each gene trap plant. Seeds were sown in ConeTainers, filled with a mixture of 67% soil-less mix and 33% coarse sand, and fertilized with 20:20:20 soluble fertilizer on days 7 and 14 after planting. In a few cases, a gene trap plant did not have seven

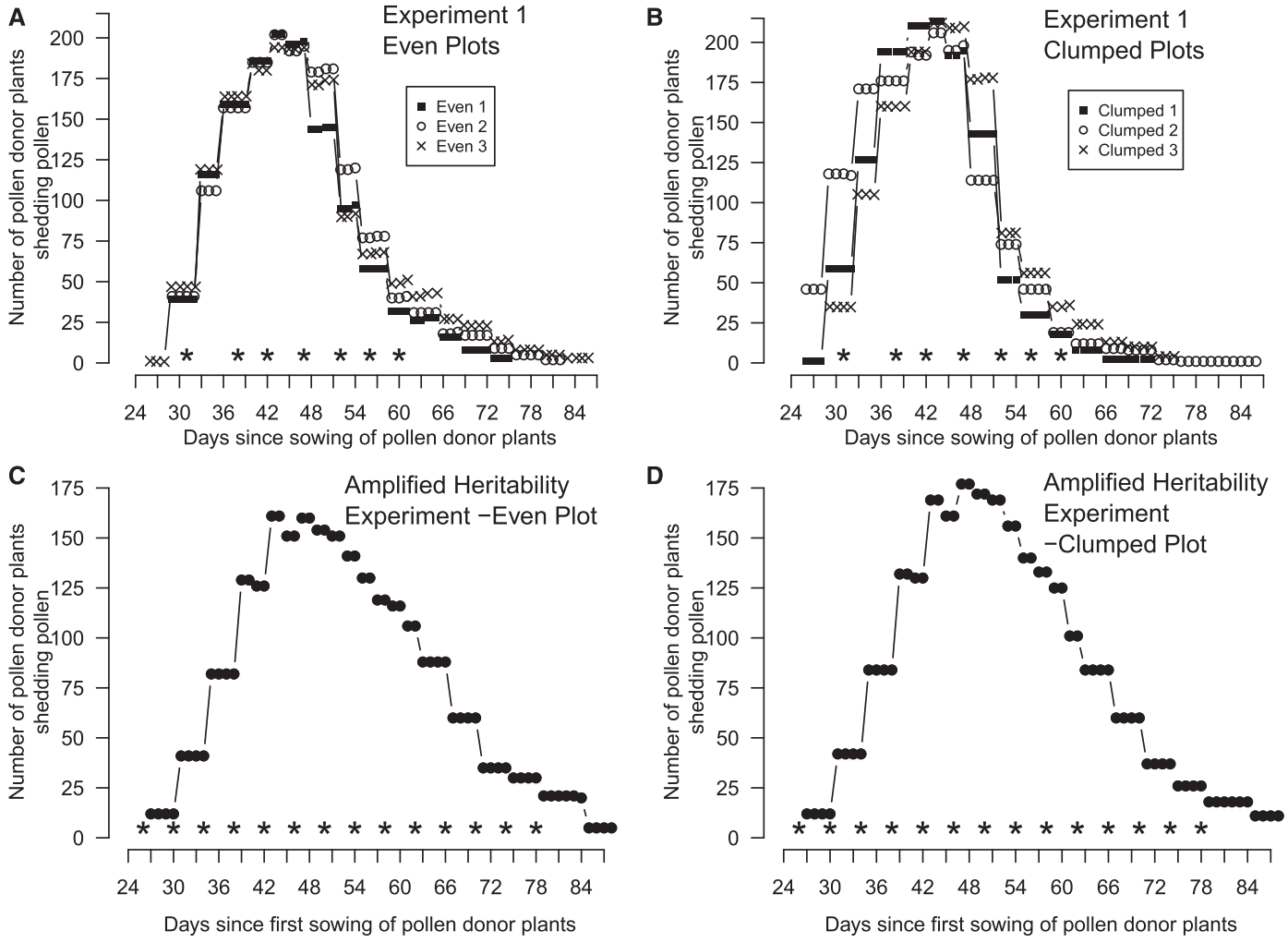

FIGURE 3 Flowering phenology of the experimental plots. In experiment one (A and B), plot plants were planted as 2-wk-old seedlings into the six plots (three plots for each spatial arrangement; see Fig. 2). In the amplified heritability experiment (C and D), we increased the heritability of flowering time by growing pollen donor plants in greenhouse conditions and plot plants with the same flowering start date as their maternal plants (see Methods). The points are the total number of pollen donor (plot) plants producing pollen on each day in each field plot (the legends distinguish each plot and its spatial arrangement). The stars are the day each male-sterile gene trap batch was placed into the plots. In experiment one (A and B), gene trap batches were placed into the plots on days 31, 38, 42, 47, 52, 56, and 60 since sowing of pollen donor. In the amplified heritability experiment (C and D), gene trap batches were placed into the plots on days 26, 30, 34, 38, 42, 46, 50, 54, 58, 62, 66, 70, 74, and 78 since first sowing of pollen donor plants.

offspring; some seeds did not germinate, or the offspring died before flowering. For this experiment, we had 2643 offspring that flowered (mean 6.1 offspring per gene trap).

Statistical analyses—We estimated the potential for assortative mating by flowering time in each plot using the Weis and Kossler (2004) approach, which quantifies the phenotypic correlation of flowering time between potential mates (ρ; Weis and Kossler's eq. 2). We documented the phenotypic shift among donors to the pollen pool through a linear regression of the pollen donor flowering time (weighted by the flower count for each pollen donor), in each plot over census date (days since sowing the pollen donor plants). Although the relationship between these two variables is not necessarily linear (e.g., Fig. 1B), the regression coefficient estimates the change in mean flowering time of pollen donors per day of season (days per day). Since the residual errors did not have constant variances, we used a robust variance estimator as implemented in the R package Sandwich 2.3-4 (Zeileis, 2006) to calculate the standard error and 95% confidence interval for the regression coefficients.

To determine whether spatial arrangement affected the mean phenotype of pollen donors, we combined all plots and constructed a regression by days since sowing of pollen donor plants, where the flowering date of the plants contributing to the pollen pool, spatial arrangement, and their interactions were covariates. We found the minimal adequate model for each experiment by stepwise backward elimination of terms using the log likelihood ratios. Plot was a random effect in the experiment one models but not in the amplified heritability experiment models since plot and spatial arrangement were confounded.

We estimated the strength of the temporal genetic structure for flowering by regressing the interval mean progeny flowering time ($\overline{z}_t^{\mathrm{O}}$, Eq. 2) over the days since sowing the pollen donor plants (specifically, the midpoint between placement and retrieval, as days since sowing) with maternal plant as a random effect. The regression coefficient reflects the average per day shift in combining ability. To examine the variance explained by the model, we used the R package MuMIn package (version 1.15.6), which estimates that R^2 value for fixed effects using the procedure of Nakagawa and Schielzeth (2013).

To determine whether spatial arrangement of the plots affected temporal genetic structure of the pollen pool, we conducted a mixed-effect regression of the progeny flowering time over the day of year, with spatial arrangement and the date × spatial arrangement interaction as fixed effects. Maternal plant nested within plot was a random effect for experiment one models, and maternal plant was a random effect for the amplified heritability experiment models. We found the minimal adequate model for each experiment by stepwise backward elimination of terms using the log likelihood ratios.

The sire and offspring generations were grown in different environments (summer-field vs. winter-greenhouse) with different photoperiods and light intensities. Shorter, dimmer winter days expand the variance in *B. rapa* flowering time (Franke et al., 2006). As a result, the regression coefficient of donor flowering time over days since sowing is not directly comparable to the coefficient for mean offspring flowering time over the days since sowing. We remedied this variance inflation by transforming offspring flowering times to match the observed variance among their parents; *z* scores were first calculated for the observed offspring data, then multiplied by the standard deviation of parental flowering time. As per Eq. 2, we then multiplied each transformed value by 2 to obtain the interval mean breeding values. This transformation puts the regressions of $\overline{z}_t^{\mathrm{O}}$ and $\overline{z}_t^{\mathrm{P}}$ over t into the same units.

As a final check on our procedures, we calculated an approximate heritability of flowering time in the field, which could be compared to values obtained by Austen and Weis (2016) for the Quebec population. To estimate heritability we used a mixed-effect linear regression of the progeny flowering time by the weighted mean flowering time of their potential sires (i.e., the regression of $\overline{z}_t^{\mathrm{O}}$ over $\overline{z}_t^{\mathrm{P}}$), which would equal $(1/2)h^2$ for the trait. Maternal plant was included as a random effect. Since we did not have exact flower counts for all of the interval midpoint dates, we used generalized additive models (gams), as implemented by the R package gam 1.12 (Hastie and Tibshirani, 1990), to interpolate the start date of the paternal plants for each midpoint date.

We conducted all data analysis in R version 3.2.3 (R Core Team, 2016). For the mixed-effect regressions, we used the R package lme4 1.1-8 (Bates et al., 2014). All standard errors and confidence intervals for the mixed-effect regressions were estimated using the package lsmeans 2.21-1 (Lenth, 2016).

RESULTS

Temporal shift in mean donor flowering time: The prospective estimate—The overall distribution and duration of flowering and pollen release was similar in all plots for both experiments (Fig. 3; Appendix S1, see Supplemental Data with this article). However, the overall shape of the flowering curves in the amplified heritability plots (Fig. 3C, D) were less skewed, and the mean flowering durations were a little longer than in the experiment one plots (22.8 and 23.4 days compared with 18.4–21.6 days in experiment one). The potential for phenological assortative mating as measured by ρ (Weis and Kossler Eq. 2) was stronger in the amplified heritability plots (0.54 and 0.64) compared to experiment one (0.36–0.52; Appendix S1). Flowering peaked 41 and 45 days after sowing pollen donors in experiment one and the amplified heritability experiment, respectively (Appendix S1).

In all plots for both experiments, we found significant shifts in the phenotype of active pollen donors over the flowering season (Table 1, Fig. 4; $P < 0.001$). The regression coefficients indicate that the mean flowering start date of pollen donors actively shedding pollen shifted by 0.387–0.506 d per day. The two plots in the amplified heritability experiment had similar regression coefficients to those in experiment one (Table 1). Thus, the potential for pollen donation shifted from early- to late-flowering plants as the season progressed in both experiments.

We found that even spacing of plants led to a stronger shift in the mean donor phenotype in experiment one (regression coefficients: 0.467 for even vs. 0.394 for clumped; Table 1, Fig. 4). The experiment-wide analysis verified the significance of the date × spatial arrangement interaction (Appendix S2). In the amplified heritability experiment, the difference between even and clumped coefficients was smaller (0.467 for even vs. 0.441 for clumped) but still significant (Appendix S3). However, spatial arrangement was not replicated in this the amplified heritability experiment, and so treatment effect per se cannot be isolated from plot effects.

Temporal genetic structure of the pollen pool: The retrospective estimate—Temporal genetic structure in the pollen pool was evident in five of six plots (all but in the clumped plot 2) from

TABLE 1. Regression coefficients of the donor mean flowering time ($\overline{z}_t^P$) and combining ability ($\overline{z}_t^O$) for mean flowering start date regressed over date within the flowering season (*t*). The donor mean over date models are the mean start date of pollen donor plants (weighted by the number of open flowers on each pollen donor) by the days since sowing of pollen donor plants (see Fig. 4). The combining ability over date are the gene trap progeny mean flowering start date (progeny flowering start date has the same standard deviation as the pollen donor plants; see Methods) by the days since sowing of the pollen donor plants (see Fig. 5). Maternal plant was modeled as a random effect.

	Donor mean			Combining ability		
Plot	Regression coefficient	95% CI	R^2	Regression coefficient	95% CI	R^2
Experiment 1						
EVEN 1	0.477‡	(0.432–0.523)	0.545	0.327‡	(0.148–0.507)	0.110
EVEN 2	0.418‡	(0.380–0.457)	0.499	0.279*	(0.057–0.503)	0.063
EVEN 3	0.506‡	(0.442–0.570)	0.542	0.335†	(0.143–0.527)	0.095
CLUMPED 1	0.397‡	(0.361–0.434)	0.445	0.337*	(0.082–0.590)	0.071
CLUMPED 2	0.398‡	(0.369–0.426)	0.504	0.136§	(−0.083–0.359)	0.021
CLUMPED 3	0.387‡	(0.352–0.421)	0.407	0.287*	(0.020–0.551)	0.041
Amplified heritability experiment						
EVEN	0.467‡	(0.447–0.487)	0.587	0.340‡	(0.254–0.426)	0.175
CLUMPED	0.441‡	(0.418–0.464)	0.491	0.343‡	(0.256–0.431)	0.200

Notes: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$, §not significant.

experiment one (Table 1). The progeny of early batches flowered earlier than those from late batches, even though maternal genotypes were randomized across all batches (significant regression coefficients ranged from 0.279 to 0.337 days per day; $P < 0.05$; Table 1, Fig. 5A, B). The regression coefficients for the amplified heritability experiment plots were steeper than in experiment one plots (0.340 and 0.343 d per day; $P < 0.001$; Table 1, Fig. 5C, D). Experiment-wide analysis did not detect an interaction between small scale spatial clumping of the pollen donors with temporal genetic structure in either experiment (Appendices S4, S5).

Relationship of mean offspring phenotype to mean pollen donor phenotype—In experiment one, average flowering time of the pollen donor during the exposure interval (i.e., $\overline{z}_t^P$) predicted the flowering time of the progeny sired over that interval ($\overline{z}_t^O$) in five of the six plots ($P < 0.05$; Table 2). The significant regression coefficients, which estimate $(1/2)h^2$, ranged from 0.274 to 0.395. The relationship of $\overline{z}_t^O$ to $\overline{z}_t^P$ was stronger in the amplified heritability experiment (regression coefficients 0.436 and 0.491; $P < 0.01$; Table 2).

DISCUSSION

The gene trap method confirmed temporal genetic structure for flowering time in the *B. rapa* plots: the mean combining ability of pollen donors shifted to later values over the flowering season (Table 1, Fig. 5). This shift is concordant with the seasonal shift in the mean phenotype of active pollen donors, as estimated from flower counts (Table 1, Fig. 4). When we amplified the genetic variation in flowering time, temporal structure was stronger (Table 1). The positive regression of breeding value over time (Table 1) is qualitatively consistent with the prediction emerging from the positive regression of mean pollen donor flowering date over time (Table 2). Surprisingly, the spatial arrangement of pollen donors (even vs. clumped) had no detectable impact on temporal population genetic structure for flowering date (Appendices S4, S5). Previous studies have found differences in bumblebees foraging behavior at similar interplant distances and spatial arrangements (e.g., Cresswell, 2000; Geslin et al., 2014). However, our plots were pollinated by a number of generalist insect pollinator taxa, (mostly bees in the Apidae and Halictidae and flies in the Syrphidae). We also examined the resulting plant mating patterns and not the foraging behavior. Therefore, a larger difference in interplant distances between the two treatments may have yielded different results and would be worth pursuing in future studies.

Comparing the prospective and retrospective estimates of temporal structure—Even though there was qualitative agreement between the retrospective and prospective estimates (Tables 1, 2), our goal was to see whether the estimates agree quantitatively under field conditions. The prospective estimate calculates the daily mean pollen donor phenotype ($\overline{z}_t^P$, Eq. 1) by assuming that every flower open on day *t* has an equal probability of exchanging pollen with every other open flower. This assumption will seldom be true because effective pollen movement in natural populations is known to vary by distance between plants (Kitamoto et al., 2006; Ison et al., 2014), coflowering species (Flanagan et al., 2011), and pollinator taxa (Greenleaf et al., 2007).

In addition, even if pollen is randomly dispersed, our retrospective estimate of temporal structure could potentially disagree with the prospective estimate because of genetic factors. The gene trap procedure estimated combining abilities between the natural population and a tester line, which could be influenced by epistatic effects on the offspring flowering time. Combining ability and breeding value are perfectly correlated under specific conditions: pollen donors and recipients are from the same breeding population, and, maternal and paternal genetic contributions are uncorrelated. The gene trap method assures the second condition is met, but not the first. Epistatic effects among genes of the FastPlant and Quebec lines could have either masked or amplified genetic effects inherited from the pollen donors. The data indicate that any masking/amplifying of genetic variance among sires was small. In experiment one, the slope of the regression of the interval mean offspring flowering dates ($\overline{z}_t^O$) over interval mean pollen donor ($\overline{z}_t^P$)—which equals $(1/2)h^2$ in the absence of bias (Table 2, Fig. 6)—falls within the range expected from independently obtained heritability estimates for the Quebec population (0.506 and 0.610; Austen and Weis, 2016). The observed slopes for the experiment one plots

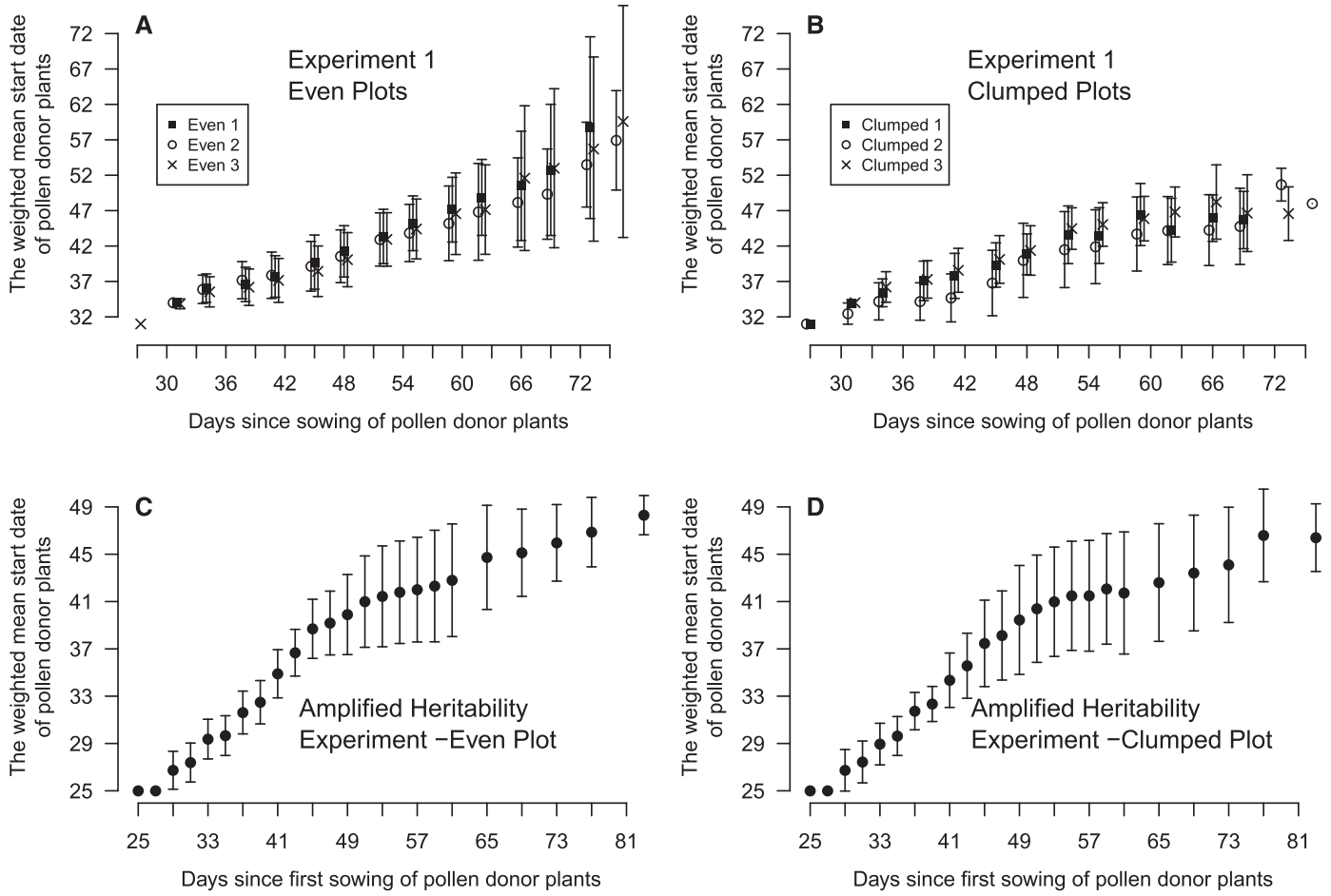

FIGURE 4 The phenotype of the pollen pool of the experimental plots. The points are the mean start date (days since sowing) of the pollen donor (plot) plants shedding pollen on each given census date weighted by the number of flowers each plant produced. In experiment one (A and B), pollen donor plants were planted as 2-wk-old seedlings into the six plots (three plots for each spatial arrangement; see Fig. 2). The legends distinguish each field plot and its spatial arrangement. In the amplified heritability experiment (C and D), we increased the heritability of flowering time (see Methods). The error bars are ± one standard deviation.

averaged 0.284 (range: 0.129–0.395; Table 2). Doubling these gives an average h^2 estimate of 0.568, which lies between the two independent predictions. This agreement indicates that bias due to dominance and epistasis is minimal.

Given this minimal genetic bias, a quantitative agreement between the retrospective and prospective estimates of temporal population structure would indicate that pollen is exchanged at random within days. In experiment one, the slope of donor mean over date averaged 0.430 days over day (range 0.387–0.506, Table 1), which puts the prospectively estimated slope of breeding value over date ($h^2 \times$ slope) at $0.568 \times 0.430 = 0.224$. This prospective estimate of temporal structure is within two standard errors of the mean retrospective estimate of 0.303 ± 0.092 (Appendix S4), suggesting that flowers received a random sample of the pollen that was in circulation on the days they were open. Although the estimated ranges overlap, the retrospective estimate is 35% larger (0.303) than the prospective estimate (0.224), thus leaving open the possibility that natural populations may actually have more temporal structure than expected simply from overlap in the flowering schedules. Interestingly, we found no difference in the retrospective temporal structure between the even and clumped plots. The mean prospective estimate of the clumped plots was 84% as large as for the even. The mean retrospective estimate was 80% as large. The fact that prospective estimate was significant and the retrospective estimate was not may simply reflect greater measurement error in estimating breeding values than in phenotypes (Appendices S2-S5).

Genome-wide consequences of temporal population structure in flowering time—In many species, individuals enter and exit the mating pool asynchronously. In addition to flowering time, examples include insect eclosion date, salmon arrival date at spawning sites, avian laying date, and parturition date in mammals (reviewed by Hendry and Day, 2005). If entry and exit dates vary, the population divides into a temporal succession of (partially) reproductively isolated mating pools. The degree of isolation depends upon the variance in starting date and the mean duration of individual mating periods (Weis et al., 2014). Temporal genetic structure for entry date and duration arises when these traits are heritable (Devaux and Lande, 2008, 2010).

There are two scenarios in which temporal genetic structure in reproductive phenology could contribute to temporal structure

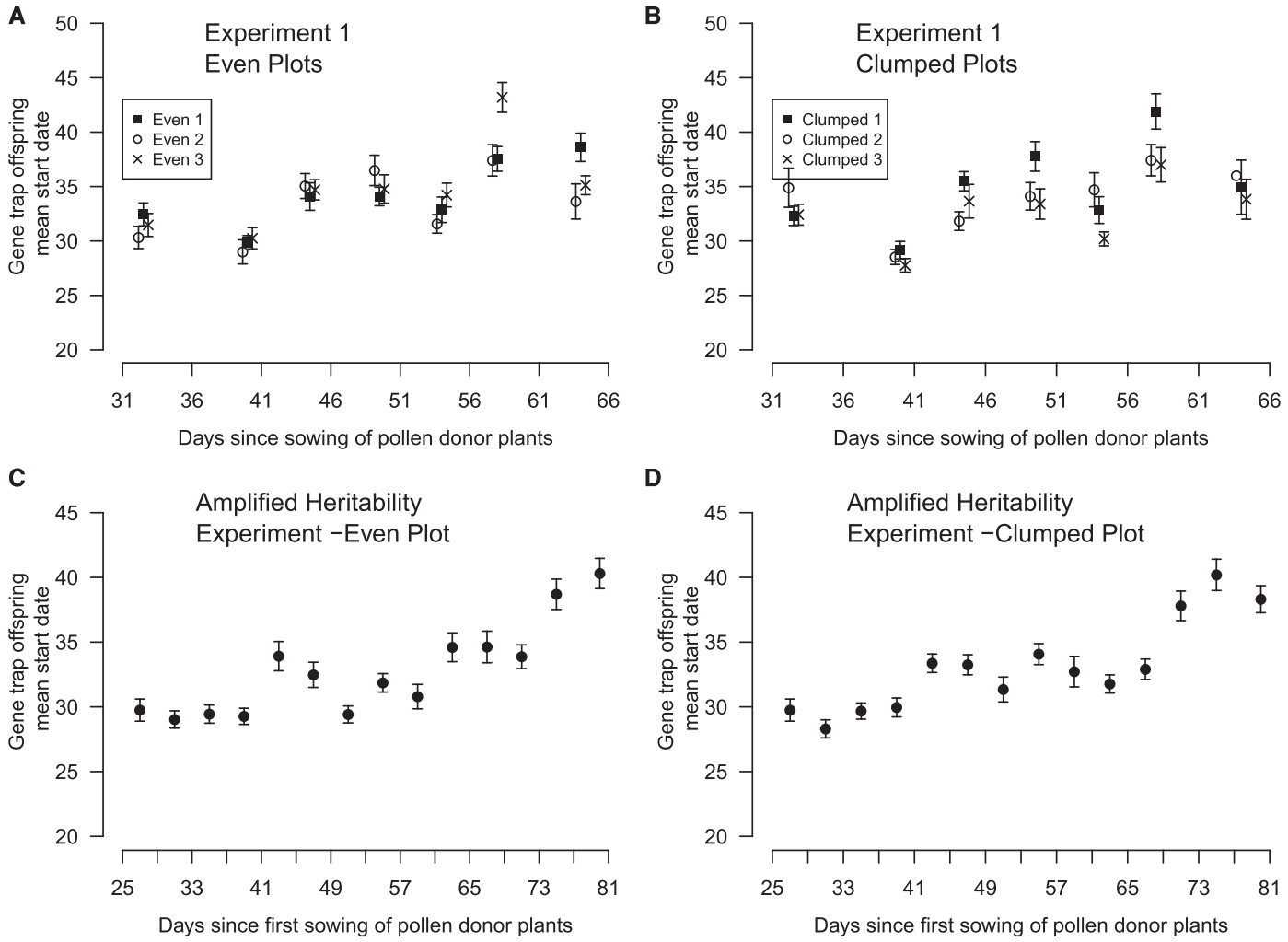

FIGURE 5 Flowering time of gene trap offspring by day. The *x*-axes are the days since sowing of the pollen donor (plot) plants. The points are the mean day each gene trap batch was in the field plotted by the mean flowering start date of the gene trap progeny for each batch (see Fig. 3 for batch interval details). Panels A and B are the progeny from experiment one. The legends distinguish each field plot and its spatial arrangement. Panels C and D are the progeny from the amplified heritability experiment where we increased the heritability of flowering time (see Methods). The error bars are ± one standard error of the mean.

across all loci. The first, described by Hendry and Day (2005), requires that mating date be highly heritable, individuals have brief mating periods, and the population as a whole has a long mating season. These conditions would push the regression of mating time breeding value over date toward a slope of 1.0 (complete turnover in the mating pool every day). In finite populations, genetic drift at neutral loci might then lead to divergence among temporal pools. Several recent studies found temporal structure at neutral loci in natural populations where individual flowering periods are short relative to the flowering season length (Daïnou et al., 2012; Suni and Whiteley, 2015). In contrast, the overlap among individual flowering schedules in most temperate species is too broad to divide populations into multiple mating pools (Rabinowitz et al., 1981; Herrera, 1986; Ison et al., 2014; Weis et al., 2014; Wadgymar et al., 2015). Yet we found that the realized temporal structure was at least as strong, and potentially stronger, than the predicted structure based solely on flowering schedules (Tables 1, 2).

The second scenario applies when breeding value for mating date is itself spatially structured. Mating between close neighbors will be more frequent than random if they are within the pollen dispersal radius *and* their flowering schedules overlap. In this scenario, temporal structure amplifies spatial structure.

TABLE 2. Regression coefficients for heritability of the pollen pool. The models are the gene trap progeny flowering start date (progeny flowering start date has the same standard deviation as the pollen donor plants; see Methods) by mean flowering start date of pollen donor plants (weighted by the number of open flowers on the pollen donors). Maternal plant is modeled as a random effect.

Plot	Regression coefficient	95% CI
Experiment 1		
EVEN 1	0.295†	0.140–0.451
EVEN 2	0.294*	0.055–0.536
EVEN 3	0.277†	0.112–0.442
CLUMPED 1	0.393†	0.117–0.668
CLUMPED 2	0.129‡	−0.092–0.355
CLUMPED 3	0.315*	0.049–0.579
Amplified Heritability Experiment		
EVEN	0.436†	0.326–0.546
CLUMPED	0.491†	0.366–0.617

Notes: *$P < 0.05$, †$P < 0.01$, ‡not significant.

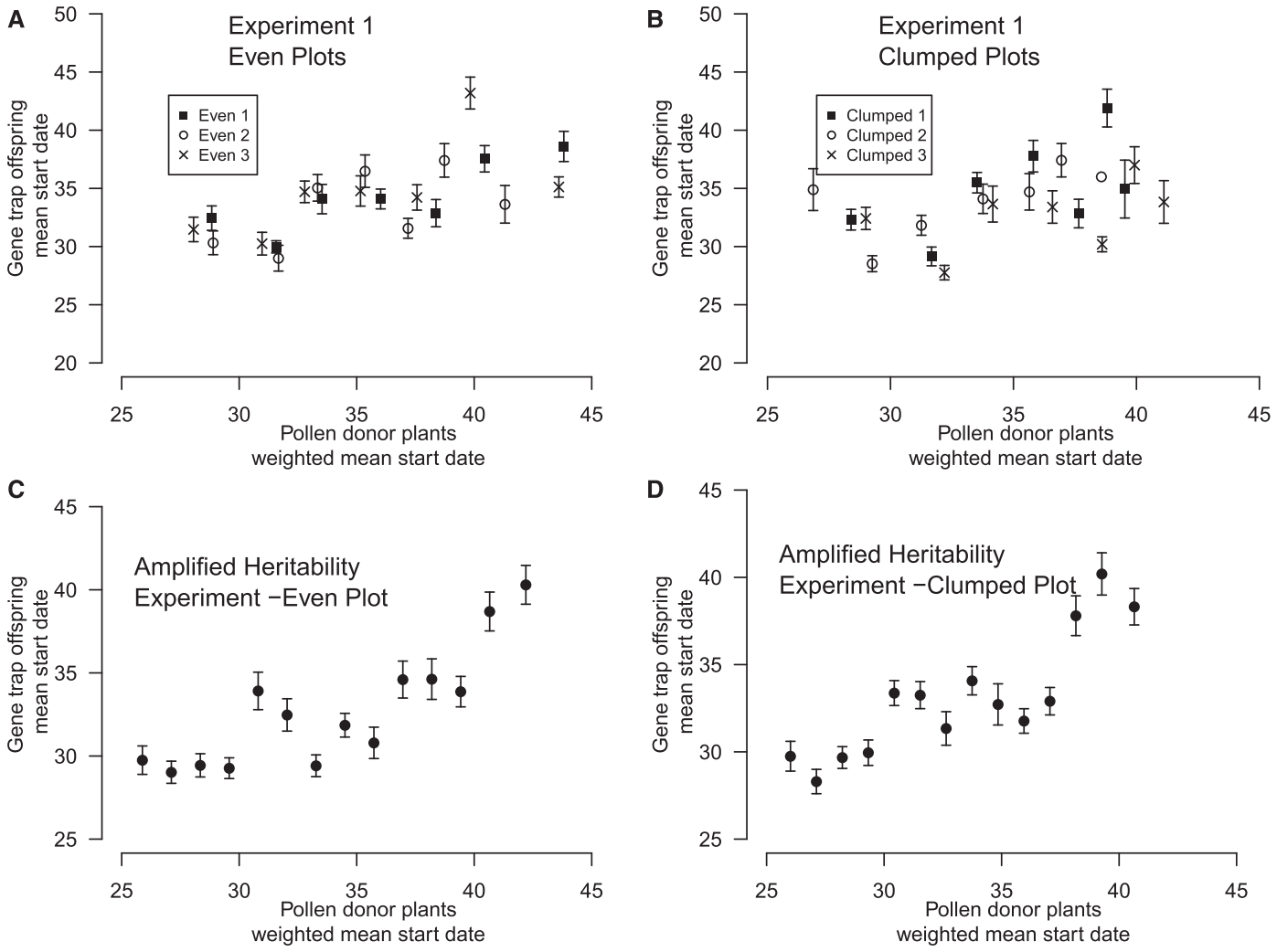

FIGURE 6 Flowering start date of gene trap offspring by phenotype of pollen pool. The *x*-axes are the mean flowering start date (days since sowing) of all pollen producing pollen donor (plot) plants in the population weighted by the number of newly opened flowers of each plant. The *y*-axes are the mean flowering start date (days since sowing) of the gene trap progeny for each batch (see Fig. 3 for batch interval details). Panels A and B are the progeny from experiment one. The legends distinguish each field plot and its spatial arrangement. Panels C and D are the progeny from the amplified heritability experiment where we increased the heritability of flowering time (see Methods). The error bars are ± one standard error of the mean.

Almeida-Neto et al. (2004) found spatial clustering of flowering phenology of the Brazilian shrub *Chromolaena odorata* consistent with spatial genetic correlation. Hirao and Kudo (2008) showed that in *Primula cuneifolia* the spatial genetic correlation at neutral loci declines more dramatically across space among individuals with nonoverlapping flowering schedules than for those that do overlap. In this regard, we believed that the pollen dispersal radius might expand at the beginning and end of the flowering season when the spatial density of flowers declines, prompting pollinators to move greater distances between visits, dampening the effect of temporal structure on spatial structure. We hypothesized that the interaction between temporal and spatial structure would vary between our two spatial arrangements because insect pollinators may vary their foraging behavior based on whether plants are uniformly dispersed compared to a more clumped distribution (Cresswell, 2000). However, we found no evidence of spatial density dampening the temporal structure (Fig. 6, Table 2) and no difference between the spatial arrangements (Appendices S4, S5).

Flowering time and adaptive temporal clines—It has been speculated that when selection on a focal trait shifts over the course of the season, temporal genetic structure can facilitate formation of adaptive temporal clines (Hendry and Day, 2005). This idea can be reformulated in terms of disruptive selection and assortative mating. For instance, Parachnowitsch and Caruso (2008) found that seed predators attack early flowering *Lobelia siphilitica* individuals more heavily than late ones, which led Parachnowitsch et al. (2012) to predict that early blooming plants should have higher levels of a latex-based defense, whereas costs of latex production favors low defense in late bloomers. Floral/seed predator attack varies across the season in a number of systems (Elzinga et al., 2007). However temporal clines in defense found for other systems appear neutral (Johnson et al., 2009) or even maladaptive (Berenbaum et al., 1986; Juenger et al., 2005). We found

that temporal population genetic structure is probably a common consequence of genetic variation in flowering time. By inducing assortative mating, it likely facilitates response to directional selection on phenology and on traits genetically correlated to phenology (see Weis, 2005). Even if temporal structure sufficient for the evolution of temporal clines is uncommon, our study gives a metric for measuring that strength and shows in principle that, given the right selective pressure, temporal clines are possible in plant populations.

DATA ACCESSIBILITY

Data files and R scripts available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.cn1c3.

ACKNOWLEDGEMENTS

We thank J. D. K. Bai, G. R. Son, D. Czosniak, J. E. Ison, O. S. Hsu, J. Jenson, and B. Anderson for assistance in the field and greenhouse. E. Austen and S. Wadgymar provided helpful feedback during project development. We thank N. and T. Dier for providing an amiable space to write and J. M. C. Weis for proofreading assistance. We also thank two anonymous referees for their helpful comments on this manuscript. Funding was provided by grants from the Canada Foundation for Innovation, the Natural Sciences and Engineering Research Council of Canada (RGPIN-2016-06540), and the University of Toronto.

LITERATURE CITED

Almeida-Neto, M., T. M. Lewinsohn, and R. H. Økland. 2004. Small-scale spatial autocorrelation and the interpretation of relationships between phenological parameters. *Journal of Vegetation Science* 15: 561–568.

Austen, E. J., J. R. K. Forrest, and A. E. Weis. 2015. Within-plant variation in reproductive investment: Consequences for selection on flowering time. *Journal of Evolutionary Biology* 28: 65–79.

Austen, E. J., and A. E. Weis. 2016. Estimating selection through male fitness: Three complementary methods illuminate the nature and causes of selection on flowering time. *Proceedings. Biological Sciences* 283: 1–9.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*. Available at http://arxiv.org/abs/1406.5823.

Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: Wild parsnips and the parsnip webworm. *Evolution* 40: 1215–1228.

Brys, R., and H. Jacquemyn. 2010. Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). *Plant Biology* 12: 597–603.

Cresswell, J. E. 2000. A comparison of bumblebees' movements in uniform and aggregated distributions of their forage plant. *Ecological Entomology* 25: 19–25.

Daïnou, K., E. Laurenty, G. Mahy, O. J. Hardy, Y. Brostaux, N. Tagg, and J.-L. Doucet. 2012. Phenological patterns in a natural population of a tropical timber tree species, *Milicia excelsa* (Moraceae): Evidence of isolation by time and its interaction with feeding strategies of dispersers. *American Journal of Botany* 99: 1453–1463.

Devaux, C., and R. Lande. 2008. Incipient allochronic speciation due to non-selective assortative mating by flowering time, mutation and genetic drift. *Proceedings. Biological Sciences* 275: 2723–2732.

Devaux, C., and R. Lande. 2010. Selection on variance in flowering time within and among individuals. *Evolution* 64: 1311–1320.

Dudash, M. R., C. Hassler, P. M. Stevens, and C. B. Fenster. 2011. Experimental floral and inflorescence trait manipulations affect pollinator preference and function in a hummingbird-pollinated plant. *American Journal of Botany* 98: 275–282.

Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: Flowering phenology and biotic interactions. *Trends in Ecology & Evolution* 22: 432–439.

Ennos, R. A., and R. K. Dodson. 1987. Pollen success, functional gender and assortative mating in an experimental plant population. *Heredity* 58: 119–126.

Falconer, D. S., and T. F. C. MacKay. 1996. Introduction to quantitative genetics, 4th ed. Longmans Green, Harlow, UK.

Felsenstein, J. 1981. Continuous-genotype models and assortative mating. *Theoretical Population Biology* 19: 341–357.

Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution* 45: 398–409.

Flanagan, R. J., R. J. Mitchell, and J. D. Karron. 2011. Effects of multiple competitors for pollination on bumblebee foraging patterns and *Mimulus ringens* reproductive success. *Oikos* 120: 200–207.

Fox, G. A. 2003. Assortative mating and plant phenology: Evolutionary and practical consequences. *Evolutionary Ecology Research* 5: 1–18.

Franke, D. M., A. G. Ellis, M. Dharjwa, M. Freshwater, M. Fujikawa, A. Padron, and A. E. Weis. 2006. A steep cline in flowering time for *Brassica rapa* in southern California: Population-level variation in the field and the greenhouse. *International Journal of Plant Sciences* 167: 83–92.

Franks, S. J. 2015. The unique and multifaceted importance of the timing of flowering. *American Journal of Botany* 102: 1401–1402.

Geber, M. A., and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. *International Journal of Plant Sciences* 164 (S3): S21–S42.

Gérard, P. R., E. K. Klein, F. Austerlitz, J. F. Fernández-Manjarrés, and N. Frascaria-Lacoste. 2006. Assortative mating and differential male mating success in an ash hybrid zone population. *BMC Evolutionary Biology* 6: 96.

Geslin, B., M. Baude, F. Mallard, and I. Dajoz. 2014. Effect of local spatial plant distribution and conspecific density on bumble bee foraging behaviour. *Ecological Entomology* 39: 334–342.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153: 589–596.

Gutierrez, M. G., and G. F. Sprague. 1959. Randomness of mating in isolated polycross plantings of maize. *Genetics* 44: 1075–1082.

Hallauer, A. R., M. J. Carena, and J. B. Miranda Filho. 2010. Testers and combining ability. *In* M. J. Carena, A. R. Hallauer, and J. B. Miranda Filho [eds.], Quantitative genetics in maize breeding, 383–423. Springer, New York, New York.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics, 2nd ed. Sinauer, Sunderland, Massachusetts, USA.

Hastie, T. J., and R. J. Tibshirani. 1990. Generalized additive models. CRC Press, Boca Raton, Florida, USA.

Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: Isolation by time and adaptation by time. *Molecular Ecology* 14: 901–916.

Herrera, J. 1986. Flowering and fruiting phenology in the coastal shrublands of Doñana, south Spain. *Plant Ecology* 68: 91–98.

Hirao, A. S., and G. Kudo. 2008. The effect of segregation of flowering time on fine-scale spatial genetic structure in an alpine-snowbed herb *Primula cuneifolia*. *Heredity* 100: 424–430.

Ison, J. L., and S. Wagenius. 2014. Both flowering time and distance to conspecific plants affect reproduction in *Echinacea angustifolia*, a common prairie perennial. *Journal of Ecology* 102: 920–929.

Ison, J. L., S. Wagenius, D. Reitz, and M. V. Ashley. 2014. Mating between *Echinacea angustifolia* (Asteraceae) individuals increases with their flowering synchrony and spatial proximity. *American Journal of Botany* 101: 180–189.

Ison, J. L. and A. E. Weis. 2017. Data from: Temporal population genetic structure in the pollen pool for flowering time: A field experiment with *Brassica rapa* (Brassicaceae). Dryad Digital Repository. doi:10.5061/dryad.cn1c3.

Johnson, M. T., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. *Journal of Evolutionary Biology* 22: 1295–1307.

Juenger, T., T. C. Morton, R. E. Miller, and J. Bergelson. 2005. Scarlet gilia resistance to insect herbivory: The effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. *Evolutionary Ecology* 19: 79–101.

Karron, J. D., and R. J. Mitchell. 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Annals of Botany* 109: 563–570.

Kirkpatrick, M. 2000. Reinforcement and divergence under assortative mating. *Proceedings. Biological Sciences* 267: 1649–1655.

Kitamoto, N., S. Ueno, A. Takenaka, Y. Tsumura, I. Washitani, and R. Ohsawa. 2006. Effect of flowering phenology on pollen flow distance and the consequences for spatial genetic structure within a population of *Primula sieboldii* (Primulaceae). *American Journal of Botany* 93: 226–233.

Lenth, R. V. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* 69: 1–33.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4: 133–142.

Parachnowitsch, A. L., and C. M. Caruso. 2008. Predispersal seed herbivores, not pollinators, exert selection on floral traits via female fitness. *Ecology* 89: 1802–1810.

Parachnowitsch, A. L., C. M. Caruso, S. A. Campbell, and A. Kessler. 2012. *Lobelia siphilitica* plants that escape herbivory in time also have reduced latex production. *PLoS One* 7: e37745.

Parker, A. J., J. L. Tran, J. L. Ison, J. D. K. Bai, A. E. Weis, and J. D. Thomson. 2015. Pollen packing affects the function of pollen on corbiculate bees but not non-corbiculate bees. *Arthropod-Plant Interactions* 9: 197–203.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.R-project.org/.

Rabinowitz, D., J. K. Rapp, V. L. Sork, B. J. Rathcke, G. A. Reese, and J. C. Weaver. 1981. Phenological properties of wind- and insect-pollinated prairie plants. *Ecology* 62: 49–56.

Rathcke, B. J., and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* 16: 179–214.

Savolainen, V., M.-C. Anstett, C. Lexer, I. Hutton, J. J. Clarkson, M. V. Norup, M. P. Powell, et al. 2006. Sympatric speciation in palms on an oceanic island. *Nature* 441: 210–213.

Schmitt, J. 1983. Density-dependent pollinator foraging, flowering phenology, and temporal pollen dispersal patterns in *Linanthus bicolor*. *Evolution* 37: 1247–1257.

Suni, S. S., and A. R. Whiteley. 2015. Genetic structure of a montane perennial plant: the influence of landscape and flowering phenology. *Conservation Genetics* 16: 1431–1442.

Thomson, J. D., and B. A. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: Implications for gene flow and reproductive success. *Evolution* 43: 657–661.

Wadgymar, S. M., E. J. Austen, M. N. Cumming, and A. E. Weis. 2015. Simultaneous pulsed flowering in a temperate legume: Causes and consequences of multimodality in the shape of floral display schedules. *Journal of Ecology* 103: 316–327.

Weis, A. E. 2005. Direct and indirect assortative mating: A multivariate approach to plant flowering schedules. *Journal of Evolutionary Biology* 18: 536–546.

Weis, A. E. 2015. Inheritance of rapid cycling in *Brassica rapa* Fast Plants: Dominance that increases with photoperiod. *International Journal of Plant Sciences* 176: 859–868.

Weis, A. E., and T. M. Kossler. 2004. Genetic variation in flowering time induces phenological assortative mating: Quantitative genetic methods applied to *Brassica rapa*. *American Journal of Botany* 91: 825–836.

Weis, A. E., E. Nardone, and G. A. Fox. 2014. The strength of assortative mating for flowering date and its basis in individual variation in flowering schedule. *Journal of Evolutionary Biology* 27: 2138–2151.

Wright, S. 1943. Isolation by distance. *Genetics* 28: 114–138.

Wright, S. 1921. Systems of mating. III. Assortative mating based on somatic resemblance. *Genetics* 6: 144–161.

Zeileis, A. 2006. Object-oriented computation of sandwich estimators. *Journal of Statistical Software* 16: 1–16.