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Simultaneous pulsed flowering in a temperate legume: causes and consequences of multimodality in the shape of floral display schedules

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Summary

1. In plants, the temporal pattern of floral displays, or display schedules, delimits an individual's mating opportunities. Thus, variation in the shape of display schedules can affect the degree of population synchrony and the strength of phenological assortative mating by flowering onset date. A good understanding of the mechanisms regulating the timing of flowering onset has been developed, but we know less about factors influencing subsequent patterns of floral display.

2. We observed unusual multimodal display schedules in temperate populations of the annual legume *Chamaecrista fasciculata*. Here, we ask whether 'flowering pulses' are simultaneous among individuals and populations and explore potential underlying mechanisms and consequences of pulsed flowering.

3. We monitored daily flower production for individual plants from genetically divergent populations during a series of field experiments that manipulated three potential influencers of display schedule shape: average daily temperature, pollinator availability and watering schedules. We measured floral longevity to isolate the contributions of flower retention and flower deployment to display schedules. We assessed relationships between flowering and environmental variables and compared estimates of population synchrony, individual synchrony and the strength of assortative mating with those of 29 unimodally flowering species from the area.

4. We observed simultaneous flowering pulses in all experiments, with peaks aligned among individuals and populations despite variation in flowering onset and/or duration. Pulses were not the result of increases in average temperature, pollinator availability or variation in watering schedules. Seasonal fluctuations in temperature correlated with floral longevity and flower deployment, suggesting that the shape of display schedules may be plastic in response to fluctuations in temperature. Average population and individual synchrony differed only slightly from those of the species with unimodal schedules, while the average strength of assortative mating for flowering onset date was strongly reduced (0.21 in *C. fasciculata* vs. 0.35 for the 29 other species).

5. *Synthesis*. Researchers should take caution in assuming that components of display schedules are genetically or developmentally correlated with flowering onset. Variation in the shape of display schedules can influence patterns of gene flow within or between populations, with potential effects on the strength of phenological assortative mating and subsequent responses to selection.

Key-words: *Chamaecrista fasciculata*, floral longevity, flower deployment, individual synchrony, phenological assortative mating, population synchrony, reproductive ecology

Introduction

The opportunities for pollen exchange among plants are dependent on temporal patterns of flower production, or flowering phenology. Coupled with other factors, including the composition of the pollinator community, the spatial layout of members of the population or the mating system of a species, synchrony in flower production among individuals can affect outcrossing rates within populations (Loveless & Hamrick 1984; Young 1988; Ims 1990; Ison *et al.* 2014). Individual variation in patterns of flowering determines the potential for non-random mating among plants with distinct flowering onset dates (phenological assortative mating), potentially influencing the efficacy of selection on flowering onset and correlated traits (Weis & Kossler 2004; Weis 2005). Despite these far-reaching effects, we have a limited understanding of the factors affecting the schedule of flowers on display across the season, or how variation in the shape of these display schedules influences the temporal structure of a plant's mating pool.

Species in temperate regions tend to flower in a unimodal fashion over the span of several weeks, with flower production

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increasing at a rapid rate to a maximum, or peak, display size, followed by a steady decline in flower number as all internal resources are diverted away from flower production and towards maturing fruit (Rabinowitz *et al.* 1981; Herrera 1986; Weis, Nardone & Fox 2014). Display schedules of this shape have been described by algebraic functions that estimate peak flowering dates and the dispersion of flower production over the season (Malo 2002; Clark & Thompson 2011). However, in perennials, the shape of individual- or population-level display schedules can be variable across years (Picó & Retana 2001). This suggests that plasticity in the symmetry of display schedules (skew), the magnitude of peak flowering (kurtosis), the number of days where flowers are produced (duration) or the number of flowering peaks (modality) may be more common than currently appreciated. In fact, a close examination of cases where individual display schedules have been tallied typically reveals short-term fluctuations in flower number about a smoother, underlying seasonal pattern (Malo 2002; Clark & Thompson 2011; Austen, Jackson & Weis 2014; Weis, Nardone & Fox 2014). This phenological 'noise' may be the result of immediate developmental responses to factors influencing flower deployment (the opening of new flowers) or floral longevity (the retention of previously open flowers), which together comprise the flowers on display each day. Fluctuations in display and deployment schedules may occur simultaneously across individuals, indicating a shared plastic response to the same external stimuli. But what causes these day-to-day fluctuations?

The display schedule can be sensitive to many factors, including environmental conditions or resource availability (Bustamante & Búrquez 2008); resource partitioning among vegetative, defensive and reproductive functions (Bazzaz *et al.* 1987); or meristem availability and allocation (Bonser & Aarssen 1996). The contribution of these factors can be teased apart experimentally (Diggle 1995); however, understanding the proximal mechanisms underlying schedule shape presents several challenges. Subtle variation in schedule shape may only be detected with fine-scale monitoring of flowering at the individual level (Miller-Rushing, Inouye & Primack 2008; Morellato *et al.* 2010). Display schedules may be influenced by environmental factors that present both seasonal trends and daily fluctuation (e.g. precipitation, temperature). These time-series data are often messy and autocorrelated, requiring special methods for analysing relationships between variables (Hudson 2010; Brown *et al.* 2011). Additionally, the daily environment can influence both flower deployment and floral longevity (Augspurger 1983; Primack 1985), requiring fine-scale data to distinguish between the contributions of each to the display schedule.

Temporal shifts in internal resource allocation can also affect the shape of display and deployment schedules (Stephenson 1981; Stanton, Bereczky & Hasbrouck 1987). Plants face a trade-off between current reproduction (resource investment in seed maturation) and future reproduction (resource investment in the production of new flowers). The potential number of flowers deployed in a given day can be inversely related to the number of fruit being matured (Primack 1978; Lloyd 1980), where a decrease in flower number in the deployment schedule reflects a temporary resource shift to fruit maturation after a period of effective pollination (Stephenson 1981). This phenomenon has been observed in the tropics, where a lack of seasonality permits flowering year-around and display schedules are often multimodal (Newstrom, Frankie & Baker 1994). The dependence of flower deployment on internal resource availability can be detected when monitoring flower number where pollinator services are limited or absent and few to no fruit are being matured.

Fluctuations in display schedules influence the probability of pollen exchange between any two individuals: plants that fluctuate in synchrony will share more mating opportunities than those fluctuating independently. The shape of display schedules dictates the degree of phenological synchrony within and among individuals, which in turn can influence rates of outcrossing and selfing via pollinator movements, or geitonogamy. Synchrony in display schedules is an essential requirement for random mating in plants. Thus, variation in schedule shape may also alter the strength of phenological assortative mating by flowering onset date in a population (Fox 2003), although this has yet to be formally tested.

In this study, we examine mechanisms contributing to, and consequences of, multimodal display schedules in temperate populations of the annual legume *Chamaecrista fasciculata* (Michx.). The occurrence of multiple flowering peaks, or pulsed flowering, is unusual at these latitudes and offers an opportunity to examine the factors that shape display schedules and how variation in shape ultimately influences synchrony and phenological assortative mating. We aim to (1) verify pulsed flowering in *Chamaecrista fasciculata*, (2) determine whether flowering pulses occur simultaneously across individuals and genetically differentiated populations, (3) assess whether flowering pulses, and the intervals between them, are the result of intermittent shifts in internal resource allocation away from flower production and towards fruit maturation, (4) establish whether flowering pulses correlate with fluctuations in abiotic variables and (5) evaluate effects of flowering pulses on synchrony and assortative mating for flowering onset date. To accomplish this, we account for variation in floral longevity to distinguish between display schedules, which include flowers of any age available for pollination, and deployment schedules, which involve the opening of new flowers each day.

Materials and methods

STUDY SPECIES

Chamaecrista fasciculata (Fabaceae, subfamily Caesalpinioideae), or the partridge pea, is a self-compatible annual legume that prefers sandy soils in prairie and disturbed habitats (Foote & Jackobs 1966). Its distribution spans the eastern half of the United States and Mexico, with the northern range limit running along the Canadian border from Minnesota to New York (Irwin & Barneby 1982).

Plants consist of a central stalk with several branches that each develop multiple compound racemes (Garrish & Lee 1989). The

flower buds produced on each raceme can be held in stasis for 4 to 10 days until blooming, resulting in multiple buds awaiting anthesis at the same time. Flowering and growth continue until first frost, and plants typically generate 100 to 800 flowers over the course of 30 to 60 days. Flowers produce no nectar, are exclusively buzz-pollinated and are reported to remain open for just 1 day (Thorp & Estes 1975).

We collected seeds from five populations of *C. fasciculata* in the fall of 2009. Two were from the U.S. mid-western states of Minnesota (MN, 44.8011°N, 92.9647°W) and Missouri (MO, 38.4979°N, 90.5610°W), while three were from the eastern states of Pennsylvania (PA, 40.1790°N, 76.7248°W), Virginia (VA, 37.5061°N, 77.7342°W) and North Carolina (NC, 35.8900°N, 79.0092°W). Where possible, three fruit were collected from each of 50–100 plants located at least 5 m apart along a transect (the approximate genetic neighbourhood size for this species, Fenster 1991).

SUMMARY OF EXPERIMENTS

We collected daily flower counts for individuals from all populations of *Chamaecrista* over the course of three common garden experiments conducted over 2 years in a field setting (Table 1). This study explores the patterns of flower deployment and floral longevity in these experiments; data addressing other questions will be reported elsewhere.

All experiments took place at the University of Toronto's field station, the Koffler Scientific Reserve at Joker's Hill (KSR, 44.0300°N, 79.5275°W). This site is just north of *Chamaecrista's* current distribution limit in eastern North America, but just within the latitudinal limit west of the Great Lakes. Each experiment included treatments that ultimately manipulated aspects of flowering phenology (Table 1). In each study, seedlings that had been planted on the same day were transplanted into the field 20 cm apart in a hexagonal array with a ring of equally spaced plants around focal individuals to absorb edge affects. All other competitors within the plots were cleared. Unless otherwise noted, all experiments began in May. With few exceptions (detailed below), flowers were counted on each individual every day until a killing frost occurred. Daily precipitation data were collected from a rain gauge monitored by Environment Canada at the nearby Buttonville Airport (43.8608°N, 79.3686°W) while temperature and humidity measurements were recorded by a weather station at KSR (HC-S3 probe, Campbell Scientific, Edmonton, Canada).

In experiment 1, we manipulated thermal regimes in order to extend the growing season to that of a latitude approximately 5° further south. Temperature has been shown to strongly advance flowering onset dates in many species (Parmesan & Yohe 2003); however, no studies have monitored subsequent patterns of flowering to see whether display schedules are similarly affected. We used infrared heaters to warm 3-metre-diameter plots by a desired amount above ambient (design per Kimball *et al.* 2008). Temperatures were monitored at the plot level with infrared radiation scans (SI-111 infrared radiometer, Campbell Scientific, Edmonton, Canada). Six of these plots were heated by 1.5 °C during the day and 3 °C at night, in accordance with diurnal warming projections (Easterling *et al.* 1997) and local warming predictions (Colombo *et al*. 2007), while six identical plots were unheated. Heated and ambient plots were otherwise exposed to natural conditions. Each plot contained five randomly selected individuals from each of four populations (Table 1).

In experiment 2, we manipulated pollinator access to plants and thus resource allocation to fruit/seed maturation. This study took place in a field that had been undisturbed for two decades. For each population, seven individuals were planted within each of six plots that either allowed or excluded pollinators. Pollinator-excluded plots were covered with a tent made of fine-mesh bridal tulle to prevent pollination. We hung a sheet of this netting on the south (sun facing) side of plots open to pollinators to account for any shading affects.

In experiment 3, we manipulated watering schedules to determine whether variation in water availability influences the shape of display schedules. We randomly assigned 12 plots to 1 of 3 watering regimes, ~91 L of water applied every 2 weeks, ~45.5 L of water applied every week or ~13 L of water applied every 2 days. Thus, all plots received the same total volume of water, but at different schedules. We staggered planting dates to obtain concurrently flowering cohorts from select populations that otherwise have minimal flowering overlap (Table 1). We planted seven seedlings from each population-cohort combination (hereafter simply referred to as population) per plot, each in their own quadrant to avoid asymmetric competition among planting groups. We excluded natural precipitation by covering plots with 2.7 × 3.7 m roofs made of clear plastic, slanted southward towards the direction of summer rain events. Rain gutters along the southern edges directed precipitation away from the plots. We measured the per cent volumetric water content (VWC) within each quadrant of each plot for a portion of the days where flowers were counted (TDR 100 Soil Moisture Metre, Spectrum Technologies, Inc., Aurora, IL, USA).

In each experiment, we counted flowers on all individuals every day. In all, we tallied over 148 000 flower observations. Experiments 1 and 2 had days of missing data (7 of 93 and 8 of 86 days, respectively). We interpolated expected flower counts from a linear function running from the day before to the day after the missed counts. There were never more than two consecutive days without data collection, so our interpolations were unlikely to distort the true pattern of flowering. Interpolated data were used in all graphs and analyses.

In experiment 3, we measured floral longevity for a subset of consecutive days. On a given day, every flower on each individual was marked with a felt tip marker on the inside of the rigid upper petal. On subsequent days, the number of flowers remained open and unwithered with colours from previous days was recorded and new flowers were counted and marked with a different colour. We used these data to measure floral longevity and to determine the proportion of newly deployed flowers contributing to display schedules. In total, the longevities of 7011 flowers were monitored. Markings did not

Table 1. A summary of several experiments conducted at the Koffler Scientific Reserve at Joker's Hill; the treatments applied, the year of study, the populations involved [Minnesota (MN), Pennsylvania (PA), Illinois (IL), Missouri (MO), Virginia (VA) and North Carolina (NC)] and the approximate number of individuals per population and treatment combination

Experiment	Treatment	Year	MN	PA	MO	VA	NC	*n*
1	± Heat	2011	X	X	X		X	30
2	± Pollination	2011	X	X	X	X	X	75
3	Watering schedule, ± Planting date	2012	X				X	84

seem to deter pollinators from visiting flowers or produce any adverse reactions in the flowers themselves (personal observation).

COMPARISON OF FLOWERING PHENOLOGIES AMONG POPULATIONS

For data from experiment 2, we used linear mixed models to determine whether pollination treatment influenced the total number of flowers produced or the flowering duration in all populations. We included pollination treatment, population and their interaction as fixed effects and plot as a random effect. In these analyses, and in subsequent models, we account for any variance heterogeneity among groups with error variance covariates as per Zuur *et al.* (2009) using the nlme package (Pinheiro *et al.* 2014) in R (R Development Core Team 2014).

We formally assessed whether flowering pulses occurred simultaneously across populations by examining the cross-correlation functions between pairs of populations, which produces correlation coefficients between time series that are aligned or shifted (lagged) by a certain number of days. This analysis is not a complete estimate of synchrony among populations; rather, it correlates patterns of flowering between populations only for the period of time where both were in flower. All correlation analyses were calculated using the proportion of total flowers in bloom each day, which standardizes flowering output across populations with different display sizes. We chose to compare populations that vary in flowering onset date, duration, genetic origin, treatments within experiments and across experiments conducted in the same year in order to capture the extent of phenological concordance between distinct groups.

Cross-correlations between time series that are themselves autocorrelated can result in inflated variances that produce erroneously large cross-correlation coefficients (Zuur *et al.* 2009; Brown *et al.* 2011). To account for autocorrelation in any of the phenological data, we applied auto-regressive integrated moving average (ARIMA) models to each time series prior to calculating cross-correlation coefficients (Box & Jenkins 1970). The order of auto-regressive and moving average terms were chosen by examining the extended sample autocorrelation functions for each time series and minimizing the Akaike information criterion (AIC). Only significant coefficients were included in the final models. All ARIMA models were analysed using the TSA package (Chan & Ripley 2012) in R.

To test whether flowering pulses were augmenting the phenological correlations between populations, we compared the cross-correlation functions of our observed data to the cross-correlation functions of simulated unimodal data. For each population, a simulated, unimodal display schedule was created by rearranging daily flower counts so that the maximum flowers on display occurred at the mid-point of the flowering duration, and the remaining flower counts were arrayed in descending order on either side of the new peak date of flowering. In this way, we preserved the total number of flowers produced, the variation in daily flowering display size (flower counts), the onset date and the duration of flowering for each population, and can compare cross-correlation functions when only the modality of the display schedule had been altered. As before, we converted data to proportions and employed ARIMA models to remove autocorrelation prior to each analysis.

If flowering pulses occur simultaneously across populations, we expect to see a strong, positive correlation coefficient at a lag of 0 days despite differences in flowering onset or duration between populations. With our multimodal data, we predict that correlations will decrease rapidly at larger lags, eventually becoming negative, because the flowering peaks of the display schedules being compared would be misaligned if shifted by more than a day or two. In contrast, comparisons of unimodal display schedules would produce correlation coefficients that varied in size and direction at larger lags, depending on the difference in flowering onset and duration between the populations being compared. Lastly, if the display schedules of different populations were completely independent, correlation coefficients would be small and less consistent in sign at all lags and in all comparisons, regardless of schedule modality.

RELATIONSHIPS BETWEEN FLOWERING PHENOLOGY AND ENVIRONMENTAL VARIABLES

We calculated cross-correlation functions lagged up to 5 days to determine whether display schedules correlate with average daily temperature, total daily precipitation and average daily humidity. Again, we fit phenological and environmental times series with ARIMA models to remove any autocorrelation from the data. In experiment 3, where natural precipitation was excluded, we analysed the relationship between display schedules and the volumetric water content of the soil within each plot. Due to data availability for soil moisture readings, we were only able to do this for the MN population planted early.

The relationship between temperature and floral longevity in experiment 3 was examined using logistic regressions. For each population, the mean proportion of flowers open for 2 days was regressed on the average temperature for the 24 h proceeding flower deployment (calculated here as the average of temperature readings taken every 15 min from 8 AM the day of flower deployment to 8 AM on the subsequent day). We used the average slope and intercept from these logistic regression equations to calculate the number of newly deployed flowers each day based on that day's average temperature. This allowed us to estimate floral deployment schedules for each population in each experiment. To examine effects of environmental variables on patterns of flower deployment, we repeated the cross-correlation analyses between deployment schedules and abiotic variables.

Where sufficient data were available, we analysed the effects of watering treatment on display schedules (for all but the NC late population) and soil moisture content (for the MN early population) in experiment 3 via generalized least squares fitted models, with watering treatment, day and their interaction included as fixed terms (again using the nlme package in R). A significant interaction term would indicate that the display schedules or soil moisture levels were variable among watering treatments throughout the growing season. We accounted for temporal autocorrelation in observations among days (nested within plot) by incorporating an auto-regressive error structure of order 1 (display schedule analysis) or an exponential correlation error structure that can account for irregularly spaced observations through time (soil moisture analysis, Zuur *et al.* 2009). Models with the lowest AIC values were selected for these analyses.

SYNCHRONY AND PHENOLOGICAL ASSORTATIVE MATING

We estimated *population synchrony* as per Weis, Nardone & Fox (2014), where synchrony information is extracted from an $n \times n$ matrix of pairwise mating opportunities, Φ, among all of the display schedules of studied individuals (n). Each matrix element of Φ_{mf} is calculated as the product of a father f's proportional contribution to the pollen pool in the population each day of the flowering season

and the number of opportunities for pollen receipt presented by mother, m (each estimated by their number of open flowers, Weis & Kossler 2004). In a perfectly asynchronous population (i.e. no plant flowers at the same time as any other), the diagonal elements of Φ are $1/n$ and the non-diagonal elements are 0. In contrast, in a perfectly synchronous population (i.e. all individuals display the same number of flowers each day), all elements equal $(1/n)^2$. The degree of synchrony among plants in a population, S_p, is calculated as the ratio of the first eigenvalue of the mating matrix, λ_1, to the sum of all n eigenvalues:

$$S_p = \lambda_1 / \Sigma\lambda \qquad \text{eqn1}$$

In the case of complete synchrony, all elements of Φ are equal; the first eigenvalue will be $1/n$ and the remaining ones will be zero. Thus, $S_p = 1$ when all plants have identical display schedules. With a completely asynchronous population, all eigenvalues are equal to $1/n$, and as per eqn 1, $S_p = 1/n$. Thus, this measure scales between $1/n$ and 1. When n is large, $1/n$ approaches 0.

We developed a measure of individual synchrony, S_i, to quantify the opportunity for geitonogamous pollen transfer (see Supporting Information, SI, for details). This measure incorporates the effects of uniformity of display schedules (scaled to total flower production) and flowering duration, such that $S_i = \mathrm{CV}_i / \sqrt{D_i}$, where CV_i is the coefficient of variation of the individual i's schedule, and D_i is the schedule duration. This equation can be easily rearranged to:

$$S_i = \frac{\sqrt{\mathrm{SS}_i}}{T_i} * \sqrt{\frac{D_i}{D_i - 1}} \qquad \text{eqn2}$$

where schedule synchrony is estimated by the sum of the squared deviation of the observed daily flower production (SS_i) relative to the total flowers counted (T_i) and corrected for schedule duration. A value of $S_i = 1$ indicates that all flowers within a plant could exchange pollen with every other flower on that plant, while $S_i = 0$ when flowers are distributed evenly across days ($\mathrm{SS}_i = 0$), thus minimizing the opportunities for geitonogamy. Synchrony is technically undefined at the limit where $D_i = 1$, but we assign this maximum possible synchrony a value of 1. We make the assumption that any open flower, regardless of age, can receive and contribute pollen equally and so conduct calculations on display schedules rather than deployment schedules.

The strength of phenological assortative mating for a given trait can be quantified by the phenotypic correlation between potential mates, ρ (Weis & Kossler 2004). Here, we estimate the potential for assortative mating by flowering onset date; however, variation in any component of schedule shape can influence an individual's mating pool (Fox 2003). We can characterize ρ by extracting the proportion of all mating opportunities in a population that occur between two individuals from the mating matrix, Φ. For hermaphrodites, like *C. fasciculata*, ρ is:

$$\rho = \frac{\sum_m \sum_f \left[\Phi_{mf}(z_m - \bar{z})(z_f - \bar{z})\right]}{\sum_m X_m (z_m - \bar{z})^2} \qquad \text{eqn3}$$

where z is the date of flowering onset, m and f represent the mother and father of a potential mating pair, ϕ_{mf} is the element of Φ corresponding to the proportion of mating opportunities between mother, m, and father, f, and X_m is the proportion of flowers in the population produced by m. When ρ = 0, the population is mating randomly with respect to flowering onset date. We make the assumptions that all flowers on display by an individual are equally likely to set seed and that all flowers open on the same day have the same potential to exchange or receive pollen.

To place our estimates of S_p, S_i and ρ into a broader context, we compare them with estimates for 29 other species naturally occurring at KSR. Most of these old-field species exhibited unimodal display schedules that are more typical of temperate regions (Weis, Nardone & Fox 2014, see Fig. S8). The data were collected in 2008 from approximately 50 individuals per species. Flowers were counted every 3 days, so individual synchrony was estimated with a modified version of eqn 2:

$$S_i = \frac{\sqrt{I_i * \mathrm{SS}_i}}{I_i * T_i} * \sqrt{\frac{I_i * C_i}{[I_i * C_i] - 1}} \qquad \text{eqn4}$$

where I_i represents the sampling interval (*e.g.* $I_i = 3$ when counts are made every 3 days) and C_i is the number of days where flowers were counted. We analysed differences in mean S_p, ρ and S_i between *Chamaecrista* populations and the KSR species using two-sample *t*-tests (if variances were equal) or Welch's two-sample *t*-tests (if variances were unequal).

Results

COMPARISON OF FLOWERING PHENOLOGIES AMONG POPULATIONS

Population-level flowering pulses were simultaneous across populations and were the result of simultaneous pulsing at the individual level. Individuals produce one or more flowering pulses in alignment with their neighbours despite differences in flowering onset and flowering duration within and among populations or treatments. We first present the observed display schedules and later present the cross-correlation analyses that formally test for simultaneity of flowering pulses.

Consider the example of the MN and MO populations in experiment 1 (Fig. 1). Under the ambient temperature regime, the former began flowering 17 days after the latter, on average, yet both share a flowering peak on day 234 and another near day 241. This is also true of plants that were artificially warmed in this experiment, where flowering pulses between heated and ambient treatments overlap despite the advancement of flowering onset in heated plots. Flowering pulses were produced simultaneously across the experiment regardless of thermal treatment or genetic origin. Similar patterns were seen between ambient and heated treatments within the PA and NC populations (Fig. S1), although the scant temporal overlap in display schedules precluded simultaneous pulsing between populations.

Overlap among populations in display schedules was enhanced in experiment 2, which included a pollinator exclusion treatment. Exclusion led to increased resource investment in flower production (Fig. 2), including an extension of flowering duration until the end of the season for the early-flowering populations (Pollination $F_{(1,\ 20)} = 129.30$, $P < 0.001$; Population $F_{(4,\ 20)} = 35.00$, $P < 0.001$; Pollination*Population $F_{(4,\ 20)} = 10.53$, $P < 0.001$), and an increase in the total number of flowers produced in all populations (Pollination

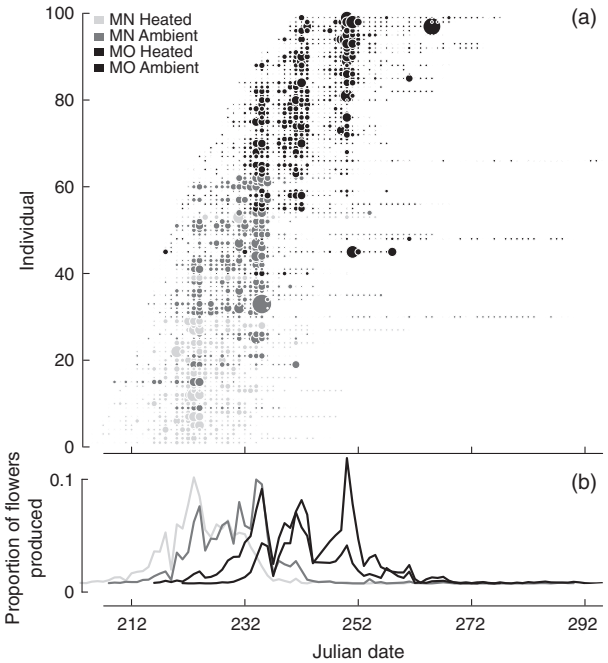

Fig. 1. (a) Individual-level display schedules from experiment 1. Individuals from the MN and MO populations from both heated and ambient treatments are staggered along the *y*-axis in order of flowering onset date. The size of each circle reflects the proportion of total flowers on display by an individual on a given day. (b) Population-level display curves for each of the same population and treatment combinations. The height of these lines reflects the proportion of total flowers on display by that group on a given day. We show data from a subset of populations for visual clarity; see Fig. S1 for the remaining data.

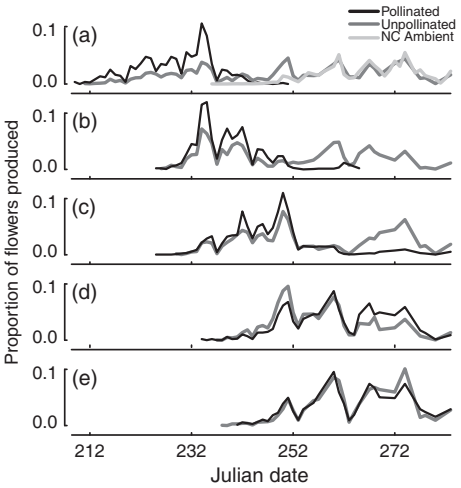

Fig. 2. The population-level display schedules for populations in experiment 2 from the open pollination (solid) and pollinator-excluded (dotted) treatments. Data are shown for the (a) MN, (b) PA, (c) MO, (d) VA and (e) NC populations. In addition, panel (a) includes the display schedule for the NC population in ambient conditions (dashed) from experiment 1 located ~0.5 km away.

$F_{(1,\ 20)} = 14.51$, $P < 0.01$; Population $F_{(4,\ 20)} = 1.51$, $P > 0.05$; Pollination*Population $F_{(4,\ 20)} = 1.40$, $P > 0.05$). Multiple, simultaneous flowering pulses were still produced when resources were not diverted towards fruit maturation. Thus, flower pulses in display schedules are not a consequence of periodic diversions of internal resources away from flower production and towards fruit maturation.

The artificially extended flowering durations in experiment 2 (Fig. 2) enabled us to observe phenological overlap between populations that were otherwise completely or partially temporally isolated. Flowering pulses in display schedules appear to be aligned between pollinated and unpollinated groups, both within and between populations during periods of overlap. The most striking example is the simultaneous pulsing of the early-flowering MN population when unpollinated in experiment 2 and the later-flowering NC population from experiment 1, which was planted 0.5 km away (shown in Fig. 2a).

Statistical support for simultaneous pulsing in display schedules is presented in Fig. 3a. Cross-correlation coefficients are shown for the 10 population and treatment comparisons with sufficient temporal overlap to allow meaningful tests. The display schedules of the various populations and experimental treatments of *Chamaecrista* are significantly positively correlated at lag 0, with a mean correlation; coefficient of $\bar{r}_0 = 0.50$ (Fig. 3a). As predicted, this positive correlation disappears when time series are misaligned by 1 day or more. Almost all pairs are weakly negatively correlated at a lag of 3 days ($\bar{r}_3 = -0.15$), suggesting that 6 days may be the most common length of time between the pulses of these display schedules. When repeating this analysis using simulated, unimodal data, all consistent associations among the display schedules of these groups disappeared ($\bar{r}_0 = 0.04$, Fig. 3b). Together, these results imply that display schedules in *Chamaecrista* are highly synchronized among these genetically differentiated populations grown under varied thermal and pollination environments and that this can be attributed to the simultaneous pulsing of floral displays.

RELATIONSHIPS BETWEEN FLOWERING PHENOLOGY AND ENVIRONMENTAL VARIABLES

Flower pulses can be the result of temporary increases in either flower deployment or floral longevity. Logistic regressions revealed a negative relationship between average daily temperature and floral longevity in experiment 3 (Fig. 4, MN early odds ratio = 0.64, $Z = -15.98$, $P < 0.001$; NC early odds ratio = 0.52, $Z = -32.22$, $P < 0.001$; MN late odds ratio = 0.62, $Z = -14.49$, $P < 0.001$), with floral longevity increasing sharply from 1 to 2 days in all populations when temperatures declined below 16–19 °C. Monitoring floral longevity allowed us to distinguish newly deployed flowers from all that were on display, and with this distinction, we constructed deployment schedules for each population. Deployment schedules are multimodal, with pulses of deployed flowers occurring simultaneously among populations (Fig. 5 and see Figs S2 and S3). Furthermore, flowers retained from previous days also appear to occur in pulses. These data suggest that floral longevity is mediated by temperature in this

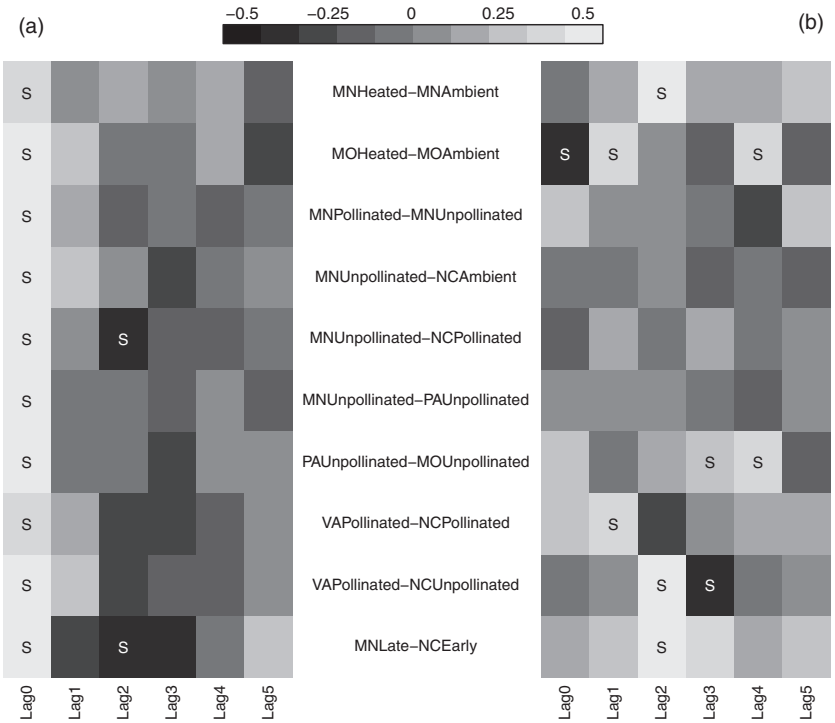

Fig. 3. Heatmaps summarizing cross-correlation coefficients between the display schedules of select populations. (a) Cross-correlations for observed, pulsed display schedules. (b) Cross-correlations for unimodal display schedule simulations. Correlation coefficients were calculated between time series lagged up to 5 days. The colour and shade of a specific box indicates the sign and magnitude of the correlation coefficient, respectively. An S signifies that the correlation was significant.

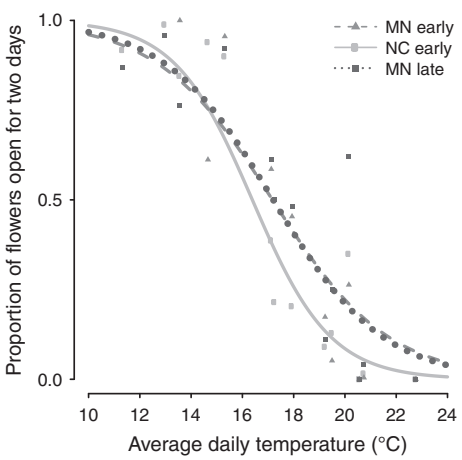

Fig. 4. Logistic regressions relating floral longevity to average daily temperatures in experiment 3. Data from all watering treatments are combined within a population and planting time because the treatments did not significantly affect flower production. Each point represents the proportion of two-day-old flowers on a given day, averaged across all individuals in a population, regressed on the average daily temperatures of the 24 h proceeding flower deployment. The adjusted r-squared values for MN early, NC early and MN late are 0.86, 0.98 and 0.62, respectively (per Naglekerke 1991).

species. Thus, pulses in display schedules are the result of pulses of deployed flowers, but can be amplified by the retention of day-old flowers when temperatures are low.

To examine the direct influence of temperature on flower deployment and retention, we calculated cross-correlation coefficients between average daily temperatures and population-level display schedules, and repeated analyses with population-level deployment schedules. When examining all flowers displayed, we observed a negative correlation (higher temperatures, fewer flowers) at a lag of 1 ($\bar{r}_1 = -0.24$) and a positive correlation at a lag of 4 ($\bar{r}_4 = 0.25$) in all populations and treatments across experiments (Fig. 6a). When accounting for temperature-mediated floral longevity, we find deployment schedules to be less consistently correlated to temperatures at a lag of 1 and 4 ($\bar{r}_1 = -0.12$, $\bar{r}_4 = 0.15$, respectively, Fig. 6b). The fluctuation in the sign of correlation coefficients as lags increase may reflect the fluctuations in average daily temperatures found in both years (Fig. 5d and see Fig. S3k for temperature data). In many populations, average daily humidity negatively correlated to both display and deployment schedules at lag 0 ($\bar{r}_0 = -0.14$ and -0.12, respectively) and lag 4 ($\bar{r}_4 = -0.17$ and -0.16, respectively), while there were no consistent relationships between precipitation and display or deployment schedules (see Figs S4 and S5).

Altering the watering schedule in experiment 3 did not affect the display schedules of any population (Fig. S6, Day*Treatment MN early $F_{(2,\ 500)} = 0.67$, $P > 0.05$; NC early $F_{(2,\ 483)} = 1.30$, $P > 0.05$; MN late $F_{(2,\ 369)} = 0.73$, $P > 0.05$). However, there was a strong negative correlation in all three treatments between VWC and display or deployment schedules at a lag of 0 ($\bar{r}_0 = -0.40$ and -0.24, respectively), as well as a positive correlation at a lag of 5 ($\bar{r}_5 = 0.36$ and 0.25, respectively, Fig. S7). Soil moisture content differed slightly among treatments throughout the season

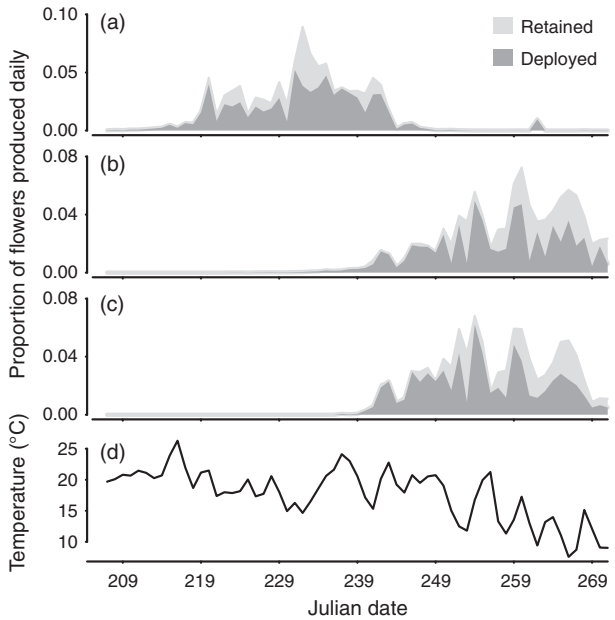

Fig. 5. The display schedules (a) MN planted early, (b) NC planted early and (c) MN planted late populations from experiment 3, and (d) average daily temperatures. Display schedules are shown with deployment schedules highlighted in dark grey and retained flowers highlighted in light grey. Deployment schedules were estimated from average daily temperatures using the logistic regression models shown in Fig. 4.

(Watering treatment $F_{(2,\ 287)} = 3.89$, $P < 0.05$; Day $F_{(1,\ 287)} = 4.42$, $P < 0.05$; Treatment*Day $F_{(2,\ 287)} = 2.58$, $P < 0.10$), with greater levels of VWC in the two-week treatment than in the one-week or control treatments. Display schedules would have differed among treatments if VWC directly influenced flower deployment or retention. It is possible that correlations between display or deployment schedules and VWC are driven by unmeasured factors that correlate with VWC (e.g. soil porosity).

SYNCHRONY AND PHENOLOGICAL ASSORTATIVE MATING

Average synchrony among populations, S_p, in *C. fasciculata* was comparable to that of natural populations of species located at KSR ($\bar{S}_p = 0.63$ vs. 0.66, respectively; $t_{43} = -0.77$, $P > 0.05$; Fig. 7a, b and see Table S1), while the average synchrony within individuals, S_i, was significantly higher ($\bar{S}_i = 0.17$ vs. 0.14, respectively; $t_{42.5} = 3.41$, $P < 0.01$; Fig. 7c, d and see Table S1), indicating a greater opportunity for geitonogamy.

Multimodal display schedules have the potential to drastically reduce S_p if flowering pulses are misaligned, and the high levels of synchrony we observed can only be maintained if individuals pulse concurrently. To confirm this, we shuffled the flowering onset dates of all individuals in each population in order to randomize the occurrence of flowering pulses among individuals. We repeated this randomization 1000 times, recalculating S_p for each population, and compare the average of these estimates to those of the KSR species. When the alignment of flowering pulses is randomized, average S_p in *Chamaecrista* significantly decreases to 0.52 ($t_{(36.2)} = -8.24$, $P < 0.001$). Variation in the onset and end dates of individual display schedules can also influence S_p in *C. fasciculata* by affecting the frequency with which early-flowering plants produce flowering pulses outside of the display schedules of late bloomers and vice versa. However, the average standard deviation in onset and end dates for populations of *Chamaecrista* were not significantly different than those of the KSR species ($t_{(24.3)} = 1.47$, $P > 0.05$ and $t_{(48)} = -1.21$, $P > 0.05$, respectively). Together, these results suggest that the levels of S_p observed in *Chamaecrista* are equivalent to those from the KSR species because flowering pulses were produced simultaneously across individuals.

The average strength of phenological assortative mating by flowering onset date was significantly lower in *Chamaecrista* than in other species ($\bar{\rho} = 0.21$ vs. 0.35, respectively; $t_{43} = -2.63$, $P < 0.01$; Fig. 7e, f and see Table S1). When flowering peaks are randomized, $\bar{\rho}$ becomes indistinguishable from that of the other species ($\bar{\rho} = 0.39$ vs. 0.35, respectively, $t_{(43)} = 0.91$, $P > 0.05$). Simultaneous flowering pulses in *C. fasciculata* may offer more mating opportunities between early- and late-flowering individuals than typically seen in the phenologies of temperate species, reducing the strength of assortative mating by flowering onset date.

Discussion

MULTIMODALITY AND DISPLAY SCHEDULE SHAPE

Display schedules in *Chamaecrista fasciculata* are multimodal, with flowering pulses produced simultaneously among individuals and populations. In temperate regions, simultaneous pulsing has only been demonstrated in several wind-pollinated *Juncus* species (Michalski & Durka 2007). *Chamaecrista* is of tropical descent, perhaps evolving from a rain forest tree to a savanna shrub prior to its colonization of temperate zones (de Souza Conceição *et al.* 2009). Multimodality in display schedules is more common in the tropics (Newstrom, Frankie & Baker 1994), and the unique pattern of flowering found in *C. fasciculata* may be explained by its tropical origin. However, in the tropics, simultaneous flowering pulses have only been formally documented in several Brazilian Myrtaceae species (Proença & Gibbs 1994). It is likely that examples of simultaneous flowering pulses, and multimodality in general, are rare simply because few studies have monitored phenology at the level of individuals (Augspurger 1983) with high enough frequency to capture fluctuations in display schedules (Miller-Rushing, Inouye & Primack 2008; Morellato *et al.* 2010).

Statistical methods have been developed for describing unimodal display schedules through fitting flexible regression functions (Malo 2002; Clark & Thompson 2011). However, adequate regression models for display schedules of other shapes may prove elusive, particularly if the number of modes is variable and they occur at irregular intervals. Several approaches have been taken to identify modality in display

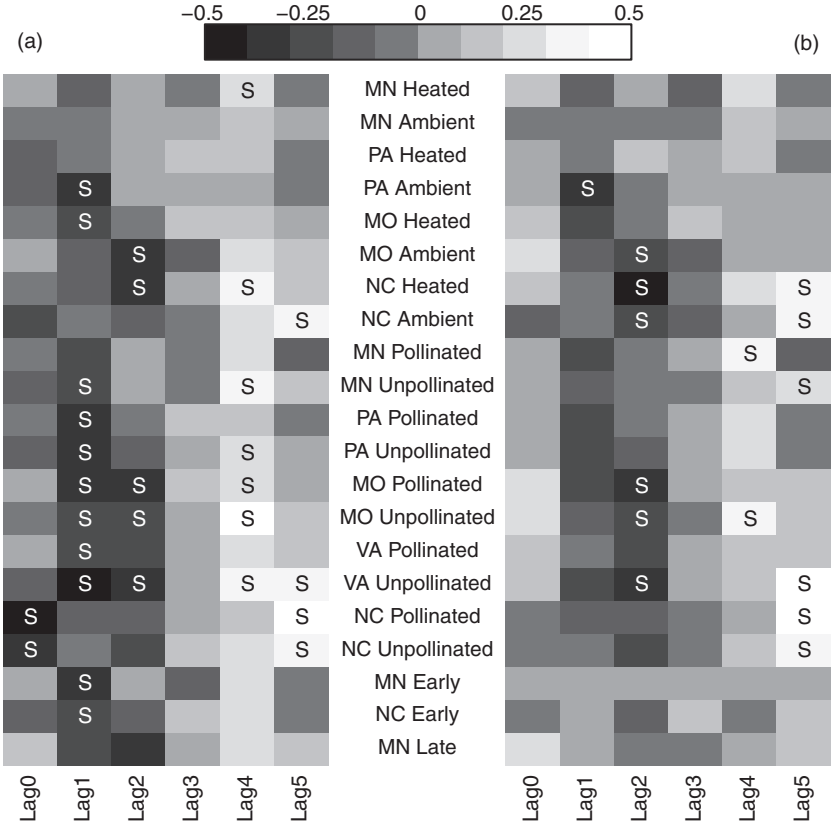

Fig. 6. Heatmaps summarizing the cross-correlation coefficients between population-level (a) display schedules or (b) deployment schedules and average daily temperatures. As in Figure 3, the colour and shade of a box reflect the sign and magnitude of the correlation coefficient, respectively, and those with an S indicate significant correlations. For each population, the number of new flowers each day was estimated from that day's average temperature using the average coefficients from the three logistic regression models in Fig. 4.

schedules, including the use of cumulative flowering density curves to examine bimodality (Aldridge *et al.* 2011), coefficients of variation to quantify temporal variability in flower production (Picó & Retana 2001; Michalski & Durka 2007) and principal coordinate analyses to distinguish between unimodal and bimodal phenologies (Austen, Jackson & Weis 2014). Each method has its own merits and limitations, and like the time-series analyses used here, many of these approaches cannot yield concrete estimates for the number of modes or the dates at which they occur (but see Aldridge *et al.* 2011). The development of methodology capable of describing variation in modality, and detecting significant departures from unimodality, may be necessary for characterizing many fine- and broad-scale phenological patterns.

CAUSES OF VARIATION IN DISPLAY AND DEPLOYMENT SCHEDULES

In *C. fasciculata*, display schedule shape is likely dictated by environmental conditions. Temperature affected the shape of display schedules by influencing the life span of individual flowers (Fig. 4), as is seen in other species (Vesprini & Pacini 2005). This may occur because cooler temperatures preserve floral tissues (Primack 1985) or because bee activity is also temperature dependent (Corbet *et al.* 1993) and flowers are left unpollinated (Blair & Wolfe 2007; Elzinga *et al.* 2007; Castro, Silveira & Navarro 2008). Additional work may distinguish between mechanisms contributing to variation in floral longevity (Yasaka, Nishiwaki & Konno 1998) and may reveal whether newly deployed and retained flowers have the potential to contribute equally to fruit production (e.g. comparable stigma receptivity, pollinator attraction).

Temperature may also influence display schedules by triggering the opening of flowers (Fig. 6b). Correlations between display schedules and temperature have been found in other temperate species where the display schedules of individuals and populations can be multimodal (Picó & Retana 2001; Michalski & Durka 2007). However, this is the first attempt to identify associations between environmental variables and flower deployment independent of display schedules. In these studies, and in ours, interannual variation in temperature profiles through the growing season may partially explain variation in modality within and across years. We observed temperatures at KSR to fluctuate while generally decreasing throughout the growing season (Fig. 5d and see Fig. S3k), and our results suggest that the degree of modality in display schedules of *C. fasciculata* may be a plastic response to temperature or a correlated variable at the time of flower bud maturation and flower opening.

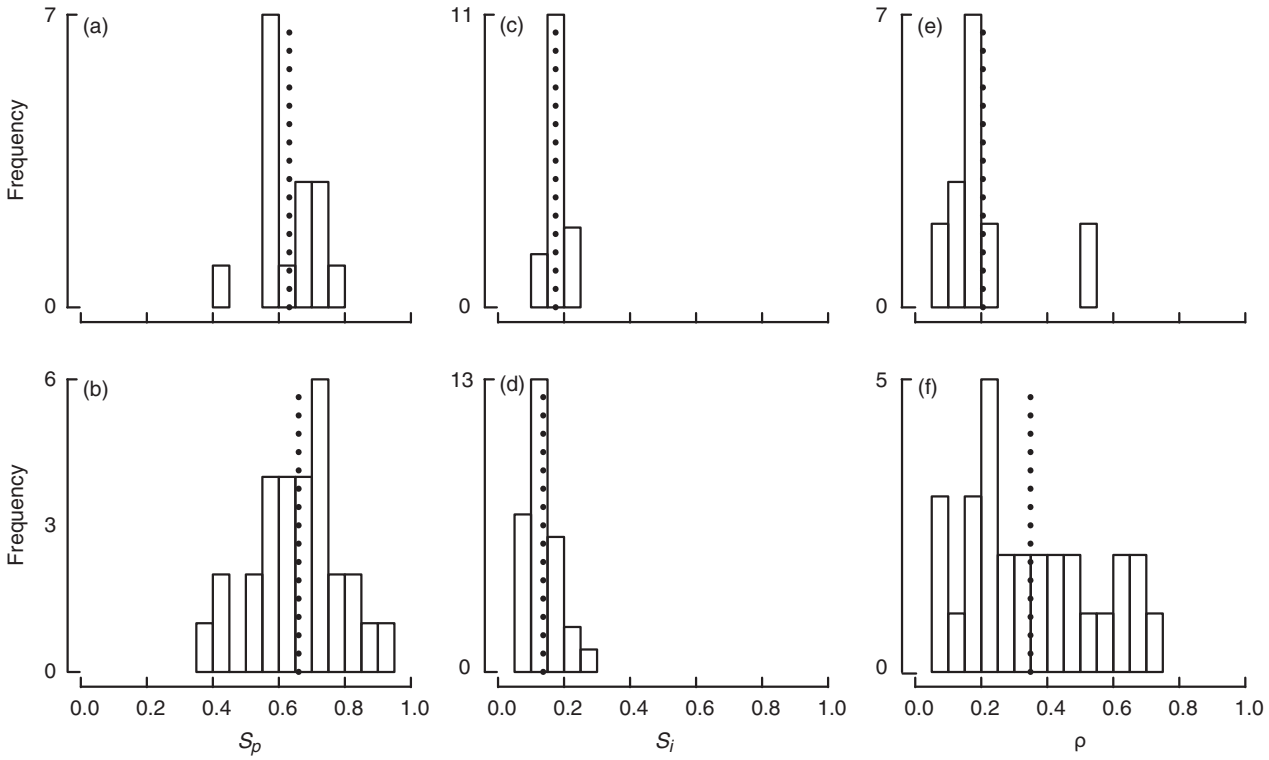

Fig. 7. Histograms showing the distribution of estimates of population synchrony (S_p), individual synchrony (S_i) and the strength of phenological assortative mating by flowering onset date (ρ) for all populations and treatments of *C. fasciculata* from experiments 1–3 (panels a, c and e, respectively) and for the 29 species located at the Koffler Scientific Reserve (panels b, d and f, respectively). Calculations were not made for treatments where pollinators were excluded. Vertical, dotted lines represent the average estimate in each panel.

Flowering pulses in *C. fasciculata* are not caused by the intermittent diversion of internal resources away from flower production and towards fruit maturation, although we found that the total number of flowers and flowering duration were resource-limited (Fig. 2). Patterns of flower and fruit production are inherently linked because both are constrained by a shared resource pool, and adjustments to resource allocation can be made via changes in flower production or through seed and fruit abortion (Stephenson 1981). Flowering and fruiting schedules can also be correlated if fruit development times are constant and flowering peaks produce subsequent pulses of maturing fruit (Rojas-Sandoval & Meléndez-Ackerman 2011). Such tight correlation does not seem to occur in *C. fasciculata*, which can hold initiated fruit in stasis for weeks until an unknown stimulus prompts the selective maturation of some fruit and abortion of others (Lee & Bazzaz 1982a,b). While patterns of flower and fruit production may be independent in *C. fasciculata*, variation in display schedule shape may influence the rate and timing of fruit and seed dispersal or predation in other species (Mahoro 2002).

POPULATION AND INDIVIDUAL SYNCHRONY

Population synchrony was, on average, indistinguishable from that of the 29 species studied at KSR (Fig. 7a,b), while estimates of individual synchrony for *C. fasciculata* were only slightly higher than the KSR species (Fig. 7c,d). If display schedule shape were the only determinant of rates of outcrossing or the occurrence of geitonogamy, this result suggests that multimodal display schedules at population and individual levels may not alter the potential for geitonogamy from that of unimodal schedules. However, we might expect large display sizes ('pulses') at the individual level to increase opportunities for geitonogamous selfing if pollinators move less frequently among individuals (Harder & Barrett 1995). On the other hand, pulsed flowering across neighbouring plants may promote outcrossing if pollinators forage among individuals with large display sizes. In *C. fasciculata,* levels of individual and population synchrony may interact with several aspects of floral morphology shown to reduce geitonogamy, including herkogamy (Webb & Lloyd 1986), enantiostyly (Fenster 1995; Jesson & Barrett 2002) and the presence of a stiff hooded petal that acts as a flight guide (Wolfe & Estes 1992). The net effect of these floral traits and of the shape of population-level display schedules may contribute to the high outcrossing rates observed in several populations of *C. fasciculata* (~80%, Fenster 1991).

The display schedules observed here may also partially explain reports of population structure in *Chamaecrista*. In this species, pollen movement is localized and populations are subdivided into small patches of related individuals (Fenster 1991). Flowering pulses in neighbouring plants produce large floral displays that may encourage pollinators to forage primarily within small groups of individuals. Coupled with short seed dispersal distances (Fenster 1991), the effects of population-level flowering pulses on pollinator movements may generate this fine-scale population structure.

PHENOLOGICAL ASSORTATIVE MATING, NATURAL SELECTION AND SCHEDULE SHAPE

Patterns of selection and assortative mating guide the evolution of phenological traits (Fox 2003). If plant mating is assortative by flowering onset, the genetic variance for flowering onset (and correlated traits) will be inflated, accelerating responses to natural selection. The shape of display schedules dictates the potential for genetic exchanges among individuals, and plasticity in schedule shape can influence the degree of phenological assortative mating within population (Weis 2005). This, in turn, can alter the effectiveness of selection on phenological (or correlated) traits (Fox 2003).

We found the average strength of phenological assortative mating by flowering onset date to be lower in *Chamaecrista* than in other species found at KSR. Simultaneous flowering pulses offer greater mating opportunities among individuals with distinct flowering onset dates, that is mating is closer to random. If display schedule shape is partially plastic, as our results suggest, and mating opportunities are environmentally dictated, the potential evolutionary responses of flowering onset date to selection may be reduced and may vary among years or among populations experiencing contrasting environmental conditions.

While the genetic and environmental influences on flowering onset are well known in several model systems (Mouradov, Cremer & Coupland 2002; Putterill, Laurie & Macknight 2004), it is often assumed that components of display schedules are genetically or developmentally correlated with flowering onset. Here, we have shown evidence that flower display and deployment may be plastic in response to temperature or a correlated variable in *Chamaecrista*, producing distinct, multimodal display schedules in alignment with the thermal regimes typically experienced at KSR. Detailed phenological data from other systems may reveal that responses to daily fluctuations in the environment are more widespread than currently appreciated. *Chamaecrista fasciculata* may prove to be an excellent candidate for understanding the ecological and evolutionary causes and consequences of variation in display schedule shape.

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Data accessibility

Data deposited in the Dryad repository: (Wadgymar *et al.* 2014).

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Supporting Information

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

Figure S1. Individual- and population-level display schedules from experiment 1 not included in the main text.

Figure S2. Display and deployment schedules for populations and treatments in experiment 1.

Figure S3. Display and deployment schedules for populations and treatments in experiment 2.

Figure S4. Heatmaps summarizing cross-correlation coefficients between display or deployment schedules and humidity.

Figure S5. Heatmaps summarizing cross-correlation coefficients between display or deployment schedules and precipitation.

Figure S6. Display schedules for the watering treatments applied in experiment 3.

Figure S7. Heatmaps summarizing cross-correlation coefficients between display or deployment schedules and volumetric water content in experiment 3.

Figure S8. Display schedules for the 29 species located at KSR.

Figure S9. Estimates of S_i as the duration of flowering increases for four example display schedules.

Figure S10. Example display schedules with variable shape, duration, and total number of flowers, and their corresponding estimates of S_i.

Figure S11. Estimates of S_i for four example display schedules as the interval between flower counts varies from 1 day to 6 days.

Table S1. Estimates of population synchrony, individual synchrony, and the strength of phenological assortative mating for all populations of *Chamaecrista fasciculata* and for the 29 species located at KSR.