

Evidence of temporal niche separation via low flowering time overlap in an old-field plant community

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Abstract

Flowering time is a trait that reflects the timing of specific resource requirements by plants. Consequently, several predictions have been made related to how species are assembled within communities according to flowering time. Strong overlap in flowering time among coexisting species may result from clustered abiotic resources, or contribute to improved pollination success. Conversely, low flowering time overlap (asynchrony) among coexisting species may reduce competition for soil, light, or pollinator resources and alleviate interspecific pollen transfer. Here, we present evidence that coexisting species in an old-field community generally overlap less in flowering time than expected under a commonly used and statistically validated null model. Flowering time asynchrony was more pronounced when abundance data were used (compared to presence-absence data), and when analyses focused on species that share bees as pollinators. Control and herbivore-exclusion plots did not differ in flowering time overlap, providing no evidence of the reduction in overlap expected to result from increased competition. Our results varied with the randomization algorithm used, emphasizing that the choice of algorithm can influence the outcome of null models. Our results varied between two years, with patterns being less clear in the second year, when both growing season and flowering times were contracted. Finally, we found evidence that further supports a previous finding that higher plot-level flowering time overlap was associated with higher proportions of introduced species. Reduced flowering time overlap among species in our focal community may promote coexistence via temporal niche differentiation and reduced competition for pollinators and other abiotic resources.

Keywords: co-flowering, coexistence, community assembly, competition, flowering synchrony

Introduction

The timing and duration of flowering is critical, greatly influencing plant reproductive success and fitness. As such, variation in flowering phenology among plant species may play a powerful role in the relative success of species, and consequently the assembly of natural communities. For these reasons, researchers have long been interested in patterns of flowering time overlap in natural communities (e.g., Anderson and Schelfhout 1980; Pleasants 1980; Fleming and Partridge 1984; Schamp et al. 2011; Dante et al. 2013). Flowering time overlap measures the degree to which plant species are synchronized in the timing of reproductive activity (also variously called flowering overlap, phenological overlap, or overlap in flowering schedules). Thus far, research examining flowering time overlap in natural communities has produced variable results (Table S1). Some studies have found strong overlap in flowering among coexisting species (11 studies), while others have found reduced overlap among coexisting species (12 studies; Rathcke and Lacey 1985; Pleasants 1990; Sargent and Ackerly 2008), and some have found patterns no different than expected by chance (3 studies). Consequently, more research is required to understand the influence of flowering time on plant community assembly.

Different predictions about flowering time overlap among coexisting species arise from multiple mechanisms

that can generate different patterns. For example, one prediction is that coexisting species should overlap less in flowering time as a form of temporal niche partitioning (Pleasants 1980; McKane et al. 1990; Zhang et al. 2017). Reduced flowering time overlap may result in less interspecific competition for resources among coexisting species (Sargent and Ackerly 2008). Coexistence among species that differ with respect to functional traits (e.g., flowering time) has also been referred to as limiting similarity (MacArthur and Levins 1967; Stubbs and Wilson 2004; Schamp et al. 2008). Limiting similarity in the form of temporal niche separation has been documented in a variety of systems (Schoener 1974). With respect to vegetation, evidence that coexisting species have reduced flowering time overlap has been documented in mountain meadows (Waser 1978; Pleasants 1980), tall grass prairie (Anderson and Schelfhout 1980), and tropical plant communities (Snow 1965; Stiles 1977). Flowering time asynchrony among coexisting species may also reduce instances of interspecific pollen transfer, which can decrease fitness (Waser 1978; Elzinga et al. 2007).

Some researchers have predicted lower flowering time asynchrony to be more likely for groups of plant species that share pollinators (e.g., bee-pollinated species; Pleasants 1980, Dante et al. 2013), because those plant species may compete for both abiotic resources and pollinators. In spite of this expectation, several researchers have found evidence that coexisting plant species overlap more in flowering time than expected (Parrish and Bazzazz

1979; Schemske 1981), even when focusing on plant species that share a group of pollinators (Dante et al. 2013: bee-pollinated species). To explain this, researchers have suggested coexisting plant species that overlap in flowering time may benefit from increased pollination rates because a more concentrated floral display attracts pollinators (Rathcke 1983; Rathcke and Lacey 1985; Elzinga et al. 2007; Knight et al. 2005; Ashman et al. 2014). For example, Augspurger (1981) discovered that flowering synchrony among tropical shrubs likely increased pollinator visitations, although that study focused on a single plant species. Other studies have also indicated that flowering time synchrony among coexisting plant species may be beneficial when pollinators are limited or the window of resource availability is narrow (Schemske 1981; Stephenson 1981; Rathcke 1988a; Ims 1990). In such circumstances, overlap in flowering time can result in a burst of seed production that can satiate floral predators and granivores, increasing the likelihood of seed survival (Smythe 1970; Janzen 1971; Beattie et al. 1973; Stephenson 1981; Mittelbach and Gross 1984). Regardless of the mechanism, the contrasting patterns of flowering time overlap in the literature highlight the need for more research.

Existing variation in observed patterns of flowering time overlap may be related to variation in approach (i.e., focal scale, analysis methods), mechanistic focus, and the particular driver that dominates a given study system. Many studies have focused analyses on a subset/guild of species in the focal community (Stiles 1977; Rathcke 1988b) or a single pair of species (Waser 1978; Schemske 1981), while others have analyzed all flowering species (e.g., Dante et al. 2013) or all bee-pollinated species (Pleasants 1980, Dante et al. 2013). Researchers have also used multiple approaches to determine whether observed flowering time overlap differs from a null expectation. For example, Pleasants (1980) tested observed flowering time overlap against a null distribution derived from a uniform distribution of flowering times, Fleming and Partridge (1984) created their null distribution by randomly placing species flowering time distributions across the growing season, and Dante et al. (2013) generated a null distribution by shuffling flowering time distributions among species. This last approach has several advantages. First, this is a strict test of whether observed overlap in samples is more pronounced compared to what might be expected if plot composition is unrelated to flowering time, which is essentially the question researchers wish to answer. Secondly, this approach maintains the overall flowering time distributions in the community, while previous methods generated null distributions using hypothetical and perhaps unrealistic flowering time distributions. A third advantage is that the null models used by Dante et al. (2013) have been shown to have acceptable type I and II error rates (Ulrich and Gotelli 2010; Lavender et al. 2016). A fourth advantage is that using multiple replicate plots to assess mean, plot-level flowering time overlap in the community, reduces sampling error. Finally, assessing flowering time overlap in small plots may be more appropriate for assessing the competition-based hypotheses above because similarly small plot sizes were recently identified as likely to reflect the consequences of species interactions (McNickle et al. 2017; Bar-Massada et al. 2018).

Several researchers have suggested that the presence of introduced species (species which are not native to the study region) may influence plant-pollinator interactions (Bjerknes et al. 2007; Bartomeus et al. 2008; Sargent and Ackerly 2008; Dante et al. 2013). For example, it has been suggested that introduced species will be most successful when they differ in niche from resident species, thereby avoiding or reducing competition (Strauss et al. 2006; Cleland et al. 2011). Consistent with this, Case (1990) found that the success of introduced species establishment was negatively correlated with the degree of competition among invading and resident plant species. Contrastingly, there is also evidence suggesting that introduced species can benefit from overlapping in flowering time with nearby native species (Dante et al. 2013). Introduced species may attract more pollinators if they grow among the expansive floral displays of native plant species. However, others have speculated that introduced species are not experiencing pollen limitation, and may not even be directly competing with native plants for pollinators (Brown and Mitchell 2001; Dietzsch et al. 2011). At this time, too few studies have expressly studied the impact of introduced species on community assembly rules related to flowering time overlap; more research is required.

In this paper, we test several specific hypotheses related to flowering time overlap among coexisting plant species. Using two years of vegetation sample data (150 plots, two plot sizes) from an old-field plant community, we tested whether coexisting plant species overlap more or less in flowering time than expected under an appropriate null model. We tested this for all species in the community, and separately for the guild of species that share bees as pollinators (Pleasants 1980; Dante et al. 2013). We examined these two guilds because plant species in this guild may compete for both abiotic resources (all species) and pollinators (species that share bees as pollinators). Because competition is an important mechanism in predictions of flowering time overlap, we compared flowering time overlap in control and low-disturbance plots (herbivore exclosure treatment). Disturbance generally lowers biomass/density, increasing the ratio of resource supply to demand, thereby reducing the influence of competition (Taylor et al. 1990); thus, competition should be more important in the assembly of species in herbivore exclusion plots. Finally, we tested whether introduced plant species tended to be more common in plots with higher flowering time overlap, as has been previously observed (Dante et al. 2013).

Methods and Materials

Study Site

We conducted this research from May to October in both 2014 and 2015 in an old-field plant community at the Ontario Forest Research Institute Arboretum, Sault Ste. Marie, Ontario (46°32' N, 84°27' W). We focused on a single, small-scale community (~1700 m²) to test for patterns of deterministic flowering time overlap among coexisting species. By focusing on a smaller, relatively homogeneous community, it is less likely that assembly rules related to flowering time overlap will be obscured by the signal of abiotic filtering, which is more likely to dominate when the focal system is large enough to include pronounced soil resource gradients/variation (Götzenberger

et al. 2012; Bar-Massada et al. 2018). Consequently, our chosen community is ideal for testing predictions related to competition, which should have a greater influence on composition when abiotic gradients are less prominent.

Our study community hosted 34 vascular plant species, all herbaceous perennials, including graminoids (16), forbs (15), and n-fixing forbs (3). Prior to 2007, the field was regularly mowed and occasionally tilled. Plots were established in 2009 within a 10 x 27 m grid of 1.5 x 1.5 m plot areas separated by 1 m laneways. Plots were randomly allocated to two treatments: 75 control plots, 75 herbivore exclusion plots (caged). Two sizes of circular quadrat were established at the centre of each plot in our treatments (50 cm and 1 m diameter).

In 2009, plots in the caged treatment were surrounded with a fence of hardware cloth (1 cm gauge) that was dug 10 cm into the ground, and extended approximately 90 cm above the ground. We covered cages for the winter using more hardware cloth to reduce access by small mammals that can move vertically through the snow-pack. The goal was for plants within caged plots to experience reduced herbivory from small mammals, which play a powerful role by consuming plant seedlings of many herbaceous species in this community (Schamp, unpublished data). We anticipate that competition will play a more prominent role in governing composition in these plots, given the reduced role of herbivore-mediated disturbance.

Flowering time assessment

We flagged 25 individual plants (ramets) of each of the 34 species throughout the study field and collected flowering time data for these throughout the growing season (925 plants). Each of these plants was observed once weekly from the start of May to the end of October (24 weeks) in each of the two study years to produce flowering time distributions for each plant species present in our plots. We generated flowering time distributions for each species as the proportion of the 25 plants of that species that flowered each week during the growing season. No plants had begun to flower prior to the start of surveys and the flowers of all plants were closed by the end of the survey period. Flowering time was considered to begin when the first flower was observed to be open on an individual plant, and was considered terminated when the anthers and stamens were withered for all flowers on a plant (Dante et al. 2013). All study species flowered within the 24 weeks of monitoring (flowering spanned 20 weeks in 2014, and 18 weeks in 2015). All animal-pollinated species in the field shared bees as pollinators (Table S2). We examined flowering time overlap for this group because it has been the focus of similar studies (Heinrich 1976; Parrish and Bazzaz 1979; Pleasants 1980; Rabinowitz et al. 1981; Dante et al. 2013; Kipling and Warren 2014), and because these species are more likely to compete for pollinators.

Plot censuses and species native status

We collected abundance data for species in each 50 cm diameter plot (150 plots) in 2014 and 2015 using weekly surveys through the growing season. We also collected presence-absence data for all 150 1 m diameter plots. Species abundance was enumerated as the number of rooted units of each species found within the quadrat (Schamp et al. 2016). Native or introduced status was determined for each of the 37 species in our study site

using the reported native status of each in Ontario, Canada as listed in the Plants Database (USDA, NRCS 2018).

Data Analysis - community flowering time overlap

We calculated flowering time distributions for each species as the proportion of individuals (out of 25) flowering per week within the community. We quantified niche overlap using Schoener's index (Schoener 1970), which has been used previously to quantify flowering time overlap (Pleasants 1980; Dante et al. 2013). We used this index to quantify plot-level overlap in flowering time for each species pair in each plot (MacArthur and Levins 1967; Schoener 1970; Pleasants 1980). Schoener's index is calculated as:

$$1 - \frac{1}{2} \sum_{k=1}^n |p_{ik} - p_{jk}|$$

where p_{ik} and p_{jk} are the proportions of individuals flowering, and i and j are different species. The proportion is calculated based on the number of individuals flowering during the k^{th} week. We used this index to measure plot-level flowering time overlap as the average pair-wise overlap in flowering time across all species in a given plot (Pleasants 1980). Because other indices of overlap exist, we also explored a second index (Pianka 1974). Results were consistent across indices, so we present only those for Schoener's index, but include as supplementary material the results using Pianka's index (Table S3).

Data Analysis - null models

To determine whether coexisting species overlap more or less in flowering time than expected by chance, we used a null modeling approach that compares our actual measure of flowering time overlap to a null distribution of the same. This null distribution was generated using an iterated process of shuffling some aspect of the data (e.g., flowering time distributions among species, species incidences in the abundance matrix), and then recalculating flowering time overlap. For our null model tests, we iterated this process 4999 to generate a null distribution with 5000 data points (the observed is included in the null distribution). We weighted all measures of pairwise flowering time overlap by abundance (50 cm plots), and also conducted analyses using presence-absence data (50 cm, 1 m plots) to compare with previous efforts (Dante et al. 2013). We analyzed flowering time overlap patterns using presence-absence data for both plot sizes to examine any possible scale-dependence of the observed patterns. Flowering time overlap was averaged across plots and compared to the null distribution of the same after the relevant randomization (described below); this is common in these types of null model tests (e.g., Stubbs and Wilson 2004; Schamp et al. 2008; Dante et al. 2013). Analogous null model approaches are known to have good type I error rates (Ulrich and Gotelli 2010; Lavender et al. 2016), as well as good power to detect patterns related to limiting similarity (Götzenberger et al. 2016). These null model tests are two-tailed, which means they can identify when flowering time overlap is significantly higher or lower than expected by chance. We calculated p-values as:

$$p = \text{MIN} [2S/(5000), 2L/(5000)].$$

Here, S and L represent the number of randomized test statistics greater than or equal to, or less than or equal to, the measured test statistic respectively (Bersier & Sugihara 1997) and 5000 represents the size of the null distribution. Notably, for each null model tests, only the smaller of the two values is reported as the p-value.

There are many different randomization algorithms possible for null models. We employed two such algorithms that make sense for this question, are both useable with abundance data, and can give us a sense of whether our results are sensitive to the choice of algorithm. The first method, the AITS algorithm (Abundance Independent Trait Shuffling), shuffles complete flowering time distributions among species in the community without replacement. The abundance matrix remains unchanged, and flowering time overlap is recalculated as the average of pairwise overlap among all pairs of species for each plot using the shuffled matrix of species flowering times. This is essentially a calculation of flowering time overlap under the assumption that species flowering times do not determine which species are found together in plots. We also included a second randomization technique, the IT algorithm (Ulrich and Gotelli 2010), which shuffles composition data (i.e. the abundance matrix), leaving flowering time distributions untouched. When the abundance matrix is shuffled, abundances are assigned to each species in each plot based on probabilities calculated from row (species) and column (plot) totals. During this shuffling process species richness of plots is unchanged and the number of plots a species is found in remains stable, which ensures flowering times associated with abundant or rare species, remain associated (i.e., abundance-flowering time relationships are preserved) (Ulrich and Gotelli 2010). All statistical tests were performed using Matlab code developed by B. Schamp and A. Jensen (The Mathworks Inc., Natick, MA).

Because flowering time overlap reflects the general timing of plant growth, it may reflect competition for abiotic resources (Dante et al. 2013), as well as for pollinator resources (Pleasants 1980). For this reason, we conducted two tests. First, we tested whether the flowering time of all flowering plant species found growing together in plots overlapped more or less than expected under our null models. These are tests for flowering time-based assembly rules that are related to temporal niche partitioning associated to abiotic resources. Second, we conducted separate analyses using only those species that share bees as pollinators (20 species in 2014; 19 species in 2015). This tested whether coexisting plant species that had the potential to compete for pollinators overlapped more or less in flowering time than expected under our null model. The IT algorithm requires abundance data, so this algorithm was only used with 50 cm plots where abundance data were collected; the AITS algorithm was run for both abundance and presence-absence data. For all algorithms, plot sizes, and guilds considered, we compared results between control and caged plots to identify whether flowering time overlap was more pronounced in plots where disturbance has been reduced (i.e. caged plots, in which competition should play a more powerful role in community assembly). All significance values were corrected for multiple testing using the False Discovery Rate method (Benjamini and Hochberg 1995).

Flowering time overlap in native vs. introduced species

An additional aim of this study was to determine whether introduced species influenced patterns of flowering time overlap. To test this, we compared plot-level effect sizes from our null model tests to the proportion of introduced plant species in each respective plot (Dante et al. 2013). Our null distributions were non-normal, so we used quantile effect sizes rather than the more common Z-scores (Bernard-Verdier et al. 2012). We assessed the degree of correlation between plot-level effect size and proportion of introduced species using non-parametric Spearman's rank correlations. As with the other tests in this study, p-values were corrected with the False Discovery Rate method (Benjamini and Hochberg 1995). These tests focused on bee-pollinated species (13 introduced; 5 native), as the expected pattern was proposed to be evidence that introduced species benefit from overlapping in flowering time with neighbouring species to enhance pollination.

Results

Flowering time overlap – full community

When all species in the community were analyzed using the 2014 abundance data (50 cm scale, 34 species) with the IT randomization algorithm, flowering time overlap was significantly less than expected under our null model ($P=0.0021$; $P=0.0027$; Table 1) for both the caged and control treatments. The same result was observed for the 2015 data, where again, both treatments showed significantly lower flowering time overlap (IT algorithm; $P < 0.05$; Table 1). The AITS randomization method, however, did not show any significant patterns for the full community data set in either year (Table 1).

Flowering time overlap – bee-pollinated species

When we analyzed those species that share bees as pollinators (50 cm scale), we found that coexisting species in both treatments in 2014 had significantly lower flowering time overlap than expected (IT algorithm), and in 2015, we found the same pattern. When we analyzed these data using the AITS algorithm, we found that coexisting species overlapped less in flowering time than expected in the caged treatment in 2014, but this pattern didn't hold for the control treatment in 2014, or for either treatment in 2015 (Table 1).

To visually compare the flowering time distribution among species, we created a bubble plot showing how many plants were flowering each week for each species across the 20 week (Fig. 1). This plot illustrates that flowering time distributions (2014) are more spread across the season for bee-pollinated species (20 week flowering duration) as compared to the remaining species (wind-pollinated species, 12 week flowering duration); this inevitably increases the potential for coexisting bee-pollinated species to overlap less in flowering time.

The influence of null model methods, treatment effects, and seasonal effects

In general, abundance data yielded different results than did results that used presence-absence data. When we conducted analyses using abundance data, we found that coexisting species generally overlapped less in flowering

time than expected; when we used presence-absence data, overlap did not differ from what was expected under our null model (Table 1). We take this as evidence that abundance data adds important information to these tests; for this reason, we focus on interpreting the results from our analyses that use abundance. We found more evidence of significant flowering asynchrony among coexisting species when we used the IT randomization algorithm (Table 1). Our treatments did not consistently differ with respect to findings related to flowering time overlap (i.e., there was no clear treatment effect; Table 1). Finally, reduced overlap in flowering time among coexisting species was more pronounced in 2014 than in 2015. This difference may result from differences in season length; 2015 was a shortened growing season, with most species displaying significantly shorter flowering periods in 2015 (Figure 3).

Patterns of Interspecific Overlap in Flowering Time (Native vs. Introduced)

In 2014, there is a positive correlation between plot-level overlap in flowering time (quantile effect size from null model test) and the proportion of introduced species in plots, although our results varied slightly between the two randomization algorithms. As with our other tests; this finding was consistent for analyses using the IT randomization (both for control and caged plots; however, the positive correlation was only found in control plots for analyses using the AITS randomization method (Table 2). There were no significant comparisons in 2015 data for either randomization algorithm.

Discussion

Flowering time overlap – full community

When all species were analyzed together, coexisting species in the community overlapped less in flowering time than expected for the majority of our null model tests. These results support predictions of temporal niche differences among coexisting species (Pleasants 1980) and provide evidence supporting the predictions that competition will limit the similarity (niche overlap) of coexisting species (i.e., limiting similarity; MacArthur and Levins 1967; Stubbs and Wilson 2004). Our findings are also consistent with those from several other studies that have found evidence of low flowering time overlap among coexisting species. These findings are consistent with the interpretation that coexisting species that differ in flowering time may avoid (or reduce) competition during a time of high resource demand (Snow 1965; Hurlbert 1970; Mosquin 1971; Heithaus 1974; Heinrich 1976; Stiles 1977; Anderson and Schelfhout 1980; Pleasants 1980; Ashton et al. 1988; Table S1).

Flowering time overlap – bee-pollinated species

Patterns of significant flowering time asynchrony were also evident within the guild of plant species that share bees as pollinators. This finding is consistent with the theory that competition among bee-pollinated species results in spatial segregation among species that overlap strongly in flowering time; reduced overlap in flowering time for these species may reduce competition for pollinators (e.g., Pleasants 1980) as well as for other resources (e.g., soil

nutrients). Our results are inconsistent with those of several other studies that have found coexisting species to overlap strongly in flowering time (e.g., Pojar 1974; Parrish and Bazzaz 1979; Schemske 1981; Rathcke 1988b; Dante et al. 2013; Fantinato et al. 2016). While competition among plant species is a viable explanation for observed flowering time asynchrony, it is also possible that low flowering time overlap reduces interspecific pollen transfer, which can have negative reproductive consequences (Mosquin 1971; Waser 1978; Pleasants 1980). It is also possible that peaks in flowering times are coincident with pollinator emergence, particularly if there are multiple specialized pollinators that have coevolved with different plant guilds. Bee pollinators are a diverse group and it remains possible that plant species that share bees as pollinators may not overlap completely in the specific bees that pollinate them.

One of the most likely explanations for the stronger pattern observed for bee-pollinated species is that these species may be competing both for pollinators and for soil resources, the combination of which is strong enough to drive the spatial separation of species that overlap significantly in flowering time. This may also explain some variation observed among studies, as different communities will likely vary with respect to both pollinator abundance and soil resource availability. However, explaining our results in terms of competition is complicated by our findings that asynchrony was not more pronounced in our herbivore exclusion treatment, in which we expected the role of competition to be greater. It is possible that herbivory impacts seedlings, and yet competitive effects observed here are largely among established plants, which small mammals generally do not feed on.

These findings are consistent with predictions related to limiting similarity, which predicts that coexisting species will differ with respect to traits related to resource acquisition (MacArthur and Levins 1967; Stubbs and Wilson 2004; Schamp et al. 2008). This evidence of limiting similarity is particularly interesting given that Götzenberger et al. (2012) found that only 18% of analyses (as of that publication date) produced support for limiting similarity. Our approach may have been better placed to find evidence of limiting similarity because it accounts for intraspecific variation in the functional trait of interest (25 individuals per species for our flowering time distributions). Some evidence suggests that intraspecific variation in functional traits can be important (e.g., Siefert et al. 2015), and that overlap-based approaches are better for testing for patterns related to limiting similarity (De Bello et al. 2013). Consequently, we recommend that researchers consider using niche overlap indices that capture intraspecific variation when investigating patterns like those examined here (e.g., trait overdispersion / divergence), particularly when dealing with discrete data as we are here.

Variation in flowering time overlap across studies: possible explanations

Not surprisingly, given the diversity of patterns found by researchers examining flowering time overlap, our results contrast with the results of some previous studies that have found evidence of flowering time synchrony (e.g., Augspurger 1981; Rathcke 1988; Dante et al. 2013). This could be due to different pressures within this particular community, as mentioned above; however, it may also be

due to methodological differences. Our findings of low flowering time overlap, for example, are most pronounced when we use abundance data rather than presence-absence data. While Dante et al. (2013) used presence-absence data, it is unlikely that this alone explains the difference between observations in the two studies. In this study, the use of presence-absence data led to no significant observed patterns in general. The use of presence-absence data has not been uncommon (e.g. Poulin et al. 1999), but as we show here, its use can influence the results, likely because without abundance data, the power of the null model randomization algorithms employed is reduced (Götzenberger et al. 2016).

Some variation in observed patterns of flowering time overlap may be due to the variety of null model analyses that researchers have employed. The chosen randomization method can affect the outcome of the null model test (Ulrich and Gotelli 2010; Götzenberger et al. 2016; Lavender et al. 2016). Consistent with this view, our results varied with randomization method. Strictly shuffling flowering time distributions among species in the community (AITS) did not yield evidence of reduced overlap in flowering time among species in the majority of tests. This method has been criticized because it allows the traits of common species to be shuffled to rare species and vice versa (Wilson and Stubbs 2012). The use of the IT randomization method (Ulrich and Gotelli 2010) addresses this specific concern, but in doing so, subtly changes the question that the null model addresses. Using IT to shuffle the abundance matrix, the test asks whether observed flowering time overlap is higher or lower than would be expected if the abundances of species are assigned to plots using the matrix row and column totals as the probability of assignment. As has been suggested elsewhere, the choice of null model algorithm requires careful consideration (Pleasant 1990; Castro-Arellano et al. 2010; Wilson and Stubbs 2012; Götzenberger et al. 2016; Lavender et al. 2016). We hope this work stimulates an effort to conduct similar tests across many data sets using a consistent set of tests.

Most studies have examined flowering time overlap within a single growing season, ignoring the potential for unusual climatic conditions to influence results. Shifts in climate impact several abiotic variables, and can influence competition and other selective pressures that affect phenological overlap (Elzinga et al. 2007). Changes in flowering time overlap across years can result from variation in the plasticity of responses among species to these year-to-year changes (Rathcke 1983). We considered two years of data and found that the strength of patterns in our results was not consistent between the two years. In 2014, the observed tendency for coexisting species to overlap less in flowering time was more pronounced. Flowering time distributions were on average shorter in 2015, in relation to a late snow-melt and shorter growing season, with shorter flowering periods for individuals in 2015 than in 2014 (Fig. 2; paired t-test: $n=37$, $t=3.014$, $P=0.0037$). This may explain why patterns of reduced flowering time overlap were not as pronounced in 2015 – an unusual year. The shorter growing season in 2015 may have restricted the amount of flowering time divergence that could occur because the completion of reproductive activities for all species was limited to a narrower window. Our results suggest that observed assembly rules can be

impacted by whether the year in which a study occurs is climatically typical, or unusual. As such, our results indicate that studies of temporal niche separation may benefit from multi-year sampling.

Another reason some flowering time studies do not produce evidence supporting limiting similarity is that variation in flowering time reflects one niche dimension, while other dimensions may also be important (Schoener 1974). Temporal niche separation may be less important in communities where functional trait variation plays a more important role. Accounting for multiple niche dimensions simultaneously may reveal clearer support for limiting similarity; we recommend that future work simultaneously explore multiple niche dimensions.

While considering our flowering time distributions (Fig. 1), we wondered whether our results for bee-pollinated species were influenced by the fact that these species, on average, flowered later in the growing season. Previous research has documented a flush of nutrients in the spring that would be available to early flowering plants (Lipson et al. 2002; Schmidt and Lipson 2004); however, as the season progresses the available nutrients are generally depleted either by uptake or leeching. Additionally, early in the growing season, plants may also be able to rely more heavily on resources stored within the root system over winter. Consequently, competition for soil resources may be more important for plant species that continue to grow and reproduce later in the growing season. While this question requires future research, we explored the possibility by conducting a post-hoc test to determine whether plots with a greater proportion of late-flowering species (onset of flowering in week eight or later) were characterized by less flowering time overlap (plot-level effect sizes for overlap). Indeed, plots with more late-flowering species had significantly lower flowering time overlap for our data in both years, regardless of treatment or null model algorithm: Spearman's Rank Correlations for 2014 fell between $-0.262 < \text{Rho} < -0.4911$ ($P < 0.05$), and for 2015 were $-0.405 < \text{Rho} < -0.719$ ($P < 0.05$). While this was somewhat outside the scope of our original aims for this research, it is clear that this possibility warrants further examination. It is possible that variation in results from past flowering time overlap studies may be explained by different communities possessing different numbers of late-flowering species.

Introduced species and flowering time overlap

We tested whether our patterns were impacted by introduced species, as has been proposed (directly and indirectly) by a number of researchers (Case 1990; Strauss et al. 2006; Cleland et al. 2011; Dante et al. 2013). We found that plot-level flowering time overlap for bee-pollinated species was significantly positively correlated with the proportion of invasive species for the 2014 season. These results are consistent with observations by Dante et al. (2013), and the interpretation that introduced species likely benefit from overlapping in flowering time with native species. Perhaps, as speculated by Dante and colleagues (2013), pollinators attracted by native floral displays are facilitating the pollination of introduced species, which have not co-evolved with native pollinators (Parker 1997; Sargent and Ackerly 2008). It is also possible that introduced species benefit from flowering closer to mid-growing season as this is when the bulk of flowering at

the community-scale occurs (Fig. 1). Such generalism may be a common feature of introduced species that is coincident with their ability to succeed in many habitats that span a wide range of environmental conditions. Mid-season flowering may be part of this success if it improves pollination and reproductive success. Future research is required to examine this possibility. There were no significant correlations in 2015, which is perhaps not surprising given the unusual climatic patterns of 2015 that we discussed above.

Conclusions

Our results contribute to a growing body of research examining trait-based community assembly rules and investigating expectations related to limiting similarity. We found evidence that coexisting species in our community overlapped less in flowering time than expected by chance. We interpret this as evidence that temporal niche partitioning contributes to the coexistence of plant species in this community. These patterns were more pronounced for bee-pollinated species, which suggests that some combination of competition for pollinators and abiotic resources may drive these patterns. Finally, we found support for the observation by Dante et al. (2013) that introduced species are more common in plots where flowering time overlap is high. This reinforces the possibility that introduced species benefit in terms of

pollination success, by flowering at the same time as their neighbours. Finally, observed flowering time asynchrony was more pronounced when abundance data were used in

combination with the IT randomization method. As such, we recommend that future research should focus on using abundance data in conjunction with multiple null model tests. We also found variation across study years, which suggests that future work should either examine multiple years or investigate whether the growing season under study is abnormal climatically.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Tables:

Table 1. Null model analysis results across treatments using two randomization methods (AITS and IT; see Methods for descriptions) for the full community, and for the bee-pollinated guild only. These results use Schoener's index, and are shown for the 50 cm scale using a) abundance data, and b) presence-absence data, and also at the c) 1m scale (presence-absence data). The comparison of the observed community overlap value to the null distribution is a two-sided test, and the lower of the two P-values is reported for each test. P-values <0.05 under synchrony indicate mean plot-level flowering overlap is significantly higher than expected, P-values <0.05 under asynchrony indicate mean plot-level flowering time overlap is significantly lower than expected (significance indicated with an asterisk). P-values were corrected using the False Discovery Rate method (Benjamini and Hochberg 1995).

a)						
Guild	Algorithm	Treatment	P-values 2014		P-values 2015	
			Synchrony	Asynchrony	Synchrony	Asynchrony
All	AITS	Caged	0.2667			0.7164
		Control	0.3318			0.4672
	IT	Caged		0.0021*		0.0016*
		Control		0.0027*		0.0016*
Bee-pollinated	AITS	Caged		0.0368*		0.2693
		Control		0.2247		0.2693
	IT	Caged		0.0013*		0.0080*
		Control		0.0013*		0.0016*
b)						
All	AITS	Caged	0.4731			0.2123
		Control	0.7284			0.1968
Bee-pollinated	AITS	Caged		0.1440		0.2123
		Control		0.1440		0.1968
c)						
Guild	Algorithm	Treatment	Synchrony	Asynchrony	Synchrony	Asynchrony
All	AITS	Caged	0.5481			0.3974
		Control	0.7692			0.3974
Bee-pollinated	AITS	Caged		0.3974		0.3974
		Control		0.3974		0.5381

Table 2. Spearman rank correlations between plot-level flowering time (quantile effect size calculated using abundance data at the 50 cm scale) and the proportion of introduced species (for species that share bees as pollinators) in a) 2014 and b) 2015. Results are shown for both null model randomization algorithms (AITS and IT; see Methods for descriptions), and values that are statistically significant at $P < 0.05$ are denoted with an asterisk. Positive rho values indicate that higher proportions of introduced species are associated with higher overlap in flowering time among plots.

a)		
	Correlation coefficients	
Randomization algorithm	Rho (caged)	Rho (control)
AITS	0.0107	0.4299*
IT	0.4414*	0.3814*
b)		
AITS	-0.0796	0.0349
IT	0.0406	0.2908

Figures

Fig. 1

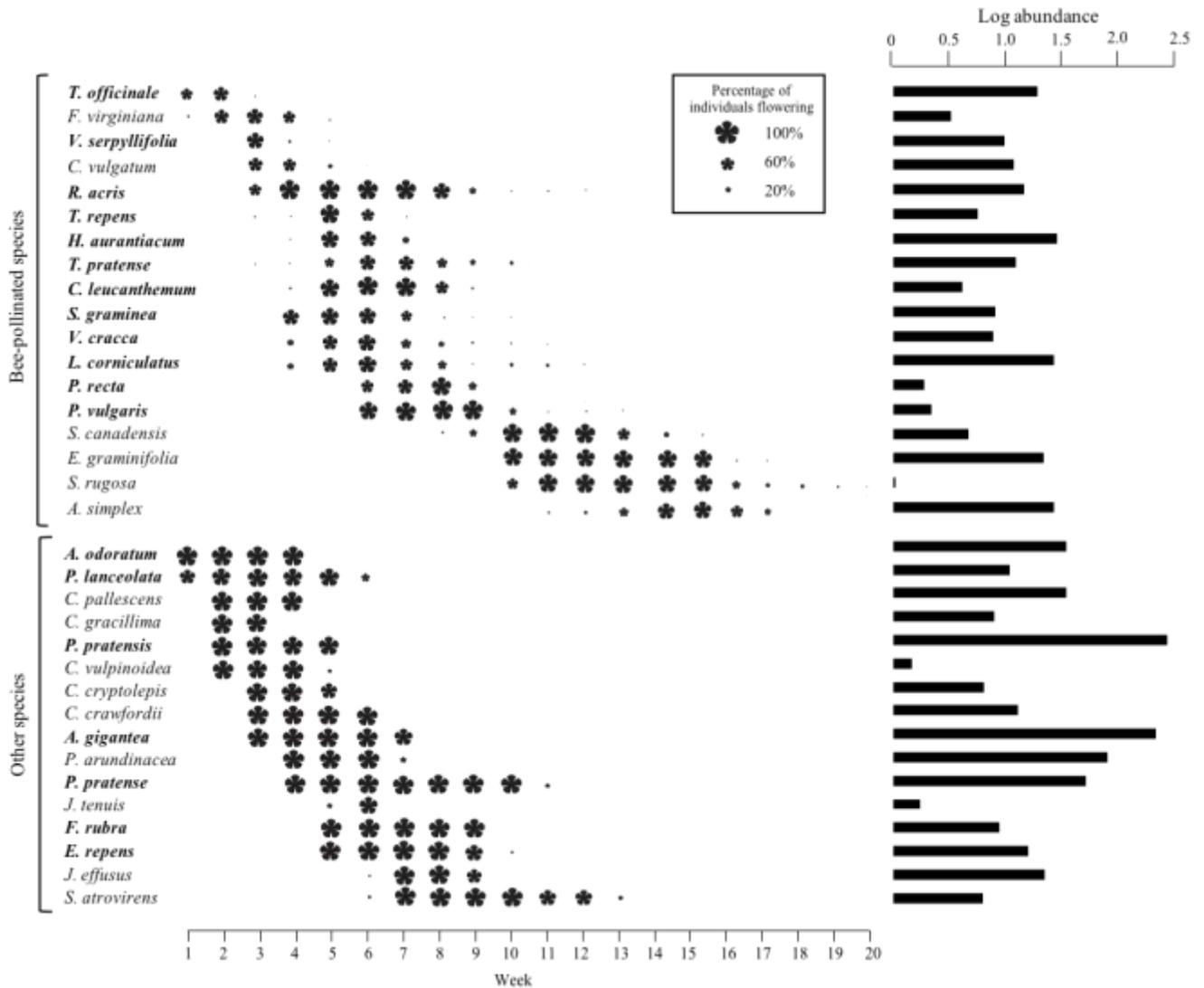


Figure 1: The 2014 flowering time distributions for each species in the study (introduced species in bold), expressed as the proportion of individual plants of each species flowering per week. The size of the flower symbol represents the proportion of individuals flowering for that species. For example, if all 25 plants of a species flower in a given week, the proportion is 100% and flower symbol is largest. For each species, we illustrate overall abundance in the study field to the right of flowering time distributions. Because of the nature of abundances across species, we illustrate the log of abundance here; most species had total abundances below 500 individuals, whereas two graminoid species had abundance values over 10,000 (*A. gigantea* (13,661) and *Poa pratensis* (16,448)). Abundance patterns for species were similar across control and caged plots; consequently, abundance here is total abundance per species across treatments. Plots for 2015 distributions are in the supplementary material (Fig. S1)

Fig. 2

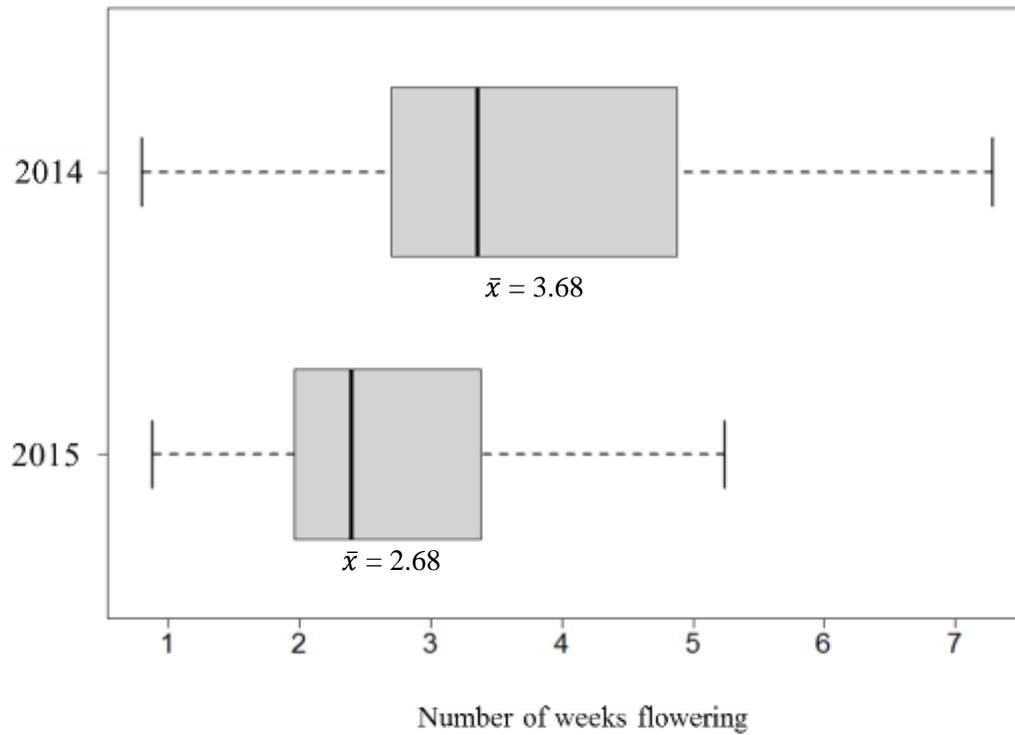


Figure 2: Boxplots of the number of weeks each species flowered on average in each of the two seasons (2014 and 2015). The mean number of weeks each species spent flowering was significantly higher in 2014 (two-sided t-test; $P = 0.0037$)

Supplementary Material

Table S1. Flowering time studies used in Fig. 1. Studies with multiple tests may be repeated if results fell into more than one category (denoted by *).

Increased overlap	No pattern	Reduced overlap
Augspurger 1981	Kipling and Warren 2014	Anderson and Schelfhout 1980
Beattie et al. 1973	Rabinowitz 1981	Ashton, Givinish and Appanah 1988
Dante et al. 2013	Wheelwright 1985	Heinrich 1976
Fantinato et al. 2016		Heithaus 1974
Parrish and Bazzazz 1979		Hurlbert 1970
Pojar 1974		Mosquin 1971
Poulin 1999 *		Pleasants 1980
Rathcke 1988b		Poulin 1999*
Schemske 1981		Smythe 1970*
Smythe 1970 *		Snow 1965
Wellstein et al. 2014		Stiles 1997
		Waser 1978

Table S2. List of species that share bees as pollinators, and their native status.

Bee-pollinated species	Native Status
<i>Aster simplex</i> ¹	Native
<i>Chrysanthemum leucanthemum</i> ²	Introduced
<i>Cirsium arvense</i> ³	Introduced
<i>Euthamia graminifolia</i> ⁴	Native
<i>Fragaria virginiana</i> ⁵	Native
<i>Hieracium aurantiacum</i> ⁶	Introduced
<i>Lotus corniculatus</i> ²	Introduced
<i>Potentilla recta</i> ⁸	Introduced
<i>Prunella vulgaris</i> ⁹	Introduced
<i>Ranunculus acris</i> ¹⁰	Introduced
<i>Sisyrinchium montanum</i> ¹¹	Native
<i>Solidago canadensis</i> ¹²	Native
<i>Solidago rugosa</i> ¹³	Native
<i>Stellaria graminea</i> ¹⁴	Introduced
<i>Taraxacum officinale</i> ⁵	Introduced
<i>Trifolium pratense</i> ¹⁵	Introduced
<i>Trifolium repens</i> ⁹	Introduced
<i>Veronica serpyllifolia</i> ¹⁶	Introduced
<i>Vicia cracca</i> ¹⁷	Introduced

¹Jones AG (1978) Observations on reproduction and phenology in some perennial asters. *Am Midl Nat* 99:184–197. doi: 10.2307/2424942

²Ginsberg HS (1983) Foraging ecology of bees in an old field. *Ecology* 64:165–175. doi: 10.2307/1937338

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Table S3. Null model analysis results across treatments using two randomization methods (AITS and IT; see Methods for descriptions) for the full community, and for the bee-pollinated guild only. These results use Pianka's index, and are shown for the 50 cm scale using a) abundance data, and b) presence-absence data, and also at the c) 1m scale (presence-absence data). The comparison of the observed community overlap value to the null distribution is a two-sided test, and the lower of the two P-values is reported for each test. P-values <0.05 under synchrony indicate mean plot-level flowering overlap is significantly higher than expected, P-values <0.05 under asynchrony indicate mean plot-level flowering time overlap is significantly lower than expected (significance indicated with an asterisk). P-values were corrected using the False Discovery Rate method (Benjamini and Hochberg 1995).

a)						
Guild	Algorithm	Treatment	P-values 2014		P-values 2015	
			Synchrony	Asynchrony	Synchrony	Asynchrony
All	AITS	Caged	0.2796			0.6984
		Control	0.3920			0.5275
	IT	Caged		0.0008*		0.0072*
		Control		0.0008*		0.0024*
Bee-pollinated	AITS	Caged		0.0435*		0.5275
		Control		0.1560		0.5275
	IT	Caged		0.0008*		0.0080*
		Control		0.0008*		0.0024*
b)						
All	AITS	Caged	0.6096			0.2656
		Control	0.7624			0.2656
Bee-pollinated	AITS	Caged		0.0872		0.2656
		Control		0.0872		0.2656
c)						
All	AITS	Caged	0.6403			0.3080
		Control	0.8684			0.3030
Bee-pollinated	AITS	Caged		0.3520		0.3030
		Control		0.2576		0.3080

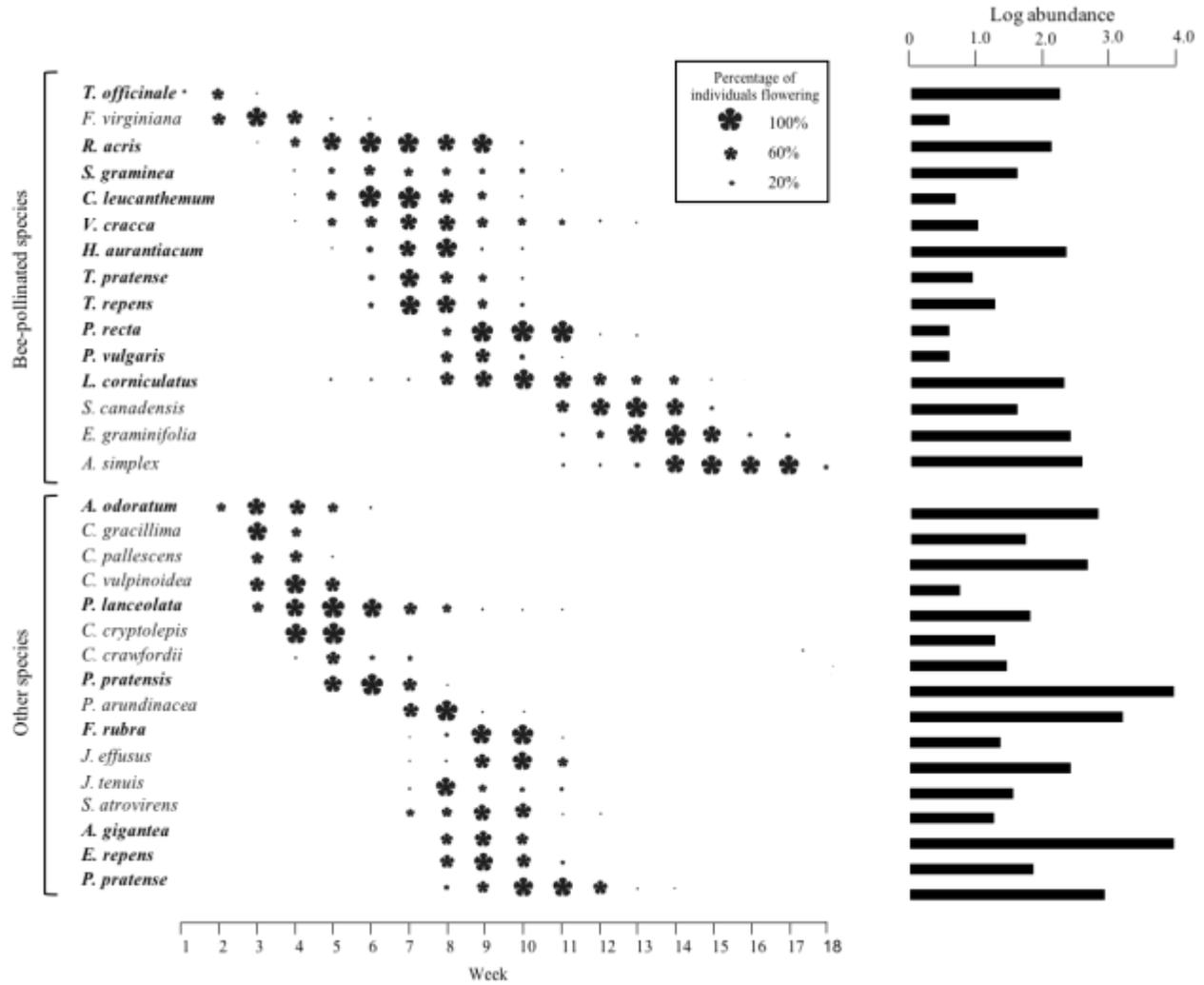


Fig. S1. The 2015 flowering time distributions for each species in the study (introduced species in bold), expressed as the proportion of individual plants of each species flowering per week. The size of the flower symbol represents the proportion of individuals flowering for that species. For example, if all 25 plants of a species flower in a given week, the proportion is 100% and flower symbol is largest. For each species, we illustrate overall abundance in the study field to the right of flowering time distributions. Because of the nature of abundances across species, we illustrate the log of abundance here. Abundance patterns for species were similar across control and caged plots; consequently, abundance here is total abundance per species across treatments.