ECOLOGY LETTERS

Ecology Letters, (2011) **14**: 69–74 doi: 10.1111/j.1461-0248.2010.01557.x

LETTER

Effects of experimental shifts in flowering phenology on plant–pollinator interactions

Nicole E. Rafferty* and Anthony R. Ives
Department of Zoology, University of Wisconsin, Madison, WI 53706, USA
**Correspondence:*
E-mail: nrafferty@wisc.edu

Abstract

Climate change has led to phenological shifts in flowering plants and insect pollinators, causing concern that these shifts will disrupt plant–pollinator mutualisms. We experimentally investigated how shifts in flowering onset affect pollinator visitation for 14 native perennial plant species, six of which have exhibited shifts to earlier flowering over the last 70 years and eight of which have not. We manipulated flowering onset in greenhouses and then observed pollinator visitation in the field. Five of six species with historically advanced flowering received more visits when flowering was experimentally advanced, whereas seven of eight species with historically unchanged flowering received fewer visits when flowering earlier. This pattern suggests that species unconstrained by pollinators have advanced their flowering, whereas species constrained by pollinators have not. In contrast to current concern about phenological mismatches disrupting plant–pollinator mutualisms, mismatches at the onset of flowering are not occurring for most of our study species.

Keywords

Climate change, flowering onset, flowering phenology, global warming, mismatch, phenology, plant–pollinator interactions, plant–pollinator mutualisms, pollination, prairie.

Ecology Letters (2011) 14: 69–74

INTRODUCTION

Temperature plays a role in determining the timing of important events in the life cycles of plants and animals, and the increasing temperatures associated with climate change have caused rapid and dramatic shifts in the phenologies of numerous and diverse organisms (Roy & Sparks 2000; Fitter & Fitter 2002; Peñuelas *et al.* 2002; Parmesan & Yohe 2003; Root *et al.* 2003; Visser & Both 2005; Menzel *et al.* 2006). Of particular interest are the effects of phenological shifts on mutualistic interactions, which often require a high degree of temporal synchrony between species. Among the most common and well-studied mutualisms are those between flowering plants and pollinators (Bronstein 1994). As flowering period and pollinator availability must coincide, changes in phenology may have a significant influence on these mutualisms (Hegland *et al.* 2009) and the valuable ecosystem services they provide (Costanza *et al.* 1997; Kearns *et al.* 1998). Yet, we have a very incomplete understanding of how interactions between plants and pollinators will be affected by the phenological shifts that accompany climate change (Hegland *et al.* 2009).

Phenological data indicate that many plant species are responding to climate change by flowering earlier (Abu-Asab *et al.* 2001; Fitter & Fitter 2002; Primack *et al.* 2004; Miller-Rushing *et al.* 2007). However, there is much variation among plant species in whether and to what extent their phenologies have shifted. For example, the date of first flowering has advanced as much as 46 days over a 30-year period in some species (Abu-Asab *et al.* 2001), whereas others have exhibited unaltered (Bradley *et al.* 1999) or even delayed flowering (Fitter & Fitter 2002). In general, species flowering early in the season have displayed the greatest advances in the onset of flowering (Fitter & Fitter 2002). Fewer data are available on pollinator responses, but the appearance dates and flight seasons of some butterflies have advanced (Roy & Sparks 2000; Peñuelas *et al.* 2002; Stefanescu *et al.* 2003). Overall, the directions and magnitudes of phenological responses to climate change are highly variable, making it difficult to forecast the impacts of altered phenologies on plant–pollinator communities.

It is similarly challenging to predict the impact of phenological shifts from the structure of plant–pollinator networks. Broad network studies have revealed that interactions between plants and pollinators tend to be asymmetrical. This tendency for specialists to interact with generalists (Bascompte *et al.* 2003; Vázquez & Aizen 2004) and the fact that most plant–pollinator interactions are diffuse (Ollerton 1996; Waser *et al.* 1996) may make it less likely for all interacting species to maintain phenological synchrony in a changing climate. However, this structure may also make synchrony less critical, as species might be buffered from the loss of some interactions, provided others remain intact (Memmott *et al.* 2004). Likewise, many community studies show that plant–pollinator interactions are naturally variable in time and space (e.g. Price *et al.* 2005; Olesen *et al.* 2008; Petanidou *et al.* 2008), suggesting that strict phenological synchrony may be unimportant.

To date, only a few studies have investigated the consequences of phenological mismatches between plants and pollinators. Opposing phenological responses of *Prunus* tree species and the butterfly *Pieris rapae* to climate changes in Japan provide evidence that plants and pollinators may not respond to the same cues at the same times (Doi *et al.* 2008). The trees responded to temperatures about a month prior to flowering, which have greatly increased, whereas the butterfly responded most to temperatures 2 weeks prior to its appearance date, which have not changed (Doi *et al.* 2008). Furthermore, plants and pollinators may respond differently to short-term temperature fluctuations. The composition of pollinators visiting an endangered plant, *Clematis socialis*, in the USA differed between years, depending on the mean monthly temperature and whether the plant flowered early or late (Wall *et al.* 2003). In Japan, an extremely warm spring caused spring ephemerals to flower 7–17 days earlier, leading to greatly reduced seed set for plants pollinated by bees, whereas fly pollinated species did not suffer (Kudo *et al.* 2004). Phenological mismatches such as these may lead to pollination depression and/or pollinator food limitation early in the flowering period. Indeed, simulations suggest that under predicted levels of warming, phenological shifts could cause 17–50% of insect pollinators to experience periods when no nectar or pollen is available (Memmott *et al.* 2007).

Of the handful of studies that have addressed the potential consequences of climate change-induced shifts in flowering phenology for plant–pollinator mutualisms, none has done so experimentally (Hegland *et al.* 2009). Experimental manipulation of flowering phenology provides a way to measure how shifts in date of first bloom (DFB) affect pollination without relying on occasional and extreme natural weather fluctuations. Here, we report on an experimental study aimed at determining how changes in first bloom date affect plant–pollinator interactions for 14 plant species native to Wisconsin, USA. We used records collected over the last 70 years (Bradley *et al.* 1999; N. Bradley, C. Bradley and A. Leopold, unpublished data) to select six species that are flowering significantly earlier in Wisconsin ('historically advanced' species) and eight species that have not shifted flowering phenology ('historically unchanged' species). We manipulated the flowering time of each species in greenhouses and placed subsets in the field before, on and after the current DFB.

We sought to determine how shifts in flowering phenology associated with climate change affect visitation rates by potential pollinators, contrasting historically advanced and unchanged plant species. The specific null hypothesis we tested is whether the identity of plant species that have experienced advanced flowering is independent of the current phenology of pollinators that they attract; this is the assumption that Memmott *et al.* (2007) used to forecast potential breakdown of plant–pollinator mutualisms. In investigating mismatches, we were not asking about differences in timing between peak flowering and pollinator peak activity. Instead, our experiments focused on the leading edge of flowering phenologies, asking whether by flowering relatively earlier, plants at first bloom are likely to flower without pollinators, or with such reduced pollinator activity that pollination may be limiting. If species receive fewer visits when flowering is experimentally made to be relatively earlier, then this would suggest that mismatches are developing. By comparing historically advanced to historically unchanged species, we can ask whether one group has the greater potential to experience mismatches based on pollinator phenology. Whether this potential is realized, however, depends on whether species are in fact experiencing advanced flowering times. Even if historically unchanged species have the greatest potential to experience mismatches due to pollinator phenology, this potential will not be realized as long as the flowering phenology remains unchanged.

METHODS

Selection of experimental taxa

We selected study species by examining a dataset that documents the dates of first bloom for plants in southern Wisconsin for two time spans: 1935–1945 and 1977–2007 (Bradley *et al.* 1999; N. Bradley, C. Bradley and A. Leopold, unpublished data). To determine which species have significantly advanced, we compared the historical

and more recent first bloom dates, restricting our analyses to species for which there were at least 4 years of data in each time span. We used *t*-tests to test for significant differences in the DFB between the two time spans for 14 perennial species with flowering seasons that extend over most of the spring and/or summer (Table 1). We found that six of these species are flowering significantly earlier, with advances of 6–13 days, and eight have not changed. The six historically advanced species tend to flower earlier in the season than the historically unchanged species. For the former, the mean DFB was calculated with data from 1977 to 2007 to yield a more accurate date that does not lag behind the current onset of flowering; for the latter, the mean DFB was calculated with all available data. We note, however, that using data from only 1977 to 2007 for the historically unchanged species yields a mean DFB that is within 1 day of the date yielded by the pooled data for all species except one (*Monarda punctata*), for which it was within 4 days and thus fell within the same week.

Flowering phenology manipulations

The overall study design involved raising plants to flower at different times in greenhouses, placing subsets of each plant species in the field and observing pollinator visits. Most of the plant species were placed in the field for at least 5 weeks, with the third week coinciding with the current mean DFB, the first and second weeks representing advanced onset of flowering, and the fourth and fifth weeks representing delayed (historical) onset of flowering (Fig. 1). As we centre the experimental flowering times around the current mean DFB, interpreting these timings differs for historically advanced vs. historically unchanged species.

Seedlings of south-eastern Wisconsin genotypes were obtained from nurseries and raised in greenhouses at the University of Wisconsin-Madison. Plants were raised in #300 (2.8 L) or #600 (6.1 L) pots in Sun Gro Metro-Mix 300 growing medium (Vancouver, British Columbia, Canada) and fertilized with Osmocote Outdoor and Indoor Smart-Release Plant Food approximately every 3 months. Plants were sprayed with pesticides only after they had been exposed to pollinators in the field, except that ultra pure oil was sprayed on occasion to control thrips, in which case we waited at least 1 week before exposing oil-sprayed plants to pollinators. Flowering was advanced by placing plants in a warmer greenhouse (24–27 °C) with supplemental lighting and delayed by placing plants in a cooler greenhouse (18–21 °C) without supplemental lighting. Supplemental lights were programmed to turn on automatically when ambient light levels were low (< 500 μmol m^{-2} s^{-1}) during the day and did not extend photoperiod. All plants were 1–2 years old when they began flowering in 2009. We recorded DFB, number of flowers produced and height for each plant.

To determine whether the timing of flowering onset altered the physiology of the plants in a way that would make them more or less attractive to pollinators, nectar quantity and sucrose content were measured for a subset of flowers from each plant of each nectar-producing species, except *Dalea candida*, *Dalea purpurea*, *Geranium maculatum* and *Phlox divaricata*. Neither *Anemone canadensis* nor *Tradescantia ohiensis* produce nectar (Douglas & Cruden 1994; Grundel *et al.* 2000). Nectar was sampled the day before plants were placed in the field at approximately the same time each week. Nectar was extracted with fresh microcapillary pipettes (Drummond Microcaps, Broomall, PA, USA), and the length of the nectar column was measured to give nectar volume. The nectar was then discharged onto a refractometer (Bellingham and Stanley Eclipse 45–81 or 45–82; Tunbridge Wells, Kent, UK) to measure sucrose content (Corbet 2003).

Study site

All field observations were conducted in the University of Wisconsin Arboretum, Madison, WI, USA. The 510 ha of the Arboretum encompass both prairie and woodland habitat. A 40-m^2 study area (43.04 °N, 89.43 °W) was established in the central portion of Curtis Prairie, a 24-ha, 75-year-old restored tallgrass prairie. Flowering phenologies of forbs occurring naturally in the study area were documented. All of the study plants occurred either in the study area or within 75–450 m, except *Astragalus canadensis*, which was not observed in 2009 but was documented within 600 m in 2005.

Plant arrays

Each week, sets of 2–5 potted plants of a species were placed at least 5 m apart in the study area in a haphazard fashion. Plants remained in the study area for 3 days. When experimental plant numbers were limited, plants were used in more than one array, and this was incorporated into the statistical analysis as repeated measures.

Field observations

Field observations were conducted for 51 days over the course of 17 weeks, from 15 April until 6 August 2009. Focal plants were observed for 10 min at a time, in haphazard order and typically at least twice per day, once in the morning and again in the afternoon between 0830 and 1700 h. Focal plant observations were conducted regardless of weather, although we discontinued observations during heavy rainfall after documenting that pollinators do not visit in such conditions. Daily weather variables (maximum temperature, minimum temperature, precipitation and wind speed) were obtained from a nearby weather station. The number of flowers or inflorescences on each study plant was also recorded each day.

For every focal observation, we recorded the start time, focal plant identity, identity of each visitor and total number of unique flowers visited by each visitor. A visitor was defined as an insect that contacted the anthers, stigma and/or nectar of a flower. Potential pollinators that were not readily identified were assigned a morphospecies code and, when possible, captured for subsequent identification.

Statistical analyses

To incorporate both fixed and random effects in the visitation data, we used general linear mixed models (GLMM; Gelman & Hill 2007). We constructed three models with different response variables, each summed over 10 min observations of focal plants: (1) the number of pollinators that visited, (2) the number of visits received (multiple visits from the same pollinator were scored when the pollinator ceased contact with the plant between visits) and (3) the number of flowers visited. As these response variables are correlated, we do not consider the results as independent evidence. For each model, the fixed effects were plant species (factor with 14 levels), time-of-day (continuous), time-of-day^2 (to account for possible nonlinear effects of time-of-day), maximum daily temperature (continuous) and precipitation (continuous). To determine the plant species-specific effect of week, we included the interaction of plant species and week-of-sample (continuous). The random effects for each model were individual plant (factor with 270 levels) and day-of-sample (factor with 51 levels). We used this statistical design because there was considerable day-to-day

Table 1 The 14 plant species used in the study, showing mean current date of first bloom (DFB) based on data from 1977 to 2007 for the six species with historically advanced flowering phenologies and on data from 1935 to 2007 for the eight species with historically unchanged flowering phenologies (N. Bradley, C. Bradley and A. Leopold, unpublished data)

Species	Family	DFB	Colour	Flowering season
Phlox divaricata L.*	Polemoniaceae	April 30 (May 11)	Blue	Late April–June
Aquilegia canadensis L.*	Ranunculaceae	May 11 (May 20)	Red, yellow	May–July
Anemone canadensis L.*	Ranunculaceae	May 27 (June 4)	White	Late May–July
Tradescantia ohiensis Raf.*	Commelinaceae	May 28 (June 3)	Blue	Late May–early August
Asclepias tuberosa L.*	Apocynaceae	June 23 (July 6)	Orange	Mid June–August
Asclepias incarnata L.*	Apocynaceae	June 29 (July 8)	Pink	Late June–mid August
Geranium maculatum L.	Geraniaceae	May 12 (May 15)	Purple	May–mid June
Astragalus canadensis L.	Fabaceae	June 1 (June 2)	Pale yellow	June–August
Verbena stricta Vent.	Verbenaceae	July 1 (July 5)	Purple	July–early October
Monarda fistulosa L.	Lamiaceae	July 4 (July 9)	Purple	July–early September
Veronicastrum virginicum (L.) Farw.	Scrophulariaceae	July 7 (July 5)	White	July–August
Dalea candida Michx. ex Willd.	Fabaceae	July 7 (July 12)	White	July–September
Dalea purpurea Vent.	Fabaceae	July 11 (July 12)	Purple	July–September
Monarda punctata L.	Lamiaceae	July 21 (July 25)	Yellow, pink	Mid July–mid September

*Indicates historically advanced plant species.
The mean historical DFB (1935–1945) is given in parentheses. Colour indicates the colour of flowers or other showy floral parts, such as bracts.

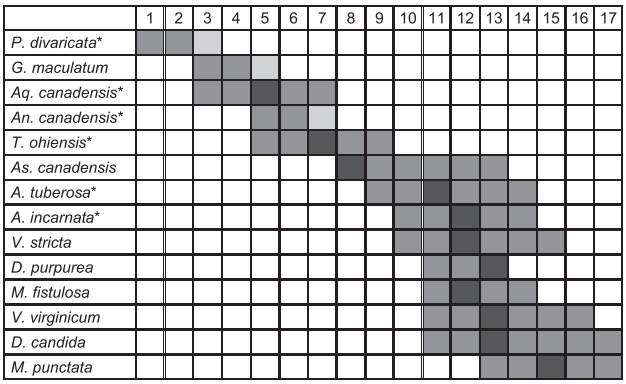

Figure 1 Schematic showing when arrays of each species were in the field for the 17 weeks of the study, from 15 April to 6 August (grey squares). The current mean week of first bloom is indicated by light grey squares if the species was not in the field at that time and by dark grey squares if the species was in the field at that time. Note that all arrays of *Phlox divaricata*, *Geranium maculatum* and *Anemone canadensis* were placed in the field before the current mean week of first bloom. *Indicates historically advanced species.

variation in the total number of pollinators we observed across all plant species, due both to the fixed weather effects we included in the model and to effects that were not completely explained by these variables. Analysing all plant species simultaneously and including a day-of-sample random effect factors out the day-to-day variation observed across plant species. The analyses assume that the changes in the response variables are monotonic through time because we are interested in whether pollination is higher or lower for plants blooming earlier. More detailed analyses that address non-monotonic changes in response variables are presented in Figure S1 and Table S1 (Supporting Information). A quasi-Poisson distribution was assumed for all analyses, as the data showed a skewed distribution with many zero values; in the quasi-Poisson distribution in the GLMM, the variance is proportional to but not necessarily equal to the mean.

After identifying which species attracted more or fewer pollinators through time, we categorized each plant species according to whether it was historically advanced or unchanged. To assess whether historically advanced vs. unchanged species showed increases vs. decreases in the number of pollinator visits, we performed a chi-square analysis with a Yates correction for small sample sizes; the Yates correction is more conservative (for our dataset) than other tests (e.g. a Fisher exact test), leading to higher computed *P*-values. We also performed a parallel analysis that accounts for the phylogenetic relationships among the 14 plant species. Specifically, we used the phylogenetic tree for the plant species with the software package RegressionV2.m that fits the data while simultaneously estimating the strength of phylogenetic signal in the residual variation measured by the parameter d (Lavin *et al.* 2008); a value of $d = 0$ indicates the absence of phylogenetic signal. For each of the three metrics of pollination, we fit the slope of the species-specific change in pollination as a function of whether or not the species was historically advanced.

The analyses above focus on the plant–pollinator interaction from the perspective of the plant rather than pollinators. We do, however, make use of data on the composition of the pollinator community (see Table S2) in interpreting plant visitation patterns. In our analyses, we use the number of pollinator visitors, the number of observed visits and the number of flowers visited as surrogates for the pollination success for plants in the field at different times (weeks). If the composition of the pollinator community changed through time, and if different types of pollinators had different efficiencies as pollinators, then the three variables we used to assess pollination might not be proportional to pollination success. Therefore, to determine whether the composition of pollinators visiting different plant species changed from week to week over the course of the experiment, we used a general linear model with a quasi-Poisson distribution (McCullagh & Nelder 1989) in which pollinators were divided into five taxonomic categories that reflect different pollinator characteristics: (1) small/medium Hymenoptera (21 morphospecies), (2) large Hymenoptera (9 morphospecies), (3) Coleoptera (13 morphospecies), (4) Lepidoptera (7 morphospecies) and (5) Diptera (11 morphospecies). For each plant species separately, we regressed the number of pollinators visiting per observation period against the pollinator taxonomic category (factor with five levels), week-of-sample (continuous), and the interaction of pollinator taxonomic category and week-of-sample. We used the contrast comparison between interactions for different taxonomic groups (e.g. between the small/medium Hymenoptera × week-of-sample interaction and the large Hymenoptera × week-of-sample interaction) to test whether the composition of the pollinator community on a given plant species changed over the course of the experiment; we used the lowest *P*-value from the pairwise contrast comparisons as the *P*-value for changes in the composition in the pollinator community. To account for multiple comparisons among the 14 species, we applied a Holm correction. In this analysis, we treated week-of-sample as a continuous fixed effect to parallel the main analysis of the changes in pollinators, visits, and numbers of flowers visited described in the preceding paragraph.

Nectar volume and sucrose content for each species were analysed separately with linear mixed models (LMM). Week-of-sample (continuous), number of flowers or inflorescences (continuous) and time-of-day (continuous) were included as fixed effects, and individual plant (factor) and flower replicate (factor) were included as random effects. The analyses were designed to assess whether nectar volume of sucrose content increased or decreased over the experimental period the plants were in the field.

All analyses were performed in R 2.10.1 (R Development Core Team 2009), using the lmer function in the lme4 package (Bates & Maechler 2009) for GLMM and LMM analyses.

RESULTS

In total, 2472 10 min focal observations were completed for the 14 plant species, for a total of 412 h and a mean of 8.2 h per day of field observation. Each plant species was observed for 2.2 h per day on average (range 0.5–7 h). A total of 10 512 visits were observed, during which 35 763 flowers were visited by 61 morphospecies of potential pollinators. On average, the flowering of all plant arrays was advanced in the greenhouse relative to the current mean DFB of wild populations. Plants placed in the field 2 weeks before, 1 week before, on, 1 week after and two or more weeks after the current mean DFB began flowering 21, 18, 16, 11 and 6 days prior to the current mean DFB of their species, respectively. For several species, the planned design of the experiment was not realized due to failure of plants to flower; these cases tended to occur for species that flower earlier, although they were not confined to either historically advanced or unchanged species. Also, when plants were available, we extended the experiment past the fifth week in a few cases (Fig. 1). We note that the documented range in DFB spanned the weeks that the experimental plants were in the field, with the exception of the third, fourth and fifth delayed sets for *As. canadensis* (weeks 11–13), the third delayed set for *Verbena stricta* (week 15), and the fourth delayed set for *D. candida* (week 17).

For all three measures of pollination, (1) the number of pollinators that visited a focal plant, (2) the total number of visits and (3) the total number of flowers visited on a focal plant, there were strong differences among plant species in the change in pollination through time. A significant increase or decrease with time (week) was detected in the number of pollinators that visited a focal plant during a 10-min observation for 12 plant species, all except *P. divaricata* and *As. canadensis* (see Table S3). For some species, the number of pollinators was greatest when flowering was advanced, whereas for others, more pollinators visited when flowering was delayed (Fig. 2). Significant increases or decreases in the total number of visits and in the number of flowers visited were detected for 11 and 6 of the 14 plant species, respectively (see Table S3).

Categorized into groups, the historically advanced plant species were significantly more likely to show a decrease through time in the number of pollinators that visited than historically unchanged plants (Table 2; Yates $\chi^2 = 4.43$, d.f. = 1, $P = 0.035$). Furthermore, phylogenetic regressions of the changes in pollination through time against whether or not the species were historically advanced supported this result for all three measures of pollination: (1) the number of pollinators [$t_{12} = 2.47$, $P < 0.03$, $d = 0$ (indicating no phylogenetic signal in the residuals)], (2) the total number of visits ($t_{12} = 2.21$, $P < 0.05$, $d = 0$) and (3) the total number of flowers visited ($t_{12} = 2.38$, $P < 0.04$, $d = 0$). These results show that for historically advanced species, the experimental forcing of flowering even earlier increased the number of and visits by pollinators. Further analyses considering non-monotonic changes in pollinator visits through time gave a similar, although statistically stronger, conclusion (see Table S1; Figure S1).

As these analyses were performed on the aggregate pollinator community, we investigated whether the pollinator community on any of the plant species changed over the duration of the experiment. Statistically significant pairwise contrasts for the pollinator-category × week-of-sample interactions were found for only *V. stricta* ($t_{20} = 5.65$, Holm-corrected $P = 0.0002$) and *Veronicastrum virginicum* ($t_{16} = 3.43$, Holm-corrected $P = 0.045$). For both species, small/medium Hymenoptera decreased in frequency over time, whereas large Hymenoptera increased, likely due to colony growth and increasing numbers of *Bombus* workers (see Figure S2). For these two species, the change in the composition of the pollinator community may change the per capita effectiveness of pollination, possibly making our metrics suspect measures of pollination services received by the plant species. Therefore, we excluded these two species from our chi-square analysis contrasting the responses of historically advanced and unchanged species. For the remaining 12 plant species, five of the six historically advanced species were visited by more pollinators when flowering was experimentally advanced, whereas all six of the historically unchanged species were visited by more pollinators when flowering was experimentally delayed (Yates $\chi^2 = 5.49$, d.f. = 1,

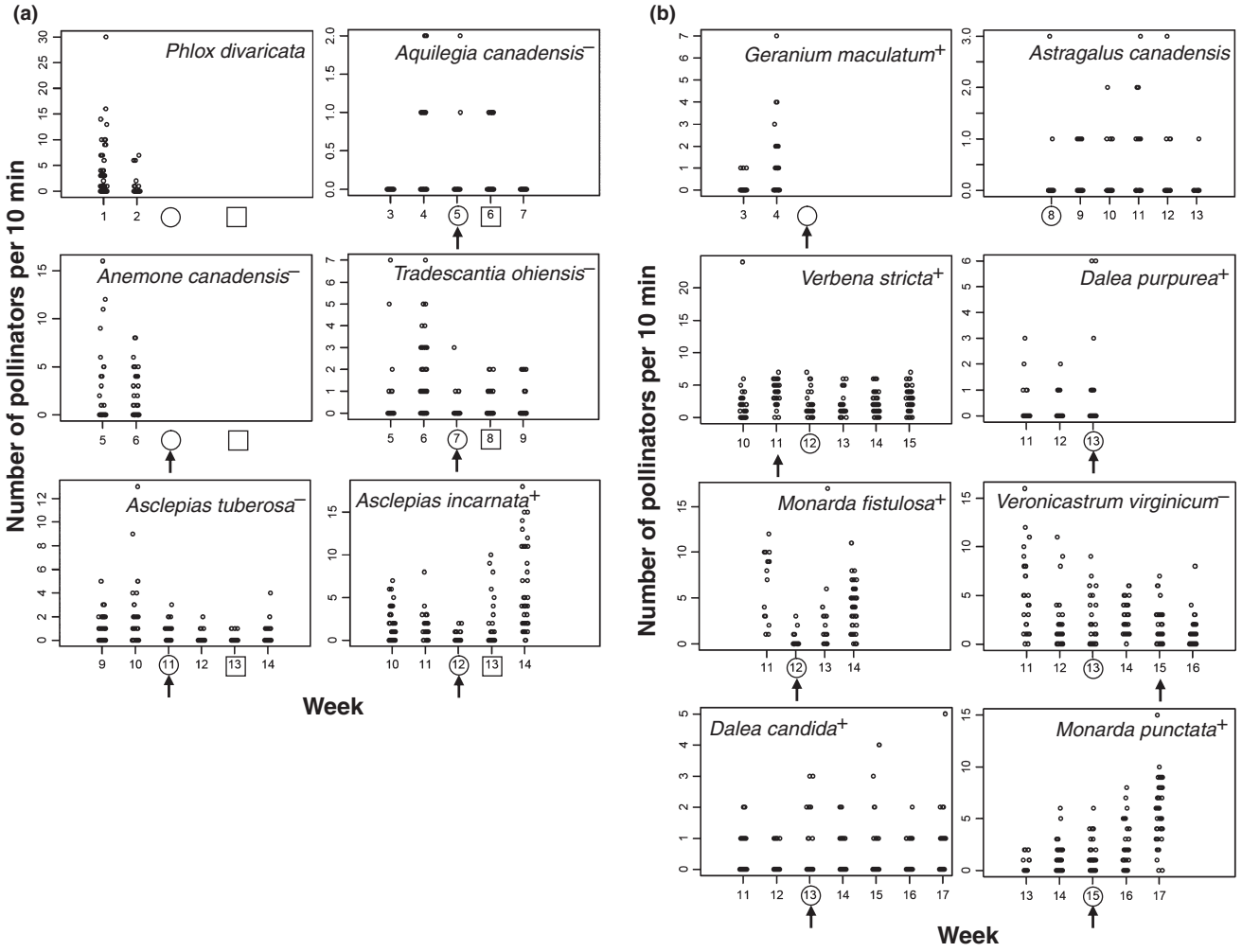

Figure 2 The number of pollinators per 10 min over the weeks that arrays of each plant species were in the field for (a) historically advanced species and (b) historically unchanged species. + and − indicate that the species-specific effect of week is significant in a positive or negative direction for that plant ($P < 0.05$). The circle indicates the current mean week of first bloom; the arrow indicates the week of first bloom in 2009 for all species for which it was documented; and the square marks the historical mean week of first bloom for the historically advanced species.

Table 2 For plant species with historically advanced vs. unchanged onset of flowering, whether the number of visiting pollinators increased or decreased through time while plants were exposed in the field

Phenology	Increase	Decrease	Total
Advanced	1 (1)	5 (5)	6 (6)
Unchanged	7 (6)	1 (0)	8 (6)

Numbers in parentheses give the results when two species (*Verbena stricta* and *Veronicastrum virginicum*) that experienced changes in their pollinator community composition over the course of the experiment are removed.

$P = 0.019$; Table 2). Removing these two plant species therefore strengthened our statistical results. Similarly, the phylogenetic regressions remained statistically significant, with no phylogenetic signal detected: (1) the number of pollinators ($t_{10} = 2.44$, $P < 0.04$, $d = 0$), (2) the total number of visits ($t_{10} = 2.24$, $P < 0.05$, $d = 0$) and (3) the total number of flowers visited ($t_{10} = 2.34$, $P < 0.05$, $d = 0$).

Nectar volume (see Table S4) and sucrose concentration (see Table S5) changed significantly for some species throughout the experiment. However, when considering all species together, there was no consistent relationship between either variable and the number of pollinators attracted. Therefore, effects of our experimental manipulation of flowering phenology on nectar volume and sucrose concentration cannot explain the temporal changes in pollinator visitation that we observed.

DISCUSSION

Our results reveal not only that shifts in flowering phenology can significantly affect pollinator visitation rates but also that the pattern of response varies among plant species. These differences are apparent even though species often were in the field at the same time, suggesting that the behaviour of pollinators, rather than weather conditions, was responsible. Despite the species-specific nature of the responses, the two groups of plant species, those with historically advanced vs. unchanged phenologies, showed strongly contrasting patterns. In most cases, species with historically advanced dates of flowering onset were visited by a greater number of pollinators when flowering was advanced, whereas species with historically unchanged dates of first bloom were visited by fewer pollinators when they were experimentally advanced. This pattern is apparent even though the 14 plant species we used display many different floral forms, ranging from highly specialized to generalized pollination mechanisms, and encompass various breeding systems, from xenogamy to delayed autonomous self-pollination. Although our phenological manipulations were not identical for each species, with some missing either the advanced or delayed treatments, the overall pattern indicates that in a broad sense, temporal mismatches between flowering onset and pollinator visitation are not occurring for most of our study plant species.

Due to the number of plants (14 species) and the number of pollinators (61 morphospecies), we did not measure pollinator effectiveness (e.g. Fishbein & Venable 1996; Ivey *et al.* 2003; Sahli & Conner 2007). Nonetheless, the effectiveness of a given pollinator can generally be inferred from visitation frequency, which is often strongly positively correlated with total pollination service (Vázquez *et al.* 2005; Sahli & Conner 2006, 2007). Furthermore, our analyses of the composition of the pollinator

community showed only two plant species for which the pollinator composition changed through time during the period plants were exposed in the field. Therefore, even though different groups of insect species might be differentially effective pollinators on different plant species, the constancy of the community through time for a given plant species implies that the response variables we measured (total number of pollinators, number of visits and number of flowers visited) remain proportional to the total pollination services received by the plant. After removing the two plant species whose pollinator communities changed, the pattern that the plant species with historically advanced flowering received higher visitation rates early in the season remained statistically significant.

Our finding that earlier flowering led to greater pollination for plants with historically advanced flowering suggests the hypothesis that historically advanced species may be unconstrained by pollinator availability; they do not suffer reduced visitation rates when warmer temperature cues initiate earlier flowering. It may be that pre-existing plant–pollinator relationships are maintained by the advance in flowering time, or new pollinators may provide a buffer. In contrast, it appears beneficial in terms of visitation frequency for the historically unchanged species to maintain their flowering phenologies to ensure temporal overlap with their pollinators. Although it is possible that rapid evolution over the last 70 years has led to differential responses of plant species to climate change depending on whether or not pollinators constrain earlier flowering, it is also possible that the patterns we observed reflect much longer term evolutionary forces. Those plant species that have earlier pollinators may have the phenotypic plasticity to shift to earlier flowering times, as suggested by spring-flowering species tending to show the strongest responses to temperature (Fitter & Fitter 2002), whereas those plant species relying on pollinators that are active later in the season may have no such plasticity.

Even though the historically advanced plants we studied are changing phenology in the right direction, it is possible that they are not shifting quickly enough to maintain overlap with their pollinators in the future. Our study was designed only to test the potential for current plant–pollinator phenological mismatches early in the flowering season, rather than investigate the underlying evolutionary explanations for whether mismatches are or are not occurring. Therefore, we can only speculate as to the underlying causes of the pattern we have documented. It is also important to note that many factors other than pollinator visitation influence first and optimal flowering time (reviewed by Elzinga *et al.* 2007). We do not equate peak visitation with any optima and recognize that more subtle mismatches may be occurring.

To predict fully the consequences of shifts in flowering phenology on plant–pollinator interactions, several additional pieces of information are needed. It would be useful to know whether advanced or delayed initiation of flowering imposes physiological constraints on fruit and seed set, or if other correlated traits might be affected. For instance, changes in flowering time can affect floral display size, reproductive rates and the growth form of the subsequent generation (Burgess *et al.* 2007; Galloway & Burgess 2009). Documenting the fitness consequences of advanced flowering over the entire flowering period is also important, as successful pollination of later flowers might make up for a lack of pollination early in the flowering period. Although flowers produced later often have lower fecundity (e.g. Stephenson 1981; Thomson 1989; Kliber & Eckert 2004), recent evidence suggests that some plants may be buffered from reproductive failure when flowering earlier in warmer years by plasticity in flowering duration and allocation of resources to successfully pollinated flowers, whether produced relatively early or late (Forrest & Thomson 2010). Furthermore, it may be important to expand our focus from one life history event, such as the initiation of flowering, to the entire life cycle of a species to integrate the effects of altered interactions at different ontogenetic stages (Yang & Rudolf 2010). Thus, documenting the fitness consequences of phenological shifts for plant species, particularly those of conservation concern, is an important future endeavour.

Despite the need for a holistic assessment of the effects of climate change, our experimental results give no indication of a growing mismatch between plant and pollinator phenologies. This is because plant species showing advanced phenologies are also those that are pollinated by insects active early in the season; a mismatch between plant and pollinator phenologies does not occur because there is a match between the response of plants to climate change and the temporal availability of their pollinators. We manipulated only the plant side of the mutualism, not the pollinator phenologies. Therefore, there remains the untested possibility that insect pollinators show differential responses to climate change, with those pollinators most likely to have advanced activity periods also matched with plant species that show advanced flowering. This type of matched phenological response of plants and pollinators may reduce the danger of climate change disrupting plant–pollinator mutualisms.

ACKNOWLEDGEMENTS

We thank K. Abbott, J. Behm, J. Boughman, T. Gilman, C. Gratton, J. Harmon, M. Helmus, R. Lindroth, K. Strier, J. Usinowicz and D. Waller for advice that improved the design of this study and B. Barton, D. Inouye and two anonymous referees for helpful comments on this manuscript. For assistance in the field and greenhouse, we are very grateful to M. Litsheim, E. Peacock, J. Rafferty, R. Rafferty, A. Smith and G. Vanderveen. We are indebted to the Aldo Leopold Foundation for allowing us to use the phenological dataset, to the UW Arboretum staff, especially B. Herrick, for facilitating our work in Curtis Prairie and to the staff of the Walnut Street Greenhouses for providing excellent plant care. This work was funded in part by the US Department of Agriculture, the Plaenert-Bascom fund and graduate research grants from the Department of Zoology, UW-Madison.

REFERENCES

Abu-Asab, M.S., Peterson, P.M., Shetler, S.G. & Orli, S.S. (2001). Earlier plant flowering in spring as a response to global warming in the Washington, DC, area. *Biodivers. Conserv.*, 10, 597–612.

Bascompte, J., Jordano, P., Melian, C.J. & Olesen, J.M. (2003). The nested assembly of plant-animal mutualistic networks. *Proc. Natl. Acad. Sci. USA*, 100, 9383–9387.

Bates, D. & Maechler, M. (2009). *Lme4: Linear Mixed-Effects Models Using S4 Classes*. R package version 0.999375-32. Available at: http://CRAN.R-project.org/package=lme4. Last accessed 31 October 2010.

Bradley, N.L., Leopold, A.C., Ross, J. & Huffaker, W. (1999). Phenological changes reflect climate change in Wisconsin. *Proc. Natl. Acad. Sci. USA*, 96, 9701–9704.

Bronstein, J.L. (1994). Our current understanding of mutualism. *Q. Rev. Biol.*, 69, 31–51.

Burgess, K.S., Etterson, J.R. & Galloway, L.F. (2007). Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity*, 99, 641–648.

Corbet, S.A. (2003). Nectar sugar content: estimating standing crop and secretion rate in the field. *Apidologie*, 34, 1–10.

Costanza, R., dArge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B. *et al.* (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387, 253–260.

Doi, H., Gordo, O. & Katano, I. (2008). Heterogeneous intra-annual climatic changes drive different phenological responses at two trophic levels. *Clim. Res.*, 36, 181–190.

Douglas, K.L. & Cruden, R.W. (1994). The reproductive biology of *Anemone canadensis* (Ranunculaceae): breeding system and facilitation of sexual selection. *Am. J. Bot.*, 81, 314–321.

Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E. & Bernasconi, G. (2007). Time after time: flowering phenology and biotic interactions. *Trends Ecol. Evol.*, 22, 432–439.

Fishbein, M. & Venable, D.L. (1996). Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology*, 77, 1061–1073.

Fitter, A.H. & Fitter, R.S.R. (2002). Rapid changes in flowering time in British plants. *Science*, 296, 1689–1691.

Forrest, J. & Thomson, J.D. (2010). Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower. *Am. J. Bot.*, 97, 38–48.

Galloway, L.F. & Burgess, K.S. (2009). Manipulation of flowering time: phenological integration and maternal effects. *Ecology*, 90, 2139–2148.

Gelman, A. & Hill, J. (2007). *Data Analysis Using Regression and Multilevel/*Hierarchical Models. Cambridge University Press, Cambridge.

Grundel, R., Pavlovic, N.B. & Sulzman, C.L. (2000). Nectar plant selection by the Karner blue butterfly (*Lycaeides melissa samuelis*) at the Indiana Dunes National Lakeshore. *Am. Midl. Nat.*, 144, 1–10.

Hegland, S.J., Nielsen, A., Lazaro, A., Bjerknes, A. & Totland, O. (2009). How does climate warming affect plant-pollinator interactions? *Ecol. Lett.*, 12, 184–195.

Ivey, C.T., Martinez, P. & Wyatt, R. (2003). Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae). *Am. J. Bot.*, 90, 214–225.

Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annu. Rev. Ecol. Syst.*, 29, 83–112.

Kliber, A. & Eckert, C.G. (2004). Sequential decline in allocation among flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology*, 89, 1675–1687.

Kudo, G., Nishikawa, Y., Kasagi, T. & Kosuge, S. (2004). Does seed production of spring ephemerals decrease when spring comes early? *Ecol. Res.*, 19, 255–259.

Lavin, S.R., Karasov, W.H., Ives, A.R., Middleton, K.M. & Garland, T. (2008). Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.*, 81, 526–550.

McCullagh, P. & Nelder, J.A. (1989). *Generalized Linear Models*, 2nd edn. Chapman and Hall, London.

Memmott, J., Waser, N.M. & Price, M.V. (2004). Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B Biol. Sci.*, 271, 2605–2611.

Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecol. Lett.*, 10, 710–717.

Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R. *et al.* (2006). European phenological response to climate change matches the warming pattern. *Global Change Biol.*, 12, 1969–1976.

Miller-Rushing, A.J., Katsuki, T., Primack, R.B., Ishii, Y., Lee, S.D. & Higuchi, H. (2007). Impact of global warming on a group of related species and their hybrids: cherry tree (Rosaceae) flowering at Mt. Takao, Japan. *Am. J. Bot.*, 94, 1470–1478.

Olesen, J.M., Bascompte, J., Elberling, H. & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, 89, 1573–1582.

Ollerton, J. (1996). Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. *J. Ecol.*, 84, 767–769.

Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37–42.

Peñuelas, J., Filella, I. & Comas, P. (2002). Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biol.*, 8, 531–544.

Petanidou, T., Kallimanis, A.S., Tzanopoulis, J., Sgardelis, S.P. & Pantis, J.D. (2008). Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecol. Lett.*, 11, 564–575.

Price, M.V., Waser, N.M., Irwin, R.E., Campbell, D.R. & Brody, A.K. (2005). Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology*, 86, 2106–2116.

Primack, D., Imbres, C., Primack, R.B., Miller-Rushing, A.J. & Del Tredici, P. (2004). Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *Am. J. Bot.*, 91, 1260–1264.

R Development Core Team (2009). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Avaialable at: http://www.R-project.org. Last accessed 31 October 2010.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. & Pounds, J.A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, 421, 57–60.

Roy, D.B. & Sparks, T.H. (2000). Phenology of British butterflies and climate change. *Global Change Biol.*, 6, 407–416.

Sahli, H.F. & Conner, J.K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oikos*, 148, 365–372.

Sahli, H.F. & Conner, J.K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *Am. J. Bot.*, 94, 203–209.

Stefanescu, C., Peñuelas, J. & Filella, I. (2003). Effects of climatic change on the phenology of butterflies in the northwest Mediterranean Basin. *Global Change Biol.*, 9, 1494–1506.

Stephenson, A.G. (1981). Flower and fruit abortion: proximate causes and ultimate functions. *Ann. Rev. Ecol. Syst.*, 12, 253–279.

Thomson, J.D. (1989). Deployment of ovules and pollen among flowers within inflorescences. *Evol. Trend. Plant.*, 3, 65–68.

Vázquez, D.P. & Aizen, M.A. (2004). Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology*, 85, 1251–1257.

Vázquez, D.P., Morris, W.F. & Jordano, P. (2005). Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol. Lett.*, 8, 1088–1094.

Visser, M.E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proc. R. Soc. Lond. B Biol. Sci.*, 272, 2561–2569.

Wall, M.A., Timmerman-Erskine, M. & Boyd, R.S. (2003). Conservation impact of climatic variability on pollination of the federally endangered plant, *Clematis socialis* (Ranunculaceae). *Southeast. Nat.*, 2, 11–24.

Waser, N.M., Chittka, L., Price, M.V., Williams, N.M. & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043–1060.

Yang, L.H. & Rudolf, V.H.W. (2010). Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecol. Lett.*, 13, 1–10.

SUPPORTING INFORMATION

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

Figure S1 Quadratic pollinator visitation curves for the 14 study plant species.
Figure S2 Frequency of visits by pollinator groups per week for *Verbena stricta* and *Veronicastrum virginicum*.
Table S1 Results of quadratic pollinator visitation analyses.
Table S2 Summary data on the number of visits by five pollinator groups to the 14 study plants.
Table S3 Results of linear pollinator visitation analyses.
Table S4 Results of nectar volume analyses.
Table S5 Results of sucrose concentration analyses.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Editor, Rebecca Irwin
Manuscript received 10 May 2010
First decision made 28 June 2010
Second decision made 5 September 2010
Manuscript accepted 12 October 2010