

Plants, pollinators and global change: the effects of invasion and flowering phenology on plant-
pollinator interactions

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Abstract

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Global environmental change is currently happening more rapidly than at any time known in Earth's previous history. The impacts of rapid biotic and abiotic change will affect multiple species interactions, including plant-pollinator interactions that are critical in terrestrial ecosystems worldwide. Invasion and climate-induced phenological shifts can have profound effects on plant-pollinator interactions that are not yet easily predicted given the current state of our knowledge. The research in this dissertation documents three ways global change will affect plant-pollinator interactions: (1) unexpectedly opposing effects of multiple shared pollinators on native and exotic plants (Chapter One); (2) surprising changes in interaction strength and direction resulting from unequal phenological shifting (Chapter Two); and (3) the emergence of new temporal patterns of floral resources in invaded communities (Chapter Three).

INTRODUCTION

Rapid environmental change in the form of biotic invasions, habitat fragmentation, pollution, climate change, and overharvesting, has reshuffled and extinguished biota on a global scale. In the process, it is not only individual species that are affected, but interactions between species—a vast network of relationships between predators and prey, seed producers and seed dispersers, pathogens and hosts, and plants and pollinators. The character and strength of these species interactions will ultimately help determine the impact of these global changes on biodiversity, community properties, and the ecosystem services that living systems provide to humans. Understanding species interactions under global change is therefore of paramount importance.

The research described in this thesis is focused on interactions between plants and pollinators. These interactions are not trivial to human welfare: famously, one of every three bites of food eaten by humans results from an animal-mediated pollination event, and between 60-90% of plants on Earth require animal pollination. Yet, the impacts of global change on plant-pollinator interactions are strong: a recent study by Burkle et. al (2013) documents the loss of >50% of bee species in a plant-pollinator network over 120 years, and the dramatic degradation of network structure and function as a result. The three chapters of this dissertation examine the effects of global change on both direct and indirect plant-pollinator interactions. Chapters One and Two examine the effects of global change on indirect interactions—that is, interactions between plants as mediated by their shared pollinators. Chapter Three looks at how altered plant communities will directly affect pollinators.

Chapter One focuses on the effects of invasion on native plants, specifically through indirect interactions with exotic plant species. Invasion creates patches of exotic-dominated vegetation interspersed with uninvaded native vegetation, producing contrasting “floral neighborhoods”. I show that native and exotic plant species interact through pollinators differently depending on whether they are found in invaded or uninvaded floral neighborhoods, and that this difference arises because their multiple shared pollinators forage differently in the different neighborhood types.

Chapter Two examines how phenology, the timing of life history events, affects indirect pollinator-mediated interactions between native and exotic plants. Flowering phenology is expected to shift in response to climate change, but unequally for different species. If flowering overlaps between native and exotic plants change, this would in turn affect the length of time during which they could interact through shared pollinators. I show that manipulating flowering phenology of exotic species leads to large, but unexpected, changes in seed produced by native species.

Chapter Three explores the effects of invasion on community-level flowering phenology, and the consequent implications for pollinator communities. I show that the composition of native and exotic floras differs with respect to traits relevant to pollinator resource provisioning (i.e., different types, amounts, and timing of floral rewards offered by plants). These differences translate to distinct patterns of community floral resource provision across the season in areas dominated by different exotic and native species, including temporal resource gaps and “resource deserts” that could negatively impact pollinators.

Neighborhood-contingent indirect interactions between native and exotic plants: multiple shared pollinators mediate reproductive success during invasions

Susan M. Waters

ABSTRACT

Native and exotic plants can influence one another's fecundity through their influence on shared pollinators. Specifically, invasion may alter abundance and composition of local floral resources, affecting pollinator visitation and ultimately causing seedset of natives in more-invaded and less-invaded floral neighborhoods to differ. Such pollinator-mediated effects of exotic plants on natives are common, but native and exotic plants often share multiple pollinators, which may differ in their responses to altered floral neighborhoods. We quantified pollinator-mediated interactions between three common forbs of western Washington prairies (native *Microseris laciniata* and *Eriophyllum lanatum* and European *Hypochaeris radicata*) in three floral neighborhoods: (i) high native and low exotic floral density, (ii) high exotic floral density and low native density, and (iii) experimentally manipulated low exotic floral density. Pollinator visitation rates varied by floral neighborhood, plant species identity, and their interaction for all three plant species. Similarly, pollinator functional groups (eusocial bees, solitary bees, and syrphid flies) contributed differing proportions of total visitation to each species depending upon neighborhood context. Consequently, in exotic neighborhoods *H. radicata* competed with native *M. laciniata*, reducing seed set, while simultaneously facilitating visitation and seed set for native *E. lanatum*. Seed set of *H. radicata* was also highest in exotic neighborhoods (with high densities of conspecifics), raising the possibility of a positive feedback between exotic abundance and success. Our results suggest that the outcome of indirect interactions between native and exotic plants depends on the density and the composition of the floral neighborhood and of the pollinator fauna, and on context-dependent pollinator foraging.

INTRODUCTION

Conservation of native plants in invaded landscapes requires an integrated understanding of how native and exotic plants interact, including their indirect interactions. Co-occurring native and exotic plants often interact indirectly through shared generalist pollinators. The foraging choices made by pollinators determine the likelihood that a native or exotic individual will receive pollinator visits, as well as the likelihood that conspecific pollen transfer will take place. Exotic plant species' effects on pollinator foraging can thus result in competitive or facilitative effects on native seed production (e.g. Caruso and Alfaro 2000, Chittka and Schürkens 2001, reviewed by Bjerknes et al. 2007, Morales and Traveset 2009), potentially influencing plant community composition in future generations. A full understanding of the impacts of exotic species on native plant communities therefore requires understanding the pollinator-mediated effects an exotic species may have on native reproductive success.

How can exotic plant species influence pollinators? Invasion by exotic plant species can alter floral neighborhoods by changing floral density and diversity (Lázaro and Totland 2010b). These neighborhood characteristics are known to influence pollinator foraging behavior (e.g., Bartomeus et al. 2008, Bosch and Waser 2001; Flanagan et al. 2010; Ghazoul 2006; Kunin 1997; Muñoz and Cavieres 1996; Yang et al. 2011). Furthermore, foraging behavior in a given floral neighborhood can be contingent on pollinator identity, as pollinator groups may behave differently in the same floral environment (Stout et al. 1998, Lázaro and Totland 2010a, 2010b). Though many studies have documented interaction between native and exotic plants through pollinator services, including the effect of floral density or diversity on pollination (e.g. Gibson et al. 2013; Lopezaiza-Mikel 2007; McKinney and Goodell 2001; Moragues and Traveset 2005), relatively few have examined how floral neighborhood influences *multiple* shared

pollinators to shape the outcome of indirect native/exotic interactions (but see Lázaro and Totland 2010b).

To help fill this gap, we investigated indirect pollinator-mediated interactions between one exotic forb and two native forbs. We examined the effect of native-dense and exotic-dense floral neighborhoods on flower visitation by three groups of pollinators, and tracked plant reproductive success. We asked three questions: (1) Does floral neighborhood affect (a) total pollinator visitation rate and (b) community composition of pollinators visiting a given plant species? (2) Does reproductive success of a given plant species depend on floral neighborhood? (3) Can the relationship between floral neighborhood and reproductive success be predicted by changes to pollinator visitation or pollinator community composition?

METHODS

Study site

We established a field experiment in Puget Trough prairies (western Washington, USA) in 2010. Formerly covering large areas from Oregon's Willamette Valley through western Washington and southwestern British Columbia, these prairies have undergone extensive fragmentation and habitat conversion, resulting in scattered remnants in small reserves (Dunwiddie et al. 2006). Puget Trough prairies are characterized by shallow, coarse soils with low nutrient levels, and are historically dominated by bunchgrasses and perennial forbs (e.g. *Festuca idahoensis*, *Camassia quamash*) (Dunwiddie et al. 2006; Ugolini and Schlichte 1973).

Species

We chose the exotic species *Hypochaeris radicata* and the native species *Microseris laciniata* and *Eriophyllum lanatum* (Asteraceae). All are perennial forbs that are abundant at our

field site. *Hypochaeris radicata* thrives even where management by mowing or burning has limited the recruitment of other invasive plants, and is often found in high densities interspersed with native forbs (S. Waters, personal observation). We expected that these species would interact via their pollinators, because (1) they share similar floral symmetry, as well as capitulum shape, size, color (to the human eye), and reward accessibility (Supplementary material Appendix 1, Fig. A1), (2) they share generalist pollinators, and (3) they overlap in flowering time (*M. laciniata* overlapped in flowering time with *H. radicata* by at least 4 weeks during spring/summer 2010, while *E. lanatum* overlapped with *H. radicata* by at least 6 weeks).

Experimental design

We established 30 5x5 m plots in an approximately 38-ha area of Glacial Heritage Preserve in Littlerock, Washington, in March 2010 in which to monitor pollinator visitation and seed set of focal species. Plots were no closer to each other than 5 m and no farther than 10 m. The area in which plots were established had been managed by mowing and burning one year previously to reduce densities of other exotic species (not included in this study). At the time of the study, the site was dominated by native forbs (but our focal exotic was still present, occasionally in high abundance). We chose 5x5 m plots as a reasonable estimate for the dimensions of a floral neighborhood that would affect reproductive success of focal plants. Tracking of individual bumble bees at the site showed that most bees moved < 3m on average for multiple flower visits before taking longer (>5m) flights (S. Waters and C. Chen, personal observation). We had previously observed eusocial bees, solitary bees, and flies visiting Asteraceae species at the site, and used bumble bee observations to scale neighborhoods because they had some of the largest foraging ranges of our pollinator species. Distances traveled by pollinators between conspecific flowers during a foraging bout are generally small; Waser (1982)

found that >85% of bee individuals visiting three nectar-providing forbs traveled less than 10 m to the next flower, regardless of body size or species. Similarly, Widén and Widén (1990) observed a mean pollen dispersal distance of 5.9 m in an herb visited by bumble bees and syrphids. Finally, Jakobsson et al. (2009) found that a substantial portion (25%) of the variation in pollinator visits and pollen deposition to two forbs was explained by the floral neighborhood within a 3m diameter circle. We used this information to scale our floral neighborhoods accordingly.

To evaluate the effect of floral neighborhood on pollinator visitation and resulting seed set, we established 3 treatments (Fig. 1), each with 10 replicate plots: (1) “Native” neighborhoods with a naturally occurring high density of focal native forbs and low density of *H. radicata*; (2) “Exotic” neighborhoods with a naturally occurring high density of *H. radicata* and low density of focal native forbs; and (3) “Clipped Exotic” neighborhoods originally with a high density of *H. radicata* and low density of natives, manipulated to produce low *H. radicata* floral density by semiweekly clipping and removal of inflorescences throughout the season. All plots contained both native species, as well as *H. radicata*, and we retained 3 *H. radicata* individuals, each with a single unclipped inflorescence, in “Clipped Exotic” plots (for experimental treatments). Both types of exotic plots were centered in areas with > 10 *H. radicata* individuals/m² (mean inflorescences/m² over season ≥ 15.5), and additionally constrained to locations where at least 3 individuals of *E. lanatum* and *M. laciniata* occurred in the center of the plot. “Native” plots were randomly interspersed with the “exotic” and “exotic clipped” plots, but centered in areas with < 4 *H. radicata* individuals/m² (mean inflorescences/m² over season < 5.6), where density of native forbs was concomitantly high.

Clipped treatments allowed us to examine the effects of reduced *H. radicata* floral density when densities of the two native flowers were also naturally low; we did not include a treatment clipping native flowers because of conservation concerns. Our treatments allowed us to compare high native/low exotic floral density, high exotic/low native floral density, and low native/low exotic floral density. By comparing native vs. clipped exotic plots, we could investigate the effect of changing native floral density only, while controlling for exotic floral density; by comparing exotic vs. clipped exotic, we could similarly investigate the effect of changing only exotic floral density while controlling for native floral density. Biweekly floral censuses of plots verified that clipping treatments and plot selection did result in the desired floral neighborhoods (Supplementary material Appendix 1, Table 1A). Finally, though floral composition was not identical among plots, the three focal species generally occupied the top three abundance ranks during our experiment. The next most abundant species, *Campanula rotundifolia*, did not share pollinators with *E. lanatum*, and reached its peak bloom after *M. laciniata* bloom was over, making it unlikely to interact with either native species through pollinators.

Pollination services

Pollinator visitation was observed in all plots for each species. We counted visitors per 10 minutes within a 1 m² square randomly selected in the plot, recording the number of inflorescences watched to estimate visitation per flower. Visits were counted only if there was contact between visitor and stigmas or anthers (Kearns and Inouye 1993). Visitors were categorized as eusocial or solitary bees, or syrphid flies (we observed no other flies and no

Lepidoptera visits). Each plot was observed a minimum of 3 and maximum of 5 times during the season, on sunny or partly sunny days, when the temperature exceeded 18° C.

To assess the importance of pollinators to plant female fecundity, we asked whether seed production depended on pollinator access to flowers. We did this by comparing seed production of three self-pollinated to three open-pollinated inflorescences for each species in each of the plots. Inflorescences were on different individual plants. The self-pollination (autogamous) treatment consisted of bagging an unopened inflorescence with a breathable nylon bag, securing it with a twist tie, and collecting seed after ample time for seed maturation. Open pollinated replicate individuals served as controls and were left unbagged. Seed was collected at the end of the season (mid July), when seed from nearby non-experimental individuals at the site had already been released and dispersed by wind. All mature seed (based on seed plumpness in *H. radicata* and on color and plumpness in *E. lanatum* and *M. laciniata*), was counted and weighed.

Statistical Analysis

We used generalized linear mixed effects models to test whether pollinator visitation rate depended on the plant species being visited (*E. lanatum* vs. *M. laciniata* vs. *H. radicata*), the neighborhood treatment in which the plant was growing (native, exotic, clipped exotic), and an interaction between the two. We repeated this analysis for rates of visitation by each pollinator functional group (eusocial bees, solitary bees and flies) as well as for total visitation rate (across all functional groups). Because the total number of flowers we watched in each plot varied, we included this as a covariate in analyses. We used a Poisson distribution (with a log-link), and designated plot as a random effect in these models, to account for non-independence of data collected from the same block and species (Crawley 2007). We explored more complicated random effect structures (e.g. species within plot random effects), as recommended by Zuur et al.

(2009), but Akaike Information Criterion (AIC) values indicated these were not necessary. For each response variable (eusocial bee, solitary bee, fly, total), we constructed five possible mixed effects models representing different combinations of explanatory variables; (1) only flowers watched (the covariate); (2) flowers watched and species identity; (3) flowers watched and neighborhood; (4) flowers watched, species and neighborhood; and (5) flowers watched, species, neighborhood and a species x neighborhood interaction. We selected the model with the lowest AIC value as the best fitting model, and used likelihood ratio tests (LRT) between this best fitting model and models missing only the explanatory variable of interest to determine significance of coefficients. We also performed post-hoc general linear hypothesis tests (using function `glht` in R, allowing for Tukey's HSD comparisons of groups in mixed effects models), to determine which treatment x species combinations were significantly different from each other.

Analysis for seed production was carried out similarly, also comparing five models: (1) a null model with only random effects; (2) neighborhood only as an explanatory variable; (3) plant species only; (4) neighborhood and species; and (5) species, neighborhood, and their interaction. Finally, we also used mixed effects models to test whether the production of seed in controls vs. pollinator exclusion treatments differed across treatment groups, again with plot as a random effect.

Generalized linear models described above allow us to determine how individual pollinator functional group visitation or total visitation is influenced by native vs. exotic floral neighborhoods, but do not allow us to determine how and whether pollinator community composition is influenced by neighborhoods (regardless of impacts on the number of visits). Therefore, we also used multinomial likelihood model fitting to determine how floral

neighborhoods and species identity influenced the proportion of eusocial bees vs. solitary bees vs. flies visiting each plant species. This could only be done for pollinator visitation data where at least one pollinator was observed, reducing our data set by approximately half. We constructed four models; (1) a null model where the proportion of pollinator functional group visits did not vary by species or neighborhood; (2) a model in which pollinator functional groups varied across species (but not neighborhoods); (3) a model in which pollinator functional groups varied across neighborhoods (but not species), and (4) a model in which the probability of observing pollinator functional groups depended both on the identity of the flower visited and on the surrounding neighborhood. We used AIC values to assess which of these four models best explained observed pollinator communities.

All analyses were performed in R 2.14.2 (R core development team 2011) using the `lmer` function in the `lme4` package (for mixed effects models), `glht` in the `multcomp` package (for post-hoc tests) and `dmvt` in the `mvtnorm` (for maximum likelihood model fitting).

RESULTS

Inflorescences in native floral neighborhoods were visited at a higher rate than in exotic neighborhoods (Fig. 2). Pollinator visitation rates were generally low, and varied by plant species, by neighborhood, and by their interaction (likelihood ratio test (LRT), $P < 0.001$, Table 1, Fig. 3). For example, *M. laciniata* received significantly more pollinator visits per inflorescence per hour than *E. lanatum* and *H. radicata* in all neighborhoods ($P < 0.001$, Tukey's HSD test for both pairwise comparisons, Fig. 3h vs. Fig 3d, l), and clipped exotic neighborhoods tended to have lower visitation per hour than both native and exotic neighborhoods, though visitation differed significantly only between clipped exotic and exotic (clipped exotic vs. exotic, $P = 0.021$; clipped exotic vs. native, $P = 0.359$; Tukey's HSD test, Fig. 2). However, neighborhood effects on pollinator visitation varied by species: visitation rates to *E. lanatum* and exotic *H. radicata* were higher in exotic neighborhoods (Fig. 3), while visitation rate to *M. laciniata* was significantly higher in native neighborhoods (Tukey's HSD test, native vs. exotic, $P < 0.001$; native vs. clipped exotic, $P = 0.015$; Fig 3a-c). Including Shannon-Wiener floral diversity in models of pollinator visitation did not improve model fit (LRT, $P = 0.746$).

Visitation rates to each plant species by individual pollinator functional groups, as well as by all pollinator groups together, were affected by plant species, by floral neighborhood, and by their interaction (Table 1). In addition, pollinator functional group composition varied by treatment: for each plant species, neighborhood had a significant effect on the community of visiting pollinators observed (LRT, see Table 1, Fig. 4). For example, flies preferentially visited *E. lanatum*, but their visitation rate depended on neighborhood. Thus, a much higher proportion of visits to *E. lanatum* in exotic neighborhoods than in other neighborhoods came from flies (Fig. 3c). Eusocial bees also visited *H. radicata* and *M. laciniata* in a neighborhood-dependent

fashion. *H. radicata* received the most frequent eusocial bee visits in exotic floral neighborhood treatments (Fig. 3h vs. 3g,i), while eusocial bee visitation to *M. laciniata* was most frequent in native neighborhoods (Fig. 3d).

Seed set per inflorescence also varied by floral neighborhood, plant species, and their interaction ($p < 0.001$ for all explanatory variables). Mean seed set for *M. laciniata* differed significantly in all neighborhoods and was higher in native than exotic and clipped exotic neighborhoods (Fig. 5, Tukey's HSD test, native vs exotic, $P < 0.001$, native vs clipped exotic, $P = 0.015$, exotic vs. clipped exotic, $P = 0.003$). Mean seed set of *E. lanatum* was highest in exotic neighborhoods, which differed significantly from clipped exotic ($P < 0.001$), but not native ($P = 0.276$) neighborhoods (Tukey's HSD test, Fig. 5). Mean seed set of *H. radicata*, by contrast, did not vary significantly among floral neighborhoods (Fig 5).

Data from pollinator exclusion treatments showed that pollinator services increased *M. laciniata* and *H. radicata* seed set regardless of neighborhood (Tukey's HSD test, selfing vs. other treatments $P < 0.001$ *H. radicata*, $P < 0.001$ *M. laciniata*). When excluding pollinators, seed production was minimal for *H. radicata* in all neighborhoods (mean = 0.30 ± 0.14 seeds per capitulum) and reduced by more than half for *M. laciniata* (mean 11.0 ± 2.3 seeds per capitulum). Pollinator exclusion treatments were not successful for *E. lanatum* because wet nylon bags broke fragile stalks, and this data was eliminated from analysis.

DISCUSSION

Overall, our study demonstrates that pollinator-mediated interactions between native and exotic plants are influenced by floral neighborhoods, affecting seed set, and consequently that neighborhood-contingent interactions are likely to be important to the conservation and restoration of native plants. Specifically, we found that pollinator visitation to co-occurring native and exotic plants varied by floral neighborhood, with exotic floral neighborhoods having both competitive and facilitative effects on pollinator visitation to natives. In addition, major pollinator functional groups shifted their visitation patterns individually by floral neighborhood. As a result, seed set of both native species was neighborhood-contingent.

We found opposite effects of the exotic floral neighborhood on visitation and seed set of the two native species (Fig. 3 d, h, I). Others have found that plants sharing pollinators can have negative, positive, or neutral indirect effects on each other's pollinator visitation (Aigner 2004; Bjerknes et al. 2007; Ghazoul 2006; Hegland and Boeke 2006; Hegland et al. 2009; Kaiser-Bunbury 2009, Lavery 1992; Moeller 2004; Morales and Traveset 2009); we observed a single exotic species exerting both positive and negative indirect effects simultaneously. Our results must be viewed with caution because our study encompasses a single pollinator community, and further studies are needed to document this phenomenon across multiple locations. However, such mixed responses by natives, if applicable across many sites and communities, could complicate restoration, as removal of exotics will therefore not necessarily benefit all native species.

The visitation and seed set patterns we observed can be explained by floral neighborhood treatments affecting pollinator groups differently, as others have also observed (Lázaro and Totland 2010b; Sieber et al. 2011; Yang et al. 2011). Syrphid flies demonstrated foraging

preferences at the neighborhood level, entering only exotic neighborhoods, where they solely visited native *E. lanatum*. In contrast, eusocial bees showed less preferential selection of foraging neighborhood, but once present, their foraging behavior differed in neighborhoods of different composition. These pollinator-specific, neighborhood-contingent behaviors resulted in opposite effects of *H. radicata* on the two natives: *H. radicata* competed with *M. laciniata* for eusocial bee pollinators, and simultaneously attracted syrphid flies to the neighborhood, where they visited only *E. lanatum*. Neighborhood-specific visitation rates by different pollinator types probably also resulted in differential pollinator contributions to seed production in different neighborhoods.

Though conspecific floral density is known to affect pollinator visitation, it is unlikely that it was the only aspect of floral neighborhood responsible for visitation to the two native species. Native plots had higher densities of *E. lanatum* and *M. laciniata* (Supplementary material Appendix 1, Table 1A), and these more attractive floral displays could attract more pollinators, particularly for *M. laciniata*. However, *E. lanatum* received significantly fewer visits in Native plots, despite higher floral densities there (Supplementary material Appendix 1, Table 1A). If this resulted from increased intraspecific competition (e.g. Wirth et al., 2011) we would have expected higher visitation in both Exotic and Clipped Exotic plots, not just the Exotic treatment, since *E. lanatum* density was low in both. Thus, we believe floral neighborhood differences in exotic, not conspecific, floral density were responsible for the patterns of pollinator visitation to *E. lanatum*. Pollinator visitation to the exotic species, *H. radicata*, was also contingent on floral neighborhood, but appeared independent of exotic (conspecific) floral density, since visitation was almost as frequent in the Clipped Exotic (low exotic/low native floral density) as in the Exotic (high exotic/low native floral density) treatments.

Neighborhood effects on seed production suggest that the net indirect effect of *H. radicata* invasions could be negative for native Puget Trough plant communities, for two reasons. First, the impacts of *H. radicata* on *M. laciniata* seed production were more strongly negative than the positive impacts of *H. radicata* on *E. lanatum* seed set. Second, the influence of exotic *H. radicata* on pollinators could result in increasingly greater per-capita seed production as its invasion proceeds, as its seed production per inflorescence was greater in exotic (already invaded) neighborhoods. Increased seed production will not necessarily lead to *H. radicata* population increase unless rates of other key processes such as seed predation, germination, seedling survival, and density-dependent flower and seed production permit (Price et al. 2008; Waser et al. 2010), but an increased seed supply nevertheless creates a potential for future population expansion. Finally, the influence of *H. radicata* invasion is not limited to the pollinator-mediated indirect interactions we have documented here, since *H. radicata*, is also known to be a strong competitor with native plant species for soil resources (S. E. Fisher, unpublished data). This could result in a positive feedback that increases invasion rates over time.

Our data paint a picture of a network in which plant-pollinator interaction strengths could be highly spatially variable and mediated by neighborhood-contingent, individualistic pollinator behaviors. One implication is that shifts in abundance of pollinator types could result in flipping of the native/exotic plant indirect interaction sign, changing the local effect of the exotic species on natives. The composition of pollinator fauna shared by coflowering plant species (including natives and exotics) is known to vary substantially from season to season (Alarcón et al. 2008, Petanidou et al. 2008), suggesting that variation in this interaction sign could be common; for example, a season with low syrphid abundance would switch the effect of exotic neighborhoods

on *E. lanatum* from positive to neutral (Fig. 3 b-d). More broadly, this suggests that recent pollinator declines could interact synergistically with invasion to produce complex and potentially unexpected impacts on native plant communities.

From an applied perspective, these neighborhood effects imply that restoration planners should consider whether exotic plants have both competitive and facilitative indirect effects on native plants of management concern. Indiscriminate elimination of an exotic species could lead to unintentional creation of floral neighborhoods not conducive to pollination of some native species, some of which may be rare to start with. This is an especially important consideration when insect-pollinated native plant populations are seed limited, as is often the case with rare plants. Removal of exotic floral resources could also have negative demographic effects on pollinators that could feed back to affect native plants in unexpected ways. Conservation practitioners should endeavor to consider pollinator-mediated indirect interactions between native and exotic plants when creating conservation and restoration plans.

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Tables

Table 1. AIC values for alternative models explaining pollinator visitation rate^a (number of visits per inflorescence per hour by solitary bees, eusocial bees, syrphid flies or all pollinators) and pollinator communities^b (proportion of visits by solitary bees, eusocial bees or flies) as a function of plant species identity and floral neighborhood (and their interaction). AIC values of best fitting models are bolded.

Model	Pollinator visitation rate ^a (number of flowers watched as a covariate)				Pollinator communities ^b
	Solitary Bees	Eusocial Bees	Syrphid Flies	Total Visits	
1. Null (intercept only)	257.79	681.40	137.03	765.17	678.42
2. Plant Species	256.08	390.93	97.89	543.72	494.64
3. Neighborhood	261.31	684.38	135.73	768.42	636.46
4. Plant Species+Neighborhood	259.76	391.18	95.03	544.15	NA
5. Plant Species*Neighborhood	245.66	353.71	91.09	499.29	436.67

^a Pollinator visitation rate models are generalized linear models with a Poisson distribution and log link, using plot as a random effect.

^b Pollinator community models are fit using multinomial likelihood methods.

Figures

Figure 1.

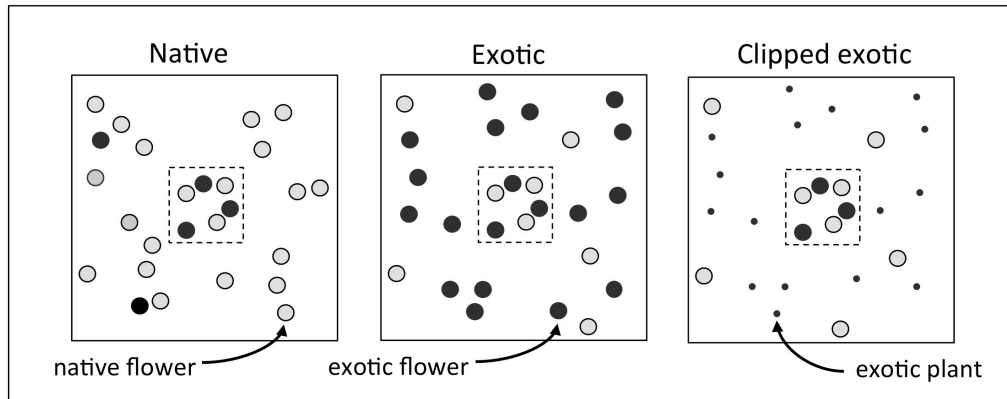


Fig. 1. Experimental design, showing focal native and exotic individuals in center of 5 x 5 m plots, in 3 different floral neighborhoods: native-dominated (high native/low exotic floral density), exotic-dominated (high exotic/low native floral density), or exotic-dominated but clipped (low exotic/low native floral density) neighborhoods.

Figure 2.

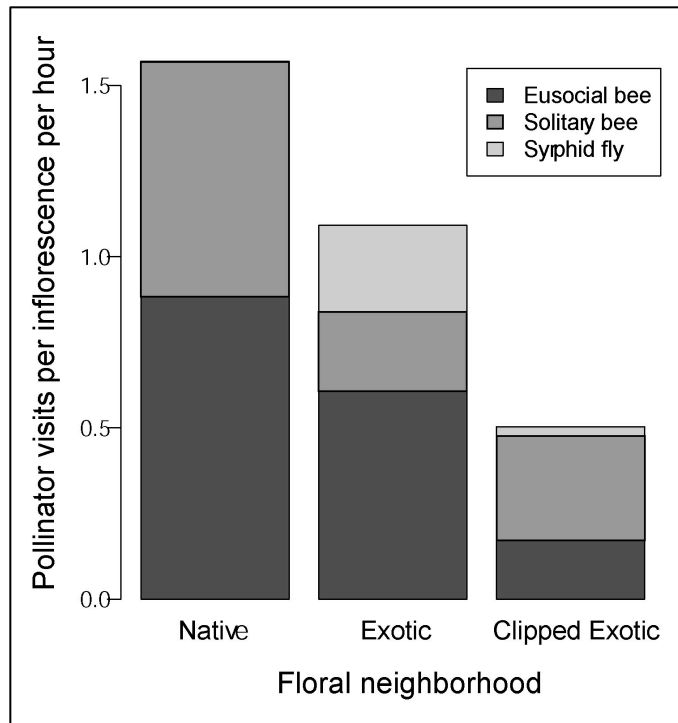


Fig. 2. Mean pollinator visitation rates per inflorescence per hour by three pollinator functional groups: eusocial bees (black), solitary bees (gray), and syrphid flies (light gray) in three floral neighborhoods (Native: high native floral density; Exotic: high exotic floral density; and Clipped Exotic: low exotic floral density produced by clipping exotic inflorescences). Visitation differed significantly between neighborhoods for most groups ($P=0.049$ eusocial bees; $P=0.933$ solitary bees; $P=0.044$ syrphid flies; $P=0.013$ all pollinators).

Figure 3.

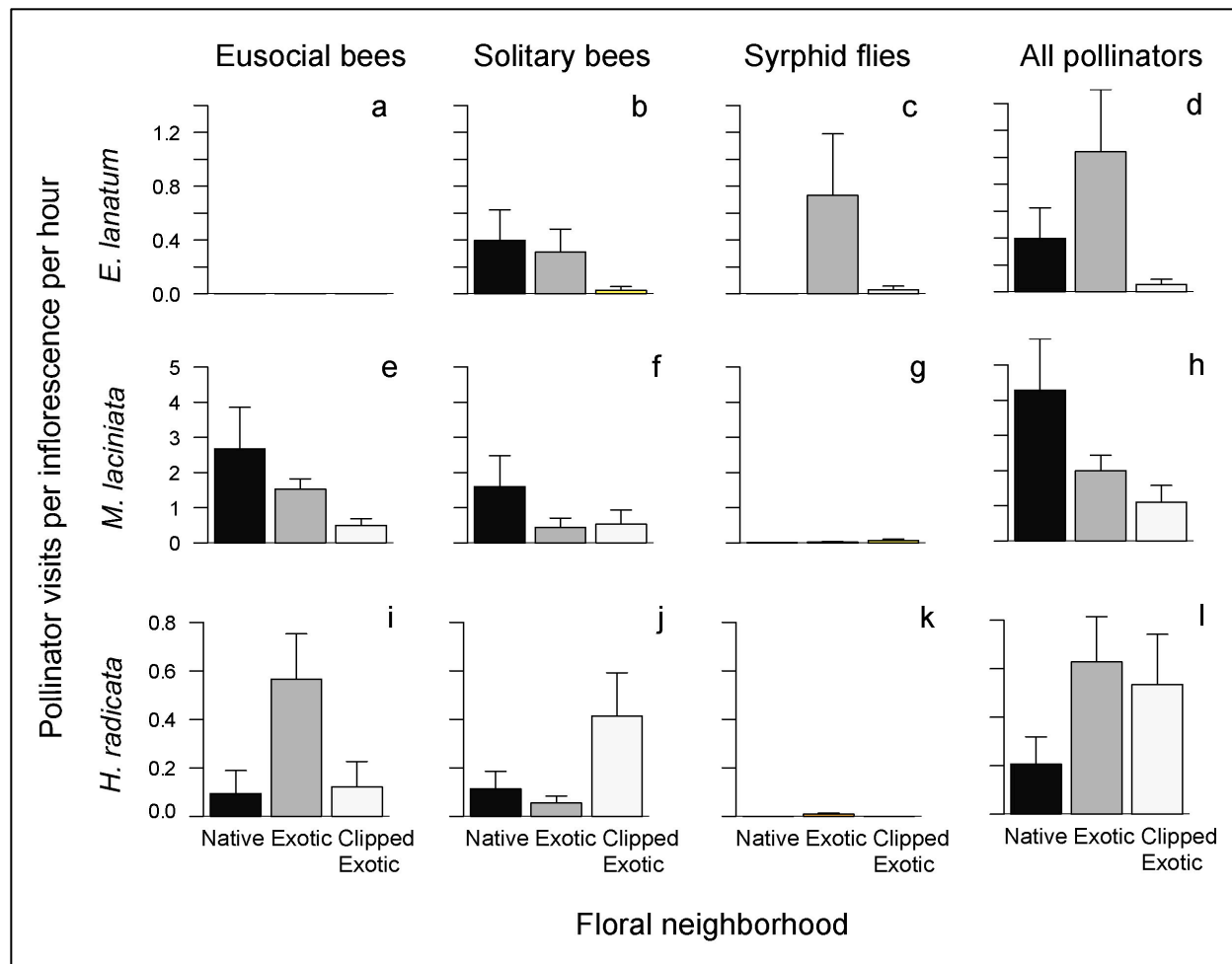


Fig. 3. Pollinator visitation rates per flower (mean \pm SE) to native prairie forbs *E. lanatum* (a-d) and *M. laciniata* (e-h) and exotic prairie forb *H. radicata* (i-l) by large eusocial bees, small solitary bees, flies, and all pollinators in the three floral neighborhoods (Black: Native, high native floral density; gray: Exotic, high exotic floral density; and light gray: Clipped Exotic, low exotic floral density produced by clipping exotic inflorescences). Total pollinator visitation differed significantly by floral neighborhood, plant species, and their interaction ($P < 0.001$ for all explanatory variables).

Figure 4.

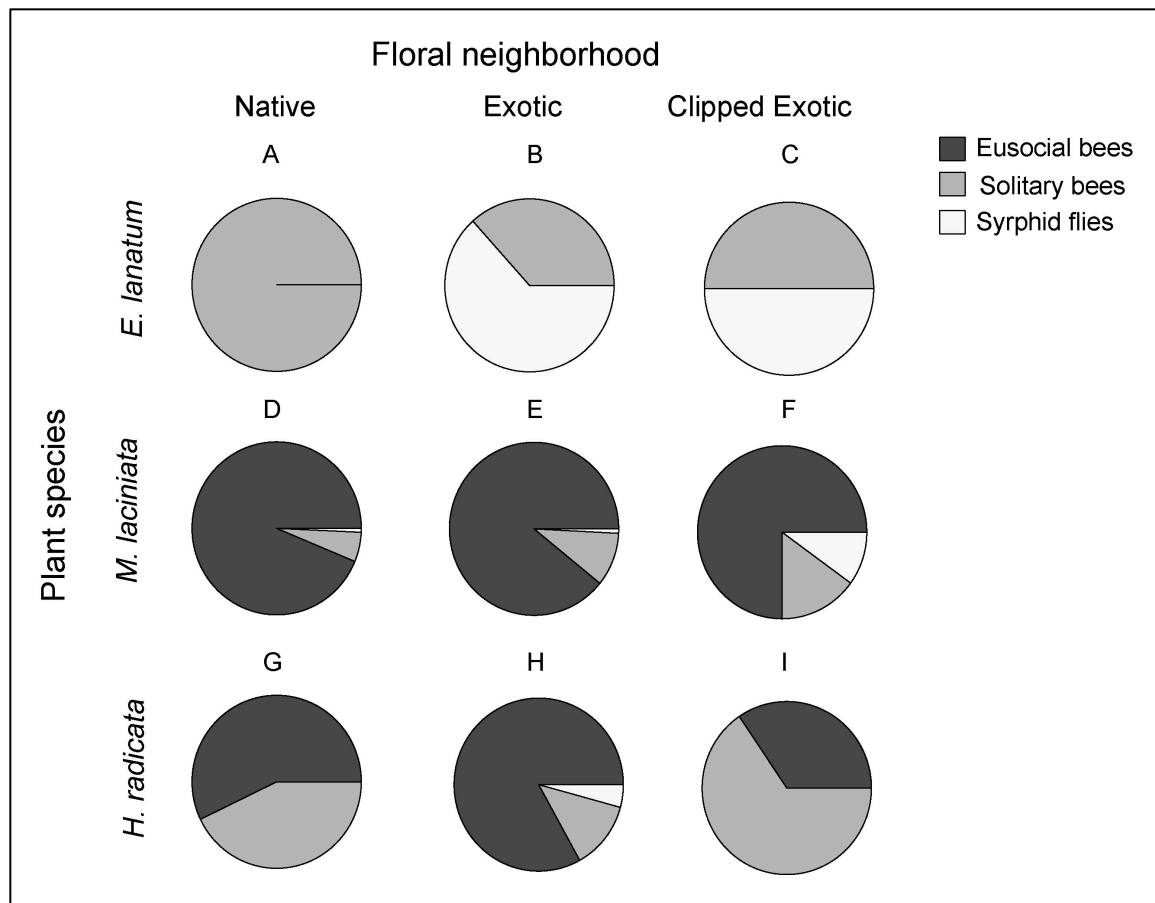


Fig. 4. Pollinator communities visiting natives *Eriophyllum lanatum* (a-c) and *Microseris laciniata* (d-f) and exotic *Hypochaeris radicata* (g-i). Pie charts represent the proportion of eusocial bees (black), solitary bees (gray) and flies (light gray) visiting each plant species in Native (a,d,g), Exotic (b,e,h) and Clipped Exotic neighborhoods (c,f,i).

Figure 5.

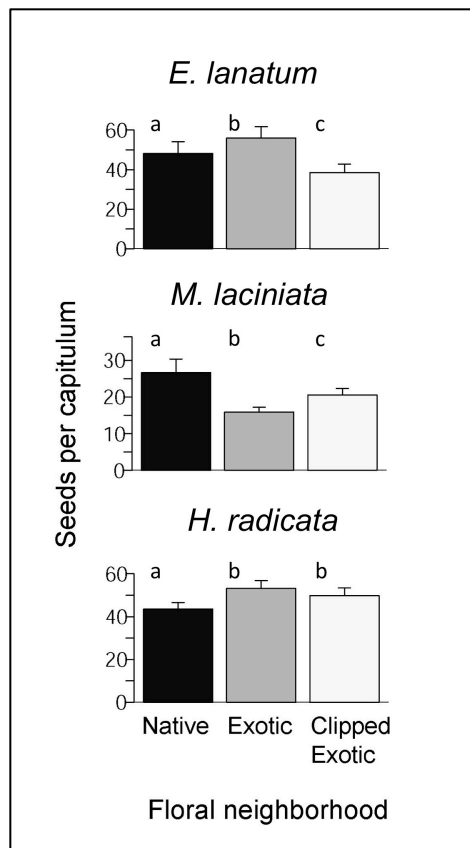


Fig. 5. Effect of floral neighborhood on seed production (mean \pm SE) for three species of prairie forbs in spring and summer 2010: (a) native *E. lanatum*; (b) native *M. laciniata*; (c) exotic *H. radicata*. Black: Native neighborhood, high native floral density; gray: Exotic neighborhood, high exotic floral density; and light gray: Clipped Exotic neighborhood, low exotic floral density produced by clipping exotic inflorescences). Seed production differed significantly by neighborhood for *M. laciniata* and *E. lanatum*, but not for *H. radicata*. Different letters indicate significant differences between neighborhoods calculated using glht/Tukey's HSD test.

Impacts of asymmetric phenological shifts on pollinator-mediated seed production

Susan M. Waters

ABSTRACT

Phenological shifts in response to climate change are ubiquitous, but are not necessarily equivalent for interacting organisms. Native and exotic plants often interact indirectly through shared pollinators, influencing each other's seed production. The strength of such pollinator-mediated interactions may depend on the duration of time such an interaction can occur, yet native and exotic plants may experience different rates of phenological shifts induced by climate change, altering that duration. To determine how individualistic changes in flowering phenologies might affect plant-plant interactions through shared pollinators, we manipulated the flowering phenology of two ubiquitous exotic plants of western Washington prairies, *Hypochaeris radicata* and *Cytisus scoparius*, relative to seven native perennial forb species whose phenologies remained unmanipulated. Specifically, we advanced and delayed the flowering of exotic species, and quantified impacts on pollinator visitation and seed set by all native species relative to an unmanipulated control. Native seed set was strongly affected by changes in exotic flowering phenology in all cases, but the magnitude and direction of effects were not predicted by the nature of the original indirect interaction (facilitative vs. neutral vs. competitive) and the change in interaction duration. We speculate that changes in pollinator behavior in response to changed floral neighborhoods are responsible for the unexpected effects. In addition to other types of ecological surprises that arise from climate change, individualistic phenological shifts have the potential to produce large and unexpected changes in indirect interaction outcomes.

INTRODUCTION

Many organisms worldwide have shown shifts in phenology over the past few decades, presumably in response to climate change (Cleland et. al 2007; Thackeray et. al 2010). While most species appear to be advancing phenology in response to warmer or earlier springs (e.g. Ellwood et. al 2013, Kauserud et. al 2010), phenological shifts among sympatric organisms are not always happening in tandem (Fitter and Fitter 2002, Post and Forchhammer 2008). Instead, long-term data sets show that the degree and direction of phenological shifts varies across trophic levels (Thackeray et. al 2010), as well as within a given trophic level in plant communities (Fitter & Fitter 2002, Willis et. al 2010). Echoing studies that draw on historical data, experiments that manipulate global change factors such as warming, CO₂, and moisture have found wide variation in the magnitude and direction of phenological responses by plants (Cleland et. al 2006, Sherry et. al 2007).

Unequal rates of phenological change in response to climate can bring about changes in how interacting organisms are aligned in time, potentially altering the strength of that interaction. Increasing phenological “mismatch” attributed to climate change has been documented in many consumer-resource systems, including caribou-arctic plants (Post & Forchhammer 2008), zooplankton-algae (Domis et. al 2005), flycatcher-caterpillar (Both 2006, Both 2009), and seabird-fish/copepod (Hipfner 2008) systems. Synchrony between direct interactors can be surprisingly important: relative phenologies of interactors can influence the likelihood of size-dependent escape from gape-limited predators (Borcherding et. al 2010), affect rates of pathogen infection (Desprez-Loustau et. al 2010) or population growth (Ozgul et. al 2010), or increase reproductive failure, resulting in population decline (Post & Forchhammer 2008). In all these cases of direct interaction, a phenological shift by one interactor alters the time envelope

available for interaction, either by removing all phenological overlap (and thus all possible interaction), or by altering the duration or seasonal timing of the overlap.

Less widely appreciated is the possibility that individualistic phenological shifts may also affect indirect interactions. Three-way (indirect) interactions such as apparent competition (Holt and Lawton 1994) and apparent facilitation may also be subject to impacts from phenological shifts by a single interactor, since a shift by just one of the three can alter the interaction time envelope (Fig. 1). Among plants, indirect interactions mediated by shared pollinators can strongly affect plant reproductive success (Traveset 2006, Kandori 2009, Waters et. al 2013). Such interactions are of special interest in invaded landscapes: sympatric native and exotic plants often share pollinators, and the foraging choices made by shared pollinators may lead to indirect competitive or facilitative effects on one or both plant species (Morales & Traveset 2009). Changes in the duration of indirect pollinator-mediated interactions could thus have strong effects on native and exotic plant fecundity, and thus on plant community composition during invasion.

How likely are disruptions of indirect interaction time envelopes? Since three, rather than two, organisms are involved, phenologically disrupted indirect interactions may be even more likely than phenological “mismatch” of direct interactions. In the case of native/exotic indirect interactions in plants, preliminary evidence is accumulating that invasive exotic plants may shift flowering phenology in response to climate more readily than native plants, thereby producing changes in time envelopes for native and exotic plants to interact through shared pollinators. For example, analysis of one long-term New England data set showed that invasive exotic plant species’ historical flowering times had advanced more rapidly than native and non-invasive exotic species (Willis et. al 2010). Exotic plant species can also show higher levels of plasticity

in phenological traits across multiple invaded environments (Monty 2013), suggesting a concomitant capacity to respond plastically to changing climate at a single site. In addition, exotic plant species expanding into a new range have often demonstrated a pre-existing capacity for local adaptation in phenological traits by developing clines in flowering time across latitudinal gradients, though whether this contrasts generally with native plants is unknown (Weber and Schmid 1998, Dlugosch and Parker 2008, Montague 2008). All of this implies that native and exotic plants are reasonable candidates for indirect interactors that may differ in the magnitude of climate change-induced phenological shifts and therefore experience future changes in interaction time envelopes. Of course, the mediators of the interactions, pollinators, may also shift phenology over time. Burkle (2013) found that a large number of interactions lost from an Illinois plant-pollinator network over time (48 of 224) were accounted for by temporal mismatch, while others have found that reductions in phenological breadth were important predictors of bee decline (Bartomeus et. al 2013, Williams et. al 2009). Phenological change in pollinator activity would also alter native/exotic plant indirect interaction time envelopes.

To explore how phenological shifting affects indirect interactions, we manipulated the phenology of one interactor, the exotic plant species, and examined the effect on pollinator-mediated interactions with multiple native plants. We asked: if flowering phenology of an exotic forb species is shifted relative to native forbs, how does this affect: (a) rate of pollinator visitation to a native plant species; (b) community composition of pollinators visiting a native plant species; and (c) seed set by a native plant species? We hypothesized that when a native/exotic plant interaction is competitive, increasing native/exotic overlap (either through advancing or delaying the exotic phenology) should lead to lower pollinator visitation, a less diverse visiting pollinator community, and lower seed set for the natives. Conversely, when the

current native/exotic plant interaction is facilitative, increasing native/exotic overlap should lead to higher pollinator visitation, a more diverse pollinator community visiting, and higher seed set for natives.

EXPERIMENTAL METHODS

Study site

We explored the effect of flowering phenology on pollinator-mediated indirect interactions in a Puget Trough prairie (western Washington, USA) in 2011. Puget Trough prairies are ideal sites for examining plant-plant pollination interactions, as native and exotic forbs co-occur, overlap in flowering phenology, and share pollinators. In addition, a relatively low diversity of pollinating insects means that changes in the foraging frequency of any one generalist taxon could produce sizeable effects on the overall number of pollinator visits to a plant. Finally, most exotic species in this system flower later than the early spring native species, meaning that advancing exotic phenology to occur earlier in the year would likely produce new phenological overlaps between native and exotic flowers and result in novel choices for pollinators.

Species

We focused on two widespread exotic prairie species, *Cytisus scoparius* (Fabaceae) and *Hypochaeris radicata* (Asteraceae). *C. scoparius* is a European shrub that can form dense monospecific stands. It flowers for approximately 3-4 weeks beginning in May, producing hundreds of bright yellow flowers. Flowers can be handled only by heavy-bodied bees (e.g. *Bombus* spp, *Apis mellifera*) and provide pollen but no nectar reward (Parker 1997). *H. radicata* is a dandelion-like forb that begins to bloom in early June, continuing for approximately 5

weeks. Its flowers provide nectar and pollen rewards and are visited by an assortment of solitary bees, as well as bumble bees.

We examined pollinator-mediated interactions between these two exotic species and seven common native forbs whose flowering phenology spans a range from early spring to midsummer (April-August): *Ranunculus occidentalis* (Ranunculaceae), *Camassia quamash* (Liliaceae), *Microseris laciniata* (Asteraceae), *Lupinus lepidus* (Fabaceae), *Eriophyllum lanatum* (Asteraceae), *Prunella vulgaris* (Lamiaceae), and *Campanula rotundifolia* (Campanulaceae). We chose to focus on native plants with a diversity of floral colors and architectures for two reasons: first, *H. radicata* and *C. scoparius* are pollination generalists, visited by multiple pollinators that may have different criteria for visitation; and second, we have observed that some pollinating insects at this site associate with *H. radicata* even when they do not visit its inflorescences (Waters, personal observation). Six of the native forbs are known to share generalist pollinators with *H. radicata* (Waters unpublished data), while five are known to be visited by heavy-bodied bumblebees that would also be capable of pollinating *C. scoparius*.

Experimental design

We established 56 5x5 m plots in Glacial Heritage Preserve in Littlerock, Washington, in March 2011. The site had been managed by mowing and burning two years previously to reduce densities of exotic grasses and *C. scoparius*, and was dominated by native forbs. Plots were organized in a randomized block design, with seven treatments in each of eight areas at the site. Within blocks, plots were randomly located, with the constraint that they must contain at least 6 naturally occurring individuals of each of the seven native species. While other (non-focal) species were present in plots, they never occurred at high densities relative to focal species. Naturally occurring *C. scoparius* was absent from all plots, and naturally occurring *H.*

radicata was present in all plots. Plots were no closer to each other than 10 m and no farther than 16 m. Foraging neighborhoods differ for pollinator taxa (e.g. Widen and Widen 1990, Stout et. al 1998); we chose these distances to reflect distinct foraging neighborhoods likely to fall within the foraging range of most pollinators at our location (Waser 1982, Jakobsson 2009, Widen and Widen 1990).

To manipulate exotic flowering phenology, we grew the two exotic species in pots in the University of Washington greenhouse until they flowered, then placed pots of flowering exotic species in prairie plots with timing that mimicked either contemporary conspecific flowering phenology at the same prairie (“Ambient”), phenology advanced by approximately 2 weeks earlier in the season (“Advanced”), or phenology delayed by approximately 2 weeks (“Delayed”). Healthy *C. scoparius* shrubs between 1m and 1.3m tall were dug from a Glacial Heritage site about ¼ mile from the study site in early March 2011, potted in 10” pots with attached prairie soil and Sunshine #4 potting soil, and placed outside the University of Washington Botany Greenhouse (about one hour’s drive from the prairie site). One third of the potted shrubs were placed in the greenhouse to induce earlier flowering, while the remainder was left outside to experience colder spring conditions. *H. radicata* seed collected at Glacial Heritage in 2010 was grown in 6” pots of Sunshine #4 soil in the greenhouse until flowering. We imposed phenological treatments by allowing potted plants to flower and then placing them in plots only at predetermined times.

We imposed six treatments: Advanced Cytisus, Ambient Cytisus, Advanced Hypochaeris, Ambient Hypochaeris, Delayed Hypochaeris, and a control. Control plots contained only the seven native species; all others contained either a single pot of flowering *C. scoparius* or four pots of flowering *H. radicata* placed in the center square meter of the plot.

Timing of placement of “Advanced” *C. scoparius* treatments was estimated by monitoring leaf-out and formation of flower buds along transects in the nearby population from which the shrubs had been dug, in combination with 2010 phenology records at the same site (Waters, unpublished). Resulting Advanced *C. scoparius* treatments had flowering potted plants in plots 23 days before ambient peak bloom (calculated as date with greatest mean number of inflorescences per square meter; peak bloom on May 16; flowering pots placed in plots for Advanced *C. scoparius* treatments on April 23). Advanced *H. radicata* treatments, whose timing was determined by a similar combination of monitoring local populations and previous year’s records, resulted in exotic blooms that were present in plots 24 days before ambient peak bloom (peak bloom on June 17; flowering pots placed in plots for Advanced *H. radicata* treatments on May 24), well before first flowering. Timing for Ambient treatments for both species was also determined by monitoring, with pots placed in plots within several days of ambient peak bloom. We were unable to maintain blooming by *C. scoparius* plants long enough to produce a Delayed treatment, but delaying *H. radicata* bloom was successful, and pots were placed in plots 20 days after ambient peak bloom (July 7). Potted *H. radicata* plants used were later bloomers rather than longer bloomers, so that the age of exotic inflorescences was unlikely to differ strongly between treatments. Comparisons between Ambient treatments and the control allowed us to assess the current indirect interaction between the exotic and native species, while comparisons between Ambient and Advanced or Delayed treatments yielded information about the effects of exotic phenological shifts on indirect interactions.

We observed pollinator visitation to each focal native species 3 times per plot per species during the bloom season. Observations were performed for ten minutes along plot edges on sunny or partly sunny days, when the temperature exceeded 65°F. We counted visitors to all

focal native individuals within the 1x1m square containing the highest density of conspecifics for that plot, and recorded the number of inflorescences observed to estimate visitation per flower. Visits were counted only if there was contact between the visitor and flower stigmas or anthers. We collected voucher specimens for all pollinators that visited frequently enough to facilitate capture, and used specimens as a reference to identify visitors to visually distinguishable morphospecies. Since floral resource availability may have a strong effect on pollinator foraging, we also surveyed floral richness and abundance biweekly in all plots as a covariate, by counting open inflorescences in 6 randomly chosen 1x1m subplots in each 5x5m plot. Floral surveys started before focal native species bloomed and continued till after all focal native flowering was complete.

We quantified seed set of individuals of each focal native species within plots, sampling three phenological types to spread sampling through the range of phenologies in the native population: early blooming (some of the first plants in plot to bloom), peak blooming, and late blooming (>80% plants stopped blooming). For each phenological type, we tagged one bud on each of two individuals in the plot and assigned them randomly to either a self-pollination or an open pollination treatment. We excluded pollinators from the inflorescences designated as self-pollinated by bagging unopened buds in tan nylon bags. We left the other three individuals (one of each phenological type) open to pollinator visits, and placed tan nylon bags over the marked inflorescence when it had senesced. We collected seed at the end of the season (more than two weeks after surrounding unmanipulated natives of the same species had set seed) and counted mature seed.

All seven species were present in each plot, but were only monitored for pollinator visitation and seed in control plots and in the subset of plots in which their bloom overlapped temporally with the bloom of the treatment exotic species.

Analysis

We analyzed data for each native species individually, since each native was exposed to a different subset of exotic phenological treatments because of its own flowering phenology and position in the season. Four native species (*M. laciniata*, *E. lanatum*, *P. vulgaris*, and *C. rotundifolia*) overlapped with only one of the two exotics, while three species, *R. occidentalis*, *C. quamash*, and *L. lepidus*, overlapped with both. We analyzed in such a way as to control for changes in the ambient available pollinator community over the season, by comparing only the phenological subset of native individuals that bloomed in all treatments simultaneously. For example, if Advanced and Ambient *H. radicata* both overlapped with native early and peak bloomers, but only Ambient overlapped with native late bloomers, we included only native early and peak bloomers' seed set when we compared the two treatments.

We used a series of linear mixed effects models with block as a random effect and a Poisson distribution to test whether rate of pollinator visitation per flower varied in response to exotic phenology treatment (including control) and abundance of floral resources as measured by number of flowers in the plot. We included all interaction terms. For the latter variable, we serially tested the effects of conspecific floral density, heterospecific floral density, and number of flowers watched during observations. For conspecific and heterospecific floral density, floral counts were averaged for each plot-observation date combination, over 6 1x1-m subsamples. Values of these variables were based on the closest floral data collection date that corresponded with each pollinator observation date (dates of pollinator observation and floral data collection in

plots differed by a maximum of 5 days and a mean of 2 days). We used likelihood ratio tests to ascertain the best fit model via AICs. One species, *M. laciniata*, was removed from analysis because the duration of its blooming season in 2011 was so short that we lacked an adequate number of pollinator observations.

We performed PERMANOVAs to examine the effect of block, exotic phenology treatments, and number of flowers in the plot (as above) on pollinator assemblages arriving at a given native flower. We chose PERMANOVA for a multivariate test because its lack of distributional assumptions is appropriate for the Poisson-distributed nature of visitation data. We used Bray-Curtis distance measures, because this measure accounts for pollinator morphospecies richness differences between samples. We standardized the data for this test by converting visits by each visitor to a proportion of the total visits per plot (row totals), and standardizing pollinator types by pollinator morphospecies maxima (column maxes). Because many plots received zero visitations during observations, we added a dummy species with a value of 1 to each plot to ensure a non-zero denominator. We created models with factors in all possible orders to determine whether sequential sums of squares influenced the significance level of any of our explanatory variables. As with the univariate tests on pollinator visitation, *M. laciniata* data was removed from the analyses because of too few observations.

We used linear mixed effects models with block as a random effect and a Poisson distribution to test whether seed set in each native species varied in response to exotic presence (before phenology manipulation), as well as in response to exotic phenology treatment and native phenology stage. In both cases we performed model selection using likelihood ratio tests and compared AIC values to choose the best fit model. In this case, model selection to ascertain the effect of phenology treatment was complicated by the fact that some native-exotic treatment

combinations were relevant for some native phenological stages, but not others. For this subset of native-exotic combinations we thus performed two sets of analyses, the set of exotic phenology treatment/native phenology stage combinations that included the maximum number of exotic phenology treatments, and the set that included the maximum number of phenology stages, and compared the results of both sets of analyses.

RESULTS

C. scoparius and *H. radicata* both interacted indirectly with a subset of the focal native forbs before phenological manipulation, with both facilitative and competitive effects. The effect of *C. scoparius* flowers on co-flowering native forbs was mostly facilitative or neutral (Fig. 3), with seed set of *M. laciniata* and *L. lepidus* increased in Ambient *C. scoparius* plots over control plots (+28.1%, $p < 0.001$, +140.0%, $p < 0.001$, LRT). The effect of *H. radicata* on native seed set was varied, with significant reductions in seed set by *M. laciniata* and *E. lanatum* in Ambient *H. radicata* plots (-15.7%, $p < 0.001$, -26.2%, $p = 0.008$, LRT) and a small but significant increase in *C. rotundifolia* seed set (+4.7%, $p < 0.001$, LRT; Fig. 4). We did not detect any indirect effects of Ambient *H. radicata* on *L. lepidus* or *P. vulgaris* (Fig. 4).

Altering *C. scoparius* and *H. radicata* flowering phenology led to five kinds of effects on native seed set (Figs. 1 and 2, Table 1): (1) novel indirect effects on native species that currently flower synchronously with one of the exotic species, but did not show any positive or negative interaction until exotic phenology was shifted; (2) novel indirect effects produced in native species that flowered synchronously with an exotic species for the first time when exotic phenology was shifted; (3) reversals of indirect effects (i.e. competitive to facilitative) due to shifted phenology; (4) increases in indirect effect size; and (5) no effect.

Advancing *C. scoparius* flowering produced novel exotic indirect effects in two native species, *R. occidentalis* and *C. quamash*. Both species are early spring bloomers that typically overlap with *C. scoparius* bloom late in their flowering seasons. Shifting *C. scoparius* earlier increased flowering overlap with both, causing significant reductions in seed set (*R. occidentalis* -37.5%, $p=0.005$, glht; *C. quamash* -14.3%, $p=0.042$, Tukey's HSD test), in comparison with Ambient *C. scoparius* plots, in which the effects of *C. scoparius* on seed set were insignificant (*R. occidentalis* -0.4%, $p=0.982$, glht; *C. quamash* +1.2%, $p=0.232$, glht). Advancing *C. scoparius* also removed flowering overlap with three native forbs; seed set of two of them (*M. laciniata* and *L. lepidus*) were significantly affected by *C. scoparius* in the Ambient Phenology treatment.

Advancing *H. radicata* created a novel temporal overlap between *H. radicata* and two native forbs, *R. occidentalis* and *C. quamash*, and this novel overlap resulted in novel indirect effects. Seed set was reduced for both species in comparison with control plots, though only *C. quamash* was significant ($\sim -44.4\%$, $p<0.001$, LRT). Advancing *H. radicata* also reversed or removed the effect of *H. radicata* on three native forbs, *M. laciniata*, *L. lepidus*, and *E. lanatum*, all of which significantly increased seed set over the Ambient *H. radicata* treatment (*M. laciniata*, +13.3%, $p<0.001$; *L. lepidus*, $\sim +67.8\%$, $p<0.001$; *E. lanatum*, +26.6%, $p=0.006$). Finally, delaying *H. radicata* flowering phenology intensified indirect effects on two additional native forbs, increasing seed set of *P. vulgaris* (+43.5%, $p<0.001$, glht) and decreasing seed set of *C. rotundifolia* (-53.6%, $p<0.001$, glht).

In many cases, observed patterns in pollinator visitation rate did not appear to be strongly related to native seed production. For example, we observed no significant changes in pollinator visitation rate to *R. occidentalis* or *C. quamash* between Ambient and Advanced *C. scoparius*

treatments, yet both species' seed set declined in Advanced plots (Fig. 3, 5). Similarly, Advanced *Hypochaeris* treatments dramatically decreased the number of visits we observed to *L. lepidus* (Fig. 6), but *L. lepidus* seed set increased significantly in the same treatments (Fig. 4). For three species, *E. lanatum*, *P. vulgaris*, and *C. rotundifolia*, treatment differences in visitation rate changes and treatment differences in seed production occurred generally in the same direction (Figs. 4, 6).

Over the whole season, the pollinator assemblages foraging at native forbs varied little in response to exotic phenology (Table 2, PERMANOVA). Pollinator assemblages arriving at native forbs differed between control plots and Ambient exotic plots for *C. scoparius*, but not for *H. radicata* (Table 2). Advancing *C. scoparius* flowering phenology did not significantly alter the pollinator community arriving at any of the co-flowering native forbs (Supplementary Table 1). Shifting *H. radicata* phenology resulted in only one significant effect, altering the composition of the pollinator community visiting *L. lepidus* in the Advanced *H. radicata* treatment.

Contrary to expectation, proportional change in native/exotic overlap did not appear strongly related to proportional change in native seed set (Fig. 7). Specifically, native species that experienced competitive indirect effects of either exotic did not consistently benefit (in terms of seed production) from a decreased phenological overlap; nor did increasing phenological overlap consistently decrease seed set (Fig. 7). Similarly, increased overlap did not increase seed set for native species whose seed set was facilitated by the exotic species, and decreased overlap did not always decrease seed set.

DISCUSSION

Altering exotic flowering phenology led to a wide range of indirect effects on native plant species (Figs. 3 and 4, Table 1). Specifically, seed set of all seven focal native forb species was changed significantly when exotic phenology was shifted. This was the case even for native species that had no pre-existing indirect positive or negative interaction with the exotic species. However, contrary to our hypotheses, prior knowledge of the nature of the pollinator mediated indirect interaction (competitive vs. facilitative) and the change in the overlap of native/exotic phenology were together not sufficient to predict the magnitude or even the sign of change in native seed set (Fig.7). Instead, shifted exotic flowering phenology led to ecological surprises with respect to native response.

What caused native seed set to respond in such diverse and unpredictable ways to exotic phenology shifts? In theory, changing the timing or duration of native/exotic flowering overlaps has the potential to alter both the ambient community of pollinators available as intermediaries between plant species and the behavior of those pollinators. Pollinator communities vary in composition and abundance seasonally, as species emerge and forage according to different schedules; as a result, shifting the native/exotic indirect interaction time envelope might cause a different community of pollinators to mediate the interactions among plants. However, we eliminated this factor in our experiment by comparing seed set of focal native individuals blooming simultaneously but experiencing altered exotic phenology, thereby controlling for differences in the ambient pollinator community. Therefore, we believe that the differences in native seed set we observed probably result from changes in pollinator foraging behavior in response to exotic phenology treatments.

One factor that could have altered pollinator foraging behavior when exotic flowering phenology was advanced or delayed is the creation of novel floral neighborhoods. The addition

of exotic flowers to plots at novel times altered floral composition, floral diversity, and floral density. All of these parameters are known to affect pollinator foraging behavior, altering pollinator foraging decisions and therefore changing the probability that a pollinator arriving at a native flower is bearing conspecific pollen. For example, Rafferty and Ives (2012) found that effectiveness of individual pollinator species (defined as probability that a visit to a flower of a given species resulted in successful pollination) changed when a plant's flowering time was artificially accelerated; the same may be true in our plots, albeit for plants experiencing the indirect effects of another species' altered blooming time.

Another way in which altered exotic phenology might have influenced pollinator behavior, and therefore seed set of natives, is by affecting pollinator learning. Changing the timing of exotic bloom alters the selection of floral resources available when individual pollinators emerge and begin to forage. Key native pollinators like bumble bees learn to 'handle' specific floral resources, and individuals often demonstrate floral constancy once they have mastered harvest from a particular floral architecture (Waser, 1986). The impact of pollinator learning and floral constancy on foraging decisions could differ if native/exotic overlap first occurred after one of the two species had been in bloom for some time, versus if pollinators first encountered both species in bloom simultaneously. For example, an early season native/exotic overlap created by an advancement in exotic phenology might provide a novel temporal choice of floral resources to a pollinator, while a later season overlap between the same two species could simply increase the length of time that shared pollinators are exposed to familiar floral choices. Timing could thus influence pollinator foraging decisions and feed back to affect native seed set, either by influencing the assemblage of pollinators arriving at a native inflorescence, or by affecting the likelihood that an arriving pollinator is carrying conspecific pollen.

The changes in visitation rates we observed to native flowers when exotic phenology was shifted were consistent with the idea that pollinator behavior influenced changes in seed set. However, these changes were neither easily predicted nor always in the same direction as changes in seed set. For example, *L. lepidus* seed set dramatically increased in Advanced *H. radicata* treatments, even though the corresponding pollinator visitation rate we observed had diminished (Figs. 5, 6). In this case, a significant difference in the pollinator assemblages visiting *L. lepidus* in the two treatments (PERMANOVA, $p=0.024$), could explain this pattern, as pollinators may differ in their efficiencies in transferring pollen, and thus a different assortment of pollinators could provide very different net benefits to plants. However, we only observed such a shift in pollinator community with *L. lepidus*. Thus, we also observed disparities between pollinator visitation rates and seed set in some species that were not explained by changes to pollinator assemblages. For example, *C. rotundifolia* was visited at a much higher rate in Delayed *H. radicata* plots than in control plots, but its seed set was significantly lower (Figs. 2, 4) even though visiting pollinator assemblages did not differ. This pattern could arise if visitors to *C. rotundifolia* in Delayed plots were more frequent, but also carried and transferred more heterospecific pollen.

Our experiment clearly demonstrates the potential for strong pollinator-mediated effects on native plants from phenological shifting by an exotic plant species, but caveats remain about applying these results to natural communities. First, our results are predicated on manipulating flowering phenology of the two most abundant exotic species in our system, while native flowering phenology remained static. Though introduced species may have more flexible phenological responses to climate change on average than native species (Willis 2010, Davidson 2011), our manipulations mimicked a relatively arbitrary set of flowering overlaps, which may

diverge widely from real future conditions. In addition, *pollinator* phenology is also subject to climate-induced changes (Bartomeus et. al 2011; Burkle et. al 2013), and this is not reflected in our experimental design. Second, our experiment shifted the onset of exotic flowering, but did not extend flowering duration. If climate change leads to extended flowering duration as well as phenological shifts (e.g. Sherry et. al 2007, Steltzer and Post 2009), species shifting earlier will likely retain their later-season indirect interactions, rather than releasing other species from late-season interactions as they did when we advanced their flowering phenology. Finally, our results for seed set of a given native in the presence of the exotic are not always applicable to the whole native population, but rather to a specific native phenological fraction that overlapped with exotic bloom, so overall population-wide responses could diverge from what we observed. Nonetheless, even with these caveats, our results demonstrate that individualistic phenological shifting by one plant species can have large indirect impacts on seed set of non-shifting species.

If future phenological shifts do lead to large changes in native seed set, they could influence plant demographics and therefore community structure. However, predicting demographic change is complicated. Increases or decreases in seed set will lead to demographic changes only if those changes are propagated to future generations, through avoidance of seed predation, successful dispersal and germination, and favorable seedling survival rates (Price et. al 2008; Waser et. al 2010). In addition, the shifting species (in this case, the exotic) might well have direct as well as indirect effects on non-shifting plant species, complicating any demographic response. For example, abiotic cues leading to accelerated flowering phenology might also accelerate other life history stages, such as seed maturation and germination (Walck et. al 2011). Earlier spring germination, leading to larger seedlings sooner, could give the shifting species a competitive advantage over non-shifters. Also, additional indirect interactions between

the shifting and non-shifting plant species could be mediated by organisms other than pollinators, such as shared herbivores, flower or seed predators, or seed dispersers, any of which could augment or counteract pollinator-mediated effects on seed set (Brody 1997).

Though future community effects remain uncertain, the large effects on seed set we documented are noteworthy, and suggest a need for further research to increase our understanding of the role of phenology in indirect interactions. For example: **(1)** How will climate change affect pollinators' phenology of emergence, foraging, and seasonal population growth? Recent studies suggest that temporal mismatches between pollinators and their food sources have already had strong negative effects on pollinator communities (Burkle et. al 2013), yet some studies suggest that plants' and pollinators' phenologies remain in synchrony (e.g. Forrest and Thomson 2011) or are overall shifting synchronously (Bartomeus et. al 2011). Detailed information about climate and pollinator phenology is lacking. **(2)** How rare are individualistic shifts in plant phenology, in which one or a few members of a plant community advance or delay phenology while others remain relatively static? Early data sets from Fitter and Fitter (2002) and Willis (2010) suggest that plant species do not all shift in tandem, but what traits influence the likelihood of a plant shifting vs. not shifting flowering phenology? **(3)** How historically common is year-to-year "flipping" of phenological precedence between two plants? How much does this affect pollinators' floral constancy and plants' seed set? **(4)** How might pollinators change their foraging behavior in response to new seasonal timing of exotic floral displays? We propose that indirect interactions will be profoundly affected by changing the phenological timing of interactors, and that answering these questions will improve our capacity to anticipate and understand some of the important biological impacts of climate

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Table 1. Types of effects of shifting exotic species flowering phenology on native species seed set. Bold values are significant to $P < 0.05$.

Effect of shifting exotic phenology	Native species	Exotic species	Exotic phenology shift	Change in native/exotic overlap when exotic phenology is shifted	Predicted effect on native seed set	Percent change in native seed set, shifted exotic vs. regular exotic phenology treatments	P	Percent change in native seed set, shifted exotic control (no exotic treatment)
Novel indirect effect: native and exotic species currently bloom synchronously, but did not show any interaction until exotic phenology was shifted	<i>R. occidentalis</i>	<i>C. scoparius</i>	Adv	increase	neutral	-37.5	0.005	
	<i>C. quamash</i>	<i>C. scoparius</i>	Adv	increase	neutral	-14.3	0.042	
Novel indirect effect: native and exotic species bloom synchronously for the first time	<i>R. occidentalis</i>	<i>H. radicata</i>	Adv	increase	unknown	NA*	NA*	
	<i>C. quamash</i>	<i>H. radicata</i>	Adv	increase	unknown	NA*	NA*	
Reversal or removal of indirect effect	<i>L. lepidus</i>	<i>C. scoparius</i>	Adv	decrease	negative	-138.6	<0.001	
	<i>M. laciniata</i>	<i>H. radicata</i>	Adv	increase	negative	13.3	<0.001	
	<i>L. lepidus</i>	<i>H. radicata</i>	Adv	increase	neutral	67.8	<0.001	
	<i>E. lanatum</i>	<i>H. radicata</i>	Adv	decrease	positive	26.6	0.006	

Increase in indirect effect size	<i>P. vulgaris</i>	<i>H. radicata</i>	Del	decrease	neutral	43.5	<0.001	
	<i>C. rotundifolia</i>	<i>H. radicata</i>	Del	decrease	neutral	-53.6	<0.001	.
No effect	<i>P. vulgaris</i>	<i>H. radicata</i>	Adv	decrease	negative	7.2	0.126	

*NA: These native forbs did not flower simultaneously with exotic inflorescences until experimental manipulation of exotic flowering phenology.

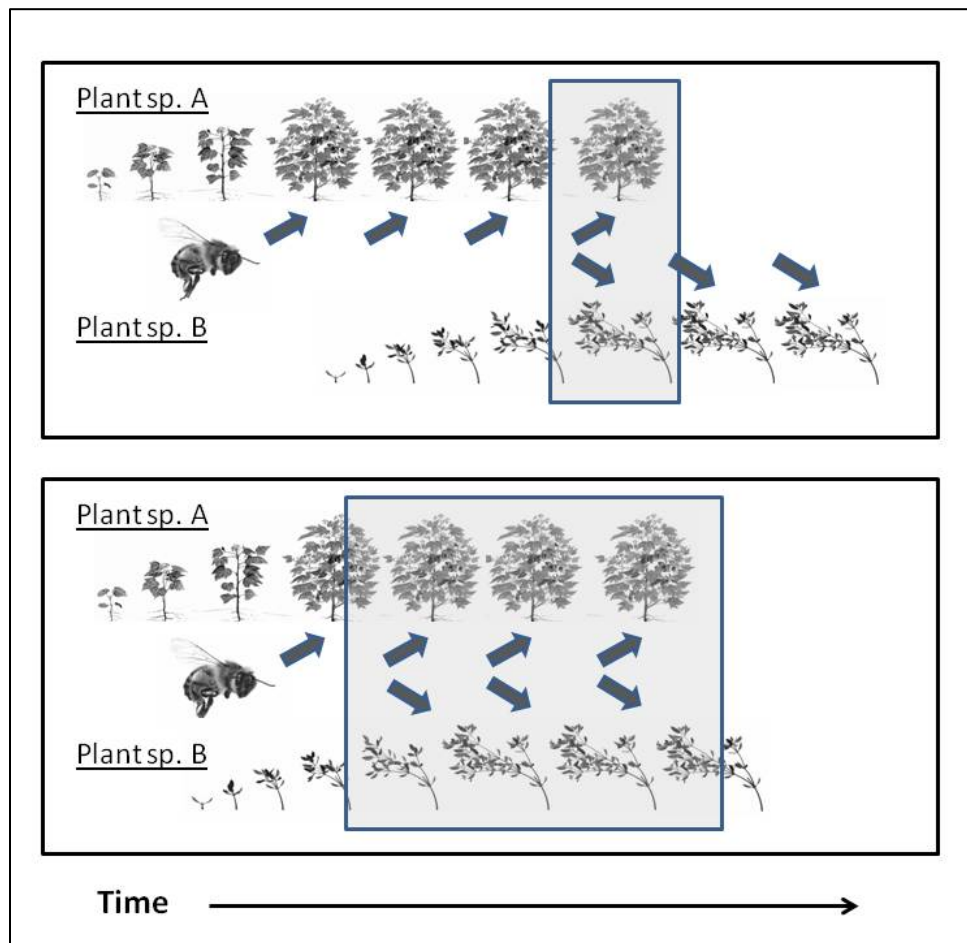


Fig. 1. **Alteration of indirect interaction time envelope by phenological shifting of one interactor.** Top panel shows early-blooming plant species A and later blooming plant species B, which share a pollinator; arrows show pollinator foraging. Because of short duration of the blooming overlap between A and B, the time for pollinator-mediated interactions between the two plants (competitive or facilitative) is short (shaded area). Bottom panel shows plant species A and pollinator maintaining static phenology, while plant species B advances flowering phenology. The resulting indirect interaction time envelope (shaded area) is longer.

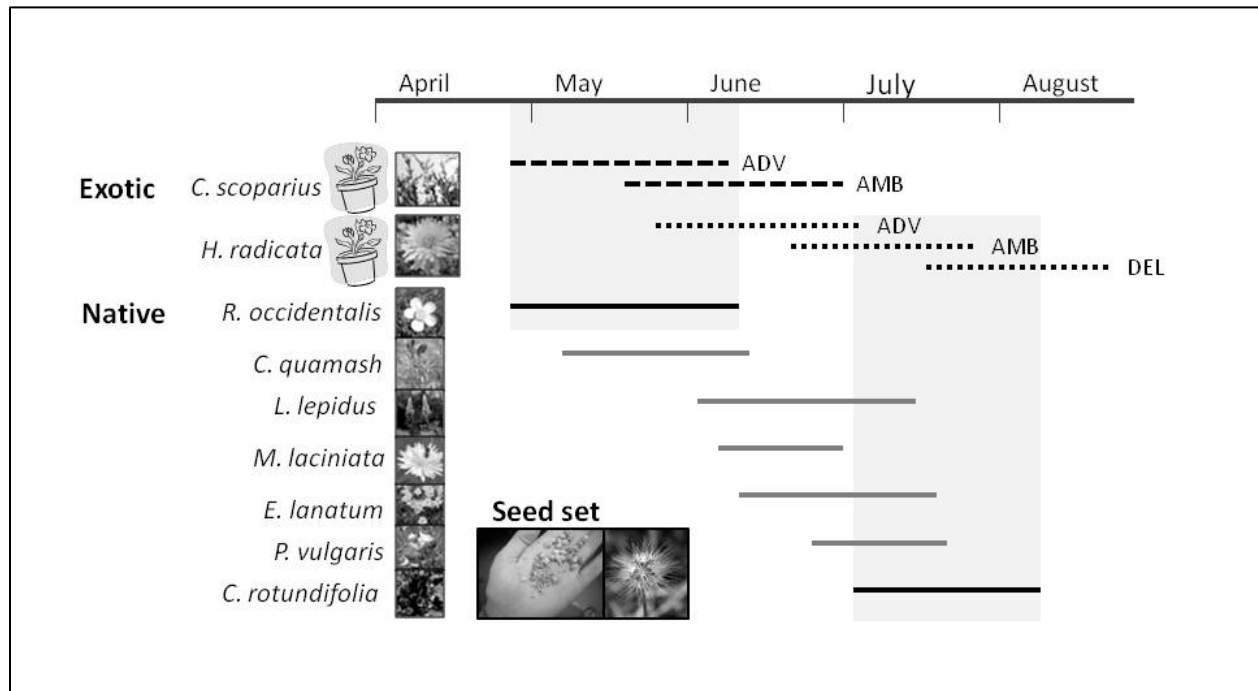


Fig. 2. Flowering phenologies of experimental native and exotic plants. Each solid line indicates approximate timing and duration of flowering for seven native forbs; dashed and dotted lines show the timing and duration of experimental exotic phenology manipulations for *C. scoparius* and *H. radicata* respectively, in which potted flowering exotics were placed in plots containing native species (ADV=advanced, AMB=ambient (unchanged), DEL=delayed). Each native forb overlaps in time with a unique subset of exotic phenology treatments. Results for each native forb are calculated only for the relevant exotic phenology treatments, in addition to the native-only control treatment; gray shaded areas show examples for natives *R. occidentalis* (overlaps with ADV and AMB *C. scoparius* and ADV *H. radicata* treatments, resulting in 4 treatments counting control) and *C. rotundifolia* (overlaps with AMB and DEL *H. radicata*, resulting in 3 treatments counting control).

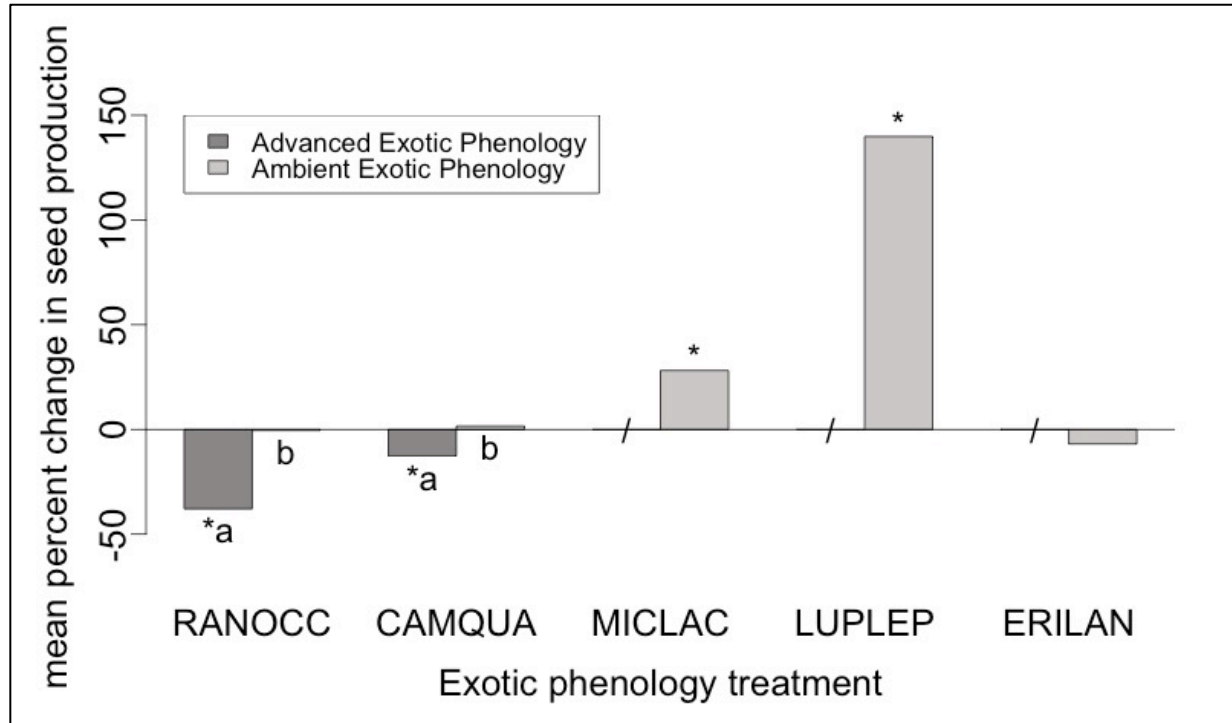


Fig. 3. Change in seed set of native plants with manipulation of *C. scoparius* phenology.

Mean percent change in seed production for five native forbs (*Ranunculus occidentalis* (RANOCC), *Camassia quamash* (CAMQUA), *Microseris laciniata* (MICLAC), *Lupinus lepidus* (LUPLEP), *Eriophyllum lanatum* (ERILAN), *Prunella vulgaris* (PRUVUL), and *Campanula rotundifolia* (CAMROT) in the presence of the exotic species *C. scoparius*, when *C. scoparius* flowering phenology is advanced vs. timed in synchrony with ambient *C. scoparius* bloom. Slash marks indicate no overlap between treatment and native species (=NA). Stars indicate a significant difference from native seed set in control (*C. scoparius* absent) plots ($P < 0.05$, LRT, glht). Different letters indicate significant differences between different exotic phenology treatments ($P < 0.05$, LRT, glht).

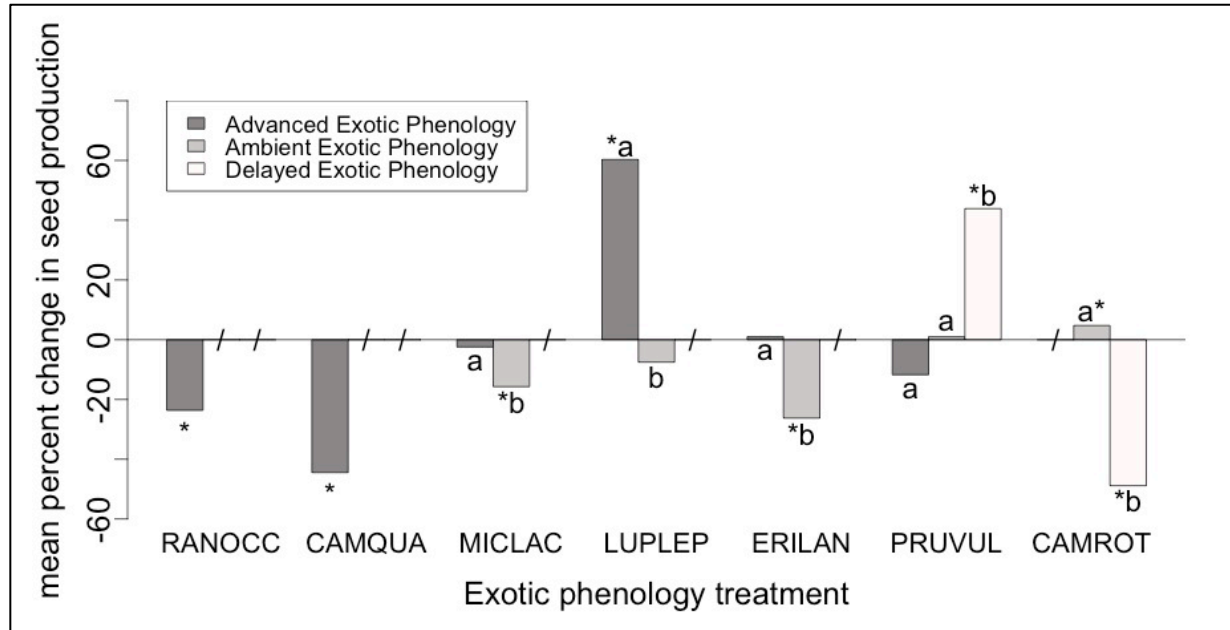


Fig. 4. Change in seed set of native plants with manipulation of *H. radicata* phenology. Mean percent change in seed production for seven native forbs (see Fig. 2) in the presence of the exotic species *H. radicata*, when *H. radicata* flowering phenology is advanced, delayed, or timed in synchrony with ambient *H. radicata* bloom. Slash marks indicate no overlap between treatment and native species (=NA). Stars indicate a significant difference from native seed set in control (*H. radicata* absent) plots ($P < 0.01$, LRT, glht). Different letters indicate significant differences between different exotic phenology treatments ($P < 0.01$, LRT, glht).

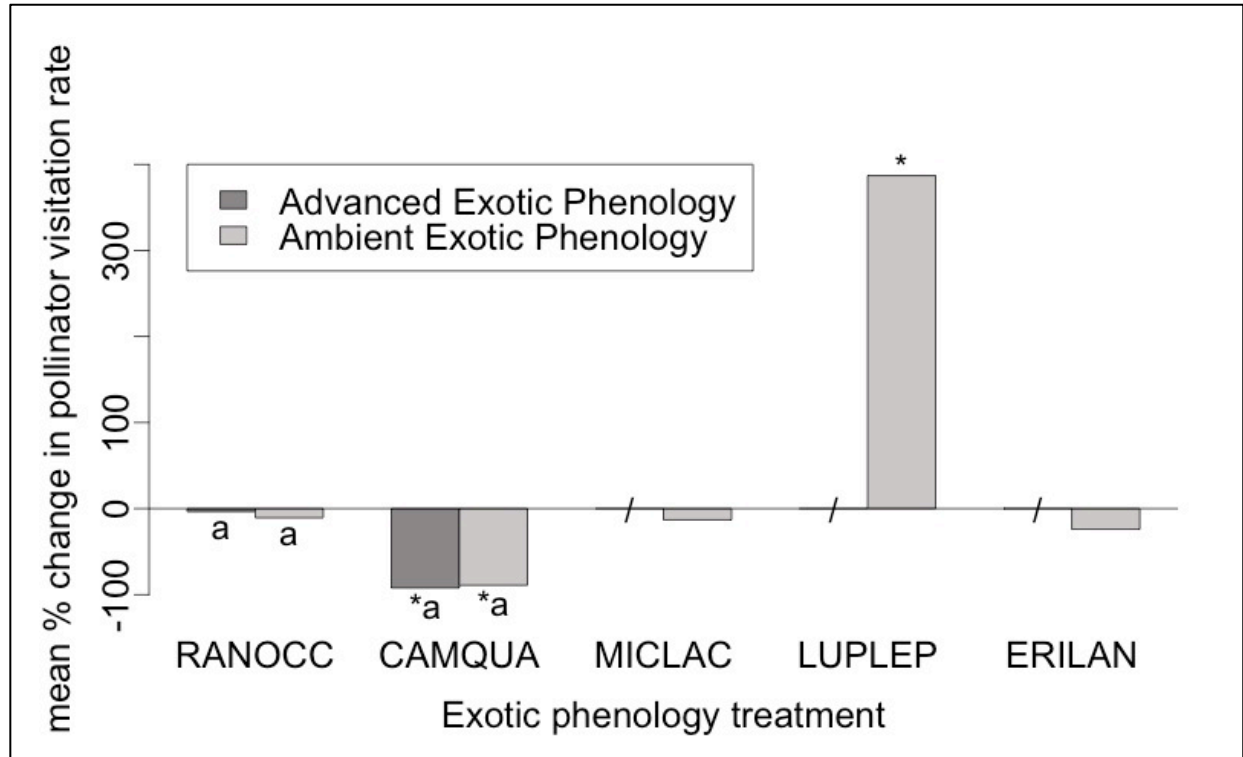


Fig. 5. **Change in pollinator visitation to native plants with change in *C. scoparius* phenology.** Mean percent change in pollinator visitation rate (in visits per inflorescence per hour) for five native forbs (see Fig. 3) in the presence of the exotic species *C. scoparius*, when *C. scoparius* flowering phenology is advanced or timed in synchrony with ambient *C. scoparius* bloom. Slash marks indicate no overlap between treatment and native species (=NA). Stars indicate a significant difference from visitation rates to native plants in control (*C. scoparius* absent) plots ($P < 0.05$, LRT, glht). Different letters indicate significant differences between different exotic phenology treatments ($P < 0.05$, LRT, glht).

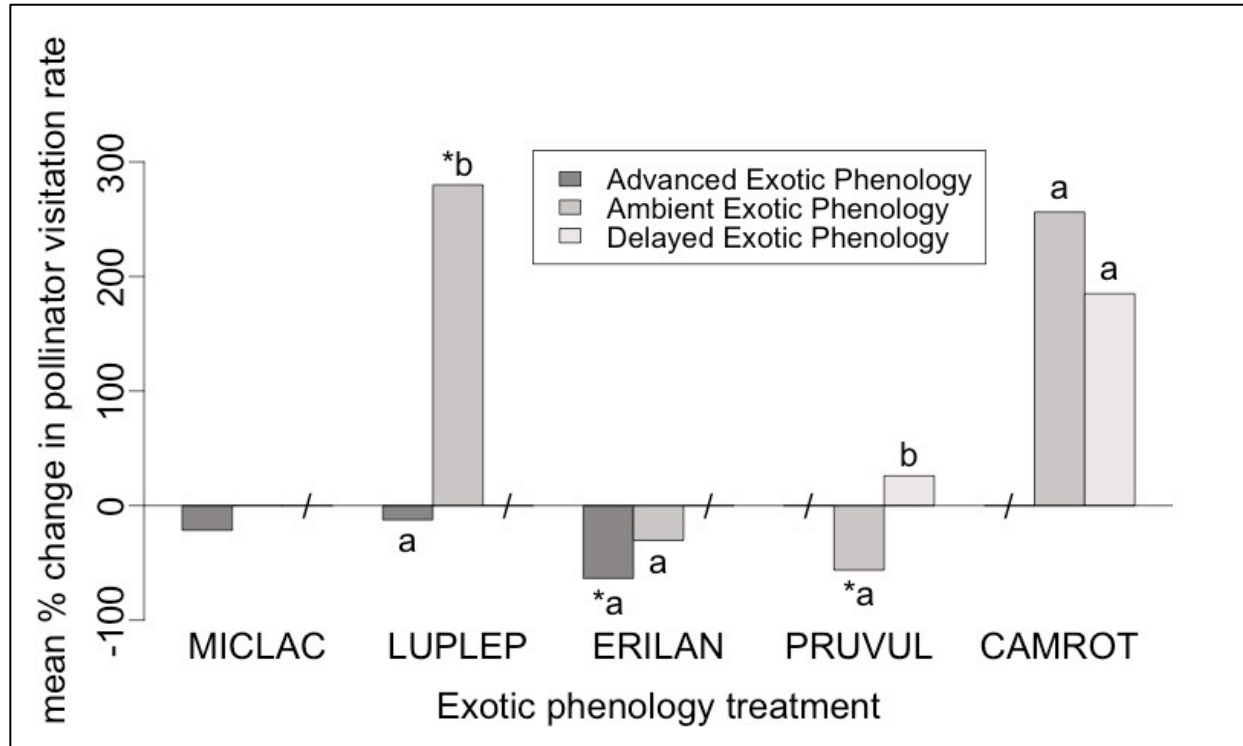


Fig. 6. **Change in pollinator visitation to native plants with change in *H. radicata* phenology.** Mean percent change in pollinator visitation rate (in visits per inflorescence per hour) for five native forbs in the presence of the exotic species *H. radicata*, when *H. radicata* flowering phenology is advanced, delayed, or timed in synchrony with ambient *H. radicata* bloom. Two additional native species were not observed (*R. occidentalis*, *C. quamash*). Slash marks indicate no overlap between treatment and native species (=NA). Stars indicate a significant difference from visitation rates to native plants in control (*H. radicata* absent) plots ($P < 0.05$, LRT, glht). Different letters indicate significant differences between different exotic phenology treatments ($P < 0.05$, LRT, glht).

Table 2. Effects of exotic phenology treatments on assemblages of pollinator morphospecies observed foraging at flowers of seven native forb species. Bold values are significant to $P < 0.05$.

Native species	Relevant overlapping exotic species	Phenology treatments compared	Change in pollinator community by exotic phenology treatment	PERMANOVA P value
<i>R. occidentalis</i>	<i>C. scoparius</i>	ADVCYT, AMBCYT	No	0.1204
<i>C. quamash</i>	<i>C. scoparius</i>	ADVCYT, AMBCYT	No	0.8894
<i>M. laciniata</i>	<i>H. radicata</i>	ADVHYP, AMBHYP	No	0.1414
<i>L. lepidus</i>	<i>H. radicata</i>	ADVHYP, AMBHYP	Yes	0.0321
<i>E. lanatum</i>	<i>H. radicata</i>	ADVHYP, AMBHYP	No	0.3687
<i>P. vulgaris</i>	<i>H. radicata</i>	ADVHYP, AMBHYP, DELHYP	No	0.0966
<i>C. rotundifolia</i>	<i>H. radicata</i>	AMBHYP, DELHYP	No	0.5997

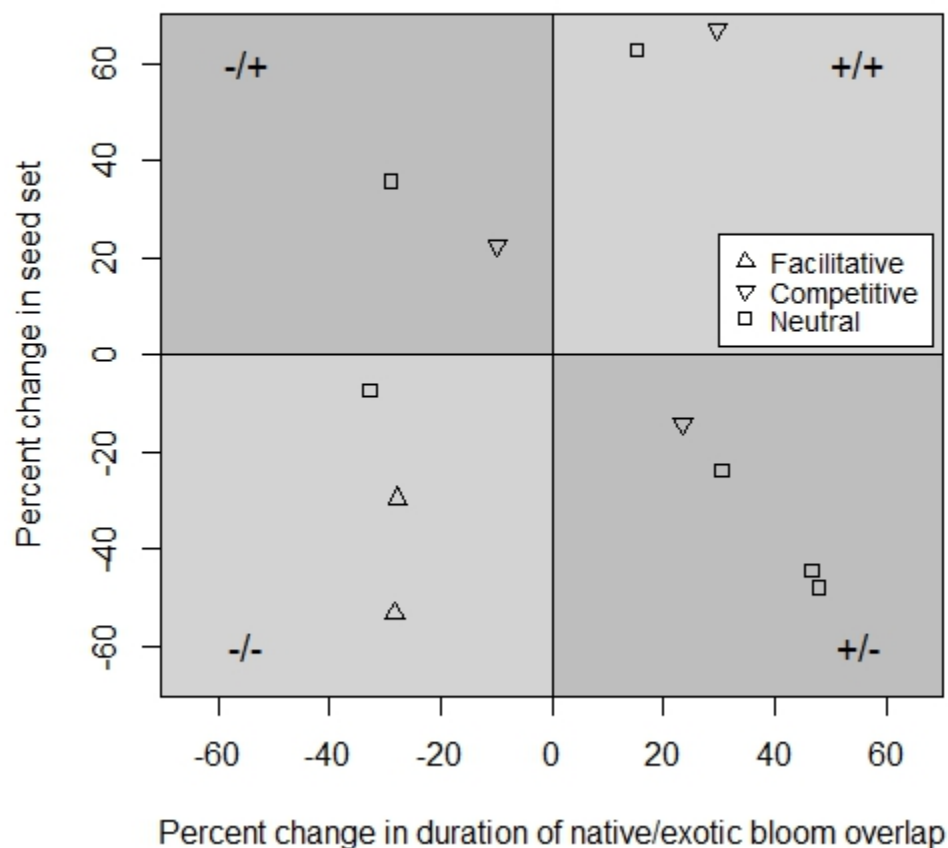


Fig. 7. **Percent change in seed set of native forbs as a function of the duration of native/exotic flowering overlap (2 exotic species, 7 native forbs).** Each point represents one of the 11 realized native/exotic overlaps in the experiment (e.g., native *P. vulgaris* and Delayed *H. radicata*; native *R. occidentalis* and Advanced *H. radicata*). Upward pointing triangles are initially facilitative indirect interactions; downward pointing triangles are initially competitive indirect interactions; squares are initially neutral indirect interactions. Under our hypothesis, points for initially facilitative relationships are expected to fall in the upper right or lower left quadrant (i.e., if the initial interaction is facilitative, increasing its duration should increase native seed set), while initially competitive relationships are expected to fall in the upper left or lower right quadrant.

Invasion by exotic plant species alters seasonal patterns of community floral resources for pollinators

Susan M. Waters

ABSTRACT

Climate-induced phenological shifts are known to induce phenological mismatches between pollinators and plants, but invasion and the resulting impoverishment of flora may produce similar patterns. Pollinators require timely availability of floral resources (nectar or pollen) throughout a season, but patterns of resource availability may be affected by invasion-induced changes in plant community composition, since native and exotic plants may differ in resource provisioning traits. To determine how invasion affects seasonal community floral resource provisioning, we first compared how native vs. exotic prairie floras' differ in traits affecting the amount, type (nectar vs. pollen) and timing of resource provision. Next, to examine how changes in species composition occurring during invasion combine with differences in resources provisioning traits to affect resource provisioning over time, we monitored floral abundance and phenology in five sites differing in species composition and in identity of dominant exotic plant species. We found that the exotic flora contained lower proportions of nectar- and pollen-providing species and a lower proportion of species provisioning two pollinator guilds (eusocial bees and Lepidoptera). Native and exotic floras did not systematically differ in the timing of flowering phenologies, but unique assortments of species at sites resulted in distinct temporal patterns of resource provision by site, including one "resource desert" at a highly invaded site and unexpected seasonal resource lows at three other sites. Invasion should be considered with climate-induced phenological shifts as a mechanism for producing "mismatch" between pollinator resource requirements and plant community resource provision.

INTRODUCTION

Phenological change in response to environmental disturbance has implications for interacting organisms. Concern about climate change has led to strong interest in the potential for phenological mismatch to influence species interactions, and many studies have documented dramatic impacts of climate-induced mismatch on populations. However, it is less well appreciated that *invasion* may also have strong effects on patterns of resource phenology in communities, with a similar potential to produce consumer-resource “mismatches”. Pollinators, which rely on flowering plants to provision them with nectar and pollen resources, may be especially affected by altered resource phenology when exotic plants invade. Recent work by Burkle (2013) attributes the loss of large numbers of pollinator species from a plant-pollinator network to temporal mismatch, making the understanding of phenological relationships between plants and pollinators especially pressing.

Invasive plants can have strong impacts on pollinators because pollinators rely on plant communities to provide the floral resources they require at the seasonal times when they are needed. For example, bees require pollen as a protein source for larval development (Müller and others 2006; Rasheed & Harder 1997a, 1997b), and most adult insect pollinators (bees, Lepidoptera, and pollinating flies) consume nectar for use as a high-energy carbohydrate as well as for other components important to adult fecundity (e.g. amino acids; Mevi-Schütz and Erhardt 2005). The timing of availability of these resources is critical to pollinator welfare; fecundity and longevity suffer if food is limited at any point during development (Boggs and Ross 1993; Boggs 2009). However, not all flowering species supply both pollen and nectar. Therefore, it is critical to pollinators

that plant species (native or exotic) supplying adequate quantities of their required resource(s) are available in their foraging habitat, and that these species bloom at the appropriate time(s) of the season to provide resources when they are needed.

However, invasion by exotic plant species displaces native plants, changing plant community composition (Hejda et al. 2009; Yurkonis 2005; Mack and others 2000). This in turn could alter the community's proportions of the resources pollinators require (Fig. 1). Plant species clearly differ in the amount, type, and timing of floral resources they offer to pollinators (Fig. 1). An exotic species' resource provisioning traits may therefore differ from those of the native individuals it competitively displaces. For example, it may provide no floral resources to pollinators (e.g., be wind pollinated, such as a grass), leading to a decline in floral resources, or it may provide a different type of resource than the native it displaces (e.g., provide mainly nectar instead of pollen, or have a floral architecture that provides nectar available mainly to short-tongued rather than long-tongued bees). Such impacts on the amount and type of floral resources could have strong effects on the abundance and composition of the pollinator community. Less appreciated is that the potential resource trait differences between native and exotic plant species could also alter seasonal patterns of floral resource provision at the community level, creating temporal resource gaps and changing the timing or magnitude of resource peaks. For example, if a native species is competitively displaced by an exotic species with an entirely different flowering phenology, the floral resources formerly contributed to the community by the native species thus disappear from the temporal progression of resources, and are replaced by the exotic floral resource *with the exotic's phenological timing*. The removal of the native's resources creates the potential for a temporal

resource gap, since even an exotic species that provides virtually the same type and amount of resource as the native it displaces may provide it at a different time of the season. Alternatively, an exotic species could displace native individuals with the same flowering phenology, but offer a different resource type, also creating a temporal gap for the native's lost resource type. Temporal patterns could also be affected by the fact that exotics often occur in very high abundance, competitively displacing large numbers of individuals from multiple native species. This could greatly reduce the resource contribution of native species at multiple time points, while concentrating the resource contribution of the exotic at a single time point. Of course, if the exotic plant species displacing native plants do not differ in their floral resources, generalist pollinators may not be affected. In short, the combination of shifts in plant species composition during invasion and differences between native and exotic flora in the amount, type and timing of floral resources could combine to influence the timing of floral resources provided by the invaded plant community (Fig. 1).

To explore the potential for changes in seasonal floral resource provisioning created by invasion, we asked whether native and exotic flora (1) provided different amounts of floral resources; (2) provided different types of floral resources (pollen/nectar), or provided resources primarily to different pollinator types; and (3) differed in their seasonal pattern of floral resource availability. Additionally, (4) we determined whether differentially invaded areas differed in seasonal patterns of pollinator resource provision as a result. Although several studies have found that exotic plants are more likely to be self-pollinated (Burns et al. 2011, Harmon-Threatt 2009), a high proportion of exotic plants at Glacial Heritage are wind-pollinated grasses (Dunwiddie et

al. 2006), so we hypothesized that the exotic flora would provide fewer floral resources. We also hypothesized that the resource-providing exotic flora would provision a different complement of pollinator types and supply a different set of resource phenologies than the native flora. Pollinator resource provisioning traits could serve as a mechanism to limit similarity in plant communities, and as an environmental filter for invading plants, but there has not been any evidence of such a phenomenon (Sargent and Ackerly 2008). Hence, we expected frequencies of resource provisioning traits in exotic flora to neither resemble nor systematically differ from native flora. Finally, we predicted that more-invaded sites, with higher proportions of exotic flowers, would contain seasonal “resource gaps”, because floral provisioning is more likely to be dominated by the phenology of the few (exotic) species that dominate.

METHODS

Study site

We explored flowering phenology patterns and pollinator provisioning in a western Washington prairie community at Glacial Heritage Preserve, Littlerock, WA. Glacial Heritage is a prairie/oak savannah historically dominated by bunchgrasses and forbs, currently experiencing invasion by a variety of exotic forbs, shrubs, and perennial sod-forming grasses, as well as incursion by native Douglas fir (*Pseudotsuga menziesii*) (Dunwiddie et al. 2006). The site is mostly open prairie dotted with occasional individual trees and more rarely, clumps of shrubs. Due to attempts to control or reverse invasion by various exotic plant species, the preserve contains areas that have undergone different restoration and management strategies (e.g. mowing, burning, and spot application of Poast grass herbicide) in different years (S. Hamman, G. Diehl, personal

communications). This has resulted in a patchwork of adjacent, similar plant communities that vary in degree of invasion (proportion of area covered by exotic species), yet draw on the same local plant species pool, experience very similar edaphic and climatic environments, and probably provide floral resources to highly overlapping assemblages of pollinators. The preserve is thus an ideal setting to examine how community provision of floral resources to pollinators can be affected by the degree of invasion and the composition of resource provisioning traits of an exotic flora.

Species

The plant community at the site comprises species from both open prairie and the edges of tree or shrub clumps. We included 48 plant species (24 native and 24 exotic) in our study; 4 were only recorded once as single individuals or in only one quadrat and were considered unlikely to contribute strongly to community dynamics. Of the remaining 44, there are 7 graminoids, 1 fern and 3 additional forbs that are self- or wind-pollinated, leaving a total of 33 insect-pollinated forbs and shrubs that could provide floral resources to pollinators. The most abundant exotic flora includes perennial grasses, an invasive shrub (*Cytisus scoparius*), and long-established exotic forbs such as *Hypochaeris radicata*, as well as recently arrived *Leucanthemum vulgare*. Common and abundant native species include *Camassia quamash*, *Ranunculus occidentalis*, *Campanula rotundifolia*, and *Lomatium utriculatum*.

The pollinator community is largely generalist, and includes native bumblebees (*Bombus* spp.), honeybees (*Apis mellifera*), halictid and andrenid solitary bees, syrphid, bombyliid, and muscid flies, beetles, and a few Lepidoptera.

Sites differing in exotic species composition

To determine how variation in the exotic plant community would influence the seasonal timing of community flowering phenology, we identified 5 sites within the preserve ranging in area from ~24 to ~38 ha, with visually obvious plant community boundaries resulting from either a road or from different management histories. Sites differed in dominant native and exotic plant cover (Table 1). Four of the five had previously been burned (sites Hyp-Leuc I, Hyp-Leuc II, Hyp-Crep, Hypochaeris; Table 1) although in different years (S. Hamman, G. Diehl, personal communications). Burning results in suppression of a major exotic species, *Cytisus scoparius*, which recolonizes the site a few years later, and can also result in flushes of bloom by some native species (e.g. *M. laciniata*; S. Hamman, G. Diehl, personal communications). All of the burned sites were also spot-treated by volunteers every summer on *Arrenatherum elatius* patches with the herbicide Poast to control spread of this exotic grass. One site was also mowed, again for *C. scoparius* control. The fifth site (*Cytisus*-dominated, Table 1) had remained unmanaged in recent years and was dominated by shoulder-height *C. scoparius* shrubs and exotic grasses; this site had not undergone mowing, burning, or Poast treatment.

We established transects of 20 1m x 1m plots at each site. Transects were created by walking along the area edge from the southwest or southeast corner for a randomly selected number of steps, then choosing a random compass direction (restricted to directions pointing into the area). We walked a randomly selected number of steps between 50 and 150 in this direction, then planted a flag to mark the center of a plot and simultaneously marked the corners of a haphazardly placed 1x1 m quadrat. If we reached

the edge of the study area (determined by coming within 5 m of a road or a clearly demarcated line of very different vegetation) we selected a new compass direction and continued the transect in zigzag. If portions of the transect crossed, we limited plot selection points so that they remained at least 10 m distant from all other plots.

Field flowering phenology

Plots were visited weekly during the flowering season to record flowering phenology and abundance, beginning as soon as flowers started to appear on April 17 and continuing until most flowers had disappeared on August 10 (17 weeks). Visits to the *Cytisus*-dominated site began later and ended earlier (due to management by fire), but no species in those plots were blooming on the first visit, so we were able to capture the beginning of flowering by all species at the site.

We counted open flowers of each species in each plot every week. For species with racemes of flowers (e.g. *Prunella vulgaris*, *Lupinus lepidus*, *Zigadenus venenosus*) we counted racemes if they bore at least one fully opened flower. We then censused 50 haphazardly selected nearby racemes of the same species from outside of our plots and averaged the number of open flowers per raceme for that species on that date, later using this number to convert our raceme counts per plot to flower counts. For species with inflorescences comprising multiple small flowers (e.g., *Leucanthemum vulgare*, *Lomatium nudicale*) we counted whole inflorescences only.

Types of resources and estimates of resource provision per flower

We used a combination of past years' field data and literature/internet searches to determine the resource type and pollinator type provisioned by each plant species. We

had pollinator visitation data available for a subset of species (8). For the remaining species, we first performed literature searches on Web of Science, using the species name in combination with search terms “pollinat*”, “pollen”, and “nectar”. We categorized species as mainly nectar-providing, nectar and pollen providing, or primarily pollen providing, based on mention of pollinator collecting behaviors, nectar measurements, or pollen measurements in the literature. We also recorded whether species provided resources to eusocial bees, solitary bees, flies, or Lepidoptera. When no literature and none of our own data was available, we also searched the web, using first the species name and then the common name with “pollen”, “nectar”, “pollinator”, “bee”, “fly”, and “butterfly”. We used images as evidence for pollinators visiting a species’ flower and perused websites from Encyclopedia of Life, local nature preserves, native plant preservation organizations, USDA PLANTS, and the Xerces Society (see Supplementary Table 1).

Calculating temporal provisioning of floral resources

Generalist pollinators could visit any plant species with the appropriate resource, but the amount of resources provided per flower will likely differ. Ideally, we would have species-specific nectar and pollen provisioning amounts available for each species, but we were unable to collect these data on a community scale. Instead, we scaled plant species’ per-flower contribution to nectar and pollen resources for pollinators based on flower traits. To estimate nectar provision, we created a resource index based on corolla diameter and corolla tube depth. We established 4 categories of corolla diameter (1= <1 cm, 2= 1-3 cm, 3= 3-5 cm, 4= >5 cm) and 2 categories of corolla tube depth estimated from flower architecture (1= very shallow (<1 mm), 2= medium (1mm-5mm), 3= deep

(>5mm). An index of nectar provision per inflorescence was then estimated by multiplying the corolla size category by the corolla tube depth category. The index of per-inflorescence pollen resources was 1 for plants visited mostly for nectar or for nectar and pollen. However, species visited mainly for pollen produce some fraction of pollen to serve as a pollinator reward rather than as functional gametes, often resulting in much higher pollen production than other species. We scaled their pollen contribution by a factor of 4. Although actual resource provisioning is likely to differ from our estimates, corolla size (diameter) and corolla tube depth are often correlated with amount of nectar or pollen reward (Cresswell and Galen 1991; Fenster et al. 2006; Gómez et al. 2008), and patterns we observed were robust to reanalysis in which we removed scaling by per-inflorescence resource quantities removed.

Analysis

To compare the resource trait composition of native and exotic floras, we first compared the proportions of resource-providing species among native vs. exotic species using chi-squared tests. We also performed chi-squared tests to compare native and exotic floras' proportions of nectar-providing species and pollen-providing species, as well as proportions of native vs. exotic species visited by eusocial bees, solitary bees, flies, or Lepidoptera. Finally, we tested for differences between the native and exotic flora in a) mean peak flowering date and b) mean first flowering date using Welch 2-sample t tests, and tested for a relationship between date of peak flowering and ln-transformed mean peak abundance in both native and exotic flora with a simple regression.

To compare differences among sites in the proportion of area covered by exotic vs. native flowers providing floral resources, we used a generalized linear mixed model with origin as an explanatory variable and site as a random effect, with the response variable logit transformed. We also compared resource provision across areas (as resource units calculated with index as described previously; number of inflorescences multiplied by per-inflorescence resource provision index) using a generalized linear mixed effects model with site, origin, (native vs. exotic), and their interaction as explanatory variables and Julian date as a random effect, specifying a Poisson distribution family.

We explored potential differences in the community composition of floral resources among site over time using PERMANOVA, specifying a Bray-Curtis distance measure, and using Julian date of observation, site, and their interaction as explanatory variables. We repeated this approach to test differences over time in composition of resources relevant to four pollinator groups (eusocial bees, solitary bees, flies, Lepidoptera, tested separately) as well as composition of nectar/pollen resources.

All tests were carried out in R version 3.0.1.

RESULTS

The native and exotic flora showed distinctly different compositions of floral resource traits relevant to pollinators. The native flora contained a higher proportion of nectar-providing species, pollen-providing species, and all floral resource-providing (entomophilous) species than the exotic flora (nectar: $X^2 = 3.49$, $df = 1$, $p\text{-value} = 0.031$; pollen: $X^2 = 5.34$, $df = 1$, $p\text{-value} = 0.010$; all resources: $X^2 = 2.78$, $df = 1$, $p\text{-value} = 0.048$, Fig. 2a). A higher proportion of native species provisioned

eusocial bees and Lepidoptera (eusocial bees: X-squared = 3.01, df = 1, p-value = 0.041; Lepidoptera: X-squared = 2.78, df = 1, p-value = 0.048; Fig 2b), but native and exotic species were equally likely to provision solitary bees or flies (solitary bees: X-squared = 2.14, df = 1, p-value = 0.072; flies: X-squared = 0.76, df = 1, p-value = 0.192; 2b). Native and exotic species also did not differ in flowering phenologies. The dispersion of peak flowering dates (PFD) and first flowering dates (FFD) across the season was indistinguishable for native and exotic flora (t-test, $p=0.7623$ PFD; $p=0.5302$ FFD; Fig 3), and there was no relationship between date of peak bloom and log mean floral abundance for species of either nativity (lm, $p=0.206$ native, $p=0.308$ exotic).

Scaled by floral abundances of native and exotic species, resource provision trait differences led to distinct temporal patterns of nectar and pollen resource availability native versus exotic species across all study sites for all pollinator groups (Fig. 4 a-d, 5 a-c). The amount of both nectar and pollen resources varied as a function of site and origin of resource (native vs. exotic), with the best fit for both resource types including an interaction between site and origin (verified by model selection, using AICs). Temporal patterns of resource availability also differed by site: differentially invaded sites differed in composition of available floral resources, and composition changed in distinct ways in each site through time (PERMANOVA, $p=0.001$ date; $p=0.001$ area; $p=0.001$ date x area interaction). Three sites (Hyp-Leuc I, Hyp-Leuc II, and Hypochaeris) showed mid-seasonal lows in nectar resources (Fig. 4e-h), while there were no seasonal nectar resource gaps per se in the most heavily invaded (Cytisus) site, where nectar resources were low throughout the season (Fig. 4e-h). Pollen resource patterns varied dramatically among sites, with the Cytisus site showing a peak in resources that dwarfed resource

peaks in the other four sites (Fig. 5e-h). Resources available to specific pollinator guilds also varied significantly by site (PERMANOVA date x area interaction $p=0.001$, eusocial bees; $p=0.001$, solitary bees; $p=0.001$, flies; $p=0.001$, Lepidoptera).

DISCUSSION

Our results suggest that invasion significantly alters community seasonal patterns of resources available for pollinators. The exotic flora provided fewer pollinator resources (both nectar and pollen) and provisioned a narrower pollinator assemblage than the native flora (Figs 2a, b). At the same time, the exotic flora covered more area than native flora (Table 1), suggesting that plant invasion causes pollinators to become more dependent on floral resources supplied by exotic species. As a group, native and exotic floras did not differ in phenology (Fig. 3a, b), but differences in their resource types interacted with phenology to produce distinctly different temporal resource assemblages for pollinators (Fig. 4a-d, Fig 5a-c). Mediated by floral abundances at specific sites, these traits produced “resource deserts” (Fig 4e-h, nectar for all pollinators at the *Cytisus* site), as well as temporal gaps and site-specific changes in the timing or magnitude of resource peaks (Figs 4e-h, 5d-f). Taken together, these effects of invasion on seasonal community-level pollinator resource provision could have negative effects on some pollinator types while selectively benefiting others, and could ultimately alter local pollinator composition.

We observed two kinds of temporal floral resource provisioning in exotic dominated plant communities that could negatively impact pollinators: resource deserts and temporal resource gaps. Relative resource deserts are produced when an invasive

exotic plant dominates a site, producing a high abundance of a single species coupled with very low abundances of other plant species. The dominance of a single species results in provision of a large amount of this species' resource type at the time of its flowering, but concomitant loss of resource type and phenological diversity of the species that were (presumably) competitively displaced. Our *Cytisus* site, the most heavily invaded site in our study, exemplified this pattern. Pollinators visiting this site had access to few nectar resources at any time of the season, and an embarrassment of pollen riches during a short period of time (Fig 4e-h, Fig. 5a). *C. scoparius* does not provision solitary bees, flies, or Lepidoptera, supplying resources to only one of the four major pollinator guilds we examined (eusocial bees). This site is thus a relative resource desert for most pollinators. Its composition is approximately duplicated in many extensive *C. scoparius*-dominated fields throughout the Puget Trough region.

We also observed temporal resource gaps, a second flowering pattern that could make trouble for pollinators, in more diverse sites where *C. scoparius* was much rarer but other exotic and native species were common (Table 1). Three sites (Hyp-Leuc I, Hyp-Leuc II, and Hypochaeris) showed a bimodal pattern of nectar provision to pollinator groups, with lows that occurred mid-season (Fig. 4e-h). This temporal reduction in nectar resources could have important impacts on pollinators if supply falls below pollinator demand, as well as potential downstream impacts on plants. There is no reason to believe that resource requirements of any pollinator guild decrease mid-season; in fact, Boggs (2009) showed that pollinator fecundity and longevity suffer if food is limited at any point during development, though the impact of a resource gap would undoubtedly depend on its duration. Effects on pollinator populations could also feed back to

influence later-blooming plant species, as sequentially flowering species may facilitate each other through maintenance of pollinator populations (Waser & Real 1979); if resource limitation led to a crash in pollinator populations at mid-season, later-blooming species would then suffer reduced visitation.

In fact, our results suggest that seasonal flowering bimodality may emerge as a general consequence of environmental change. Most studies show continuous phenological sequences of flowering within a season, without gaps (whether flowering is aggregated, random, or dispersed; Anderson and Schelfhout 1980, Wilke and Irwin 2010, Totland 1993, Petanidou et al. 1995, Osada et al. 2003, Craine et al. 2012, but see Catorci et al. 2012). However, a recent study tracking phenological shifting in response to climate change documents increasing bimodality of flowering phenology over time (Aldridge et al. 2012) analogous to what we found in exotic dominated sites. Additionally, warming experiments have also led to increasing bimodality due to divergent phenological shifts in communities (Sherry et al. 2007).

The temporal resource gaps we observed resulted from the fact that the native flora lacked species filling key phenological niches, which in turn were not filled by the exotic flora that presumably displaced these species. Resource lows corresponded to a gap in native species' dates of peak bloom, when earlier-blooming species' floral abundance was decreasing but later-peaking species were at the onset of flowering (Fig. 3). The two members of the exotic flora whose flowering peaks occurred in the gap did not sufficiently compensate, because one (*C. scoparius*) supplied no nectar, while the other (*V. sativa*) occurred only in low abundance. We lack historical data on floral abundance and composition at the preserve that would allow us to quantify past

community patterns, but Glacial Heritage was restored over the past 15-20 years from a state of heavy *C. scoparius* invasion closely resembling the composition of the *Cytisus* site (E. Delvin, G. Diehl, personal communications), and has very low plant diversity compared with other extant prairie fragments (R. Gilbert, personal communication). It is therefore possible that the assortment of plants here represents an impoverished flora that no longer contains the phenological diversity the historical community would have had. However, without such data, this must remain speculative.

How likely is it that the phenological resource patterns we observed influence pollinators? The answer to this question is complicated by pollinator mobility. Differences in local resource provision patterns mediated by exotics will only affect pollinator success if pollinators cannot compensate for local gaps in resources by their mobility. All pollinator guilds at the preserve are highly mobile, and most can probably travel freely between our experimental sites, as well as fly to areas farther afield where floral composition is different. This means that a temporal resource limitation in one area would be most likely to have an impact only if it a) increased energy demand for foraging by causing pollinators to fly farther in search of resources; or b) happened concurrently with widespread floral resource limitation, across an area equal to or larger than pollinators' foraging ranges. We speculate that both possibilities are plausible at Glacial Heritage, given that Puget Trough prairies are now limited to only 1% of their historical geographical extent (Dunwiddie et al. 2006), and that the preserve is surrounded by agricultural land and housing tracts.

Resource deserts and temporal resource gaps could make pollinator populations more vulnerable. Many pollinators are in decline (Biesmeijer et al. 2006; Goulson et al.

2008; Potts et al. 2010). Relative resource specialists, such as many Lepidoptera, could suffer from invasion if their preferred floral resource types and phenologies disappear and are not replaced. Generalists are affected too. Among bees, declines are particularly steep in bumblebees (*Bombus* spp), which require a sequence of food resources over a growing season; those with narrower foraging phenology and diet breadth are most strongly affected (Bartomeus et al. 2013). A recent analysis of changes in a plant-pollination network over approximately a century shows that temporal and spatial mismatches between pollinators and their plant resources account for a large proportion of links lost (Burkle et al. 2013). These data suggest that phenological resource patterns in a community can be important. We suggest that resource-mediated impacts of invasion on pollinators are likely wherever plant communities experience either a) dominance by a single invasive plant species (with extreme reduction in floral diversity and a consequent “resource desert”), or b) an impoverished flora, such as might occur after invasion by multiple species or other forms of habitat degradation, even following restoration, that is likely to contain seasonal resource gaps from unfilled phenological niches.

Finally, recent evidence suggests that climate change is likely to shift both plant flowering phenology and timing of pollinator emergence (Bartomeus et al. 2011, Cleland et al. 2007). In a memorable study, Memmott et al. (2007) simulated shifting of plant phenologies in a community, and found that generalist pollinators lost 17-50% of available resources as well as substantial diet breadth; shifting created both resource gaps and earlier cessation of community flowering. We suggest that invasion is a mechanism that may already produce similar patterns in some systems, and urge more research on how community flowering patterns through seasonal time impact pollinators.

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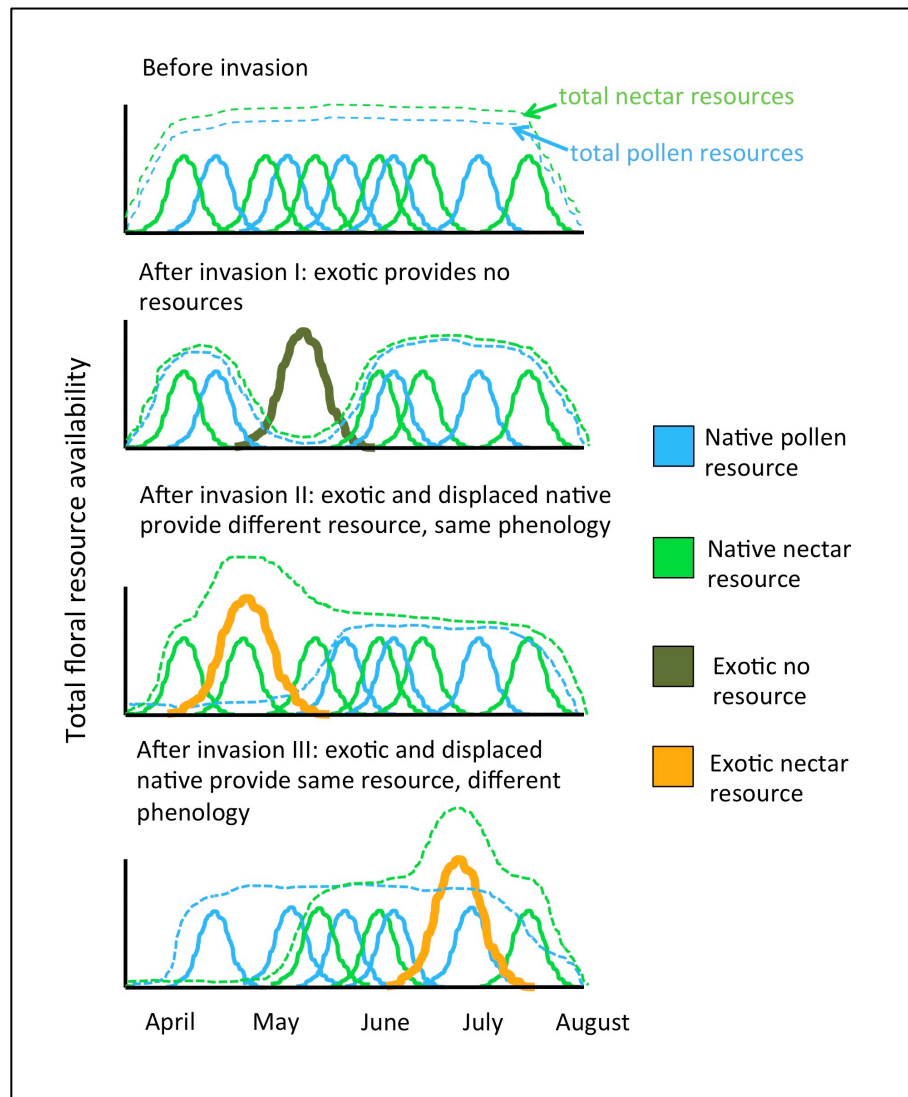


Fig. 1. Three hypothetical changes to community patterns of nectar and pollen provision resulting from invasion. Each curve represents one species' flowering. Before invasion: hypothetical community has native nectar and pollen-providing species blooming throughout the season, providing consistent availability of both resource types. After invasion I: The exotic species provides no floral resources (e.g., is a grass or is autogamously self-pollinated), and displaces resource-providing native species. The result is temporal resource gaps in both nectar and pollen. After invasion II: The exotic species provides nectar, and blooms at the same time as native species it displaces, but displaces native species providing pollen. There is a new temporal nectar peak and a simultaneous gap in pollen provision. After invasion III: The exotic species provides nectar, and displaces native nectar-providing species, but blooms at a different time. There is an early-season gap in nectar provision and a late-season peak in nectar provision.

Site name (dominant exotic species)	Mean percent exotic cover	Mean proportion of exotic cover providing no floral resources	Mean proportion of exotic cover providing nectar resources	Mean proportion of exotic cover providing pollen resources	Florally dominant species	Main resource type(s)	Poll. type(s) visiting
Hyp-Leuc I	69.4	0.46	0.51	0.48	<i>H. radicata</i>	np	ESF
					<i>R. occidentalis</i>	np	SF
					<i>L. vulgare</i>	np	SFL
Hyp-Leuc II	73.0	0.60	0.35	0.35	<i>H. radicata</i>	np	ESF
					<i>C. rotundifolia</i>	np	ESF
					<i>L. vulgare</i>	np	SFL
Hyp-Crepis	70.4	0.35	0.62	0.54	<i>H. radicata</i>	np	ESF
					<i>C. capillaris</i>	np	ESF
					<i>C. rotundifolia</i>	np	ESF
Hypochaeris	81.5	0.79	0.20	0.18	<i>H. radicata</i>	np	ESF
					<i>L. utriculatum</i>	np	SFL
					<i>C. rotundifolia</i>	np	ESF
Cytisus	89.8	0.60	0.05	0.40	<i>C. scoparius</i>	p	E

Table 1. Characteristics of five sites at which temporal patterns of floral resource provisioning for pollinators was monitored. N=20 quadrats per site. Species designated as florally dominant flowered in the largest number of quadrats per site; exotic species are bolded. Main resource types: np=nectar and pollen, p= pollen only (species can provide both or just one). Pollinator types: E=eusocial bees; S=solitary bees; F=flies; L=Lepidoptera.

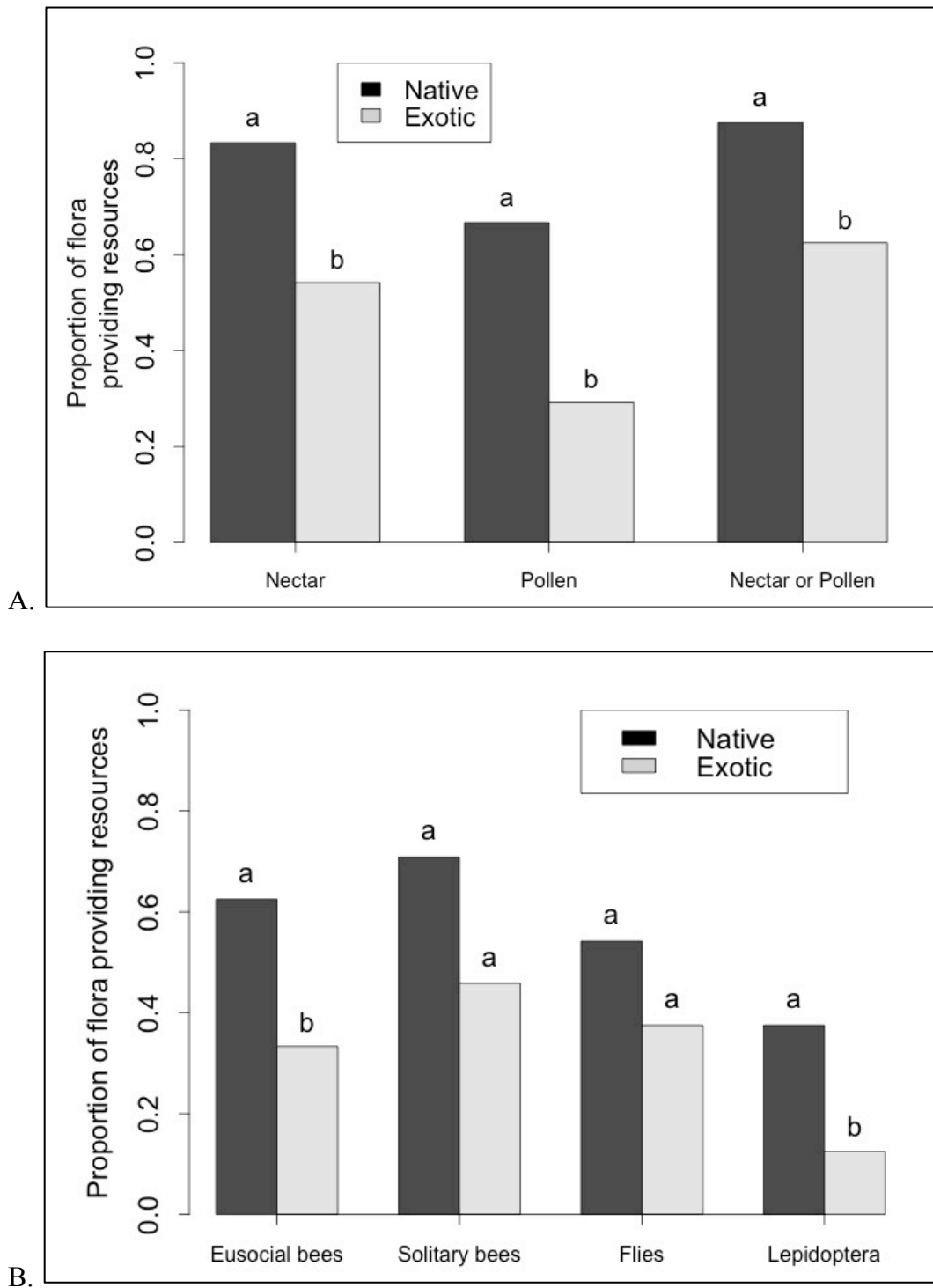


Fig. 2. A) Proportions of native and exotic flora providing nectar resources, pollen resources, or both. B) Proportions of native and exotic flora provisioning four pollinator guilds. Paired bars with different letters over them are significantly different at the $\alpha < 0.05$ level.

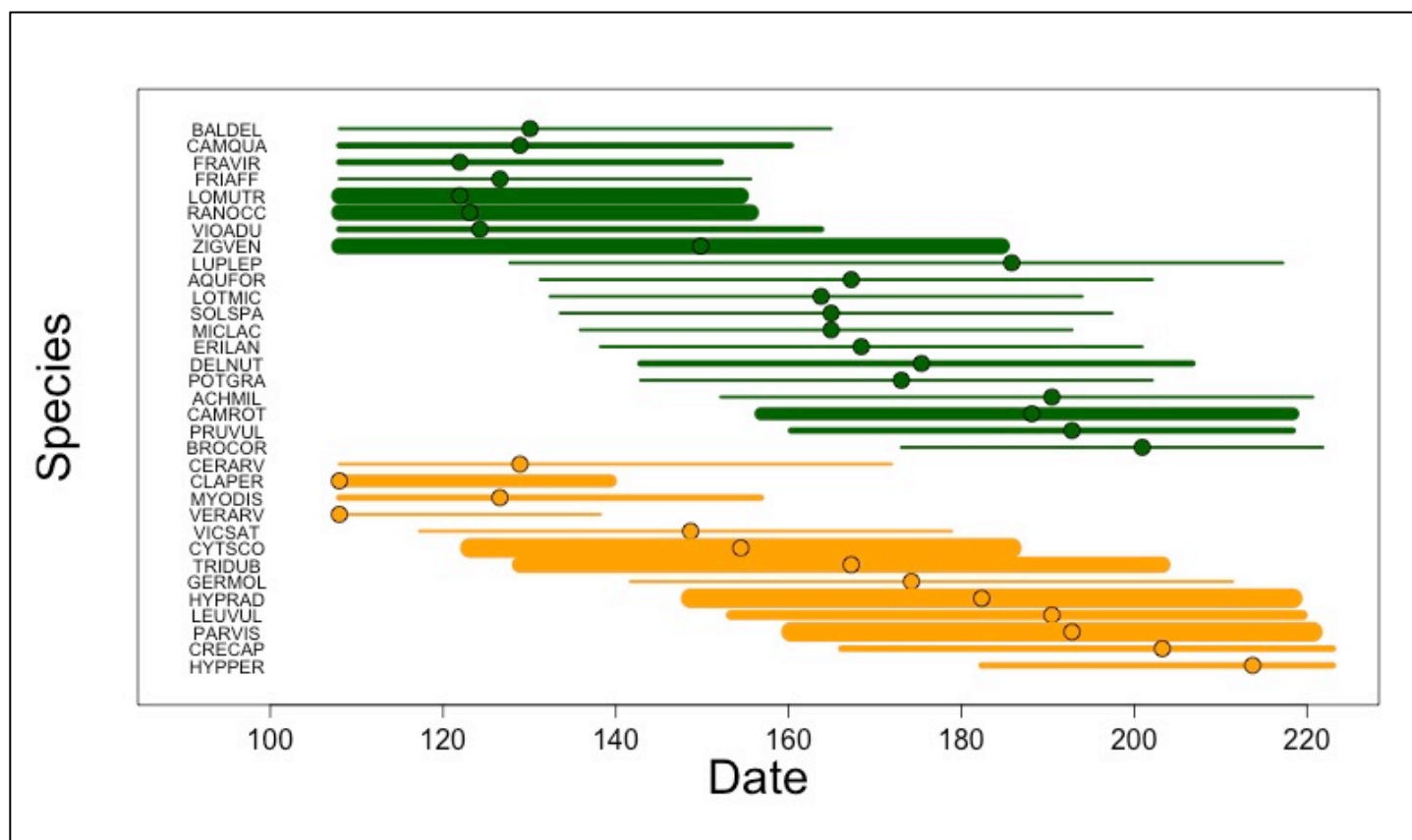


Fig. 3. Duration and peak flowering dates for all native and exotic plant species providing floral resources (green=native, orange=exotic). Lines depict time period during which 90% of floral abundance occurred for each species; points mark peak abundance. Line thickness is scaled by overall seasonal mean floral abundance of each species. Species are coded with the first three letters of genus and species names.

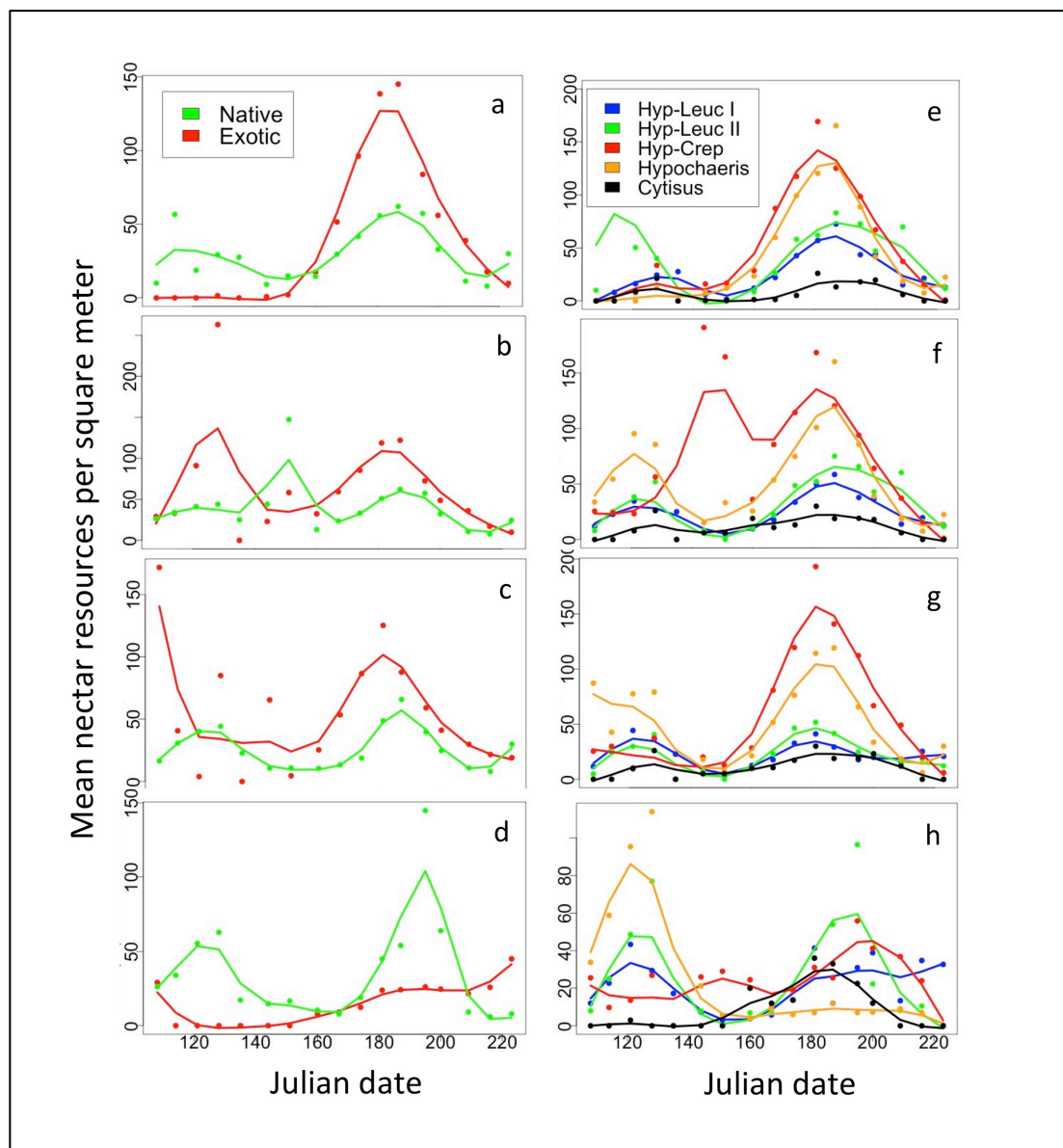


Fig. 4. a-d: Mean availability of nectar resources through time provided by native vs. exotic flora (pooled across 5 sites) to a) eusocial bees; b) solitary bees; c) flies; d) Lepidoptera. e-g: Mean availability of nectar resources through time at 5 sites differing in species composition and identity of dominant exotic plant species (see Table 1). e) eusocial bees; f) solitary bees; g) flies; h) Lepidoptera. Weekly resource provision is estimated by multiplying mean floral abundance (of each species accessible to a given pollinator group) by a factor that scales per-inflorescence resource availability according to inflorescence traits (see Methods). Curves are splines.

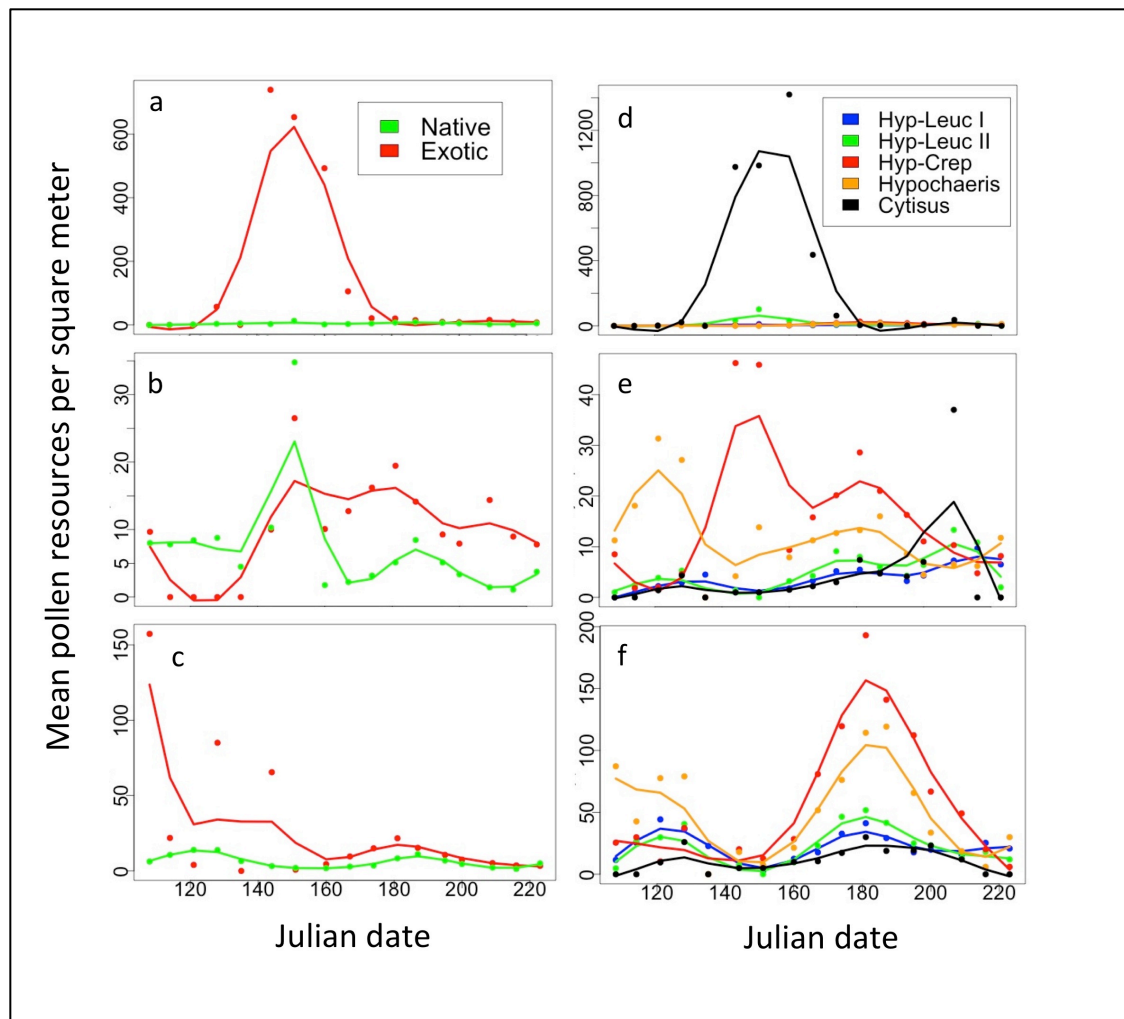


Fig. 5. a-c: Mean availability of pollen resources through time provided by native vs. exotic flora (pooled across 5 sites) to a) eusocial bees; b) solitary bees; c) flies. d-f: Mean availability of pollen resources through time at 5 sites differing in species composition and identity of dominant exotic plant species (see Table 1). d) eusocial bees; e) solitary bees; f) flies. Weekly resource provision is estimated by multiplying mean floral abundance (of each species accessible to a given pollinator group) by a factor that scales per-inflorescence resource availability according to inflorescence traits (see Methods). Curves are splines.

SUMMARY OF THE DISSERTATION

Global environmental change is happening at the fastest pace in the history of the Earth. For humans to cope with this change, understand it, and perhaps in some cases take actions to reverse it, we must understand the ways in which it alters the world as we have known it. My research shows that invasion and climate-induced phenological shifts can have profound effects on plant-pollinator interactions, effects that are not yet easily predicted given the current state of our knowledge. The unexpectedly opposing effects of multiple shared pollinators on native and exotic plants (Chapter One), the surprising changes in interaction strength and direction resulting from unequal phenological shifting (Chapter Two), and the emergence of new temporal patterns of floral resources in invaded communities (Chapter Three) are all exemplars of ecological surprises we can expect from global change. Yet, these particular ecological surprises arise largely because of an incomplete understanding of the system, not because of intrinsic environmental stochasticity or unpredictability.

Research on plants and pollinators undoubtedly arose because of humans' curiosity about the natural world, perhaps heightened by our aesthetic appreciation of both the structural beauty and the intellectual appeal of this complex, dynamic system. Plant-pollinator systems face serious global threats, yet paradoxically, in the responses of plants and pollinators to these threats, we can glimpse the mechanisms that knit the system together. In the best of possible worlds, the insights that arise from research on global change will help us to comprehend plant-pollinator interactions more fully, deepening our appreciation of their beauty and importance, and motivating us to balance human priorities so as to meet global change in the most productive way we can.

