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THE INFLUENCE OF EXOTIC PLANT INVASION ON NATIVE BEE ABUNDANCE AND

DEMOGRAPHY, AND THE POLLINATION AND ABUNDANCE OF NATIVE PLANTS

By

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ABSTRACT

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Flowering plants and bees are fundamentally linked. Bees rely wholly on floral resources for food and many native plants rely on bees to assist in reproduction. Despite this fundamental connection, how plants and their bee pollinators influence one another's abundance remains unclear. Also unclear is how invasion by exotic flowering plants disrupts bee- native plant relationships. I examined how the invasive plant, *Euphorbia esula* (Euphorbiaceae) influenced the native annual forb, *Clarkia pulchella* (Onagraceae) by altering pollinator visitation (i.e., indirect effects) and through direct resource competition. I observed pollinator visitation and estimated the extent to which plant reproduction was limited by pollen receipt (i.e., pollinator visits) in invaded and native-dominated sites. Though pollinator visitation was strongly reduced in invaded sites, plants were only weakly pollen limited. In contrast, *E. esula* removal experiments indicated that the effect of resource competition on *C. pulchella* fitness was very strong. Seed addition experiments indicated that changes in fecundity resulting from ameliorating competition for pollinators could influence future plant abundance, however these effects were dwarfed by the effects of resource competition.

To determine if strong reductions in pollinator visitation in invaded sites was mirrored by changes in entire bee communities, I quantified bee communities in sites dominated by *E. esula* and native-dominated sites. Bee abundance, richness and diversity were reduced in invaded sites. Thus, though native bees foraged on *E. esula* flowers, the net effect of invasion on bee communities was negative. However, because bees are highly mobile, patterns of forager abundance in relation to floral abundance may be misleading. Such patterns may be the result of bees moving to high resource patches rather than any fundamental change in bee abundance based on altered demographic processes. To examine this issue, I quantified the influence of floral resources and plant invasion on the demography of the native solitary bee, *Osmia lignaria* (Megachilidae). I placed nest blocks and preemergent cocoons in 27 sites that varied along a floral resource gradient and in extent of invasion, and found that nesting and fecundity were positively correlated with the abundance and richness of the native forb community and that nesting decreased with increasing exotic forb species richness. Despite increased parasitism in high-resource native-dominated sites, a marginally positive relationship between native forb species richness and bee population growth rates remained, suggesting that floral resources can positively influence bee demography.

Finally, via a literature review, I examined whether excessive harvesting of pollen by bees has led to the evolution of chemical defense of pollen. While bees are generally perceived as mutualists, they also act as herbivores, collecting substantial quantities of pollen to rear larvae, and the loss of pollen to consumption by bees has the potential to decrease plant fitness. Many morphological features of flowers are interpreted to serve in defense of pollen from excessive harvesting, and there is growing evidence that pollen can contain secondary compounds that may be an additional means of pollen defense.

TABLE OF CONTENTS

CHAPTER ONE

INDIRECT COMPETITION FOR POLLINATORS IS WEAK

COMPARED TO DIRECT RESOURCE COMPETITION:

POLLINATION AND PERFORMANCE IN THE FACE OF AN INVADER

Abstract

Invasive plants have the potential to reduce native plant abundance through both direct and indirect interactions. Direct interactions, such as competition for soil resources, and indirect interactions, such as competition for shared pollinators, have been shown to influence native plant performance, however, we know much less about how these interactions influence native plant abundance in the field. While direct competitive interactions are often assumed to drive declines in native abundance, an evaluation of their influence relative to indirect mechanisms is needed to more fully understand invasive plant impacts. We quantified the direct effects of resource competition by the invasive perennial forb, *Euphorbia esula* (Euphorbiaceae), on the recruitment, subsequent performance, and ultimate adult abundance of the native annual, *Clarkia pulchella* (Onagraceae). We contrast these direct effects with those that indirectly resulted from competition for shared pollinators. Although *E. esula* dramatically reduced pollinator visitation to *C. pulchella*, plants were only weakly pollen-limited. Pollen supplementation increased the number of seeds per fruit from 41.28 to 46.38. Seed addition experiments revealed that the impacts of ameliorating pollen limitation only increased potential recruitment by 12.3%. In contrast, seed addition experiments that ameliorated direct competition with *E. esula* resulted in an increase in potential future recruitment of 574%. Our results show that while the indirect effects of competition for pollinators can influence plant abundance, its effects are dwarfed by the magnitude of direct effects of competition for resources.

Introduction

Invasive plants often have well documented negative impacts on native plant abundance (reviews by Levine et al. 2003; Vilá et al. 2011). These impacts are most commonly thought to derive from invasives outcompeting natives for resources such as nutrients or light (Melgoza et al. 1990; D'Antonio and Mahall 1991; Gorchov and Trisel 2003; Vilá and Weiner 2004; Combs et al. 2011), however, indirect interactions between invasives and natives, which can be mediated by soil microbes (Klironomos 2002; Stinson et al. 2006), herbivores (White et al. 2006; Meiners 2007; Orrock et al. 2008), or pollinators (Bjerknes et al. 2007; Morales and Traveset 2009) may also play a role. A key question concerns what the relative strength of direct versus indirect effects of invasives on natives might be. If native abundance is commonly reduced by direct resource competition with invasives, then management efforts to ameliorate this competition might be profitable (Alpert 2010; Blumenthal et al. 2010). Alternatively, if indirect effects are implicated in native decline, then the impact of invasives may be more far reaching, and insidious, than currently appreciated. Thus, a consideration of the indirect effects of invasives on natives may be necessary in order to predict how native plants respond to invasion and restoration.

Evidence for direct competitive effects of invasives on natives comes in part from field studies that have manipulated exotic abundance around focal native plants and found negative effects on native plant performance (Levine et al. 2003; MacDougall and Turkington 2005; Biggerstaff and Beck 2007; Coleman and Levine 2007; Denoth and Myers 2007; Cipollini et al. 2008). Yet whether these negative effects on native plant growth or reproduction are responsible for driving declines in native plant abundance has seldom been evaluated empirically (but see Williams and Crone 2006; Maron and Marler 2008). As such, there is a need for field

experiments that evaluate the population-level consequences of competition between invasives and natives.

In addition to direct competition for abiotic resources, plants can also engage in indirect exploitative competition, in which the resources plants compete for are other species, such as shared pollinators (Rathcke 1983; Wooten 1994; Palmer et al. 2003; Bjerknes et al. 2007; Morales and Traveset 2009; Kaiser-Bunbury et al. 2011). Plant species that flower simultaneously often share pollinators (Bronstein 1994; Waser et al. 1996), which can reduce seed production if it results in either a reduction in pollinator visitation (Bjerknes et al. 2007; Morales and Traveset 2009) or an increase in deposition of heterospecific pollen grains on stigmas (reviewed in Morales and Traveset 2008). Pollinator-sharing can also have positive outcomes (i.e., facilitation), whereby visitation rates are increased in the presence of another species due to enhanced attractiveness of the flowering patch (Moeller 2004; Ghazoul 2006) or when multiple flowering species jointly support larger populations of resident pollinators (Moeller 2004). However, in the case of pollinator-sharing between exotic and native forbs, reduced visitation to natives is the most common outcome because exotics often have enhanced pollinator attractiveness relative to co-flowering native species (Brown et al. 2002; Moragues and Traveset 2005; Munoz and Cavieres 2008; Kandori et al. 2009; Morales and Traveset 2009). These competitive effects are exacerbated by the numerical dominance of invasives relative to natives (Bjerknes et al. 2007; Morales and Traveset 2009).

The implications of reduced pollinator visits resulting from competition for shared pollinators on native seed set has been less well-studied, but, when examined, effects are usually negative (reviewed by Morales and Traveset 2009). However, while previous studies are useful in indicating whether indirect competition is occurring, they do not typically reveal whether

reductions in native seed set affect native plant abundance. Linkages between seed production and recruitment need not be strong as they are often decoupled by compensatory density dependent factors such as seedling survival. Such factors may limit the extent to which changes in fecundity translate to changes at the population level (Ashman et al. 2004; Price et al. 2008; Feldman and Morris 2011; Horvitz et al. 2011).

While indirect interactions between native and invasive plants via shared pollinators have been the focus of much attention over the last decade (reviews by Bjerknes et al. 2007; Morales and Traveset 2008; Morales and Traveset 2009), these interactions do not occur in isolation. Rather, competition for pollinators and competition for other resources occur simultaneously and are fundamentally linked (Campbell and Halama 1993). First, competition for resources could limit the extent to which changes in reproduction due to pollen receipt influence plant abundance by decreasing recruitment, survival, or flowering. Second, seed production is influenced by both pollen receipt and resource availability (Haig and Westoby 1988; Campbell and Halama 1993; Burd 1994; Ashman et al. 2004; Burkle and Irwin 2008). Seed production may be limited by pollen receipt when the supply of pollen grains is inadequate (i.e., pollen- limitation) or the quantity and quality of pollen may be more than adequate, but seed production may be limited by resources (i.e., resource-limitation). Moreover, there need not be a dichotomy between resource and pollen-limitation. For example, pollination and resource levels may affect different components of female fitness such as seed production and flower number, or resource levels may influence floral attractiveness with possible implications for pollination (Zimmerman 1983; Zimmerman and Pyke 1988; Campbell and Halama 1993; Munoz et al. 2005; Burkle and Irwin 2008). Most studies of pollen limitation of natives in invaded sites have compared seed production for plants growing near and at variable distances from competitors (but see Chittka

and Schürkens 2001; Brown et al. 2002; Cariveau and Norton 2009; Bartomeus et al. 2010; Flanagan et al. 2010). However, plants growing near competitors engage not only in competition for pollinators, but also competition for resources. Attributing reductions in seed production to reduced visitation requires the assumption that seed production is solely limited by pollen receipt. However, plants growing near the competitor may produce fewer seeds due to resource constraints resulting from resource competition.

Here, we ask how the invasive forb, *Euphorbia esula* indirectly and directly influences the abundance of the annual native forb, *Clarkia pulchella*. We examined pollinator visitation and the degree to which *C. pulchella* seed production is limited by pollen-receipt in invaded and native-dominated communities. We also examined the direct effects of competition by *E. esula* on *C. pulchella* recruitment and performance. We then used these data to evaluate both indirect and direct impacts of an invasive forb on native plant abundance.

Methods

Study Design

We selected fifteen grassland sites in western Montana. Seven sites were heavily invaded by *E. esula* (Euphorbiaceae) and eight were dominated by native forbs. *E. esula* is a Eurasian perennial that invades grasslands throughout the northern Great Plains and Rocky Mountains. It spreads clonally via rhizomes and often occurs at high densities within invaded sites. It produces abundant inflorescences of reduced female and male flowers (cyathia) beginning in late May and continuing for several weeks. Copious nectar is produced by glands at the base of each inflorescence and is attractive to a variety of pollinators, including native bees, honeybees *(Apis mellifera),* and flies. Native sites were characterized by abundant cover of the

perennial bunchgrasses (*Festuca idahoensis* and *Festuca scabrella)*. Abundant forbs included

Balsamorhiza sagittata (Asteraceae), *Achillea millefolium* (Asteraceae), and *Lupinus sericeus*

(Fabaceae). At all sites there were low numbers of other exotic species that co-flower with *C.*

pulchella. We performed different experiments and observations across the fifteen sites

(Appendix A).

  C. pulchella is a native annual, distributed from British Columbia south through Northern

California and east through South Dakota. *C. pulchella* is self compatible, though protandry and

herkogamy promote outcrossing (Lewis 1953). Individuals produce one to fifteen flowers in

mid-summer that are primarily pollinated by solitary bees. Capsules are formed in late July and

early August. Seeds germinate rapidly when exposed to moisture and proper temperatures in the

field, thus the seed bank likely contributes little to population dynamics (Lewis 1953; Newman

and Pilson 1997).

Indirect Effects: Pollinator Visitation and Pollen limitation

  To determine how *E. esula* invasion influenced pollinator visitation to *C. pulchella*, we

grew individuals from seed to flowering in pots and observed pollinator visitation to *C. pulchella*

at six invaded sites and five native sites in 2010. To eliminate the influence of background

variation in *C. pulchella* abundance on visitation, we used only sites that lacked naturally

occurring *C. pulchella* populations. At each site, we placed three arrays 10 m apart from one

another. Each array contained three potted plants which were placed 0.5 m apart. During site

visits, we observed each array for fifteen minutes. Our goal was to visit each site on four

separate days, however this was not possible at all sites due to inclement weather during the

period when *C. pulchella* was in bloom. As a result, one native and one invaded site were

observed on three days, and one native and one invaded site were observed twice. All

observations took place between 22-June-2010 and 29-June-2010, between the hours of 09:00 and 15:00. We observed arrays only on rain-free days when temperatures were greater than 14ºC. Observers sat 1 m from the array and recorded all pollinator visits to *C. pulchella* flowers and the number of open flowers on each plant. Visits were counted only if the insect made contact with reproductive parts of the flower. To estimate pollinator activity at the sites, we hand-netted solitary bees, which were the only observed visitors to *C. pulchella,* within 30 m of the array for 45 minutes following one randomly chosen observation period.

We examined the extent to which *C. pulchella* reproduction was limited by pollen-receipt (i.e., pollen limitation) in 2011 at three native sites and three invaded sites. Pollen limitation is generally assessed by comparing seed production under ambient pollen loads to seed production under experimentally supplemented pollen loads. We used naturally-occurring *C. pulchella* at one invaded and all three native sites. At two invaded sites we used plants that recruited from seeds that were experimentally added to plots in fall 2010 (see below). At the three native and three invaded sites, we haphazardly selected plants and randomly assigned half of these to receive supplemental pollen. On these we marked one receptive flower and supplemented pollen to the stigma and on the remaining plants we marked a single flower but did not add pollen. At all three native sites and one invaded site we selected sixty plants, while at two invaded sites with low *C. pulchella* abundance, we were only able to select 28 and 21 plants, respectively. At each site, we collected supplemental pollen from dehisced anthers of plants at least 5 m from recipient plants. We collected marked fruits prior to dehiscence in late July, and counted the number of filled seeds. Within a plant, supplementing pollen to a single flower did not affect subsequent flower production (J.D. Palladini, unpublished data) and progressive hand pollination of all flowers in a congeneric species did not influence flower production (Moeller 2004),

suggesting that our estimates of pollen limitation may be minimally biased by resource reallocation. However, it remains possible that *C. pulchella* plants reallocated resources to supplemented flowers, in which case our design will tend to overestimate the magnitude of pollen limitation (Knight et al. 2006).

Direct Effects: Recruitment and Performance

We examined the direct competitive effects of *E. esula* on *C. pulchella* recruitment, performance and ultimate abundance by adding seeds to subplots embedded within 1 m radius circular plots that either had *E. esula* experimentally removed from them or left intact. We placed twenty plots at randomly selected locations within two heavily invaded sites lacking natural *C. pulchella* populations. Ten of these plots were randomly assigned to receive the *E. esula*-removal treatment, while the other half remained non-manipulated controls. Pre-treatment density of *E. esula* stems did not differ between experimental and control plots ($F = 2.213$, $P = 0.143$), or between sites ($F = 2.056$, $P < 0.157$). We removed *E. esula* from treatment plots in spring 2010 when *E. esula* was just beginning to appear above ground. Because *E. esula* establishes dense underground rhizomes, manual removal would have greatly disturbed soils, thus we used chemical means of removal. We applied 1.8% glyphosphate (Roundup RTU®) to the tips of *E. esula* stems within a 1.0 m radius circular plot while avoiding damage to other plant species present, and removed dead *E. esula* stems from the site.

In each plot, we established three evenly spaced 15 cm x 15 cm subplots separated by at least 15 cm. In fall 2010, we added ten, fifty or 100 *C. pulchella* seeds to a randomly selected subplot. These seed density levels were designed to encompass densities lower than, approximately equal to, and greater than observed seed production in invaded sites. We purchased seeds from a local native seed supplier (who grew his plants from seeds collected at a

source population that was approximately 20 km from our study sites). In spring and summer 2011 we recorded the number of recruits that survived to flower in each subplot. As well, we measured plant height (at peak plant size) to the nearest cm and counted the total number of flowers produced per plant. For comparison, we also counted the number of flowers produced on plants in one additional *C. pulchella* population that naturally occurred with *E. esula,* and at three native sites.

Analysis

All analyses were run with the statistical software R 2.13.2 (R Development Core Team 2011). Gaussian mixed effects models were fitted with the function lme (library: nlme; Pinheiro et al. 2011) and Poisson mixed effect models were fitted with the function lmer (library: lme4; Bates et al. 2011).

We examined whether the number of pollinator visits per flower differed between invaded and native sites using a generalized linear mixed effects model (GLMM, Gaussian family/identity link), with observation date nested in array, and array nested in site. We used a Mann-Whitney U-test to examine possible differences in bee activity between invaded and native sites. To determine whether the supplemental pollen treatment increased seed production and whether invasion influenced the magnitude of pollen limitation, we used GLMM (Gaussian family/identity link) with treatment, invasion status, and the interaction between the two as fixed effects, and site as a random effect.

We used GLMM (Poisson family/log link) to determine the extent to which increasing seed density increased *C. pulchella* recruitment, and whether the presence of *E. esula* influenced this pattern. *E. esula* removal was a whole-plot factor and seed density was a subplot factor in a split-plot design. Seed density was included as a categorical variable rather than a continuous

variable due to the small number of seed densities used. We included seed density, treatment (*E. esula*-removal v. control), and density × treatment as fixed effects, and plot in treatment in site as a random effect. We determined whether *C. pulchella* height was influenced by the *E. esula* removal treatment by using GLMM (Gaussian family/identity link), with treatment as a fixed effect and plot nested in site as a random effect. A similar model was used to examine differences in flower number among *E. esula* removal and control treatments, as well as native sites. Testing fixed effects was done with Wald tests. Finally we used GLMM (Gaussian family/identity link) to examine whether *C. pulchella* height and flower number were related to *C. pulchella* density, with the number of recruits in each subplot as a fixed factor and plot in site as a random factor.

We then used these data to evaluate the influence of indirect and direct effects of *E. esula* on *C. pulchella* abundance. Where treatment effects were significant, we used treatment means to simulate amelioration of pollen limitation (i.e., indirect effects) and direct competitive effects in combination and alone to determine how the number of predicted recruits is influenced by *E. esula*. We also compared these to the expected number of recruits in native sites using observed flower number and ambient seed production in native sites. Because seed additions were not performed in native sites, we used the observed recruitment estimated from seed additions in invaded sites. Because our calculations begin with a single plant, the number of recruits expected is equivalent to the population growth rate.

Results

Indirect Effects: Pollinator Visitation and Pollen limitation

C. pulchella growing with *E. esula* received 90% fewer pollinator visits ($\bar{x} = 0.07$ visits per flower in a 15 minute period) compared to plants growing in native-dominated sites ($\bar{x} = 0.72$

11

visits per flower; $F_{(1, 9)} = 6.54$, $P = 0.0001$, Fig. 1A), despite the fact that there was no difference in bee activity between *E. esula* invaded and native sites (Z = 16.0, N=10, $P = 0.547$).

Flowers receiving experimentally supplemented pollen produced more seeds than those receiving ambient pollen ($F_{(1, 274)} = 4.15$, $P = 0.042$, Fig. 1B). However, plants were not more pollen-limited in invaded than uninvaded sites (treatment x invasion status: $F_{(1, 273)} = 0.05$, $P = 0.816$), and across sites, supplemental pollen increased seed production by only 10.6%. Pollen limitation tended to be more variable at invaded sites than at native sites; one invaded population was strongly pollen-limited while the other two invaded populations showed little or no evidence of pollen limitation. In contrast, at native sites the increase in seed production with supplemental pollen was consistent among sites. There was no overall difference in the number of seeds produced per flower between invaded and native sites ($F_{(1, 4)} = 0.98$, $P = 0.376$).

Direct Effects: Recruitment and Performance

Our *E. esula* removal treatment was effective in reducing the number of *E. esula* stems in treatment plots compared to control plots ($t_{(10)} = -4.71$, $P < 0.001$). The number of *C. pulchella* plants recruiting and surviving to flower across treatments increased from ten to fifty seeds added ($z = -3.68$, N=51, $P < 0.001$), and differences in recruitment between fifty and 100 seed subplots were non-significant ($z = 1.06$, N = 51, $P = 0.287$). Removal of *E. esula* increased recruitment across seed densities ($z = -2.54$, N = 51, $P = 0.011$, Fig. 2) but there was no significant seed density × removal treatment interaction. The mean recruitment rate across seed densities in *E. esula* removal treatments was 0.026% compared to 0.053% in control plots.

E. esula strongly affected the performance of *C. pulchella* target plants (Fig. 3). Individuals in *E. esula*-removal plots averaged 12.9 cm in height, compared to 7.5 cm in plots containing *E. esula* ($F_{(1, 11)} = 24.96$, $P < 0.001$). Flower production also differed among

treatments ($F_{(2, 15)} = 10.75$, $P = 0.001$). Flowering was greater in *E. esula* removal plots compared to control plots (3.8 and 1.1 flowers per plant, respectively, $t_{(15)} = -4.2$, $P < 0.001$), and was greater still in native sites (4.82 flowers per plant, $t_{(15)} = 3.19$, $P = 0.006$). There was no evidence of density-dependent reductions in *C. pulchella* performance. Neither height ($F_{(1, 101)} = 0.33$, $P = 0.562$) nor flower number ($F_{(1, 101)} = 1.74$, $P = 0.190$) were related to the number of *C. pulchella* plants in each subplot.

Comparison of direct and indirect effects

Using treatment means to simulate amelioration of pollen limitation (i.e., indirect effects) in invaded sites only increased potential recruitment from 1.22 plants to 1.37, an increase of 12.3% (Table 1). In contrast, using treatment means to simulate amelioration of direct competitive effects on flower number and recruitment rates resulted in expected recruitment of 8.25 plants, an increase in of 574%.

Discussion

The sharing of pollinators with co-flowering species has the potential to reduce or even increase pollinator visitation (Levin and Anderson 1970; Waser 1983; Campbell 1985; Moeller 2004; Ghazoul 2006). While interactions between natives that share pollinators with invasive species, on average, reduce visitation and seed production (Morales and Traveset 2009), whether this indirect competition is meaningful for native plant populations has remained untested. This is particularly important in the case of interactions between native and invasive plant species, because direct competitive effects of invasive plants are often strong (Levine et al. 2003; Maron and Marler 2008). *E. esula* competes with *C. pulchella* for both pollinator visits and resources. However, because plants were only weakly pollen-limited, competition for pollinators has

limited potential to influence *C. pulchella* abundance. In contrast, competition for resources strongly reduced both the number of *C. pulchella* plants that recruit into the population and flower production. Ameliorating direct effects of resource competition on recruitment and adult performance could increase future abundance by 574%.

The number of studies demonstrating competitive effects of exotics on natives for pollinators has increased dramatically over the last decade (reviewed in Morales and Traveset 2009; Bartomeus et al. 2010; Flanagan et al. 2010; McKinney and Goodell 2011; Takakura et al. 2011). However, we have lacked studies examining whether changes in pollinator visitation and its effects on seed input have significant impacts on plant abundance. Changes in seed production may not translate to changes in plant abundance due to a number of compensatory, density-dependent processes (Ashman et al. 2004; Price et al. 2008; Feldman and Morris 2011; Horvitz et al. 2011). We found that pollinator visitation to *C. pulchella* was greatly reduced in sites containing *E. esula* compared to native-dominated sites. Reductions in visitation could contribute to reduced seed production in invaded sites, because in general, reproduction of *C. pulchella* is limited by pollen receipt, and because increases in seed input increased *C. pulchella* recruitment. However, because the magnitude of pollen limitation in our system is relatively weak and because *E. esula* suppresses *C. pulchella* recruitment, increases in abundance are small.

Across sites, supplemental pollen increased seed set for *C. pulchella* by only 10.6%, compared to an average increase of 42% for studies generally examining the magnitude of pollen limitation (Ashman *et al.* 2004). Few studies investigating the influence of plant invasion on seed production have estimated pollen limitation. Rather, most studies have compared seed production for natives growing near and at varying distances from an invader. However, these results may be confounded with direct effects of competition for soil resources. Reductions in

seed production may result from competition for pollinators; however it is also possible that resource competition limits seed production. Of the few studies using potted plants to isolate indirect effects of competition for pollinators, results have ranged from no effect on seed set (Cariveau and Norton 2009; Bartomeus et al. 2010) to reductions of 25% to 40% (Chittka and Schürkens 2001; Brown et al. 2002; Flanagan et al. 2010).

The relatively small effect of supplementing pollen to *C. pulchella* may be the result of self-pollination in invaded sites where visitation was rare. Though *C. pulchella* has morphological features that promote outcrossing, flowers may self-pollinate in the absence of pollinators (Newman and Pilson 1997), and competition for pollinator services can select for self-pollination (Levin 1972; Fishman and Wyatt 1999; Moeller and Geber 2005). Both reduced visitation and interspecific pollen transfer have the potential to select for traits that favor self-pollination (Fishman and Wyatt 1999; Knight 2004) such as reduced herkogamy (spatial separation of anthers and stigma), reduced dichogamy (temporal separation of anther dehiscence and stigma receptivity), and reduced corolla size (Wyatt 1983). If rates of self-pollination are greater in invaded sites where pollinator visits are rare, there may be consequences for population persistence in invaded sites due to reductions in genetic variation. Newman and Pilson (1997) found that genetic variation strongly influenced germination and survival for *C. pulchella,* and that decreased genetic effective population size increased the probability of population extinction over only three generations.

We assessed pollinator visitation and pollen limitation in different years (2010 and 2011, respectively). It is possible that the degree of pollen-limitation did not differ between invaded and native sites in 2011 because pollinator visitation patterns between site types were equivalent in 2011. However, we tested whether relative pollinator visitation rates were consistent between

years by re-observing visitation at a subset of sites in 2011 (three native and three invaded), and found that visitation rates did not differ between years (J.D. Palladini, *unpublished data*). As well, *C. pulchella* abundance was notably lower at two invaded sites used to assess pollen limitation. Because floral density can influence pollination success (Groom 1998; Knight 2003), it is possible that the pollen limitation we observed was influenced by plant density. However, the site with the lowest abundance showed no evidence of pollen limitation, suggesting the plant abundance per se, is not a primary factor influencing pollen limitation across sites.

In contrast to indirect effects, direct effects of competition with *E. esula* were strong. Both recruitment and the number of flowers per plant increased when *E. esula* was removed. Vilá et al. (2011) found that only 14% of field investigations of impacts of exotic plant used manipulative experiments. However, when examined, exotic plants significantly reduced growth and reproduction of native plant species (Levine et al. 2003; MacDougall and Turkington 2005; Biggerstaff and Beck 2007; Coleman and Levine 2007; Denoth and Myers 2007; Cipollini et al. 2008; Vilá et al. 2011). Our study is unique in that we use experimental removals to examine not only the impacts of *E. esula* on adult plant fitness but also its effects on subsequent recruitment and abundance of *C. pulchella*. Strong resource competition reduced recruitment and also lowered flower number, with much greater consequences for whole plant seed production than limited pollen receipt, consistent with research demonstrating that resource levels strongly influence plant fitness (Campbell and Halama 1993; Munoz et al. 2005; Ne'eman et al. 2006; Burkle and Irwin 2008). Because reduced recruitment and adult performance are likely outcomes of competition between exotics and invasive forbs, even changes in the number of seeds per fruit of 25% to 40% (Chittka and Schürkens 2001; Brown et al. 2002; Flanagan et al. 2010), may result in only trivial changes in plant abundance. For example, in order for the

effects of competition for pollinators to equal effects of resource competition, *E. esula* would need to reduce ambient seed production by *C. pulchella* to 7 seeds per fruit, 83% fewer than we observed.

This study demonstrates that indirect interactions involving competition for shared pollinators between and invasive and native plant may have consequences for plant abundance. However, these effects are weak compared to the effects of direct resource competition. Thus, restoration efforts that ameliorate resource competition would likely benefit *C. pulchella.* Future studies that examine the relative importance of indirect and direct effects will provide further insight in to the mechanisms by which invasives reduce native abundance and enable managers to better predict how native plants will respond to restoration efforts.

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Table 1. Reproductive output, recruitment rate, and projected future number of recruits (assumes N_t=1) for *C. pulchella* in native and invaded plots with ambient or supplemental pollen (i.e., with and without competition for pollinators) and when *E. esula* is present or removed from within 1.0 m (i.e., with and without competition for resources). Recruitment is the mean number of seeds added that survived to flower across seed densities. Because no seed additions were performed in native sites, recruitment rate from *E. esula* removal plots in invaded sites is used.

Invasion status	± supp. pollen	± *E. esula*	Flowers/ plant	Seeds/ flower	Seeds/ plant	Recruitment	Nt+1
Native	-	-	4.82	41.28	198.97	0.053	10.55
Invaded	-	+	1.14	41.28	47.06	0.026	1.22
Invaded	+	+	1.14	46.38	52.87	0.026	1.37
Invaded	-	-	3.77	41.28	155.63	0.053	8.25
Invaded	+	-	3.77	46.38	174.85	0.053	9.27

Figure 1 Influence of invasion by *E. esula* on: a) mean number of pollinator visits per *C. pulchella* flower over a fifteen minute observation period and b) seeds produced in invaded and native sites given ambient and supplemental pollen receipt. Solid bars are mean seed production under ambient conditions; open bars are seed production when pollen is supplemental. Vertical lines indicate +1 SEM
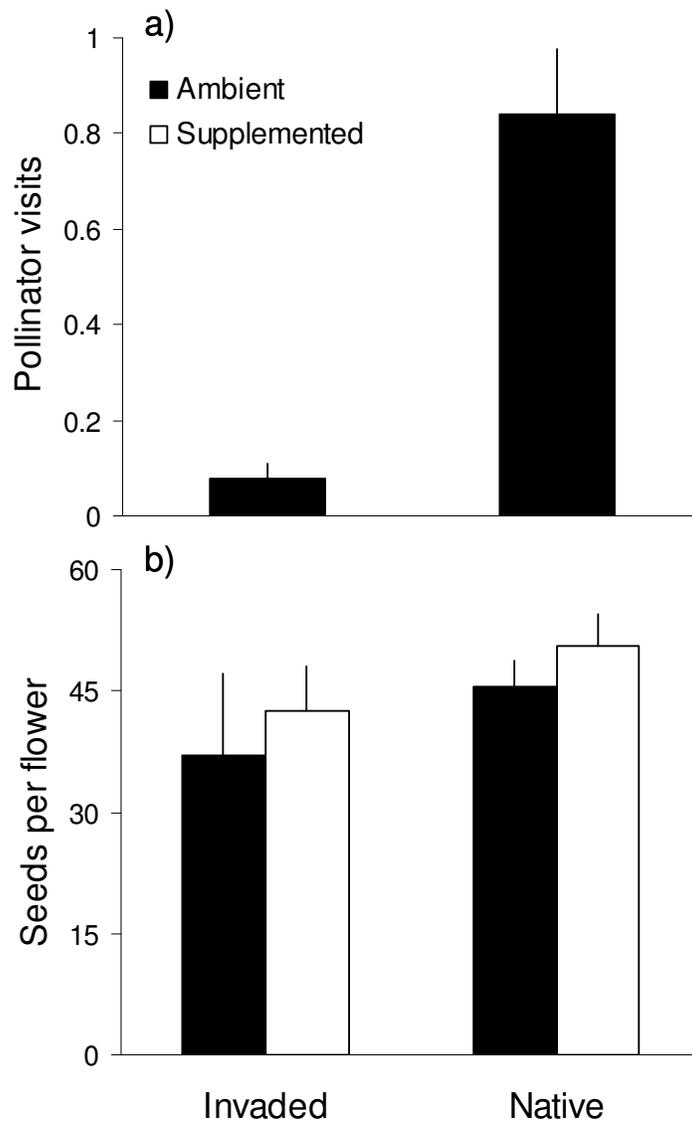
Figure 2 Number of *C. pulchella* plants (mean ± SE) surviving to flower as a function of seed density in plots with *E. esula* removed or present
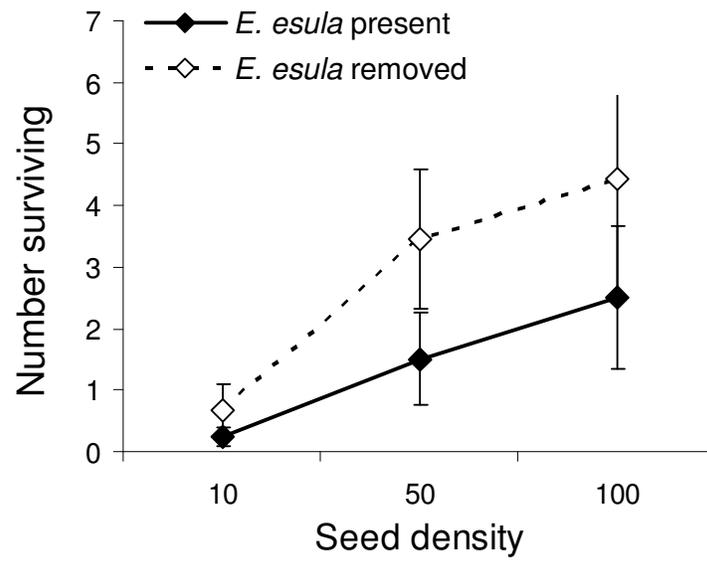
Figure 3 Response of *C. pulchella* to removal of *E. esula* a) height, b) flower number, as well as

flower number in native sites. Bars are means + SE
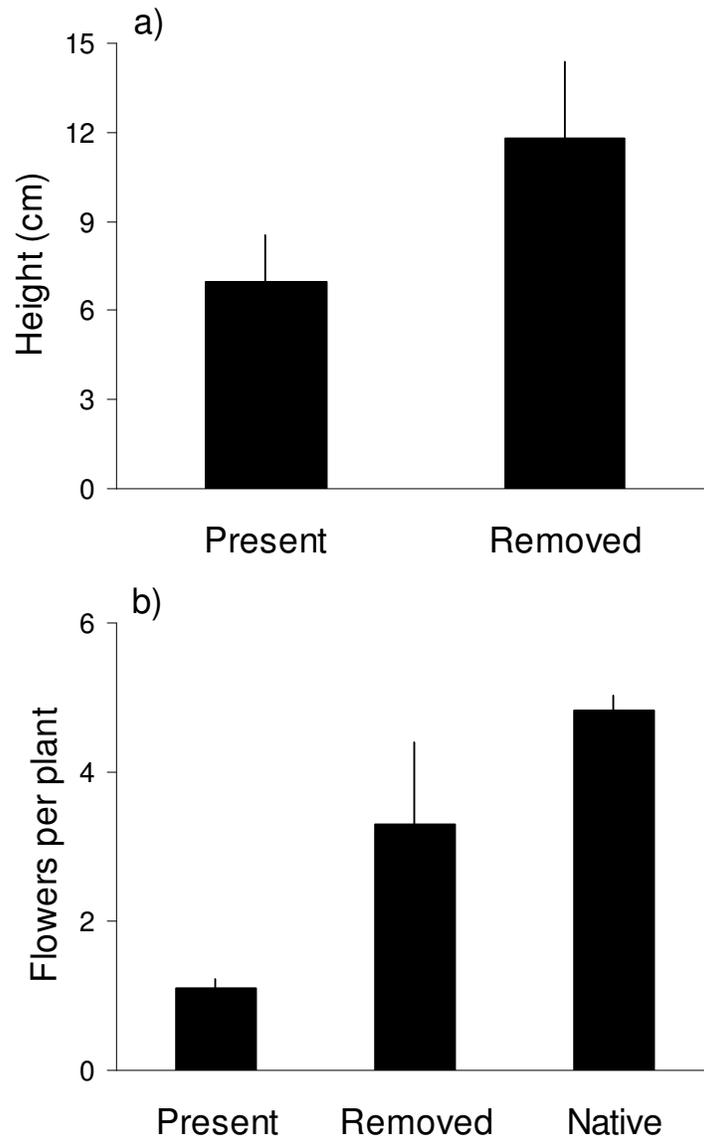
Appendix A

Site Name	Invasion Status	Latitude (°N)	Longitude (°W)	Pollinator observations	Pollen supplementation	Recruitment rate	Flower number
Bandy Ranch	native	47.04	113.14	X			
Big Madison	native	46.57	114.23		X[b]		X
Blackfoot-Clearwater Game Range	native	47.02	113.21	X			
Elk Ridge	invaded	46.54	113.56	X			
Grant Creek 1	invaded	46.56	114.00	X	X[a]	X	X
Grant Creek 2	invaded	46.56	114.01		X[b]		X
Kleinschmidt Flat	native	46.58	113.03	X			
Lower Madison	native	46.57	114.24		X[b]		X
Mountain View	invaded	46.53	113.58	X			
National Wildlife Refuge	invaded	46.55	114.00		X[a]		
Ninemile Praire	native	46.56	113.27	X			
Petty Pasture	native	45.56	114.25		X[b]		X
Rattlesnake	invaded	46.54	113.58	X		X	X
Salmon Lake	invaded	47.06	113.25	X			
Schroeder Settlement	native	46.41	114.00	X			

[a] experimental population
[b] naturally-occurring population

CHAPTER TWO

EXOTIC PLANT INVASION REDUCES ABUNDANCE, RICHNESS AND DIVERSITY OF

NATIVE BEES IN MONTANA GRASSLANDS

Abstract:

Invasive plants are a great threat to rare insect species in North America and dramatically alter interactions between plants and pollinators. However, very little is known about how plant invasion affects native bees, which are the dominant pollinator in most ecosystems. Plant invasion could influence bees in opposing ways. Because bees are often more abundant in sites with greater floral resources, invasion by exotic plants that offer pollen and nectar rewards could benefit native bees by increasing overall floral resource availability. However, floral community composition plays an important role in influencing bee richness and diversity. Exotic plant invasion often reduces native plant abundance and diversity, thus many native pollinators may be harmed by the loss of native floral host plants. We compared native bee abundance, richness, diversity, and community composition in 20 western Montana grasslands, half of which were dominated by native-forbs and half of which were heavily invaded by *Euphorbia esula,* an exotic forb that is highly attractive to many native pollinators. We found that bee communities differed in composition between the two site types, and invaded sites had lower abundance, richness and diversity. Thus, despite the use of *E. esula* floral resources in invaded sites, the effect of invasion on bee communities was negative.

Introduction:

Bees are essential pollinators in many ecosystems (Michener 2000) and provide valuable ecosystem services in both native and agricultural landscapes (Buchmann and Nabhan 1996, Linder 1998, Klein et al. 2007). Widely reported declines in native bee populations have raised awareness of the importance of maintaining native pollinators and the ecosystem services they provide (Buchmann and Nabhan 1996, Allen-Wardell et al. 1998, Sao Paulo Declaration on Pollinators 1999, Biesmeijer et al. 2006, U.S. National Resource Council 2007, Potts et al. 2010). These declines and related efforts to maintain native bee populations in the face of environmental change highlight the importance of understanding the basic factors that influence bee communities. Knowledge of how changes in floral communities influence bees will help predict the response of bees to environmental changes such as exotic plant invasion, and will assist in attempts to maintain and restore bee communities.

Because bees are wholly dependent on floral resources for food, bee abundance is often positively correlated with floral abundance (Heithaus 1974, Banaszak 1996, Potts et al. 2003a). Indeed, floral resources are commonly thought to limit bee populations (Williams and Kremen 2007, Roulston and Goodell 2011, Palladini and Maron forthcoming-b), although nest availability can also be limiting (Steffan-Dewenter and Schiele 2008). If bee populations are limited by floral resources and bee abundance increases with floral abundance, then disturbances that serve to increase available floral resources may benefit native bees. One such disturbance is exotic plant invasion. Because invasive plants are often present at high densities, those offering abundant nectar and pollen may increase the overall availability of floral resources within a site (Westphal et al. 2003, Kleijn and van Langevelde 2006, Bjerknes et al. 2007, Tepedino et al. 2008). Moreover, native bees often forage on the flowers of invasive forbs (e.g., Tepedino et al.

2008, Williams et al. 2011), suggesting that high density populations of invasive forbs could subsidize bee populations, thereby increasing their abundance (Bjerknes et al. 2007, Tepedino et al. 2008) or at least maintain their populations in disturbed landscapes where intact natural habitat has been lost (Williams et al. 2011).

However, bee communities might be influenced by more than abundance of floral resources alone; the species composition of the floral community might critically influence bee diversity. For example, we know that increasing plant diversity increases arthropod herbivore diversity (Pimental 1961, Root 1973, Siemann et al. 1998). Similarly, bee richness and diversity are often correlated with floral richness and diversity (Heithaus 1974, Gathmann et al. 1994, Banaszak 1996, Potts et al. 2003a). Thus, to the extent that invasive plants reduce native plant richness (Levine 1999, Vilà et al. 2011) and shift communities from diverse mixed native assemblages to species poor assemblages dominated by a single exotic species (Lonsdale 1999, Ortega and Pearson 2005), invasion could have strong impacts on bee communities, despite increasing total resource availability. Invasive plants are recognized as one of the leading threats to the conservation of rare insects, principally because they replace native species upon which insects feed (Wagner and Van Driesche 2010).

Floral community composition plays a large role in organizing bee communities (Potts et al. 2003a) in large part because individual bee species have characteristic foraging requirements (O'Toole and Raw 1991). In particular, trophic specialists that harvest pollen from a small group of plant taxa could be particularly at risk at sites that are highly invaded. While generalist bees may respond positively to increases on overall floral abundance, specialists, which can account for 15% to 60% of bee species in a given community (Minckley and Roulston 2006), will respond only to changes in the abundance of their particular host species (Williams et al. 2010).

Thus, while some bee species may benefit from the increase in floral resources that can accompany plant invasion, many specialist native bee species will be unable to take advantage of the floral resources provided by invasive plants (Traveset and Richardson 2006, Stout and Morales 2009, Potts et al. 2010). Moreover, many specialists may be indirectly harmed by plant invasion if it results in the loss of native forbs on which they are dependent (Stout and Morales 2009, Wagner and Van Driesche 2010, Roulston and Goodell 2011). Finally, even generalist bees may not benefit from invasion because of phenological mis-matches between invasive forbs and native bees (Stout and Morales 2009) or because nectar and pollen may be morphologically inaccessible (Corbet et al. 2001, Goulson 2003, Liu and Pemberton 2009), chemically protected, or of lower nutritional quality (Stout and Morales 2009).

The goal of this study is to determine whether floral resources offered by an invasive forb benefit or maintain native bee communities, or alternatively, whether declines in native forb richness that accompany invasion indirectly harm native bee communities. We examined native bee communities in grasslands of western Montana in sites invaded by *Euphorbia esula* (Euphorbiaceae), which is highly attractive to a variety of pollinators, and sites dominated by native forbs, and asked how *E. esula* invasion influences native bee abundance, richness, diversity, and community composition.

Methods:

Study System

Euphorbia esula is a Eurasian perennial that has invaded millions of hectares throughout the northern Great Plains and Rocky Mountains (Duncan *et al.* 2004). It spreads clonally via rhizomes and often occurs at high densities within invaded sites and can dramatically reduce the

abundance and diversity of native plants (Atwater et al. 2011). It produces abundant

inflorescences of reduced female and male flowers (cyathia) beginning in late May and

continuing for several weeks. Glands at the base of each inflorescence produce copious amounts

of nectar that attracts a variety of pollinators including native bees, honeybees (*Apis mellifera*),

and flies. Pollinator visitation to native plants that co-occur with *E. esula* is often reduced

(Larson et al. 2006, Montgomery 2009, Palladini and Maron forthcoming-a), which could be the

result of pollinator preference for *E. esula* compared to native flowers (e.g., Montgomery 2009),

reduced attraction to floral patches that contain *E. esula,* or overall reductions in pollinator

abundance in invaded sites. *E. esula* invasion occurred relatively recently in the Missoula Valley,

and it is estimated that dense patches becamse common only within the last 15 years (Atwater et

al. 2011).

Twenty grassland sites were selected on lands owned or managed by the Lolo National

Forest, Montana Fish, Wildlife and Parks, Missoula County Parks and Recreation, the University

of Montana, the National Wildlife Federation, and private landowners. Sites supported

intermountain bunchgrass plant communities with scatterings of ponderosa pine (*Pinus

ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*). All sites were greater than 10 hectares and

in many cases were bounded by dense, contiguous mixed conifer forest, agricultural lands, or

urban areas. Cattle grazing has occurred historically at the sites but does not occur presently.

Ten sites were heavily invaded by *E. esula* and contained a suite of other exotic forbs such as

Sisymbrium altissimum (Brassicaceae), *Tragopogon dubius* (Asteraceae), and *Linaria dalmatica*

(Scrophulariaceae), as well as exotic annual grasses (*Bromus tectorum, Bromus japonicus*).

Native forbs were present at invaded sites, though many occurred at low abundance. Ten sites

were native dominated, with the forbs *Balsamorhiza sagittata* (Asteraceae), *Achillea millefolium*

(Asteraceae), *Lupinus sericeus*. (Fabaceae), *Phacelia hastata* (Hydrophyllaceae), and *Erigeron*

spp. (Asteraceae) being the most common. The native perennial bunchgrasses (*Festuca*

idahoensis and *Festuca scabrella*) were also abundant in native-dominated sites. Sites were

sampled once in either 2009 or 2010. No sites were sampled during both years. While we

cannot rule out that invaded and native sites differ in some aspect that has influenced the pattern

of invasion and thus underlying bee communities, invaded and native sites were both distributed

across the study area and invaded and native sites often occurred within 1.5 km of one another.

We chose native sites where the general plant community resembled the native component

remaining in invaded patches. All sites were southwest facing, moderately sloped, and ranged in

elevation from 1000 to 1300 m. Because *E. esula* reproduction is primarily clonal, we presume

that sites lacking *E. esula* represent suitable habitat and that populations simply have yet to

establish.

Vegetation surveys

To quantify gross differences in floral abundance and richness among sites, we estimated

the abundance of native and exotic flowers (or inflorescences for species that produce reduced

flowers, e.g. Asteraceae, *Euphorbia*) inside a circular plot (radius=100 m) in the center of each

site. Because bees are likely to respond only to large changes in floral abundance (Williams and

Tepedino 2003, Williams and Kremen 2007), we estimated the number of flowers or

inflorescences for each species in abundance categories (0-9, 10-99, 100-999, 1000-4999, 5000-

9999, 10,000-49,999, and > 50,000). These broad categories of floral abundance have been

shown to capture larger-scale variation in forb availability among sites (Williams and Kremen

2007, Palladini and Maron forthcoming-b). Floral abundance was estimated by a single observer

to ensure uniformity across sites and species. We surveyed only those forb species that were in

bloom during the peak of bee activity (mid-May through mid-July), thus early and late season flowering forbs were excluded.

Pollinator surveys

We sampled bees at each site once in early June and again in late June or early July, the period of peak flowering by native plants (and *E. esula*, the dominant exotic). Three parallel 30 m transects spaced 6 m apart were established in the middle of each site, minimizing the distance to site boundaries such as roads, contiguous conifer forest or riparian corridors. On each sampling day, a single collector slowly walked each transect for 30 minutes for a total of 90 minutes of sampling per site. Collectors netted bees from all flowering plants as well as those in flight within 1 m on either side of the transect. We did not collect data on the managed, non-native honeybee (*A. mellifera*). Surveys took place between the hours of 09:00 and 15:00 only on rain-free days when temperatures were greater than 16ºC. Pan traps were not used due to concerns that they overestimate bee abundance at sites with low floral cover (Roulston et al. 2007). Individuals were pinned and identified to the lowest taxonomic level possible. In some cases, a lack of available species keys for difficult groups resulted in identification only to the level of morphospecies. Data from the two sampling periods were pooled prior to analysis.

Analyses:

Unless otherwise noted, all analyses were run with the statistical software R.2.13.2 (R Development Core Team 2011). We used t-tests to examine differences in native, exotic and total forb richness and abundance in invaded and native sites. To examine how bee abundance was affected by *E. esula* invasion, we used a general linear model (Poisson family, log link). To

estimate bee richness at both the genus and species levels, we used sample-based rarefaction. We generated rarefaction curves for each site (1000 sub-samples, EcoSim Professional v1.2d; Entsminger 2012) and then rescaled curves by plotting accumulated richness against the accumulated number of individuals. Rarified richness estimates at common abundance values were compared in invaded and native sites using t-tests, as were observed richness and Shannon-Wiender diversity and evenness indices.

We then examined how floral abundance and richness of exotic and native forbs influenced bee abundance and estimated genus and species-level richness using general linear models. We examined main effects of the following variables, referred to henceforth as "floral resources"): native floral abundance, native forb species richness, exotic floral abundance, exotic forb species richness, total floral abundance, and total forb species richness. Nonsignificant parameters were removed in a stepwise approach from the model until all parameters were significant (Crawley 2002). Abundance models were constructed using a Poisson distribution (log link) while richness and diversity models incorporated a Gaussian distribution (identity link).

Finally, we assessed differences in bee communities at the genus and species-level composition among invaded and native sites using nonmetric multidimensional scaling (NMS) with the Sorensen distance measure (PC-ORD version 6; McCune and Mefford 2011). We used NMS because of its effectiveness in assessing non-normal data sets (McCune and Grace 2002; McCune and Mefford 2011). The slow and thorough "autopilot function" (maximum iterations = 500, 250 real runs, 250 randomized runs) was used to select the optimum dimensionality for the ordination. We used a multi-response permutation procedure (MRPP) with the Sorenson distance measure to explore forb community differences between native and invaded sites. Species and genera present in two or fewer sites and sites containing fewer than three individuals

were discarded, leaving a dataset of 18 sites and 39 species for the species-level ordination and 18 sites and 14 genera for the genus-level ordination.

Results:

Invaded sites had lower native plant species richness ($t = -4.398$, $p < 0.001$) and reduced richness of all forb species ($t = -3.883$, $p = 0.001$) compared to native-dominated sites (Fig. 1A). Reduced native species richness at invaded sites translated to lower native floral abundance ($t = -3.669$, $p = 0.004$) and higher exotic floral abundance ($t = 5.655$, $p < 0.001$, Fig. 1B) than what occurred at native-dominated sites. However, total floral abundance did not differ between invaded and native site types ($t = -0.305$, $p = 0.764$).

We collected 389 individual bees across all sites and identified 46 species and an additional 32 morphospecies (see Appendix A for a list of species observed at each site). Individuals were categorized as morphospecies for the genera *Lasioglossum, Osmia, Nomada, Melissodes,* and *Sphecodes*, for which there is no reliable taxonomic key for western species. Total bee abundance was reduced by 49% in invaded sites ($z = 6.31$, $p < 0.001$, Fig. 2A), as was observed genus (43% decline, $t = -2.72$, $p = 0.014$) and species richness (45% decline, t = -2.552, $p = 0.010$, Fig. 2B). Bee abundance was best predicted by both native forb species richness ($z = 2.413$, $p = 0.015$) and native forb abundance ($z = 4.595$, $p < 0.001$). Observed bee genus and species richness were best predicted by native forb richness ($t = 2.577$, $p = 0.02$; $t = 4.373$, p <0.001).

Estimates of genus-level bee richness generated by rarefaction were reduced in invaded sites when assessed at a common abundance value ($t = -3.276$, $p = 0.004$, Fig. 3A), while differences in estimated species-level bee richness were marginally significant ($t = -1.751$, $p = $

0.052, Fig. 3B). Estimated genus-level bee richness decreased with increasing exotic forb abundance (t = -3.17, p = 0.005). Despite being reduced in invade sites, estimated bee species richness was not significantly related to any measured floral resource characteristic.

Native bee diversity was reduced in invaded sites (t = 1.853, p = 0.040) while evenness did not differ between the two site types (t = -0.164, p = 0.435). Native bee diversity was best predicted by native forb species richness (t = 3.431, p = 0.003). Of 30 rare species that were represented by only a single individual, 21 occurred in native sites while only 9 occurred in invaded sites.

For the bee species-level ordination, the best NMS solution was a 2-dimensional model that captured 70.1% of the variation, with axes 1 and 2 explained 40.1% and 70.1% of the variation, respectively (Fig. 4). Invaded and native sites formed distinct groupings (A = 0.025), p = 0.022), indicating that, at the bee species-level, community composition differed between sites dominated by *E. esula* and sites dominated by native plants. Axis 2 ordination scores were weakly correlated with exotic forb abundance (R = 0.425, p = 0.078). PC-Ord was not able to identify a suitable NMS genus-level ordination solution.

Discussion:

Invasion by *E. esula* significantly reduced the abundance, richness, and diversity of native bees compared to native-dominated sites. Changes in bee communities were associated with diminished native floral resources in invaded sites, and suggest that restoring invaded sites to a condition more closely resembling native-dominated sites would benefit native pollinators. Invasive plants have negative effects on butterflies (Valtonen et al. 2006, de Groot et al. 2007, Skórka et al. 2007, Morón et al. 2009), and hoverflies (Morón et al. 2009), and our observations

add to a growing body of research demonstrating that the overall effect of plant invasion on native bees is also negative. Hopwood (2008) found that roadsides restored with native forbs had greater bee abundance and species richness compared to weedy roadsides. Similarly, Morón et al. (2009) found that invasion by *Solidago canadensis* (Asteraceae) reduced bee abundance, species richness and diversity. Because bees are mobile and can forage over large distances, it is possible that changes in abundance are actually the result of a concentration of foraging efforts in areas with more flowers (Pyke 1984). However, where demographic effects have been examined, proximity to or availability of floral resources has been shown to positively influence solitary bee fecundity (Minckley et al. 1994, Williams and Kremen 2007, Palladini and Maron forthcoming-b), and high levels of invasion reduce nesting, fecundity and population growth in the solitary bee *Osmia lignaria* (Palladini and Maron forthcoming-b).

Our results are not consistent with predictions that invasion by exotic forbs will increase the number of native bees by subsidizing food resources (Tepedino et al. 2008). Though native bees foraged on the flowers of *E. esula*, for both nectar and pollen (J. Palladini and D.Venturella, personal observations), the net influence of invasion on many aspects of the native bee community was negative. Of documented interactions between bees and flowers across sites, 26% involved one of the three most common exotics, *E. esula*, *S. altissimum,* and *T. dubius,* and in the invaded sites 80% of interactions involved an exotic. However, despite using exotic floral resources, their net effect on native bees in our system was negative. Williams et al. (2011) found that, while native bees used exotic floral resources, they did not prefer them over natives. However, the authors found no effect of exotic plant abundance or richness on bee abundance or richness. We found that native bee communities in invaded sites clearly differed from those in native-dominated site, but some bee species persisted despite invasion. It is possible that the

presence of the invasive forb benefits particular native species in highly degraded landscapes altered by agricultural intensification (Williams et al. 2011), urbanization, or invasion by non-rewarding exotic grasses.

We attempted to minimize differences between sites in abiotic factors and underlying plant community type so as to isolate the influence of *E. esula* invasion on bee communities. It is possible that an additional unidentified factor may have confounded the presumed effect of *E. esula*, though sites were chosen to be similar with regards to slope, aspect, elevation, grazing history, and underlying plant community composition. In addition, detection probability may have differed among site types. While habitat structure was similar among site types, the increased abundance of flowers and pollinators in native-dominated sites may have diminished our ability to sample these sites as fully compared to invaded sites, where activity was lower and most observed bees were sampled. In this case, differences between invaded and native-dominated sites may have been underestimated.

There is growing evidence that invasive plants negatively influence native bees (Hopwood 2008, Morón et al. 2009, Hanula and Horn 2011), but the driving mechanism may not be the novelty of the invasive plant *per se*, but rather the associated declines in native forb richness and diversity that often accompany plant invasion (D'Antonio and Vitousek 1992, Levine 1999, Mack et al. 2000, Sala et al. 2000, Alvarez and Cushman 2002, Ortega and Pearson 2005, Flory and Clay 2010, Vilà et al. 2011). By shifting communities from a diverse native assemblages to species poor assemblages (Lonsdale 1999, Ortega and Pearson 2005), invasion results in the loss of bees' floral hosts. Because bee richness and diversity increase with forb species richness and diversity (Gathmann et al. 1994, Potts et al. 2003a), disturbances that result in a loss of native diversity and dominance by a small number of native forb species are likely to

have similar consequences (Traveset and Richardson 2006, Roulston and Goodell 2011). For example, changes in bee abundance and diversity following fire and changes associated with grazing mirror changes in floral abundance and diversity (Carvell 2002, Kruess and Tscharntke 2002, Potts et al. 2003b, Vulliamy et al. 2006, Xie et al. 2008, Kearns and Oliveras 2009).

Reductions in bee abundance that accompany plant invasion could have consequences for pollination of native plants in invaded sites. The majority of plant species rely on animal pollinators for sexual reproduction (Linder 1998) and bees are the major pollinators of wild plants (Linder 1998). Pollinator visitation to native forbs is often reduced in invaded sites (Morales and Traveset 2009, Bartomeus et al. 2010, Flanagan et al. 2010, McKinney and Goodell 2011, Takakura et al. 2011). These reductions are thought to result primarily from enhanced pollinator attractiveness relative to co-flowering natives (Brown et al. 2002, Moragues and Traveset 2005, Munoz and Cavieres 2008, Kandori et al. 2009, Morales and Traveset 2009) and are exacerbated by the numerical dominance of the invader (Bjerknes et al. 2007, Morales and Traveset 2009). Our results suggest that reduced bee abundance in invaded sites or reduced attractiveness of an invaded site relative to native-dominated sites may play an important but presently underappreciated role in diminished visitation, though it is possible that changes in abundance observed.

Invasive plant species are a threat to the biodiversity of a range of native taxa (Duncan et al. 2004, Pimental et al. 2005, Wagner and Van Driesche 2010). Despite the use of exotic forbs by native pollinators (Williams et al. 2011), the loss of native floral resources that accompanies plant invasion can reduce the abundance and richness of native pollinators (Wagner and Van Driesche 2010), with possible consequences for pollination, fitness and persistence of native plants (Bjerknes et al. 2007, Morales and Traveset 2009, Palladini and Maron forthcoming-a).

Our results suggest that restoring invaded sites to a condition more closely resembling native-dominated grasslands will likely benefit native pollinator populations and restore pollination services. However, many efforts to control invasive forb populations result in reductions not only in the target species, but in native forb populations as well (Rinella et al. 2009, Ortega and Pearson 2010). Because bee abundance and richness are positively related to native forb richness and abundance (Heithaus 1974, Gathmann et al. 1994, Banaszak 1996, Potts et al. 2003a), control efforts that further degrade native forb communities will not benefit native pollinators (Pearson and Ortega, Zavaleta et al. 2001, Williams et al. 2011). Restoration programs must seek to reestablish native forbs in invaded sites in order to maintain abundant native bees.

Acknowledgements

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Figure 1. Mean (± 1 SE) species richness (A) and abundance (B) of forbs in invaded and native-dominated. Filled bars represent native forb species and open bars represent exotic forbs.
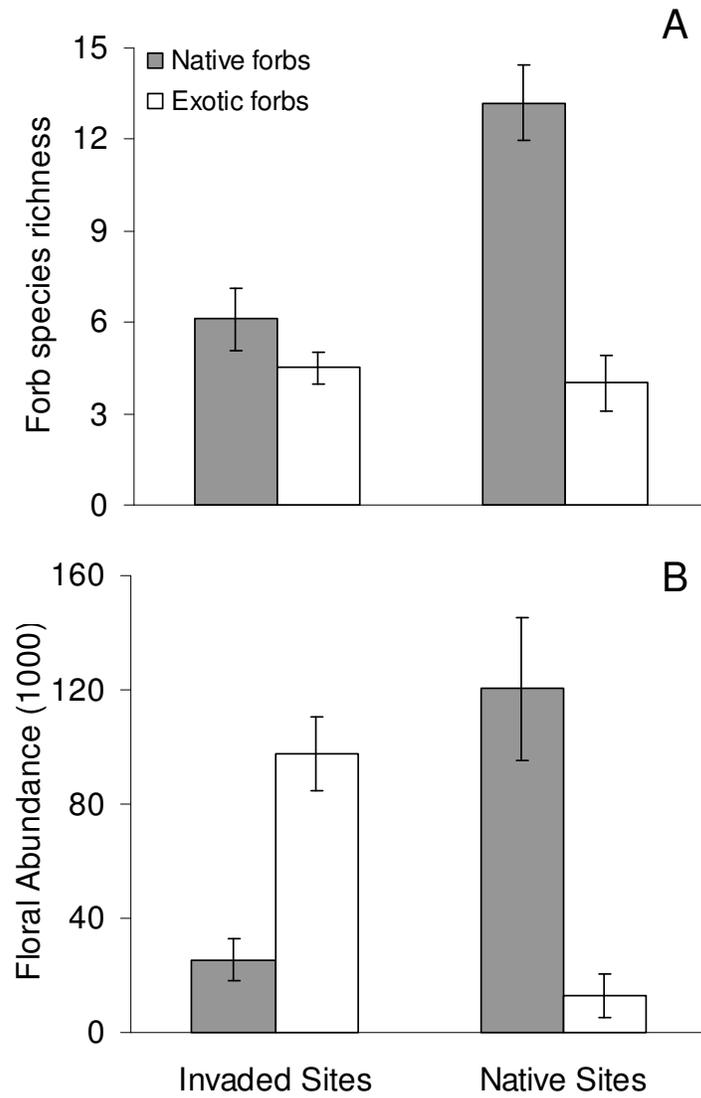
Figure 2. Mean (± 1 SE) total bee abundance (A) and observed richness (B) in invaded and native-dominated sites.
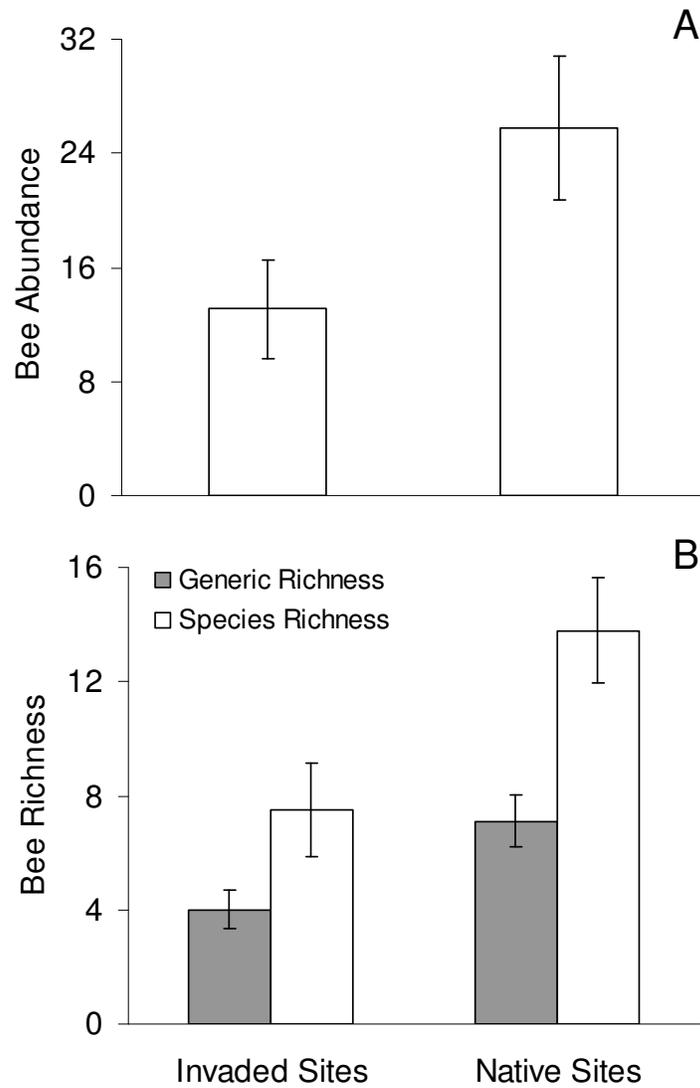
Figure 3. Individual-based rarefaction curves generated separately for each population in native dominated (solid lines) and invaded sites (dashed lines) at the genus (A) and species (B) levels. Grey vertical line indicates common abundance value at which differences in richness were assessed. Inset graphs show the same rarefaction curves at low abundance values to allow better resolution of curves for individual sites.
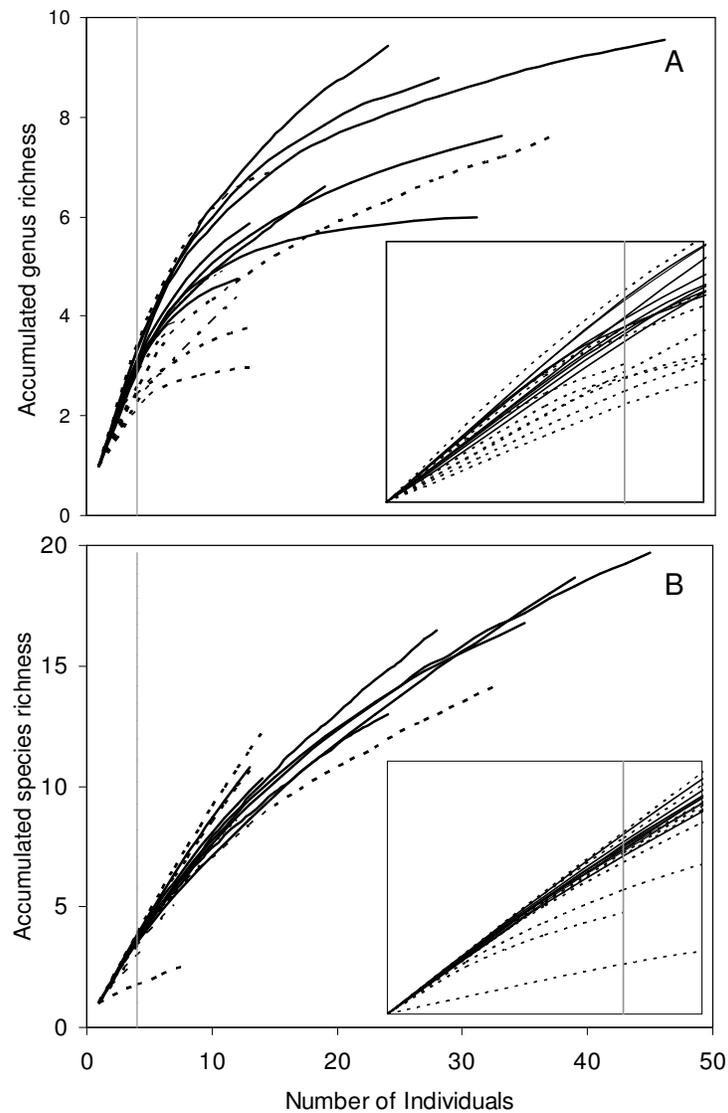
Figure 4. Nonmetric multidimensional scaling ordination of bee species in invaded (filled circles) and native-dominated (open circles) sites. Large symbols represent means (±1 SE).
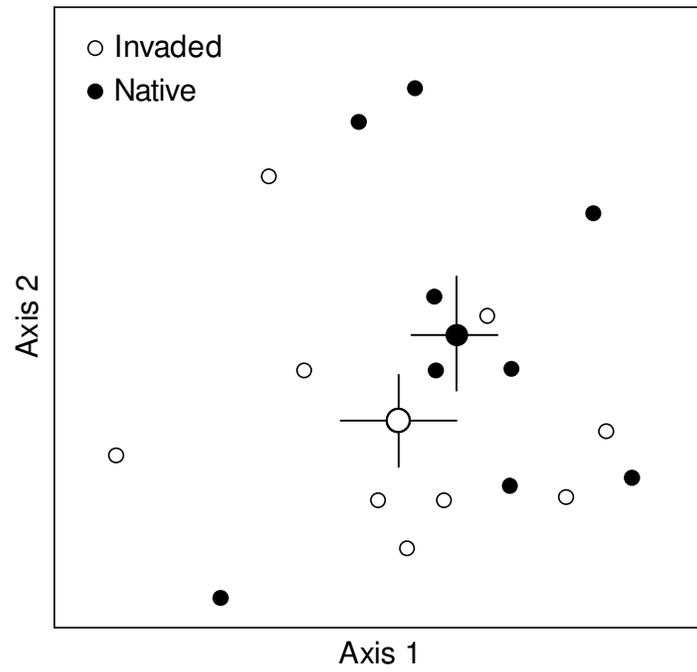
Appendix A. Complete list of species observed in native-dominated sites and sites invaded by *E. esula*

Species	Native	Invaded	Totaled Across Sites
Agapostemon texanus	8	1	9
Agapostemon virescens	0	4	4
Andrena 1	1	9	10
Andrena 2	2	1	3
Andrena 3	1	5	6
Andrena 4	0	1	1
Andrena 5	2	1	3
Andrena 6	0	4	4
Andrena amphibola	1	0	1
Andrena angustitarsata	0	1	1
Andrena carlini	1	0	1
Andrena chapmanae	0	2	2
Andrena crataegi	0	1	1
Andrena cupreotincta	2	1	3
Andrena lupinorum	0	2	2
Andrena milwaukeensis	3	1	4
Andrena miranda	1	0	1
Andrena nigrocaerula	10	4	14
Andrena perplexa	0	2	2
Andrena prunorum prunorum	3	4	7
Andrena robertsonii	0	1	1
Andrena thaspii	4	5	9
Andrena w-scripta	1	3	4
Anthophora ursina	14	0	14
Ashmeadiella cactorum	1	0	1
Bombus 1	1	1	2
Bombus appositus	1	0	1
Bombus bifarius	6	3	9
Bombus huntii	0	1	1
Bombus insularis	5	4	9
Bombus nevadensis	2	0	2
Bombus ruficinctus	5	0	5
Bombus ternarius	2	0	2
Ceratina 1	0	1	1
Ceratina neomexicana	2	0	2
Chelostoma 1	2	0	2
Coelioxys sadilis	1	0	1
Colletes kincaidii	1	0	1
Dufourea maura	2	0	2
Halictus confusus	2	1	3
Halictus farinosus	1	1	2
Halictus ligatus	6	4	10
Halictus rubicundus	10	4	14
Halictus tripartitus	7	6	13
Hesperapis carinata	5	0	5

Species	Native	Invaded	Totaled Across Sites
Heterosaurus 1	2	0	2
Lasioglossum 1	12	1	13
Lasioglossum 2	8	3	11
Lasioglossum 3	1	1	2
Lasioglossum ssp. Dialictus 1	22	23	45
Megachile apicalis	1	0	1
Megachile dentitarsus	4	1	5
Megachile melanophaea	2	7	9
Megachile perhirta	14	0	14
Megachile pugnata	1	0	1
Melecta pacifica	2	1	3
Melissodes 1	8	0	8
Nomada 1	2	7	9
Nomada 2	0	1	1
Oreopasites sciptuli	0	1	1
Osmia 1	9	0	9
Osmia 2	1	0	1
Osmia 3	18	3	21
Osmia 4	1	0	1
Osmia 5	5	0	5
Osmia 6	3	0	3
Osmia 7	1	0	1
Osmia 8	0	1	1
Osmia 9	0	2	2
Osmia 10	4	0	4
Osmia 11	1	0	1
Osmia 12	1	0	1
Panurginus 1	11	0	11
Psuedopanurgus nebrascensis	1	0	1
Sphecodes	1	0	1
Sphecodes 2	1	1	2
Stellis labiata	1	0	1
Stellis lateralis	1	0	1

CHAPTER THREE

DEMOGRAPHIC RESPONSES OF A SOLITARY BEE TO FLORAL RESOURCE

GRADIENTS CREATED BY NATIVE AND INVASIVE PLANTS

Abstract: Native bee abundance has long been assumed to be limited by floral resources. This paradigm has been established in large measure because more bees are often found in areas supporting greater floral abundance. However, whether enhanced bee abundance is due to their attraction to resource-rich sites or greater local demographic performance in sites supporting high floral abundance is usually unknown. Factors other than floral resources per se, such as availability of nest sites, pressure from natural enemies, or whether floral resources are from a mixed native or mostly monodominant exotic assemblage might influence demography and hence abundance. We examined how the demography of the native solitary bee *Osmia lignaria* varied along a gradient in floral resource abundance. We released male and female bees alongside a nest block at 27 grassland sites in western Montana (USA) that varied in floral abundance and extent to which they were invaded by exotic forbs. We monitored nest construction and the fate of eggs and larvae within each nest. The number of nests established was positively related to native forb abundance and was negatively related to exotic forb species richness. Per capita fecundity was positively related to native forb species richness. In sites with abundant native forbs, the brood parasite *Tricrania stansburyi*, was a significant cause of offspring mortality. Despite increased parasitism in high-resource native-dominated sites, a marginally positive relationship between native forb species richness and bee population growth rates remained. Together these results suggest that floral resources can positively influence bee demography, although floral identity can partially offset this effect.

Introduction

Bees are the primary pollinators in most terrestrial ecosystems (Michener 2000) and provide valuable ecosystem services in both native and agricultural landscapes (Buchmann and Nabhan 1996, Linder 1998, Klein et al. 2007). Despite their importance, we know surprisingly little about how fundamental factors such as food availability and natural enemies influence bee population dynamics. Floral resources have typically been thought to be of primary importance in affecting bee abundance, yet the demographic effects of floral resources on bees are surprisingly understudied (Palmer et al. 2003, Roulston and Goodell 2011). Determining how floral resources influence particular demographic responses in bees will increase our basic understanding of controls over bee dynamics and assist in our ability to predict bee response to environmental changes such as habitat fragmentation, climate change, and exotic plant invasion (Roulston and Goodell 2011).

The vast majority of work exploring relationships between bees and floral resources has examined differences in bee abundance, species richness, or diversity across sites that vary in floral cover. Although bee abundance generally increases with floral cover (Steffan-Dewenter and Tscharntke 2000, Potts et al. 2003, Westphal et al. 2003, Hopwood 2008), whether these patterns are underpinned by changes in bee demography remains unclear. Correlations between floral abundance and bee abundance do not necessarily imply that bee populations are limited by floral resources. Bees are highly mobile and the observation that more bees forage in areas with more abundant flowers could be the result of foraging preferences (Pyke 1984); bees may simply concentrate their foraging efforts in sites with more flowers. Where demographic effects have been examined, proximity and seasonal availability to floral resources has been shown to positively influence solitary bee fecundity (Minckley et al. 1994, Williams and Kremen 2007).

However, other limiting resources or pressure from natural enemies might influence whether bee populations respond positively to an increase in floral resources. Indeed, it has been suggested that availability of nest sites may be limiting for some populations of solitary bees (Potts and Willmer 1997, Wuellner 1999, Potts et al. 2005, Steffan-Dewenter and Schiele 2008), and that flowers are frequently available in excess (Steffan-Dewenter and Schiele 2008). Distinguishing between these alternatives requires disentangling behavioral effects of abundant floral resources on bees from their demographic and population-level effects.

Natural enemies may also play an important yet underappreciated role in regulating bee populations. Natural enemies include predators that attack adult bees while foraging (Stubblefield et al. 1993, Galeotti and Inglisa 2001), predators and parasites that attack offspring (Zammit et al. 2008), and cleptoparasites (i.e., brood parasites) that attack the stored food provisions of brood cells (Wcislo and Cane 1996, Schmid-Hempel 1998). While these natural enemies can reduce the performance of honey bees (vanEngelsdorp et al. 2009) and bumble bees (Dukas 2005, Williams and Osborne 2009), the influence of natural enemies on native solitary bee populations have rarely been examined directly (Roulston and Goodell 2011). In particular, brood parasites, which develop inside brood chambers of bees, killing eggs and consuming pollen provisioned for larval development, are often regarded as having little influence on bee dynamics (Wcislo and Cane 1996, Goodell 2003, Steffan-Dewenter and Schiele 2008, Roulston and Goodell 2011), despite often accounting for a large share of documented mortality (Vicens et al. 1994, Steffan-Dewenter and Schiele 2008).

Interestingly, the impacts of brood parasites might either exacerbate or counterbalance the demographic impacts of floral resources on bee demography. For example, scant floral resources increased parasitism of *Osmia pumila* Cresson (Hymenoptera: Megachilidae) by

Sapyga centrata Say (Hymenoptera: Sapygidea) in cage experiments (Roulston and Goodell 2011). Alternatively, if brood parasites themselves depend directly on floral resources, parasitism could counterbalance increases in fecundity. For example, *Tricrania stansburyi* Haldeman (Meloidae), common brood parasites of *Osmia lignaria*, enter bee nests as larvae on the bodies of bees. Larvae are thought to congregate on flowers and grasp the legs of foraging bees. The presence of particular plant taxa that are preferred oviposition sites and species upon which larvae transfer to foraging bees may play a critical role in this parasite-bee relationship (Torchio and Bosch 1992).

If floral resources or natural enemies play a large role in influencing bee population dynamics, then environmental changes that alter these factors could strongly contribute to changes in bee abundance. Biological invasion by exotic plants may be particularly problematic in this regard, because highly abundant and competitive invasives often reduce the local abundance and diversity of native plants (D'Antonio and Vitousek 1992, Mack et al. 2000, Sala et al. 2000, Vilá et al. 2011), while increasing abundance of novel species that may not serve as resources for native bees (Stout and Morales 2009). The influence of invasive plant species on the pollination of native plants has received considerable attention (reviewed in Bjerknes et al. 2007, Morales and Traveset 2009), but we know little about how invasive plants influence bee population dynamics, and even studies attempting to relate plant invasion and bee abundance are rare (but see Moron et al. 2009). Some argue that the influence of invasive plant species on native bees should be positive because many invasive forbs produce abundant flowers with plentiful nectar or pollen which may supplement native bees (Westphal et al. 2003, Kleijn and van Langevelde 2006, Bjerknes et al. 2007, Tepedino et al. 2008). However, surveys indicate that bees may be less abundant in sites invaded by exotic plants (Hopwood 2008, Moron et al.

2009), and there are several reasons why many bee species may not benefit from invasive exotics (Stout and Morales 2009). For example, there may be phenological mis-matches between invasive forbs and native bees (Stout and Morales 2009), or nectar and pollen provided by the invasive forb may be morphologically inaccessible (Corbet et al. 2001, Goulson 2003, Liu and Pemberton 2009), chemically protected, or of lower nutritional quality (Stout and Morales 2009). If invasive plants reduce diversity and abundance of native forbs on which bees are highly dependent, then plant invasion may reduce bee abundance (Stout and Morales 2009). Finally, plant invasion and associated changes in forb communities might indirectly alter bee demography by affecting the populations or behaviors of bee natural enemies. For example, Goodell (2003) found that reductions in floral availability increased rates of brood parasitism for *Osmia pumila* by the cleptoparasitic wasp *Sapyga centrata*, possibly because the nest was more frequently left undefended by females (Goodell 2003).

Here we ask how native floral resources and invasion by several abundant exotic forbs influence nest establishment, parasitism rates, offspring production, offspring survival, and overall population growth rates of the native solitary bee, *Osmia lignaria propinqua* Cresson (Megachilidae), in grasslands of western Montana. We monitored *O. lignaria* demography across a steep gradient of forb abundance and species richness, ranging from native-dominated/ high floral resource grassland sites, to exotic-dominated/high resource sites, to sites with very low floral abundance. In addition to estimating how floral resources influence bee demography and brood parasitism, we quantified how floral resources influenced bee foraging, and identified pollen species used to provision offspring. This enabled us to determine whether exotic plant pollen contributed to fecundity and whether patterns of pollen collection influenced offspring survival.

Methods

Study Sites

We selected 27 grassland sites distributed across Missoula, Ravalli and Powell counties in western Montana that spanned a gradient of floral resource abundance and extent of exotic plant invasion. Sites were at least 1.5 km apart and were located on lands owned or managed by the Lolo National Forest, Montana Fish, Wildlife and Parks, Missoula County Parks and Recreation, the University of Montana, the National Wildlife Federation, and private landowners. Sites ranged in elevation from 1000 to 1300 m and supported intermountain bunchgrass plant communities with scatterings of ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*). All sites were greater than 10 hectares, and were bounded by dense, contiguous mixed conifer forest, agricultural lands, or urban areas. To quantify gross differences in floral abundance, richness and invasion among sites, we placed a circular plot (radius=100 m) in the center of each site and within this plot we estimated native and exotic forb species richness and the abundance of native and exotic flowers (or inflorescences for species that produce reduced flower, e.g. Asteraceae, *Euphorbia*). Because each site contained relatively uniform cover type, estimates from sampling plots characterize the plant community at the overall site. The number of flowers or inflorescences for each species was estimated in abundance categories (0-9, 10-99, 100-999, 1000-4999, 5000-9999, 10,000-49,999, and > 50,000), as bees are likely to respond only to large changes in floral abundance (Williams and Tepedino 2003, Williams and Kremen 2007). Differences in floral abundance based on these broad categories is designed to roughly capture variation in forb availability among sites (Williams and Kremen 2007). Floral abundance was estimated three times during the nesting season between late May and mid-July

in 2009, 2010 and 2011 by a single observer to ensure uniformity across sites and species, and the peak abundances of each species were used in analyses. We only estimated floral abundance of those forb species that were in bloom during the nesting season; early and late season flowering forbs were excluded. *Euphorbia esula* (Euphorbiaceae), *Sisymbrium altissimum* (Brassicaceae), and *Tragopogon dubius* (Asteraceae) were the three most dominant exotic forbs at the majority of invaded sites (these species made up an average of 62.9%, 21.5%, and 10.8% of exotic flowers among sites, respectively). Native forbs were present at invaded sites, though many occurred at low abundance. Common forbs in native-dominated sites included *Balsamorhiza sagittata* (Asteraceae), *Achillea millefolium* (Asteraceae), *Lupinus spp.* (Fabaceae), *Phacelia spp.* (Hydrophyllaceae), and *Erigeron spp.* (Asteraceae). See Appendix A for the complete list of native and exotic forb species across sites.

Bee Demography

O. lignaria propinqua is a solitary bee native to western North America. It is a generalist forager that collects pollen from a variety of plant taxa (Cripps and Rust 1989). Females build nests in existing cavities and will readily nest in holes drilled into wood blocks. Each nest consists of a linear arrangement of brood chambers. Inside of each chamber, the female places a provision of pollen and nectar and lays a single egg. Chambers are separated with mud partitions, and females place a mud cap at the entrance to the nest once it is complete (Phillips and Klostermeyer 1978). We placed one 48-hole nest block in each grassland site, maximizing the distance to grassland boundaries such as forest edges. Each nest block was 15 cm x 35 cm, and contained a grid of 4 by 12 drilled holes that were 4 cm long and 8 mm in diameter and were lined with removable paper liners. A small cardboard box with a single exit hole and containing 16 preemergent female and 16 preemergent male cocoons was secured to the bottom of each nest

block. Cocoons were obtained from two commercial sources within the Rocky Mountains and were mixed before being placed in emergence boxes. Because nest blocks placed in the field are often not used by naturally occurring bees, the addition of preemergent cocoons ensured the presence of a nesting population that could be monitored (Williams and Kremen 2007).

Nests and cocoons were placed in the field in mid to late May 2009 and 2010. This timing was just before the peak in flowering and native bee activity at the study sites and 2 to 3 weeks later than naturally occurring *Osmia* became active in the region (J.D. Palladini, *personal observation).* Males emerged within 1-2 days, and females emerged within days afterwards. Mated females began nesting within one week after emergence boxes were placed in the field, and additional pre-emergent cocoons were set out to replace those that failed to emerge after one week. Nesting progress was monitored through mid-July when activity ceased. To determine whether wild *O. lignaria* individuals might influence nesting in our experimental nest boxes, in 2009 we placed nest blocks at eight additional sites where we did not add preemergent cocoons. None of these nest boxes received any nesting activity. In 2010 we again used four of these sites but added preemergent cocoons next to nest boxes, which resulted in nest construction at all of these sites. Thus, we assume that the *O. lignaria* that nested in our experimental boxes were from the pool of individuals that we released and not from pre-existing wild individuals. We examined bee demography at 16 sites in 2009 and 11 additional sites in 2010 for a total of 27 sites. No sites were used during both years.

In 2009, we monitored nests once every three days. All females were color-marked on the thorax with paint pens so we could determine how frequently single individuals constructed multiple nests. Since we did not observe any marked females constructing multiple nests, we assume that the number of nests gives an estimation of the number of nesting females, and that

the number of brood cells per nest is an estimation of per capita female fecundity, though it is possible that females constructed additional nests in locations other than the nest block. Nest blocks were observed for a minimum of 60 minutes, and the duration of foraging bouts was recorded. Females forage for pollen and nectar as well as for mud, which is used to separate individual brood chambers. These two foraging trip types were generally distinguishable in the field (Phillips and Klostermeyer 1978). If the trip was not easily categorized into a food or mud foraging trip, it was not used in analyses. We recorded the length of only a single food trip per female per observation date, even in cases where females made multiple trips within an observation period.

In 2010, we monitored nest blocks once per week. Each block was observed for 30 minutes for signs of nest construction. To determine plant species used as pollen sources, we sampled pollen from a subset of brood chambers after nest completion in July at all sites, with the exception of one site that had no completed chambers. We carefully removed straws from the nest block, and made a slit in the straw through which we could extract a small amount of pollen with fine-tipped forceps. We sampled chambers in the rear, middle and front of the nest when possible so that we could observe pollen use throughout the nesting season. The straw was then sealed and placed back into the nest block. Pollen was stored in 70% EtOH before being stained with safranin (Fultz 2005) and identified to the genus level using light microscopy, though in many cases only one species in a given genus was present at the sites, and thus species-level identification was inferred. We counted approximately 250 pollen grains from each provision. We compared pollen collected from brood chambers to a reference pollen library constructed from forbs flowering at our study sites. Pollen was identified to the genus

level. Pollen samples and digitized photos are maintained as a reference at the University of Montana.

We removed nest blocks from the field in early autumn. At this time, surviving offspring overwinter in pupal cocoons as dormant adults. We removed nests from individual holes in each nest block by extracting the straw liner and then assessed the fate of each offspring in each nest. Some brood chambers contained intact pollen provisions but no evidence of *O. lignaria* offspring, which likely resulted from mortality of eggs or very early larvae. Other brood chambers, however, contained intact larvae that had either survived to the overwintering stage or died prior to completing development.

Tricrania stansburyi (blister beetle) was the dominant brood parasite in our system. Adults emerging from host nests disperse via flight, have not been observed to feed, and live for one to two weeks. After mating, females in lab settings have been shown to lay several hundred eggs, though, to our knowledge, oviposition in natural settings has not been observed. Parasitism occurs through phoresy of the first instar (triangulin), with the triangulin transported to the nest by female bees which they latch onto as the bees forage on flowers. After entrance to the nest, triangulins combat one another until a single survivor consumes the bee egg and pollen provision. *T. stansburyi* overwinter within the host nest, emerging in the spring.

Torchio and Bosch (1992) found that parasitism in a greenhouse was strongly tied to the presence of a particular plant, *Borago officinalis* (Boraginaceae), where larvae were suspected to congregate. Observations of managed *O. lignaria* populations suggest that *Hydrophyllum capitatum* (Hydrophyllaceae) may also harbor larvae (Torchio and Bosch 1992). We hypothesized that, in our system, larvae may grasp bees while foraging on *Phacelia spp.* (Hydrophyllaceae), because it is a relative of *H. capitatum* and is a common pollen host for *O.*

lignaria in our system. To assess this, we examined relationships between pollen provisioning

and parasitism within a brood chamber. To increase our sample size above what we were able to

obtain in 2010, floral abundance was estimated and nest blocks and pre-emergent cocoons were

placed at 18 additional sites in summer 2011 that had not previously been examined in either

2009 or 2010. We removed individual nest straws from the field upon nest completion, and

transferred offspring to individual plastic vials (2.0 ml, Perfector Scientific Cryo-Store,

Atascadero, CA) for rearing in the laboratory. We sampled and identified pollen provisions

using methods detailed above, and recorded the presence of parasites. In total, we sampled and

identified pollen provisions from 181 brood cells from 2010 and 2011. Demographic data from

the 18 additional 2011 nests were not analyzed for this study.

Analyses

We used generalized linear models (GLM) and generalized linear mixed models

(GLMM) to analyze the influence of species richness and floral abundance on bee demographic

processes. Unless otherwise noted, we examined main effects of the following variables

(referred to henceforth as "floral resources"): native forb species richness, exotic forb species

richness, native forb abundance, exotic forb abundance, and the individual and combined

abundance of the primary plant species whose pollen was used for larval provisions. We did not

include total forb abundance or richness because these were highly correlated with native and

exotic floral attributes (see Appendix B). Nonsignificant parameters were removed in a stepwise

approach from the model until all parameters were significant (Crawley 2002). Because native

forb species richness and abundance were correlated (R = 0.48, p = 0.01, Appendix B), as were

exotic forb species richness and abundance (R=0.48, p = 0.01), these should be interpreted as

metrics of overall native or exotic floral resource availability rather than distinct floral

community attributes to which bees differentially respond. When year was included as a random

effect, the estimated variance was consistently small, thus year was excluded from reported

models. All analyses were run with the statistical software R 2.13.2 (R Development Core Team

2011). Gaussian mixed effects models were fitted with the function lme (library: nlme; Pinheiro

et al. 2011). Poisson and binomial mixed effect models were fitted with the function lmer

(library: lme4; Bates et al. 2011).

We examined the influence of floral resources on the number of nests initiated per site

using GLM (Gaussian family/identity link). A nest was counted if we saw females completing

foraging trips or if mud and pollen were present at the rear of the nest. In some cases, no cells

were complete, but nests had clearly been initiated and thus the nest was included. We used

GLMM (Poisson family/log link) to explore how floral resources and site (a random factor)

influenced the number of offspring per nest (i.e., the number of completed, provisioned brood

chambers). To determine how floral resources influenced foraging in 2009, the average trip

length per observation date for each site was modeled using GLMM (Gaussian family/identity

link), with the addition of Julian date as a fixed factor and site as a random effect. We used a

simple linear model to determine the relationship between nesting duration in 2009 (i.e., the

number of days between the first and last signs of nesting activity for sites with $\geq$ 3 nests) and

native forb species richness.

The number of offspring surviving to the adult stage was modeled using GLMM

(binomial family/logit link) with site and nest as random effects. We found that 17.7% of

offspring died prior to the adult stage for unknown reasons that could have been due to our

sampling the pollen provisions. This mortality was not related to any site characteristics

examined. Because we could not rule out the possibility that mortality may have been caused by

researcher interference, we removed these offspring when estimating how survival rates were correlated to site characteristics. Relationships between site-based *T. stansburyi* parasitism rates (i.e., the proportion of brood chambers containing *T. stansburyi*) and floral resources were examined using GLM models. To explore whether parasitism rates increased with host density, we used a linear model to analyze relationships between parasitism rate and both the number of nesting females and the total number of offspring produced. To explore relationships between parasitism and pollen provisioning within individual brood chambers, we used GLMM (binomial family/logit link). Fixed effects included the proportion of pollen in each brood chamber coming from each pollen source, while site and nest were random effects. We also used a Chi-squared goodness-of-fit test to determine whether the presence or absence of different pollen types in a brood cell influenced incidence of parasitism. Finally, we explored the net effect of floral richness on bee population growth rates (λ). We calculated λ by multiplying the total number of offspring produced at each site by the survival estimate at that site, and then divided this estimated N_{t+1} by 32, the initial population size placed at each site. We used linear models to examine how native and exotic forb species richness and abundance influenced λ.

Results

The number of nests initiated in each nest block increased with native forb abundance (t = 3.591, p < 0.001, n =27) and decreased with increasing exotic forb richness (t = -2.082, p = 0.048, n = 27). The number of offspring per nest increased with increasing native forb species richness (z = 2.352, p = 0.018, n = 140, Fig. 1). The relationship between foraging trip length and native forb species richness was marginally negative (*t*= -2.030, p = 0.063, n = 42), suggesting that increases in fecundity in high native-richness sites resulted from increased rates of daily provisioning. The average pollen collecting trip lasted 35 minutes, and trip length also

increased with Julian date (t = 3.916, p < 0.001, n = 42), likely due to the senescence of native forbs during the mid to late summer dry season. Most nests were completed in late June, and average nesting duration across sites was 23 days. Nesting duration decreased with increasing native forb species richness (t = -4.060, p = 0.003, n =12). Provisions contained pollen from an average of 3.24 pollen types. Three plant taxa accounted for 94.7% of pollen identified in pollen provisions: *Lupinus sericeus.* (Fabaceae, 44.3%), *Phacelia spp.* (Hydrophyllaceae, 34.0%), and *Amelanchier alnifolia* (Rosaceae, 16.4%). However, individual and combined abundance of these three main pollen sources at sites did not predict any metric of *O. lignaria* demography including rates of parasitism.

Surprisingly, we found that the probability of survival increased with increasing exotic forb species richness (z = 2.122, p = 0.033, n = 300, Fig. 2) and rates of parasitism by *T. stansburyi* increased with native forb abundance (t = 3.044, R^2 = 0.316, p = 0.006, n = 22). Parasitism accounted for 0% to 66% of mortality, depending on site. Overall, brood parasites were present in 30.1% of *O. lignaria* brood chambers, with *T. stansburyi* alone found in 20.8% of brood chambers. Other brood parasites included *Stellis* (Megachilidae, 4.5% of brood chambers) as well as unidentified waps and flies. Increasing rates of parasitism with abundant native forbs could result from the presence of particular plant taxa that play a critical role in the parasite-bee relationship. However, we found no relationship between either presence or proportional abundance of particular pollen types and the probability of parasitism within a brood chamber. Rates of parasitism by *T. stansburyi* were positively related to the number of nesting females at a site (t = 2.639, R^2 = 0.317, p = 0.018, n =22) as well as the number of brood cells produced (t = 2.849, R^2 = 0.351, p = 0.012, n = 22).

The conflicting positive effect of native forb richness on bee reproduction and negative effect on bee survival (due to increasing brood parasitism) led to only a marginally significant positive relationship between native forb richness and bee population growth rates ($t = 1.965$, $R^2 = 0.134$, $P = 0.060$, n = 27, Fig. 3).

Discussion

Bee nest initiation and fecundity were strongly correlated with native floral resources across multiple sites. Mortality caused by brood parasites limited the extent to which gains in reproduction at sites with greater native floral resources translated directly to enhanced population growth, although a weak relationship between bee population growth and native forb species richness remained. These results highlight the importance of understanding the joint influence of multiple factors, such as resources and natural enemies, in affecting bee demography (Steffan-Dewenter and Schiele 2008, Roulston and Goodell 2011).

O. lignaria responded differently to native and exotic floral resources. Reduced nest establishment in sites with high exotic forb richness and low native abundance may have been a consequence of either high rates of pre-nesting adult mortality or dispersal by females to higher quality sites. It has been suggested that invasive plant species with abundant flowers may positively influence native bees (Westphal et al. 2003, Kleijn and van Langevelde 2006, Bjerknes et al. 2007, Tepedino et al. 2008), but despite the observation that *O. lignaria* commonly foraged on exotics for nectar, bees did not use any exotic pollen for larval provisioning. These results bolster observational studies showing greater solitary bee abundances in native versus exotic-dominated sites (Hopwood 2008, Moron 2009, J.D. Palladini, *unpublished data*) and point to underlying demographic processes as a causal mechanism explaining these patterns. Our results contrast those that have found that mass-flowering exotic

plant species benefit populations of highly generalized bumble bees (Westphal et al. 2003, Herrmann et al. 2007). It may be that solitary and social bees respond differently to plant invasion, as sociality is known to influence bees' response to a number of disturbance types (Winfree et al. 2009, Williams et al. 2010).

Observations of foraging trip length suggest a possible mechanism behind increases in fecundity in sites with high native forb richness; females in sites with high forb-species richness spent less time on each foraging bout. This may have allowed them to make more trips per day and provision offspring at a faster rate than was the case for bees foraging in more invaded sites. (Minckley et al. 1994, Zurbuchen et al. 2010). Females in low-richness sites likely spent more time seeking out resources, and despite an increase in nesting duration in these sites, fecundity was still reduced as is predicted for central-place foragers (Minckley et al. 1994, Cresswell et al. 2000, Zurbuchen et al. 2010).

The observation that nesting duration was reduced in high-richness sites is intriguing, and to our knowledge has not been reported in other studies. One possible explanation is that sites with few native forb species were also those that were heavily invaded by exotics that tended to flower later in the summer (J.D. Palladini, *personal observations)*. Nectar provided by late season exotics may have fueled females later into the summer compared to native-dominated sites that lacked nectar-rich late season forbs, as has been proposed by Tepedino et al. (2008). A second possibility is that females are egg-limited (Rosenheim 1996, Neff 2008) and reached this limitation more quickly in native-dominated sites where young were provisioned more rapidly. There was no evidence that increased nesting duration, per se, resulted in increased output (J.D. Palladini, *unpublished analysis*), suggesting that to some extent, producing offspring over a longer period of time may compensate for low availability of floral resources, though it is

possible that females constructed nests elsewhere after completing observed nests in the nest blocks. If this is the case, overall nesting duration may not have been reduced in high-resource sites and per capita fecundity may have been underestimated.

Individual and combined abundance of the three primary pollen sources (*L. sericeus, Phacelia spp.,* and *A. alnifolia*) did not predict nesting or fecundity better than overall native forb species richness. It may be that patches of these pollen sources were utilized outside of our sampling plot radius since *O. lignaria* commonly forage at distances greater than 100 m (Rust 1990, Greenleaf et al. 2007), though sites contained relatively uniform cover type. Alternatively, there may not be a strict relationship between the abundance of particular pollen sources and bee demography because despite strong preferences for particular taxa, bees displayed great flexibility in using other sources when preferred sources were locally rare, as has been observed in other studies (Rust 1990, Williams and Tepedino 2003, Kraemer and Favi 2005). Thus, while *O. lignaria* populations were impacted by declines in native forb species richness, they were not affected by changes in native plant community composition that retained native richness. Because native forb abundance and native forb richness were correlated at our study sites we are unable to disentangle their effects. However, it is possible that increased native forb species richness, per se increases bee demography by increasing the likelihood that a suitable pollen source will be present.

Incidence of brood parasitism by *T. stansburyi,* the leading cause of mortality, increased in sites with greater native forb abundance. This produced a surprising, positive relationship between offspring survival and exotic forb species richness. We hypothesized that *T. stansburyi* larvae grasped *O. lignaria* while foraging on *Phacelia spp.* because it is a relative of other species suspected to harbor *T. stansburyi* larvae (Torchio and Bosch 1992)and is a common

pollen host for *O. lignaria.* Thus, reduced abundance of *Phacelia spp.* resulting from invasion could be responsible for decreased parasitism in invaded sites. However, we found no relationship between the abundance of *Phacelia spp.* and site-based parasitism rates, nor was there a relationship between the abundance of *Phacelia spp.* pollen within brood chambers and parasitism rates within those chambers.

Rather than host plant availability driving higher parasitism rates at sites with abundant native forbs, the greater abundance of bees at these sites may have produced this pattern (Vandermeer and Goldberg 2003). Rates of parasitism were related to the number of brood cells produced as well as the number of nesting females at a site. We do not know how the background abundance of hosts or parasites might vary among sites with variable native floral richness or abundance. Nonetheless, the positive relationship between *O. lignaria* offspring numbers and parasitism rates suggest that parasitism is density-dependent and could play a role in regulating bee populations at high densities (Vandermeer and Goldberg 2003). Other studies seeking to link parasitism to population size in bees have produced mixed results, including both density-dependence and inverse density dependence (Rosenheim 1990, Antonini et al. 2003, Steffan-Dewenter and Schiele 2008), which may be due, in part, to varying strategies by parasites. For example, Steffan-Dewenter and Schiele (2008) found that parasitism on *Osmia rufa* was reduced at high densities, likely because the dominant brood parasite, the drosophilid fly *Cacoxenus indagator*, patrolled nest entrances and requires females to be absent to enter the nest. High densities of nesting females inhibited the ability of the parasites to enter nests. These results contrast to ours, since the dominant parasite in our study does not enter while foraging females are away, but rather enters the nest on the bodies of females. We speculate that

abundant host bees in high-resource sites may contribute to the build-up of parasites at these sites, with resulting increased rates of parasitism.

Ultimately, however, the negative impacts of increased parasitism in high-resources sites did not outweigh the positive influence of native forb species richness on nesting and fecundity. As a result, population growth rates were marginally positively related to native forb species richness. This relationship was primarily driven by the strong increase in the number of nests constructed, as fecundity was less strongly influenced by native forbs. Since our estimates of population growth rate (λ) are all less than 1.0, it suggests that experimental bee populations would decline across generations at all of our sites. However, these values should be interpreted with caution. Since dispersal of *O. lignaria* from release sites is known to be greater when bees emerge from loose cocoons rather than natal nests (Torchio 1985), we have likely underestimated λ. Thus, although the absolute values of our lambda estimates are likely too low, the strength of our approach is that it enables us to integrate various components of demography and determine how population growth varies among sites.

Our observations that both nest initiation and per capita fecundity increase with native forb species richness support the hypothesis that bee populations in natural settings are food-limited. However, we recognize that our metrics of resource availability across sites are crude. That is, native forb species richness and abundance were correlated, and therefore we can not be certain what precise attributes of floral resources across the landscape bees are responding to. Regardless, our results are consistent with patterns demonstrated elsewhere. For example, the specialist solitary bee *Dieunomia triangulifera* (Halictidae) also increased reproduction when flowers of its pollen host were abundant (Minckley et al. 1994). As well, Williams and Kremen (2007) found that *O. lignaria* nest establishment and fecundity were reduced at sites isolated

81

from natural habitat containing pollen resources, though interestingly were unrelated to floral resources within the nesting site(Williams and Kremen 2007). One difference between the two studies may be the availability and use of floral resources outside of the immediate study area. In our study, sites were clearly bounded by unsuitable habitat such as dense conifer forest that likely deterred movement out of the grassland and contained few resources, whereas in Williams and Kremen (2007), the more open agricultural, chaparral, and oak woodland habitats permitted use of off-site resources, obscuring the relationship between bee reproduction and floral resources. There is also indirect evidence for food limitation of bee populations from studies of competition. Experimental or natural increases in the abundance of competitively dominant bees, such as honey bees (*Apis mellifera)* or bumblebees (*Bombus spp.)*, can alter behavior of competitively inferior bee species (Inouye 1978, Hingston and McQuillan 1998), or reduce forager densities (Roubik 1978, Bowers 1986). In contrast, Steffan-Dewenter and Schiele (2008) observed population growth rates over several years and found that wild *Osmia rufa* Linnaeus (Hymenoptera: Megachilidae) populations in an orchard meadow were not food limited, but rather were suppressed by inadequate nest sites. It may be that orchards possess particularly high densities of floral resources or low densities of nest sites compared to natural habitats, and that food limitation of bee reproduction in natural habitat is common.

Bees play critical roles as pollinators in many habitats (Buchmann and Nabhan 1996, Linder 1998, Klein et al. 2007), and recent declines have sparked interest in preserving native bee populations in both agricultural and natural areas (Kremen et al. 2002, Steffan-Dewenter et al. 2005, Biesmeijer et al. 2006). Floral resources have long been assumed to be the primary factor influencing bee population dynamics, yet data on how variation in floral resources affects demographic processes of solitary bees has been lacking. Our results support the hypothesis that

82

bee populations in natural habitats are food limited, and despite suggestions that exotic plants may subsidize the diet of native bees, benefiting bee populations, we found that *O. lignaria* responded negatively to invasion. Our results suggest that conserving native bees will require the maintenance of native forbs and ameliorating the negative effects of exotic plants on native plant communities.

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Figure 1. Influence of native forb richness on the number of offspring produced in each nest (filled circles, points jittered). Squares indicate site means (± 1 SE). Line shows function fitted to individual nests, with site as a random factor using GLMM (Poisson family/log link)
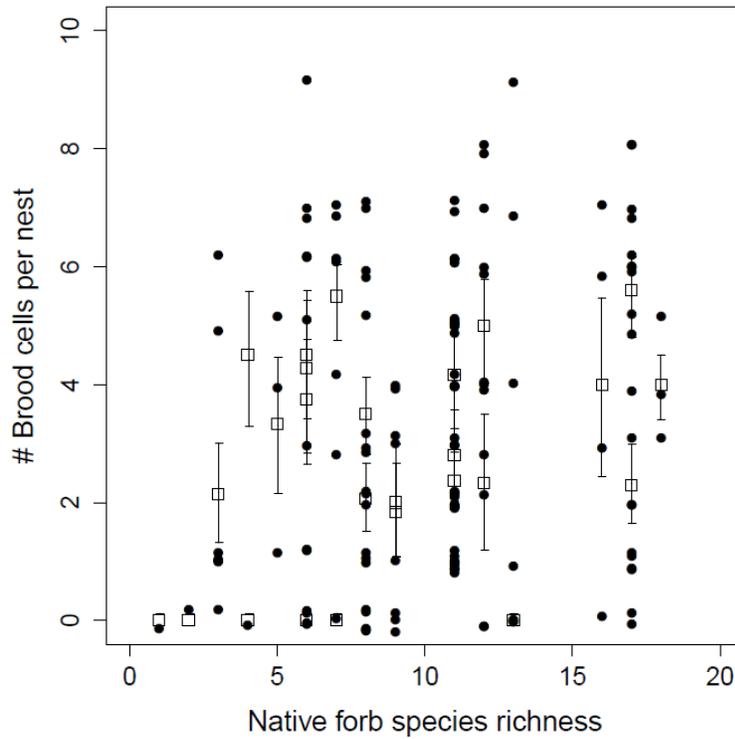
Figure 2. Bubble plot of survival probability plotted against exotic forb species richness. Bubble area is in proportion to the number of individuals that survived or died. Line shows fitted survival function.
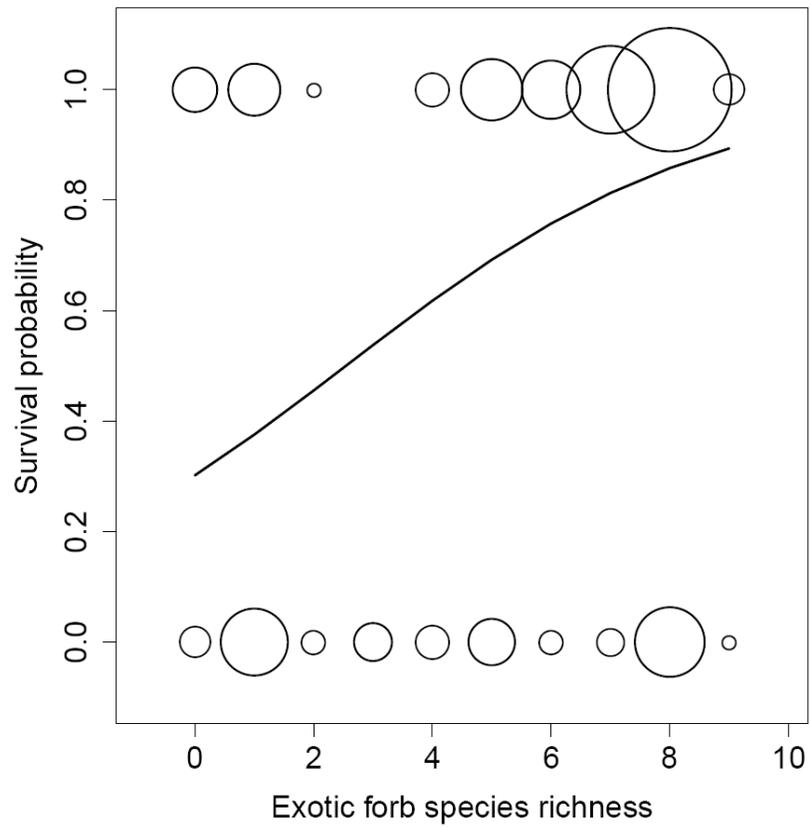
Figure 3. Population growth rates (lambda) increased with native forb species richness
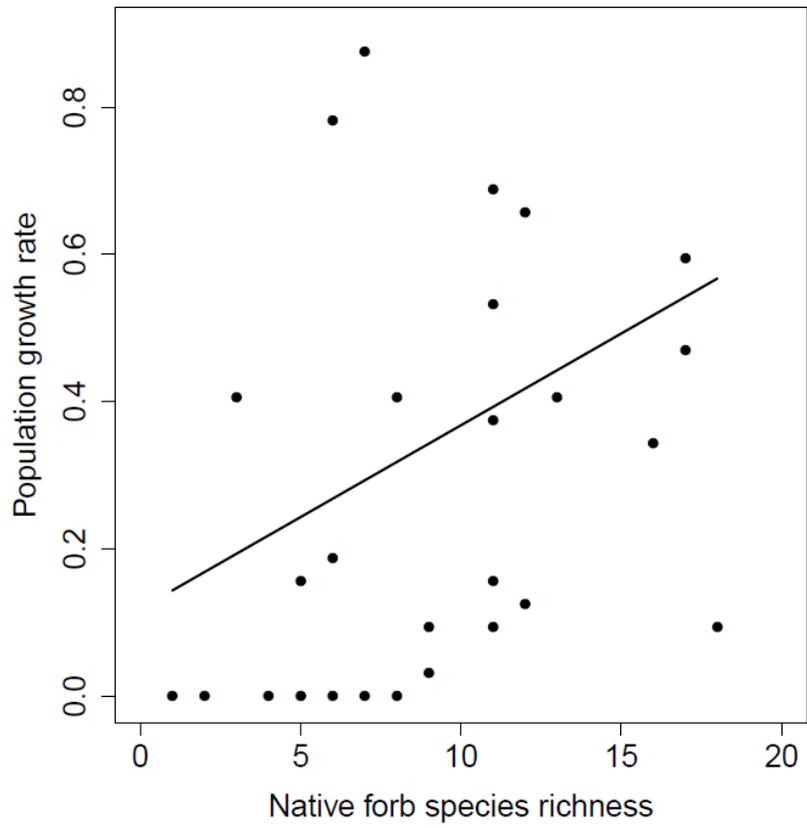
Appendix A. List of native and exotic forbs in flower during *Osmia lignaria* flight season. For each site type (native, intermediate, invaded), numbers give the percentage of sites in which the species was located (e.g., *Achillea millefolium* was found in 83% of native sites, 91% of intermediate, and 90% of invaded sites).

	Native	Intermediate	Invaded
Native Forbs			
Achillia millefolium	83	91	90
Allium cernuum	16	0	0
Amelanchier alnofolia	16	0	20
Antenaria rosea	33	0	20
Apocynum androsaemifolium	16	0	20
Arenaria capillaris	50	27	0
Arnica sororia	16	27	20
Aster ericoides	0	0	20
Astragalus miser	33	18	10
Astragalus inflexus	16	36	0
Balsamorhiza sagittata	66	63	60
Campanula rotundifolia	0	0	0
Castilleja sp.	66	36	20
Chrysopsis villosa	16	18	80
Clarkia pulchella	16	0	10
Erigeron spp.	100	51	40
Eriogonum umbellatum	83	0	10
Gaillardia aristata	83	64	50
Geranium viscosisimum	33	0	20
Geum triflorum	16	18	0
Lewisia rediviva	66	18	0
Lupinus sericeus	83	90	70
Microsteris gracilis	0	27	0
Monarda fistulosa	0	27	10
Orthocarpus tenuifolius	50	0	10
Penstemon sp.	16	45	20
Phacelia hastata	16	18	10
Phacelia linearis	50	63	10
Philadelphus lewisii	16	9	10
Potentilla arguta	50	0	0
Purshia tridentata	50	45	0
Senecio triangularis	33	9	0
Symphoricarpus albus	16	0	10
Zigadenus venenosus	33	9	0
Exotic Forbs			
Cirsium vulgare	0	0	10
Cynoglossum officinale	16	9	10
Euphorbia esula	33	100	90
Potentilla recta	33	18	40
Linaria dalmatica	0	18	30
Sisymbrium altissimum	50	64	40
Tragopogon dubius	50	90	100
Vicia cracca	0	18	0

Appendix B. Characterizing vegetation communities

  We explored relationships among floral attributes using Pearson's correlation tests. Native forb abundance and native richness were positively correlated as were exotic forb abundance and richness (Table B1). Total forb abundance and richness were highly correlated with several native and exotic floral attributes, thus we excluded these variables from further analyses of bee demographic performance. Because native forb species richness and abundance were correlated, as were exotic forb species richness and abundance, these should be interpreted as metrics of overall native or exotic floral resource availability rather than distinct floral community attributes to which bees differentially respond.

  We assessed differences in species composition among invaded and native sites, using nonmetric multidimensional scaling (NMS) with the Sorensen distance measure (PC-ORD version 6; McCune and Mefford 2011). We used NMS because of its effectiveness in assessing non-normal data sets (McCune and Grace 2002; McCune and Mefford 2011). The slow and thorough "autopilot function" was used to select the optimum dimensionality for the ordination. We used a multi-response permutation procedure (MRPP) with the Sorenson distance measure to explore forb community differences between native, intermediate, and invaded sites. Using the proportion of floral abundance that was native or exotic in origin, sites were categorized into three types along natural breakpoints in the data set as native-dominated (>70% native), invaded (<30% native), or intermediate (31% to 69% native). One invaded site with only 2 species was dropped from this analysis, and only species which occurred in at least 2 sites were included, giving a dataset of 26 sites and 45 species. Indicator species analysis was used to identify species with an affinity to one of the three site types (i.e., native, invaded and intermediate), and

relationships between the abundance of the indicator species and ordination scores were examined using Pearson's correlation tests.

The best NMS solution was a 3-dimensional model that captured 88.9% of the variation (Fig. B1). Axes 1, 2, and 3 explained 43.9%, 27.2% and 17.8% of the variation, respectively. All three site types formed distinct groupings (Native v Invaded: $R = 0.254$, $p < 0.001$; Native v. Intermediate: $R = 0.092$, $p = 0.004$; Invaded v. Intermediate: $R = 0.164$, $p < 0.001$). Of the 42 species used in the analysis, 9 were significant indicators of site type. *E. esula* ($p < 0.011$) and *C. villosa* ($p = 0.003$) were indicators of invaded sites, *L. sericeus* ($p = 0.017$) and *T. dubius* ($p = 0.023$) were indicators of intermediate sites, and *Eriogonum umbellatum* ($p < 0.001$), *Lewisia rediviva* ($p < 0.001$), *Potentilla arguta* ($p = 0.007$), *Orthocarpus tenuifolius* ($p = 0.022$), and *Castilleja spp.* ($p = 0.040$) were indicators of native sites. Figure B1 indicates relationships between abundance of indicator species and ordination scores.

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Figure B1. Nonmetric multidimensional scaling ordination of forb species in native,

intermediate and invaded sites. Axes 2 and 3 are both plotted against Axis 1. Vectors represent

significant correlations (p <0.10) between ordination scores and abundance of indicator species.
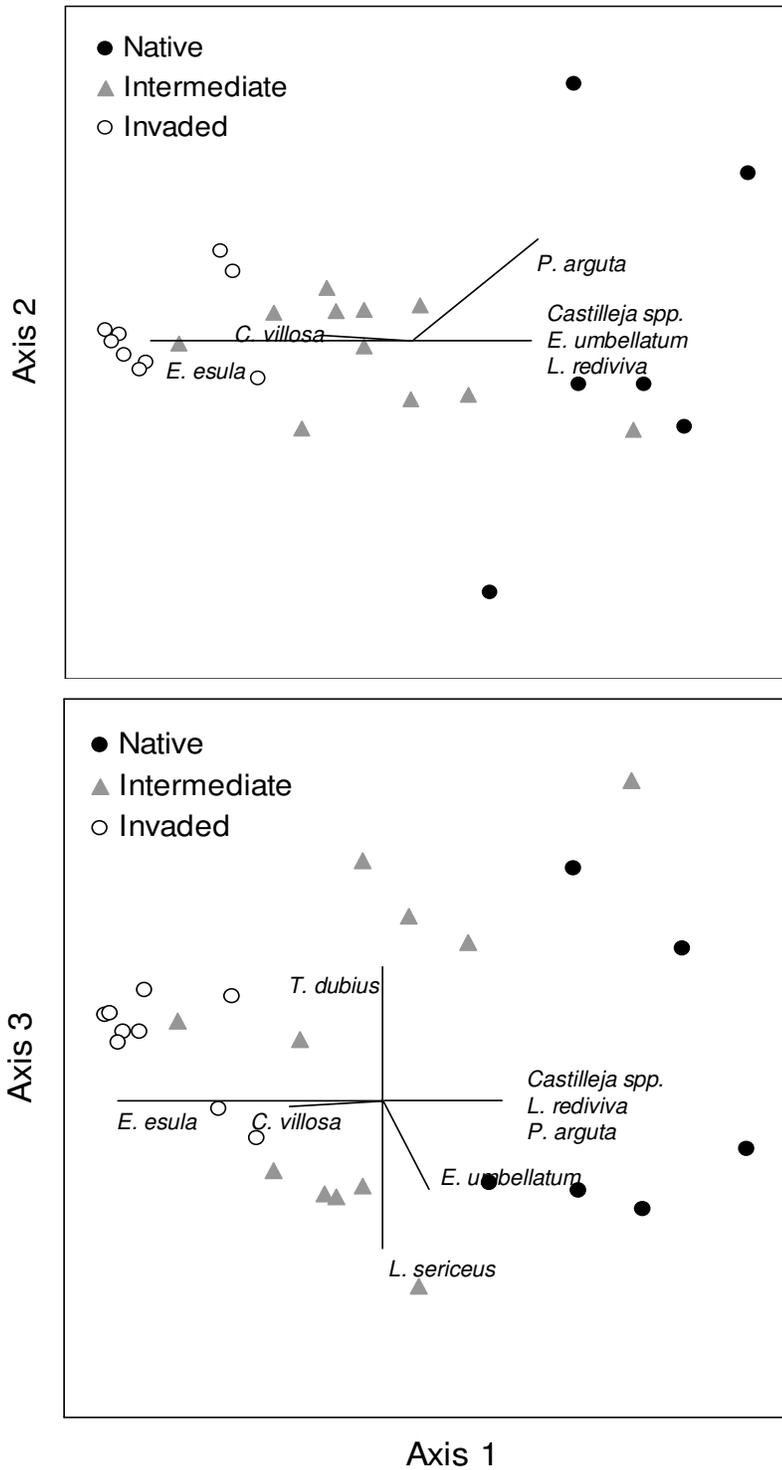
Table B1. Pearson correlation coefficients for relationships among forb community attributes.

	Native abundance	Native richness	Exotic abundance	Exotic richness	Total abundance	Total richness
Native abundance	...	0.48**	-0.16	0.43*	0.74***	0.71***
Native richness	...	...	-0.41*	-0.22	0.13	0.84***
Exotic abundance	...	...	...	0.48**	0.55***	-0.13
Exotic richness	...	...	...	...	0.69***	0.34
Total abundance	...	...	...	...	...	0.51***
Total richness	...	...	...	...	...	...

* $p \leq 0.10$

** $p \leq 0.05$

*** $p \leq 0.01$

CHAPTER FOUR

POLLEN DEFENSE: DO PLANTS CHEMICALLY PROTECT POLLEN FROM

CONSUMPTION BY BEES?

Abstract

Bees are the major pollinators of wild plants. While bees are generally perceived as mutualists, they also collect substantial quantities of pollen to rear larvae. Pollen consumption by bees may be an underappreciated contributor to pollen limitation of plant reproduction and has the potential to result in a substantial loss of fitness. Here I review what is known about pollen sequestration by bees, its potential influence on the evolution of defensive traits in plants to protect pollen from excessive harvesting, and how this might promote specialist vs. generalist pollinators. While pollen has historically been viewed as an easy-to-use source of protein for bees, there is growing evidence that pollen can contain toxins that kill some bee species but have no lethal effects on specialized bees. Many morphological features of flowers are interpreted to serve in defense of pollen from excessive harvesting, and secondary compounds in pollen may be an additional means of pollen defense. Future studies should directly explore the influence of toxic pollen on bee behavior, development and trophic specialization, as well as the extent to which pollen consumption and presence of secondary compounds in pollen affect plant fitness.

Introduction

Most of the world's plant species rely on animal pollinators for sexual reproduction, and bees are the major pollinators of wild plants and crops (Buchmann and Nabhan 1996, Klein et al. 2007, Linder 1998). Although bees are generally perceived as mutualists, bee-plant relationships are complex, and may be better described as "mutual exploitation" in which plants lure bees with floral nectar as a reward for pollination services and bees seek nectar and pollen for consumption (Westerkamp 1996). Unlike nectar, which serves solely as a pollinator reward and is offered by the majority of animal-pollinated plants, pollen is rarely offered as a reward but needs to be made accessible so that it will be transferred to pollinators incidentally (Westerkamp 1996). Thus, pollen is a major source of conflict in bee-plant relationships; plants must expose pollen so it will contact visiting bees, however exposed pollen is vulnerable to substantial harvest by adult bees and consumption by bee larvae.

Bees require large amounts of pollen to rear larvae, often leaving little left for pollination (Müller et al. 2006, Schlindwein et al. 2005). Reproduction in angiosperms is often constrained due to lack of adequate pollen receipt (i.e. pollen limitation), with evidence for pollen limitation found in 63-73% of examined plant species (Ashman et al. 2004, Burd 1994). Adding supplemental pollen to stigmas typically increases fruit set by an average of 67% (Larson and Barrett 2000) and seed production by 42% (Ashman et al. 2004) .   While the ubiquity and importance of pollen limitation has long been recognized, pollen consumption by bees has only recently been suggested as a potential driver of pollen limitation (Hargreaves et al. 2009). Given the large amounts of pollen that are sequestered by bees, plants may possess adaptations to restrict pollen collection and thus limit the likelihood of associated pollen-limited reductions in fitness. Although many morphological features of flowers have been interpreted in this light

(Erbar and Leins 1995, Johnson and Edwards 2000, Praz et al. 2008, Schlindwein et al. 2005, Verhoeven and Venter 2001, Westerkamp 1997), whether plants deploy chemical compounds to defend pollen is unclear. Pollen has historically been viewed as an easy-to-use protein source that is readily digestible for bees, but there is growing evidence that the sequestering of large amounts of pollen by bees may have selected for chemical traits that may protect pollen from excessive harvesting and consumption. Here I: 1) evaluate what is known regarding the magnitude of pollen removal by bees, 2) review evidence that plants use chemical means to defend pollen, and 3) explore the influence of toxic pollen on specialization by bees and plant-bee relationships.

Bees Sequester Substantial Amounts of Pollen

Unlike hummingbirds, butterflies (except *Heliconius*), or moths, which visit flowers to collect nectar and only incidentally remove pollen, bees (and pollen wasps, Masaridae) actively harvest pollen to rear their offspring. Pollen provides an essential protein source to developing larvae, while adult bees primarily consume nectar for carbohydrates and water (Westerkamp 1996). In order to provision young, bees can remove a large portion (70-90%) of the pollen presented by a flower in a single visit (Dunham 1939, Harder and Thomson 1989, Strickler 1979). This pollen is quickly groomed into specialized pollen-carrying structures such as dense branched hairs (scopae) or hairless basket-like areas (corbiculae). Pollen that is carried in scopae or corbiculae is generally unavailable to serve in pollination (Thomson 1986, Morris et al. 1995, Harder and Wilson 1998, Schlindwein et al. 2005, but see Barthell and Knops 1997, Moeller 2005). As a result, the proportion of pollen removed from a flower that is transferred to other flowers is very small (Harder and Thomson 1989, Morris et al. 1995, Thomson 1986). For

example, in one estimate, bees deposited only 0.6% of pollen removed from a flower to the stigmas of subsequently visited flowers (Harder and Thomson 1989). Thus, bees may be more selfish consumers of pollen than is generally appreciated.

Demand for pollen by individual bees is high. Bees often require the entire pollen content of many flowers in order to provision a single offspring. For example, *Dieunomia triangulifera,* a specialist on the annual sunflower *Helianthus annuus* (Asteraceae), requires the pollen from an entire head inflorescence to provision just over three brood cells (Minckley et al. 1994). The specialist bee *Ptilothrix plumata* requires the entire pollen content of 28 – 40 flowers of *Pavonia cancellata* (Malvaceae) to provision a single brood cell (Schlindwein and Martins 2000). Similarly, the specialist bee *Chelostoma rapunculi* needs the pollen content of 36-79 flowers of *Campanula rapunculus* (Campanulaceae) in order to rear a single offspring (Schlindwein et al. 2005). In the most comprehensive study to date, Müller *et al*. (2006) directly measured the amount of pollen required to rear offspring for 14 European specialist bee species, and used body size to estimate the requirements for 27 additional species. To produce a single brood cell, the large bee, *Hoplitus adunca* (dry mass = 22.6 mg) required the pollen content of 140 flowers of *Echium vulgare* (Boraginaceae) and over 1000 *Onobrychis viciifolia* (Fabaceae) flowers are likely required to rear a single offspring for *Megachile parietina* (dry mass = 80 mg). Of the 41 species considered, 85% required the entire pollen content of over 30 flowers to rear just a single offspring.

Since solitary bees require large amounts of pollen to provision each offspring, and on average produce between 10 and 30 brood cells annually (Müller et al. 2006), summed across entire bee populations, a large proportion of the pollen produced by entire plant populations may be used by bees for offspring provisioning. Only one study has explicitly examined how the

pollen requirements of individual bees scale up to impact the availability of pollen remaining for

plant reproduction at the level of plant populations. Schlindwein et al. (2005) estimated that

95.5% of the pollen grains in a population of *Campanula rapunculus* (Campanulaceae) were

collected by bees to feed offspring, and only 3.7% remained available to contribute to pollination,

though plants were not pollen-limited.   Müller *et al.* (2006) suggest that in some cases, plant

populations may be too small for many bee species to obtain enough pollen for reproduction.

From the perspective of the plant, the large pollen requirements of bees could substantially

reduce plant fitness (Lau and Galloway 2004, except see Schlindwein et al. 2005).

Pollen Consumption and Plant Reproduction

As a consequence of the high pollen demand by bees, the amount of pollen made

unavailable for pollination may be larger than appreciated. As such, there is substantial potential

for collection of pollen by bees to be a driver of pollen-limitation and reduced siring success. It

is generally accepted that reproduction by plants is often limited by pollen receipt (Ashman et al.

2004, Burd 1994, Larson and Barrett 2000). Several factors have been proposed to explain the

prevalence of pollen-limitation among angiosperms, including ecological perturbations (Knight

et al. 2005) and the production of excess ovules as a bet-hedging strategy (Burd 1995; Knight et

al. 2005). Herbivores may also increase pollen-limitation (Ashman et al. 2004, Irwin et al. 2004,

Mothershead and Marquis 2000, Strauss and Whittall 2006), however few studies have explored

links between pollen consuming bees and reproductive success.

Pollen consumption by non-native honey bees can decrease female fitness of bee-

pollinated plants (do Carmo et al. 2004, Gross and Mackay 1998, Torezan-Silingardi and Del-

Claro 1998). Only one study has explored how pollen consumption by legitimate native

pollinators influenced seed production and male fitness (Lau and Galloway 2004). Further

research is needed that quantifies the degree to which loss of pollen to consumption by bees

drives reductions in plant fitness. While not conclusive, also instructive would be studies

exploring whether bee plants are generally more pollen-limited than plants pollinated by birds or

lepidopterans, which generally consume only nectar. Indeed, plants producing nectar rewards

are less pollen-limited than plants that produce only pollen (Larson and Barrett 2000), which is

consistent with the hypothesis that pollen harvesting reduces plant fitness if one assumes that

visitors to nectariferous flowers are more likely seeking nectar rather than pollen and thus the

amount of pollen lost to consumption per visit is lower.

Plant Defense of Pollen

The high demand for pollen by bees, coupled with widespread pollen limitation, suggests

that plants should possess traits that reduce pollen loss, and a number of morphological traits

have been interpreted in this light, including: 1) anthers that are positioned in narrow floral tubes

(Thorp 1979), bilabiate flowers (Westerkamp and Classen-Bockhoff 2007), or specialized keel

flowers (Westerkamp 1997) that make harvesting pollen difficult, 2) pollen contained in

poricidal anthers that can only be accessed by a subset of bees capable of vibrating the anthers

(Buchmann 1983, Harder and Barclay 1994), 3) flowers that release only small amounts of

pollen at a time, thus preventing over-collection of pollen by a single floral visitor (Erbar and

Leins 1995, Schlindwein et al. 2005), and 4) pollen packaged into discrete, inedible units that are

attached to the bodies of visiting bees (i.e., pollinia; Johnson and Edwards 2000, Verhoeven and

Venter 2001). Since many of these traits are often present in species for which bees are the

primary pollinator, they may be adaptations that have arisen to prevent or minimize pollen

harvesting by bees (Westerkamp 1997). Morphological features that discourage pollen collection, however, cannot be so extreme as to reduce successful pollen transfer.

Because pollen actively harvested by bees is generally made unavailable for pollination (Harder and Wilson 1998, Morris et al. 1995, Schlindwein et al. 2005, Thomson 1986), any pollen to be transferred between flowers is generally deposited onto bees incidentally (Thorp 1979, Westerkamp 1996). Thus, anthers cannot be hidden and must be sufficiently exposed to allow contact with the bodies of visiting bees (Roulston et al. 2000, Westerkamp 1996). The dilemma of how to prevent harvesting of pollen while at the same time facilitating pollen transfer may best be solved by non-morphological defensive strategies, such as the presence of toxins (Roulston et al. 2000). Pollen compounds and their putative function in affecting plant-bee relationships have received relatively little attention compared to secondary compounds found in vegetative tissues and floral nectar. Whether selection independently operates on pollen chemistry to prevent over-consumption of pollen is unknown but intriguing. Increasing evidence suggests that pollen chemistry has important effects on bee behavior and development, and potentially influences trophic specialization by bees (Dobson and Bergström 2000, Müller and Kuhlmann 2008, Praz et al. 2008, Sedivy et al. 2013).

Secondary compounds in pollen that influence bee behavior and development are primarily contained in the pollenkitt, a lipid coat surrounding the pollen grains of most angiosperms. While the pollenkitt is adhesive and enables pollen clumping during dispersal, the functions of the compounds contained within the pollenkitt layer are not completely understood. It is possible that compounds contained in pollen function in protection against ultraviolet light or microbial degradation, or are a non-adaptive, pleiotropic consequence of the presence of secondary compounds produced to defend against herbivores or nectar robbers (Kessler and

107

Halitschke 2009). Alternatively, these compounds may be adaptations to protect pollen from

pollen feeders, including bees. A first step in determining whether this may be the case is

understanding how these compounds influence bee behavior and development. Below I

summarize this research. For simplicity and for continuity with the literature (Hargreaves et al.

2009, Müller et al. 2006, Praz et al. 2008, Williams 2003) I will sometimes refer to pollen that

deters collection or depresses bee growth or survival as "toxic pollen", which should be

distinguished from nutritionally inadequate pollen (Roulston and Cane 2000, Roulston and Cane

2002), and the compounds present in the pollen as "toxins", even though we may not know that

these compounds cause toxicity.

Toxic Pollen

Bees are deterred and even killed by pollen containing high concentrations of numerous

secondary compounds. Numerous alkaloids are present in pollen of several angiosperm genera,

and at least 27 volatiles alone have been identified in angiosperm families, often in species-

specific combinations that influence attraction and in some cases serve in defense (Dobson and

Bergstrom 2000). Honey bees avoid pollen with high concentrations of cyanogenic glycosides

and phenolics if other pollen sources are available (Liu et al. 2007, London-Shafir et al. 2003).

The α-methyl ketones detected in the pollen of *Rosa rugosa* (Rosaceae), when used in isolation,

deter landing by bumblebees (Dobson et al. 1999). More strikingly, there is growing evidence

that pollen from numerous plant taxa is lethal to bee larvae. For example, honey bee larvae are

killed by pollen from at least 16 genera (Lundgren 2009). Solitary bees, which comprise the vast

majority of bee diversity, have received less attention yet lethal effects of pollen have been

clearly demonstrated for a growing number of taxa (Table 1).

Ranunculaceae pollen, which is high in protoanemonin, is toxic to two bee species which do not normally feed on Ranunculaceae pollen (Praz et al. 2008). Protoanemonin is poisonous to vertebrates and shows insecticidal activity (Jurgens and Dotterl 2004). Protoanemonin clearly deters herbivory in leaf tissues, thus its presence in pollen could be a pleiotropic effect. However Bergström et al. (1995) found that protoanemonin is the most abundant volatile in pollen of *Ranunculus acris*, and that it is more abundant in pollen than in other floral tissues. Jurgens and Dötterl (2004) compared levels of protoanemonin among several bee-pollinated taxa in the family Ranunculaceae. Protoanemonin is particularly abundant in the pollen of two genera that offer nectar rewards, *Ranunculus* and *Pulsatilla*, perhaps serving to defend pollen from consumption. Interestingly *Anemone sylvestris*, which is bee-pollinated but produces no nectar, does not contain abundant protoanemonin. In this case, pollen is the sole reward for visiting bees, and as such does not appear to be chemically protected by protoanemonin. Though *Ranunculus* pollen was lethal as a novel pollen source to two bee species, *Chelostoma rapunculi,* a *Campanula* specialist, and *Heriades truncorum,* an Asteraceae specialist (Praz et al. 2008), when ranunculin, the prescursor of protoanemonin, was added to pollen provisions normally tolerated by *C. rapunculi* and *H. truncorum*, it was lethal only in concentrations exceeding that found in *Ranunculus* pollen (Sedivy et al. 2012). This suggests that another still unknown toxic pollen compound or a lack of essential nutrients in the pollen causes mortality of bees not specialized on *Ranunculus* pollen (Sedivy et al. 2012).

Pyrrolizidine alkaloids, a potent defense against herbivores (Biller et al. 1994, Hartmann and Ober 2000, Hartmann and Zimmer 1986, Wink 1993), are present at high levels within pollen of plants in the genus *Echium* (Boraginaceae) (Boppré et al. 2005). Nutritional analyses of *Echium* pollen suggest that it has high crude protein content, does not lack essential amino

acids, and thus should be nutritionally sufficient to permit bee development (Somerville and

Nicol 2006). However, it is toxic to bees that typically do not collect pollen from plants in the

genus *Echium* (Praz et al. 2008).

Like *Echium*, many Asteroideae species contain pyrrolizidine alkaloids in pollen (Boppré

et al. 2008). Three bee species for which Asteroideae is not the normal host failed to develop or

had longer development time when fed pollen from this subfamily (Praz et al. 2008). As well,

three generalist bees failed to develop on diets of pure Asteroideae pollen (Guirguis and Brindley

1974, Levin and Haydak 1957), or had decreased larval mass with increasing proportions of

Asteroideae pollen in the diet (Williams 2003). Although particular compounds present in high

quantities in pollen are implicated in affecting bee development, it is usually unclear whether

chemical constituents or other factors such as low nutritional quality (Roulston and Cane 2000)

contribute to poor larval performance. The effects of individual compounds in pollen have

seldom been isolated and tested on larval bees.

Interestingly, pollen that is lethal to some bee species may have no negative effects on

other, often specialized, bee taxa, and even some generalists vary in their ability to survive on

different pollen sources. For example, Asteraceae are toxic to several bee taxa yet this family

supports numerous specialized bee species. *Ranunculus* pollen, which contains protoanemonin,

is toxic to honey bees, two nonadapted solitary bee specialists, and two generalist solitary bees,

yet it supports development of two generalists and one specialist (Nepi and Pacini 1997, Praz et

al. 2008, Sedivy et al. 2011, Westrich 1989 as cited by Praz et al. 2008). While the increased

levels of protoanemonin might be lethal to some bee taxa, others clearly have physiological

mechanisms to cope with its presence, and it may even play a role in attracting specialized bees

(Bergström et al. 1995). Similarly, *Echium* (Boraginaceae) pollen supports the development of

an *Echium* specialist, but is toxic to some specialist and generalist bee species (Praz et al. 2008, Sedivy et al. 2011). Surprisingly, *Heriades truncorum*, an Asteroideae specialist develops normally on *Echium* pollen (Praz et al. 2008). Like *Echium,* Asteroideae contains pyrrolizidine alkaloids, thus this bee likely possesses physiological adaptations that allow it to cope with these toxins in its Asteroideae hosts and in nonhost *Echium* pollen (Müller and Kuhlmann 2008).

Toxic Pollen and Trophic Specialization

　　Toxic pollen could play a crucial yet underappreciated role in patterns of trophic specialization among bees. Secondary compounds in vegetative tissues clearly influence herbivore specialization (Berenbaum 1981, Berenbaum 1981, Berenbaum 1981, Berenbaum 1983, Cornell and Hawkins 2003, Feeny 1976, Gilbert 1971, Rhoades and Cates 1976), and secondary compounds that are repellant to most animals are tolerated, and in many cases trigger feeding by certain specialist herbivores (Bernays and Chapman 1994, Dethier 1941, Dethier 1954, Thorsteinson 1960). For example, *Manduca sexta* will feed only on foliage containing indioside D (del Campo et al. 2001), and glucosinolates in the Brassicaceae family stimulate feeding by caterpillars of the specialized butterfly subfamily Pierinae (del Campo et al. 2001). Might toxins in pollen similarly influence bee-plant relationships?

　　Floral tissues of basal angiosperm taxa contain numerous monoterpenes that are similar to compounds used to deter herbivores in both angiosperms and conifers (reviewed in Pellmyr and Thien 1986). Thus, early bee taxa likely possessed physiological mechanisms to circumvent these toxins. If physiological tradeoffs limit the ability of bees to metabolize multiple compounds efficiently (Cornell and Hawkins 2003), than early bee taxa might have been forced to specialize on a restricted set of host plants with similar toxins. Indeed, there is evidence that a

specialized pollen diet is the ancestral condition for bees (Danforth et al. 2006). The basal clades

of most bee families contain a high proportion of specialists (Wcislo and Cane 1996) and

specialization remains widespread among extant bee species (Minckley and Roulston 2006).

Transitions from generalization to specialization and vice versa are relatively rare (reviewed in

Praz et al. 2008), and speciation events within highly specialized lineages usually involve shifts

to a closely related host, perhaps because closely-related plants have similar pollen chemistry

(Minckley and Roulston 2006, Sedivy et al. 2013). While specialization may be favored under

certain conditions (reviewed by Minckley and Roulston 2006), the large quantities of pollen

required for reproduction should select for the ability to use numerous plant hosts (Praz et al.

2008), particularly if competition for pollen is high or host abundance is variable. However,

physiological tradeoffs and the presence of toxins in pollen could reinforce specialization and

inhibit frequent evolution of a generalized pollen-feeding strategy (Praz et al. 2008).

The influence of toxic pollen on specialization and evolution has not been explored

directly. However, use of Asteraceae pollen by specialist and generalist bees in the genus

Colletes reveals an interesting pattern (Müller and Kuhlmann 2008). Asteraceae is the second

largest family of flowering plants, with 1600 genera and 23,000 species worldwide. Members of

this family occur in nearly all terrestrial habitats, flower from early spring through fall, and

produce copious amounts of pollen and nectar. Resources from within the flowers are easy to

access, and extracting pollen does not require specialized morphology. However, the great

majority of bees that collect pollen from Asteraceae are specialists and it is only of marginal

importance as a pollen source for generalists (Hurd et al. 1980, Müller and Kuhlmann 2008).

This pattern may be explained by pollen chemistry. Plants in the Asteraceae, and particularly the

subfamily Asteroideae, are known to possess unfavorable or protective properties that make

112

them difficult to digest. Pyrrolizidine alkaloids are found in honey produced by honey bees foraging on Asteroideae flowers (Deinzer et al. 1977; Edgar, Roeder and Molyneux 2002), and occur in pollen (Boppre et al. 2008). The development of three nonadapted specialist and three generalist bee species failed or was impaired when fed pollen from this subfamily (Levin and Haydak 1957, Guirguis and Brindley 1974; Williams 2003, Praz et al. 2008). While use of Asteroideae pollen might require physiological adaptations to detoxify toxic compounds (Müller and Kuhlmann 2008), given the ubiquity of Asteroideae flowers, selection should favor the evolution of such physiological adaptations. The physiological-efficiency hypothesis suggests that bees adapting physiologically to secondary chemistry of Asteroideae might be unable to efficiently use alternative hosts (Cornell and Hawkins 2003).

Müller and Kuhlman (2008) examined patterns of host plant use by 60 species in the bee genus *Colletes* (Colletidae). Of these, 14 harvested pollen exclusively from the subfamily Asteroideae. Only 7 of 34 pollen generalists harvested any Asteroideae pollen, and when Asteroideae pollen was harvested by generalists, it comprised only 2.7% of pollen loads. Three of the seven generalists that collected Asteroideae pollen are closely related and are likely derived from Asteroideae pollen specialists. Asteroideae flowers are ubiquitous in terrestrial habitats and lack morphological features that make pollen removal difficult. Why are members of the Asteroideae such an important pollen source for specialist bees but very rarely used by generalist bees? It may be that Asteraceae pollen requires specialized physiological adaptations to detoxify secondary compounds in the pollen or overcome nutrient deficiencies. At this point, we are unable to differentiate these two possibilities.

Secondary compounds in pollen may also explain patterns of specialization among osmiine bees on the plant families Boraginaceae and Fabaceae. Sedivy et al. (2013) found that

many bees of the *Annosmia-Hoplitis* group (Megachilidae) that specializied within the Boraginaceae plant family included plant species with morphologically different flower types that required a variety of morphological and behavioral adaptations to exploit them. If bees are able to harvest pollen from such morphologically distinct plant taxa, why do they specialize within the family rather than also utilizing taxa outside of the Boraginaceae? Sedivy et al. (2013) propose that physiological adaptations to plant alkaloids permit harvesting of pollen from the diverse taxa within Boraginaceae and underlie fidelity within the family, as they are unable to tolerate the secondary compounds in alternative hosts (Cornell and Hawkins 2003). They suggest that shared secondary compounds that can be detoxified by the same physiological tools may explain this pattern. In addition, they observed that bees not dependent on Boraginaceae were highly dependent on Fabaceae; several specialists collected only pollen from Fabaceae and all generalists used Fabaceae in addition to Boraginaceae. This is paradoxical because Fabaceae and Boraginaceae are not closely related and are morphologically distinct, requiring different pollen-harvesting techniques. Again, the authors suggest that the presence of similar secondary compounds in pollen that can be detoxified using the same physiological tools may underlie this pattern.

Benefits to Plants

Since toxins in pollen, such as protoanemonin and pyrrolizidine alkaloids, are often present in vegetative tissues, it is unclear whether toxins in pollen represent a pleiotropic effect of selection on vegetative defensive traits or whether they are under selection by bees to reduce pollen consumption. While there are often correlations between levels of secondary compounds in vegetative tissues and in floral rewards (reviewed in Kessler and Halitschke 2009), numerous

studies suggest that not all examples of toxic pollen result from simple pleiotropy. For example, many toxins are distinct in pollen or are present in greater amounts than in other floral tissues (Dobson and Bergstrom 2000; London-Shafir et al. 2003; Kessler and Halitschke 2009). In some cases, these contrasts are extreme. For example, London-Shafir et al. (2003) found that levels of amygdalin in almond flowers were over 250 times greater in pollen compared to nectar, while it was not present in leaves (Dicenta et al. 2002). More strikingly, Kessler and Halitschke (2009) found that while there were correlations between several phenolics in leaves and pollen which may suggest pleiotropy, pollen contained novel coumaroyl derivatives that weren't present in leaf tissues.

If not due to pleiotropy, the presence of compounds in pollen that deter pollen-feeding bees is intriguing. It is also counter-intuitive, since any trait that deters pollen collection by bees might decrease visitation and ultimately plant reproductive success. Thus, why should pollen that has deterrent effects on behavior and development of bees evolve in plants that use pollen feeding-bees as pollinators? This question could similarly be posed in the case of morphological adaptations that prevent over-harvest of pollen. Such adaptations often limit the ability of bees to harvest floral rewards, possibly at the cost of restricting pollen flow and reproduction. However, morphological adaptations may influence the quantity of pollen that is removed by bees (e.g., dispensing mechanisms), or restrict access to pollen by particular bee taxa (e.g., long corolla tubes that limit access by bees with short mouthparts). Similarly, secondary compounds present in pollen might serve to either reduce overall pollen harvest by an individual (as with dispensing mechanisms) or colony, or restrict visitation to a subset of bee taxa (Jurgens and Dötterl et al. 1995, Müller and Kuhlmann 2008).

Toxins in pollen might reduce the total pollen harvested by an individual bee or colony by encouraging mixing of foods to dilute toxins. Generalist herbivores often mix foods (Freeland and Janzen 1974), and in some cases herbivores have enhanced performance on mixed diets (Singer 2001; Karban et al. 2010). Food mixing may benefit herbivores by enabling them to balance nutrients (Pulliam 1975) or dilute secondary compounds present in plant tissues (i.e., the Toxin Dilution Hypothesis; Freeland and Janzen 1974). While these two hypotheses have been difficult to separate empirically, Singer et al. (2002) showed that *Grammia geneura* caterpillars switch foods to dilute uptake of secondary compounds. Secondary compounds in fleshy fruits are similarly proposed to induce frugivores to leave fruiting plants early in a feeding bout (Cipollini and Levey 1997, Sorenson 1983). Secondary compounds in pollen might similarly trigger switching of food sources by generalist bees such as honey bees (London-Shafir et al. 2003). Pollen mixing by bees would result in reduced pollen feeding on an individual plant species and perhaps flowers within an individual plant. It is unclear in the case of pollen-collecting bees what would trigger switching behavior, because adult foraging bees do not consumer the pollen themselves and thus have no means of testing its quality (Westerkamp 1996). However, bees sometimes actively remove pollen from their bodies that is picked-up incidentally (Cazier and Linsley 1974, Hurd et al. 1980, Pick and Schlindwein 2011) suggesting that bees may have some ability to perceive pollen quality or chemistry without consumption.

Toxic pollen could also benefit plants by restricting access to pollen by a subset of bee taxa. Secondary compounds in vegetative tissues strongly influence the identity of herbivores that use plant resources. For example, chemical compounds that repel generalist herbivores are often used as host-finding cues by specialist herbivores (del Campo et al. 2001, Van der Meijden 1996). Similarly, pollen compounds might aid specialists in locating their host plant. In

addition, these compounds might simultaneously deter visitation and pollen collection by generalists incapable of physiologically coping with pollen toxins. When present, specialist bees can be high-quality partners relative to generalist bees (Lindsey 1984, Motten et al. 1981, Stucky and Beckmann 1982, Tepedino 1981). Thus the attraction of specialist bees that results from pollen compounds may benefit plant reproduction. Alternatively, if there is high temporal or spatial variation in specialist abundance, generalist bees can be better partners (Barrows 1976, Bernhardt and Weston 1996, Olsen 1997, Zavortink 1992). In these cases, reduced fitness associated with pollen consumption by specialist bees should select for escalating defensive toxins. However, direct demonstrations that variation in pollen toxicity produces variation in plant fitness are lacking.

Future Research Priorities

In recent years there has been increasing interest in the conflicting selection pressures on floral traits exerted by enemies and mutualists (Irwin et al. 2004; Strauss and Whitall 2006; Hargreaves et al, 2009; Kessler and Halitschke 2009). Bees serve both of these roles; they are necessary for pollination, yet they sequester and consume large amounts of pollen. For this reason, many plants face a dilemma in that they must make pollen available to facilitate pollen transfer, yet simultaneously prevent harvest of pollen that reduces fitness (Westerkamp 1996). While many morphological features of flowers are interpreted to serve in defense of pollen form over-harvest (Erbar and Leins 1995, Johnson and Edwards 2000, Praz et al. 2008, Schlindwein et al. 2005, Verhoeven and Venter 2001, Westerkamp 1997), secondary compounds in pollen may be an additional yet presently unappreciated mode of pollen defense. A variety of compounds in pollen clearly deter collection (Dobson et al. 1999, Liu et al. 2007, London-Shafir et al. 2003)

and kill larvae (Lundgren 2009, Praz et al. 2008), and these compounds very likely influence specialization by bees on particularly pollen sources (Müller and Kuhlmann 2008). However, many key questions remain.

First, while pollen has historically been viewed as an easy-to-use protein source that is readily digestible for bees, it is clear that pollen contains an array of secondary compounds. Pollen chemistry has not historically been studied apart from overall floral chemistry, but despite this, the number of compounds detected in pollen is growing rapidly. Less clear, however, is the relationship between pollen chemistry and overall plant chemistry. While numerous studies have found correlations between secondary compounds in vegetative tissues and floral nectar (reviewed by Kessler and Halitschke 2009), only a handful have examined chemistry of pollen and how it relates to other tissues (Boppré et al. 2005, London-Shafir et al. 2003, Pernal and Currie 2002).

Second, the influence of pollen chemistry on bee behavior, development, and evolution should be more directly explored and distinguished from nutrient deficiencies, which can be detrimental to developing bees (Roulston and Cane 2002). Amino acid profiles vary among species with taxonomically related species having similar compositions (Wieiner et al. 2010), and at the species level, pollen of plants hosting specialists contained significantly lower amounts of amino acids, though this was not true at the family level (Wieiner et al. 2010). To tease apart the influence of toxins and nutrient deficiencies, one could isolate a particular pollen compound and add this to a benign pollen source normally tolerated by a particular bee species (e.g. Sedivy et al. 2012). A phylogenetic perspective on toxic pollen will also enhance our understanding of how toxins influence host shifts and the evolution of specialist and generalist bees. For example, do plants that commonly host specialist bees posses more toxic pollen than

those primarily hosting generalists? With regards to plant diversity, Ehrlich and Raven (1964) proposed that plants are able to rapidly diversify once a novel defense reduces pressure from herbivores. Are increases in pollen defense associated with increases in plant diversity?

Third, we know little regarding the magnitude of pollen consumption by bees at the level of plant populations, and how this influences plant fitness. Most work explores only removal by a single, specialized bee species, thus we know nothing about the magnitude of pollen removal by generalist bees, or the combined effects of multiple bee species harvesting pollen from a single plant species. Moreover, only a single study (Schlindwein et al. 2005) has investigated how consumption scales up to affect the amount of pollen that remains available for pollination in at the level of plant populations.   It is also unclear how pollen consumption by native bees influences pollen-limitation and male fitness. Studies of how pollen-limitation is influenced by pollen vector may be useful. For example, we know that plants producing nectar rewards, which presumably suffer lower loss of pollen to consumption per visit, are less pollen-limited than plants that produce only pollen rewards (Larson and Barrett 2000). If bee-pollinated plants lose more pollen to consumption than plants adapted for pollination by other vectors, are they in general more pollen-limited?   More useful yet difficult will be work that explicitly examines how pollen consumption influences plant fitness. While a handful of studies have attempted this, nearly all have involved pollen consumption by nonnative honeybees, and often the plant in question is not adapted for pollination by bees (reviewed in Hargreaves et al. 2009). Manipulating antagonistic pollen removal while maintaining visitation and pollen transfer is likely not possible in most systems but could be explored using modeling approaches. This will be will crucial to understanding whether consumption of pollen by bees influences plant fitness.

Fourth, to assert that toxic pollen evolved in response to selection by pollen consumers, we must explore whether secondary compounds in pollen have heritable variability, and whether this variation affects fitness. While variation in pollen chemistry within a species has not been demonstrated, there is heritable variation of pollen compounds occurring in other plant tissues. For example, levels of amygdalin in almond (*Amygdalis communis)* fruit are controlled by a single gene with two alleles, 'S' dominant gives low levels of amygdalin, 's' recessive gives high levels, and heterozygotes are intermediate (Dicenta et al. 2002, Vargas et al. 2001). If amygdalin in pollen is under similar control, one could compare visitation and fitness of plants with different phenotypes.

If variation in pollen chemistry is shown to influence pollen collection and plant fitness, and is under selection by pollen-consuming bees, than we can begin to explore patterns of pollen chemistry in relation to ecological factors. For example, we might predict that plants producing nectar rewards invest more in chemical defense of pollen (Jurgens and Dötterl et al. 1995; Frolich et al. 2005; Kessler and Halitschke 2009), and that outcrossing plants contain higher levels of defense than plants with autonomous self-pollination (i.e., self pollination that occurs spontaneously without the need of a pollen vector). In addition, plant resources may influence investment in pollen defense, as is the case for defense against vegetative herbivory (Coley et al. 1985, Fine et al. 2006). Pollen is rich in nitrogen, and soil nitrogen can limit pollen production, grain size, and performance (Lau and Galloway 2004). Plants growing in nitrogen-rich soils might readily give up pollen to bees if production of pollen is relatively inexpensive, while plants in nitrogen-poor soils might invest more heavily in defense of pollen.

Pollen consumption by bees is substantial, yet its impact on plant fitness is poorly understood. If toxic pollen reduces consumption of pollen by bees, it could directly benefit plant

fitness. In addition to its influence on plant reproduction, toxic pollen may also strongly influence patterns of specialization by bees. Given the prevalence of plant-bee interactions, these topics warrant more direct consideration as well as integration into the broader topic of plant-defense theory and plant-herbivore interactions.

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Table 1. Studies examining the response of solitary bees when reared on novel pollen hosts.

Species	Generalist or Specialist Host	Novel Host	Development	Source
Megachile rotundata	Generalist	*Pyrrocoma* (Asteraceae)	Failed	Guirguis and Brindley 1974
Osmia bicornis	Generalist	*Echium* (Boraginaceae)	Failed	Sedivy et al. (2011)
		Ranunculus (Ranunculaceae)	Normal	
		Sinapis (Brassicaceae)	Normal	
		Tanacetum (Asteraceae)	Failed	
Osmia cornuta	Generalist	*Echium* (Boraginaceae)	Normal	Sedivy et al. (2011)
		Ranunculus (Ranunculaceae)	Failed*	
		Sinapis (Brassicaceae)	Normal	
		Tanacetum (Asteraceae)	Failed	
Osmia lignaria	Generalist	Asteroideae (Asteraceae)	Failed	Levin and Haydak 1957
		Heliantheae (Asteraceae)	Smaller	Williams 2003
		Brassica (Brassicaceae)	Smaller	Williams 2003
Chelostoma florisomne	*Ranunculus* (Ranunculaceae)	*Tanacetum* (Asteraceae)	Failed	Praz et al. 2008
		Campanula (Campanulaceae)	Normal**	
		Brassica (Brassicaceae)	Normal	
Chelostoma rapunculi	*Campanula* Campanulaceae	*Ranunculus* (Ranunculaceae)	Failed	Praz et al. 2008
		Buphthalmum (Asteraceae)	Failed	
		Echium (Boraginceae)	Failed	
		Sinapis (Brassicaceae)	Failed	
Heriades truncorum	Asteraceae	*Camanula* (Campanulaceae)	Normal	Praz et al. 2008
		Ranunculus (Ranunculaceae)	Failed	
		Echium (Boraginaceae)	Normal	
		Sinapis (Brassicaceae)	Longer	
Hoplitis adunca	*Echium* (Boraginaceae)	*Buphthalmum* (Asteraceae)	Failed	Praz et al. 2008
Lasioglossum galpinsiae	*Oenothera,* (Onagraceae)	*Medicago* (Fabaceae)	Normal	Bohart and Youssef 1976
Osmia californica	Asteraceae	*Phacelia* (Hydrophyllaceae)	Normal	Williams 2003
		Brassica (Brassicaceae)	Normal	

* two larvae survived but were reduced in size

**late mortality due to fungal infection unrelated to pollen host