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COMPETITIVE CONTEXT DRIVES POLLINATOR BEHAVIOR: LINKING FORAGING PLASTICITY, NATURAL POLLEN DEPOSITION, AND PLANT REPRODUCTION

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Abstract

Competitive Context Drives Pollinator Behavior: Linking Foraging Plasticity, Natural Pollen Deposition, And Plant Reproduction

With ongoing global pollinator declines it is important to understand the functional impact of pollinator species losses. While network-based simulation models of pollinator declines predict that plant communities will be robust to losses of pollinator species, these predictions have never been tested empirically. In four chapters, my dissertation uses both empirical and modeling approaches to explore the impacts of losing pollinator species in alpine plant communities.

First, I test the hypothesis that interspecific interactions among pollinators (rather than fixed species roles) dynamically alter the functional contribution of species in a community and that these dynamic roles can sometimes reduce plant reproductive function. Experimental removal of the most abundant bumble bee pollinator species from an alpine bumblebee community led to a reduction in floral fidelity in the remaining pollinators. Importantly, this behavioral response in the remaining pollinators reduced plant reproduction by increasing the transfer of ineffective heterospecific pollen.

Second, I evaluate how patterns of naturally deposited heterospecific pollen relate to the reproductive output of *Delphinium barbeyi,* a common subalpine perennial herb in the Rocky Mountains. I found a significant negative interaction between conspecific pollen and the amount of heterospecific pollen whereby the

relationship between conspecific pollen and viable seed production became weaker with increasing heterospecific pollen deposition on stigmas.

Third, I explore how traits—specifically pollinator tongue length, which dictates pollinator resource partitioning—influences how pollinators responded to reduced interspecific competition. I found that bees vary in their baseline floral fidelity and that their tongue length explained a large part of this variation. Bees with shorter tongues moved between plant species (floral infidelity) more often than bees with longer tongues. I did not find significant variation in how bee species responded to reduced interspecific competition, but rather saw a guild-wide reduction in floral fidelity in response to the removal of the dominant bee species. Interestingly, I found that competitive context determines the floral fidelity of the pollinators in a community. In this case, as the tongue length of the most abundant bee increases, the site level foraging fidelity decreases.

Network model simulations predict plant populations will be resilient when faced with pollinator extinctions. Importantly, these models are built on the assumption that all interactions in networks are positive, potentially overestimating the resilience of plant-pollinator networks. A wide range of studies (including my field work) have shown that many pollinators are actually ineffective at pollinating plant species they visit, and can therefore have *negative* consequences for plant reproduction. Fourth, I used interaction network simulations to understand how the addition of negative interactions impacts the predictions of resilience when plant communities are faced with pollinator species losses. I found that the addition of

negative interactions makes networks less robust to pollinator extinctions, but not always in the same way. This work suggests metrics specific to interaction networks may be important in determining how robust plant-pollinator interactions are to extinctions.

Finally I provide overview on some of the causes and consequences of pollinator decline in the US and offer insight in to how my basic research in to pollinator ecology can help pinpoint vulnerable species and communities that should be targeted for conservation efforts.

To PTH and LJH — the brightest lights at the end of the tunnel.

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Introduction:

Globally, native pollinator species are facing both range contractions and population declines due to a combination of threats including; habitat loss, disease, agricultural intensification and global climate change (Kearns et al. 1998, Biesmeijer et al. 2006, Potts et al. 2010). While we still do not have sufficient long term data to understand the extent to which pollinator declines will impact the plants that rely on them for reproduction, some studies suggest that plants and pollinators are facing concurrent declines (Memmott et al. 2004, Biesmeijer et al. 2006, Memmott et al. 2007, Kaiser-Bunbury et al. 2010, Potts et al. 2010). Still, recent network modeling work based on pollination networks suggest that plant communities will be highly resilient to linked extinctions (plant extinctions that result from pollinator extinctions) when faced with pollinator declines (Memmott et al. 2004, Bascompte et al. 2006, Memmott et al. 2007, Bastolla et al. 2009). This is because pollination networks have an asymmetric structure (i.e., specialists tend to interact with generalists rather than other specialists) and because these networks are dominated by generalist interactions. Generalism is thought to add functional redundancy. That is, when a pollinator is lost from the community there is another that can fill its functional role. Simulations of pollinator species losses suggest that the asymmetric, generalist-dominated structure of pollination networks should provide extreme resilience to extinctions of either plants or pollinators (Memmott et al. 2004, Bascompte et al. 2006, Memmott et al. 2007, Bastolla et al. 2009). These predictions of resilience are promising, but have not been tested

empirically. My PhD research is focused on understanding the implications of pollinator species losses.

Chapter 1: Single pollinator species losses reduce floral fidelity and plant reproductive function. This study shows that a reduction in interspecific competition (through targeted removals of the most abundant bee species) leads to a reduction in floral fidelity of the remaining bee pollinators and ultimately a reduction in plant reproductive output, even when other effective pollinators remain in the system. This chapter was published in the Proceedings of the National Academy of Sciences (Brosi and Briggs 2013)

Chapter 2: Heterospecific pollen deposition in *Delphinium barbeyi*: linking stigmatic pollen loads to reproductive output in the field. While a reduction in plant reproductive output was documented in Chapter 1, we did not directly link pollen deposition on a stigma to seed set in the same carpel (flower). This chapter takes a comparative field approach to explore how patterns of naturally deposited heterospecific pollen (HP) relate to the reproductive output of *Delphinium barbeyi,* a common subalpine perennial herb in the Rocky Mountains. I directly linked stigmatic pollen deposition to seed set in individual carpels in the field. Results show that there is a significant negative interaction between conspecific pollen and HP amount whereby the relationship between conspecific pollen and viable seed production

became weaker with increasing HP deposition on stigmas. This chapter was published in Annals of Botany (Briggs et al. 2015).

Chapter 3: Reponses of pollinators to reductions in interspecific competition are context-dependent and correlated with bee tongue length. This study moves beyond the community-wide response to pollinator losses described in Chapter 1, and explores both if species vary in their response to pollinator loss and if traits—specifically pollinator tongue length, help predict how pollinators will respond to a change in interspecific competition interactions.

Chapter 4: Resilience of Plant Pollinator Networks. Network model simulations predict plant populations will be resilient when faced with pollinator extinctions. Importantly, these models are built on the assumption that all interactions in network are positive, potentially overestimating the resilience of plant-pollinator networks. A wide range of studies (including my field work described in chapters 1-3) have shown that many flower visitors are actually ineffective at pollinating plant species they visit, and therefore can have *negative* consequences for plant reproduction. This chapter builds on the three empirical natural science chapters described above and uses trinary (1,0,-1 interaction network simulations to understand how the assumption of all positive interactions impacts the predictions of resilience when plant communities are faced with pollinator species losses.

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Single pollinator species losses reduce floral fidelity and plant reproductive function

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ABSTRACT

Understanding the functional impacts of pollinator species losses on plant populations is critical given ongoing pollinator declines. Simulation models of pollination networks suggest that plant communities will be resilient to losing many or even most of the pollinator species in an ecosystem. These predictions, however, have not been tested empirically and implicitly assume that pollination efficiency is unaffected by interactions with interspecific competitors. By contrast, ecological theory and data from a wide range of ecosystems show that interspecific competition can drive variation in ecological specialization over short timescales via behavioral or morphological plasticity, though the potential ecosystem functional implications of such changes in specialization remain unexplored. We conducted manipulative field experiments in which we temporarily removed single pollinator species from study plots, to test the hypothesis that interactions between pollinator species can shape individual species' functional roles via changes in foraging specialization. We show that loss of a single pollinator species reduces floral fidelity (short-term specialization) in the remaining pollinators, with significant ecosystem functional implications in terms of reduced plant reproductive output, even when potentially effective pollinators remained in the system. Our results suggest that ongoing pollinator declines may have more serious negative implications for plant communities than is currently assumed. More broadly, we show that the individual functional contributions of species can be dynamic and shaped by the community of interspecific competitors, thereby documenting a new mechanism for how

biodiversity can drive ecosystem services and functions, with potential relevance to a wide range of taxa and systems.

INTRODUCTION

Global pollinator declines are ongoing (Biesmeijer et al. 2006, Potts et al. 2010), leading to concerns about the functional impacts of pollinator species losses on both pollination-dependent native plants (Ollerton et al. 2011) and crop production (Klein et al. 2007). Network-based simulation models of pollinator species losses, however, predict that major functional impacts on plant species persistence will not typically result until many, or even most, pollinator species are lost from a system (e.g. Memmott et al. 2004, 2007, Kaiser-Bunbury et al. 2010), but these results have not been tested empirically. These predictions are based in part on redundancies in pollination networks: most plant species are visited by several pollinator species, contributing to the robustness of such networks (5). This robustness, however, is dependent on the implicit assumption that the functional roles of pollinator species are static. If interspecific interactions can dynamically alter species' functional contributions—such as the efficacy of a particular plant-pollinator relationship—pollinator species losses may have greater cascading impacts on plant communities than predicted by current models.

Such dynamic changes in species functional roles are predicted by the ecological literature on interspecific competition and specialization, but have not been studied in the context of pollination or other ecosystem functions and services. Competition between species has long been known to affect specialization in a wide range of taxa and ecosystems over both evolutionary and ecological timescales (Fretwell and

Calver 1969, Pimm et al. 1985, Futuyma and Moreno 1988, Rosenzweig 1991, Bolnick et al. 2010). When interspecific competition increases, each species in a system tends to specialize ecologically. By contrast, when intraspecific competition increases, individuals within that species tend to become more ecologically generalized. When considered over evolutionary time, this process can lead to niche differentiation (Futuyma and Moreno 1988), resulting in static ecological complementarity among species, a well-documented mechanism for positive biodiversity-ecosystem function relationships (Hooper et al. 2005, Cardinale 2011). Over ecological timescales, however, interspecific competition can lead to *dynamic* specialization (Pimm et al. 1985, Rosenzweig 1991, Bolnick et al. 2010) via phenotypic plasticity, either behavioral or morphological. In the case of pollination, short-term pollinator foraging specialization on particular plant species, or "floral fidelity", is critical because transfer of conspecific pollen must occur in order for fertilization to take place. Reductions in floral fidelity can have deleterious effects on plant reproduction (Morales and Traveset 2008, "Effects of multiple competitors for pollination on bumblebee foraging patterns and Mimulus ringens reproductive success" 2010, Arceo-Gómez and Ashman 2011). Floral fidelity may be shaped by interspecific interactions because most pollinator species are generalists with labile foraging patterns. Despite the potential importance of dynamic specialization, its contributions to ecosystem services and functions has not been considered to date.

Here, we assessed the impacts of single-pollinator species losses on dynamic specialization (floral fidelity) and pollination function using novel field manipulations. Our manipulations temporarily and non-destructively removed the most abundant bumble bee (Hymenoptera: Apidae: *Bombus*) species from field plots using targeted aerial netting. The identity of the target species varied between plots, and we manipulated 6 *Bombus* species, or more than half of the 11 species in the system. We assessed each individual plot in both a control and a manipulated state, allowing us to hold the plant community constant within our comparisons. We assessed how (a) dynamic specialization (floral fidelity) of bumble bees changed between the control state and the manipulated state of each plot, and followed the linked changes through the steps of the pollination process in terms of (b) bee pollen carriage, (c) deposition of pollen on floral stigmas, and finally (d) plant reproductive function, i.e. seed output (Fig. 1). We tracked the pollination process in a larkspur, *Delphinium barbeyi* (Ranunculaceae), an abundant wildflower that is visited by at least 10 of the 11 bumble bee species in the system.

RESULTS

Rates of floral fidelity, proportions of conspecific pollen carriage and deposition, and seed production significantly decreased in the manipulated state—with a single pollinator species removed—relative to the control state. We assessed floral fidelity on a per-plant-visit basis (proportion of individual foraging movements that were within-species vs. between-species) and on a per-bee basis (proportion of bees that

visited only a single plant species, versus those that foraged on more than one plant species). On a per-plant-visit basis, foraging movements between individual plants of different species increased by an average of 156% in manipulated plots relative to controls, based on observation of >23,500 between-plant foraging movements in 736 individual bumble bees ($P = 6.35 \times 10^{-6}$, GLMM; $N = 20$ sites with control/manipulation pairs, Table 1). On a per-bee basis, the percentage of individual bees visiting only one species of plant within a single foraging bout decreased from 77.7% to 66.4% in the control relative to the manipulated state of each plot (Fig. 2a, Table 1) in the same 736 individual bees ($P = 0.000748$, generalized linear mixed-effects model "GLMM", see Methods; $N = 20$ sites). Patterns of pollen carriage also reflected decreased floral fidelity: bumble bees in the manipulated state carried 17.5% more mixed-species pollen loads relative to controls ($P = 0.040$, GLMM, pollen loads from 254 bees, $N = 15$ sites; Fig 2b, Table 1). The proportion of conspecific pollen deposited on *D. barbeyi* stigmas concurrently decreased from 61% to 56% in control vs. the manipulated state ($P = 2.67 \times 10^{-7}$, GLMM, counts of >47,000 pollen grains from 129 plants in $N = 5$ sites; Fig 2c, Table 1). These changes in specialization behavior, pollen carriage, and stigmatic deposition were ultimately reflected in decreased ecosystem function, i.e. a significant reduction in seed production in *D. barbeyi* in the manipulated relative to control state of each plot ($P = 0.0331$, GLMM, counts of 1,599 developed seeds in 192 plants in $N = 5$ sites; Fig 3, Table 1). Based on GLMM model coefficients and mean *Bombus* species richness and abundance, single-pollinator species removals reduced mean seed count per flower by 32.0%.

Our results also provide support that the effects of the manipulations on plant reproductive function were driven by differences in pollinator species richness, rather than changes in abundance. The single pollinator species removal manipulations reduced *Bombus* relative abundance (not just species richness) on average, though the difference was marginally non-significant ($P = 0.059$, paired t-test, $N = 20$ site pairs). Individuals of non-target *Bombus* species could freely enter the plots, and abundance effects were highly variable (mean paired abundance changes in manipulated relative to control plots = -11.4%; range: -88.6% to +225%). In 7 of the 20 sites, *Bombus* abundance was greater in the manipulated state relative to the control (despite the removal of the target species), allowing us to statistically control for changes in *Bombus* abundance due to the manipulations. Bumble bee abundance was not significantly related to either of the two floral fidelity outcomes or to pollen carriage (Table 1). The relationships between *Bombus* abundance and both stigmatic pollen deposition and seed set, while statistically significant, were *negative* with small model coefficients (Table 1), i.e. indicating that increased bumble bee abundance was associated with slightly lower conspecific pollen deposition and seed production. Thus, the pattern of reduced ecosystem function in the manipulations is not likely driven by abundance changes.

DISCUSSION

Our results provide the first demonstration of interspecific interactions dynamically changing the ecosystem functional roles of particular species. Specifically, we show that pollination function can be reduced with the removal of a single pollinator species, despite the fact that other potentially effective pollinator species remained in the system. This empirical finding contrasts with prevailing predictions of network robustness to pollinator species losses, based on simulation models that do not account for changes in the efficacy of particular plant-pollinator relationships. The reduction in plant reproductive function that we observed is associated with dynamic changes in pollinator behavior, through the steps shown in Fig. 1. When we removed an interspecific competitor, remaining pollinators broadened their foraging strategies to include flowers that the removed species had previously focused on. This dietary broadening within a single foraging trip means that remaining pollinators show less fidelity to particular plant species. Reduced floral fidelity translates functionally into less conspecific pollen carried by bees and transferred between individual plants of the same species, which was ultimately reflected in decreased plant seed production in our manipulated sites. Thus, our results show that the ecosystem functional contributions of bee species in our system are not fixed, but instead are dynamic and dependent on interactions with competing species.

These findings highlight a new mechanism for how biodiversity can shape ecosystem services and functions. Complementarity—in which different species perform distinct

fixed roles in different components of ecosystem function—is the primary accepted mechanism for biodiversity-ecosystem function (BDEF) patterns (Hooper et al. 2005, Cardinale 2011). In contrast, we show a role for biodiversity *per se* in ecosystem function, in shaping dynamic specialization and its functional consequences over short (ecological or behavioral) timescales. While we focused on specialization and did not assess dynamic complementarity in our experiments, complementarity and specialization are often ecologically intertwined (Blüthgen and Klein 2011) and there is evidence for interspecific competition shaping specialization in ways that likely increase dynamic complementarity (Morse 1977). The mechanism of interspecific competition driving dynamic specialization is widespread both taxonomically and geographically (Pimm et al. 1985, Rosenzweig 1991, Bolnick et al. 2010), supporting the idea that our results likely extend to other BDEF relationships more generally.

If dynamic specialization and/or complementarity drive some ecosystem functions, however, why has this result not been uncovered in the hundreds of studies on BDEF relationships? Three factors might contribute. First, the vast majority of BDEF studies have focused on plants and other sessile autotrophs (Hooper et al. 2005, 2012), while much of the work on competition and dynamic specialization has focused on animals (Fretwell and Calver 1969, Pimm et al. 1985, Rosenzweig 1991). Given the rapid behavioral plasticity of animals in response to interspecific competition (Bolnick et al. 2010), dynamic specialization and/or complementarity may be especially important for animal-driven ecosystem functions. Still, there is recent evidence of dynamic

specialization in plant communities driven by morphological phenotypic plasticity (Ashton et al. 2010, "Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits" 2012). Second, most BDEF studies cover relatively short time scales; but longer-timescale experiments, especially for plants, may allow for more dynamic specialization to occur, and may explain in part the result that greater biomass "overyielding" in species-rich treatments relative to monocultures often occurs only after months or years in long-term BDEF experiments ("Diversity and Productivity in a Long-Term Grassland Experiment" 2001, "Overyielding among plant functional groups in a long-term experiment" 2003, "Impacts of plant diversity on biomass production increase through time because of species complementarity." 2007). Third, the bulk of BDEF experiments are designed such that species identities and abundances are tightly controlled. While such studies are undoubtedly of great value for mechanistically untangling BDEF relationships, manipulative experiments under natural field conditions (Selmants et al. 2012), especially in terms of interspecific competition regimes, may allow for stronger dynamic responses and thus greater impacts on ecosystem function.

As we have demonstrated, losses of a single pollinator species lead to reductions in dynamic specialization, driven by interspecific interactions, that in turn drive significant negative effects on ecosystem functioning in terms of plant reproduction. Our work thus suggests that ongoing pollinator declines could already be causing negative impacts on plant populations, in contrast to the network-based simulation

models that predict plant communities will be robust to pollinator species losses. To prevent disruptions of pollination and other critical ecosystem functions and services, we must move beyond assuming that the functional roles of species are static and work to understand how and under what conditions phenotypic plasticity and interspecific competition can interact to drive dynamic changes in ecosystem services and functions.

MATERIALS AND METHODS

STUDY AREA AND SITE SELECTION

We worked in 20 plots in the landscape surrounding the Rocky Mountain Biological Laboratory (38°57.5′N, 106°59.3′W, 2900 m a.s.l.), in the Gunnison National Forest, western Colorado, USA. We selected 20 × 20 m plots in sites with comparable densities of bumble bees and floral resources. Sites were separated by a minimum distance of 1 km. We collected data over two summer growing seasons, in 2010 and 2011.

MANIPULATIONS

We assessed each plot in a control and then a manipulated state, with one day in between; we kept the interval between control and manipulated states short because of the rapid turnover in flowering phenology in our high-altitude system, allowing us to keep the plant community constant in our control-manipulation comparisons. We conducted experiments in each plot only once per year. We used targeted aerial

netting to remove the most abundant *Bombus* species in each plot. Manipulated bees were kept alive in individual vials in a shaded, cool container until the end of the study period (several hours) and then released. After removing all visible individuals of the target species, we waited 1-hr before collecting data. One member of the field team patrolled the site boundaries to prevent bees of the target species from moving into the plot.

FORAGING OBSERVATIONS

We directly followed the foraging sequences of *Bombus* individuals, recording the identity of each plant species visited in a sequence. We followed individual bees in the order in which they were first seen. We discontinued an observation when the bee was lost from sight, when it ventured more than 5 m outside of the plot, when it had been observed for 10 full minutes, or when we had tallied 100 individual plants visited. We discarded observations of fewer than five plants visited.

BOMBUS INVENTORY

We inventoried *Bombus* species richness and abundance using non-destructive aerial netting, with two field team members netting for a 20-minute period, not including handling time (the time from when a bee was in the net until it was in a closed vial). To avoid double-counting, each bee was kept in an individual glass vial, identified to species, and kept in a cool, dark location until the inventory time period was over, at which point bees were released.

BEE POLLEN LOAD REMOVAL AND ANALYSIS

We removed pollen loads from 15 bumble bees in each site, selected haphazardly from those sampled during the *Bombus* inventory, in both control and manipulated states. We induced cold anesthesia by placing the glass vial containing the bee on ice, and removed pollen non-destructively under a stereoscope set up in the field, using cubes of fuchsin jelly (*Techniques for pollination biologists* 1993). We mounted the pollen-containing jelly on microscope slides and later assessed floral fidelity in each pollen load by identifying the pollen types contained within. We counted pollen loads as monospecific if >95% of pollen grains represented a single species, and as heterospecific otherwise.

MEASURING STIGMATIC POLLEN DEPOSITION AND SEED SET

We assessed the effects of our manipulations on stigmatic pollen deposition and seed production in *Delphinium barbeyi* (Ranunculaceae), a common, long-lived perennial herbaceous wildflower in our plots that is pollinated by several species of bumble bees. We pre-bagged racemes containing immature floral buds of *D. barbeyi* in each plot 48-72 hours before experiments. We opened 15 separate bags containing mature, virgin flowers in the control and the manipulation experimental periods, and re-closed the bags at the end of a standardized 4-hr period. We returned to the site 3-4 days after the treatment to harvest stigmas (allowing sufficient time for pollen tube growth), and 7-15 days later to harvest fruits after they had matured. Stigmas were removed

from the flower and mounted on a slide with fuschin jelly in the field to avoid any loss of pollen in transit. We counted both total number of pollen grains as well as proportion of delphinium and non-delphinium pollen grains on the stigmas. We dissected mature fruits and counted developed and undeveloped seeds in the laboratory.

DATA ANALYSIS

Data collected within a study site are not independent: bees and plants are likely to be closely related genetically, and environmental conditions, floral resources, and competitive community context are all similar. To address this lack of independence and prevent pseudoreplication, we used generalized linear mixed-effects models (GLMMs) with site as random effect. Thus, the site represents the level of experimental replication across all of our statistical models. We included three fixed effects in all models: experimental state (manipulated vs. control), *Bombus* species richness, and *Bombus* abundance, allowing us to statistically control for the changes in abundance in our manipulations, as well as the differing levels of initial *Bombus* species richness in each plot. We assessed full models to maintain consistency and comparability among the different analyses.

Our data on floral fidelity, pollen carriage, and stigmatic pollen deposition were measured in a binomial fashion: individual bees either displayed fidelity (i.e. visited only one species) or infidelity; foraging transitions were either to a conspecific or to a

heterospecific plant; pollen loads were either "pure" (monospecific, and thus displaying fidelity) or else "mixed" (heterospecific, and thus displaying infidelity); and pollen grains on stigmas were identified as either conspecific (i.e., pollen of *D. barbeyi*) or heterospecific. We used GLMMs with binomial errors to model these response variables. For all of these outcomes, the data were overdispersed (i.e. greater variance relative to the expectation in a binomial distribution), and so we included an individual-level random effect in the model to compensate for the over-dispersion (Elston et al. 2001). We used the *lme4* package for the *R* statistical programming language to conduct binomial-errors GLMMs.

Seed production is a count variable, and plants with insufficient pollination do not produce seeds, resulting in zero counts. Seed count data were both highly over-dispersed and zero-inflated relative to a Poisson distribution, so we modeled seed production with a zero-inflated negative binomial distribution using the *glmmADMB* library for *R*.

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AUTHOR CONTRIBUTIONS

B.J.B. and H.M.B. designed research, performed research, analyzed data, and wrote the paper.

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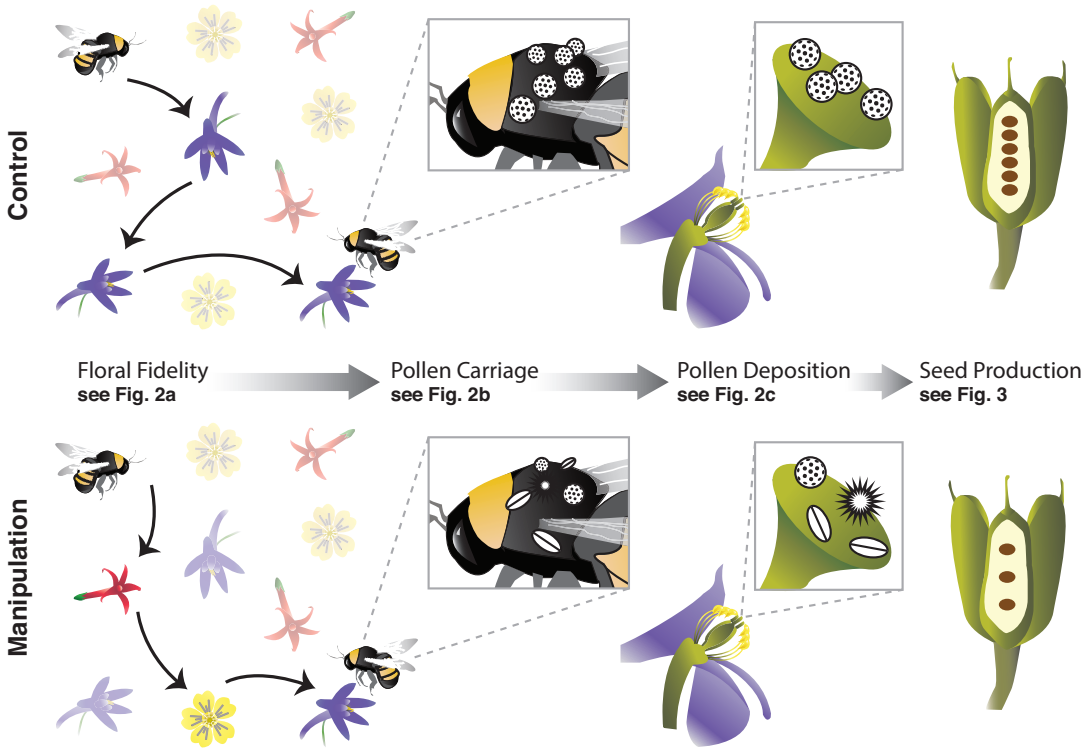

Figure 1. Floral Fidelity and Pollination Function
Displays the steps of the process by which the single-species removal experiments lead to changes in plant reproductive function in the manipulated (bottom) versus the control (top) state in each plot. Species removals lead to reductions in floral fidelity in bee foraging, a lower proportion of conspecific pollen carried by bees and transferred to floral stigmas, and ultimately reduced seed set.

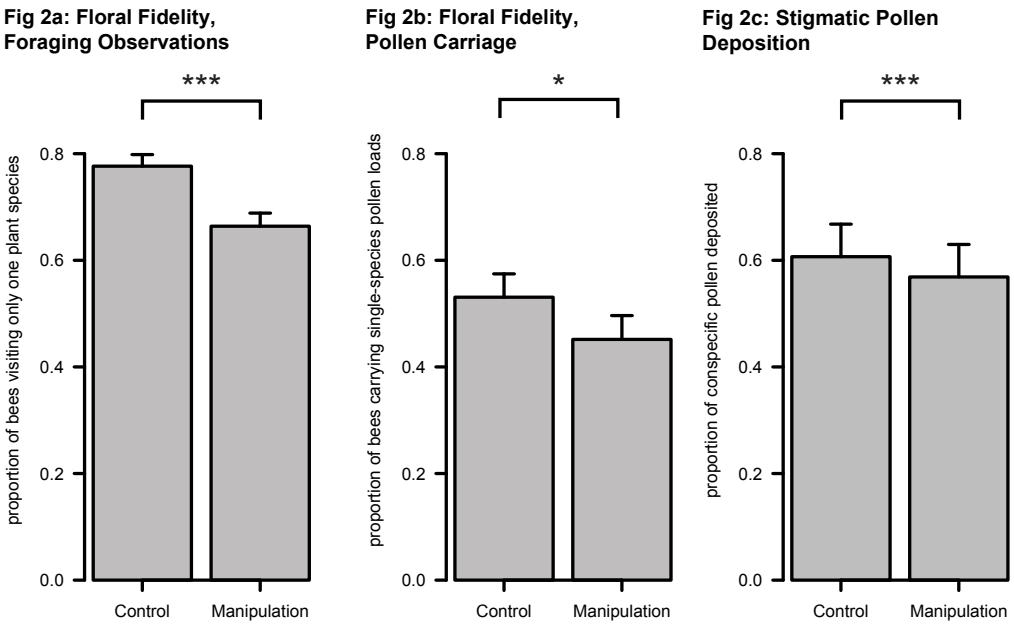

Figure 2. Floral Fidelity, Pollen Carriage, and Stigmatic Pollen Deposition

Fig 2a: Per-bee floral fidelity based on foraging observations, i.e. the relative proportions of bees displaying fidelity (visiting only one species of plant) in control vs. manipulated plots. Bars show means + S.E.M., based on observations of 736 individual bees in 20 sites; GLMM $P = 0.000748$. **Fig 2b:** Floral fidelity based on pollen carriage, i.e. the relative proportions of bees displaying fidelity (carrying pollen from only one species of plant) in control vs. manipulated plots. Bars show means + S.E.M., based on observations of 254 individual bees in 15 sites; GLMM $P = 0.040$. **Fig 2c:** Stigmatic pollen deposition expressed as the proportion of conspecific pollen in bee-carried pollen loads. Bars show means + SEM, based on counts of >47,000 pollen grains from 129 plants in $N = 5$ sites, GLMM $P = 2.67 \times 10^{-7}$

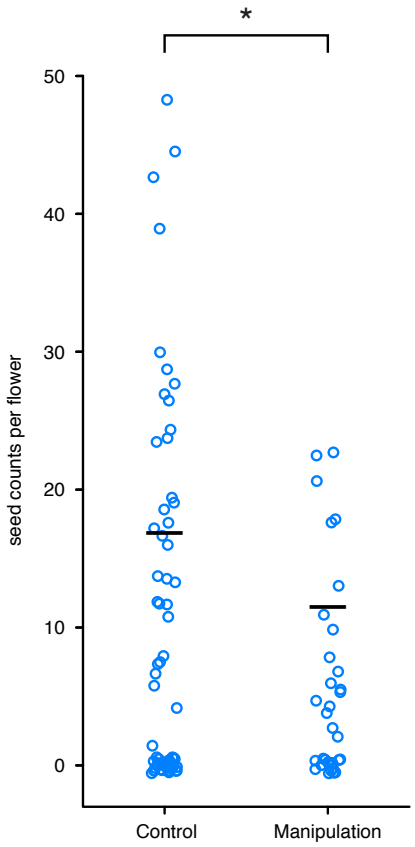

Figure 3. Seed Production

Fig 3: Seed counts per flower, for sites where *Bombus* species richness = 5. This value for species richness allows for comparison between the control and manipulated state, as the only value with more than one site represented in each state. It is also the median and modal value of species richness. Raw seed production values per flower are shown, and are jittered to allow for display of overlapping points; horizontal black bars represent mean values predicted by the GLMM, assuming mean values of *Bombus* abundance. GLMM $P = 0.033$, based on counts of 1,599 developed seeds in 192 plants in 5 sites.

Table 1. Statistical Results.
Results of Generalized Linear Mixed-Effects Models with binomial errors (floral fidelity results) and negative binomial errors with zero-inflation (seed set results). For the "Manipulation" line, the model coefficient reports the difference moving from control to manipulated plots.

	Foraging by plant				Foraging by bee				Pollen Carriage				Stigmatic Pollen Deposition				Seed Set			
	Coeff.	St. Err.	*P*-val		Coeff.	St. Err.	*P*-val		Coeff.	St. Err.	*P*-val		Coeff.	St. Err.	*P*-val		Coeff.	St. Err.	*P*-val	
(Intercept)	-5.416	0.647	< 2e-16	***	-1.715	0.580	0.003	**	1.226	0.791	0.121		-2.860	0.576	6.77e-07	***	1.352	0.619	0.029	*
Manipulation	-0.978	0.223	1.12e-05	***	-0.689	0.204	0.001	***	-0.598	0.291	0.040	*	-1.062	0.206	2.67e-07	***	-0.386	0.181	0.033	*
Bombus abundance	0.008	0.007	0.253		0.021	0.124	0.863		0.011	0.010	0.307		-0.046	0.007	5.95e-12	***	-0.011	0.005	0.017	*
Bombus richness	-0.055	0.132	0.675		0.004	0.006	0.489		-0.379	0.187	0.043	*	1.068	0.118	< 2e-16	***	0.396	0.122	0.001	**

Heterospecific pollen deposition in *Delphinium barbeyi*: linking stigmatic pollen loads to reproductive output in the field

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ABSTRACT

- *Background and Aims:* Most pollinators are generalists and therefore are likely to transfer heterospecific pollen among co-flowering plants. Most work on the impacts of heterospecific pollen deposition on plant fecundity has utilized hand-pollination experiments in greenhouse settings, and we continue to know very little about the reproductive effects of heterospecific pollen in field settings.
- *Methods:* We explored how patterns of naturally deposited heterospecific pollen relate to the reproductive output of *Delphinium barbeyi,* a common subalpine perennial herb in the Rocky Mountains (USA). We assessed a wide range of naturally occurring heterospecific pollen proportions and pollen load sizes, and linked stigmatic pollen deposition directly to seed set in individual carpels in the field.
- *Key Results:* We found that heterospecific pollen deposition in *D. barbeyi* is common, but typically found at low levels across stigmas collected in our sites. Neither conspecific nor heterospecific pollen deposition was related to carpel abortion. By contrast, we saw a significant positive relationship between conspecific pollen amount and viable seed production, as well as a significant negative interaction between the effects of conspecific pollen and heterospecific pollen amount, whereby the effect of conspecific pollen on viable seed production became weaker with greater heterospecific deposition on stigmas.

- *Conclusions:* To our knowledge, this is the first demonstration of a relationship between heterospecific pollen and seed production in a field setting. In addition, it is the first report of an interaction between conspecific and heterospecific pollen quantities on seed production. These findings, taken with the results from other studies, suggest that greenhouse hand-pollination studies and field studies should be more tightly integrated in future work to better understand how heterospecific pollen transfer can be detrimental for plant reproduction.

INTRODUCTION

Most pollinators are generalist foragers that can switch between plant species within a single foraging bout (Waser et al., 1996; Brosi and Briggs, 2013). This sharing of pollinators among plant species within a community can lead to the transfer of heterospecific pollen to plant stigmas. Such heterospecific pollen deposition is highly variable in nature (Ashman and Arceo-Gomez, 2013), and can represent a substantial percentage of total pollen on a stigma, often more than 50% of grains (Ashman and Arceo-Gomez, 2013). Stigmatic heterospecific pollen can negatively impact plant reproductive function (reviewed in Morales and Traveset, 2008; Mitchell et al., 2009; and Ashman and Arceo-Gomez, 2013), but we continue to have a limited understanding of the magnitude and mechanisms of those impacts, particularly under field conditions.

Most of what we know about the reproductive effects of heterospecific pollen comes from hand-pollination studies, which have primarily focused on mechanisms of reproductive disruption (reviewed in Morales and Traveset, 2008). Heterospecific pollen can reduce reproductive output by physically blocking conspecific pollen from adhering to the stigma (Caruso and Alfaro, 2000); by driving stigma closure (e.g. Waser and Fugate 1986); by producing allelochemicals that limit subsequent pollen germination (Thomson, 1981; Kanchan and Jayachandra, 1980; Murphy and Aarssen, 1995); by interfering with pollen tube growth in the style (Arceo-Gómez and Ashman, 2011), and by usurping ovules, especially among closely related plant species (Harder and Cruzan, 1993; Burgess et al., 2008).

There is reason to suspect that these hand-pollination results, which come primarily from greenhouse or potted-plant studies, may not reflect the reality of field situations. In nature, we would expect a range of proportions of heterospecific pollen deposited on stigmas, as well as a range of pollen from different plant species, with different amounts of diversity on stigmas. Most hand-pollination experiments have applied fixed heterospecific:conspecific pollen ratios to stigmas, and the results on plant reproduction are variable, with some finding detrimental effects of heterospecific pollen (Thomson, 1981; Brown et al., 2002; Larson et al., 2006) and others showing no impact (Kohn and Waser, 1985; Caruso and Alfaro, 2000). Most of these studies used only one heterospecific pollen species and in proportions not necessarily common in nature, making it difficult to apply inference from these results to plant reproduction in the field. Moreover, we know of only one study (Thomson 1981) that assessed the impact of heterospecific pollen over a continuous range of experimental heterospecific pollen proportions—a situation that is likely common in nature—and that was a hand-pollination study that held conspecific pollen quantity constant, using a single heterospecific pollen donor species. In addition, the greenhouse or potted-plant context of most or all of these studies may not translate to the field in terms of possible interactions between resource limitation and heterospecific pollen deposition. For example, heterospecific pollen may have larger impacts on seed set in plants that are water- or nutrient- stressed relative to plants that are not facing serious resource limitation. Thus, while we know from field studies that heterospecific pollen deposition is common in nature, and from hand-

pollination studies understand some of the mechanisms by which it can disrupt plant reproduction, the extent to which heterospecific pollen impacts plant reproduction in the field remains poorly understood.

Another gap in the literature on heterospecific pollen is assessment of possible interactions between heterospecific and conspecific pollen, i.e. if the impact of a fixed amount of heterospecific pollen has varying impacts on plant reproduction depending on conspecific pollen deposition. Such interactions could arise from mechanisms either driven by the heterospecific pollen or driven by the plant on which the heterospecific pollen is deposited. Mechanisms mediated by heterospecific pollen include stigma clogging, stylar clogging, allelopathic inhibition, and ovule usurpation (Morales and Traveset 2008). One common feature of these mechanisms is that heterospecific pollen is likely to have stronger impacts if it is deposited before conspecific pollen, especially if it is in more contact with the receptive stigmatic surface (Waser and Fugate 1986, Morales and Traveset 2008). In most realistic scenarios of pollen deposition, the more heterospecific pollen that is proportionally present on a stigma, the greater the chance that it arrived early, enhancing its negative impact, whereas with more conspecific pollen the chance of an early arrival and concomitant deleterious effects is reduced. Second, there is at least one documented mechanism driven by the plant receiving the heterospecific pollen, which is stigma closure, i.e. stigmatic lobes closing in response to heterospecific pollen, effectively ruling out subsequent seed production in that flower (Waser and Fugate, 1986; Morales and Traveset, 2008), and plants could also hypothetically drive active

inhibition of pollen tube growth (both conspecific and heterospecific); or ovule or carpel abortion, in response to heterospecific pollen. To effect such active mechanisms, flowers must be able to detect the presence of heterospecific pollen. If they can also detect quantity or proportion of heterospecific pollen grains, plants could potentially use that signal when actively disrupting pollination at various points in the process (stigma closure; inhibition of pollen tube growth; ovule or carpel abortion), ultimately as a means to conserve resources by not investing in flowers that may have low-quality seed production or quality.

In this study, to begin to understand the impact of heterospecific pollen in natural systems, we used a field approach linking stigmatic pollen deposition to seed set in the same individual carpels in wild plants that had been naturally pollinated (Waser and Price, 1991a; b). In contrast to hand-pollination studies, this approach allowed us to assess stigmatic pollen loads varying greatly in conspecific pollen and heterospecific pollen quantities (but varying by definition over a range that is found in nature), while achieving a relatively large sample size and replication across space. This approach also allowed us to assess interactions between conspecific and heterospecific pollen in assessing plant reproduction.

We examined how the total amount of naturally deposited heterospecific pollen co-varied with the reproductive output of *Delphinium barbeyi* Huth (Ranunculaceae), a common subalpine flower species in the Rocky Mountains of Colorado, USA. *D. barbeyi* receives visits from several species of bumble bees (Apidae: *Bombus*) that are known to visit many co-flowering species within a

community (Elliott and Irwin, 2009; Brosi and Briggs, 2013). We asked the following specific questions: (1) How variable is heterospecific pollen deposition in naturally occurring *D. barbeyi* populations? (2) Is conspecific pollen and/or heterospecific pollen deposition related to whether or not a carpel will abort? (3) How are the amounts of conspecific pollen and heterospecific pollen deposition related to seed production in (non-aborted) carpels? We hypothesized that there would be a positive relationship between heterospecific pollen deposition and carpel abortion rates and a negative relationship between heterospecific pollen deposition and seed set. In particular, we predicted that the effect of heterospecific pollen would vary depending on conspecific pollen deposition, with heterospecific pollen having a larger negative impact on stigmas with lower conspecific pollen deposition.

MATERIALS AND METHODS

Plant Study System—

D. barbeyi is a long-lived perennial herb found in sub-alpine meadows in western North America. *Delphinium barbeyi* flowers are protandrous and deep purple in color with elongated corollas. A flower generally has three carpels. *Delphinium barbeyi* plants are typically large with multiple racemes per plant. The total number flowers per plant vary greatly, but the plants in our plots had an average of 12.3 flowers per raceme (median = 11, max = 52, min = 1,). *D. barbeyi* is generally in bloom from mid-June through late July in our field sites and is a predominately outcrossing but self-compatible species (Williams et al., 2001). The primary pollinators of

Delphinium barbeyi are worker bumble bees (particularly *Bombus appositus* and *B. nevadensis*). *D. barbeyi* is known to receive heterospecific pollen, as the majority of its pollinators are generalist bumble bees, all of which also visit several other co-flowering species (Elliott and Irwin, 2009; Brosi and Briggs, 2013). While hummingbirds are common in this system and are important pollinators of the early season congener *Delphinium nuttallianum*, we made only one observation of a S*elasphorus rufus* hummingbird visiting *D. barbeyi* in our sites in thousands of hours of field time (H. Briggs, *pers obs*.).

Field Sites—

We worked in five study sites in subalpine meadows surrounding the Rocky Mountain Biological Laboratory in Gothic, Colorado (38° 57.5′N, 106°59.3′W, 2740 - 3065 m above sea level), in the Gunnison National Forest, western Colorado, United States. Study sites were located at least 1 km apart. We selected sites with comparable densities of *D. barbeyi* (i.e. those in which *D. barbeyi* was the most abundant or the second most abundant plant species). The sites all occur within 10 km of each other and were similar in terms of both biotic and abiotic conditions (though it was beyond the scope of this study to quantify the abiotic conditions of each site). Each site had on average 9.2 plant species in bloom at the time of data collection (median = 10, max = 12, min = 5). Each site consisted of a single 20 × 20m plot, which was chosen within the site to maximize *D. barbeyi* density.

Stigma And Seed Collection And Quantification—

We collected data from each site once during the summer of 2013. At each plot, we bagged 50 *D. barbeyi* racemes (from different plant individuals) with incipient flower buds. Two to three days later, we opened 15 to 20 bags to expose female receptive but un-pollinated flowers, and labeled two receptive flowers per raceme. We allowed pollination to occur uninhibited for a standardized four-hour period before reclosing the bags (Brosi and Briggs, 2013). *D. barbeyi* flowers are typically female-receptive for approximately two days (Nickolas Waser, *pers. comm*.) Some of our stigmas had extremely large pollen loads and our expectation is that if we had assessed stigmas over the full 48-hour period of receptivity, we may have seen a few additional individuals with slightly larger pollen loads. Still, we expect that we captured most of the variation in pollen deposition that is naturally present in the system with a four-hour visitation window.

We followed a protocol to allow us to link pollen deposition and seed set in the same individual carpels in the field (Waser and Price, 1991a; b). Approximately 4 days after each receptive flower was pollinated (i.e. after pollen tube growth and fertilization had occurred and flowers had wilted, but while the germinated pollen exines were still adhering to the stigma) we harvested floral stigmas, carefully leaving remaining floral structures undamaged). At this time we also marked individual developing carpels (typically three per flower) with a permanent marker. We mounted the harvested stigmas from each plot on slides with fuchsin jelly in the field (Kearns and Inouye, 1993). In the laboratory, we counted the total numbers of conspecific (*D.*

barbeyi) and heterospecific pollen grains on each stigma. With the help of pollen reference collections housed at the Rocky Mountain Biological Laboratory, we were able to differentiate *D. barbeyi* pollen from the heterospecific pollen on our slides. It was beyond the scope of this study to identify the heterospecific pollen grains to species. Finally, we returned to each site seven to 15 days later to collect the mature fruits. We dissected the fruits and counted the developed and undeveloped seeds per ovary, allowing us to match the total seed set per carpel with the pollen load of each previously harvested stigma.

Data Analysis—

We examined how well total heterospecific pollen predicted the reproductive output in *D. barbeyi* by running two primary analyses: first, an assessment of the proportion of carpels that had fully aborted (i.e. contained no developed seeds); and second, an assessment of seed set in carpels that had not aborted (i.e., those with one or more developed seeds). We ran these analyses separately because different factors (e.g., resource limitation) may drive a plant to fully abort a carpel versus invest in it (Burd, 1998). In addition, we assessed the colinearity between conspecific and heterospecific pollen deposition using the non-parametric Spearman's rho. We used a non-parametric test because the data did not meet assumptions of linearity. Because the correlation was low ($\rho = 0.001$) and not statistically significant ($p = 0.86$), we used both as covariates. We conducted all analyses using the R statistical programming language .

We used generalized linear mixed-effects models (GLMMs) because of hierarchical lack of independence in our data (Bolker et al., 2009). Plants within sites are not independent given similar resource conditions and are likely genetically related. Similarly, flowers within a plant clearly do not represent independent samples. Thus, we included nested random effects in the model, with flower nested within plant nested within site. We assessed carpel abortion as counts of aborted vs. non-aborted carpels, and seed production as the counts of developed (fertilized) seeds vs. undeveloped seeds, both of which are structurally binomial variables. Relative to a binomial distribution, however, our data for both analyses were overdispersed which we corrected by including an individual-level (i.e. carpel-level) random effect (Elston et al., 2001). We ran GLMMs with binomial-errors using the default logit link in the 'lme4' package for R (Bates et al., 2012).

We assessed the relative contribution of the quantities of heterospecific and conspecific pollen on reproductive output and carpel abortion using a model-comparison framework. In these models conspecific pollen and heterospecific pollen, as well as their interaction, are the fixed effects that we assess in different combinations. We specifically compared five mixed-effects models: (1) full model including an interaction between the two fixed effects (conspecific pollen and heterospecific pollen); (2) additive model (in which the fixed effects are added but interactions are not assessed); (3) amount of conspecific pollen only; (4) amount of heterospecific pollen only; and (5) a model including no fixed effects (i.e. random effects only). For all analyses, we used AICc, Akaike's Information Criterion

corrected for small sample size (Burnham et al., 2010), to determine the best model(s). We reported all models within two **Δ**AICc points of the best model. We used the 'AICcmodavg' package in R (Mazerolle, 2012) for model selection.

We excluded observations in which there was seed set with either zero stigmatic pollen recorded (3 carpels of 619), or zero proportion of conspecific pollen (12 carpels). We assume that these observations were due to loss of stigmatic pollen in the field between fertilization and stigma collection.

RESULTS

Overview—

We counted over 2,400 seeds and linked reproductive output to pollen deposition in 604 carpels from 216 flowers on 131 plants. Each non-aborted carpel produced a mean of 6 viable seeds (median = 5; range = 0–22; standard deviation = 5.12). Of the 604 carpels we examined, 510 (85%) of them had at least some heterospecific pollen on the stigma (median = 3 pollen grains; mean = 4.6; range = 0–222; standard deviation = 10.85, Fig. 1) and 211 (35%) had fully aborted. Many of the stigmas in our study received more conspecific pollen (median = 11 pollen grains; mean = 33.6; range = 0-656; standard deviation = 57.30) than is strictly necessary for fertilization based on ovule number (median ovule number per carpel = 10; mean = 8.7; range = 0-26; standard deviation = 7.26). Finally, conspecific pollen and heterospecific pollen counts were not correlated at the level of the stigma (Spearman's *rho* = 0.001; p = 0.86; Fig. 2).

Carpel Abortion—

We found three candidate models that were indistinguishable (i.e. within two ΔAIC points) as the best predictors of carpel abortion: 1) a random-effects only model (lowest AIC); 2) a model with conspecific pollen as the only fixed effect; and 3) a model with heterospecific pollen as the only fixed effect (see table 1 for model comparison). For the second and third-ranked models, neither conspecific pollen amount ($p = 0.90$) nor heterospecific pollen amount ($p = 0.97$; see Table 1 for model coefficients) was significantly associated with carpel abortion. Thus, neither heterospecific nor conspecific pollen deposition seems to be a primary driver of carpel abortion in *D. barbeyi* in our system.

Seed Production—

The model that best explained the probability of viable seed production in non-aborted carpels was the full model that included an interaction term between heterospecific and conspecific pollen quantities (Fig. 3, Table 1). This model included a significant positive relationship between conspecific pollen amount and viable seed production; no significant effect of heterospecific pollen on its own; and a significant negative interaction between conspecific pollen and heterospecific pollen amount (Fig. 3, also see Table 1 for model coefficients) such that the effect of conspecific pollen deposition on viable seed production gets weaker (i.e. shallower slope) with greater heterospecific pollen deposition. For example, according to the model predictions, if a stigma had 54 grains of conspecific pollen and no heterospecific

pollen, 60% of the seeds would be viable. By contrast, for a stigma with the same 54 grains of conspecific pollen but also 50 grains of heterospecific pollen, the model predicts that only 45% of the seeds would be viable.

DISCUSSION

To understand the association between naturally deposited heterospecific pollen and seed production, we used a field approach linking stigmatic pollen deposition to seed set in the same individual carpels in wild plants. Heterospecific pollen deposition was highly variable on *D. barbeyi* stigmas in the field and while some degree of heterospecific pollen was found on most sampled flowers, it was typically present in low amounts (Fig. 1). Neither heterospecific pollen nor conspecific pollen was a good predictor of carpel abortion in our sites. Conspecific pollen deposition was positively related to viable seed production, but we also found a significant negative interaction between heterospecific pollen and conspecific pollen. That is, with increasing heterospecific pollen, the positive relationship between conspecific pollen deposition and viable seed production in *D. barberyi* became weaker (Fig. 3).

We found that heterospecific pollen on *D. barbeyi* stigmas is widespread, highly variable, and typically occurs at relatively low levels. In terms of variability, heterospecific pollen represented anywhere from 0 to 97% of the grains present in a pollen load. The widespread nature of heterospecific pollen deposition is reflected by the fact that 85% of stigmas had some heterospecific pollen present. Still, most stigmas received only low levels of heterospecific pollen deposition (median of only

3 grains per stigma, relative to 11 grains for conspecific pollen). These results are broadly consistent with patterns reported in a comprehensive review of studies assessing the impact of heterospecific pollen transfer, including 77 species from 17 different sources (Ashman and Arceo-Gomez, 2013). This review found that, although receipt of heterospecific pollen was variable, all species received at least some heterospecific pollen on their stigmas. Similarly, in a community-wide analysis of pollen transfer, Fang and Huang (2013) found that of the 57 plant species they surveyed, heterospecific pollen deposition was common but highly variable, representing 0-66% of the total pollen on stigmas. The co-flowering plant community and pollinator behavior are just two of the factors that might influence variation in HP receipt between plants of the same species within and between sites. Variation in heterospecific pollen receipt within species or even within a population obviously has important implications for evolution of coping mechanisms and remains an area ripe for more research (Ashman and Arceo-Gómez 2013).

A large proportion of carpels aborted in our study (nearly 35%), yet contrary to our expectations we found that neither heterospecific pollen nor conspecific pollen was a good predictor of carpel abortion in *D. barbeyi*. Thus, it seems likely that resource limitation, rather than pollen limitation, drove carpel abortion in our system. Extrinsic factors such as weather conditions, flower phenology, herbivory, competition (both intra- and inter-specific), and disease can lead to within plant-variation in carpel abortion (Burd, 1998; Niesenbaum, 1999; Marshall et al., 2010). Furthermore, intrinsic factors including architecture (i.e. location of flower on the

raceme), plant size, developmental constraints or allocation strategies are also known to affect patterns of carpel abortion (Delph, 1986; 1993; Guitian et al., 1996; Burd, 1998).

In contrast to carpel abortion, both conspecific pollen and heterospecific pollen played a role in seed production in *D. barberyi*. As expected, conspecific pollen deposition on its own was positively related to viable seed production. Heterospecific pollen on its own did not affect seed set, but there was a significant negative heterospecific pollen × conspecific pollen interaction. In other words, with greater heterospecific pollen deposition, a fixed amount of conspecific pollen would result in lower seed set. Because this was a correlational study, we were not able to tease apart other factors that may have lead to a decrease in seed production such as diversity of heterospecific pollen. To our knowledge, this is the first demonstration of an interaction between heterospecific pollen and conspecific pollen in their effects on plant reproduction. We hypothesized that we would find such an interaction as we expected that the negative effects of heterospecific pollen might occur only in the context of conspecific pollen. In a case comparing deposition of a small vs. a large heterospecific pollen load in stigmas with no conspecific pollen should yield the same result: zero seeds produced. Following the same logic, we would expect the impact of depositing a medium-sized load of heterospecific pollen on a stigma would differ in stigmas with just a few conspecific pollen grains versus a large conspecific pollen load (relative to ovule number). We are aware of only one previous assessment of the statistical interaction between stigmatic pollen load quantity and the proportion of

heterospecific pollen grains (Arceo-Gómez and Ashman, 2011). This study found no evidence for a pollen quantity × proportion heterospecific pollen interaction, but the study design was oriented at a different objective (assessing diversity of heterospecific pollen donors) and thus included just two heterospecific pollen load sizes, which corresponded to 28% and 16% of total pollen load proportions. Finally, we were not able to distinguish between self and outcrossed conspecific pollen in this study. Self pollen has been shown to intensify the negative impact of HP receipt in *Mimulus guttatus* and self pollen has been shown to produce fewer seeds in *D. barbeyi* (Williams et al. 2001, Arceo-Gómez and Ashman 2014). More studies examining how mixed mating plant species cope with HP receipt would be valuable, particularly in a field setting where plants may receive HP from a variety of plant species.

Putting this result in the context of previous findings highlights that there has been a gulf between field studies and hand-pollination studies in greenhouse settings, a gulf that, if bridged, would improve our understanding of the effects of heterospecific pollen transfer in nature. Future work in hand-pollination studies should contribute to bridging the gap by integrating what we have learned from field studies so far. First, hand-pollination studies should be designed using parameters explicitly drawn from field studies, in terms of heterospecific and conspecific pollen amounts, and heterospecific pollen diversity, considering not only means but also variability. To date, most hand-pollination studies have used only one proportion of heterospecific pollen (typically 50%), and we are aware of only one hand-pollination

study (Thomson, 1981) that specifically applied a full range of heterospecific pollen deposition. Second, the results from our field study, particularly our finding of a heterospecific pollen × conspecific pollen interaction in seed production, highlights the advantage of understanding this result mechanistically, via hand-pollination studies that vary the quantity of both conspecific pollen and heterospecific pollen factorially. Third, our finding that neither conspecific pollen nor heterospecific pollen was strongly linked to carpel abortion rates underscores that hand-pollination studies that control both pollen and resource limitation (for example, with limited watering and/or limited soil nutrients) in a greenhouse setting could greatly improve our understanding of how these factors interact.

Similarly, field studies on the effects of heterospecific pollen can look to hand-pollination experiments—with their typically more mechanistic focus—for inspiration. A critical first step is to increase the number of field studies that directly link pollen deposition and seed production within the same carpel (Waser and Price, 1991a; b). To our knowledge the work we present here was the first time that this approach has been applied to understanding the effects of heterospecific pollen, which should be repeated in a wide range of plant species with different mating systems (Ashman and Arceo-Gomez, 2013). Another particular need is for field studies that assess multiple sites. We continue to have a poor understanding of how plant genotype and environmental factors interact to shape the effects of heterospecific pollen, and work along environmental gradients could be informative, especially in disentangling the relative effects of pollen vs. resource limitation in

shaping seed set. Similarly, experimental approaches to assessing the relative effects of resource vs. pollen limitation in the context of heterospecific pollen deposition in field settings (e.g. with watering and/or fertilizer treatments) would also be a valuable research direction. Finally, hand-pollination and field approaches should be explicitly integrated by conducting more hand-pollination experiments in field settings.

Our understanding of the impact of heterospecific pollen deposition is growing, but there is still much to learn about the way that co-flowering plants interact through pollinator sharing and how heterospecific pollen deposition impacts plant reproductive fitness. In a changing world where we can expect to see both increasing disruptions in pollination (Biesmeijer et al., 2006; Potts et al., 2010; Brosi and Briggs, 2013) as well as the emergence of new interactions via introduced species and climate change, studies that unify both field studies and controlled hand-pollination studies will allow us to better understand the implications of heterospecific pollen deposition for reproductive output in natural plant communities.

Author contributions

H.M.B. & B.J.B designed the study, H.M.B collected data with the assistance of those mentioned in the Acknowledgements and L. M. Anderson, L. M. Atalla, A. M. D & E.K.D, H.M.B analyzed the data, and wrote the paper.

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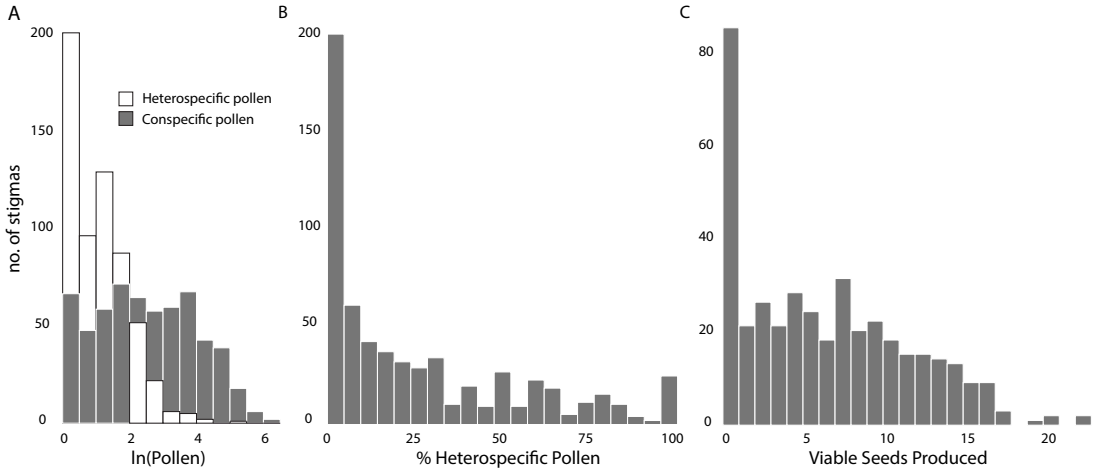

Figure 1. Natural Pollen Deposition in *Delphinium barbeyi*. Panel A shows the total amount of heterospecific and conspecific pollen naturally deposited on stigmas in the field. Panel B shows the total amount heterospecific pollen/total pollen deposited on stigmas in the field and Panel C shows the total number of viable seeds that were produced in the non-aborted carpels.

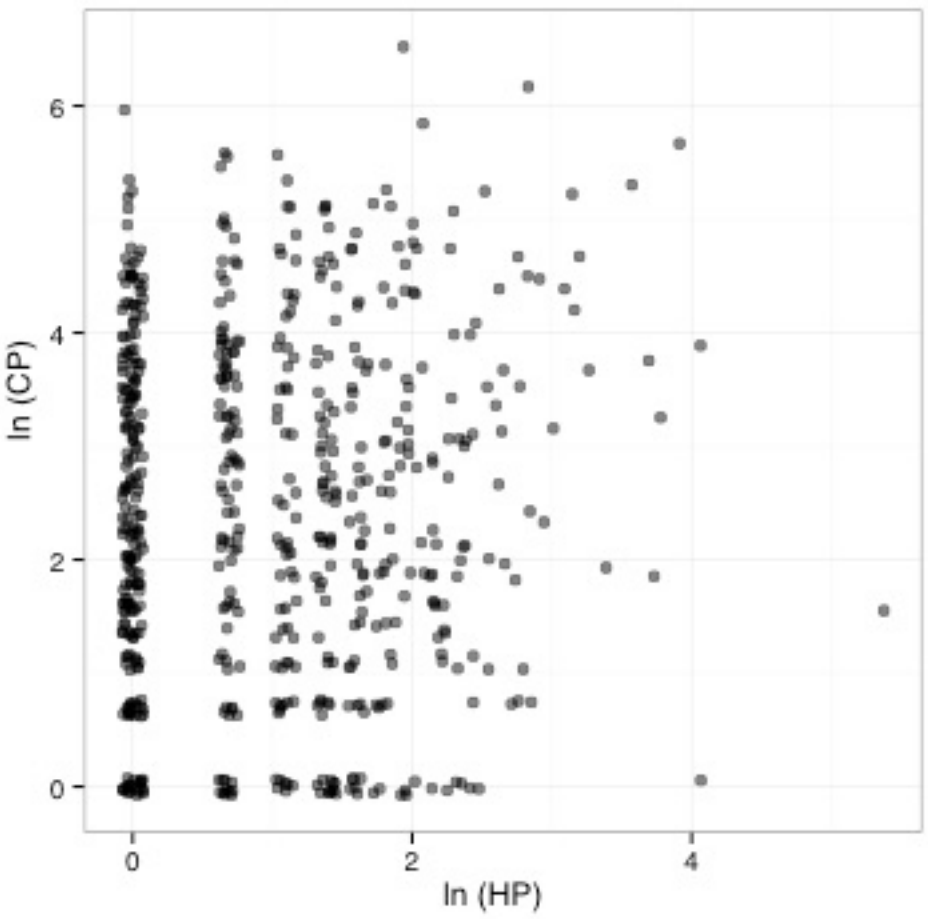

Figure 2. Relationship between heterospecific pollen and conspecific pollen. Counts of conspecific and heterospecific pollen are shown here with natural-log transformation to better visually depict the relationship, and are jittered to improve visualization of overlapping data points. The two variables are uncorrelated (raw data, non-parametric Spearman's rho = 0.007, $P = 0.86$).

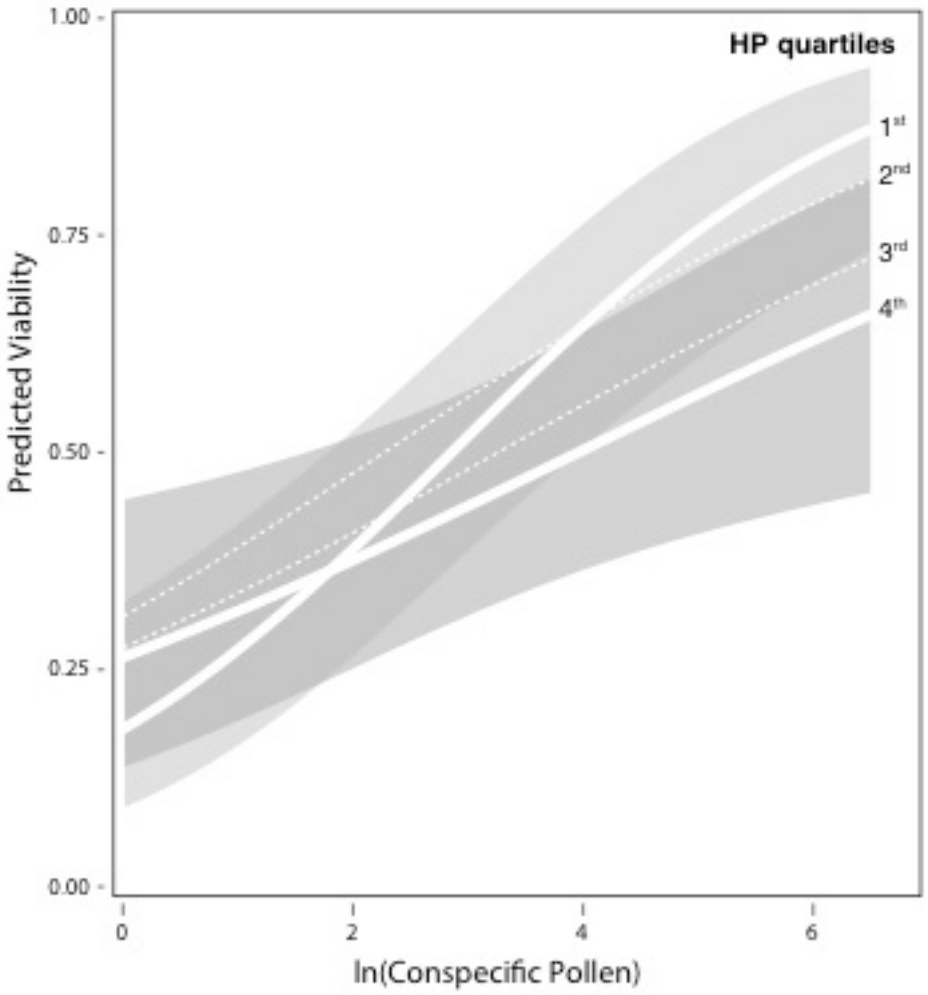

Figure 3. Effect of conspecific and heterospecific pollen deposition on probability of viable seed production. Lines depict the best-fit estimate from the GLMM that describes interaction between conspecific and heterospecific pollen deposition and viable seed production. 1st and 4th quartiles are solid white lines with 95% confidence intervals while 2nd and 3rd quartiles are depicted as thin dashed lines without confidence intervals for clarity. Note the difference between the fits for the 1st and 4th quartiles at the higher values of the x-axis. For the purpose of visualization we used ln(conspecific pollen) in this model to depict a more even spread across the conspecific pollen axis. See table 1 for the estimates based on continuous variation in heterospecific pollen. Heterospecific pollen quartiles: 1st = 0.0-1.0, 2nd = 1.0-2.5, 3rd = 2.5-5.0, 4th = 5.0-222.0

Table 1. Results of generalized linear mixed-effects models with binomial errors (viable seed production and carpel abortion results). *P < 0.05; ***P < 0.001.

Candidate Models	K	AICc	rAIC	AICcWt	Cum Wt
1.Seed Production in Non-Aborted Carpels					
a. proportion viable seeds~ HP*CP	8	1727.74	0	0.91	0.91
b. proportion viable seeds~ HP+CP	7	1733.86	6.11	0.04	0.96
2. Carpel Abortion					
a. prob of not aborted~full random effects	5	243.52	0	0.5	0.5
b. prob of not aborted ~ CP	6	245.18	1.66	0.22	0.72
c. prob of not aborted ~ HP	6	245.74	2.23	0.17	0.89

Best Model Results	Fixed Effects	Coeff.	SE	*P* value
1.Seed Production in Non-Aborted Carpels				
a. proportion viable seeds~ HP*CP	(Intercept)	-0.207	0.308	0.501
	HP	0.006	0.014	0.692
	CP	0.006	0.002	0.001***
	HP:CP	-0.0004524	0.0001786	0.011 *
2. Carpel Abortion				
b. prob of not aborted ~ (full random effects)	(Intercept)	12.609	6.490	0.052*

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Trait Driven Competitive Context Influences Bee Foraging Behavior

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Introduction

Pollinator foraging behavior directly impacts plant reproductive output and ecosystem function through the transfer of pollen between plant individuals within a single foraging bout. If pollinators move between different plant species they can transfer heterospecific pollen, potentially reducing reproductive output (Ashman and Arceo-Gomez 2013, Briggs et al. 2015). Given the functional significance of pollinator resource use, it is important to understand the factors driving pollinator foraging behavior. The plants that pollinators forage on is a function of a number of factors including innate and learned preference, morphological traits, as well as direct and indirect competition with other pollinators in the community (Pimm et al. 1985, Cnaani et al. 2006, Stang et al. 2009, Brosi and Briggs 2013). This study focuses on how competitive interactions between pollinators shape pollinator foraging behavior.

Decades of research has demonstrated that interspecific competition can influence pollinator foraging behavior. Largely in line with expectations derived from ecological theory, the range of resources used by a species contracts as the strength of interspecific competition increases {Inouye:1978to, Morse:1977vq, Pimm:1985vc, Hubbell:1978wk, Frund:2013gy, Brosi:2013hn, Bolnick:2010ij}. In one example, Pimm et al. (1985) found that in the presence of a dominant competitor, two other hummingbird species spent more time at a less rewarding feeder. In contrast, *without* interspecific competition, all three hummingbird species visited a feeder with high sucrose concentrations. Brosi and Briggs (2013) found that after a release from

interspecific competition, bumble bees decreased their floral fidelity (pollinators moved more often between plant species within a single foraging bout). These changes in foraging behavior led to a significant decrease in reproductive output in a common alpine plant species. Fründ et al. (2013) provide another example in which pollinators' flower preferences can be flexible, and depend on community context (i.e., interspecific competition with other pollinator species present). In their system, as competition between pollinator species increased, the species reduced their niche overlap by shifting to new plant species, which resulted in increased plant reproduction. Thus, we know bees respond to competition and often do so strongly, but we do not know if bee species vary in their response to competition in complex assemblages of bee species or what traits are important in determining how they will respond.

Trait based differences between species may reduce interspecific competition and maintain diversity within a community (Grant 2006, Mayfield and Levine 2010). Generally, we still have a poor understanding of which traits influence the outcome of competition and community structure (McGill et al. 2006, Messier et al. 2010, HilleRisLambers et al. 2012) but some animal traits such as body size (Wells 1988) or bill size/shape (Wiens and Rotenberry 1981, Grant 2006) and plant traits related to resource acquisition, such as root depth (Stubbs and Bastow 2004, Adler et al. 2010), can limit competition thus encouraging species coexistence. Our study aims to explore the role of traits in mediating competition within a community context.

Bumble bee (*Bombus*) communities provide an excellent system in which to empirically explore how trait differences drive foraging plasticity in response to interspecific competition, all within a community context. *Bombus* assemblages are often species-rich, and sympatric species typically have substantial overlap in their life history requirements (Goulson et al. 2008). Furthermore, traits that affect resource acquisition and foraging efficiency can influence how species partition resources within a community (Abrams and Chen 2002, Grant 2006). Tongue length is a trait that directly determines which resources a bumble bee can access and how resource selection varies among species (Heinrich 1976, Inouye 1978, McGill et al. 2006, Stang et al. 2009). In general, long-tongued bumble bee foragers visit flowers with deep corollas and short-tongued bumble bees forage on shallow flowers (Heinrich 1976, Stang et al. 2006, McGill et al. 2006). Still, bumble bees are known to be labile in their foraging patterns if more rewarding floral resources become available or the competitive landscape shifts (Inouye 1978, Gegear and Thomson 2004, Brosi and Briggs 2013). Tongue length appears to be important for structuring foraging preferences but we are lacking experimental work that evaluates how traits such as tongue length influence pollinators' response to competition.

We systematically manipulated interspecific competition in bumble bee communities through targeted single species removals and examined patterns of species-specific plasticity in resource use in the remaining pollinators. Specifically we examined to what extent tongue length explains species-specific difference in the pollinators' foraging behavior in response to release from competition. Our sites are

self assembled communities with natural diversity varying in plant and bee community composition. Utilizing removals in these natural communities allowed us to examine if the identity of the most abundant bee species, (i.e. competitive context) influences bee foraging behavior. We focused on the foraging response of the remaining bees in the community with respect to floral fidelity, or, within plant species movements within a single foraging bout. Floral fidelity is critical for many plants species' reproductive success because transfer of conspecific pollen must occur in order for fertilization to take place.

We asked specifically:

1. How do bee species vary in their *baseline* floral fidelity (irrespective of the manipulations)
2. How to bee species vary in their behavioral *responses* (via floral fidelity) to the reduction in interspecific competition?
3. To what extent does tongue length explain variation in species-specific foraging behavior?
4. Does the tongue length of the most abundant bee (i.e. competitive context) influence the floral fidelity of the other bees in the community?

Methods:

Study Sites: We worked in 28 subalpine meadow sites in the landscape surrounding the Rocky Mountain Biological Laboratory (38° 57.5′N, 106°59.3′W, 2,900 m above sea level), in the Gunnison National Forest, western Colorado, United States. Each

site consisted of a 20 × 20-m plot, all with the same dominant plant species (*Delphinium barbeyi*) to minimize, as much as possible, plant-community-driven differences in foraging behavior. A minimum distance of 1 km separated any two sites. We collected data over three summer growing seasons (June-August), in 2010, 2011 and 2013.

Manipulations. We assessed each plot in a control state, waited one day, and then assessed in a manipulated state. We kept the interval between control and manipulated states short because of the rapid turnover in flowering phenology in our high-altitude system, allowing us to keep the plant community constant in our control–manipulation comparisons (Langenheim 1962, Brosi and Briggs 2013). Manipulations reduce interspecific competition through the temporary, non-destructive removal of the most abundant bumblebee species in each plot. We determined the most abundant bee via inventory of *Bombus* species richness and abundance on the control day using nondestructive aerial netting, with two field team members netting for a 20-min period, not including handling time (the time from when a bee was in the net until it was in a closed vial). To avoid double-counting, we kept each bee in an individual glass vial, identified to species, and kept in a cool, dark cooler until the inventory time period was over, at which point bees were released.

On the manipulation day we removed the most abundant bee species (as determined two days previously in the control state). The removals were accomplished through targeted hand-netting, and we minimized disturbance of other bees and vegetation by

carefully placing the insect net over entire inflorescence and allowing bee individuals to fly up into the net (Inouye 1978). Captured bees were transferred to vials and placed in a cooler during the manipulation and released unharmed afterward. We used as much time as necessary to remove essentially all individuals of the target species from the sample plot and immediately adjacent area (typically in 1-2 hours we would achieve 98% removal). We left a period of at least 30 minutes between manipulative bee removals and subsequent sampling to minimize the impact of the disturbance on the foraging activities of other bees. We recorded both the abundance of removed (captured) individuals, as well as the number of un-captured "escapees" that were observed during bee sampling. We assessed resource use (plant species visited within a single foraging bout) in each site in both a control and a manipulated state. Each site was only used once in a manipulated and controlled state per year (i.e., no sites were re-sampled within a single season).

Foraging observations: We directly followed the foraging sequences of *Bombus* individuals in both the control and manipulated states. We recorded the identity of each plant species visited in a foraging sequence. We discontinued an observation when the bee was lost from sight, when it ventured more than 5 m outside of the plot, when it had been observed for 10 full minutes, or when we had tallied 100 individual plants visited. We discarded observations of bees that visited fewer than five plants. The number of individuals observed per/state/site varied due to bee abundance (mean = 33 individuals per site; range = 8-54).

Tongue Length measurements: The data on proboscis lengths of workers was taken from published measurements of bumblebees collected on the Front Range of the Colorado Rocky Mountains and overlap with the sites and species that we used for this study (i.e., east of the Continental Divide) (Inouye 1980). Measurements indicate the sum of the individual lengths of the prementum and glossa. The mean tongue length of each bumble bee species was assigned to each individual bee and used in the trait analysis ([see Table 1 for tongue lengths] (Inouye 1978, 1980, Pyke 1982).

Data analysis

Quantifying floral fidelity:

Floral fidelity was measured as the binomial counts of individual bee foraging movements that were conspecific (within plant species) vs. heterospecific (between plant species) (Brosi and Briggs 2013). We used GLMMs with binomial errors using the logit link in the lme4 package for R to model the floral fidelity response variable (Bates et al. 2012). Data from individual bees foraging within a site cannot be considered independent (bees within a site are likely to be closely related genetically and environmental conditions are similar) and therefore we used site as a random effect (Bolker et al. 2009). Relative to a binomial distribution, our data were overdispersed, which we corrected by including an individual-level (i.e,. bee individual) random effect (Elston et al. 2001). We used the R statistical programming language (R Core Development Team 2012) for all models.

Species Specific differences:

We first ran a null model (M0) that included only the two structural random effects: one to control for pseudoreplication for site and another to correct for over-dispersion in the binomial response variable (see above).

M0: fidelity~ (1|site) + (1|unique bee ID)

These structural terms were retained in all models. We then added state (control or manipulation) as a fixed effect to M0, giving M1 below, to assess whether the removal of the most abundant bee had a guild-wide impact on foraging fidelity.

M1: fidelity~ state + (1|site) + (1|unique bee ID)

We compared M1 and M0 through a likelihood ratio test (LRT) and retained state as a fixed effect in all subsequent models (see Results, table 2). Next, we estimated the magnitude of between-bee species variation in foraging dynamics by adding bee species as a random intercepts term with state to M1 yielding M2 below:

M2: fidelity~ state + (1|site) + (1|unique bee ID) + (1+state|bee species).

This model allowed us to estimate how much bee species differ in both their base-line fidelity (i.e. intercept) and in their response to the manipulation (i.e. slope) by

estimating a variance term for each. To see if the random effects associated with bee species improved the model fit, we compared models M1 and M2 using LRT and by computing differences in the values of Akaike's Information Criterion (ΔAIC) between the models.

Traits:

To understand if bee species-level traits explain the species-specific differences in foraging behavior, we added tongue length as a continuous fixed factor to M2 yielding M3 and M3' below. Our approach was two-fold: first, we examined whether there are *base-line differences* between species level floral fidelity (regardless of state; M3) and then we tested for species level differences in the response to experimental removal of the dominant bee species (M3'). Models were compared using LRT and by computing differences in the values of Akaike's Information Criterion (ΔAIC).

M3 fidelity~ state + tongue length + (1|site) + (1|unique bee ID) + (1+state|bee species)

M3': fidelity~ state*tongue length + (1|site) + (1|unique bee ID) + (1+state|bee species)

Baseline differences in species specific fidelity:

To assess the baseline differences in species-specific floral fidelity we compared a model (M3 that included tongue length as an additive fixed effect to the model without tongue length (M2). We compared the variance estimate for bee species from model M2 to that of M3: $C_\alpha = 1 - (\text{Var}(\alpha_{M2})/\text{Var}(\alpha_{M3'}))$ following Jamil (2013):, where α is the random intercept estimate for bee species (irrespective of state). If $C_\alpha > 0$, it suggests inter-specific differences in base-line fidelity can be explained by differences in their tongue lengths.

Response to reduction of interspecific competition:

We examined whether species-level traits explain variation in the *response* of the different bee species to our manipulations. We do so by including tongue length as a fixed interaction term with state in M3.

This model captures the extent to which the response to state co-varies with tongue length. Because bee species with similar tongue lengths would have more similar responses to state, a substantial effect of tongue length on fidelity would reduce the variance estimate for the bee species-by-state random slope. We compared the magnitudes of the variance estimates for the random bee species slope between M3' and a model with tongue length included only as an additive main effect (M3). We did this by calculating the relative change in variance between the interaction trait model (M3') and the additive trait model (M3), following Jamil (2013): $C_\beta = 1 - (\text{Var}(\beta_{M3})/\text{Var}(\beta_{M3'}))$, where β is the random slope estimate for bee species (with

respect to state). If $C_{\beta} > 0$, variance among bees can be said to relate to their traits. Reductions in the variance estimates for the bee species random intercept reveal the amount that the included traits factor explains between-species differences in fidelity (Jamil et al. 2013).

Site level attributes:

Finally, we explored if community context influenced species-specific foraging responses. We compared M4, which included the tongue length of the removed (and most abundant) bee species in the site as a continuous fixed factor, to model M3 (without removed tongue length).

M4: fidelity~ state + tongue length + manip. tongue length + (1|site) + (1|unique bee ID) + (1+state|bee species)

In the same way we compared the variance estimates between species above, we compared variance estimates for the site intercept from M4 to that from M3. If $C_{\alpha} > 0$, then our interpretation is that manipulated tongue length explains site-to-site variation in bees' floral fidelity (over and above species-level differences). In order to determine whether the drop in variance to zero after the addition of the tongue length of the removed bee was an artifact of the model structure, we ran a permutation test by randomly reassigning the values for tongue length of the removed bee species across sites. Each value was permuted in proportion to its occurrence in the observed

data. We then used the permuted data to calculate a distribution of C_α from comparing M3 to M4. The number of replicate permutations was 1000.

Results:

Species-specific differences:

The addition of manipulated state as a fixed effect in M1 confirmed that the removal of the most abundant bee resulted in a guild-wide reduction in foraging fidelity (Coeff = 0.93, SE = 0.22, $p = 3.87 \times 10^{-5}$, see full model results in Table 2). Furthermore, we found that M1 was a significantly better model when compared to the M0 (ΔAIC = 15, LRT: $p = 3.2 \times 10^{-5}$), we therefore retained state as well as the structural random effects (site and bee individual) in all subsequent models.

There was only slight interspecific variation in the *response* to the manipulation that is not already captured by the main effect of state (variation in the random slope term for bee species = 0.10, see table 2). In contrast, there appears to be substantial interspecific variation in the *baseline* floral fidelity of bees (variation in the random intercept term for bee species = 0.24, see table 2). When bee species was added both as a random slope with respect to state and as a random intercept yielding M2, we found it to be a better model than M1 (ΔAIC = 26, LRT: $p = 4.8 \times 10^{-7}$). The fixed effect of state remained highly significant (Coeff = 0.72, SE = 0.26, $p = 0.005$, see Table 2) in this model.

Traits:

Baseline differences in species specific fidelity:

The addition of tongue length as a fixed effect in M3 largely explained the *baseline* variation in inter-specific floral fidelity (when compared to M2). The baseline fidelity of bees with shorter tongues is less than that of bees with longer tongues (i.e. short-tongued bees move between plant species more often). The variance estimate for the random intercept term for bee species is much smaller in M3 than in M2, with tongue length explaining ~72% of the species-specific differences in base-line fidelity (see Table 2, Figs. 1,2). Furthermore, M3 is a significantly better model than M2 (ΔAIC = 2, LRT: $p = 0.043$, Table 2), indicating the importance of tongue length as an explanatory factor for floral fidelity.

Response to reduction of interspecific competition:

Tongue length, when modeled as a species-level trait, does not explain variation in the *response* of the different bee species to our manipulations. The random slope term for bee species is nearly identical between model M3' (tongue length and state included as a fixed interaction term) and M3 (the additive trait model; $C_\beta = -0.048$; see Table 2 for model descriptions). Still, the fixed effect tongue length is always statistically significant (M3' Coeff = -0.24, SE = 0.11, $p = 0.022$ and M3 Coeff = -0.243, SE = 0.102, $p = 0.017$) both with nearly identical negative coefficients in the two models, indicating that shorter tongue bees have decreasing floral fidelity irrespective of state. Additionally, there is neither a statistically significant main

effect of state, nor a state*tongue length interaction in M3' (Coeff = 0.844, SE = 1.15, $p = 0.46$) but the main effect of state was retained in the additive model (Coeff = 0.734, SE = 0.276, $p = 0.007$). Finally, M3 is only slightly better than M3' (ΔAIC = 2, LRT: $p = 0.926$). See Table 2 for full model results.

Site level attributes:

Site level variation in bumble bee floral fidelity is largely explained by the tongue length of the most abundant bee species (i.e., the species removed experimentally) (Fig. 1. Fidelity decreased more when longer-tongued bee species were removed, relative to shorter-tongued species We found that adding the tongue length of the removed bee as a fixed effect, (a site-level attribute), largely accounts for site-to-site variation in bees' floral fidelity (over and above species-level differences). When we compared the variance estimate for the site intercept from M4 to that from M3, the estimate dropped to zero, suggesting that the model did not fit a parameter because there is no variance left to explain (see Table 2 for variance terms). The three fixed effects, state, tongue length and the tongue length of the removed bee species, are also highly significant in M4 (Coeff = 0.937, SE = 0.242, $p = 0.0001$, Coeff = 0.734, SE = 0.276, $p = 7.46 \text{ X } 10^{-8}$, Coeff = 0.301, SE = 0.081, $p = 0.0001$, respectively). Finally, M4 is a much better model than M3 (ΔAIC = 7, LRT: $p = 0.003$) justifying the retention of the site-level tongue length factor (see Fig.1).

We assessed whether the drop in variance to zero after the addition of the tongue length of the removed bee was an artifact of the model structure. We did so by

randomly permuting the values of tongue length across sites and then recalculating the relative change in variance (C_α) between M3 and each permuted version of M4. The permutation test showed that the distribution of C_α was bimodal. The first mode was centered on zero suggesting that the majority of permuted models showed no change in variance. The observed C_α fell in the second mode, and 4% of the permutations fell above this value (see Fig. S1).

Discussion:

Our results demonstrate that bees vary in their floral fidelity and that tongue length explains a large part of this variation. Bees with shorter tongues move between plant species (floral infidelity) more often than bees with longer tongues. We did not find significant variation in the response of bee species to a reduction in interspecific competition, but rather saw a guild-wide reduction in floral fidelity in response to the removal of the dominant bee species (following Brosi and Briggs 2013). Finally, our results suggest that tongue length of the most abundant bee species, a site-level attribute, explains much of the site-to-site variation in pollinator foraging behavior. In particular, we found that as the tongue length of the most abundant bee (i.e. the species that was experimentally removed) increases, the site level foraging fidelity decreases.

We found that bumble bee species vary in the degree to which they move between different plant species within a single foraging bout, and tongue length explains much of the variation. Some suggest that long tongued bees should exhibit broader resource

usage patterns because their traits permit them access to a wider range of flower types (Santamaría and Rodríguez-Gironés 2007) . In contrast, short-tongued bees should act as specialists, with a more restricted range of resource use options, rarely able to access the nectar at the base of the flowers with long corollas (Harder 1985, Graham and Jones 1996). Our results suggest the opposite pattern, that shorter tongue bees are more labile with their foraging patterns and on average move between plant species within a single foraging bout more often than longer tongue bees. We suggest the following interpretation, because long tongues enable bees to access flowers with better rewards (e.g. long-corolla flowers) and maintain a monopoly on those rewards, they may have less incentive than short-tongued species to move between plant species while foraging. While longer tongue bumble bees are capable of foraging on flowers with short corollas (Heinrich 1976, Plowright and Plowright 1997) it would provide less energetic gain, potentially making behavioral plasticity less profitable (Inouye 1980). In contrast, the shorter-tongued bees in our system tend to have smaller bodies and are more likely to depend on resources within a more restricted foraging range (Westphal et al. 2006). These limitations could favor a labile foraging habit, with shorter tongue bees constantly assessing the resource availability and competitive context in their community. As such, shorter-tongued bees more readily switch between plant species.

While we saw little variation in species-specific foraging responses to our manipulations, we found an overall reduction in floral fidelity across sites after the removal of the most abundant bee species. Our results build on the findings of Brosi

and Briggs (2013), reaffirming a guild-wide reduction in floral fidelity in response to a reduction in interspecific competition. This study adds an additional two years, 8 sites and 165 bee individuals to our previous study, confirming that the guild-wide results found in Brosi and Briggs (2013) are robust.

Variation in bumble bee floral fidelity is largely explained by the tongue length of the most abundant (i.e. removed) bee species in each site. This means that pollinator foraging behavior is context dependent and is determined (at least in part) by the most abundant bee species. In general, short tongued bees exhibit lower floral fidelity than long tongued bees, and when they are in a site that has a long tongued bee removal, their reduction in floral fidelity is magnified.

Bumble bees are large bodied insects that require many floral resources to keep their hive growing throughout the (often short) growing season. As such, we might expect strong competition between these species, and tongue length, arguably the trait most relevant for resource acquisition, could dictate how resources are partitioned within a community, ultimately drive the assembly of bumble bees within communities (Heinrich 1976, Harmon-Threatt and Ackerly 2013). Pyke (1982) proposed that bumble-bee species with similar tongue lengths could not exist in his altitudinal alpine transects presumably because the bees compete for floral resources. But later studies did not support this pattern (Ranta 1982, Goulson et al. 2008), and, as in our study, found that bumble bee species with similar tongue lengths co-ocurred within a community. This has left researchers to wonder if the coexistence of many bee species with substantial overlap in their life history requirements is possible

because bumble bee species compete for something other than flower resources (i.e. nesting sites) allowing so many similar species to co-occur (Goulson et al. 2008). Our study suggests that in our system, bumble bees do in fact compete for floral resources and that longer tongue bees seem to elicit competition that is experienced across the range of trait values seen in our sites (see Table 1). The willingness of short tongue bees to exhibit behavioral plasticity may allow for such a large number of seemingly similar bee species to coexist in a community. Future work should examine the extent to which this plasticity is adaptive and assess the fitness costs (or benefits) that may result from the willingness to switch floral resources in response to a reduction in interspecific competition.

Most pollinators are generalist foragers that can switch between plant species within a single foraging bout (Waser et al. 1996, Brosi and Briggs 2013). When pollinators move between plant species, they can transfer heterospecific pollen to plant stigmas which in turn can reduce plant reproduction (Morales and Traveset 2008) (Mitchell et al. 2009) (Ashman and Arceo-Gomez 2013, Briggs et al. 2015). We found an effect in which competition from a long tongued bee changes the foraging behavior of the rest of the bees in a way that could be detrimental to plant reproduction. From a plant's perspective, not only do short tongue bees exhibit behavior that likely results in the transfer of heterospecific pollen, but when short tongue bees are in communities in which a longer tongue bee is most abundant, they exhibit even greater floral infidelity, making the likelihood of heterospecific pollen deposition even greater (see Fig. 2).

Pollinator species are on the decline globally (Potts et al. 2010). Bumble bees in particular are experiencing population range contractions due to climate change (Kerr et al. 2015) as well reductions in abundance due to disease (Cameron et al. 2011), agricultural intensification and pesticide use (Goulson et al. 2015). In a changing world where we are likely to experience an emergence of new interactions via range shifts, introduced species and climate change, exploring how the competitive landscape shapes foraging plasticity will help us generalize to other plant pollinator systems and begin to better predict the functional implications of competitive interactions.

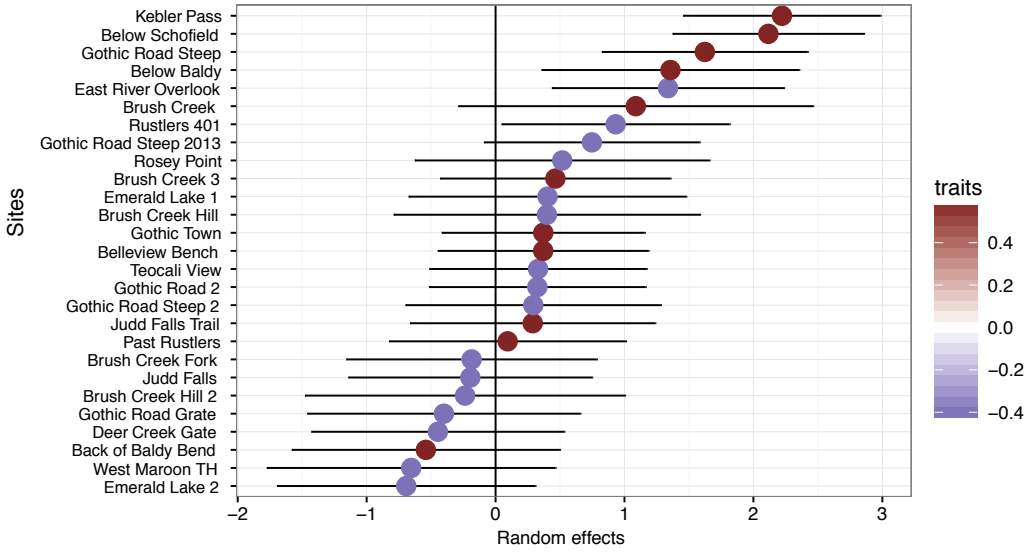

Figure 1: **Random intercept coefficient estimates for each site from M3.** Higher values indicate positive deviation from global model intercept. Some sites have higher are higher deviance from the mean compared to other sites, and the value on the x axis represents this deviation. The colors represent tongue length of the manipulated bee at each of the sites and are mean centered. Error bars represent 95% CIs. Note that sites with positive deviation (i.e. higher than average infidelity among bees) tended to be those where the removed bee species had longer tongue lengths (red dots).

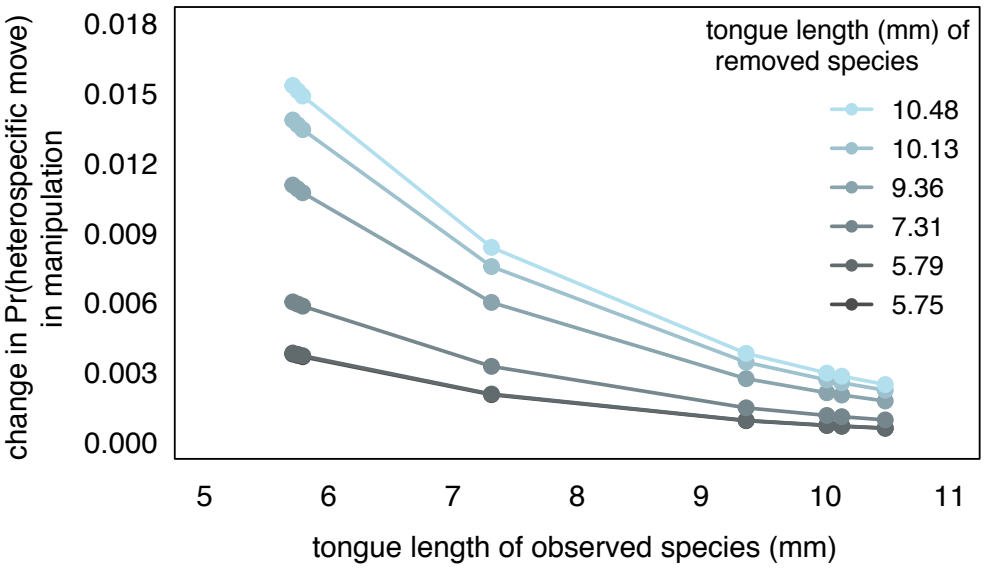

Figure 2: Predicted values of the three fixed effects (tongue length, manipulated tongue length and state) from M4. Y axis values represent the increase in heterospecific moves in the manipulation compared to the control state. Each line represents predicted values for each tongue length (responding species) in the context of the tongue length of each manipulated (most abundant) species.

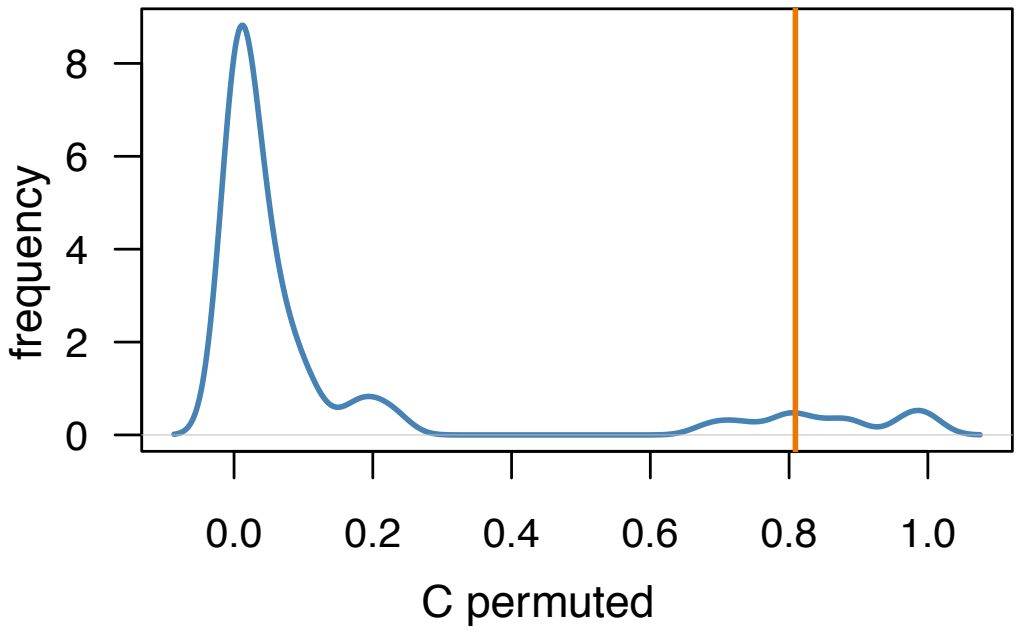

Figure S1. Random permutation of tongue length values across sites. Displayed is the relative change in variance (C_α) between M3 and each permuted version of M4. Orange line is the observed value for the unpermuted data. 1000 replicate permutations.

Table 1: Tongue lengths of sympatric *Bombus* spp. Mean and standard deviation of 50 individuals per species from Inouye (1976).

Bombus Species	mean mm	sd	N Sites in which spp. was removed
B. occidentalis	5.71	0.25	0
B. bifarius	5.75	0.37	6
B. sylvicola	5.79	0.58	1
B. flavifrons	7.31	0.8	9
B. balteatus	9.36	0.62	4
B. californicus	10.01	0.75	0
B. nevadensis	10.13	0.68	2
B. apposititus	10.48	0.95	5

Table 2: Results of generalized linear mixed-effects models with binomial errors. TL = tongue length MTL = manipulated tongue length (i.e. tongue length of the species removed from each site); R^2C = describes the proportion of variance explained by both the fixed and random factors; R^2M describes the proportion of variance explained by the fixed factors alone; LRT = likelihood ratio test.

	Fixed effects				Random effects			Model Comparison			
model	state	TL	state*TL	MTL	Species α/ß	Site	Individual	AIC	R^2C	R^2M	LRT
MO	--	--	--	--	--	0.88	5.24	2079	0.093	0	--
M1	0.93***	--	--	--	--	0.93	5.00	2064	0.0122	0.022	0:1, p = 3.2 x 10-5
M2	0.72**	--	--	--	0.24/0.10	1.11	3.90	2038	0.014	0.19	1:2, p = 4.8 x 10-7
M3	0.844	–0.24*	–0.013	--	0.06/0.12	0.89	4.17	2038	0.042	0.169	3:3', p = 0.92
M3'	0.73**	–0.24*	--	--	0.06/0.12	0.89	4.17	2036	0.041	0.168	2:3, p = 0.04
M4	0.937***	–0.38***	--	0.301***	0/0	0	0.00	2029	0.11	0.12	3:4, p = 0.003

*P < 0.05; **P < 0.01; ***P < 0.001

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Not all interactions are positive: testing the robustness of plant pollinator networks to extinction simulations after incorporating negative interactions.

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INTRODUCTION

Pollinator losses are increasing across the globe which could have potentially strong negative effects on the plants that rely on them for pollination (Biesmeijer et al. 2006, Potts et al. 2010). Network-based simulations suggest that plant communities will be very robust to pollinator extinctions (i.e. secondary plant extinctions resulting from pollinator extinctions) (Memmott et al. 2004, 2007, Kaiser-Bunbury et al. 2010). This robustness is likely driven by two features of network structure. First, pollination networks are dominated by *generalist* interactions; most plants and pollinators each interact with several species from the other group over the course of their lives (Waser et al. 1996). Second, pollination networks have a *nested* structure in which specialist plants and pollinators tend to interact with a subset of the species in the other group that generalists interact with (Bascompte et al. 2003, 2006). Nestedness leads to an asymmetric interaction structure where specialists from one group tend to act with generalists, not specialists, from the other, which could reduce linked extinctions if specialists are vulnerable to stochastic extinctions (Bascompte et al. 2006, Bascompte and Jordano 2007). One feature of binary-graph plant-pollinator simulation models that may overestimate network robustness is that all interactions in a binary-graph network are positive. A typical binary-graph simulation modeling approach is that if at least one link remains between a plant and a pollinator, the plant will continue to persist (Memmott et al. 2004, 2007).

While the assumption of "all interactions positive" is a reasonable starting point in a mutualistic network model, empirical evidence suggests at least two ways

in which pollinators can have negative consequences for the reproduction of plants they interact with (Irwin et al. 2010, Ashman and Arceo-Gomez 2013, Brosi and Briggs 2013). First, the benefit that pollinators have on plant reproduction is sensitive to how "faithful" pollinators are to particular plant species in a single foraging bout. Most pollinators are generalist foragers that can, in some contexts, switch between plant species within a single foraging bout (Waser et al. 1996, Brosi and Briggs 2013). When pollinators are promiscuous within a single foraging bout, they may transfer heterospecific pollen to floral stigmas, which can have negative effects on both male and female elements of plant reproduction (Morales and Traveset 2008, Flanagan et al. 2011, Arceo-Gómez and Ashman 2011, Brosi and Briggs 2013). While heterospecific pollen deposition is highly variable in nature (Ashman and Arceo-Gomez 2013), it can represent a substantial percentage of total pollen on a stigma, often more than 50% of grains (Ashman and Arceo-Gomez 2013).

Second, there is the extreme example of an antagonistic interaction from pollinators wherein the visitors do not visit the flower "legitimately" but rather pierce holes in a flower's corolla (or utilize a hole that has already been made) to access the nectar rewards without ever touching the reproductive parts of the flower and therefore not acting as a pollinator (Bronstein 2001, Genini et al. 2010, Irwin et al. 2010). Some researchers suggest that most all flowering plants with accommodating floral architecture (i.e. flowers that are tubular or with nectar spurs) will experience some degree of nectar robbing (Barrows 1980, Irwin & Maloof 2002, Irwin et al. 2010). Furthermore, if a pollinator possesses mouthparts capable of robbing, they are

likely to act as both legitimate pollinators on some plant species and primary or secondary robbers on others (Irwin et al. 2010). On the other side of the interaction, some plants produce chemicals in pollen or nectar that that can be harmful to the development of the bees that visit their flowers, reducing bee fitness (Praz et al. 2008, Sedivy et al. 2011, Haider et al. 2012).

It has long been recognized that exploitation of mutualisms ("cheating") is commonplace and can have substantial impacts on the evolutionary persistence of mutualisms (Bronstein 1994, Richardson 2004, Bronstein et al. 2006, Chamberlain et al. 2014). While our understanding of the extent to which antagonistic interactions between plants and their pollinators is not complete, the examples listed above are common enough that the inclusion of such demonstrated negative interactions on network dynamics and how they might impact the robustness of interactions to extinctions warrants exploration.

Recent network studies have begun to explore interactions in a continuous, rather than binary positive-or-not framework (comparison between the two in Kaiser-Bunbury, Vieira and Neto 2015). While such studies do not take the potential for negative interactions into account, these models allow for some pollinators to be "better" than others in the services they provide (Ballantyne et al. 2015) or, in the case of Vieira and Neto (2015), to vary the amount of dependence that the mutualistic partners have on one another.

Here, we build on binary network simulation modeling approaches to assess

how negative interactions impact the effects of pollinator species losses on plant species persistence, i.e. network robustness. In previous simulations where all network interactions are considered positive, the removal of a given pollinator species could result only in the loss of one or more plants. By contrast, after incorporating negative interactions, extinction cascades, (i.e. a second round of pollinator extinctions) also become possible (though see Vieira and Almeida-Neto (2015), who produce extinction cascades in an all positive framework by relaxing the assumption that extinctions only take place after all partners are lost). In other words, if the removal of a pollinator species causes plant extinctions, those losses can tip the balance of interactions toward the negative for remaining pollinator species, which can then go extinct, in turn potentially leading to further plant species losses, thus an extinction cascade. Our study examines the overall robustness to extinctions in two ways (1) *R,* or, the area under each extinction curve (see methods for details) and (2) extinction cascade length –i.e. higher order extinctions that occur beyond the induced pollinator knockout and the resulting plant extinction.

To our knowledge there is only one network study that incorporates the possibility for negative interactions between plants and pollinators (Campbell et al. 2012) while examining the robustness of the network when faced with extinctions This model classifies interactions as either mutually beneficial or beneficial for one species and detrimental to the other. This is in contrast to our model that examines robustness of networks with all positive interactions to those that incorporate negative interactions (that are negative for both parties involved). Importantly the Campbell

model focuses on hypothetical (rather than empirical) networks and does not directly evaluate the role of assigned negative interactions in determining the robustness of the network to extinctions and furthermore does not compare networks with and without negative interactions. Our method of incorporating negative interactions in to empirical networks will allow for higher order extinction cascades, giving us a more realistic impression of what might happen to a network after pollinator extinctions. This is not the case in extinction simulations that simply allow for asymmetric positive interactions (though see Vieira and Almeida-Neto (2015), as noted above).

We examined the effects of two factors on network robustness: (1) the proportion of negative interactions in the network, including an all-interactions-positive control; and (2) the order of extinction, random pollinator losses vs. specialist-to-generalist vs. generalist-to-specialist. Removing specialists first could be the most probable extinction sequence (Dunne et al. 2002) as specialist pollinators also tend to be the rarest species (e.g. Vazquez and Aizen 2003), but see (Aizen et al. 2012) who show that loss of specialists can accelerate the rate of species loss). By contrast, generalists are thought to be the "backbone" of networks and when highly connected nodes are lost, networks are expected to collapse rather quickly (Dunne et al. 2002, Tylianakis et al. 2010, Albert et al. 2013). While losing generalist pollinators first from a network may seem unlikely, we have seen rapid declines and range contractions (leading to local extinctions) in several highly generalist bumble bee species which had previously been abundant (Goulson et al. 2008, Meeus et al. 2011).

First, we hypothesized that increasing the proportion of negative interactions would lead to both a decrease in the robustness of the network (*R,* or the area under the extinction curve) and greater number of extinction cascades. Second, we hypothesized that inclusion of negative interactions would not change the effects of extinction order relative to all-interactions-positive networks, in which specialist-to-generalist pollinator species removals had the least impact on plant extinctions, generalist-to-specialist removals had the most, and random removals intermediate between the two (Memmott et al. 2004, 2007).

METHODS

Empirical networks

Following previous binary network assessments of robustness (Memmott et al. 2004, 2007), we used empirical networks to conduct our robustness assessments. We selected three plant-pollinator networks of varying size and connectance that represent a range of natural plant-pollinator interactions; as in previous assessments, this selection is not meant to be exhaustive (Memmott et al. 2004, 2007, Valdovinos et al. 2012). 1) The Clemens and Long (1923) network was collected on Pikes Peak, Rocky Mountains, Colorado USA. This is by far the largest with 97 plant species forming 918 unique pairwise interactions with 275 pollinator species. Data were collected in various subalpine habitats at 2500 m elevation over 11 years (Clements & Long 1923). 2) The Arroyo et al (1982) network data were collected at an elevation between 2200m and 2600m between 1980 and 1981 in the alpine (Andean) zone of

Cordon del Cepo in Central Chile. The network is medium in size with 87 plant species forming 372 unique pairwise interactions with 98 pollinator species. 3) The Dupont network (2003) is the smallest as data were collected on the sub-alpine desert above 2000 m on the island of Tenerife, Canary Islands. Data were collect between May 7 and June 7, 2001. The network consists of 11 plant species forming 109 unique pairwise interactions with 38 pollinator species. These networks were retrieved from the NCEAS Interaction Web Database (http:// www.nceas.ucsb.edu/interactionweb).

Assignment of negative interactions

We randomly assigned negative values to the existing interactions in each binary empirical network (non-existing interactions were not subject to negative assignment). Negative interactions were set at a value of -1, i.e. equivalent magnitude to positive interactions. Thus, each possible interaction in a network could have a value of -1, 0, or +1. We assumed that the presence and sign of interactions were symmetric between plants and pollinators following network literature that assumes quantitative interaction strengths are symmetric (Okuyama and Holland 2008, Holland and Hastings 2008). We assessed four different proportions of negative interactions: 0 (control), 0.05, 0.10, and 0.15, with 50 replicate configurations of negative values for each network at each proportion of negative values. We thus used a total of 3 (empirical networks) × 4 (proportion negative interactions) × 50 (replicate configurations) = 600 networks. We only used configurations of negative interactions that were initially stable, i.e. which

would not automatically lead to extinctions in the absence of perturbations. As a stability condition throughout our simulations, we assumed that plants and pollinators need a positive balance of interactions, that is, a row or column sum of greater than or equal to one. By definition, this automatically excluded all highly specialist nodes (i.e. those two or fewer interactions) from being assigned a negative interaction. While the proportion of negative interactions in empirical networks has not been studied to our knowledge, the maximum value we used (15% negative) represents a practical upper limit of negative interaction assignment that allows for stable network configurations without excessive search times.

Extinction simulations

We simulated extinctions by sequentially removing pollinator species one at a time (i.e. pollinator "knockouts") and recording the number of plant species that were left with a positive sum of pollinator interactions. Plant species left with an interaction sum less than or equal to zero were then considered extinct and removed from the network due to assumed failure to sexually reproduce. Next, we evaluated if the secondary removal of those plant species left a pollinator species with an interaction sum less than or equal to zero. If yes, they were then considered extinct and removed from the network (these cycles of extinctions are referred to throughout the text as extinction cascades). This cycle continued until all plant and pollinator species were left with an interaction sum greater than 0 at which point the simulation moved on to

the next pollinator species knockout. Knockouts were repeated until all of the plant species were lost from the network. We carried out the extinction simulations separately for each of the aforementioned 600 network configurations and each of the three extinction orders.

Analysis of network robustness metrics

We evaluated network robustness via two response variables: (1) *R* (the area under the curve of extinction) – this is a quantitative measure of robustness of a network following a species knockout (extinction). *R* is a simple calculation of the sum of the remaining plant species at each time step along the extinction simulation. *R* is standardized by its maximum value which equals the starting number of plants * the starting number of pollinators (Burgos et al. 2007). *R* was calculated for each of the 50 simulations per order and proportion negative for all three networks. (2) Extinction cascades – the number of higher order extinction cycles that take place after a single pollinator species knockout.

R: area under the extinction curve.

We used binomial GLMs with a logit link function to network robustness. In the first set of models (models R1–3) we assessed the effect of increasing proportion negative interactions, the order of extinctions (main effects) and the interaction between proportion of negative interactions and order of extinction on network robustness (*R*) for each of the three networks. The second set of models (R4–R6) assessed the

overall difference in robustness between the three networks for a given extinction order, as well as the relationship between increasing proportion of negative interactions and robustness (i.e. the interaction terms) for each extinction order.

Total number of extinction cascades.

Cascade length is defined as the higher order extinctions that occurred beyond the induced pollinator knockout and the resulting plant extinction(s). A cascade of length 1 results when such plant extinctions lead directly to subsequent pollinator extinction(s), while a cascade of length 2 indicates additional subsequent plant extinction(s). We calculated the total number of cascade events that occurred across the entire knockout sequence for each of the 50 replicate network configurations for each set of starting networks using GLMs with Poisson errors and a log link function. This analysis directly mirrors the modeling approach for robustness (R) discussed above, comprising two sets of models. The first set compared the effect of negative interactions, extinction order, and their interaction within each network (models C1–3), while the second set of models compared the effect of negative interactions, network ID, and their interaction, within each extinction order (models C4–6).

RESULTS

Network Robustness, R.

The first set of models (models R1–3, Table 1) made comparisons across rows for each column (i.e. each network) of Fig. 1, comparing how extinction order affected R

(i.e. the main effect of extinction order) as well as the impact of negative interactions on *R* (i.e. the interaction terms). When compared to random extinction order, simulations with generalists removed first show the lowest overall *R* (i.e. the intercepts are lower; $p<10^{-5}$, Table 1, Fig 2), while removing specialists first has the least impact on lowering network robustness ($p<10^{-5}$, Table 1, Fig 2). This pattern held across all three networks. In the Dupont network, extinction order did not impact the magnitude of the effect of negative interactions on *R* (interaction terms both $p>0.4$, Table 1, Fig 2). In contrast, in the remaining two networks, when generalists were removed first, the effect of increasing negative interactions was reduced relative to the random extinction order (interaction terms both $p<10^{-5}$, models R2–3; Table 1, Fig 2). In both the Arroyo and Clemens networks, the effect of negative interactions was magnified when specialists were removed first compared to the random extinction order simulations (interaction terms both $p<10^{-5}$, Table 1, Fig 2).

The second set of models (Table 2) made comparisons across columns for each row of Fig. 1. Specifically, for a given extinction order, models R4–6 compared the overall differences between networks in robustness to pollinator extinctions (i.e. main effect of network ID) as well as the relationship between negative interactions and robustness (i.e. the interaction terms). For the simulations with both random and generalists-first extinction orders, the Arroyo and Clemens networks both exhibited lower overall *R* (main effects $p<10^{-5}$, models R4–5, Table 2) as well as a larger effect of negative interactions on reducing *R* (interaction terms both $p<10^{-5}$, Table 2) compared to the Dupont network. For the specialist-first extinction order simulations,

the three networks exhibited similar overall *R*, although Arroyo was slightly less robust than Dupont in general (Arroyo main effect p=0.04; model R6, Table 2). Negative interactions had a larger negative impact on *R* in the Arroyo and Clemens networks compared to Dupont (interaction terms $p<10^{-5}$, model R6, Table 2).

Extinction cascades

The first set of models (models C1–3, Table 3, Fig. 3) made comparisons across rows for each column (i.e. each network) of Fig. 3, comparing both how extinction order as well as negative interactions impact the total number of extinction cascades. For the Dupont network, extinction order did not impact the total number of extinction cascades (order main effects, $p>0.1$, model C1, Table 3), and the effect of negative interactions was consistent for all extinction orders (interaction terms $p>0.5$). For the Clemens network simulations, different extinction orders yielded different total number of cascades: for the generalist first order, the total number of cascades was higher than in the other two orders (main effect $p<10^{-5}$), while the specialists first simulations showed the lowest number of cascades (main effect $p<10^{-5}$, model C2, Table 3). Negative interactions had no effect on total extinction cascades for the Clemens network (main effect and interactions, $p>0.5$). In contrast, in the Arroyo network, negative interactions increased total extinction cascades similarly across all extinction orders (main effect $p<10^{-5}$, interactions $p>0.1$, model C3, Table 3). In addition, when compared to the random extinction order, more extinction cascades

were observed in the generalist first order and fewer in the specialist first order (main effects $p<0.05$, model C3, Table 3), just as in the Clemens network.

The second set of models (Table 4) made comparisons across columns for each row of Fig. 3. Specifically, for a given extinction order, models C4–6 compared the overall differences between networks in total number of extinction cascades (i.e. main effect of network ID) as well as the relationship between negative interactions and total number of extinction cascades (i.e. the interaction terms). For both the random and generalists-first extinction orders, the Arroyo and Clemens networks both exhibited a greater number of extinction cascades compared to Dupont (main effects $p<0.05$, models C4–5, Table 4). The differences in total cascades for the specialist-first order differed only slightly (Clemens, $p<0.05$) or not at all (Arroyo, $p=0.2$) when compared to the Dupont network (model C6, Table 4).

The effect of negative interactions on total cascades differed both between networks and between extinction orders: in generalist- and specialist-first simulations, both Clemens (interaction terms $p<0.003$) and Arroyo (interaction terms $p<0.002$) networks exhibited significantly weaker effects of negative interactions on total cascades compared to the Dupont network (models C5–6, Table 4). In fact, as noted above, there was no effect of negative interactions on total extinction cascades for the Clemens network for any of the extinction orders (Table 3). For the Arroyo network, however, the extent to which negative interactions increased total extinction cascades was similar to the Dupont network in the random extinction order simulations ($p>0.1$).

DISCUSSION

Our study examined how both the incorporation of negative interactions into networks as well as order of extinction impacted robustness and total number of extinction cascades in three empirical networks. As expected, we found that incorporating negative interactions leads to lower network robustness via an increased rate of plant species loss (as compared to networks with only positive interactions) in all three of the networks. Furthermore, when compared to random extinction order, simulations with generalist removed first show the lowest overall robustness whereas the removal of specialists first has the least impact on lowering network robustness. This is true for all three networks and followed our expectations based on previous network extinction simulations (Memmott et al. 2004, 2007).

While some of the results from our simulations were predictable or even mathematical inevitabilities (i.e. addition of negative interactions would make networks less robust to extinction), not all of our results could be predicted *a priori*. We did not expect the networks to behave idiosyncratically with respect to how negative interactions and extinction order impacted both *R* and the total number of extinction cascades. Specifically, we found that in the smallest of the networks (Dupont), extinction order did not impact the magnitude of the effect of negative interactions on network robustness. The effect of removing generalist-first from the two larger networks (Arroyo and Clemens) seemed to supersede the impact of negative interactions, leading us to conclude that the impact of losing generalists can,

in some cases, be so detrimental so as to make inclusion of negative interactions irrelevant.

While extinction cascades can only take place in networks that incorporate negative interactions, the impact of negative interactions on total cascade length was unpredictable. Neither increasing negative interactions nor extinction order were a good predictor for how many extinction cascades networks would take place in the simulations. Total number of extinction cascades is likely relate to structural properties unique to each network and warrant further exploration.

It difficult to draw conclusions as to why these distinct differences between networks were seen, as this study did not include an exhaustive exploration of other network properties that may influence network robustness and total extinction cascades. Future studies should focus on selecting networks with systematically varying properties such as; network size, nestedness, and connectance. These properties each have the potential to influence the impact that negative interactions have on network robustness. Nestedness is a network property that has been identified in nearly all empirical networks. It is the tendency of specialists to interact with generalists in the network (Bascompte et al. 2003). We would expect that an increase in nestedness could make the networks more robust to extinctions due to the redundancy in the number of pollinators available per plant. Still, Campbell et al. (2012) found that high values of nestedness actually can have the opposite effect, and can decrease robustness after the loss of a single species, in some extreme cases lead to the total collapse of the highly nested community. In our simulations, networks

with more generalists will be inherently more vulnerable (depending on extinction order) because true specialists were excluded from assignment of negative interactions (see methods for full explanation of why). While we do not expect this limitation to impact our results dramatically—as true specialists that only interact with one or two plant species are uncommon—to fully explore the role of nestedness one would need to develop a method of assigning negative interactions that distributes the negatives evenly among the specialists and generalists.

Connectance is simply the proportion of realized links in a network (Jordano et al. 1987). In this study we, by chance, selected a range of network size and connectance. Still, we do not have any replication for the different levels of connectance and so it is impossible to draw any conclusions regarding this structural property. We expect that, following many plant-pollinator network and food web extinction simulations, higher connectance would lead to greater robustness (Dunne and Williams 2009, Lever et al. 2014). However, the addition of negative interactions could complicate the interactions, making highly connected interaction networks vulnerable if positive interactions were lost, leaving only negative interactors (Vieira and Neto 2015). Future work exploring the role of both nestedness and connectance in networks that incorporate a range of negative interactions could help improve understanding of the extent to which these commonly used structural metrics predict robustness in networks.

In all three networks, removing pollinator species from generalist to specialist first has a larger impact on the robustness of the network than with the order of specialist to generalist removals. This pattern has been noted in previous studies (Memmott et al. 2007 and 2009) though ours is the first study to examine the role of extinction order after incorporating negative interactions in the networks. Memmott et al. (2007 and 2009) noted from their study (including only positive interactions) that while robustness was impacted when species were removed from generalist to specialist, the effect was not as dramatic as expected or as reported in food web studies when the most linked interactors are removed (Dunne et al. 2002, Curtsdotter et al. 2011). In our case, adding negative interactions in to the network made the network less robust, resulting in patterns more like those of food web studies where removal of the most linked species causes a collapse to low richness (Dunne et al. 2002, Srinivasan et al 2007). Future studies should focus on exploring the impact of higher proportions of negative as well as networks of varying properties to determine how and why networks are most vulnerable and to understand if order of extinction matters only in specific cases.

Extinction cascade length, or, the number of additional species losses triggered by initial pollinator extinctions is a property unique to simulations that incorporate negative interactions in to the network. As far as we know this is the first study to incorporate negative interactions in to plant pollinator networks in an effort to assess network robustness to pollinator extinctions. This makes it impossible to compare the role of extinction cascades on network robustness to other studies

examining the same question. Still, our results indicate that extinction cascades play an important role in our simulations but that networks behave idiosyncratically. Understanding if variation in total cascade length is biologically meaningful and whether total cascade length helps us understand how vulnerable a network is to extinctions will require further exploration. The focus should be to pinpoint properties that make networks more vulnerable to cascades and the point at which networks are most vulnerable (i.e. after a single extinction or not until many pollinators have been lost from the network).

Our exploration into the role of negative interactions in extinction simulations lead us to question the expectation that plant-pollinators interactions will be robust to extinctions. If by chance we lose the "good" pollinators from communities leaving less effective or detrimental pollinators (Irwin et al. 2010, Ashman and Arceo-Gomez 2013, Brosi and Briggs 2013) then we may see a series of additional extinctions or population declines. These results certainly warrant future explorations in to the role of variation in interaction quality and the potential to impact communities through extinction cascades.

While not exhaustive, this study is an important exercise in assessing how one key assumption in plant-pollinator simulation models may overestimate network robustness. There are other studies that are making an effort to incorporate more biologically realistic interactions into network studies (Ne'eman et al 2010, Vieira and Neto 2015), though not through the addition of negative interactions. Vieira and Neto (2015) expand on traditional extinction models (Memmott 2004, Memmott

2007) by allowing for variation in how much one species relies on the other (pollinators have high reliance on plants as they need nectar to survive but some plants can rely on self-pollination if they fail to receive pollination services) and variation in the assumption species can persist even if only a minor, weakly interacting partner is present. They find that the relaxing the first assumption leads to a decrease in robustness and the second assumption leads to an increase in robustness. Moving beyond the expectation that all interactions between plants and their pollinators are strictly positive will help us to explore not only the robustness of networks to extinctions, but also has the potential to help us further understand how and why mutualisms are maintained when faced with exploitation (Bronstein 2001, Irwin et al. 2010, Chamberlain et al. 2014).

Campbell et al. (2012) is the only other study to incorporate negative interactions into network simulations. They do so by identifying interactions as either mutually beneficial or beneficial for one species and detrimental to the other. While they do not directly assess how these antagonistic interactions impact network robustness when faced with extinctions, they do reveal the "critical species" in their simulations—those species that cause significant community collapse when removed—are species that tend to have asymmetric interaction direction. Their results suggest that when these critical species are lost, the network is left with an abundance of negative interactions in their absence, which can lead to further extinctions and often collapse. An important next step in assessing the robustness of plant-pollinator networks to extinctions would be to build networks that incorporate

variation in negative interactions (rather than just a binary assessment) and complete similar extinction simulations. We expect that a more continuous interaction space will lead to similar results that we found in this study, with just a slower rate in loss of robustness due to variation in interaction strength (both negative and positive).

In recent years there has been a substantial effort to incorporate realistic dynamics in to ecological network models. These efforts have revealed that the incorporation of community dynamics such as population sizes and interaction strengths and topological dynamics such as re-wiring (i.e. the potential for species to alter their interactions after species are lost from the network) can offer a more realistic assessment of the robustness of networks faced with extinctions. (Fortuna and Bascompte 2006, Kaiser-Bunbury et al. 2010, Valdovinos et al. 2012) While the inclusion of population and topological dynamics were beyond the scope of this study, we acknowledge that such dynamics could potentially add to our understanding of how negative interactions impact network robustness. For example, incorporating migration into our models could make the networks more robust to extinctions if the species moving in were positive interactors. However, one could imagine a scenario where a less effective pollinator such as a honey bee moves in after an extinction event of a more effective pollinator (Thomson 2004). In this scenario, a plant species would be rescued from the loss of interaction but could experience reproductive loss with the new, less effective, pollinator. Valdovinos et al (2012) find that allowing for adaptive foraging (i.e. when variation in resource availability leads to changes in foraging effort that result in fitness benefits), leads to enhanced network robustness

against species extinctions. This is due to an increase in the floral resources extracted by the specialist pollinators as well as an increase in the rates of visitation to specialist plants in the network. Allowing for adaptive foraging in our simulations would likely make the networks more robust to extinctions (in spite of the addition of negative interactions), although, this robustness would likely vary depending on extinction order (i.e. when specialist are removed first, then adaptive foraging may not lead to more robust networks). Finally, allowing for re-wiring to a new interaction partner after experiencing either extinction or fluctuations in partner availability has been shown to lead to more robust networks (Kaiser-Bunbury et al. 2010, Thierry et al. 2011, Ramos-Jiliberto et al. 2012). Still, if plants re-wire to a less effective pollinator they may suffer reproductive consequences. Future studies exploring such dynamics could substantially add to our understanding of how incorporating negative interactions in to plant pollinator network models impact network robustness when faced with species loss. Habitat loss and degradation are leading to global pollinator losses (Biesmeijer et al. 2006; Potts et al.2010). Given the central importance of pollination in food production and the maintenance of biodiversity and ecosystem function, it is imperative that we come to a predictive understanding of how pollinator losses will affect plant-pollinator systems. Evidence suggests that losing even a few pollinators can have a strong negative effect on the plants that rely on pollination for reproduction (Biesmeijer et al. 2006, Potts et al. 2010, Brosi and Briggs 2013). In the absence of empirical studies, network-based simulations left researchers hopeful that plant communities would be robust to pollinator extinctions (Memmott et al. 2004,

2007, Kaiser-Bunbury et al. 2010) Our study is a step in showing that these simulation models may overestimate network robustness by assuming that all interactions in the networks are positive. By incorporating more realistic representations of the interactions that take place in a plant-pollinator community, then we are more likely to identify properties of networks that determine robustness to extinctions, helping direct conservation efforts. Future studies that improve on predictive models that will allow us to anticipate likely changes in pollination services, and help us designing strategies to maximize ecosystem resilience will be essential to preserving pollination services.

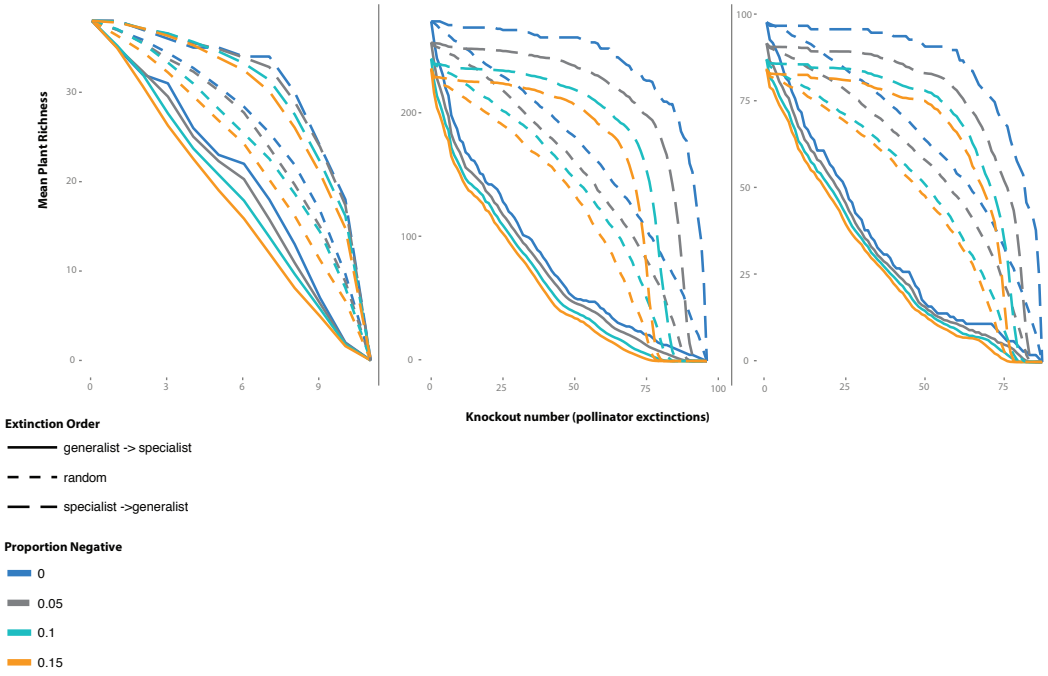

Figure 1: Extinction patterns for the three pollination networks (a) Dupont, (b) Arroyo and (c) Clemens. Lines represent average of 50 simulations for each given proportion negative and extinction order.

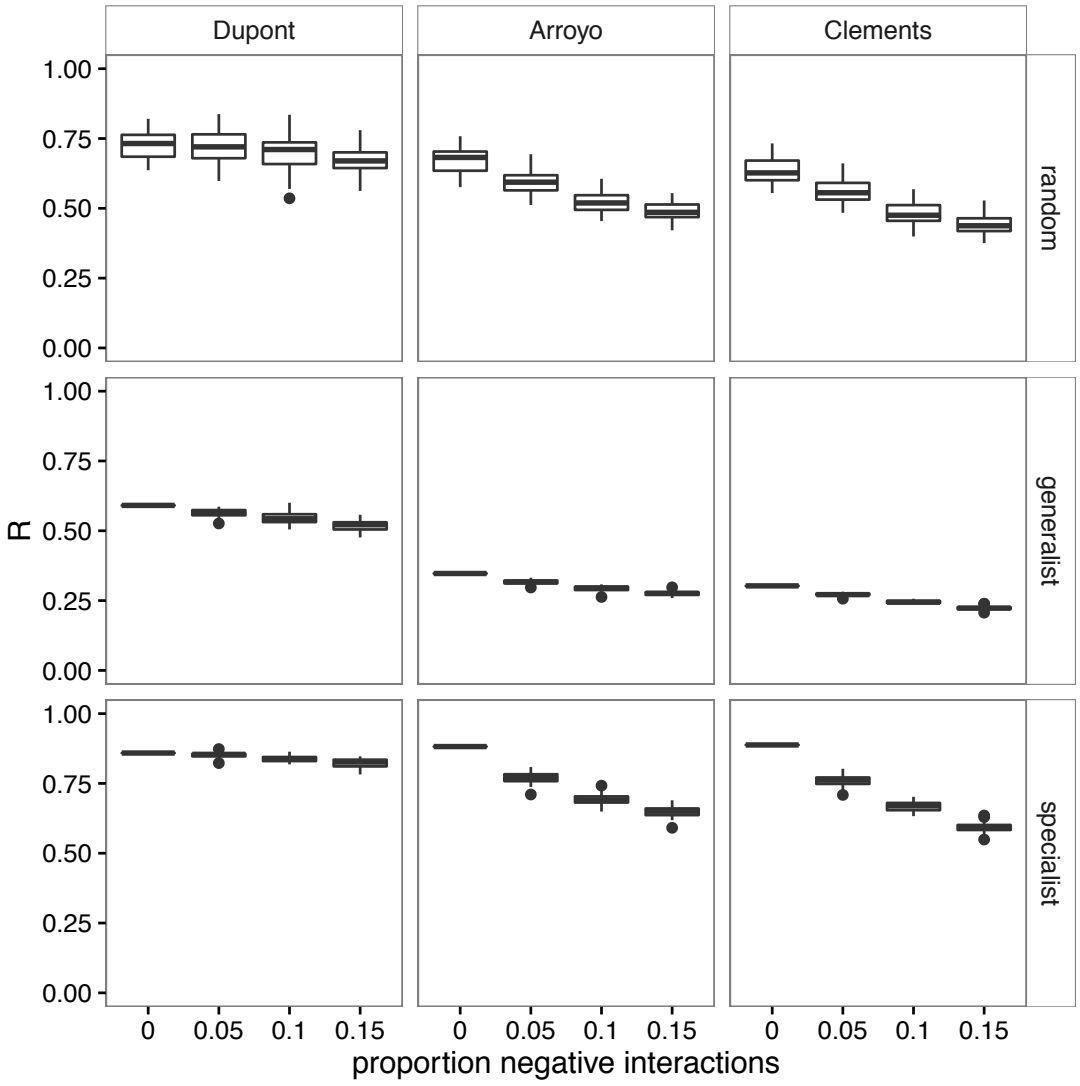

Fig. 2. Effects of negative interactions on network robustness during pollinator knockout simulations for different extinction orders and starting networks. Data shown are boxplots displaying median, 50%, and 95% quantiles of robustness (*R*) for the 50 simulations for each proportion negative across the three simulated extinction orders (row headers), and starting network IDs (column headers). Data points beyond ± 95% quantiles are displayed as points.

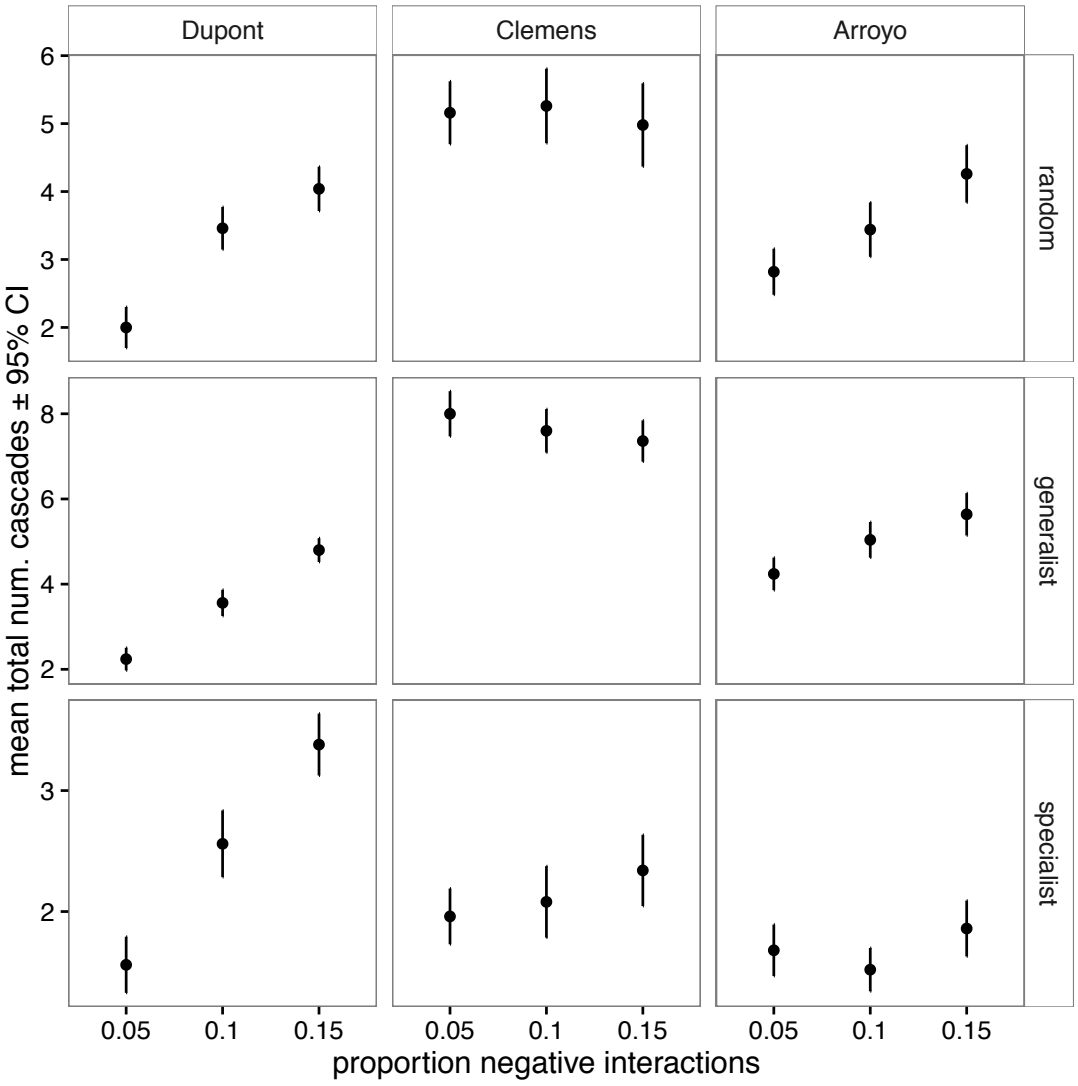

Fig. 3. Effects of negative interactions on total number of extinction cascades occurring during pollinator knockout simulations for different extinction orders and starting networks. Points indicate the average across 50 extinction simulations of the total number of cascades summed across entire knockout sequence. Error bars indicate ± 95% confidence limits on the estimate of the mean ($2*\sqrt{(\sigma/n)}$) calculated from the results from the n = 50 replicate simulations. Total cascades are presented for each proportion negative interaction across three simulated extinction orders (row headers) and starting network IDs (column headers).

Table 1: Results of network robustness (*R*) GLMs for each network separately.

Model Number	Network		Estimate	Std. Error	Z value	P value
R1	Dupont	Intercept	0.999	0.013	77.21	$p<10^{-5}$
		PNI	-1.76	0.136	-12.958	$p<10^{-5}$
		Generalist First	-0.637	0.017	-36.48	$p<10^{-5}$
		Specialist First	0.822	0.021	39.246	$p<10^{-5}$
		PNI*Generalist First	-0.153	0.184	-0.829	0.47
		PNI*Specialist First	-0.088	0.218	-0.404	0.686
R2	Clemens	Intercept	0.519	0.001	347.052	$p<10^{-5}$
		PNI	-5.333	0.016	-335.789	$p<10^{-5}$
		Generalist First	-1.359	0.002	-619.182	$p<10^{-5}$
		Specialist First	1.309	0.002	531.221	$p<10^{-5}$
		PNI*Generalist First	2.564	0.024	107.368	$p<10^{-5}$
		PNI*Specialist First	-4.988	0.025	-202.52	$p<10^{-5}$
R3	Arroyo	Intercept	0.665	0.003	248.79	$p<10^{-5}$
		PNI	-5.099	0.028	-181.276	$p<10^{-5}$
		Generalist First	-1.31	0.004	-343.465	$p<10^{-5}$
		Specialist First	1.122	0.004	257.603	$p<10^{-5}$
		PNI*Generalist First	2.876	0.041	70.217	$p<10^{-5}$
		PNI*Specialist First	-3.436	0.044	-78.825	$p<10^{-5}$

PNI = proportion of negative interactions.

Table 2: Results of network robustness (*R*) GLMs for each extinction order separately.

Model Number	Order		Estimate	Std. Error	Z value	P value
R4	Generalist First	Intercept	0.362	0.012	30.86	$p<10^{-5}$
		PNI	-1.913	0.125	-15.329	$p<10^{-5}$
		Arroyo	-1.007	0.012	-83.6	$p<10^{-5}$
		Clemens	1.202	0.012	-101.543	$p<10^{-5}$
		PNI*Arroyo	-0.311	0.128	2.421	*0.015*
		PNI*Clements	-0.856	0.126	-6.794	$p<10^{-5}$
R5	Random	Intercept	0.999	0.013	77.211	$p<10^{-5}$
		PNI	-1.76	0.136	-12.958	$p<10^{-5}$
		Arroyo	0.334	0.013	-25.268	$p<10^{-5}$
		Clemens	-0.481	0.013	36.903	$p<10^{-5}$
		PNI*Arroyo	-3.339	0.139	-24.079	$p<10^{-5}$
		PNI*Clements	3.573	0.137	-26.133	$p<10^{-5}$
R6	Specialist First	Intercept	1.822	0.016	110.54	$p<10^{-5}$
		PNI	-1.848	0.17	-10.839	$p<10^{-5}$
		Arroyo	-0.034	0.017	-2.021	*0.043*
		Clemens	0.005	0.017	0.325	*0.745*
		PNI*Arroyo	-6.687	0.174	-38497	$p<10^{-5}$
		PNI*Clements	-8.472	0.172	-49.398	$p<10^{-5}$

PNI = proportion of negative interactions.

Table 3. GLM results for total number of cascades vs. PNI for each network separately.

Model number	Network	Variable	Estimate	Std. Error	Z value	P value
C1	Dupont	Intercept	0.461	0.136	3.398	0.001
		PNI	6.557	1.154	5.681	0
		Generalist First	0.015	0.189	0.077	0.939
		Specialist First	-0.336	0.207	-1.625	0.104
		PNI * Generalist First	0.853	1.595	0.535	0.593
		PNI * Specialist First	0.89	1.745	0.51	0.61
C2	Clemens	Intercept	1.671	0.095	17.61	0
		PNI	-0.351	0.883	-0.397	0.691
		Generalist First	0.447	0.122	3.659	0
		Specialist First	-1.098	0.179	-6.121	0
		PNI * Generalist First	-0.486	1.141	-0.426	0.67
		PNI * Specialist First	2.14	1.633	1.31	0.19
C3	Arroyo	Intercept	0.827	0.123	6.706	0
		PNI	4.136	1.079	3.831	0
		Generalist First	0.488	0.16	3.06	0.002
		Specialist First	-0.412	0.209	-1.969	0.049
		PNI * Generalist First	-1.311	1.406	-0.932	0.351
		PNI * Specialist First	-3.068	1.882	-1.631	0.103

Table 4. GLM results for total number of cascades vs. PNI for each order separately.

Model number	Order	Variable	Estimate	Std. Error	Z value	P value
C4	Random	Intercept	0.461	0.136	3.398	0.001
		PNI	6.557	1.154	5.681	0
		Clemens	1.209	0.166	7.3	0
		Arroyo	0.365	0.183	1.992	0.046
		PNI * Clement	-6.908	1.453	-4.754	0
		PNI * Arroyo	-2.421	1.58	-1.532	0.125
C5	Generalist	Intercept	0.476	0.131	3.64	0
		PNI	7.41	1.101	6.733	0
		Clemens	1.642	0.152	10.815	0
		Arroyo	0.839	0.165	5.074	0
		PNI * Clement	-8.246	1.317	-6.262	0
		PNI * Arroyo	-4.586	1.423	-3.224	0.001
C6	Specialist	Intercept	0.126	0.156	0.809	0.418
		PNI	7.447	1.309	5.69	0
		Clemens	0.447	0.218	2.054	0.04
		Arroyo	0.289	0.23	1.259	0.208
		PNI * Clement	-5.658	1.898	-2.981	0.003
		PNI * Arroyo	-6.379	2.022	-3.155	0.002

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Discussion:

Pollination is an invaluable ecosystem service and ecological function that is required for the reproduction of many wild plant species as well as many economically important agricultural crops. It is estimated that over 87% of the world's wild plant species are visited by pollinators (Ollerton et al. 2011). Insect mediated pollination is also important for agricultural production around the world. Globally, approximately 80 economically important crop species utilize pollination services, though the degree to which they depend on pollination varies (Klein et al. 2007).

Growers in the US rely primarily on the pollination services of managed hives of *Apis mellifera,* the honey bee, which were brought from Europe to the US in the late 1700s (NRC 2007). The non-native European honey bee is historically the most important managed crop pollinator worldwide (Losey and Vaughn 2006). In recent years honeybee colonies have succumbed to disease leading to decline in the number of hives available for pollination services and subsequent fluctuation in the price of rental services (Neumann and Carreck 2010). The amount that farmers pay for pollination services varies by crop and location, more labor intensive crops at peak season cost more for services (Champetier et al. 2015). Almond crops require intensive pollination services and the price that North American almond growers pay for hive rental has increased from $35 per hive in the early 1990s to around $150 per hive in 2016 (a 2.3-fold increase in adjusted dollars) (*beesource* n.d.). Access to honey bee pollination is increasingly unreliable, making our dependence on a single

pollinator species a profound problem, as growers ultimately suffer a shortage of pollination services (Buchmann & Nabhan 1997, NRC 2007, Winfree et al. 2009).

Of the ~8,000 native bee species in the US, growers actively utilize only 11 of these as managed crop pollinators (Michener 2000, Ricketts et al. 2008). Recent data suggest that native bees can "buffer" or even replace lost pollination services in agricultural production due to honey bee losses (Williams and Adam 2001, Kremen et al. 2007, Otterstatter and Thomson 2008a, Ricketts et al. 2008). Maintenance of native bee diversity is essential because bee communities fluctuate seasonally and reliance on one species could lead to inefficient crop pollination (Kremen 2005, Greenleaf and Kremen 2006, Winter et al. 2006, Koch et al. 2015). Recent work has shown that crop yields are greater and more consistent when pollinator richness is high compared to honey bee visitation as native bees are often more efficient pollinators (Javorek et al. 2002, Klein et al. 2007, Winfree et al. 2007a, Ne'eman et al. 2010, Garibaldi et al. 2013). Furthermore, as shown in Chapter 1, reductions in pollinator richness can decrease pollinators' floral fidelity, which in turn can reduce plant reproduction. As such, native pollinator diversity can benefit both agricultural and wild-plant reproductive output.

The use of native bees for crop pollination requirements seems encouraging, but growing evidence suggests that native bee populations are experiencing population declines and contractions (America and National Research Council 2007, Potts et al. 2010, Ollerton et al. 2014). Bee and hoverfly abundance and diversity are declining in the Netherlands (Biesmeijer et al. 2006, Keil et al. 2011), as have bumble bees and

butterflies in the United States and Europe over the last twenty years (Steffan-Dewenter and Tscharntke 2000, Goulson et al. 2008). Unfortunately, monitoring pollinators and the plants that depend on them has been patchy and inconsistent, making it very difficult to assess the status of pollinator populations and even more difficult to predict how pollinators will respond to ongoing anthropogenic environmental change. The disruption of pollination function has the potential to result in severe and far-reaching impacts on biodiversity, the persistence of ecosystem function, and the ecosystem services upon which human communities depend.

Over the last fifteen years, researchers have reached a general consensus regarding the major causes of pollinator decline. Habitat loss, degradation and fragmentation, intensive agricultural practices including pesticide application, pathogen and pests as well as global climate change are believed to be the greatest contributors to pollinator declines (America and National Research Council 2007, Potts et al. 2010, Goulson et al. 2015). Additional factors including lack of genetic diversity and large-scale transportation of honey bee hives and the impact of invasive species will not be discussed here. Most importantly there is no single factor that drives the decline of pollinators, both native and domesticated. Rather, a synergistic effect of multiple stressors threatens the health of pollinators worldwide. Below is an overview of the major impediments to pollinator health and suggestions on how to alleviate the threats in an effort to restore pollinator populations in the US.

Habitat Loss, Degradation, and Fragmentation: Current State of Knowledge

Habitat loss, degradation, and fragmentation are among the greatest the best-studied drivers of pollinator declines (Rathcke and Jules 1993, Cane 2001, Fortuna and Bascompte 2006, Winfree et al. 2009, Albrecht et al. 2014). Pollinator habitat has been lost and degraded through urbanization/suburbanization and agricultural expansion (Potts et al. 2010, Williams and Winfree 2013). In many cases these changes in land-use lead to a reduction in nesting sites, sufficient floral resources, or access to clean water (Winfree et al. 2007b, 2009, Williams and Winfree 2013) which could each have negative impacts on pollinator populations.

While pollinator response to land-use change is highly variable and often difficult to characterize, some useful trends have been identified (de Miranda and Genersch 2010, Winfree et al. 2011). Pollinator body size, a trait that is highly correlated with foraging range, seems to shape how bees respond to habitat degradation (Greenleaf et al. 2007, Benjamin et al. 2014). In a study examining the role of landscape context on the distribution of native pollinators, Steffan-Dewenter et al. (2002) found that pollinator species diversity and density decreased linearly with decreasing proportion of natural habitat, making it difficult to establish a threshold at which pollinator populations will be negatively impacted by habitat degradation. Importantly, solitary bees, honey bees and bumble bees all responded to landscape structure in different ways. Smaller solitary bees responded to their landscape at smaller scales (i.e. natural habitat must be available within 750m to support solitary bees) whereas bumble bees and honey bees responded to landscape context at larger

scales (~3000m), likely because these larger social bees can fly longer distances to seek necessary resources.

Another factor that has been shown to influence the degree to which bees respond to habitat degradation is foraging generalization. The majority of bee species are generalists, visiting many flower species throughout their lifetime to collect both pollen and nectar (though some bees are oligolectic pollen specialists, collecting pollen from specific plant genera or families) (Waser et al. 1996, Graystock et al. 2016). Specialist bees are less common, but are more strongly affected by habitat degradation when compared to generalist bees (Goulson et al. 2008, Burkle et al. 2013, Rader et al. 2014).

Finally, nesting requirements also seem to influence how bees respond to habitat degradation. Bees vary greatly in their nesting needs, but some requirements include abandoned rodent nests (as is the case with most bumble bees), stumps and logs (Rincon et al. 1999), as well as plant products such as resins and leaf material (Roubik 2006). Nesting requirements seem to be one of the best predictors of how bees will respond to land-use change. Bee species that build nests above ground are more negatively impacted by isolation from natural habitat as well as by agricultural intensification, while ground nesting bees are sensitive to compacted soils common in urban landscapes as well as tilling and mowing in agricultural landscapes (Williams et al. 2010, Geib et al. 2015).

While urbanization often leads to degraded pollinator habitat, urban environments are capable of providing nesting sites and floral resources for

pollinators. A recent study out of the UK suggests that some urban habitats can harbor pollinator abundance and diversity and similar to that of surrounding pasture and nature reserves (Baldock et al. 2015). Furthermore, once pollinators are drawn to the non-crop resources found in urban landscaping, they can deliver invaluable pollination services to urban gardens (Klee et al. 2007, Otti and Schmid-Hempel 2007, Lowenstein et al. 2015).

Habitat fragmentation is also a major threat to pollinator populations. Reduction in the size of habitat patches as well as increasing isolation generally leads to lowered pollinator richness and abundances as well as changes in species composition (Aizen and Feinsinger 1994, Bommarco et al. 2010, Garibaldi et al. 2011). Still, not all pollinators respond to habitat fragmentation in the same way. Scale (including size, distance to nearest habitat patch, and shape) is exceedingly important in determining how pollinators will be impacted by habitat fragmentation (Steffan-Dewenter et al. 2002, Bommarco et al. 2010) .

Size of habitat fragments is known to be important for species richness and abundance (MacArthur and Wilson 1967). Small habitat fragments often contain fewer pollinator species and in lower abundances and the plants that require pollination are more likely to be pollen limited compared to larger fragments that can support more continuous plant populations (Aguilar et al. 2006). As with habitat degradation, studies have sought to understand if traits can help explain how different bee species will respond to habitat fragmentation (Williams et al. 2010, Fürst et al. 2014). A study done by Bommarco et al. 2010 (2010) revealed that smaller bodied

pollinators were more heavily impacted by habitat fragmentation and that the small patches in their study supported only large bodied generalist species, probably because large bees can fly longer distances in search of resources.

Habitat Loss, Degradation, and Fragmentation: Management and Policy Actions

While it is difficult to make predictions about how land-use change will impact specific groups of pollinators, many parties, including the federal government, non-governmental organizations (NGOs) as well as researchers and citizen scientists, are working to mitigate pollinator declines through pollinator population monitoring coupled with preservation and restoration of pollinator habitat. Habitat restoration and/or preservation is perhaps the most impactful way to protect native pollinators, as safe nesting sites and abundant floral resources allow pollinators to remain healthy and withstand multiple stressors.

Pollinator habitat is lost primarily through urbanization/suburbanization and agricultural expansion/intensification (Potts et al. 2010). Organizations such as the Xerces Society are focused on disseminating useable information to land-users regarding preservation and restoration of pollinator habitat. The Xerces Society leads workshops throughout the country teaching landowners how to plant hedgerows and "conservation cover", or create meadows focused on attracting pollinators. Additionally, they publish free plant guides for most regions in the US, providing examples of which plants provide the best forage for native pollinators and selling seeds of these pollinator-attractive plants to expedite the planting process (Black et al. 2001, Winter et al. 2006). Since 2004 the Xerces Society has trained over 40,000

individuals and helped restore or protect ~ 200,000 acres of pollinator habitat (Xerces Society Annual Report, 2014). The Xerces Society is the largest pollinator conservation NGO, but there is a multitude of smaller groups doing similar work to mitigate the effects of habitat degradation.

The detrimental effects of habitat fragmentation can be ameliorated in similar ways to habitat degradation. While preserving "pristine" tracts of native habitat is becoming less and less likely in most human-dominated landscapes, ensuring the land between native habitat (often agricultural fields) is pollinator friendly will allow for movement between patches. The Xerces Society worked with lawmakers to help draft the 2014 Farm Bill Act, which allowed USDA to enact a farmer incentive pollinator conservation program. Building off the 2008 Farm Bill, the 2014 Farm Bill encourages the creation of pollinator habitat through a variety of programs including the Conservation Stewardship Program, a payment for practices program. This program offers farmers monetary incentives to conserve or restore pollinator habitat around their fields (rangeland, grassland and private forest are among the other land-use types eligible for this program) (Vaughan and Skinner 2008). Agroecosystems that are managed in a sustainable way, incorporating pollinator attractive plants and eliminating harmful on-farm practices, can play an important role in buffering species loss and allowing dispersal between forest fragments (Philpott et al. 2008, Perfecto and Vandermeer 2008, Briggs et al. 2013, M'Gonigle et al. 2015).

To understand the extent to which pollinators are impacted by the various

factors examined in this discussion, we need comprehensive monitoring of pollinator populations. Several organizations (including the Xerces Society) have enacted robust citizen scientist monitoring programs that allow everyday gardeners and hikers to survey the pollinators they see in their backyards and parks, and report those findings. The citizen science program "The Great Sunflower Project", has been gathering abundance and diversity data on pollinators since 2008, and makes these data accessible online. Data produced by citizen observations could help researchers and land-use managers evaluate the success of restoration and preservation projects and determine if habitat fragmentation is impacting pollinator populations. Still, the ability of the citizen science programs to capture full and accurate information about diversity and abundance of pollinators is limited and should be paired with studies done by professional researchers (Kremen et al. 2011).

In conclusion, our understanding of how habitat loss and land-use change impact pollinators is growing and it seems that small fragments that are disconnected from native pollinator habitat will lead to the loss of small solitary bees and dietary specialists (Steffan-Dewenter and Tscharntke 2002, Watanabe 2008, Winfree et al. 2009, Bommarco et al. 2010, Rosenkranz et al. 2010). Efforts should continue to focus on restoring or creating pollinator habitat as well as enhancing the area between native habitats to maintain healthy pollinator populations. Perhaps most importantly, healthy pollinator habitat will allow pollinators to escape many other current pressures including pesticide use and increased exposure to parasites and pathogens (Potts et al. 2010).

Pesticides: Current State of Knowledge

Numerous pesticides are used around the world to combat agricultural pests. The neonicotinoid insecticides, including imidacloprid, clothianidin, thiamethoxam and thiacloprid, are among the most commonly applied insecticides (Elbert et al. 2008, Ruhman et al. 2016). Neonicotinoids have been identified as one of the potential causes of pollinator declines (Lundin et al. 2015) making application highly controversial in the US and Europe. The role of neonicotinoid application in pollinator declines will be the focus of this section. The effect of other commonly used pesticides on pollinator health has been reviewed many times and will not be covered in this summary (Richards 1993, Thompson 2003, Desneux et al. 2007)

Since the early 1990s, use of neonicotinoid pesticides has increased in frequency, with application in 2013 exceeding 2.0 million pounds (Lundin et al. 2015). These pesticides are popular because they are so effective at controlling unwanted pests in agricultural crops, including common cereal and oilseed rape and many other fruit and vegetable crops in the US and Europe (Goulson 2013, Lundin et al. 2015). Neonicotinoids bind to nicotinic acetylcholine receptors in the central nervous system of insects and cause nervous system stimulation at low concentrations and at high concentrations cause receptor blockage, paralysis and death (Goulson 2013, Zeng et al. 2013).Many of the crops that utilize neonicotinoids for pest management are utilized by both native and honey bee pollinators for pollen and nectar, often causing unintended harm to these beneficial insects (Ruhman et al.

2016). Applied as a either a seed or foliar treatment, neonicotinoids are systemic, and can accumulate in the soil, plant tissue and flower tissue including nectar and pollen (Cresswell 2011, Pisa et al. 2015, Ruhman et al. 2016). The half-life of neonicotinoids in soils can be greater than 1,000 days, leading to long-term bioaccumulation after repeated applications (Goulson 2013, Lundin et al. 2015, Pisa et al. 2015)

Most of the studies assessing the impact of neonicotinoids on pollinators have taken place in the laboratory and are focused on honey bees. A meta-analysis suggested that when imidacloprid (a particular neonicotinoid insecticide) was applied to honeybees in the lab at concentrations that would likely be experienced in the field (~2ppb), mortality was low (Cresswell 2011). Still, while direct mortality from exposure may be low, the detrimental effects experienced through sublethal exposure may impact a pollinator's ability to forage, groom and reproduce (Goulson 2015). Yang et al. (2012) applied the pesticide to honeybee larvae and demonstrated that once the larvae were adult bees, they had difficulty learning to forage on new flowers. Williams et al. (2015) demonstrated that field realistic exposure to neonicotinoids led to severely deformed anatomy in honey bee queens, suggesting that sublethal exposure could have impacts on colonies through reduced reproductive success. While have clear evidence that neonicotinoids are detrimental to honey bees, in some cases native bees have been shown to be more negatively impacted, making extrapolation between native and non-native bees difficult (Rundlöf et al. 2015).

While evidence on the impact of neonicotinoids on native bees is still lacking, recent studies suggest that like honey bees, field level exposure can lead to an impaired performance in bumble bees. This decrease in foraging rate can lead to a reduction in seed set and fruit size in the crops they are visiting, (Stanley et al. 2015) as well as a decrease in nest growth (Whitehorn et al. 2012). Rundolf et al. (2015) provides one of the first examples of how neonicotinoids can directly impact native pollinators in the field. The study included eight paired field sites, half sown with seeds coated with neonicotinoid and a common fungicide and the other half sown with seeds coated only in fungicide. They found that those fields sown with neonicotinoids showed lower densities of native bees (both bumble bees and solitary bees, reduced nesting in solitary bees common to the area, and a reduction in bumble bee colony growth and reproduction. Studies that describe the impact of pesticide application on pollinators in the field are essential in helping researchers and policy makers predict the realistic consequences of pesticide application.

Finally, there is growing evidence that the application of pesticides make some bees more susceptible to parasites (Williams and Osborne 2009, Goulson et al. 2015). Fauser-Misslin et al. (2014) show that, when neonicotinoids are applied to a bumble bee colony via chronic food exposure, not only does the colony exhibit reduced worker production and shortened life-span, but the queens are more susceptible to parasite infection, threatening the health of the entire colony. Understanding the extent to which multiple threats (for example habitat loss and pesticide use) interact, exacerbating the impact on pollinator health is an important

next step in pollination research.

Pesticides: Management and Policy Actions

Pesticide use is a common, and some argue necessary, practice for limiting pest outbreaks in intensive agriculture throughout the world. Regulating the use of pesticides that negatively impact pollinators is an essential step in insuring that we maintain the delivery of an invaluable ecosystem service.

Pesticide use in the European Union is highly regulated and requires thorough assessment (including specific study designs) prior to crop application (EFSA 2013). The European Food Safety Authority (EFSA) assessed the impact of neonicotinoids on bee health (both honey bees and wild bees) and determined that three common pesticides in the neonicotinoid family, thiamethoxam, clothianidin and imidacloprid were too harmful to be used by growers. The three pesticides were banned for two years from use on flowering crops such as corn, oilseed rape, and sunflowers (EFSA 2013). The EFSA is currently re-examining the risk of neonicotinoid use and a new report is due out on 2017. The three pesticides remain on a temporary ban (EFSA 2013).

Similar regulations in the United States do not yet exist, but as of 2016 the Environmental Protection Agency was reviewing the risk of all neonicotinoids pesticides simultaneously in an effort to complete a comprehensive determination of the safety of this class of pesticides (Ruhman et al. 2016). The assessment is taking place over 2016-2017 and determinations are expected to be made in 2017.

Given the overwhelming evidence that neonotinoids are detrimental to honey bee health, I argue that the US should follow the EU and temporarily ban pesticide use until more research can be done examining the impact on native bees. Furthermore, tougher regulations should be put in to place to register a pesticide for use on crops that are visited by pollinators. The risk-assessment analyses required for pesticide registration are historically done on honey bees, with the assumption that honey bees would act as a good bioindicator for all pollinators. It is not yet clear whether the impacts on honey bees can be extrapolated to effects on other native bees, but it is likely that the effects are not highly transferable. A recent study suggests that the impact of neonicotinoids is species-specific and the lethal dose can vary dramatically among bee species (Pisa et al. 2015). The impact of neonicotinoids will depend a great deal on what the bee species forages for, when they forage (i.e. during pesticide application) and their body size (Sandrock et al. 2014). As such, more rigorous testing on the impacts of neonicotinoids on native bees should be required before the EPA approves pesticides for application on flowering plants. Additionally, studies need to move beyond lethal dose assessment to gather more information on how extended exposure to pesticides can impact the essential daily functions of pollinators including foraging, learning, grooming, mating and reproduction. These studies could be used to improve the EPA certification program, forcing manufacturers to describe how their chemicals will impact native pollinators, rather than just the honey bee.

In conclusion, application of neonicotinoids is common in the US and subsequently many pollinators (both native and domesticated) are being exposed to potentially harmful pesticides. While there is a consensus that application of neonotinoids can be detrimental to both native bees and honey bees, we lack realistic and sufficiently replicated studies to determine the concentration and the extent to which these pesticides impact pollinator growth and reproduction as well as delivery of pollination services.

Parasites and Pathogens: Current State of Knowledge

While pollinators are naturally susceptible to a large number of parasites and pathogens throughout their life, their susceptibility increases when they are stressed (Freestone et al. 2008). In recent years we have seen a decline in commercial honey bee populations in the US (Neumann and Carreck 2010). While the declines cannot be blamed on any one cause, parasites and pathogens are thought to be a major factor threatening honey bee health.

The parasite, *Varroa destructor*, came to the US in the late 1980's and is often described as the greatest threat to honey bee colony health (Guzman-Novoa et al. 2010). *Varroa* mites attach to honey bees and extract the hemolymph (Rosenkranz et al. 2010), making the bees more susceptible to disease and ultimately shortening their lifespan (Moore et al. 2015). Since the discovery of *Varroa* mites in honey bee colonies, around twenty new bee viruses have also been discovered, usually in association with the *Varroa* mites (Moore et al. 2015).

Some of the most important pathogens of honey bees include deformed wing (DWV) virus, foulbrood, and *Nosema* fungi (Watanabe 2008, Rosenkranz et al. 2010). The deformed wing virus (DWV) occurs in the majority of honeybee colonies but the bees remain largely asymptomatic unless the colony is also experiencing an outbreak of *Varroa* mites in which case the bees exhibit deformed wings (de Miranda and Genersch 2010). DWV symptoms include early death in pupae, deformed wings and shortened abdomen in adult bees (Dietemann et al. 2012). Most bees die within 3 days of contracting DWV, often leading to colony collapse (de Miranda and Genersch 2010). DWV is usually treated through treating Varroa infestations, though as I discuss below, treatment is often ineffective (Dietemann et al. 2012).

Nosema disease in US honeybees is caused by both the *Nosema apis* and *Nosema ceranae* fungus. *Nosema* are intracellular parasites that bees ingest as spores, and then the parasite attacks the middle intestine of workers, queens and drones, eventually killing the affected insects. Nosema disease was loosely linked to Colony Collapse Disorder in 2007, when many of the dead bees tested positive for *Nosema* (Cox-Foster et al. 2007). Since then, many healthy colonies have also tested postivive for *Nosema*, leaving researchers to suspect that the parasite is likely taking advantage of a weakness in the insects' immune systems, caused by something other than *Nosema* (Klee et al. 2007).

Increasing agricultural intensification has put strong demands on domesticated pollinators to provide year-round pollination services(vanEngelsdorp et al. 2008). The increased use of domesticated pollinators has put wild bees in direct contact with the

pathogens and parasites described above (Otterstatter and Thomson 2008a, Ollerton et al. 2011). Pathogen spillover from domesticated bees to wild bees has received a great deal of attention due to the rapid decline of honeybee colonies and the subsequent decline of many bumblebee species. It was been shown in laboratory settings that transmission of pathogens and parasites between domesticated honeybees and wild bumblebees is possible (the opposite is also true, though fewer studies have been conducted on the bumble bee to honey bee transmission) (Graystock et al. 2013, 2014, Fürst et al. 2014). In the field, bumble bees that forage in close proximity to honey bees have been shown to have similar levels of DWV (Graystock et al. 2016). Honey bees can be asymptomatic while carrying (and transferring) DWV, while the bumble bees can experience a reduction in survival via reduced foraging (Graystock et al. 2016).

Domesticated bumble bee colonies are used regularly to pollinate crops such as tomatoes, peppers, blueberries and strawberries (Goulson et al. 2008). There are a number of pathogens that have been shown to be transferred from domesticated to wild bumble bees include tracheal mites and a different *Nosema* parasite, *Nosema bombi* (Meeus et al. 2011). The decline of wild bumble bee species was suggested to be driven by the pathogen *Nosema bombi*, a pathogen also found regularly in managed bumble bee colonies (Winter et al. 2006). It was hypothesized that domesticated *Bombus occidentalis*, which tested positive for high *N. bombi* levels, was transferring the pathogen to wild bumble bees that foraged in the same area. These wild bumble bees exhibited similarly high *N. bombi* levels and were

experiencing dramatic population declines. The link between native bumble bee declines and *N. bombi* transferred from domesticated colonies have since been refuted, as *N. bombi* was shown to be present in the US prior to the dramatic population declines (Cameron et al. 2011, 2016). The pathogens that are transferred from domesticated to wild bees are not always lethal, but as is the case with pesticides, infection can weaken the colonies, making them more susceptible to pathogens and/or making it more difficult to forage efficiently and to reproduce (Otterstatter and Thomson 2008a). *Nosema bombi* has been shown to reduce the fitness of the bumble bee queens, reduce the survival rate of workers, and leave wild colonies weak and compromised (Klee et al. 2007, Otti and Schmid-Hempel 2007).

Parasites and Pathogens: Management and Policy Actions

The protection of wild bees will require interventions ranging from international regulations to improved husbandry skills to limit the mixing of wild and domesticated bees. Evidence suggests that commercial market for pollination services by domesticated bees (both honey bees and bumble bees) brings wild native bees into contact with novel and often-harmful pathogens (Sherman et al. 1998, Klee et al. 2007, Cornman et al. 2009, Fürst et al. 2014). While we are just beginning to understand the extent to which bee species can cross-infect one another, we know that it is possible and that interventions must take place to prevent such spillovers.

Some states such as Oregon and California have banned the importation of non-native pollinator species (with the exception of honey bees) and countries such as

Mexico and Japan have started screening the bumble bees that enter their countries before allowing them to be used as agricultural pollinators (Winter et al. 2006). In order to minimize the transfer of pathogens between native and domesticated bees, interested parties should develop unified international regulations eliminating the movement of exotic parasites to new countries. Furthermore, while screening bees for possible pathogens is costly, and we are limited by the technology and expertise required to perform such screens, screening will play an essential role in making sure that infected bees are not allowed to be introduced in to the US (or moved out of the US) (Graystock et al. 2016). Recent development of molecular screening tools may allow for more accurate screening and may allow more efficient high throughput screening . International funding should support a centered effort to discover more efficient and cost effective methods of identifying pathogens.

Less sophisticated prevention measures are also promising in helping to reduce pathogen spread between domesticated and native bees. In Japan, the simple technique of placing screens around greenhouses and making entrances to the greenhouse impermeable for bumble bees has allowed farmers to eliminate many domesticated escapees, though results are anecdotal and the technique has not been tested rigorously (Winter et al. 2006).

Global Climate Change: Current State of Knowledge

Changing temperatures associated with global climate change (GCC) has the potential to impact many ecological processes and interactions through range shifts

and/or change in phenology. Phenology, the timing of life cycle events, is triggered by environmental cues that allow species respond to optimal conditions (Ibáñez et al. 2010, Rafferty and Ives 2011). Climate change can decouple organisms from the environmental cues that help them predict optimal conditions for their life cycle (Visser and Holleman 2001). While some species may have enough genetic variability to adapt to a new climate regimes, others may not (Burgess et al. 2007, Miller-Rushing and Inouye 2009). Plant phenology can evolve in response to climate change (Burgess et al. 2007), as can insect phenology (Van Asch et al. 2007)but we lack sufficient evidence concerning how animals and plants are responding to changing climate, and whether these two groups are responding synchronously (Miller-Rushing et al. 2007). In some cases plants have exhibited early phenological advancement relative to their animal pollinators, resulting in a phenological mismatch (Doi et al. 2008, Forrest and Thomson 2010, McKinney et al. 2012) while in other cases both plants and their pollinators seem to be changing at the same or similar rate (Bartomeus et al. 2011).

Phenological data indicate that the first flowering date of some plant species has advanced in recent years (Miller-Rushing et al. 2007, Rafferty et al. 2013, CaraDonna et al. 2014, Gezon et al. 2016). Early flowering can be detrimental to plants for both abiotic reasons such as early-season frost damage (Inouye 2008), as well as biotic reasons such as reduction in outcrossing due to lack of pollinators, both of which have been shown to lower plant reproduction in some plant species (Forrest and Thomson 2010, Gezon et al. 2016). The phenology of early-flowering plants

appears to be changing more quickly than late-flowering plants in response to warming temperatures (Menzel et al. 2006, Miller-Rushing and Primack 2008), and both late and early flowering plants are more susceptible to frost at high elevations compared to mid-season flowers (Inouye 2008, Gezon et al. 2016). Miller-Rushing and Inouye (2009), showed that the phenologies of early-flowering species in Gothic, Colorado change more rapidly in response to climate and other abiotic cues than do late-flowering species. In the same high-alpine community, Caradonna and Inouye (2015) found a slight phylogenetic signal in first-flowering date for 60 alpine species, but the strength of the signal was highly variable. Furthermore, no signal was detected for response to temperature and/or snowmelt. There is a great deal of variation in how plant species respond to climate change, and in some cases, plants will remain unaltered, or even have delayed phenology (CaraDonna et al. 2014).

While temperature is an important cue in the phenology of insects (at least in temperate zones), few studies have examined phenology of insects independent of the plants that the insects are visiting (Taylor 1986, Forrest and Thomson 2011). Still, there is emerging evidence that pollinators respond to temperature in similar ways to the plants that they pollinate (Forrest and Thomson 2011, Pyke et al. 2016). The potential for mismatches has less obvious consequences for pollinators, as most pollinators are lifetime generalists meaning they may switch to new plant species if they become mismatched with their old mutualist partners (Waser et al. 1996, Burkle et al. 2013). Still, migratory pollinators such as hummingbirds will be highly susceptible to GCC if their migrations become asynchronous with flowering plants

that provide nectar throughout their journey (McKinney et al. 2012). Furthermore, pollinators that are the first to emerge each season, such as bumble bee queens could experience severe consequences if they emerge without floral resources or if the floral resources have all received frost damage (Inouye 2008, Gezon et al. 2016). Furthermore, pollinators that exhibit a hesitation to switch floral preferences, such as the long tongue bumble bees described in my chapter 3, may be vulnerable to shifts in plant phenology if they are left without their preferred plant.

Empirical evidence on how GCC impacts phenological mismatching of pollinators and the plants they visit is growing. Future studies that move beyond species-specific interactions, allowing us to make generalizations about specific characteristics that make plant-pollinator interactions more vulnerable to GCC will be especially valuable.

Global Climate Change: Management and Policy Actions

It is beyond the scope of this discussion to deliver management and policy suggestions of how to mitigate the impacts of global climate change on pollinators and plants. Still, climate trends from the last forty years are expected to continue into the foreseeable future, and phenological shifts are likely to continue as well (CaraDonna et al. 2014). Given that pollinators and plants are experiencing a number of simultaneous stressors, shifting phenologies and potential mismatches could deliver the final blow for already vulnerable species. Extra focus should be put on the identifying the most threatened species and ensuring protection of habitats and

resources for these species so that more readily cope with changing climate. Reserves and/or protected areas that are designed specifically for high elevation expansion would help protect the high alpine plant-pollinator interactions that are particularly susceptible to climate change (Hülber et al. 2016).

Conclusion:

Mutualistic plant-pollinator interactions are important drivers of community structure and stability (Okuyama and Holland 2008, Mitchell et al. 2009)and the anthropogenic disturbances outlined in this discussion leave researchers questioning the fate of these important interactions. There is a general consensus that greater species richness is beneficial to communities as it leads to complementarity and robustness against perturbations (whether they be of anthropogenic origin or otherwise) (Hooper et al. 2005, Cardinale et al. 2012). Native pollinator diversity is no exception, and has been shown to be beneficial to plant reproduction in both agricultural and natural field observational studies (Kremen et al. 2002, Veddeler et al. 2006, Hoehn et al. 2008, Mallinger and Gratton 2015). This positive relationship between richness and plant function holds true in studies that directly manipulate pollinator species richness (Albrecht et al. 2012, Brosi and Briggs 2013, Fründ et al. 2013).

This discussion has outlined some of the most pressing threats to pollinator health, but the threats are numerous and far reaching (Potts et al. 2010). With global pollinator populations on the decline, it is increasingly important to understand the functional implications of losing pollinator richness for both agricultural crops as well

as natural plant communities. There is already evidence that plant-pollinator communities are changing, be it through species losses caused by acute stressors or range shifts and novel species interactions caused by global climate change (Harte and Shaw 1995, Burkle et al. 2013, Miller-Struttmann et al. 2015). Species differ in their functional importance in a given community and they also differ in their vulnerability (Fontaine et al. 2006, Burkle et al. 2013). Understanding species' roles in a community context will help us both predict the impact of species losses as well as the outcome of novel species interactions. Furthermore, understanding the role of species in different community contexts will help us make specific management decisions based on functionally important and specifically vulnerable species. Here I discuss how my dissertation work improves on our understanding of the implications of pollinator loss.

One way to explore the impact of species loss on community function is to manually (and temporarily) remove species from communities and assess the impact. My dissertation work utilized pollinator removal experiments to gain insight in to how plant-pollinator interactions are impacted by pollinator species loss. In this work I showed that the removal of the most abundant pollinator species can lead to a reduction in pollinator fidelity, which in turn can translate to the transfer of heterospecific pollen, and ultimately a decrease in plant reproduction (Brosi and Briggs 2013). While the removals in my study were targeted on the most abundant species, and were only temporary, this work suggests that plant-pollinator communities might be more vulnerable to pollinator losses than previous studies

suggested (Dunne et al. 2002, Memmott et al. 2004). Future work should focus on comparing other realistic species losses and their effects on different ecosystem functions. In a recent study, the experimental removal of the most abundant seed-dispersing ant had little impact on seed-dispersal function, revealing that some mutualistic interactions might be more resilient to species loss than others (Timóteo et al. 2016). Once we gather a collection of studies that explore the role of species losses, we can begin to make generalizations about what makes a system more or less resilient to species loss as well as identify properties about the removed species that might lead to greater or lesser functional impacts when removed.

Understanding how traits drive community and ecosystem functional relationships will help us to predict the impacts of biodiversity loss. Furthermore, identifying traits that make species more or less vulnerable to extinction will help establish targeted conservation. As mentioned above, traits such as pollinator body size, dietary generalization, as well as nesting requirements have been useful in predicting how pollinators will respond to habitat loss and fragmentation. Large bodied bees, in general, are less vulnerable to habitat destruction, presumably because they can fly longer distances to seek resources (Steffan-Dewenter and Tscharntke 2002). This finding has encouraged land-managers to establish conservation corridors of suitable pollinator habitat to help mitigate loss of small-bodied bees. Ground nesting bees have been shown to be especially vulnerable to farm management practices such as tilling and mowing (Winfree et al. 2009). This information can help encourage farmers to mitigate their management practices in an effort to protect

ground-nesting bees, and encourage pollination on their farm. In chapter 3, I found that long tongued bumble bees were more faithful to plant species, meaning that they were less likely than short tongue bees to explore multiple plant species in a single foraging bout. While this is good news for the plant species that rely on them for pollination, it might mean that the long tongued bees are less flexible, and therefore might be more vulnerable to changes in plant community driven by climate change (CaraDonna et al. 2014). Ensuring that communities retain plant species that are attractive to long tongue bumble bees may mediate the negative effects of climate change on these important pollinators.

Network studies provide a useful tool for exploring complex biotic interactions. Network simulation models can be useful in helping us explore the implications of pollinator losses plant communities. A series of network model simulations have predicted that plant populations will be resilient when faced with pollinator extinctions (Memmott et al. 2004, Kaiser-Bunbury et al. 2010). Importantly, these models are built on the assumption that all interactions in network are positive, potentially overestimating the resilience of plant-pollinator networks. A wide range of studies (including my field work described in chapters 1-3) have shown that many flower visitors are actually ineffective at pollinating plant species they visit, and therefore can have *negative* consequences for plant reproduction. In chapter 4, I explored whether the addition of negative interactions impacted network robustness. My extinction simulations revealed that while the addition of negative interactions generally decreased network robustness when faced with extinctions, the three

networks behaved idiosyncratically with respect to how negative interactions and extinction order impacted robustness. In the smallest network, extinction order (generalist-first, specialist-first or random) did not impact the magnitude of the effect of negative interactions on network robustness *yet* the effect of removing generalist-first from the two larger networks seemed to supersede the impact of negative interactions. This reveals that in some networks, the impact of losing generalists can be so detrimental so as to negate the potential negative impact of the inclusion of negative interactions.

Identifying metrics that make certain plant-pollinator networks more vulnerable to extinctions could be useful to conservation practitioners when determining priority habitats or communities. Still, a number of network studies have explored how network structure influences network robustness when faced with perturbations including species extinctions (Bascompte and Jordano 2007) and to my knowledge very few, if any, of these studies have been used in conservation planning. Kaiser-Bunbury and Bluthgen (2015) recently proposed a new framework in which network ecology could be applied to conservation practices. The authors suggest seven quantitative metrics that describe network properties—hierarchically arranged by network, species and guild levels— that can be used to describe changes in networks and help to determine conservation priorities. Metrics include the following; network level metrics: interaction diversity and interaction evenness, species level metric: partner diversity, guild level metric: vulnerability/generality, and distribution metrics that are useful in characterizing the distribution of interactions in a network,

namely, the uniqueness of the interactions. These metrics include specialization indices d' and H_2', and modularity (see Kaiser-Bunbury and Blüthgen 2015) for detailed description of each metric). The authors emphasize that both network ecologists and conservation practitioners should work together to define conservation goals, design experiments, and monitor the outcomes. While still untested, this framework offers an innovative and collaborative approach to addressing the complex problem of understanding first, how plant-pollinator communities will be impacted by pollinator losses and then, formulating a plan for combating those impacts.

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