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UNIVERSITY OF CALIFORNIA,
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Mechanisms for pollinator-mediated interactions between native and invasive plants

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Daniela Bruckman

Dissertation Committee:
Professor Diane R. Campbell, Chair
Professor Stephen G. Weller
Associate Professor Kailen A. Mooney

2015

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CURRICULUM VITAE

Daniela Bruckman

EDUCATION:

- 2015 Ph.D. Ecology and Evolutionary Biology, University of California, Irvine
2006 B.S. Biology, University of California, Santa Cruz

APPOINTMENTS:

- 2009-2015 Doctoral research, pollination ecology; University of California, Irvine
2015 Adjunct biology professor, Santa Ana College (Santa Ana, CA)

SELECTED ACADEMIC HONORS:

- 2009 AGEP Competitive Edge Summer Scholarship Program (NSF sponsored)
2011 NSF GK-12 Fellowship
2012 Center for Environmental Biology Research Fellowship, University of California-Irvine
2012 Lake Forest Garden Club Scholarship (Lake Forest, CA)
2013 GAANN (Graduate Assistance in Areas of National Need) Fellowship, University of California-Irvine.

PUBLICATIONS:

- Bruckman, D** and Campbell, DR. 2014. Floral neighborhood influences pollinator assemblages and effective pollination in a native plant. *Oecologia*. 176(2): 465-476
Bruckman, D and Campbell, DR. Timing of invasive pollen deposition influences pollen tube growth and seed set in a native plant. *in review*

PRESENTATIONS:

- 2009: **AGEP (Alliance for Graduate Education and the Professoriate) Symposium, UC Irvine:** *Does Brassica nigra have detrimental effects on the reproductive success of native plant species?;* talk
2012: **American Academy of Arts and Sciences annual meeting, Vancouver, BC:** *The effects of floral neighborhood on the reproductive success of Phacelia parryi and implications for invasive weed control;* poster
2013: **Ecological Society of America annual meeting, Minneapolis, MN:** *The effects of floral neighborhood and an invasive plant on the pollination of Phacelia parryi ;* poster
2013: **California Invasive Plant Council annual symposium, Lake Arrowhead, CA:** *The effects of invasive pollen on the seed set of a native plant;* poster

ABSTRACT OF THE DISSERTATION

Pollinator-Mediated Interactions Among Native and Invasive Plants

By

Daniela Bruckman

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2015

Dr. Diane R. Campbell, Chair

Pollinators represent a means by which different plant species may interact and influence one another's reproductive fitness. The pollinator-mediated plant interactions that occur between native and exotic plants are of particular interest due to the increasing frequency of plant invasions worldwide. While effects of invasive plant species on native plant pollination have been documented, the mechanisms that drive such interactions are poorly understood. This dissertation focuses on the mechanisms of pollinator-mediated interactions between native and invasive plants through the study of *(i)* the effects of floral neighborhood on the pollination of a native plant (Chapter 1), *(ii)* the influence of invasive pollen deposited on native stigmas (Chapter 2), and *(iii)* the effects of an exotic plant on native reproductive fitness over the course of an invasion (Chapter 3).

I explored how the heterospecific floral neighborhood affects pollinator visitation and composition of pollinator assemblages for native plant, *Phacelia parryi*. Through observations of pollination in natural patches of *P. parryi*, I found that floral neighborhood changed pollinator assemblage composition and that native bees were superior pollinators compared to nonnative honeybees (Chapter 1). Next, a series of hand pollination experiments was used to examine how

pollen from the invasive *Brassica nigra* influences pre- and post-fertilization stages in *Phacelia parryi*. Mixed pollen applications resulted in deleterious effects on both seed set and pollen tube growth when compared to pure conspecific pollen deposition (Chapter 2). Finally, I tested the effects of invasive *B. nigra* abundance on the reproductive fitness of *Phacelia parryi* by simulating four stages in invasion and measuring pollinator visitation, pollen deposition and seed set. Native individuals near the invasion and within areas of low invasive density showed the highest reproductive fitness resulting from facilitation of pollinator visits, while natives within areas of high invasive density showed high levels of invasive pollen deposition. Isolation from the invasive reduced native fitness as a result of low pollinator visitation and conspecific pollen receipt (Chapter 3). Collectively, these results underscore the importance of determining the mechanisms for pollinator-mediated interactions between native and exotic plants and present valuable information for the mitigation of invasive plant species.

INTRODUCTION

Plants and pollinators embody a classic mutualism in which plants provide nutritional resources for the animals that visit them and, in return, plant reproduction is facilitated through the dispersal of pollen to conspecifics. With nearly 90% of angiosperms being pollinated, at least in part, by animals (Ollerton et al. 2011), the study of this intimate relationship is vital for our understanding of plant reproduction. Because of the sessile nature of plants, pollinators also represent a means by which different plant species can interact and influence each other's reproductive fitness. Such pollinator-mediated interactions between different plant species may be facilitative (Feldman et al. 2004, Moeller 2004, Ghazoul 2006) or competitive (Waser 1978a, Campbell and Motten 1985, Mitchell et al. 2009) and function through a number of direct and indirect mechanisms.

One plant species may alter the reproductive fitness of another species by modifying the foraging behavior of pollinators. For example, an attractive plant may monopolize the attention of pollinators and attract visits away from cohabiting species (eg. Waser 1978a). Conversely, heterospecific plants could act as facilitators that increase the attractiveness of a foraging patch (Feldman et al. 2004, Molina-Montenegro et al. 2008). Furthermore, when pollinators move between flowers of different species, the quality of floral visits may become compromised through increasing rates of interspecific pollen transfer (Waser 1978b, Morales and Travaset 2008). Finally, heterospecific plants could change the mix of insects that visit other species in an area (Larson et al. 2006), leading to a change in the effectiveness of the local pollen vectors.

Non-native plant species may have a more pronounced effect on the pollination of neighboring plants than sympatric natives since they often occur at exceptionally high densities

and may produce large, showy floral displays (eg. Chittka and Schurkens 2001; Brown et al. 2002). Dense stands of flowering exotic plants have the potential to usurp pollinator attention away from natives (Bjerknes et al. 2007) and may inundate native communities with alien pollen (Lopezaraiza-Mikel et al. 2007). Facilitation of natives by invasives may also be possible (Chittka and Schurkens 2001, Moragues and Traveset 2005, Bartomeus et al. 2008, McKinney and Goodell 2010, Sun et al. 2013) since the presence of a showy competitor can increase the total resource availability in a foraging patch (Ghazoul 2006). Furthermore, invasive plant species have the potential to alter the pollination of sympatric natives by attracting disproportionately high numbers of generalist pollinators to a foraging patch (Memmott & Waser 2002, Rejmanek et al. 2005).

Though several studies have shown that invasive species can affect native reproductive success (eg. Chittka and Schürkens 2001, Brown et al. 2002, Moragues and Traveset 2005, reviewed by Bjerknes et al. 2007, Munoz and Cavieres 2008, Aizen et al. 2008, Flanagan et al. 2010, McKinney and Goodell 2010), there is still a need for comprehensive assessments of the mechanisms involved. Moreover, little is known about how the effects of exotic plants on native pollination may change as density increases over the course of an invasion. Recent studies have demonstrated that competitive effects among sympatric species vary with relative plant abundance (Caruso 2002, Ghazoul 2006, Takakura et al. 2009), however, few have looked specifically at the role of invasive plant abundance (Munoz and Cavieres 2008, Flanagan et al. 2010, Dietzsch et al 2011, King and Sargent 2012).

Southern California's coastal ecosystems are heavily invaded by exotic annual plants. One introduced plant species, *Brassica nigra* (black mustard, Brassicaceae), is especially ubiquitous in the coastal ranges of Orange County, California, forming dense stands of yellow

flowers. *Brassica nigra* contains glucosinolate chemical defenses (Traw, 2002) that can deter herbivores and reduce mycorrhizal abundance in the soil, and produces small seeds, which allow it to evade granivory. It reduces native plant establishment through allelopathic interactions (Bell & Muller 1973) and through effects on the activity of small mammal consumers of native plants (Orrock et al. 2008). *B. nigra* is a winter annual forb that is visited by several species of insect pollinators (Conner and Neumeier 1995) and flowers primarily between the months of February and May in southern California, overlapping substantially with the phenology of several native plant species. One such species is *Phacelia parryi* (Boraginaceae), an annual herb found in coastal sage scrub and chaparral ecosystems throughout southern and Baja California.

For my dissertation research, I use *Phacelia parryi* to investigate the mechanisms through which pollinator-mediated plant interactions function. I incorporate *Brassica nigra* as a model invasive plant species to examine the effects of alien species on the pollination of native plants. The goal of this work was to address the following questions:

- (1) What are the rates of self-compatibility and autogamy in *Phacelia parryi*?
- (2) How does floral neighborhood affect pollinator visitation to *Phacelia parryi* and the composition of its visitor assemblage?
- (3) How do different groups of insect visitors compare to each other in terms of pollinator effectiveness and overall pollinator importance for *Phacelia parryi*?
- (4) To what extent do *Phacelia parryi* and *Brassica nigra* share pollinators?
- (5) Does the deposition of invasive *Brassica nigra* pollen on the stigma influence seed set and pollen tube growth in *Phacelia parryi*?

(6) Does the stage of a plant invasion have an effect on the reproductive success of *Phacelia parryi*?

In Chapter 1 I determine the rates of self-compatibility and autogamy in native plant, *Phacelia parryi* (question 1). I also explore how the heterospecific floral neighborhood affects pollinator visitation and composition of pollinator assemblages for *P. parryi* (question 2). Finally, I assess the relative effectiveness of different insect visitors to interpret the potential effects on plant fitness of shifts in pollinator assemblage composition (question 3). In Chapter 2 I investigate how pollen from the invasive plant *Brassica nigra* influences pre- and post-fertilization stages in the native plant *P. parryi*, through a series of hand pollination experiments (question 5). An observational field study is also included to determine the extent to which *P. parryi* and *B. nigra* share pollinators (question 4). In Chapter 3 I use a field experiment to simulate four stages in invasion and test whether the presence and density of *B. nigra* influences pollinator visitation, pollen deposition (conspecific and invasive) and seed set in *P. parryi* (question 6).

Collectively, this research demonstrates that the effects of *B. nigra* on *P. parryi* reproductive fitness are contingent upon the mechanism of pollinator-mediated plant interaction. *Brassica nigra* reduces *P. parryi* fitness through heterospecific pollen transfer and potentially by modifying local flower communities, which may shift pollinator assemblages towards a higher percentage of less effective pollinators. However, for otherwise isolated plants of *P. parryi*, *B. nigra* may also increase pollinator visitation when nearby or present at low densities, resulting in potential facilitation.

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CHAPTER 1: Floral neighborhood influences pollinator assemblages and effective pollination in a native plant

INTRODUCTION

Plant fitness often depends on interactions with other types of vegetation in the surrounding environment, and many of these interactions occur via limiting resources or relationships with organisms at higher trophic levels (Strauss & Irwin 2004). Pollinators represent an important intermediary by which different plant species can influence each others' reproductive fitness. Plant neighbors may modify pollinator behavior either by attracting visits away from a focal species (eg. Waser 1978, Campbell & Motten 1985) or by acting as facilitators that increase the attractiveness of a foraging patch (Feldman et al. 2004, Molina-Montenegro et al. 2008). Heterospecifics may also interfere with successful pollination through interspecific pollen transfer (reviewed in: Morales and Traveset 2008, Ashman and Arceo-Gomez 2013), resulting in heterospecific pollen deposition on stigmas and conspecific pollen loss (Campbell and Motten 1985, Feinsinger and Tiebout 1991, Murcia and Feinsinger 1996, Bell et al. 2005). Furthermore, floral neighbors can modify the diversity and composition of visitors that plants receive (Moeller 2005, Lázaro et al. 2009) leading to potential changes in the effectiveness of local pollinator assemblages.

Floral neighborhoods often consist of a mixture of native and non-native species. Non-native neighbors, specifically, have been shown to compete for pollinator services with native plants (eg. Chittka and Schürkens 2001, Brown et al. 2002, Moragues and Traveset 2005, reviewed by Bjerknes et al. 2007, Munoz and Cavieres 2008, Aizen et al. 2008, Flanagan et al. 2010, McKinney and Goodell 2011) and may have a more pronounced effect than sympatric

natives on the mix of pollinators that visit a target species especially when present in high abundance (Larson et al. 2006). The lack of coevolved, specialized mutualisms of invasive plant species means that generalist exotic plants have the potential to alter the pollination of sympatric natives by attracting high proportions of generalist pollinators to a foraging patch (Memmott & Waser, 2002, Rejmanek et al. 2005). Moreover, there is evidence to suggest that alien plant species are more often associated with alien floral visitors than with native visitors (Morales and Aizen 2006). Native neighbors can also impact pollinator assemblages, as floral neighborhoods high in native species diversity sometimes attract a more diverse collection of pollinators than neighborhoods with low floral diversity (Potts et al. 2003, Hegland & Boeke 2006).

Successful pollination of native plants could be impacted not only by non-native plants but also by invasion of non-native pollinators. Although the effectiveness of native and non-native pollinators have not been systematically compared, many plant species are visited by a wide variety of pollinator taxa (Waser et al. 1996, Bascompte et al. 2003), and those taxa can vary greatly in ability to pollinate a particular plant species (reviewed by Ne'eman et al. 2010). Given the disparity in effectiveness among floral visitors, shifts in pollinator assemblages have the potential to reduce plant reproductive fitness if more effective pollinator taxa are lost or replaced by visitors of inferior quality. Recent declines in pollinator populations have been reported worldwide (Kearns et al. 1998, Kremen and Ricketts, 2000) and there is much interest in how pollinator diversity and potential pollinator extinction may affect the reproduction and persistence of native and rare plants (Bond 1994, Biesmeijer et al. 2006). Introduced bees such as the European honeybee (*Apis mellifera*) in its non-native range are highly polylectic (Goulson 2003) and although known to be very effective at pollinating a variety of plant species (Huryn 1997), are sometimes poor pollen vectors for wild (Paton 1993, Aizen & Feinsinger 1994,

Roubik 1996, Gross & Mackay 1998) and agricultural (Willmer et al. 1994, Richards 1996, Canto-Aguilar & Parra-Tabla 2000, Thomson and Goodell 2001, Garibaldi et al. 2013) plants as compared to native pollinators.

Although several studies have examined how floral neighborhood influences the composition of pollinator assemblages visiting a target species (eg. Hegland and Boeke 2006, Larson et al. 2006, Lazaro et al. 2009, Flanagan et al. 2010), these studies have not taken the next step to see whether such a change in visitor assemblage leads to differences in pollinator effectiveness. The goal of this study was to determine how the heterospecific floral neighborhood affects overall pollinator visitation, the composition of pollinator assemblages, and the per-visit effectiveness of those visitors for a plant native to Southern California coastal sage scrub, *Phacelia parryi*. As the non-native *Brassica nigra* is an especially common invader in this habitat, we specifically tested for effects of its abundance in the floral neighborhood. By examining how different types of insect visitors compared to each other in terms of pollinator effectiveness, we could interpret the potential consequences of modifications in pollinator assemblages. We used two measures of effectiveness: transfer of conspecific pollen in a single visit and seed set from a single visit. To help interpret the effectiveness of each visitor, we also determined the extent to which *P. parryi* is self-compatible.

Using both observational and manipulative methods, we examined the following questions: 1. What are the rates of self-compatibility and autogamy in the native plant, *Phacelia parryi*? 2. How does floral neighborhood affect overall visitation rate to *P. parryi* and the composition of its visitor assemblage? 3. How do these different groups of insect visitors compare to each other in terms of pollinator effectiveness and overall pollinator importance?

MATERIALS AND METHODS

Study Species

Phacelia parryi Torr. (Boraginaceae) is an annual herb found in coastal sage scrub and chaparral ecosystems throughout southern and Baja California and is especially common on open, recently burned slopes. In our field sites in Orange County, California, *P. parryi* flowers from late February to early May. The inflorescence is a helicoid cyme of bell-shaped flowers each 1 to 2 centimeters long. It has showy violet to royal blue flowers that last 3-4 days. Daily floral display size depends on plant size and can vary from just a few individual flowers to several dozen. There is no previous information in the literature regarding the self-compatibility or rate of autogamous selfing in *P. parryi*.

Black mustard (*Brassica nigra* (L.) W. D. J. Koch) was used in this study due to its high abundance in the floral neighborhood surrounding *P. parryi*. It is a ubiquitous annual weed of Mediterranean origin that occurs in disturbed areas throughout North America (Westman et al. 1999). Black mustard plants have numerous terminal inflorescences of yellow, hermaphroditic, self-incompatible flowers that attract a variety of insect pollinators (Conner & Neumeier 1995). In *B. nigra*'s invasive range it often grows in large, dense stands and secretes allelochemicals that inhibit germination of neighboring plant species (Bell & Muller 1973). *B. nigra* is a winter annual forb and flowers primarily between the months of February and May in southern California, overlapping substantially with the phenology of *P. parryi*.

Test of self-compatibility in *P. parryi*

To determine if *P. parryi* is self-compatible, we conducted a hand-pollination experiment in a pollinator-free greenhouse at the University of California, Irvine. The four levels of the hand pollination treatment were outcross pollen-emasculated, self pollen-emasculated, self

pollen-intact, and unmanipulated. The flowers in the outcross pollen treatment were emasculated to ensure that no self pollen contacted the stigma. The two self pollen treatments were compared to test for effects of removing anthers. Unmanipulated flowers were neither emasculated nor hand-pollinated.

In January 2012, *P. parryi* seeds were germinated in plug trays and after 4 weeks, seedlings were transplanted into individual 2-gallon pots. Once flowers were produced, hand pollinations were made between 24 March and 26 April 2012. Emasculations were performed on large purple buds 2 days prior to pollination. Because *P. parryi* exhibits protandry, pollinations were made once flowers were in female phase and the stigma was protruding past the anthers. Pollen was transferred from anthers to stigmas using a wooden toothpick. For outcross pollinations, pollen was collected from one flower on each of 3 donor plants to minimize any effect of donor identity on pollination and seed set. Self pollination treatments were performed by using 3 flowers on the same plant as donors. A total of 304 *P. parryi* flowers were pollinated on 25 plants and each individual *P. parryi* plant contained at least 8 experimental flowers - two to five for each treatment level, depending on the number of available flowers. Each treated flower was scored for fruit production and number of seeds per fruit (if present) approximately one month after pollination.

A randomized block ANOVA was performed on the means of seeds produced per fruit in each treatment for each plant, with plant identity used as the block in the model. The identity of the recipient plant was included in the model to control for any variation in pollen deposition or seed set among plants. To compare fruit set among treatments, randomized block ANOVA was performed on the arcsine transformed proportion of fruits set for experimental flowers in each treatment on each plant. For both dependent variables, a priori contrasts were used to compare

outcross pollinations with the average of the two self-pollination treatments to test for self-incompatibility or early acting inbreeding depression. Unmanipulated flowers were compared with self-pollinations to test for autogamous fertilization, and intact and emasculated self-pollinations were compared to test for effects of anther removal.

To determine if selfing versus outcrossing influenced the number of pollen tubes formed, in February 2014, two flowers from each of 14 *P. parryi* plants were emasculated and hand pollinated with either outcross or self pollen. Outcrossed flowers were pollinated with pollen collected from one flower of 3 different donor plants while selfed flowers were pollinated with pollen collected from 3 different flowers on the same individual. Each experimental plant contained one flower of each hand-pollination treatment. Experimental flowers were collected 24 hours after pollination, cleared in 10 N NaOH for two hours, and stained in decolorized aniline blue (Kearns and Inouye 1993). Individual stigmas were mounted onto microscope slides after 2-3 days in the stain and examined under a fluorescent light microscope. We quantified pollen tube growth by counting the total number of fluorescing pollen tubes present at the base of each stigma. Randomized block ANOVA was used to compare the number of pollen tubes present in styles pollinated with outcross-pollen versus self-pollen with plant identity used as the block in the model.

Effects of floral neighborhood on visitation to *P. parryi*

In spring of 2011, patches of naturally occurring *Phacelia parryi* were used to examine the effects of floral neighborhood on pollinator visitation rate and composition of pollinator assemblages. This study was conducted within the OC Parks natural preserve in Orange County, California. We took advantage of a particularly good flowering year for *P. parryi*; in 2011 a fire had recently passed through the area (Santiago fire of October 2007) and winter precipitation

was above average. Drought conditions prevailed in the subsequent years and flowering was too low to permit such a study. Although this study was conducted in an exceptional flowering year, it does not necessarily represent a rare occurrence. Wildfires are relatively common in southern California and are becoming more so as a result of climate change (Westerling et al. 2011). Moreover, high variability in precipitation is also characteristic of this geographical region where an average rainfall of 11 inches (Western Regional Climate Center 2013) is typically punctuated by both drought and El Niño events. A total of 24 observational plots were established by finding all useable patches of *P. parryi* within a 9-km² area (33° 44' 01" N, 117° 42' 20" W). Our field sites are dominated by coastal sage scrub habitat, characterized by low-growing aromatic, drought-deciduous shrubs adapted to the semi-arid Mediterranean climate of the coastal lowlands (Schoenherr 1992).

For each plot, a 1-meter² area centered around a patch of flowering *P. parryi* was used as the pollinator observation area. Open *P. parryi* flowers were counted before each observation period, with the number varying between 11 and 186. Floral neighborhood was quantified by counting the number of open flowers of *B. nigra* and all other heterospecifics found within 0.5 meters of the central observation plot, making a total neighborhood area of 2 m x 2 m. The minimum distance between observation plots was 5m. Flowering heterospecifics other than *B. nigra* found in the floral neighborhoods included both native species (*Encelia californica*, *Lotus scoparius*, *Phacelia cicutaria*, *Lupinus microcarpus*, *Salvia mellifera*) and nonnative species (*Erodium cicutarium*, *Melilotus indicus*, *Vicia villosa*).

Observations of pollination lasted 30 minutes. We defined a pollination event as any contact of an insect with anthers or stigmas on an individual flower. We recorded the insect visitor to the lowest field-identifiable taxonomic category. At the end of the 30 minutes, we

moved on to the next plot, chosen at random, and the process was repeated. Each plot was observed between 1 and 3 times for the duration of the study. Pollinator observations were conducted between 9:00 and 15:00 on 17 days between 17 February and 4 May 2011. We did not conduct observations if it was raining or if temperatures were below 12 °C. We recorded 2981 individual insect visits over 54 half-hour observation periods.

Samples of insect pollinators were collected for identification at each area where visitation was assessed approximately once per week during the field season. Observers were trained to identify common bee and fly families prior to beginning work and pollinators were usually identified to the generic level. *Apis mellifera* was compared to native insects throughout this study due to several characteristics that set it apart from other floral visitors. *A. mellifera*'s nonnative origin means that it is a relatively novel member of the plant-pollinator network in our system and it tends to be a generalist in its interactions with plants. It also displays distinct foraging behaviors due to its eusocial nature and is often numerically abundant in the coastal habitats of southern California.

Visitation rates by honeybees and native insects were calculated for each observation period as the number of visits/flower/hour. Proportion of visits made by honeybees was also calculated for each observation period. Each of these dependent variables was regressed onto the density of *Brassica nigra* and density of other heterospecific species found in the floral neighborhood during each observation period using a multiple linear regression analysis. Date was used as an additional independent variable in our model to examine changes in pollinator visits over time. Separate regression analyses were run using proportion of visits made by honeybees as a dependent variable with the following three independent variables: total heterospecific floral density, native heterospecific floral density and nonnative heterospecific

floral density within the floral neighborhood. Date was also added as an independent variable to each of these analyses. For these analyses, residuals were approximately normally distributed.

Comparison of pollinator effectiveness

In order to quantify the relative pollinator effectiveness of common floral visitors to *P. parryi*, we employed two field methods. The first approach involved examining the number of pollen grains deposited on the stigma per single visit (eg. Primack and Silander 1975, Waser and Price 1990, Suzuki et al. 2002). This technique assesses the quality of flower visitors as pollen vectors, but it does not take into account qualitative differences in the viability or compatibility of the pollen. The second method evaluated pollinator effectiveness by measuring final seed set and has the advantage of describing pollinator performance in terms of plant reproductive success but inevitably includes biases that result from post-pollination processes. Combining pollen deposition and seed set metrics resulting from a single visit to a virgin flower may be a useful way to overcome the drawbacks found in either approach.

In spring of 2012, *Phacelia parryi* individuals growing at the Irvine Ranch Conservancy's native plant farm (33° 44' 32" N, 117° 44' 4" W) were used to examine the relative effectiveness of insect pollinators at transferring conspecific pollen. The 12.5-acre farm contains 43 native plant species. *P. parryi* at the farm grew along one 200-ft. crop row and spacing between plant rows was approximately 1 m. Virgin *P. parryi* flower buds were emasculated to prevent self-pollination, tagged with sewing thread and bagged with fine mesh jewelry bags to exclude pollinator contact with stigmas. After two days when buds had opened and flowers were in the female phase, bags were removed and each flower was allowed one visit by a single insect. Stigmas were then collected, stained with basic fuchsin gel (Kearns and Inouye 1993) and examined under a microscope to determine the number of conspecific and

heterospecific pollen grains deposited by each type of visitor. To determine whether pollen deposition occurred without pollinator contact, control flowers were emasculated, bagged and collected without allowing an insect visit. Insect visitors were identified “on the wing” and were grouped into four categories: honeybees (non-native), bumble bees (native), other bees (native), and control. Species in the ‘other bees’ category include all native bees other than bumblebees and were comprised of several solitary bee species including *Ceratina arizonensis* (Apidae) as well as two species of sweat bee in the genus *Lasioglossum* (Halictidae) (Table 1). Observations were conducted between 9:00 and 15:00. A total of 88 insect-visited stigmas and 16 control stigmas were collected and stained over a period of ten weeks between March and May of 2012.

This study was followed by a similar experiment in spring of 2013 with the aim of comparing seed set, rather than pollen loads, in flowers singly visited by different insects. Once again, virgin buds at the native plant farm were tagged and bagged two days prior to pollinator observations. However, only half of the experimental flowers were emasculated, because some pollinators are unlikely to visit flowers lacking pollen rewards. Once an insect had visited a flower, it was immediately re-bagged and allowed to set fruit. For controls, emasculated and intact flowers were uncovered and re-bagged without visitation. Bumblebees were not observed at field sites during 2013, so insect visitors were grouped into three categories: honeybees (non-native), native bees, and control. The composition of native bee taxa was distinct from the previous year with a higher presence of bees from the genus *Anthophora* (Apidae)(Table 1). Once fruits matured over a period of 3 to 4 weeks, they were collected and dried and seeds were counted. A total of 68 intact (58 insect-visited, 10 control) and 56 emasculated (43 insect-visited, 13 control) flowers were included in the data set. Observations were conducted between 9:00 and 15:00 from 29 March to 25 April 2013.

Conspecific and heterospecific pollen deposition resulting from single visits was compared among flowers visited by honeybees, bumble bees, 'other bees' and controls. These comparisons were performed using a one-way ANOVA with a priori contrasts comparing pollen deposition of honeybees with native bees, 'other bees' with bumblebees and insect-visited flowers with control flowers. For intact flowers, seed set was compared among flowers visited by honeybees, native bees and controls using a generalized linear model with a zero-inflated Poisson distribution due to the large proportion of flowers that failed to produce a fruit. This analysis was supplemented with a priori contrasts comparing honeybees with native bees and insect-visited flowers with control flowers. Emasculated flowers only made fruit when visited by native bees, so we used instead a contingency analysis with a likelihood-ratio chi-square to determine whether the frequency of setting fruit depends on the type of insect visitor.

Comparison of Pollinator Importance

To compare the overall pollinator importance of honeybees, bumble bees and other bees, we multiplied the visitation rates from the floral neighborhood observations by the single-visit pollinator effectiveness, measured in conspecific pollen deposition, for each pollinator type. These values were averaged across all pollinator observation periods and divided by the total amount of predicted pollen receipt to determine the percentage of pollen transferred by each pollinator type. Seed set was not used as a metric for pollinator importance since we did not have sufficient overlap in pollinator types between the 2011 floral neighborhood observations and the 2013 pollinator effectiveness observations.

RESULTS

Test of self-compatibility in *P. parryi*

Hand pollination treatment had a significant effect on proportion of fruit set (randomized block ANOVA: $F_{3,72} = 48.98$, $P < 0.0001$; Table 2) with the unmanipulated treatment producing fruit less than half as often as the three other treatments (a priori contrast in one-way ANOVA: $P < 0.0001$; Fig. 1B). Fruit production did not, however, differ between the self-pollination and outcross-pollination treatments (Table 2). A fruit was made more than 90% of the time regardless of whether flowers were selfed or outcrossed. Hand pollination treatment also had a significant effect on seed set per fruit (randomized block ANOVA: $F_{3,61} = 6.22$, $P < 0.0001$; Table 2). Seed set per fruit was highest in the outcross pollination treatment (Fig. 1A), which averaged more than 3 times as many seeds as the unmanipulated flowers and approximately 27% more seeds than both self pollination treatments ($P < 0.05$; Table 2). Self-emasculated and self-intact treatments had similar amounts of fruit production and seed set ($P > 0.05$; Fig. 1, Table 2), suggesting no effect of emasculation on either of these variables. Number of pollen tubes reaching the base of the style was not significantly different between self-pollinated and cross-pollinated *P. parryi* flowers (randomized block ANOVA: $F_{1,27} = 0.93$, $P > 0.05$).

Effects of floral neighborhood on visitation

The majority of visits to *P. parryi* were made by *A. mellifera*, which accounted for over 83% of all visits over the course of the 2011 floral neighborhood study (Table 3). Native bees accounted for 12.2 % of all visits, of which the two most common visitors were sweat bees in the genus *Lasioglossum* (Halictidae) and the yellow-faced bumblebee, *Bombus vosnesenskii*, comprising 5.3% and 1.7% of visits, respectively. Native flies in the families Syrphidae and Bombyliidae each accounted for approximately 2% of visits. Honeybees were the only known

nonnative pollinator and visited three times more flowers per foraging bout (mean = 6.38) during observation periods than did other bees (mean = 2.13) or flies (mean = 2.21), and more than twice as many as bumblebees (mean = 2.7) (one way ANOVA; $F_{3,122} = 13.81$, $P < 0.0001$). Native plants comprised an average of 35.5% of the open flowers in the floral neighborhoods while nonnative plants made up an average of 64.5% of all open flowers, of which 41% was represented by *B. nigra*. Density of *B. nigra* in the floral neighborhood had no detectable effect on total visitation rate to *P. parryi* or on the proportion of native rather than non-native insect visitors (Table 4). Increasing density of other heterospecific flowers in the neighborhood decreased the proportion of flower visits made by the non-native *A. mellifera* (multiple regression; $F_{3,55} = 2.23$, $P = 0.0312$) but had no effect on total visitation rate (Table 4). The proportion of visits made by *A. mellifera* also decreased as total floral density within floral neighborhood increased (multiple regression; $F_{2,55} = 3.37$, $P = 0.0136$) but was not significantly influenced by density of either native (multiple regression; $F_{2,55} = 0.81$, $P > 0.05$) or nonnative (multiple regression; $F_{2,55} = 1.54$, $P > 0.05$) heterospecific flowers only.

Comparison of pollinator effectiveness

In a single visit, native pollinators deposited significantly more conspecific pollen on *P. parryi* stigmas than did *A. mellifera* (a priori contrast in one-way ANOVA $P = 0.025$; overall ANOVA: $F_{3,99} = 14.37$, $P < 0.001$; Fig. 2A). Flowers visited once by native insects received, on average, twice as many conspecific pollen grains as flowers visited by *A. mellifera*. Control stigmas had only small numbers of pollen grains, indicating that contamination of unvisited flowers was low (a priori contrast with the average for visited flowers, $P = 0.0004$). Both subcategories of native visitors (other bees and bumblebees) deposited conspecific pollen at a nearly identical rate. All groups of pollinators transferred similar amounts of heterospecific

pollen (one-way ANOVA: $F_{3,99} = 1.22$, $P = 0.31$; Fig. 3B), depositing on average one pollen grain.

Seed set resulting from a single visit followed a similar pattern, with a visit by a native bee leading to more seeds than a visit by *A. mellifera* to an intact flower (generalized linear model with zero-inflated Poisson, $P = 0.008$). Emasculated flowers only produced seed when visited by native pollinators. For intact flowers, native bee visits resulted in 45% more seeds than *A. mellifera* visits, whereas for emasculated flowers, *A. mellifera* visits failed to produce any seeds at all even though native bee visits resulted in an average of 11.3 seeds. Control flowers produced few seeds in intact flowers (mean = 0.7) and no seed set in emasculated flowers.

Comparison of pollinator importance

Since 83% of all visits to *P. parryi* flowers were made by *A. mellifera* during our floral neighborhood observations, we estimated that they accounted for about 75% of conspecific pollen transfer during that year even though they deposit half as much conspecific pollen as native bees do in a single visit. Other bees (native bees other than bumblebees) were responsible for about 22% of conspecific pollen transfer while bumblebees, which made only 1.7% of flower visits during floral neighborhood observations, accounted for about 2% of all pollen transfer when weighted by single visit effectiveness.

DISCUSSION

Increasing density of heterospecific species other than invasive *B. nigra* in the floral neighborhood resulted in a decreased proportion of floral visits to *P. parryi* from the non-native honeybee, *A. mellifera*, and therefore, a greater proportion of visits from native pollinators. In order to determine how shifts in pollinator assemblages may affect the reproductive fitness of the

focal plant, *P. parryi*, we compared pollinator effectiveness among different types of insects and found that native bees were more effective pollinators than honeybees. Thus, the total number of floral visits did not change with alteration of the neighborhood, but visits by more effective native insects increased at the expense of visits by less effective *A. mellifera*. In principle this shift should decrease total pollen receipt and seed set. We did not attempt to calculate an expected effect on reproductive success because we lacked effectiveness data for some of the visitors to *P. parryi* and because the relative frequency of visits by *A. mellifera* varied so greatly between the effectiveness study and the one year when natural visitation rates could be measured. Nevertheless, our results suggest that the loss of other native and nonnative heterospecifics due to encroachment from highly invasive plants like *B. nigra* could result in decreased reproductive fitness in *P. parryi* due to less effective pollination. The potential for floral neighborhood to modify pollinator assemblages may be a common phenomenon (Hegland and Boeke 2006, Larson et al. 2006, Lazaro et al. 2009), however the consequences of these modifications for plant pollination are poorly understood.

Impacts of floral neighborhood on pollinator assemblages

The increase in the proportion of visits from native pollinators in patches with higher densities of ‘other heterospecific’ plant species may be explained by the response of native bees to the overall floral density of the local plant community. Pollinators are often attracted to large, dense floral displays, a behavior that maximizes their net rate of energy gain (Covich 1974, Heinrich and Raven 1972, Eickwort & Ginsberg 1980), and a floral neighborhood with high floral density may act as a pollinator “magnet” for a focal plant species (Laverly 1992, Feldman 2004). We were not able to determine whether these visitation patterns were a response to native heterospecific plant species, non-natives, or both, perhaps because of low statistical power. For

example, we computed a retrospective power of 22% for our model testing how well native plant density within the floral neighborhood predicts the proportion of visits by *A. mellifera* with a sample size of 56 and the estimated partial correlation of -0.159. In order to have power of 80% to detect a relationship of this strength we would have needed a sample size of 305. Although we could not distinguish whether the response was specifically to natives or non-natives, in other studies native insects responded to the local density of one or a few other native plant species. For example, the densities of several halictid bee species were positively and linearly related to the density of native *Convolvulus arvensis* flowers (Waddington 1976). Since solitary bees generally have smaller maximum foraging distances than social species of bees (Gathmann and Tschamtkke 2002, Greenleaf et al. 2007), they may be more likely to concentrate their foraging efforts in areas where nutritional rewards are maximized. Restricted foraging distances may also mean that solitary bees, in particular, tend to create nests in areas with high floral density that provide abundant and stable resources necessary for survival and reproduction. Finally, it is possible that both floral density and native bee visitation increased in tandem due to a common cause, such as optimal microhabitat for both plants and pollinators.

We did not detect any direct effects of the presence and abundance of *B. nigra* on visitation to *P. parryi*. However, *B. nigra* has the potential to influence indirectly visitation of native plants like *P. parryi* by displacing other heterospecific plant species that appear to attract native pollinators. *B. nigra* is an exotic species that has thoroughly invaded coastal habitats of California (Muller 1969). Its success as an invader depends in part on allelopathic compounds (allyl-glucosinolate) found within its tissues (Bell and Muller 1973, Weston 1996), which exist at higher levels in invasive populations compared to populations in its native range (Oduor et al. 2011). *B. nigra* suppresses seed germination of native grasses that occur in its immediate

surroundings (Muller 1969) through leached toxins from decomposing stems and leaves (Bell and Muller 1973, Lankau and Strauss 2007) and shows strong competitive dominance over co-occurring California native forbs (Lankau 2008). Still, the allelopathic and competitive impacts of *B. nigra* on the particular plant species examined in this study are not known.

In general, animal-pollinated exotic weeds tend to attract high densities of nonnative pollinators such as *A. mellifera* (Morales and Aizen 2006). One study by Conner and Neumeier (1995) found that large populations of *B. nigra* were primarily visited by honeybees, compared to smaller populations, where solitary bees were more common. This suggests that as *B. nigra* densities become greater, they have the potential to attract pollinator assemblages that are dominated by generalist, nonnative bees which may also impact the pollination of sympatric native plant species.

Pollinator effectiveness for *P. parryi*

Shifts in visitor assemblages may be important for plant reproductive success even if total visitation rate is unaffected because shifts toward less effective pollinators will decrease overall visit quality. For either visitation quantity or quality to affect reproductive success, flowers cannot be completely autogamous. The data from our single-visit study showed that a visit is required for fruit production in *P. parryi*. Moreover, our greenhouse study indicated that outcrossing leads to higher seed set than self-pollination, possibly because of early-onset inbreeding depression since the number of pollen tubes reaching the base of the style did not differ between these treatments. In order for visitor assemblages to influence plant reproductive success, insects also need to vary in their effectiveness as pollen vectors. In this study, introduced honeybees were less effective pollinators of *P. parryi* than their native counterparts. Solitary bees and bumblebees transferred more conspecific pollen in a single visit than non-

native, *A. mellifera*. Single visits from solitary bees also resulted in twice as many seeds in intact flowers as did honeybee visits, suggesting that the high relative effectiveness of solitary bees at transferring conspecific pollen carries over to final female reproductive success.

Pollinator effectiveness has rarely been studied in coastal sage scrub ecosystems but in other habitats, several studies have found nonnative honeybees to be inferior to native bees at pollinating wild plants (eg. Roubik 1996, Aizen and Feinsinger 1994, Gross and Mackay 1998, Rymer 2005). For example, Gross and Macakay (1998) demonstrated that honeybees were poor pollinators of Australian native *Melastoma affine*, depositing less pollen and resulting in lower fruit set, on a per visit basis than native visitors. Yet other studies reveal that honeybees are effective pollinators of many plants, including those they did not coevolve with (reviewed by Hurn 1997; Freitas and Paxton 1998, Watts et al. 2012). Here, *A. mellifera* transferred approximately half as much conspecific pollen as native bees did in a single visit (Fig. 2) however, its abundance more than compensated for this in terms of pollinator importance, suggesting that both honeybees and native bees may now be key pollinators for *P. parryi*. Nevertheless, there are some important foraging behavior differences between native bees and *A. mellifera* that may curtail its apparent value as a pollen vector for *P. parryi*.

Honeybees often adopt a ‘sideworking’ foraging behavior where they feed by perching on flower petals to reach a nectary from the side (Roberts 1945). This posture can reduce pollination success because in many cases, only peripheral contact is made with the anthers and none with the stigmas (McGregor 1976, Westerkamp 1991, Thomson and Goodell 2001). We often observed this behavior in our study when honeybees visiting *P. parryi* were foraging for nectar rather than pollen. The effect of this behavior is apparent in our results from the 2013 single visit observations; emasculated *P. parryi* flowers visited by honeybees failed to produce

any seeds. It is unclear what proportion of honeybees that forage on *P. parryi* exhibit sideworking behavior or what proportion is collecting nectar at any given time since we did not quantify this behavior, and studies on apple pollination have found a great variation in the frequency of sideworking visits (Thomson and Goodell 2001, Benedek and Nyeki 1996).

Both honeybees and bumblebees exhibit high levels of floral constancy (Heinrich 1979, Hill et al. 1997, Wells and Wells 1983, Waser 1986), a behavior that can be beneficial in terms of reducing heterospecific pollen transfer. However, honeybees often make fewer interplant movements than other types of pollinators (McGregor et al. 1959, Heinrich and Raven 1972, Silander and Primack 1978, Westerkamp 1991, Keys et al. 1995, Rymer 2005), which can lead to high levels of geitonogamous self-pollination. In our floral neighborhood study, this type of foraging behavior was often observed when honeybees visited many flowers during a single foraging bout within an observation patch, while solitary bees generally visited one or two flowers in the patch before moving on to another foraging location. The consequences of infrequent interplant movements may be more evident in self-incompatible plant species but may also have negative consequences for species like *P. parryi* that exhibit signs of inbreeding depression.

The relative abundance of *A. mellifera* could vary more than native bees spatially and temporally in native habitats since their populations are often determined by anthropogenic activity. Honeybees may be especially common in areas close to agricultural development, particularly, for fruit crops where supplemental honeybee colonies are used for the pollination of orchards. Our floral neighborhood pollinator observations included a very high relative abundance of honeybee pollinators (>80%), which contributed to the overall importance of *A. mellifera* as a pollen vector for *P. parryi*. However, honeybee abundance may be lower than this

in other areas where *P. parryi* is common, therefore, the importance of native bees should not be overlooked. Moreover, recent regional declines of honeybees across the USA (Natural Research Council 2006, vanEngelsdorp et al. 2008) further imply that reliance of wild plants on honeybees as a principal pollinator may be unsustainable. On the other hand, bumblebees were in very low abundance during our floral neighborhood observations but were in relatively high abundance during our 2012 single visit observations suggesting that their importance as a pollen vector for *P. parryi* may have been underestimated in our study. Bumblebees were as efficient as solitary bees at transferring conspecific pollen and they represent a potentially important pollinating resource for *P. parryi*.

Pollinators of plant species in coastal sage scrub are surprisingly unstudied in comparison with other habitats. Our findings represent a direct measure of pollinator performance for native plants in southern California coastal sage scrub habitat and will provide insight for restoration and conservation efforts. For example, our study indicates the value of solitary bees as pollinators for a native plant. Increasing nesting sites for native bees has been implemented through restoration of roadsides and other disturbed habitats (Hopwood 2008, Steffan-Dewenter & Schiele 2008) and may be a useful approach to mitigate native pollinator declines in California coastal habitats.

Conclusions

We have shown that floral neighborhood can influence the composition of pollinator assemblages that visit a native plant and that changes in local flower communities have the potential to affect native plant reproductive success through shifts in these assemblages towards a higher percentage of less effective pollinators. The results of this study illustrate the importance of considering not only how visitation is influenced by heterospecific plant species

but also how effectiveness of the pollinator assemblage can be altered. In a rare comparison of pollinator effectiveness in coastal sage scrub habitat, native bees were more effective at pollinating *P. parryi* on a single visit basis than honeybees, but high abundance of honeybees suggests that *P. parryi* may rely on both native and non-native pollinators for seed set. Future studies of native and invasive non-native plants should consider the possibility of indirect interactions in which displacement of favored host plants for native pollinators reduces their importance as pollinators of focal natives.

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TABLE 1.1. List of insect species that visited *P. parryi* during single-visit observations.

Dependent Variable	Insect Species	Pollinator Type	Number of Visits
Pollen Deposition	<i>Apis mellifera</i>	honey bee	33
	<i>Bombus californica</i>	bumble bee	2
	<i>Bombus vosnesenskii</i>	bumble bee	26
	<i>Anthidium</i> sp.	solitary bee	1
	<i>Ceratina</i> sp.	solitary bee	3
	<i>Hylaeus</i> sp.	solitary bee	1
	<i>Lasioglossum</i> spp.	bee	20
	<i>Osmia</i> sp.	solitary bee	1
Seed Set - Intact	<i>Apis mellifera</i>	honey bee	24
	<i>Agapostemon</i> sp.	solitary bee	3
	<i>Anthidium</i> sp.	solitary bee	3
	<i>Anthophora</i> sp.	solitary bee	9
	<i>Lasioglossum</i> spp.	bee	23
Seed Set - Emasculated	<i>Apis mellifera</i>	honey bee	30
	<i>Anthidium</i> sp.	solitary bee	1
	<i>Anthophora</i> sp.	solitary bee	6
	<i>Lasioglossum</i> spp.	solitary bee	9

TABLE 1.2. ANOVA for hand-pollination experiment including a priori contrasts

Dependent variable	Source	df	MS	F	P
% fruit produced	treatment	3	4.305	48.98	<0.0001
	Self vs control	1	9.252	105.27	<0.0001
	Outcross vs self	1	0.019	0.22	0.639
	Emasculated vs intact	1	0.058	0.66	0.419
	Plant	24	0.138	1.57	0.075
	Error	72	0.088		
Seed set per fruit	treatment	3	8.787	23.33	<0.0001
	Self vs control	1	19.447	51.63	<0.0001
	Outcross vs self	1	2.498	6.63	0.011
	Emasculated vs intact	1	0.001	0	0.951
	Plant	24	1.460	3.88	<0.0001
	Error	165	0.377		

TABLE 1.3. Identity and frequency of insect visitors during observations of *Phacelia parryi*

Insect Species	Number of Visits	% of Visits
Order Hymenoptera		
<i>Apis mellifera</i>	2501	83.9
<i>Lasioglossum</i> spp.	158	5.3
<i>Bombus vosnesenskii</i>	51	1.71
<i>Chelostoma</i> sp.	39	1.3
<i>Anthophora pacifica</i>	34	1.14
<i>Ceratina arizonensis</i>	23	0.77
<i>Agapostemon</i> sp.	15	0.5
<i>Anthophora californica</i>	8	0.26
<i>Macrophya</i> sp.	7	0.23
<i>Hylaeus</i> sp.	7	0.23
<i>Sphecodes</i> sp.	7	0.23
<i>Colletes</i> sp.	4	0.13
<i>Andrena</i> sp.	4	0.13
<i>Eucera</i> sp.	3	0.1
<i>Pseudomasaris</i> sp.	3	0.1
<i>Osmia</i> sp.	2	0.07
Order Diptera		
<i>Bombylius</i> spp.	62	2.08
<i>Eupeodes</i> sp.	28	0.94
unidentified syrphid flies	19	0.64
<i>Allograpta</i> spp.	3	0.1
<i>Sphaerophoria</i> sp.	2	0.07
<i>Copestylum</i> spp.	1	0.03

TABLE 1.4. Linear regression analysis for pollinator response variables in floral neighborhood study

Dependent Variable	Parameter	Estimate	St. Error	t value	Pr> t
Total visitation rate	Intercept	0.316	0.256	1.23	0.223
	<i>B. nigra</i>	-0.001	0.001	-1.55	0.127
	Other heterospecifics	0.0001	0.001	0.11	0.913
	Date	0.002	0.003	0.75	0.454
Proportion visits by <i>A. mellifera</i>	Intercept	0.183	.246	.74	0.459
	<i>B. nigra</i>	-0.001	.001	-1.99	0.052
	Other heterospecifics	-0.002	.001	-2.21	0.031*
	Date	0.005	.002	1.86	0.069

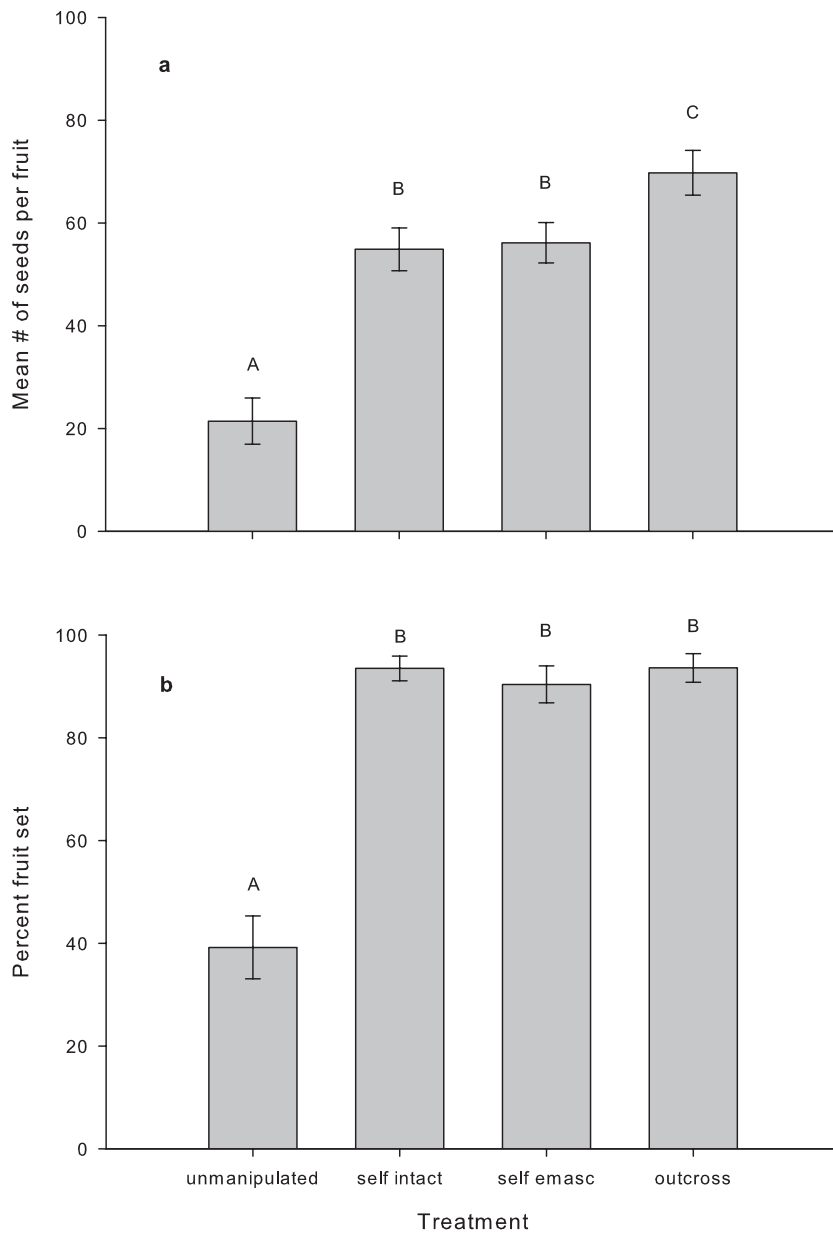


Figure 1.1 Average number of seeds per fruit (a) and percentage of flowers that produced fruit (b) compared in different hand-pollination treatments. Different uppercase letters represent statistical differences among treatments based on Tukey multiple comparisons. Error bars represent +/- 1 SE.

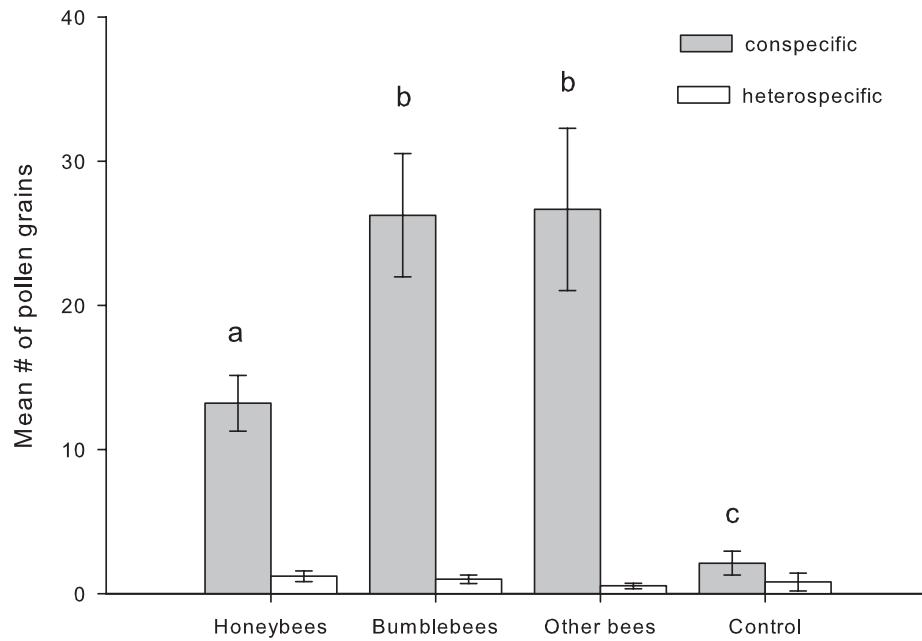


Figure 1.2. Comparison of mean (± 1 SE) conspecific (grey bars) and heterospecific (white bars) pollen loads of single visits from various insect visitors to *P. parryi*. A different lowercase letter represents a significant difference among groups. All bees included in the ‘other bees’ category are native to our study system.

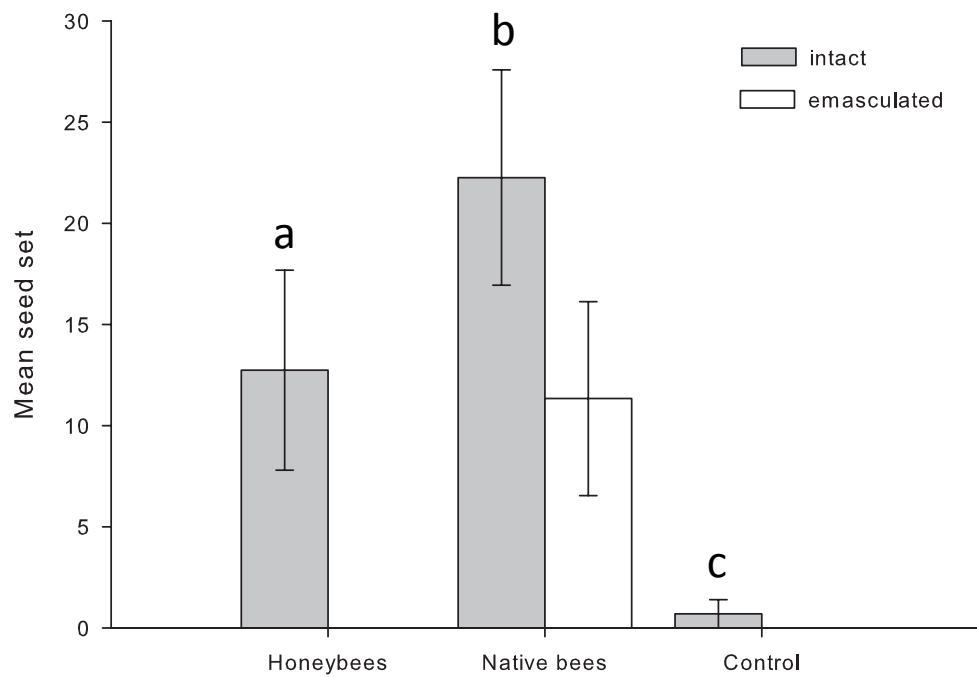


Figure 1.3. Comparison of mean (± 1 SE) seed set resulting from single visits by insect visitors to intact (grey bars) and emasculated (white bar) *P. parryi* flowers. No seeds were produced in emasculated flowers from honeybee or control visits. Groups with different lowercase letters differ significantly.

CHAPTER 2: Timing of invasive pollen deposition influences pollen tube growth and seed set in a native plant

INTRODUCTION

Invasive plants may pose a critical threat to native plant reproduction through interference of plant-pollinator mutualisms (Traveset and Richardson 2006, Bjerknes et al. 2007). The presence of exotic plants within a community can impede the pollination process by influencing the quantity and quality of pollinator visits that native plants receive (eg. Chittka and Schürkens 2001; Brown et al. 2002; Larson et al. 2006). While changes in visitation quantity are determined by pollinator preference, visitation quality may be reduced through interspecific pollen transfer that occurs when pollinators move pollen from one plant species to another as a consequence of mixed visits (reviewed in: Morales and Traveset 2008; Ashman and Arceo-Gomez 2013). In plant communities infested with exotic species, interspecific pollen transfer may be an important mechanism for decreased reproductive success in native species (Traveset and Richardson 2006; Bjerknes et al. 2007).

In order for an invasive plant species to influence the reproductive fitness of a native species through interspecific pollen transfer, both species must have similar flowering phenology and share pollinators. Furthermore, the intensity of interspecific pollen movement will be influenced by pollinator foraging patterns (eg. floral constancy) (Waser 1986; Gegeer and Lavery 2005) and the layering of pollen grains on the body (Price and Waser 1982; Kohn and Waser 1985), which will determine the pattern of heterospecific pollen deposition on a stigma. If pollen is successfully transferred from one species to another, there must be a cost to female or male fitness as a result of heterospecific pollen deposition or conspecific pollen loss (eg. Waser

and Fugate 1986; Galen and Gregory 1989; Harder et al. 1992; Brown and Mitchell 2001; Moragues and Traveset 2005). Here, we focus primarily on how deposition of invasive pollen influences reproductive success in a native plant.

The placement of heterospecific pollen on a stigma may depress reproduction through a variety of mechanisms that act at different stages of the fertilization process. Foreign pollen may interfere with stigma receptivity through stigma clogging (Galen and Gregory 1989) or stigma closing (Waser and Fugate 1986), whereas pollen allelopathy may reduce pollen germination and growth through chemical interference (Sukhada and Jayachandra 1980; Thomson et al. 1981; Murphy and Aarssen 1995). In closely related co-flowering species, heterospecific pollen may influence reproduction through usurpation of ovules and hybridization (Harder 1992; Brown and Mitchell 2001).

Previous studies suggest that exotic plants often share pollinators with sympatric natives (Grabas and Lavery 1999; Brown et al. 2002) and that invasive pollen readily integrates into native plant-pollinator networks (Memmott and Waser 2002; Vila et al. 2009), making the potential for invasive pollen deposition high. However, few studies (Brown and Mitchell 2001; Jacobsson et al. 2008; Matsumoto et al. 2010; Tscheulin et al. 2009; Da Silva and Sargent 2011) have gone beyond looking at the amounts of alien pollen on native stigmas by directly testing the impact of invasive pollen on native reproduction.

Invasive plants may act as superlative competitors since they can differ from native species as heterospecific pollen donors in various ways. Invasive species often occur at exceptionally high densities and may produce large, showy floral displays (eg. Chittka and Schurkens 2001; Brown et al. 2002), creating the potential for exotic pollen to dominate pollen transfer webs (Lopezaraiza-Mikel et al. 2007). Since invasive plants are novel members of a

plant community, the coevolutionary relationships necessary to form specialized relationships with animal pollinators are not present, thus, mutualistic interactions are mostly with generalist species (Morales and Aizen 2006). Such interactions provide more opportunities for pollinator sharing between alien and native plant species and may further increase the frequency of heterospecific pollen transfer. Finally, native plant species may not be well adapted to the presence of alien pollen, as it poses the potential for novel pollen-pollen or pollen-pistil interactions.

The timing of foreign pollen receipt may also be an important factor in determining reproductive success. An inconstant pollinator may either deposit mixed pollen loads that expose a flower stigma to simultaneous placement of conspecific and heterospecific pollen, or may deposit pure heterospecific pollen if it has just visited several flowers of another plant species. A visit depositing pure heterospecific pollen followed by a more constant pollinator foraging on conspecific flowers would result in sequential deposition of foreign and conspecific pollen. Such variation in the pattern of interspecific pollen deposition may be significant for successful pollination. For example, pollen originating from *Delphinium nelsonii* caused a reduction in seed set in *Ipomopsis aggregata* only when applied prior to conspecific pollen (Waser and Fugate 1986). Caruso and Alfaro (2000) later built upon these results, showing that *Castilleja linariaefolia* pollen also reduced seed set in *I. aggregata* when applied prior to, but not simultaneously with conspecific pollen, suggesting the presence of a priority effect. It is possible that priority effects are common in plant communities where heterospecific pollen transfer is high, but few studies (Kohn and Waser 1985; Waser and Fugate 1986; Kwak and Jennersten 1991; Caruso and Alfaro 2000) have investigated this phenomenon with no known research involving invasive plant species.

In this study, we examined the effects of pollen of the invasive species *Brassica nigra* on the reproductive success of a native plant, *Phacelia parryi*. The timing of pollination and amount of *B. nigra* pollen on stigmas was manipulated using hand pollination experiments to investigate how invasive pollen influences pre- and post-fertilization stages of reproduction. The two plant species used in our experiments co-occur in southern California coastal habitats and display spatial and phenological overlap. Pollen from invasive *B. nigra* is often found on native *P. parryi* stigmas, particularly in areas of high infestation (Bruckman and Campbell, in preparation). The specific questions we address here are: (1) to what extent do *P. parryi* and *B. nigra* share pollinators? (2) Does the presence of *B. nigra* pollen on the stigma influence seed set and pollen tube growth in native *P. parryi*? (3) Do seed set and pollen tube growth in *P. parryi* vary in response to differences in timing of *B. nigra* pollen deposition onto the stigma?

MATERIALS AND METHODS:

Study Species

Black mustard (*Brassica nigra* (L.) W. D. J. Koch) was used in this study as a model invasive species. It is a ubiquitous annual weed of Mediterranean origin that occurs in disturbed areas throughout North America (Westman et al. 1999) and is particularly common in the coastal and grassland habitats of southern California. *B. nigra* plants have numerous terminal inflorescences of yellow, hermaphroditic, self-incompatible flowers that attract a variety of insect pollinators (Conner and Neumeier 1995). In its invasive range *B. nigra* often grows in large, dense stands and secretes allelochemicals that inhibit germination of neighboring plant species (Bell and Muller 1973).

Phacelia parryi Torr. (Boraginaceae) is an annual herb native to coastal sage scrub and chaparral ecosystems throughout southern and Baja California and is especially common on open, recently burned slopes. Flowers are self-compatible but exhibit signs of inbreeding depression when self-pollinated, suggesting the importance of pollinators for transfer of outcross pollen (Bruckman and Campbell 2014). The inflorescence is a helicoid cyme of showy, violet to royal blue, bell-shaped flowers each 1 to 2 centimeters long that last 3-4 days. *B. nigra* and *P. parryi* exhibit substantial spatial and phenological overlap in coastal habitats in southern California.

Assessment of pollinator sharing

To determine the degree of pollinator sharing between *P. parryi* and *B. nigra*, natural populations of both plant species were observed for 30-minute intervals to determine the frequency of visitation by different types of insect pollinators. Observations were conducted on 1-m² plots of each species between 0900h and 1500h on 8 days in April of 2011 within the OC Parks natural preserve in Orange County, California (33° 44' 01" N, 117° 42' 20" W). Each observation plot was a minimum of 5 meters away from any other observation plot. During a given observation period, one plot of each *P. parryi* and *B. nigra* was observed simultaneously to control for temporal variation. A visit was counted when an insect contacted either the anthers or stigma of a flower. Pollinators were recorded to the lowest field-identifiable taxonomic category. A total of 2946 floral visits were made over 24 paired 30-minute observation periods on 15 different plots of each plant species. The extent of pollinator sharing between *P. parryi* and *B. nigra* was estimated using Pianka's (1973) niche overlap index, which takes into account the relative visitation frequency of each pollinator species visiting both *P. parryi* and *B. nigra* flowers.

The effects of invasive pollen on seed set in *P. parryi*

To determine whether pollen deposition from invasive plant *B. nigra* affects seed set in native plant *P. parryi*, we conducted a hand-pollination experiment in a pollinator-free greenhouse at the University of California, Irvine. The four levels of hand-pollination were: conspecific pollen only, simultaneous deposition of conspecific and invasive pollen, conspecific pollen followed 3 hours later by *B. nigra* pollen (hereafter invasive pollen), and invasive pollen followed 3 hours later by conspecific pollen. The conspecific only treatment served as a control. The other treatments were included to determine whether the timing of *B. nigra* pollen deposition influenced seed set in *P. parryi*.

Plants of *P. parryi* used for pollination experiments were germinated in November 2012 in plug trays and transplanted into individual 2-gallon pots 4 weeks later. *B. nigra* plants were also grown from seed and maintained in pots in greenhouse facilities to serve as a source of invasive pollen for experiments. Once *P. parryi* flowers were produced, hand pollinations were made between 10 January and 10 February 2013. Since *P. parryi* is self-compatible, all experimental flowers were emasculated prior to pollination to prevent deposition of self-pollen prior to the controlled treatment. Emasculations were performed on large purple buds 2 days prior to pollination. Because *P. parryi* exhibits protandry, pollinations were made once flowers were in female phase and the stigma was protruding past the anthers. Pollen was transferred from anthers to stigmas using a wooden toothpick. For conspecific pollinations, pollen was collected from one flower on each of 3 different *P. parryi* donor plants to ensure outcrossing and to minimize any effect of donor identity on pollination and seed set. For pollinations using invasive species, pollen was collected from three different *B. nigra* flowers to provide approximately equal amounts of pollen for each pollen application. For simultaneous pollen

applications, 5 anthers were removed from 3 different flowers of both *P. parryi* and *B. nigra* individuals and both types of pollen were mixed together in a small Petri dish prior to application. The first set of pollinations, including all treatments, was performed between 0800 and 1000 h. When required, the second set of pollinations was performed between 1100 and 1300 h, 3 hours following the first pollinations. Each of 28 individual *P. parryi* plants contained 8 experimental flowers, two for each treatment level, making a total of 224 *P. parryi* experimental flowers. Each treated flower was scored for fruit production and number of seeds approximately one month after pollination.

A randomized block ANOVA was performed on the means of seeds produced per flower and seeds produced per fruit in each treatment for each plant, with plant identity used as the block in the model. The identity of the recipient plants was included in the model to control for any variation in ovule number or resources for seed production among plants. For both dependent variables, an a priori contrast was used to compare control flowers with the average of the three mixed pollen treatments. A Tukey multiple comparisons test was also used to determine any differences in seed set among specific pollination treatments.

In order to determine the relative amounts of pollen applied to each treatment during our hand pollinations, 40 flowers from 10 different plants (1 for each treatment on each plant) were emasculated and hand pollinated in the manner described above. Stigmas were collected immediately after pollinations and stained with basic fuschin gel (Kearns and Inouye 1993). Each stigma was examined under a compound microscope at 100x magnification to determine the number of conspecific and *B. nigra* pollen grains present. *P. parryi* and *B. nigra* pollen grains were distinguishable due to differences in size and external morphology. A one-way

ANOVA was used to compare the relative amounts of conspecific and *B. nigra* pollen deposited on *P. parryi* stigmas across hand-pollination treatments.

The effects of invasive pollen on pollen tube growth in *P. parryi*

In order to assess how invasive pollen deposition may influence pre-fertilization events in the native plant *P. parryi*, another hand-pollination experiment was carried out to examine conspecific pollen tube growth within *P. parryi* styles. In February 2014, we propagated 19 individual *P. parryi* plants within the UC Irvine greenhouses and conducted hand-pollination treatments identical to the ones described above. For this study however, we did not allow flowers to set seed but harvested all experimental flowers 24 hours after pollinations were completed. Flowers were detached from plants just below the ovary, and later the corolla, calyx and anthers were removed to leave only the style and stigma for examination. Each style was then stained for viewing using fluorescence microscopy (Kearns and Inouye 1993). After harvesting, styles were immediately placed in 10 N NaOH at room temperature for two hours, washed with deionized water and then placed in decolorized aniline blue (0.1% aniline blue in 0.1 M K₃PO₄) and stored for 24-72 hours at 4 degrees C.

Each stained style was mounted on a microscope slide with 3-4 drops of aniline blue solution and squashed so that individual pollen tubes could be more readily observed. *In vivo* pollen performance was assessed by quantifying pollen tubes at the base of each style, where the highest density of individual tubes was most visible. Conspecific and *B. nigra* pollen grains were counted on each stigma and the number of tubes was divided by the number of conspecific pollen grains on the stigma to determine the ratio of *P. parryi* pollen tubes to pollen grains for each style. A total of 150 *P. parryi* styles were included in our data set.

A randomized block ANOVA was performed on the means of pollen tubes per style for each treatment for each plant, with plant identity used as the block in the model. A randomized block ANOVA was also performed to analyze the proportion of pollen grains that resulted in pollen tubes among hand-pollination treatments since some flowers inevitably received more pollen than others during pollinations. Out of 150 cases in total, 6 had more pollen tubes than pollen grains (presumably due to occasional grains falling off in the preparation procedure), and the proportion of pollen grains resulting in tubes was set to one in these instances. Pollen tube number was regressed onto *B. nigra* pollen grain number to determine whether invasive pollen grain density on the stigma had an overall effect on pollen tube growth.

RESULTS

Assessment of pollinator sharing

Both *P. parryi* and *B. nigra* were visited by a diversity of insect taxa including eusocial bees, solitary bees and syrphid flies (Table 1). The European honeybee, *Apis mellifera*, was the most common visitor to both *P. parryi* and *B. nigra* and made up approximately three quarters of all visits for both species. Sweat bees of the genus *Lasioglossum* were the next most common visitors to both species and represented 8% and 14% of visits to *P. parryi* and *B. nigra*, respectively. Consequently, the extent of pollinator sharing between *P. parryi* and *B. nigra* was very high, with a Pianka's niche overlap value of 97.2% overall and 74.2% when excluding honeybees.

The effects of invasive pollen on seed set in *P. parryi*

Hand-pollination treatment had a significant effect on seed set per flower (randomized block ANOVA; $F_{3,80} = 7.89$, $P = 0.001$; Table 2) with the simultaneous deposition of conspecific

and invasive pollen resulting in the lowest number of seeds (Fig. 1). The control treatment produced significantly higher seed set per flower than the average of the three mixed pollen treatments (a priori contrast in one-way ANOVA; $P = 0.001$; Table 2). Hand-pollination treatment also had a significant effect on the number of seeds per fruit (randomized block ANOVA; $F_{3,68} = 3.16$, $P = 0.03$; Table 2) with the control treatment also resulting in higher seed set than the average of the mixed pollen treatments (a priori contrast in one-way ANOVA; $P = 0.01$; Table 2). Each of the four hand-pollination treatments resulted in similar amounts of conspecific pollen (one-way ANOVA, $P > 0.05$) and invasive pollen (one way ANOVA, $P > 0.05$; Fig. 2).

The effects of invasive pollen on pollen tube growth in *P. parryi*

Hand-pollination treatment had a significant effect on the number of pollen tubes present at the base of *P. parryi* styles (randomized block ANOVA; $F_{3,54} = 70.32$, $P < 0.0001$; Table 3) with the control treatment containing more than three times as many pollen tubes as the mixed-pollen treatments where invasive pollen was applied either simultaneously or before conspecific pollen (Fig. 3). Flowers that were pollinated with conspecific pollen before invasive pollen produced an intermediate number of pollen tubes (Fig. 3). The proportion of conspecific pollen grains that resulted in pollen tubes was also significantly influenced by hand-pollination treatment (randomized block ANOVA; $F_{3,54} = 75.32$, $P < 0.0001$; Table 3), although, in this case, flowers pollinated with conspecific pollen only and conspecific prior to invasive pollen displayed a statistically similar pollen tube to pollen grain ratio, and were significantly higher than the other two treatments. Furthermore, the number of pollen tubes decreased significantly with greater quantities of invasive pollen on the stigma (regression; $F_{1,148} = 38.99$; $P < 0.0001$; Fig. 4).

Pollen tubes originating from *B. nigra* pollen grains were not observed growing past the stigmatic surface of *P. parryi* styles.

DISCUSSION

Brassica nigra is an exotic species that grows in high-density monospecific stands and has thoroughly invaded coastal habitats throughout California (Muller 1969). *B. nigra*'s exceptionally high abundance in native plant communities may therefore pose a substantial threat to plants like *P. parryi* if it inundates pollen transfer webs with invasive pollen and interferes with pollination. In this study, *B. nigra* was found to share pollinators to a high degree with *P. parryi* (Table 1), demonstrating the potential for interplant movements among species if individual pollinators are not entirely constant in their foraging behavior. Furthermore, other field studies (Bruckman and Campbell, in preparation) show that *B. nigra* pollen is commonly found on *P. parryi* stigmas in areas of high infestation, presenting further evidence that interspecific pollen movement between these species occurs in nature. Here we showed that such deposition of *B. nigra* pollen onto the stigma reduces both pollen tube growth and seed set in *P. parryi*.

The detrimental effects of *B. nigra* pollen on *P. parryi* reproductive success were demonstrated in this study when pollination with only conspecific pollen resulted in significantly higher numbers of pollen tubes within *P. parryi* styles than mixed pollen treatments. Our findings also indicate the presence of a priority effect, as flowers pollinated with conspecific pollen prior to invasive pollen contained significantly higher numbers of pollen tubes than did the other mixed pollen treatments. In general, flowers with higher numbers of invasive pollen

grains contained lower numbers of pollen tubes, suggesting that *B. nigra* may interfere with *P. parryi* pollination as a result of heterospecific pollen transfer.

Seed set was reduced in *P. parryi* only when invasive and conspecific pollen were applied simultaneously to *P. parryi* stigmas. This result is contrary to the outcomes of several other studies on different plant species. Application of heterospecific pollen prior to conspecific pollen has often had a greater negative effect on seed set than simultaneous application (eg. Kohn and Waser 1985; Waser and Fugate 1986; Caruso and Alfaro 2000), due to mechanisms such as stigma closure or loss of stigmatic receptivity. For example, application of *Delphinium nelsonii* pollen on *Ipomopsis aggregata* stigmas caused stigmatic lobes to close within 1.5-6 hours after application (Waser and Fugate 1986). In the case of *P. parryi*, loss of stigmatic receptivity is unlikely since seed set was not significantly reduced when heterospecific pollen was applied prior to conspecific pollen. Rather, our results could be interpreted by considering interactions between interspecific pollen grains on the stigma.

Heterospecific pollen grains may show the strongest physical interference when applied concurrently with conspecific grains since both types of pollen will be fresh and viable upon deposition. In the case where *B. nigra* pollen is applied prior to conspecific pollen, the time lag between applications may be sufficient for the alien pollen to wither or diminish in size before the arrival of conspecific pollen. It is also possible that potential chemical interference of *B. nigra* pollen due to allelopathy is most intense during simultaneous deposition with conspecific pollen and that these effects may diminish over the course of the three-hour time lag. Pollen allelopathy is a possible explanation since *B. nigra* is known to release soluble growth inhibitors through root exudates and foliar leaching (Bell and Muller 1973; Weston 1996) and at least one other species in the genus *Brassica* is thought to have allelopathic pollen (Murphy 1992).

Pollen tube number was equally reduced in flowers that received invasive pollen concurrently and prior to conspecific pollen. This result is contradictory to that of the 2013 seed set experiment, which demonstrated that *P. parryi* reproductive success is only influenced when *B. nigra* pollen is applied simultaneously with conspecific pollen. There may be several explanations for the discrepancy in outcomes of our two experiments. One possibility is that the deleterious effects on seed set may be modified at stages following pollen tube germination and growth down the style. For example, pollen tube growth in the simultaneous application treatment may have decreased in the areas of the style beyond our examination or once inside the ovary, leading to low ovule fertilization. Another possibility is that detrimental effects of the simultaneous invasive pollen deposition treatment are taking place beyond the time frame of 24 hours after pollination. If conspecific pollen grains normally continue to germinate and grow for more than a day after pollination, we may not have accurately quantified ultimate pollen tube growth for each treatment. Finally, because experiments were carried out in different years, there is the possibility that slightly different environmental conditions influenced the outcome of our results. Since *B. nigra* and *P. parryi* are not closely related species and we did not observe *B. nigra* pollen tubes growing down *P. parryi* styles, ovule usurpation by *B. nigra* is not a plausible explanation for the decrease observed in *P. parryi* seed set.

The results of this study bolster a growing sum of evidence showing that heterospecific pollen transfer is an important mechanism by which invasive plants compete for pollination with native species (Brown and Mitchell 2001; Matsumoto et al. 2010; Da Silva and Sargent 2011). One explanation for the detrimental effects of alien pollen involves exotic pollen acting as a ‘novel weapon’ (Callaway and Ridenour 2004; Bais et al. 2003) in native plant communities. Plant species that have an evolutionary history of pollinator sharing may show a high tolerance

for heterospecific pollen receipt. Conversely, an introduced species may pose more deleterious interspecific interactions, to which established community members are not well adapted. A recent review of the fitness consequences of heterospecific pollen receipt by Ashman and Arceo-Gomez (2013) found that species with the most detrimental effects as heterospecific pollen donors are indeed alien plants.

This study has also highlighted the importance in timing of invasive pollen deposition. In nature, the probability that heterospecific pollen will be deposited on a stigma in a particular pattern depends largely on pollinator flower constancy (Waser 1986, Gegear and Lavery 2005) and the configuration of pollen layering on a pollinator's body (Price and Waser 1982; Kohn and Waser 1985). In our system, the pattern by which pollinators deposit invasive and conspecific pollen on *P. parryi* stigmas is not known. However, *B. nigra* pollen is found on *P. parryi* stigmas in the field, particularly in areas of high infestation and in amounts similar to the range of invasive pollen grains applied to experimental flowers in this study. In a field experiment in 2014, *P. parryi* flowers surrounded by high densities of *B. nigra* flowers (1000+ flowers within a 1-m² area) had an average of 8.7 grains of *B. nigra* pollen on their stigmas, and 90% of experimental flowers contained at least some *B. nigra* pollen (Bruckman and Campbell, in preparation). This evidence suggests that shared pollinators are switching between plant species at least some of the time while foraging. Since detrimental effects on reproductive success were found when conspecific and invasive pollen were deposited simultaneously, it would be useful to determine how often common pollinators are carrying both types of pollen in a given foraging bout.

In conclusion, this research represents one of few studies that explore how invasive pollen deposition influences native reproductive success. We have shown that the exotic plant

Brassica nigra has detrimental effects on pollen tube growth and seed set in native *Phacelia parryi*, which adds to a growing sum of evidence pointing to heterospecific pollen transfer as a critical mechanism of competition between alien and native plants. We have also demonstrated that the timing of invasive pollen deposition can be an important factor in determining the degree to which native reproductive success will be depressed. Future research on invasive pollen transfer in native plant communities should focus on the patterns of pollen placement by pollinators on native stigmas as well as the implications for evolutionary responses of native species to invasive pollen deposition.

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TABLE 2.1. List of pollinator taxa including number of individuals observed (N) and percentage (%) of total visits for each pollinator taxon observed to visit individuals of native species, *Phacelia parryi* and invasive species, *Brassica nigra*.

	<i>P. parryi</i>		<i>B. nigra</i>	
	N	%	N	%
Order Hymenoptera				
<i>Apis mellifera</i> (Apidae)	1077	78.7	1212	76.8
<i>Lasioglossum</i> spp. (Halictidae)	115	8.4	224	14.2
<i>Bombus</i> spp. (Apidae)	33	2.4	0	0
<i>Chelostoma</i> sp. (Megachilidae)	26	1.9	0	0
<i>Ceratina</i> sp. (Apidae)	23	1.7	0	0
<i>Hylaeus</i> sp. (Colletidae)	7	0.5	0	0
<i>Anthophora californica</i> (Apidae)	6	0.4	0	0
<i>Colletes</i> sp. (Colletidae)	0	0	67	4.2
Order Diptera				
<i>Bombylius</i> spp. (Bombyliidae)	35	2.6	0	0
<i>Eupeodes</i> sp. (Syrphidae)	25	1.8	15	1
Unidentified Syrphidae	18	1.3	25	1.6
<i>Allogaptra obliqua</i> (Syrphidae)	3	0.2	22	1.4
<i>Copestylum</i> spp. (Syrphidae)	1	0.1	12	0.8
Total	1369	100	1577	100

TABLE 2.2. ANOVA for hand-pollination experiment examining seed set including a priori contrasts

Dependent Variable	Source	DF	MS	F	<i>P</i>
Seeds per flower	Plant	27	2259.469	2.71	0.0003
	Treatment	3	6571.857	7.89	0.0001
	P-only vs average of 3 mixes	1	9106.248	10.94	0.0014
	Error	80	832.477		
Seeds per fruit	Plant	27	1816.872	2.02	0.0104
	Treatment	3	2841.362	3.16	0.0302
	P-only vs average of 3 mixes	1	5971.58	6.64	0.0122
	Error	68	899.996		

Table 2.3. ANOVA for hand-pollination experiment examining pollen tube growth including a priori contrasts

Dependent variable	Source	DF	MS	F	<i>P</i>
Total pollen tubes	Plant	18	256.66	1.54	0.112
	Treatment	3	11712.4	70.32	< 0.0001
	P only vs. average of 3 mixes	1	20700.3	120.5	< 0.0001
	Error	54	166.56		
Proportion pollen tubes	Plant	18	0.029	2.44	0.0059
	Treatment	3	0.894	75.32	< 0.0001
	P only vs. average of 3 mixes	1	1.22	102.86	< 0.0001
	Error	54	0.012		

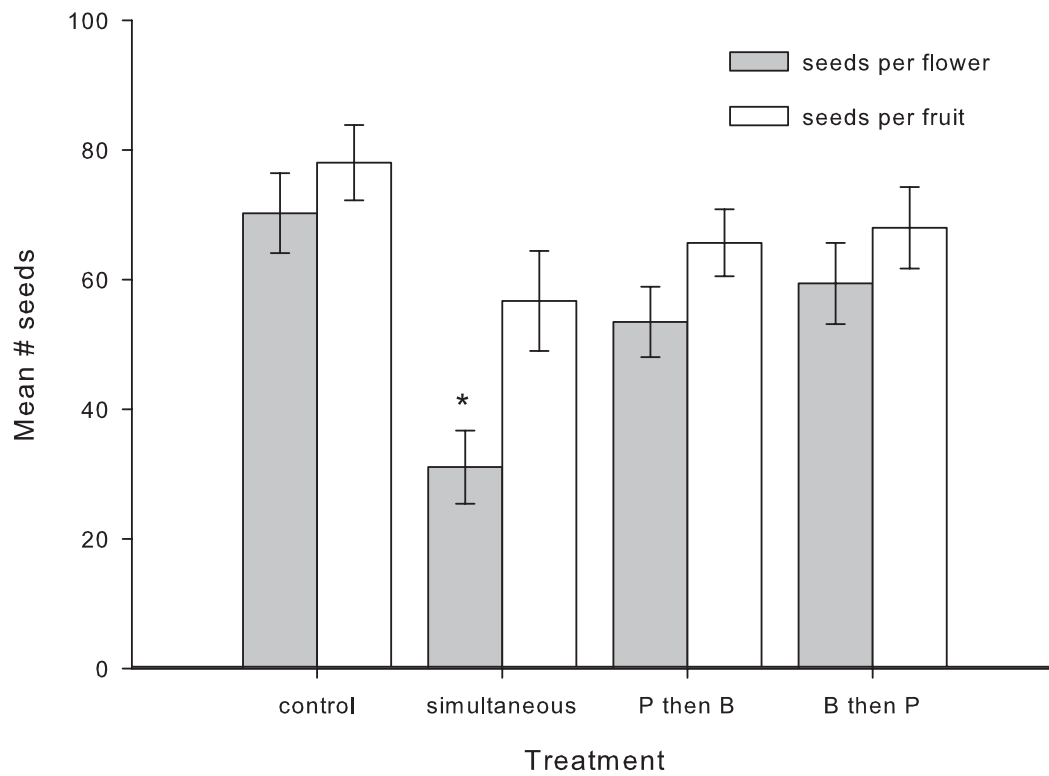


Figure 2.1 Average number of seeds per flower (grey bars) and seeds per fruit (white bars) compared in different hand-pollination treatments. Asterisk represents statistical difference from other treatments based on Tukey multiple comparisons. Error bars represent +/- 1 SE.

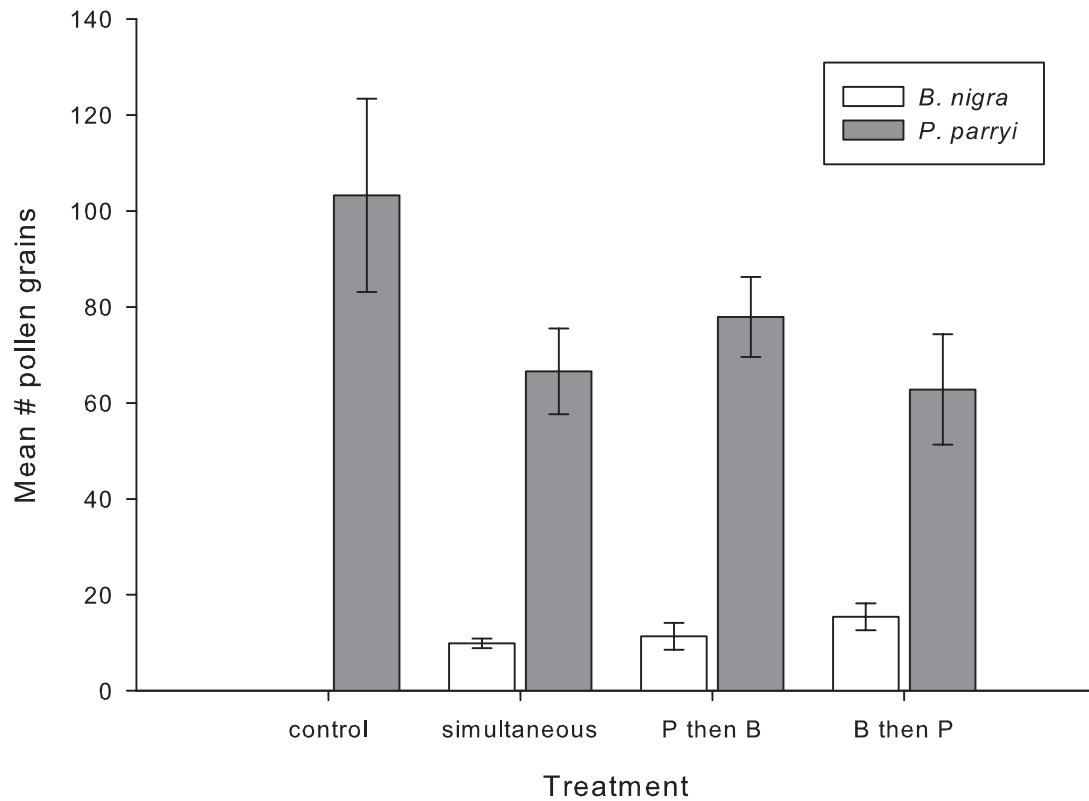


Figure 2.2 Average number of conspecific and *B. nigra* pollen grains deposited on *P. parryi* stigmas in 2013 hand-pollination treatments. No statistically significant difference found among treatments based on one-way ANOVA. Error bars represent +/- 1 SE.

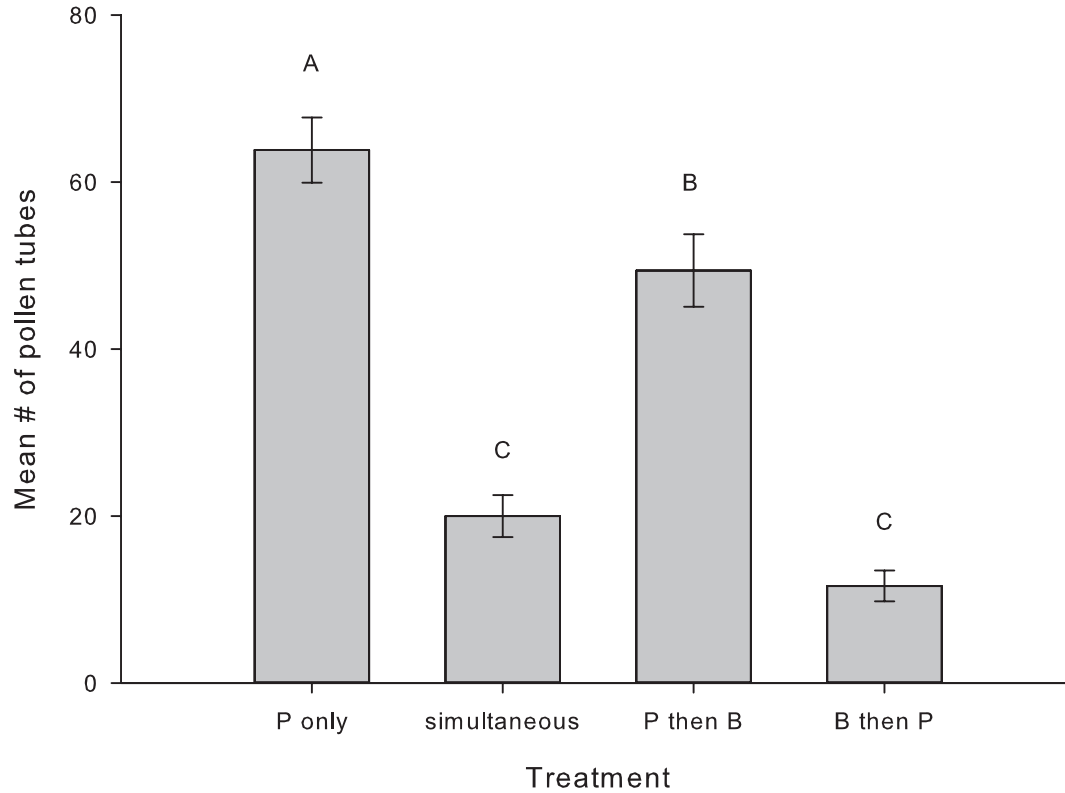


Figure 2.3. Average number of conspecific pollen tubes quantified at the base of *P. parryi* styles compared in different hand-pollination treatments. Different uppercase letters represent statistical differences among treatments based on Tukey multiple comparisons. Error bars represent +/- 1 SE.

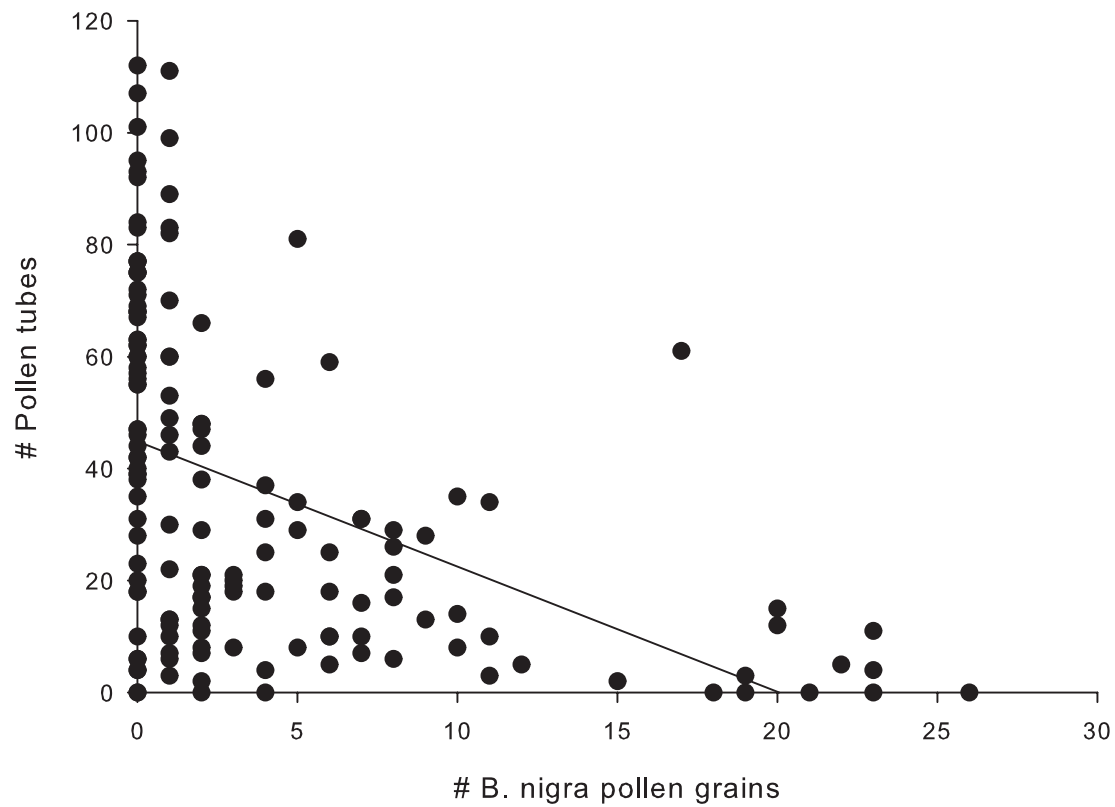


Figure 2.4. Regression of *P. parryi* pollen tube number on *B. nigra* pollen grain number for experimental *P. parryi* styles. Line indicates best-fit; $y = -2.2362x + 44.879$, $r^2 = 0.21$, $P < 0.0001$.

CHAPTER 3: Pollination of a native plant changes over the course of biological invasion: a field experiment

INTRODUCTION:

Exotic plants may inhibit the growth of native species by competing for vegetative resources (Levine et al. 2003, Gioria and Osborne 2014), but they may also impact the plant-pollinator mutualisms that many native plants rely on for successful reproduction (Traveset and Richardson 2006, Bjerknes et al. 2007). Exotic plant species may decrease native pollination and reproductive success via two important mechanisms. First, alien plants may influence the quantity of pollinator visits native plants receive by usurping pollinator attention and attracting visitors away from native flowers (eg. Chittka and Schurkens 2001, Brown et al. 2002, Totland et al. 2006). Secondly, as invasive species become more abundant, they may interfere with pollinator visit quality by increasing interspecific pollen transfer (Morales and Traveset 2008), resulting in increased amounts of heterospecific pollen placement on native stigmas and loss of native pollen to exotics (Feinsinger and Tiebout 1991, Bell et al. 2005; Murcia and Feinsinger 1996, Morales and Traveset 2008).

Exotic plants are likely to have varying effects on a native community at different stages of the invasion process (Melbourne et al. 2007). During the initial period of alien plant establishment, density is usually low and may have little influence on pollinator behavior and therefore, native reproductive success. However, as invasive abundance increases, the pollinator-mediated interactions between natives and exotics may change. For example, pollinator visit quantity may simply decrease as a linear function of invasive plant density if competition gradually becomes more intense (eg. Muñoz and Caveires 2008, Flanagan et al. 2010). Alternatively, invasive effects on pollinator visits may shift from facilitative to

competitive when exotic plants act as “magnet species” (Thomson 1978, Moeller 2004, Molina-Montenegro et al. 2008) at low to moderate densities, but eventually outcompete natives for pollinator attention once their abundance becomes overwhelmingly high (Rathke 1983, Mitchell et al. 2009). Pollinator visit quality on the other hand, is expected to decrease monotonically with increasing invasive density (Morales and Traveset 2008).

Native reproductive fitness may also vary with distance to an invasion. Given that the mobility of animal pollinators allows for plants to interact even when species are not completely sympatric, native plant pollination may be influenced by invasive species that have not yet infiltrated a plant community. Individuals near to an invasive species may experience greater fitness reductions than distant individuals if competition is strong and, similarly, facilitative interactions may be more pronounced near the leading edge of an invasion if interactions between species are positive. Moreover, interactions between species may be more complex depending on spatial scale. For example, showy competitors could facilitate visitation by attracting pollinators from outside the floral neighborhood, yet might compete on a local scale via mechanisms like interspecific pollen transfer (Morales and Traveset 2009, Seifan et al. 2014).

Recent studies have demonstrated that competition among sympatric species for pollination may be influenced by relative plant abundance (eg. Caruso 2002, Ghazoul 2006, Takakura et al. 2009, Seifan et al. 2014), however few have looked specifically at the role of invasive plants (Munoz and Cavieres 2008, Flanagan et al. 2010, Dietzsch et al 2011, King and Sargent 2012, Sun et al. 2013). Fewer still used both an experimental approach and seed set data as a measure of reproductive fitness (Muñoz and Cavieres 2008, Flanagan et al. 2010, Sun et al. 2013). These three studies examined the effect of invasive abundance (either absolute or relative to the native), but did not consider the impact of distance to the nearest invasive for plants in control

treatments. Furthermore, none of these studies examined how foreign pollen receipt on native stigmas was influenced by invasive plant density. Quantifying interspecific pollen transfer between alien and native plants is essential for identifying the potential mechanisms of competition between species since reductions in native visit quality are possible even when the frequency of pollinator visits is not affected (Waser 1983; Larson et al. 2006; Lopezaraiza-Mikel et al. 2007).

In this study we experimentally test whether variation in abundance of the invasive species *Brassica nigra* (Brassicaceae) has an effect on the reproductive success of the sympatric native, *Phacelia parryi* (Boraginaceae). By manipulating the quantity of *B. nigra* flowers around potted *P. parryi* individuals in a field setting, we simulated four stages in invasion: native plants far from the invasion, native plants near the invasion, the invasive species intermixed with the native species at low density, and the invasive species intermixed with the native species at high density. We measured 1) pollinator visitation to the native species; 2) the extent of invasive pollen deposition on native stigmas; and 3) native reproductive fitness (ie. seed set). In doing so we provide unusual mechanistic insights into pollinator-mediated interactions between native and exotic plants over the course of a plant invasion.

MATERIALS AND METHODS

Study Species

Phacelia parryi Torr. (Boraginaceae) is an annual herb native to coastal sage scrub and chaparral ecosystems throughout southern and Baja California and is especially common on open, recently burned slopes. Flowers are self-compatible but exhibit signs of inbreeding depression when self-pollinated, suggesting that this species relies on pollinators for transfer of

outcross pollen (Bruckman and Campbell 2014). In Orange County, California, *P. parryi* flowers from late February to early May. The inflorescence is a helicoid cyme of bell-shaped flowers each 1 to 2 centimeters long. The showy violet to royal blue flowers last 3-4 days. Daily floral display size depends on plant size and can vary from just a few individual flowers to several dozen.

Black mustard (*Brassica nigra* (L.) W. D. J. Koch) was used in this study as a model invasive species and is often found growing near *P. parryi*. It is a ubiquitous annual weed of Mediterranean origin that occurs in disturbed areas throughout North America (Westman et al. 1999). Black mustard plants have numerous terminal inflorescences of yellow, hermaphroditic, self-incompatible flowers that attract a variety of insect pollinators (Conner & Neumeier 1995). In *B. nigra*'s invasive range stands are often large and dense and secrete allelochemicals that inhibit germination of neighboring plant species (Bell & Muller 1973). *Brassica nigra* is a winter annual forb and flowers primarily between the months of February and May in southern California, overlapping substantially with the phenology of *P. parryi*. Although individual *B. nigra* flowers are small, the floral display of a single plant may contain several hundred flowers.

Experimental Design

In spring of 2013, 37 potted *P. parryi* plants were used in a field experiment at the UC Irvine Arboretum (33° 39' 49" N, 117° 51' 10" W) to determine the effects of *B. nigra* on native pollination. A 38m x 25m area of bare ground was hand-seeded with *B. nigra*. It was then manually thinned to create patches (ranging from approximately 5m² to 25m²) that represented four interspersed invasive treatments, each replicated five times. Between 1 and 3 potted *P. parryi* plants were placed within each patch, separated by a minimum of 3 m. "Low density" patches contained between 50 and 500 *B. nigra* flowers per meter² while "high density"

treatments contained more than 1,000 flowers per meter². In both of those treatments, *P. parryi* pots were placed so that the appropriate *B. nigra* density surrounded them by at least 0.5 meters on every side. “Near” treatments contained no *B. nigra* plants within the patch with the potted plants of *P. parryi* within 2.8 m of an invasive stand, and “far” treatments also contained no *B. nigra* in the patch with the *P. parryi* pots ranging from 5.2 m to 6.5 m to the invasive. The purpose of the latter two treatments was to investigate whether distance from invasive patches influenced reproductive success in *P. parryi*. The number of open *P. parryi* flowers on individual experimental plants ranged from 30 to 112. *B. nigra* densities were maintained by thinning and removing plants as needed throughout the course of the study. Flowering plants other than *B. nigra* were weeded out of field plots to control for effects that other flowers may have on pollinator activity.

The experiment was repeated in spring of 2014 with 29 potted *P. parryi* plants and patches were established in the same manner described above. Due to the severe drought conditions of 2014, the study site was irrigated to maintain flowering populations of *B. nigra* for the duration of our experiment. The addition of water in this year increased average *B. nigra* floral display size in high-density treatments from 1250 flowers in 2013 to 1675 flowers in 2014. Invasive densities in low-density treatments remained similar between years.

Pollinator Visitation

Pollinator observations were conducted between 8:00 and 15:00 on 12 days between 3-April and 1-May in 2013 and on 8 days between 8-April and 22-April in 2014. We did not conduct observations if it was raining or if temperatures were below 12 °C. During each observation period, one individual *P. parryi* plant from each of the four invasion treatments was

observed simultaneously by a different research participant in order to control for temporal variation in pollinator visitation. Observations of pollination lasted 30 minutes, and we defined a pollination event as any contact of an insect with anthers or stigmas on an individual *P. parryi* flower. We recorded the insect visitor to the lowest field-identifiable taxonomic category. At the end of the 30 minutes, we moved on to the next four *P. parryi* individuals, chosen at random, and the process was repeated. Each individual *P. parryi* plant was observed between 1 and 5 times for the duration of the study. We recorded 219 individual insect foraging bouts (775 visits) to 37 *P. parryi* plants over 128 half-hour observation periods in 2013 and 381 individual insect foraging bouts (2347 visits) to 29 *P. parryi* plants over 67 half-hour observation periods in 2014.

Honeybee, bumblebee (genus *Bombus*), solitary bee (families Halictidae and Apidae), syrphid fly (family Syrphidae) and total pollinator visitation rates were compared among invasive density treatments by calculating the mean visitation rates of each plant in each treatment. Plant means were then averaged to obtain a mean visitation rate for each experimental patch. Visitation rates were calculated as the number of visits per flower per hour. Since visitor composition was different between the two years of the study, we analyzed the years separately. We used a one-way ANOVA supplemented with Tukey Post-hoc tests to compare the four treatments with patch as the unit of replication.

Pollen Deposition

On 10-April-2013, two flowers from each of 27 individual *P. parryi* plants from all treatments (6 far, 6 high, 7 low, 8 near) were tagged as mature virgin buds. These flowers were exposed to open pollination and harvested 48 hours later, once flowers had opened and reached female phase. Stigmas were then stained with basic fuchsin gel (Kearns and Inouye 1993) and examined under a compound microscope to determine the number of conspecific and *B. nigra*

pollen grains that had been deposited by insect visitors. The same procedure was carried out on 18-April-2014, with four flowers sampled per plant on 21 *P. parryi* individuals.

Pollen deposition on *P. parryi* stigmas was compared among invasion treatments by calculating the mean number of conspecific and *B. nigra* pollen grains on the stigmas of each plant for each patch. Patch means were then compared among treatments using a two-way ANOVA with year and treatment as factors. Least squares means for the four treatments were compared with a Tukey Post-hoc adjustment. Pollen quantities were log transformed prior to analysis to meet the assumption of normality.

Seed Set

At the beginning of both field seasons, three flower buds were tagged on each experimental *P. parryi* plant to examine the effect of invasive density on seed set. All buds were tagged on the same day during both field seasons to control for temporal variation. If tagged flowers set fruit, seed capsules were collected approximately three weeks later and seeds were counted. The final number of fruits collected per plant varied from 0 to 3 due to the loss of individual plants and/or branches that occurred over the course of the study. In 2013, we collected a total of 80 fruits from 31 *P. parryi* plants while in 2014, we collected a total of 67 fruits from 23 *P. parryi* plants. Seed set was first averaged across flowers on a plant and then over plants to yield a mean patch seed set. The data were then analyzed using a two-way ANOVA with Tukey post-hoc adjustments as described above under Pollen Deposition experiment.

Predicted Effect of Pollen Deposition on Pollen Tube Growth

In order to assess further how invasive pollen deposition may influence *P. parryi* reproductive success, data from earlier research were used to predict how conspecific and *B.*

nigra pollen deposition might influence pollen tube growth in the *P. parryi* plants used in this field study. In a previous experiment (Chapter 2, Bruckman and Campbell, in review) we used a series of hand-pollinations to compare *P. parryi* pollen tube growth in flowers pollinated with pure conspecific pollen and flowers pollinated with a combination of conspecific and *B. nigra* pollen loads. Pollen tube growth in *P. parryi* was influenced by both conspecific and invasive pollen deposition and the detrimental effects of *B. nigra* pollen on *P. parryi* pollen tube growth were strong when conspecific pollen deposition was also high, but weak when conspecific pollen loads were small. Multiple regression on our hand-pollination data yielded the following fit to with the number of pollen tubes as a function of number of conspecific pollen grains, number of *B. nigra* pollen grains, and the interaction term of the two preceding variables:

$$\text{pollen tubes} = 0.541 (\text{conspecific}) + 0.035 (B. \textit{nigra}) - 0.026 (\text{conspecific} * B. \textit{nigra})$$

We then used the fitted regression equation (above) to predict how pollen tube numbers would compare among invasive treatments from this study given the pollen quantities measured on experimental *P. parryi* stigmas (described above under Pollen Deposition).

RESULTS

Pollinator visitation

The European honeybee *Apis mellifera* dominated pollinator visitation to *P. parryi* in 2013, making approximately 95% of all flower visits (Table 1), with the remaining 5% of flower visits divided between native bees in the genera *Lasioglossum*, *Ceratina* and *Bombus*. Pollinator

visitation rates in 2013 were not significantly influenced by invasion treatment ($F_{3,19} = 0.63$, $P = 0.61$). In 2014, the pollinator assemblage was more diverse, with bumblebees most common and making up 53.4% of all flower visits, while honeybees, solitary bees and syrphid flies comprised 35.1%, 8%, and 3.5% of visits, respectively (Table 1). Invasion treatment had a significant effect on total pollinator visitation rate to *P. parryi* in 2014 (one way ANOVA; $F_{3,19} = 3.64$, $P = 0.035$) with plants in the ‘near’ treatment receiving approximately three times as many visits/flower/hour as those in the ‘far’ treatment (Fig. 1). *P. parryi* plants in the ‘near’ treatment received approximately 7 times more honeybee visits/flower/hour as those in the ‘high’ treatment (significant at 0.05 level with Tukey post-hoc test; Fig. 1, Table 2). Visitation by bumblebees, solitary bees and syrphid flies were similar across invasive density treatments in 2014.

Pollen Deposition

Invasive plant treatment influenced both conspecific and heterospecific pollen deposition. For conspecific deposition, there was a significant year by treatment interaction ($F_{3,24} = 5.36$, $P = 0.0057$), so we also analyzed results separately by year using one-way ANOVA. In 2013, no differences across treatment were detected ($P = 0.16$). In 2014, treatments differed in conspecific pollen deposition ($F_{3,10} = 6.29$, $P = 0.0114$) with flowers in the ‘far’ treatment receiving approximately 10 times fewer conspecific pollen grains on average than flowers in either the ‘near’ or ‘low’ invasive density treatments (Fig. 2). Plants in the high invasive density treatment showed a trend for lower conspecific pollen compared to plants in the ‘near’ and ‘low’ treatments, but the differences were not statistically significant (Fig. 2). For *B. nigra* pollen deposition, the effect of treatment did not vary between years (interaction $F_{3,24} = 1.39$, $P = 0.2709$). Deposition of invasive pollen increased monotonically from the ‘far’ to ‘near’ to ‘low’ to ‘high’ density treatment, with significant differences in all cases except between the ‘far’ and ‘near’ and between

the 'near' and 'low' treatments (Fig. 2). Both conspecific and *B. nigra* pollen showed higher deposition on *P. parryi* flowers in 2014 than in 2013 ($P = 0.0014$ and $P = 0.0475$, respectively; Fig. 2), consistent with the higher pollinator visitation rates that year (Fig. 1).

Seed Set:

Seed set in *Phacelia parryi* varied both with invasive plant treatment and with year (two-way ANOVA treatment: $F_{3,27} = 4.10$, $P = 0.0161$, year: $F_{1,27} = 6.25$, $P = 0.0188$; Fig. 3). Flowers on plants in the 'low' and 'near' treatments produced approximately 2.5 times as many seeds as those on plants in the 'far' density treatment (Fig. 3). 'High' density plants produced an intermediate number of seeds that was statistically indistinguishable from the other treatments (Fig. 3).

Predicted Effect of Pollen Deposition on Pollen Tube Growth:

Predicted pollen tube numbers were highest for *P. parryi* plants in the near and low treatments (mean = 23.8 and 23.9, respectively) and lowest in far treatment plants (mean = 7.4) (Fig. 4). High invasive density treatment *P. parryi* individuals showed an intermediate number of predicted pollen tubes (mean = 12.2) (Fig. 4).

DISCUSSION

Pollinator mediated interactions between native and invasive plant species may change over the course of a plant invasion (eg. King and Sargent 2012). As alien plant populations establish and spread through host communities, native reproductive success will likely be affected first by the distance to an invasion and later to increasing invasive densities. In this

study, we found that pollinator visitation, pollen deposition, and seed set in a native plant were all influenced by the simulated stage of exotic plant invasion. Native individuals near invasive patches and within areas of low invasive density showed the highest reproductive fitness in our experiments, resulting from facilitation of pollinator visits. However, natives within areas of high invasive density received high levels of heterospecific pollen deposition, which tends to lower seed set. Isolation from an invasive patch was also detrimental to native fitness as individuals located relatively far from exotics suffered reduced seed set likely due to low pollinator visitation and conspecific pollen receipt.

Pollinator visitation rates to *P. parryi* were significantly influenced by invasive density treatment in our 2014 field season but not in 2013. Differences between years are likely attributable to the more diverse and evenly distributed insect species in the pollinator communities visiting *P. parryi* and higher native pollinator presence in 2014 (Table 1). It is not clear what factors beyond natural annual variation may have influenced pollinator assemblage composition between years, however, the addition of supplemental water to field plots in drought-stricken 2014 may have had an effect on the overall attractiveness of our field sites by creating an “oasis” of green vegetation within an otherwise parched landscape.

In 2014, *P. parryi* individuals in ‘near’ treatments received significantly more pollinator visits than individuals in the ‘far’ treatments. This finding suggests that *P. parryi* plants in close proximity to a patch of this showy invasive species (but not within it) may reap the benefits of being within an attractive environment without necessarily experiencing high levels of competition for pollinator attention. *P. parryi* individuals situated within ‘far’ treatments however, suffered reductions in pollinator visitation, perhaps being overlooked by pollinators traveling to areas of higher flower concentrations. Visitation specifically by honeybees was also

influenced by *B. nigra* abundance in our study, and *P. parryi* individuals within ‘high’ treatments received significantly lower honeybee visitation than individuals in the ‘near’ treatments.

Honeybees foraged frequently on *B. nigra* flowers and it is possible that in very high densities, *B. nigra* may usurp the attention of highly constant pollinators like honeybees (Free 1963) as a result of overwhelming abundance or by visually obstructing the view of *P. parryi* floral displays. Bumblebee visitation, conversely, was unaffected by *B. nigra* density. Bumblebees, which were abundant in 2014, were not frequently observed visiting *B. nigra* flowers (personal observation).

Despite the evidence indicating total pollinator visitation rate was not reduced in the presence of a high density of invasives, frequent pollinator visitation may not necessarily translate into high reproductive fitness. In both our 2013 and 2014 field seasons, we found that *P. parryi* stigmas within high invasive density treatments received significantly more *B. nigra* pollen than *P. parryi* plants in all other treatments (Fig. 2). Sun et al. (2013) found similar results when *Sonchus arvensis* visitation was facilitated by high densities of invasive, *Solidago canadensis*, yet seed set did not follow the same trend. The high levels of invasive pollen and low levels of conspecific pollen they found on pollinator bodies suggested that high visitation rates were counteracted by interspecific pollen transfer. The deposition of foreign pollen on stigmas can potentially reduce reproductive success by interfering with pollen germination, pollen tube growth or ovule fertilization (Morales and Traveset 2008). In a previous study (Bruckman and Campbell, in review), we found that *B. nigra* pollen does indeed impair seed production in *P. parryi*. Through a series of hand pollinations we demonstrated that the simultaneous placement of mixed *B. nigra* and conspecific pollen loads on *P. parryi* stigmas resulted in a decrease in both pollen tube growth and seed set compared to pure conspecific

pollen loads, suggesting that visitation quality may be just as important as visitation quantity for *P. parryi*.

Conspecific pollen deposition in *P. parryi* was not influenced by invasive density treatment during our 2013 field season, however, in 2014, *P. parryi* stigmas in ‘far’ treatments received significantly fewer conspecific pollen grains, on average, compared to plants that were placed within or close to *B. nigra* patches. The reduction in conspecific pollen receipt for flowers relatively isolated from patches of *B. nigra* appear to reflect the low pollinator visitation rates recorded during field observations. ‘Far’ treatment plants received particularly low visitation from native pollinators (Table 2) such as bumblebees and solitary bees, which are more effective at transferring *P. parryi* pollen than nonnative honeybees (Bruckman and Campbell 2014).

P. parryi seed set was influenced by invasive density treatment over both our 2013 and 2014 field seasons with low seed quantities found in ‘far’ *P. parryi* individuals. The reduction in reproductive success in *P. parryi* plants isolated from *B. nigra* patches is not surprising given the infrequent pollinator visitation and low levels of conspecific pollen deposition we observed. However, in an attempt to control for the effects of *B. nigra*, we removed all other heterospecific flowers from our study site, which may have resulted in a somewhat unrealistic distribution of floral resources. Therefore, it is worth noting that in a more natural setting, flowers from other intervening plant species may attenuate the negative effects of isolation observed in this study. ‘High’ treatment natives produced a quantity of seeds intermediate between ‘far’ plants and ‘near’/‘low’ plants indicating the costs likely associated with high heterospecific pollen deposition. Our calculations of predicted pollen tube numbers provide a mechanisms for this pattern in seed set, with low pollen tube numbers predicted for ‘far’ treatment *P. parryi* plants, high pollen tube numbers predicted in ‘near’ and ‘low’ treatment plants, and intermediate

numbers of pollen tubes predicted for ‘high’ treatment plants as a result of high heterospecific deposition in these areas of high invasive infestation.

The facilitation we observed in this study appears to be functioning through the effects *B. nigra* has on pollinator visitation quantity. In general, pollinators preferentially visit large flower patches that maximize their foraging efficiency (Silander and Primack 1978, Schaffer and Schaffer 1979, Thomson et al. 1982, Eckhart 1991, Goulson 1999). Exotic plants with large, showy floral displays such as *B. nigra* may, therefore, attract pollinators from the surrounding habitat, leaving solitary plants less likely to receive pollinator services. In our study, *P. parryi* plants within or near a patch of *B. nigra* received more frequent insect visits, higher quantities of conspecific pollen deposition and greater seed set than plants that were relatively isolated. Other studies investigating the effects of invasive density on native pollination have also found evidence of facilitation, particularly at low densities (Muñoz and Cavieres 2008, Sun et al. 2013). Still, a few studies demonstrate that native pollination decreases with increasing exotic density (Flanagan et al. 2010, Dietzsch et al. 2011, King and Sargent 2012) suggesting the direction of native-invasive interactions may be context dependent and that further studies are needed in order to draw more general conclusions.

Our study reveals how pollinator-mediated interactions between invasive and native plant species could change over the course of an invasion. We found that *P. parryi* suffers fitness reductions when relatively isolated from *B. nigra* and when surrounded by high densities of it. This finding suggests that exotic plants may impair native pollination most in late stages of the invasion process through effects of heterospecific pollen deposition and possibly at the beginning of the invasion process if large stands of *B. nigra* attract pollinators away from smaller native patches on a relatively large spatial scale. The results from this experiment demonstrate changes

in pollination of *P. parryi* within the context of variable invasive presence and density, however this represents a specific scenario that does not include populations of other sympatric flowering plants. In a more natural setting, *B. nigra* may influence *P. parryi* reproductive success through a different mechanism. In a previous study (Bruckman and Campbell 2014), we found that the loss of other native and nonnative heterospecifics due to encroachment from highly invasive plants like *B. nigra* could result in decreased reproductive fitness in *P. parryi* due to less effective pollination.

Conclusions:

The findings from this study demonstrate how ecological relationships may change over the course of a biological invasion. The pollination and reproductive fitness of native, *Phacelia parryi* varied under different simulated stages of plant invasion. Furthermore, the effects of invasive plant density on native pollination appeared to be contingent upon the mechanism of pollinator competition. Although pollinator visit quantity in *P. parryi* was positively influenced by near proximity to the invasive plant, *Brassica nigra*, native pollinator visit quality was reduced under conditions of high infestation. Our seed set data suggest that the facilitative effects of our model invasive may outweigh the harm of heterospecific pollen deposition, however, the trends toward low native pollen deposition and low native seed set under conditions of high invasive density could potentially be magnified under distinct environmental circumstances. Future research should focus on how pollinator foraging patterns change over the course of plant invasions.

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TABLE 3.1. List of insect pollinator taxa, number of individual visits (N) and percentage (%) of total number of visits made by each insect taxon to experimental *P. parryi* plants in 2013 and 2014 field seasons.

Year	Insect Type	N	%
2013	<i>Apis mellifera</i>	738	95.2
	<i>Bombus spp.</i>	5	0.7
	solitary bees	32	4.1
	total	775	
2014	<i>Apis mellifera</i>	823	35.1
	<i>Bombus spp.</i>	1253	53.4
	solitary bees	189	8
	syrphid flies	82	3.5
	total	2347	

TABLE 3.2. Proportion of visits made by different types of insect pollinators to *Phacelia parryi* plants for invasive density treatments in 2013 and 2014 field seasons.

Year	Treatment	honeybees	bumblebees	solitary bees	syrphid flies
2013	High	0.96	0	0.04	0
	Low	0.96	0.02	0.02	0
	Near	0.93	0	0.07	0
	Far	1	0	0	0
2014	High	0.13	0.78	0.08	0.01
	Low	0.37	0.51	0.1	0.03
	Near	0.41	0.46	0.1	0.03
	Far	0.58	0.25	0.06	0.11

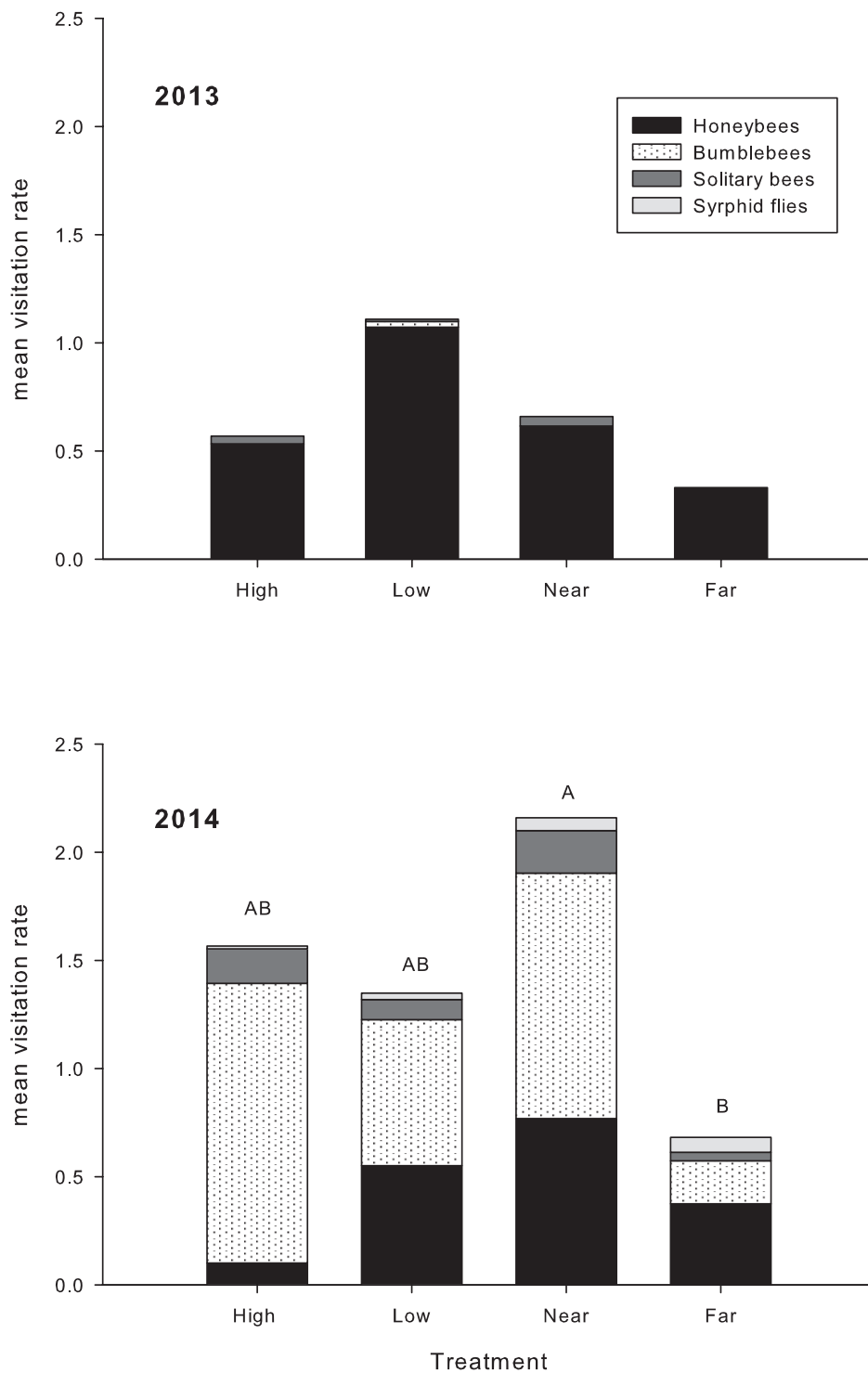


Figure 3.1. Comparison of mean visitation rates by different types of pollinators to *P. parryi* for each invasive treatment for 2013 and 2014 field seasons. Different uppercase letters in 2014 represent statistical differences among treatments based on Tukey multiple comparisons.

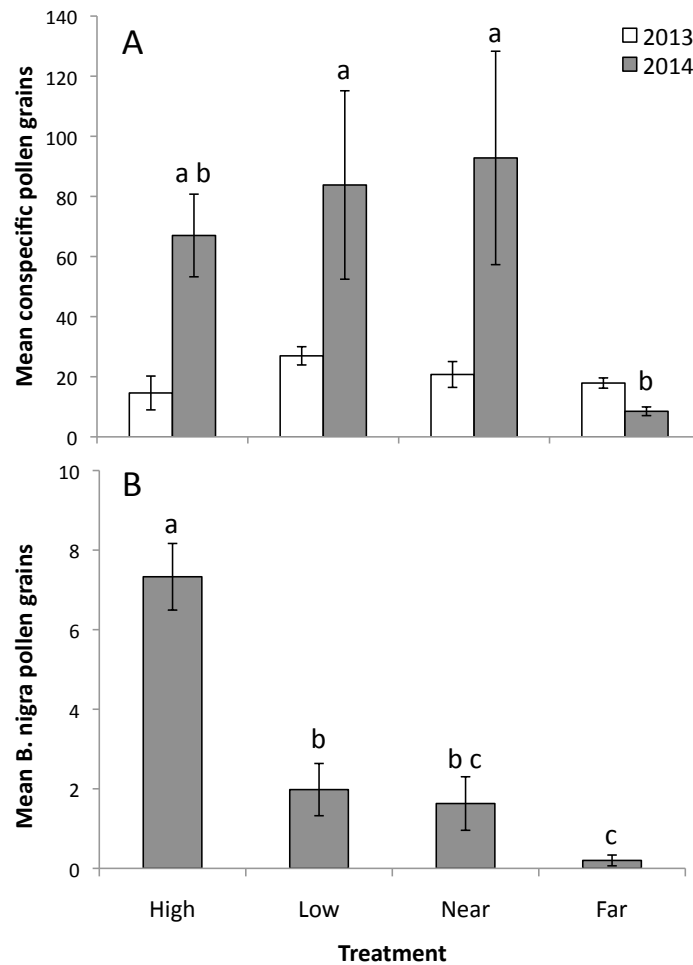


Figure 3.2 Comparison of pollen deposition on *P. parryi* stigmas among invasive treatments. Conspecific pollen deposition (A) reflects separate analyses for 2013 and 2014 field seasons while *B. nigra* pollen deposition (B) reflects combined 2013 and 2014 data. Different lowercase letters represent statistical differences among treatments based on Tukey multiple comparisons. Error bars represent +/- 1 SE.

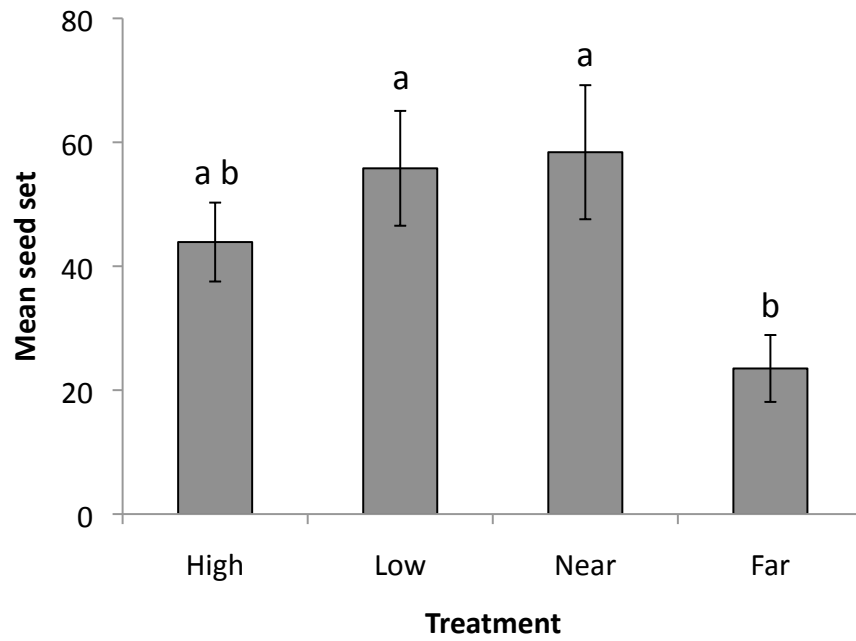


Figure 3.3. Comparison of mean seed set for *P. parryi* among invasive treatments reflecting combined 2013 and 2014 data. Different lowercase letters represent statistical differences among treatments based on Tukey multiple comparisons. Error bars represent +/- 1 SE.

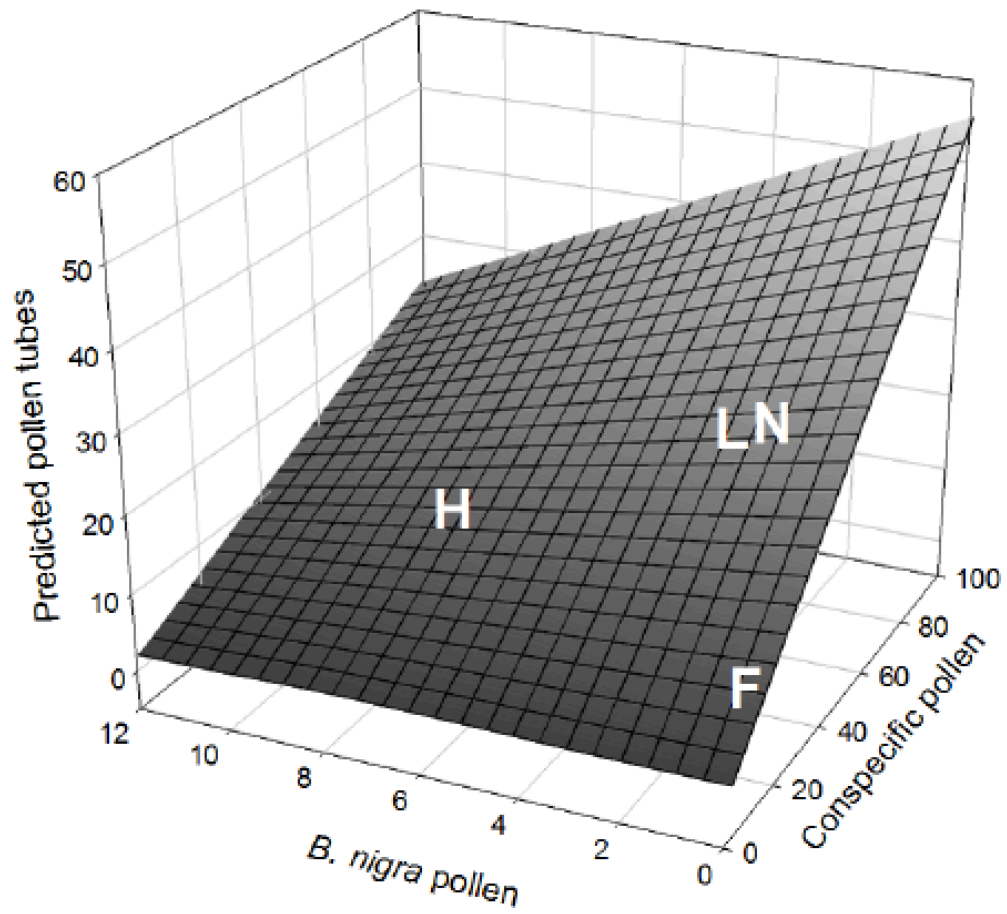


Figure 3.4. Predicted *P. parryi* pollen tube quantities as a function of conspecific and *B. nigra* pollen deposition. Letters represent average predicted pollen tubes for high (H), low (L), near (N) and far (F) invasive density treatments.