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Negative effects of heterospecific pollen receipt vary with abiotic conditions: ecological and evolutionary implications

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● **Background and Aims** Studies that have evaluated the effects of heterospecific pollen (HP) receipt on plant reproductive success have generally overlooked the variability of the natural abiotic environment in which plants grow. Variability in abiotic conditions, such as light and water availability, has the potential to affect pollen–stigma interactions (i.e. conspecific pollen germination and performance), which will probably influence the effects of HP receipt. Thus, a more complete understanding of the extent, strength and consequences of plant–plant interactions via HP transfer requires better consideration of the range of abiotic conditions in which these interactions occur. This study addresses this issue by evaluating the effects of two HP donors (*Tamonea curassavica* and *Angelonia angustifolia*) on the reproductive success of *Cuphea gaumeri*, an endemic species of the Yucatan Peninsula.
● **Methods** Mixed (conspecific pollen and HP) and pure (conspecific pollen only) hand-pollinations were conducted under varying conditions of water and light availability in a full factorial design. Reproductive success was measured as the number of pollen tubes that reached the bottom of the style.
● **Key Results** Only one of the two HP donors had a significant effect on *C. gaumeri* reproductive success, but this effect was dependent on water and light availability. Specifically, HP receipt caused a decrease in pollen tube growth, but only when the availability of water, light or both was low, and not when the availability of both resources was high.
● **Conclusions** The results show that the outcome of interspecific post-pollination interactions via HP transfer can be context-dependent and vary with abiotic conditions, thus suggesting that abiotic effects in natural populations may be under-estimated. Such context-dependency could lead to spatial and temporal mosaics in the ecological and evolutionary consequences of post-pollination interactions.

Key words: Co-flowering species*, Cuphea gaumeri*, heterospecific pollen, pollen tube growth, pollination ecology, pollinator sharing, post-pollination interactions, reproductive success, Yucatan Peninsula, *Tamonea curassavica*, *Angelonia angustifolia*, *Cuphea gaumeri*.

INTRODUCTION

Plants rarely occur alone and often co-occur and co-flower with other plant species in a community, establishing intricate networks of indirect interactions via pollinators (e.g. Olesen and Jordano, 2002; Bascompte *et al.*, 2006; Bascompte and Jordano, 2007; Campos-Navarrete *et al.*, 2013; Albrecht *et al.*, 2014). Understanding the mechanisms and factors that influence the outcome of interspecific plant interactions as well as their importance in generating (i.e. floral evolution; e.g. Caruso, 2000; Mitchell *et al.*, 2009; Hopkins and Rausher, 2012) and organizing (i.e. community assembly; e.g. Ghazoul, 2006; Lazaro and Totland, 2010; McEwen and Vamosi, 2010; de Jager *et al.*, 2011) plant diversity is thus crucial if we aim to better comprehend the ecological processes that shape plant communities in nature (Geber and Moeller, 2006; Sargent and Ackerly, 2008). While the ecology and evolutionary importance of pre-pollination interactions (i.e. facilitation and competition for pollinators) have been well studied (e.g. Fishman and Wyatt, 1999; Moeller, 2004; Ghazoul, 2006; Caruso, 2000; Mitchell *et al.*, 2009), much less is known about the ecology

and evolutionary consequences of the post-pollination interactions that take place via pollen-style and/or pollen–pollen interactions on the stigma (but see Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013). These post-pollination interactions have the potential to reinforce or undermine pre-pollination interactions (Galen and Gregory, 1989; Sargent and Ackerly, 2008) and thus can play a central role in evolutionary and community assembly processes (Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013). However, the exact mechanisms, consequences and potential modifiers of post-pollination interactions are still poorly understood. Studies of post-pollination interactions are becoming even more important as we seek to predict how plants will respond to human disturbances that are gradually altering the composition of plant and pollinator communities and the extent to which they interact, directly and indirectly (e.g. Traveset and Richardson, 2006; Bjerknes *et al.*, 2007; Memmott *et al.*, 2007; Hegland *et al.*, 2009).

Detailed studies of pollen transfer between species (hereafter, 'heterospecific pollen [HP] transfer') within and across

communities have revealed that post-pollination interactions can be far more common and intense than previously acknowledged (e.g. Montgomery and Rathcke, 2012; Ashman and Arceo-Gómez, 2013; Fang and Huang, 2013). For instance, a review of the incidence and magnitude of HP transfer across species showed that plant species can receive HP in >70 % of its flowers and in amounts that surpass 60 % of the total pollen load (Ashman and Arceo-Gómez, 2013). Furthermore, HP receipt reduces seed production by 20 %, on average (Ashman and Arceo-Gómez, 2013), and in some species can cause complete reproductive failure (Thomson *et al.*, 1982) by physically or chemically interfering with conspecific pollen performance (reviewed in Morales and Traveset, 2008). What is more, recent studies have shown that the negative effects of HP receipt can increase with the diversity of the HP load (Arceo-Gómez and Ashman, 2011), be greater on self-pollen compared with outcross conspecific pollen (Arceo-Gómez and Ashman, 2014) and be stronger if HP is deposited prior to conspecific pollen (Waser and Fugate, 1986; but see Arceo-Gómez and Ashman, 2014). However, our current understanding of HP effects comes primarily from studies that have evaluated these effects in plants under the same environmental conditions (reviewed in Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013) or through *in vitro* testing of pollen extracts interfering with fertilization success (e.g. Sukhada and Jayachandra, 1980; Murphy and Aarssen, 1995). These type of studies have been the norm, even though plants typically experience wide environmental variability in nature, both spatially and temporally (Chapin *et al.*, 1987; Davis *et al.*, 2000), which can directly impact pollination success (Herrera, 1995). In fact, the availability of water (Lush *et al.*, 1998) and light (e.g. Feng *et al.*, 2000; Campbell *et al.*, 2001) as well as temperature (Lankinen, 2001) can influence conspecific pollen germination and pollen tube growth. For instance, limited water and light availability caused a decrease in pollen germination rate in *Nicotiana alata* (Lush *et al.*, 1998) and pollen tube growth in some wheat cultivars (Campbell *et al.*, 2001), respectively. Thus, it would be logical to expect that not only conspecific pollen performance but also its competitive ability (i.e. capacity to respond against interference by HP), and thus the effects of HP would vary with variation in abiotic conditions, with HP effects being stronger (e.g. reduced conspecific pollen tube growth and seed production) in stressful abiotic conditions (i.e. when light and water are scarce). However, to our knowledge, this has never been tested and thus the full extent of HP effects in natural communities has not yet been explored.

Furthermore, the negative effects of HP receipt have been proposed as a strong selective force promoting floral evolution and shaping co-flowering communities (e.g. Levin and Anderson, 1970; Waser, 1978; Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013), but variation in HP effects with varying abiotic conditions could lead to temporal and spatial variability in its strength as an evolutionary force within and among populations. Thus, in order to have a better understanding of the ecological and evolutionary consequences of post-pollination interactions we need to evaluate how variation in HP effects relates to variation in abiotic conditions. In this study we aim to fill this gap in our understanding of post-pollination interactions by testing the effects of two HP donors (*Angelonia angustifolia* and *Tamonea curassavica*) on the reproductive success of *Cuphea gaumeri* under different light and water regimes. In doing so, we will reveal the potential for spatial and temporal mosaics in the outcome of plant–plant post-pollination interactions. We ask the following specific questions: (1) Does HP receipt decrease pollen tube growth in *C. gaumeri*? (2) Does the effect vary by HP donor? (3) Does the effect depend on the availability of water, light, or both?

MATERIALS AND METHODS

Study system

We used *Cuphea gaumeri* (Lythraceae), a self-compatible, insect-pollinated (mainly bees and butterflies) shrub endemic to the Yucatan Peninsula in Mexico (Celaya, 2012), as the pollen recipient. *Cuphea gaumeri* has small tubular flowers (∼6–8 mm long, 4–8 mm wide), floral display ranges from ten to 80 open flowers per plant, and fruits are dry and can produce up to 20 seeds (Celaya, 2012). This species can self-pollinate but produces very few seeds in the absence of pollinators (V. Parra-Tabla, pers. comm.). *Cuphea gaumeri* experiences wide heterogeneity, particularly in light and water availability, in the macro- and micro-environmental conditions in which it grows along its entire distribution range. For instance, it can be found in shaded environments below the canopy or near to sources of surface water that accumulate during the rainy season (up to 1000 mm month^{-1}), as well as in fully open, rocky and dry areas (<100 mm month^{-1}; Parra-Tabla *et al.*, 2015).

As HP donors we used *Angelonia angustifolia* (Scrophulariaceae) and *Tamonea curassavica* (Verbenaceae), two self-compatible, insect-pollinated, non-endemic shrubs that grow in the dry scrublands along the north coast of the Yucatan Peninsula in Mexico. Both HP donors grow and bloom synchronously with the focal species during the rainy season (July–November; Alonso *et al.*, 2013; Parra-Tabla *et al.*, 2015). All three species have similar flower colour (all purple flowers) and shape (all zygomorphic flowers; Alonso *et al.*, 2013) and are considered generalists in their pollination system. Pollinator sharing among all three species is high; pollinators mainly include bees and butterflies, although the plants can also be visited by flies and wasps, among other insects (V. Parra-Tabla *et al.*, unpubl. res.). All three species are never found together; *C. gaumeri* only coexists with one of the two HP donors (*A. angustifolia* or *T. curassavica*) at a time along its distribution range (Alonso *et al.*, 2013; Parra-Tabla *et al.*, 2015). High levels of natural HP transfer have been observed between *C. gaumeri* and the two HP donors (Ashman *et al.*, unpubl. res.) and previous evidence suggests that *C. gaumeri* seed production decreases in the presence of *A. angustifolia* and *T. curassavica* (Celaya, 2012).

Plant material and experimental design

To evaluate the effects of HP receipt, water and light availability and their interaction on *C. gaumeri* reproductive success, we conducted an experiment of full factorial design with three factors (hand-pollination, water and light treatments) of two levels each (control [conspecific pollen only] vs. mixed [conspecific pollen + HP] pollinations, low vs. high water

availability and low vs. high light exposure). However, since *C. gaumeri* coexists separately with each HP donor at different sites, we conducted two separate experiments, one to evaluate the effect of each HP donor. In each experiment we only used *C. gaumeri* plants that naturally co-flower with the respective HP donor since HP effects can be greater in recipient plants with no previous coexistence history with the HP donor (Arceo-Gómez *et al.*, UADY, Yucatan, Mexico, unpubl. res.), and this could have obscured the interpretation of our results. Thus, for each experiment we had 80 *C. gaumeri* plants that served as pollen recipients and 30 as conspecific pollen donors, as well as 20 HP donors. All plants were grown from seeds collected from two natural populations, Chicxulub (21° 08′ 13″ N; 89° 30′ 35″ W), where *C. gaumeri* grows along with *A. angustifolia*, and Dzemul (21° 17′ 31·92″ N; 89° 19′ 43·81″ W), where *C. gaumeri* and *T. curassavica* grow together. All pollen recipient plants were kept in mesh enclosures (see section Light and water treatments, below) for the duration of the experiment at the University of Yucatan, Mexico, and this prevented natural pollen deposition by pollinators.

Hand-pollination treatments

To evaluate the effect of each HP donor on the reproductive success of *C. gaumeri*, we conducted three hand-pollination treatments as follows: (1) *C. gaumeri* pollen only, (2) *C. gaumeri* plus *A. angustifolia* pollen and (3) *C. gaumeri* plus *T. curassavica* pollen. Mixed pollen loads were created based on the mean number of pollen grains per anther in each species (Arceo-Gómez and Ashman, 2011) for a 50/50 conspecific–HP mix, as in previous studies on HP effects (reviewed in Morales and Traveset, 2008). Pollen grains per anther were counted using a particle counter (Beckman Coulter Z Series). Mean ± s.e. for the number of pollen grains per anther for each species were as follows: 1017 ± 400 for *C. gaumeri*, 3060 ± 194 for *A. angustifolia* and 317 ± 117 for *T. curassavica*. A set of *C. gaumeri* plants different from the ones used as pollen recipients were used as conspecific pollen donors (i.e. only outcross conspecific pollen was used). Pollen donors were watered and kept in greenhouse conditions under natural light and free of pollinators, but were not manipulated otherwise. Anthers for all pollination treatments were collected 1 d in advance and stored at room temperature in microcentrifuge tubes overnight to let them dehisce. New pollination treatments were created daily and these were not used for more than 1 d. The success of our 50/50 conspecific–HP mixes was assessed by estimating the percentage of HP from the total pollen load in a subset of pollinated flowers ($n = 20$) that received the mixed pollen treatment in experiments with *A. angustifolia* (mean HP 48·8 %, range 40–57 %) and *T. curassavica* (mean HP 49 %, range 43–57 %) as the HP donor. We applied each pollination treatment (i.e. control and mixed)to separate plants to control for the effects of resource reallocation among treated flowers on the same plant (Zimmerman and Pyke, 1988; Knight *et al.*, 2006). Thus, out of 80 recipients in each experiment 40 were randomly chosen to receive the control and 40 to receive the mixed pollen load treatment. Each pollination treatment was applied to three separate flowers on each plant using a toothpick. Flowers were emasculated before being hand-pollinated to avoid autonomous self-pollination. Since mixed pollen loads only contained 50 % conspecific pollen, we applied larger pollen loads (by pollinating flowers twice) to flowers that received the mixed treatment to account for differences in total conspecific pollen load size between the control and HP-treated flowers. Pollen load sizes were later verified by counting pollen grains on a subset of pollinated stigmas and no difference in conspecific pollen load size was observed between the control (mean ± s.e. $26 \pm 9\cdot5$ grains) and the HP-treated ($27 \pm 14\cdot4$ grains) flowers ($F < 1\cdot7$, $P > 0\cdot2$ $n = 13$ for both experiments). Thus, any potential differences in reproductive success between control and HP-treated flowers would not be due to differences in total conspecific pollen load size. Average conspecific pollen loads in both pollination treatments were greater than the average number of ovules in *C. gaumeri* (i.e. 20) and were also similar to natural pollen loads observed on stigmas of this species ($29\cdot9 \pm 4\cdot5$; Alonso *et al.*, 2013). Since *C. gaumeri* experiences high levels of pollen limitation in natural conditions, meaning that it typically requires more pollen to fertilize all ovules (Alonso *et al.*, 2013), it is unlikely that stigma clogging occurred as a result of our experimental pollinations. Furthermore, no evidence of stigma clogging (e.g. overlying grains, saturated stigma surface) was observed after inspecting stigmas of pollinated flowers under the microscope.

Light and water treatments

Each hand-pollination treatment (in the two experiments) was randomly paired with a light and water availability treatment. Plants were assigned to two light conditions, high (120·9 W m^{-2}) and low (60·4 W m^{-2}) solar radiation. These treatments were achieved by covering all plants, within a light treatment, with a greenhouse mesh that provided 20 or 70 % shade. Plants on each light treatment were in turn assigned to one of two water treatments, high ($\sim$500 mm month^{-1}) and low ($\sim$2·41 mm month^{-1}) water availability. The high water availability treatment was achieved by watering plants daily, while plants in the low water availability treatment only received the natural rainfall that occurred at the time of the experiment. These levels of light and water availability were chosen to reflect the range of macro- and micro-environmental conditions experienced by *C. gaumeri* in nature. Variation in micro-environmental conditions among *C. gaumeri* individuals within a population can be as drastic (e.g. between <1 and >50 mm month^{-1} of water availability) as among-population differences due to the high spatial heterogeneity of the habitats in which this species grows (V. Parra-Tabla, unpubl. res.) and this can have important consequences for plant reproductive success (e.g. Herrera, 1995).

Estimation of reproductive success

We used the number of pollen tubes that reached the bottom of the style (hereafter, pollen tube success) as an estimate of female reproductive success. Pollen tube success has been described as a good indicator of pre-zygotic pollination success; it is often highly correlated with seed production and thus can be considered a reliable estimator of reproductive success (e.g. Winsor *et al.*, 1987; Waser and Price, 1991; Arceo-Gómez and Ashman, 2014). To evaluate pollen tube success we collected

styles from all three hand-pollinated flowers in each plant and stored them in 70 % ethanol. Styles from each flower were later decolorized and stained with aniline blue (Dafni, 1992; Alonso *et al*., 2013) and the number of pollen tubes at the base of the style was counted using a fluorescence microscope (Nikon e200©). Since multiple flowers were hand-pollinated on each plant (see section Hand-pollination treatments, above), we estimated the average number of pollen tubes at the base of the style per treatment for each plant.

Data analyses

To evaluate whether HP receipt, water and light availability or their interaction affected *C. gaumeri* female reproductive success we performed a mixed model (proc mixed in SAS; SAS Institute, 2010) with pollination, water and light treatments and their interactions as fixed factors. Variation due to individual plants was included as a random effect in the model. We performed the same model for both experiments (one for each HP donor). When the interactions were significant we conducted comparisons between levels across factors using the 'slice' statement in SAS with Bonferroni correction to adjust for multiple comparisons. Pollen tube data were transformed (square root $+ 0.5$) in order to meet assumptions of normality of the residuals. For clarity of interpretation, raw means $\pm$ s.e. are reported throughout the article.

RESULTS

When pollen mixes using *T. curassavica* as the HP donor were applied we observed an overall 45 % decrease in pollen tube success (2.9 ± 0.4) compared with pollinations with pure conspecific pollen loads(control, 5.3 ± 0.4; Table 1). Pollen tube success was also reduced by 38 % when plants were exposed to low (3.1 ± 0.5) compared with high (5 ± 0.4) light conditions (Table 1). More importantly, however, the HP effect depended on the availability of light and water (significant three-way interaction; Table 1). Multiple comparisons showed that while HP receipt reduced pollen tube success by between 54 and

64 % compared with the control when light, water or both were at low levels ($F_{1,54} > 4.0$, $P < 0.05$ for all comparisons; Fig. 1), no difference between the two pollination treatments (control vs. mixed) was observed when the availability of both light and water was high ($F_{1,54} = 0.01$, $P = 0.9$; Fig. 1). No other factor or interaction was significant when *T. curassavica* was used as the HP donor (Table 1).
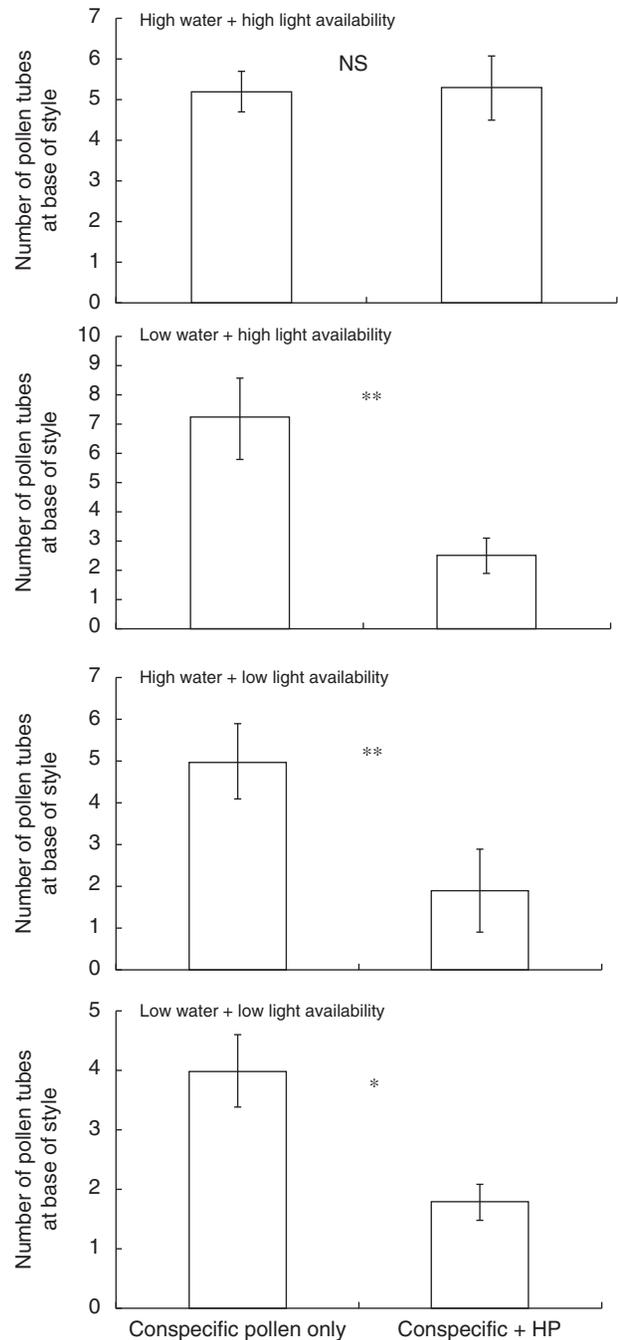

FIG. 1. Mean $\pm$ s.e. number of *C. gaumeri* pollen tubes at the base of the style when pure (conspecific pollen only) and mixed (conspecific $+ T. curassavica$ [HP]) pollen loads were applied and when the availability of light, water or both was low and when both resources were abundant. $*P < 0.05$; $**P < 0.01$; NS, not significant.

TABLE 1. *ANOVA results for the effects of pollination treatment (conspecific pollen only vs. conspecific $+$ heterospecific pollen), light (high vs. low) and water availability (high vs. low) and their interactions on the number of* C. gaumeri *pollen tubes that reached the bottom of the style. Results for the effects of each of the two heterospecific pollen donors evaluated are presented separately. Significant effects are noted in bold face.*

Source	Heterospecific pollen donor					
	Tamonea curassavica			*Angelonia angustifolia*		
	d.f.$_{63}$	F	P	d.f.$_{63}$	F	P
Pollen	1	19·8	**0·001**	1	0·9	0·3
Light	1	11·1	**0·001**	1	2·6	0·1
Water	1	1·1	0·2	1	0·01	0·9
Pollen × light	1	0·6	0·4	1	0·1	0·6
Pollen × water	1	1·4	0·2	1	0·5	0·4
Light × water	1	0·2	0·5	1	5	**0·02**
Pollen × light × water	1	5·8	**0·01**	1	3	0·08

When *A. angustifolia* was used as the HP donor we found no effect of pollen (control, 4.6 ± 0.5; mixed, 3.9 ± 0.4), light (high, 4.9 ± 0.5; low, 3.7 ± 0.4) or water (high, 4.2 ± 0.4; low, 4.4 ± 0.5) treatments on pollen tube success (Table 1). However, we did observe a significant effect of light × water treatment interaction (Table 1). Multiple comparisons showed that low light availability reduced pollen tube success by 40 %, but only when water availability was low ($F_{1,54} = 6.8$, $P = 0.01$; Fig. 2) and not when it was high ($F_{1,54} = 0.16$, $P = 0.6$; Fig. 2). No other interaction was found to be significant (Table 1).

DISCUSSION

Context-dependency of heterospecific pollen effects

This study demonstrates that abiotic conditions such as water and light availability can play an important role in determining the outcome of interspecific post-pollination interactions. Specifically, we show that HP receipt, in this case from *T. curassavica*, decreases *C. gaumeri* pollen tube success only when the availabilities of water, light or both are low and not when both are abundant (Fig. 1), thus confirming our prediction that not only conspecific pollen performance can be reduced under adverse external conditions (Lush *et al*., 1998; Campbell *et al*., 2001) but also its competitive ability against HP. For instance, impaired pollen germination in the absence of sufficient light and/or water could limit its response against physical or chemical interference by HP. Overall, this result strongly indicates that HP effects can depend on the abiotic environment in which plants grow, a possibility that so far has not been sufficiently explored. Studies have demonstrated wide variability in HP effects (reviewed in Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013), ranging from severe reductions in the reproductive success of recipient plants (e.g. Thomson *et al*., 1982) to no effect at all (e.g. Kohn and Waser, 1985). However, these studies might have overlooked the natural range of abiotic conditions in which these plants exist, which can affect the outcome, and thus may have misrepresented the full extent and strength of HP effects in natural communities, presumably underestimating its effects (assuming plants were kept in high-resource conditions; Herrera, 1995). For instance, in Kohn and Waser's (1985) pioneer study *Delphinium nelsonii* (Ranunculaceae) pollen had no effect on the reproductive success of *Ipomopsis aggregata* (Polemoniaceae) when deposited on stigmas of plants that were kept in pots and in homogeneous greenhouse conditions. However, *I. aggregata* is a widespread herb in western USA, where it coexists with *D. nelsonii* and experiences high environmental variability (Grant and Wilken, 1986; Wu and Campbell, 2006). Thus, it is possible that HP effects may exist and vary along the entire distribution range of *I. aggregata* depending on the environmental conditions encountered by each population. In fact, it has been noted that the effects of *D. nelsonii* pollen on *I. aggregata*'s reproductive success can be variable among years (N. Waser, University of California Riverside, CR, CA, USA, pers. comm.; see also Waser, 1978; Waser and Fugate, 1986), and here we show that this variation could be at least partially attributed to inter-year variability in resource availability. Our focal species, *C. gaumeri*, also has a widespread distribution range along the north coast of the Yucatan Peninsula, where it coexists with *T. curassavica* and is exposed to wide variation in resource and environmental conditions (Parra-Tabla *et al*., 2015). Specifically, *C. gaumeri* can grow in a wide range of habitats that range from open scrubland with high light exposure to deciduous tropical forest, which tend to be dryer and more shaded environments (Celaya, 2012; Parra-Tabla *et al*., 2015). Thus, variation in HP effects among populations of this species in natural conditions is highly likely. Our results thus highlight the importance of considering the natural range of abiotic conditions experienced by plants when evaluating the effects of HP receipt if we aim to fully understand the extent and strength of its effects in natural plant populations.
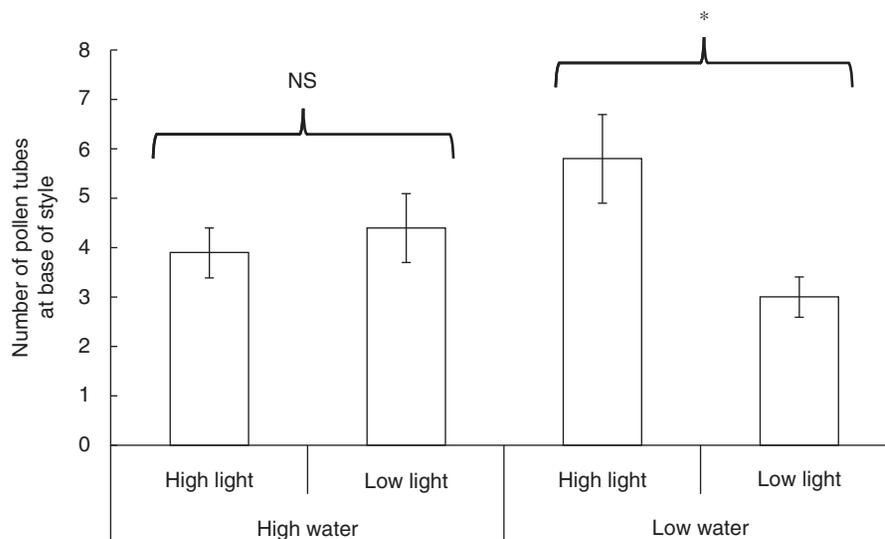
FIG. 2. Mean ± s.e. number of *C. gaumeri* pollen tubes at the base of the style in plants exposed to high and low water and light availability treatments. This result is only for plants in the experiment where *A. angustifolia* was used as the HP donor. *$P < 0.05$; NS, not significant.

It is also worth mentioning that we found no effect of *A. angustifolia* pollen on *C. gaumeri* pollen tube success. This result adds to the evidence suggesting that HP effects can be highly species-specific (Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013). However, the underlying causes of among-species variation in HP effects are still unknown and thus remain an important and interesting avenue of research (Ashman and Arceo-Gómez, 2013). In this case, since we used the same HP recipient for both experiments, the lack of an HP effect is likely associated with traits of the HP donor (*A. angustifolia*) or due to the interaction between donor and recipient traits (for a detailed description of such traits see Ashman and Arceo-Gómez, 2013). Interestingly, we did find a light × water treatment interaction, corroborating that abiotic conditions can have a strong influence on conspecific pollen performance.

Ecological and evolutionary implications

It has been suggested that the effects of HP receipt can have important implications for floral evolution and community assembly (Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013). Specifically, these authors have suggested that HP receipt can drive the evolution of traits that confer tolerance of its negative effects, such as stigma area and style length, or cause divergence in flowering phenology (e.g. Waser, 1978) and traits that reduce pollinator sharing among species and thus help avoid HP transfer (Ashman and Arceo-Gómez, 2013). However, our results suggest that the strength of HP receipt as an evolutionary force can vary temporally and spatially within and among populations. Thus, the evolutionary implications of HP receipt should also be evaluated in the context of the range of abiotic conditions in which plants exist. For instance, if there is high temporal variation in environmental conditions, and thus in HP effects within populations, it could relax the effects of natural selection and contribute to the persistence of traits that confer vulnerability to HP effects (for examples of such traits see Ashman and Arceo-Gómez, 2013) that could even lead to maladaptations (Thompson, 1999). On the other hand, if high spatial variability in environmental conditions (i.e. across populations) exists it could intensify selection in some populations and not others, thus causing among-population divergence in traits that mediate HP effects. The latter could represent a particular case of diffuse selection (Janzen, 1980) driven by spatial and temporal variability in abiotic conditions. Furthermore, variability in HP effects could allow the establishment and persistence of plant species within communities that would not be present if detrimental effects of HP were spatially and temporally consistent, potentially influencing the structure of co-flowering communities. Although we are aware that our discussion here is speculative, we believe these are interesting avenues of research that are relevant in light of our findings, but that have been little explored and deserve further attention. We propose that only by taking into account the variability in the environmental context in which plants occur will we have a complete understanding of the ecological and evolutionary implications of the effects of HP receipt in natural communities.

Finally, *C. gaumeri* is an endemic species found in small populations along a narrow distribution range, where its reproduction is strongly limited by pollen availability (Alonso *et al.*, 2013; Parra-Tabla *et al.*, 2015). Here we show that HP receipt has the potential to exacerbate the effects of pollen limitation, further decreasing reproductive success in this species, which could seriously threaten its persistence in natural populations. We then emphasize that studies that evaluate the effects of HP receipt should pay particular attention to its effects on endemic species, given the high vulnerability of their populations to small variations in reproductive success (Alonso *et al.*, 2010).

Conclusions

To our knowledge, this is the first study to show evidence for potential spatial and temporal mosaics in the outcome of post-pollination interactions due to variability in abiotic conditions. Field studies that evaluate the effect of HP receipt, from the same HP donor, across multiple communities exposed to different environmental conditions (in space or time) would be desirable to confirm the context-dependency of plant–plant post-pollination interactions and its ecological and evolutionary consequences. Finally, we stress the importance of incorporating the full complexity of natural systems, particularly when evaluating species interactions, if we aim to better understand the ecological and evolutionary dynamics of natural populations.

LITERATURE CITED

Albrecht M, Padrón, B Bartomeus, I, Traveset A. 2014. Consequences of plant invasions on compartmentalization and species' roles in plant–pollinator networks. *Proceedings of the Royal Society of London B, Biological Sciences* **281**: 20140773.

Alonso C, Vamosi JC, Knight TM, Steets JA, Ashman TL. 2010. Is reproduction of endemic plant species particularly pollen limited in biodiversity hotspots? *Oikos* **119**: 1192–1200.

Alonso C, Navarro-Fernández CM, Arceo-Gómez G, Meindl GA, Parra-Tabla V, Ashman TL. 2013. Among-species differences in pollen quality and quantity limitation: implications for endemics in biodiverse hotspots. *Annals of Botany* **112**: 1461–1469.

Arceo-Gómez G, Ashman TL. 2011. Heterospecific pollen deposition: does diversity alter the consequences? *New Phytologist* **192**: 738–746.

Arceo-Gómez G, Ashman TL. 2014. Heterospecific pollen receipt affects self pollen more than outcross pollen: implications for mixed-mating plants. *Ecology* **95**: 2946–2952.

Ashman TL, Arceo-Gómez G. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany* **100**: 1061–1070.

Bascompte J, Jordano P. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution and Systematics* **38**: 567–593.

Bascompte J, Jordano P, Olesen JM. 2006. Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* **312**: 431–433.

Bjerknes AL, Totland Ø, Hegland SJ, Nielsen A. 2007. Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation* **138**: 1–12.

Campbell A, Griffin W, Burritt D, Conner A. 2001. The importance of light intensity for pollen tube growth and embryo survival in wheat × maize crosses. *Annals of Botany* **87**: 517–522.

Campos-Navarrete MJ, Parra-Tabla V, Ramos-Zapata J, Díaz-Castelazo C, Reyes-Novelo E. 2013. Structure of plant–Hymenoptera networks in two coastal shrub sites in Mexico. *Arthropod-Plant Interactions* **7**: 607–617.

Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* **54**: 1546–1557.

Celaya IN. 2012. *Efecto de la co-floracion sobre el exito reproductivo de la especie endemica Cuphea gaumeri Koehne (Lythraceae) en la costa norte de Yucatán, México*. BS Thesis, University of Yucatan, Mexico.

Chapin FS, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. *Bioscience* **37**: 49–57.

Dafni A. 1992. Pollination ecology: a practical approach. Oxford: Oxford University Press.

Davis MA, Grime JP, Thompson K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* **88**: 528–534.

Fang Q, Huang SQ. 2013. A directed network analysis of heterospecific pollen transfer in a biodiverse community. *Ecology* **94**: 1176–1185.

Feng H, An L, Tan L, Hou Z, Wang X. 2000. Effect of enhanced ultraviolet-B radiation on pollen germination and tube growth of 19 taxa *in vitro*. *Environmental and Experimental Botany* **43**: 45–53.

Fishman L, Wyatt R. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* **53**: 1723–1733.

Galen C, Gregory T. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia* **81**: 120–123.

Geber MA, Moeller DA. 2006. Pollinator responses to plant communities and implications for reproductive character evolution. In: SC Barrett. ed. *Ecology and evolution of flowers*. Oxford: Oxford University Press, 102–119.

Ghazoul J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology* **94**: 295–304.

Grant V, Wilken DH. 1986. Taxonomy of the *Ipomopsis aggregata* group (Polemoniaceae). *Botanical Gazette* **147**: 359–371.

Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* **12**: 184–195.

Herrera CM. 1995. Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* **76**: 1516–1524.

Hopkins R, Rausher MD. 2012. Pollinator-mediated selection on flower color allele drives reinforcement. *Science* **335**: 1090–1092.

de Jager ML, Dreyer LL, Ellis AG. 2011. Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* **166**: 543–553.

Janzen DH. 1980. When is it coevolution? *Evolution* **34**: 611–612.

Knight TM, Steets JA, Ashman TL. 2006. A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *American Journal of Botany* **93**: 271–277.

Kohn JR, Waser NM. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *American Journal of Botany* **72**: 1144–1148.

Lankinen Å. 2001. *In vitro* pollen competitive ability in *Viola tricolor*: temperature and pollen donor effects. *Oecologia* **128**: 492–498.

Lazaro A, Totland Ø. 2010. Local floral composition and the behaviour of pollinators: attraction to and foraging within experimental patches. *Ecological Entomology* **35**: 652–661.

Levin DA, Anderson WW. 1970. Competition for pollinators between simultaneously flowering species. *American Naturalist* **104**: 455–467.

Lush WM, Grieser F, Wolters-Arts M. 1998. Directional guidance of *Nicotiana alata* pollen tubes *in vitro* and on the stigma. *Plant Physiology* **118**: 733–741.

McEwen JR, Vamosi JC. 2010. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society of London B, Biological Sciences* **227**: 2957–2965.

Memmott J, Craze PG, Waser NM, Price MV. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* **10**: 710–717.

Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD. 2009. New frontiers in competition for pollination. *Annals of Botany* **103**: 1403–1413.

Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* **85**: 3289–3301.

Montgomery BR, Rathcke BJ. 2012. Effects of floral restrictiveness and stigma size on heterospecific pollen receipt in a prairie community. *Oecologia* **168**: 449–458.

Morales CL, Traveset A. 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Science* **27**: 221–238.

Murphy SD, Aarssen LW. 1995. *In vitro* allelopathic effects of pollen from three *Hieracium* species (Asteraceae) and pollen transfer to sympatric Fabaceae. *American Journal of Botany* **82**: 37–45.

Olesen JM, Jordano P. 2002. Geographic patterns in plant-pollinator mutualistic networks. *Ecology* **83**: 2416–2424.

Parra-Tabla V, Celaya C, Téllez P, Salinas L, Abdala-Roberts L, Alonso C. 2015. Fenología de floración y polinización en herbáceas de la Reserva de la Biósfera Ria Lagartos. In: SJ Ramos. ed. *Ecología funcional de la Reserva de la Biósfera de Ria Lagartos*. Merida, Yucatan, Mexico: UADY-CONANP, in press.

Sargent RD, Ackerly DD. 2008. Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution* **23**: 123–130.

SAS Institute. 2010. SAS/IML software. Version 9.2. SAS Institute, Cary, NC, USA.

Sukhada DK, Jayachandra. 1980. Pollen allelopathy: a new phenomenon. *New Phytologist* **84:** 739–746.

Thompson JN. 1999. Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist* **153**: S1–S14.

Thomson JD, Andrews BJ, Plowright R. 1982. The effect of a foreign pollen on ovule development in *Diervilla lonicera* (Caprifoliaceae). *New Phytologist* **90**: 777–783.

Traveset A, Richardson DM. 2006. Biological invasions as disruptors of plant reproductive mutualisms. *Trends in Ecology and Evolution* **21**: 208–216.

Waser NM. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* **59**: 934–944.

Waser NM, Fugate ML. 1986. Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* **70**: 573–577.

Waser NM, Price MV. 1991. Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set. *Ecology* **72**: 171–179.

Winsor J, Davis L, Stephenson A. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigor in *Cucurbita pepo*. *American Naturalist* **129**: 643–656.

Wu CA, Campbell DR. 2006. Environmental stressors differentially affect leaf ecophysiological responses in two *Ipomopsis* species and their hybrids. *Oecologia* **148**: 202–212.

Zimmerman M, Pyke GH. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* **131**: 723–738.