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Can plants evolve tolerance mechanisms to heterospecific pollen effects? An experimental test of the adaptive potential in *Clarkia* species

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Flowering plants do not occur alone and often grow in mixed-species communities where pollinator sharing is high and interactions via pollinators can occur at pre- and post-pollination stages. While the causes and consequences of pre-pollination interactions have been well studied little is known about post-pollination interactions via heterospecific pollen (HP) receipt, and even less about the evolutionary implications of these interactions. In particular, the degree to which plants can evolve tolerance mechanisms to the negative effects of HP receipt has received little attention. Here, we aim to fill this gap in our understanding of post-pollination interactions by experimentally testing whether two co-flowering *Clarkia* species can evolve HP tolerance, and whether tolerance to specific HP 'genotypes' (fine-scale local adaptation to HP) occurs. We find that *Clarkia* species vary in their tolerance to HP effects. Furthermore, conspecific pollen performance and the magnitude of HP effects were related to the recipient's history of exposure to HP in *C. xantiana* but not in *C. speciosa*. Specifically, better conspecific pollen performance and smaller HP effects were observed in populations of *C. xantiana* plants with previous exposure to HP compared to populations without such exposure. These results suggest that plants may have the potential to evolve tolerance mechanisms to HP effects but that these may occur not from the female (stigma, style) but from the male (pollen) perspective, a possibility that is often overlooked. We find no evidence for fine-scale local adaptation to HP receipt. Studies that evaluate the adaptive potential of plants to the negative effects of HP receipt are an important first step in understanding the evolutionary consequences of plant–plant post-pollination interactions. Such knowledge is in turn crucial for deciphering the role of plant–pollinator interactions in driving floral evolution and the composition of co-flowering communities.

The recent explosion of analytical techniques to evaluate community-wide patterns of species interactions (Ackerly and Cornwell 2007, Bascompte and Jordano 2007, Dormann et al. 2009, Kraft and Ackerly 2010) has stimulated studies of interaction networks between plants and their pollinators (Olesen and Jordano 2002, Bascompte et al. 2003, Bascompte and Jordano 2007, Olesen et al. 2007, Albrecht et al. 2010). These studies have revealed complex networks of interactions where generalization in the use of flower resources by pollinators, and of pollinators by plants, is widespread (Olesen and Jordano 2002, Bascompte et al. 2003, Bascompte and Jordano 2007, Martín González et al. 2010, Burkle et al. 2013). This observed high degree of pollinator sharing has reinvigorated interest in the mechanisms by which plant species interact via pollinators, and the role that these interactions play in the evolution of floral traits and in the assembly of co-flowering plant communities (Caruso 2000, Brown et al. 2002, Geber and Moeller 2006, Sargent and Ackerly 2008, Mitchell et al. 2009, McEwen and Vamosi 2010, Sargent et al. 2011). Plant–plant interactions via pollinators can occur at two stages of the pollination process: 1) pre-pollination, by altering pollinator foraging behavior through facilitation (Moeller 2004, Ghazoul 2006) or competition (Caruso 2000, Mitchell et al. 2009), and 2) post-pollination, via pollen-pistil and/or pollen–pollen interactions on the stigma (reviewed by Morales and Traveset 2008). Although studies of pre-pollination interactions are accumulating (Rathcke 1983, Caruso 2000, Brown et al. 2002, Bell et al. 2005, Ghazoul 2006, Sargent and Ackerly 2008, Mitchell et al. 2009, de Jager et al. 2011), much less is known about the ecological and evolutionary consequences of post-pollination interactions (but see Morales and Traveset 2008, Hopkins and Rausher 2012, Ashman and Arceo-Gómez 2013). A complete understanding of interactions at both pollination stages is crucial in predicting plant responses to anthropogenic disturbances to the degree of co-flowering and pollinator sharing in nature (Traveset and Richardson 2006, Bjerknes et al. 2007, Memmott et al. 2007, Hegland et al. 2009, Forrest et al. 2010).

It has been proposed that individual pollinators often show high constancy to flowers of the same species during a foraging bout (Waser 1986, Goulson and Cory 1993, Chittka et al. 1999), and that heterospecific pollen (hereafter, HP) transfer by shared pollinators is thus rare in natural communities (Morales and Traveset 2008). However, pioneering studies (Macior 1970, Feinsinger et al. 1986, Grabas and Laverty 1999) as well as more recent work on patterns of HP transfer has shown that the incidence and magnitude of HP transfer in communities can be high (Montgomery and Rathcke 2012, Fang and Huang 2013), occurring in more than 50% of flowers and constituting more than 60% of the total pollen load on stigmas in some species (Ashman and Arceo-Gómez 2013). Furthermore, the effect of HP on the reproductive success of recipient plants can be strong, reducing seed production by an average of 20% (Ashman and Arceo-Gómez 2013) and sometimes resulting in complete reproductive failure (Thomson et al. 1982). Variation in the effect of HP receipt across plant species is also high, with effect sizes ranging from 0.01 to –4.7, but the underlying causes of this variation are unknown (Ashman and Arceo-Gómez 2013). Nevertheless, evidence so far suggests that HP receipt has the potential to act as a strong agent of selection promoting the evolution of flowering traits that diminish its negative effects (Kay and Schemske 2008, Hopkins and Rausher 2012). Heterospecific pollen receipt could also act as an important biotic filter restricting community membership for some species (Ashman and Arceo-Gómez 2013), but these predictions remain untested (but see Briscoe Runquist and Stanton 2013). A more complete understanding of the effects and the ecological and evolutionary consequences of post-pollination plant–plant interactions is thus necessary if we want to fully assess their role as a generating and organizing force of plant diversity in nature.

Recent progress has been made in understanding the ecological determinants of HP effects. In particular, we now know that HP load diversity (Arceo-Gómez and Ashman 2011), conspecific pollen sources (Arceo-Gómez and Ashman 2014) and timing of HP arrival to the stigma (Waser and Fugate 1986) all play important roles as modifiers of the effects of HP receipt. However, an important gap in our understanding of post-pollination interactions concerns the evolutionary implications of HP receipt (but see Kay and Schemske 2008, Hopkins and Rausher 2012). A recent study has proposed potential traits that could moderate the negative effects of HP receipt and thus may be under strong selection in natural populations (Ashman and Arceo-Gómez 2013). Although the latter study serves as an important guide to potential targets of selection, we still do not know whether plants can evolve tolerance (e.g. the degree to which reproductive success is not affected) to the negative effects of HP receipt. Thus, before exploring the underlying mechanisms and potential traits under selection, an important first step would be to evaluate HP effects on plants of the same species, with and without a previous history of coexistence with the HP donor. Smaller HP effects in plants that have previously coexisted with the HP donor compared to plants that have not would indicate that plants can evolve tolerance to HP. If tolerance to HP is found to depend on plant's history of exposure, we will then have the basis to examine specific traits and mechanisms conferring tolerance to HP effects. Furthermore, testing for fine-scale local adaption to the effects of HP (adaptation to local HP 'genotypes'; Kay and Schemske 2008) would shed light on the strength of HP transfer as an evolutionary force within populations. If plants have the potential to adapt to specific 'genotypes' of HP donors then the extent to which HP transfer influences microevolutionary process within populations and promotes population divergence could be far greater than previously thought.

In this study we aim to fill this gap in our understanding of post-pollination interactions by testing experimentally if plants have the potential to evolve HP tolerance by comparing HP effects on plants of two species of *Clarkia* (Onagraceae) in populations of both species with and without previous coexistence history with the HP donor. We further test for fine-scale local adaptation to HP receipt by evaluating HP effects of HP donors from the same and from a different locality as the recipients. We ask the following specific questions: does HP receipt decrease reproductive success of *C. xantiana* and *C. speciosa*? Does the effect depend on the *Clarkia* species' previous coexistence history with the HP donor (flowering context) and/or their interaction? Does the effect of HP receipt depend on HP source (same versus different locality as the recipient) and does this effect vary by *Clarkia* species? *Clarkia* is good system in which to ask these questions because 1) multiple *Clarkia* species frequently co-occur and co-flower at sites (Lewis 1953, MacSwain et al. 1973), 2) the species composition of *Clarkia* composition remains quite stable from year to year (Lewis 1953, Geber unpubl.), 3) *Clarkia* species often flower late in the spring when few other plant species are flowering, and 4) cross-pollination in most outcrossing *Clarkia* is effected by solitary bee pollinators, many of which are specialists that visit only *Clarkia* plants for pollen and nectar (McSwain et al. 1973, Moeller 2004). As a result of these conditions, HP receipt is likely to be greatest among co-flowering congeners within sites.

Material and methods

Study species

We conducted the study using two *Clarkia* species, *C. xantiana* and *C. speciosa*. *Clarkia xantiana* and *C. speciosa* are both bee pollinated, hermaphroditic, self-compatible, annual species endemic to central–southern California (Eckhart et al. 2006, Moeller 2004). Both species are highly outcrossing (Moeller 2004). *Clarkia xantiana*, in particular, has a high degree of herkogamy and protandry, which precludes autonomous self-fertilization and thus is highly dependent on pollinators for successful reproduction (Runions and Geber 2000, Moeller 2006). *Clarkia xantiana* and *C. speciosa* coexist and co-flower along much of their natural distribution range in the Kern River Basin (Moeller 2004). High levels of pollinator sharing have been observed between these two *Clarkia* species (Moeller 2004, Singh 2014), which are mainly visited by 10 oligolectic bee species from the families *Andrenidae*, *Apidae*, *Dasypodaidae*, *Halictidae* and *Megachilidae* that forage only on *Clarkia* species for nectar and pollen (MacSwain et al. 1973, Moeller 2005, 2006, Eckhart et al. 2006). Pollen transfer between *C. xantiana* and *C. speciosa* has also been observed in the field (A. James and J. Brokaw pers. comm.) but no hybridization occurs (Lewis and Lewis

1955). *Clarkia xantiana* and *speciosa* produce an average of 20 and 25 seeds per fruit, respectively (up to 80 seeds maximum) under natural conditions (Geber unpubl.).

Experimental design

In order to evaluate the effects of HP transfer between *C. xantiana* and *C. speciosa* and whether these effects depend on the degree of previous exposure (e.g. flowering overlap), to the HP donor we selected three types of sites with the following flowering contexts: 1) *C. xantiana* flowering alone, 2) *C. speciosa* flowering alone and 3) both *Clarkia* species flowering together ('mixed'). Two sets of the three sites were selected, each set along a different road (locality) in the Greenhorn Mountains of the southern Sierra Nevada mountain range (Kern County, California). Sites within each locality were separated by at least 2 km and localities were separated by ca 6 km. Furthermore, *Clarkia* populations were located within a matrix composed of other grassland species and urban areas and thus little interaction via pollinators is expected among them. Species composition in these *Clarkia* communities has remained the same over 20 years (Geber unpubl.) and thus flowering context is presumed to be quite constant over time. We collected 33 inflorescences per species/site, each inflorescence from a different plant and with at least three flower buds per inflorescence to serve as pollen recipients. An additional ~150 inflorescences were collected from each species/site to serve as pollen donors. All inflorescences were kept in water and in enclosed common garden conditions for the duration of the experiment to prevent pollen transfer by pollinators.

Hand pollinations

Three hand pollination treatments were performed on three different flowers on each inflorescence per species per site (792 total hand pollinations) as follows: 1) control (only outcross conspecific pollen from population of origin), 2) outcross conspecific pollen plus HP from the mixed site in the same locality (hereafter, H1) and 3) outcross conspecific pollen plus HP from the mixed site in the different locality as the recipient (hereafter, H2). This design allowed us to test if plants have the potential to adapt to tolerate the negative effects of HP receipt by comparing its effects in 'alone' versus 'mixed' populations. It further allowed us to test if tolerance to HP receipt can occur at a fine scale by comparing the effects of HP donors from the same and from a different locality (H1 versus H2) as the recipient in 'mixed' populations.

Pure pollen loads were obtained by pooling four conspecific anthers, each from a different flower and donor plant. Mixed pollen loads were obtained by mixing pollen from four conspecific anthers (from different flowers and donors) and one heterospecific anther for an approximately 20% HP load which is representative of natural HP loads on flowers (Ashman and Arceo-Gómez 2013). We created pollen mixes assuming both *Clarkia* species have a similar number of pollen grains per anther since anthers of the two species do not differ in size ($F = 2.68$, $p > 0.05$, $n = 40$; data not shown) and pollen grains from the two species are indistinguishable from each other in size and shape (Geber unpubl.). Pollen pools for pure and mixed loads were created one day in advance and left overnight in microcentrifuge tubes at room temperature for anther dehiscence (Arceo-Gómez and Ashman 2011). Tubes were then stored in a refrigerator but no pollen mix was used for more than two days. Previous work showed that pollen remains viable over this period of time.

Hand pollination treatments were applied in random order to flowers in each inflorescence as flowers opened until all treatments were applied. All flowers were emasculated before being hand pollinated to avoid autonomous self-pollination. Since mixed-species pollen loads (H1, H2) contain 20% HP, we applied larger pollen loads to flowers that received H1 and H2 treatments so as to equalize the amount of conspecific pollen applied in control and HP treatments. Thus, control pollinations received on average 249 ± 21.2 pollen grains ($n = 20$) while H1 and H2 received 407 ± 49.2 and 377 ± 26.4 pollen grains per flower respectively ($n = 20$ each). Thus, mixed pollen loads (H1, H2) were 35% larger than pure conspecific loads ($p < 0.05$) and thus receive an estimated of 326 (H1) and 302 (H2) conspecific pollen grains on average. There was no difference in pollen load size between H1 and H2 treatments ($p = 0.9$). Consequently, any differences observed among control and H1 and H2 pollination treatments were not due to differences in conspecific pollen load size. All styles were collected one day after being hand pollinated and stored in microcentrifuge tubes with 70% ethanol. Pollen tubes typically reach the ovary after 8 h. (Briscoe Runquist et al. 2014), and thus a 24 h period was sufficient time for full pollen tube growth. Styles were softened and then stained with decolorized aniline blue following Arceo-Gómez and Ashman (2011), and the number of pollen tubes at the base of the style was counted using a fluorescence microscope. Since no interspecific pollen tube growth has been observed in previous pollinations between these two species (Geber unpubl.) only conspecific pollen tubes were counted at the base of the style. The number of pollen tubes at the base of the style is a good estimator of reproductive success as it is correlated with seed production in these species ($r = 0.5$, $p < 0.001$).

Data analyses

In order to test if HP receipt decreases pollen tube success (e.g. the number of pollen tubes at the base of the style) and if the effect depends on *Clarkia* species, previous exposure to the HP donor and/or their interaction, we conducted a mixed effects ANOVA (proc mixed; SAS 2010) with hand-pollination treatment (control versus mixed pollinations [pooled effect of H1 and H2]), species, population flowering context (alone versus mixed species sites) and their interaction as fixed factors. Locality and plant (inflorescence) nested within locality were considered random factors in the model but the significance of their effects was not tested. To test for fine-scale local adaptation to HP receipt we used a mixed effects ANOVA to compare the effects of H1 versus H2 on pollen tube success using plants from mixed populations. We confined the comparison of H1 and H2 effects to populations from mixed sites because it is only these populations that have chronic exposure to HP and might therefore evolve tolerance to local heterospecific pollen (H1), but have less tolerance to heterospecific pollen from another

Table 1. ANOVA results for the effects of species, co-flowering context (alone versus mixed species sites), pollination treatment (conspecific only versus mixed species pollen loads) and their interactions on the number of pollen tubes that reach the base of the style (pollen tube success). Values in bold are statistically significant.

Source	DF	F	p
Species	1, 675	49.8	**<.0001**
Co-flowering context	1, 675	3.9	**0.04**
Pollination treatment	1, 675	61.6	**<.0001**
Species × Context	1, 675	3.9	**0.04**
Context × Treatment	1, 675	0.2	0.6
Species × Treatment	1, 675	10	**0.001**
Species × Context × Treatment	1, 675	0.2	0.6

locality (H2). Species, HP treatment (H1 versus H2) and their interaction were considered fixed factors while locality and plant nested within locality were included as random factors in the model. We conducted a posteriori multiple comparisons with a Bonferroni correction to compare between levels across different factors when necessary. The residuals from both models were normally distributed ($p > 0.05$).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.hb2bt> (Arceo-Gómez et al. 2015).

Results

Pollen tube success differed between the two *Clarkia* species, with *C. xantiana* having significantly more pollen tubes reaching the base of the style (mean ± SE: 39 ± 1) than *C. speciosa* (30.7 ± 1; Table 1) across all treatments. Furthermore, HP significantly affected conspecific pollen tube success but the magnitude of the effect varied by *Clarkia* species (Table 1). While HP receipt reduced conspecific pollen tube success by 32% in *C. xantiana* (control versus conspecific-HP mix: $t = 7.8$, $p < 0.001$) this decrease was only 20% in *C. speciosa* ($t = 3.2$, $p < 0.05$; Fig. 1).

Co-flowering context also had a significant effect on pollen tube success but this effect also depended on the species (Table 1). Multiple comparisons showed that conspecific pollen tube success was 15% greater in plants from 'mixed' compared to plants from 'alone' populations across pollination treatments, but this effect was only observed in *C. xantiana* ($t = 2.8$, $p = 0.02$) and not in *C. speciosa* ($t = 0.1$, $p = 0.9$; Fig. 2; also see Supplementary material Appendix 1). However, no significant interactions were found between flowering context and pollination treatment, or among the three factors (*Clarkia* species, flowering context and pollination treatment; Table 1).

Finally, when we tested for fine-scale local adaptation to HP we did not find significant differences in the effects of HP on conspecific pollen tube success when we used HP from the same and from a different locality as the pollen recipient (H1 versus H2: $F_{1,178} = 0.8$, $p = 0.3$), and this was the same for both Clarkia species ($F_{1,178} = 1.6$, $p = 0.2$; also see Supplementary material Appendix 1).

Discussion

While the evolution of floral traits in response to altered pollinator attraction has been widely explored (Caruso 2000, Brown et al. 2002, Sargent and Ackerly 2008, Mitchell et al. 2009) the evolutionary consequences of plant–plant interactions via HP transfer in mixed-species co-flowering communities has received less attention (but see Kay and Schemske 2008, Hopkins and Rausher 2012). Here we present partial evidence suggesting that plants may have the potential to evolve tolerance to HP effects; this result provides the impetus to identify the specific floral adaptations underlying HP tolerance. We also show that the degree of tolerance to HP effects is species-specific and may depend

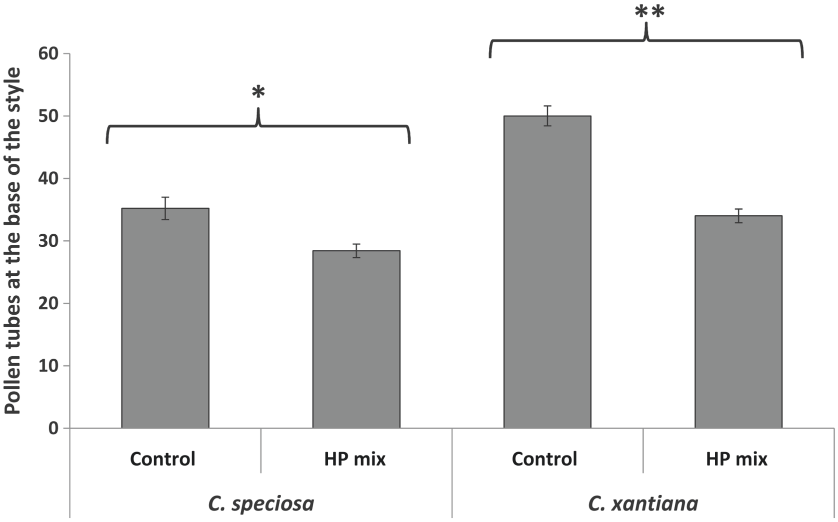

Figure 1. Mean (± SE) for the number of pollen tubes that reach the base of the style in *C. speciosa* and *C. xantiana* flowers when pollinated with pure conspecific pollen loads (control) and a conspecific-heterospecific pollen mix (HP mix). Asterisk denotes significant differences: one asterisk denotes $p < 0.05$, two asterisks denotes $p < 0.001$.

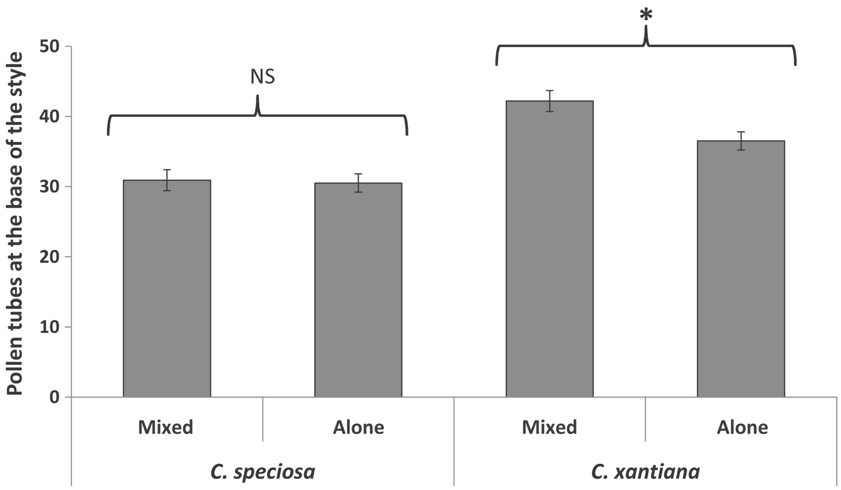

Figure 2. Mean (± SE) for the number of pollen tubes that reach the base of the style in *C. speciosa* and *C. xantiana* flowers from plants collected at 'alone' (no other *Clarkia* species flowering) and 'mixed' (*C. speciosa* and *C. xantiana* co-flowering) species sites, averaged across all three pollination treatments. Asterisk denotes significant differences at $p < 0.05$, NS = not significantly different.

on the species' history of exposure to HP receipt. We discuss these results and their ecological implications in more detail below.

Conspecific pollen performance and the evolution of tolerance to heterospecific pollen effects

We found greater conspecific pollen tube success in *C. xantiana* plants from populations with previous exposure to the HP donor (mixed) compared to plants from populations without exposure (alone). However, the greater pollen tube success in *C. xantiana* from mixed populations was observed across all pollination treatments, including the control treatment with conspecific pollen exclusively (i.e. there was no significant pollination treatment by flowering context interaction). Thus, our results provide no evidence for evolved HP tolerance, at least at the level of the stigma, and suggest that differences in conspecific pollen tube success could be due to other differences among populations that might have affected pollen performance and were not evaluated in this study (e.g. abiotic conditions and resource availability). For example, reductions in water and light availability have been shown to negatively affect pollen germination (Lush et al. 1998) and pollen tube growth rate (Campbell et al. 2001). In addition, it is also possible that the availability of these resources varied consistently between alone and mixed sites. It is also likely that our low replication at the site level limited our ability to observe a significant interaction between population context (alone versus mixed species sites) and pollination treatment (control [conspecific pollen only] versus mixed [conspecific + HP]). For instance, the reduction in conspecific pollen success in plants from 'alone' relative to 'mixed' species sites was nearly twice as large in mixed pollen treatments (17%, $p = 0.06$) than in control pollinations (10%, $p = 0.4$) but because effect sizes seem to be small larger sample sizes would be required to detect statistical significance (Wahlsten 1991). A power test showed that at least twice the sample size (66 plants site^{-1}) would have been necessary to provide a 50% probability of detecting a significant interaction. Another limitation of this study could have been the small spatial scale at which it was conducted, since the studied populations were not more than 6 km apart. Perhaps the stability of the historical co-flowering context and thus the likelihood of finding an effect might have been greater at larger spatial scales. However, an alternative (and potentially more interesting) explanation for our results is that adaptation to HP effects occurs in the male gametophyte (pollen) in addition to, or instead of, in the female sporophyte (stigma and style). For instance, environments where male gametophytes are frequently exposed to competition from heterospecific grains might impose stronger selective pressures on pollen performance compared to environments where HP is absent (Ashman and Arceo-Gómez 2013). Thus, adaptation in the male gametophyte (pollen) to succeed in highly competitive pollination environments presents an alternative that has been considered far less frequently. Enhanced pollen performance in environments with high chronic levels of HP transfer could then explain why pollen grains from 'alone' populations performed poorly in comparison with pollen grains from 'mixed' populations. However, the role of HP in driving selection on pollen performance in a manner perhaps similar to that of conspecific pollen competition (Mazer et al. 2010) still needs to be explored. Studies designed to separate HP tolerance from the male and female perspective are thus necessary to fully assess the adaptive potential of plants to HP effects.

We did not find any evidence of fine-scale local adaptation to HP effects as no differences were observed in the effect of HP on conspecific pollen success between HP donors from the same versus a different locality as the recipient. This result suggests that *C. xantiana* plants do not have the capacity to tolerate specific genotypes of HP donors or perhaps that local adaptation to HP effects may occur at broader spatial scales than the ones examined in this study. Thus, more studies are needed in other plant taxa and conducted at different spatial scales in order to assess the generality of these findings and to better comprehend the strength of HP receipt as an evolutionary force within populations.

Differential heterospecific pollen effects between *Clarkia* species

While extensive variation in HP effects among plant species has been reported, the ultimate causes of this variation are not fully known (Ashman and Arceo-Gómez 2013). Thus, it is not totally surprising that the magnitude of the effect of HP receipt on pollen tube success differed between *C. xantiana* and *C. speciosa*, being 12% stronger in the former compared to the latter (across all populations [mixed and alone]). To date, much of the variation in HP effects has been attributed to methodological differences among studies, such as timing of HP application, size of HP load and differences in the response variables measured (e.g. seed set, pollen tube success; Morales and Traveset 2008). However, in this study, where both species were treated and measured in the same way, none of these factors are likely to have contributed to variation in HP effects. Our findings suggest that small differences in traits that mediate HP effects, such as style length, stigma type (wet or dry) and stigma area (Ashman and Arceo-Gómez 2013), may contribute to interspecific differences in HP effects in *Clarkia*. In fact, stigmas tend to be larger in *C. speciosa* compared to *C. xantiana* (Geber unpubl.), a trait that has been proposed to confer greater tolerance to HP effects (Ashman and Arceo-Gómez 2013). Furthermore, the species differ in flower shape and orientation, which may result in differential exposure to heterospecific pollen. *Clarkia speciosa* has more exposed flowers (bowl-shaped, erect flowers with radial symmetry, and larger and more exposed stigmas) that bees approach from above and dive into to reach the nectar and pollen, resulting in pollinator contact with the stigma. By contrast, *C. xantiana* has more restricted flowers (open-faced, with bilateral symmetry) that bees approach from the front and side. Thus, even though pollinator sharing exists between these two *Clarkia* species, HP transfer may not occur in both directions with the same frequency. A higher degree of exposure of *C. speciosa* stigmas could lead to a higher incidence of HP receipt, which in turn could select for greater tolerance to HP effects (Ashman and Arceo-Gómez 2013). Nevertheless, quantification of the magnitude and frequency of HP receipt by these two species in natural populations is needed in order to have a better understanding of their differential susceptibility to HP effects. Given the strong HP effect in *C. xantiana* we might expect one of three outcomes when this species grows in mixed species communities: 1) HP receipt is minimized through the evolution of avoidance mechanisms, 2) HP tolerance mechanisms evolve, or 3) both. In fact, pollinator partitioning between *C. speciosa* and *C. xantiana* has been observed (Singh 2014) which may lead to HP avoidance; and in this study we show partial evidence suggesting greater tolerance to HP effects in *C. xantiana* plants from mixed-species communities.

Pollinator facilitation by co-flowering congeners, including *C. speciosa*, has been reported in *C. xantiana* (Moeller 2005). Facilitation between plant species via shared pollinators should only be maintained when the benefits to seed set through increased visitation are not outweighed by the costs of receiving greater amounts of HP on stigmas (Geber and Moeller 2006). Thus, higher reproductive success of *C. xantiana* in the presence of *C. speciosa* would only be possible if HP receipt by the former from the latter is minimal or if tolerance mechanisms to HP effects evolve. Whether HP avoidance and/or tolerance are necessary conditions for the establishment and persistence of pollinator-mediated facilitation, however, remains unknown and is an interesting and promising avenue for future research. What is certain is that our understanding of the traits and mechanisms that mediate HP effects within and across species is still in its infancy and more studies are needed to fully comprehend the puzzling variation observed and thus take our knowledge of HP effects from a descriptive to a predictive stage.

Conclusions

In this study we give evidence of variation in *C. xantiana* conspecific pollen performance and tolerance of HP effects that could be, at least, partially related to the population's history of exposure to HP. Conspecific pollen in populations without a history of exposure to HP produce fewer pollen tubes, especially in the presence of HP, compared with pollen from populations with a history of exposure to HP. The latter results highlight the importance of evaluating tolerance to HP effects not only from the perspective of the female sporophyte but also of the male gametophyte, a phenomenon that so far has been overlooked. We also find that congeneric species of *Clarkia* vary in their tolerance of HP transfer, and suggest that this variation may be due to differences in flower shape affecting flower handling by bees, which should impact the likelihood of HP receipt. However, as this is, to our knowledge, one of the first studies (also see Kay and Schemske 2008) to experimentally test the adaptive potential of plants to tolerate the negative effects of HP receipt (as defined by Morales and Traveset 2008) it is clear that more studies are needed to understand the causes of variation in, and mechanisms of, response to HP receipt (Huang et al. 2015). Studies like the one presented here are important as a first step in understanding the evolutionary consequences of plant–plant post-pollination interactions and are the basis for later assessing the potential traits and mechanisms involved. To date, the study of plant–plant interactions via pollinators has been almost entirely dominated by studies of pre-pollination interactions. However, because the outcomes of pre-pollination interactions can be influenced by interactions that take place on the stigma after pollination has occurred (Galen and Gregory 1989, Sargent and Ackerly 2008), we must begin to address the causes and consequences of interactions at both pollination stages if we are to fully comprehend the role of plant–pollinator interactions in generating and organizing biodiversity.

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Supplementary material (available online as Appendix oik-02594 at <www.oikosjournal.org/appendix/oik-02594>). Appendix 1.