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Co-Flowering Community Effects on the Relative Contribution of Pollen Quantity and Quality Limitation to the Reproductive Success of Four Clarkia Species

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Co-Flowering Community Effects on the Relative Contribution of Pollen Quantity and Quality Limitation to the Reproductive Success of Four *Clarkia* Species

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A thesis

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the faculty of the Department of Biological Science

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Master of Science in Biology

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by

Emma Moore

August 2023

Committee Chair, Dr. Arceo-Gomez

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Keywords: pollination, community ecology, ecology, plant ecology

ABSTRACT

Co-Flowering Community Effects on the Relative Contribution of Pollen Quantity and Quality Limitation to the Reproductive Success of Four *Clarkia* Species

by

Emma Moore

Pollen limitation occurs due to low quantity or quality of pollen delivered to stigmas. Diverse communities where pollinator sharing is common and can influence quality and quantity aspects of pollen limitation. Co-flowering can attract larger numbers of pollinators or they can compete for pollinators affecting pollen loads. Here, we used populations of four *Clarkia* to evaluate how changes in co-flowering communities impact pollen quantity and quality limitation. All Clarkia species differ in the amount of pollen grains received and pollen tubes formed. Pollen quantity and quality varied among individuals within a population compared to individuals across populations or species. Differences in pollen limitation may depend on characteristics rather than co-flowering. This study emphasizes the need to fully evaluate pollen limitation and how these vary across species. This knowledge is key to understanding the processes that mediate plant reproductive success in nature and how plants will respond to human disturbances.

DEDICATION

This is dedicated to my Mom and Dad, and Dr. Arceo-Gomez who helped me through this masters thesis. Thank you

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Thank you to Gerado Arceo-Gomez, Tia Lynn Ashman, Kate Eisen and Monica Geber for your data and specimen contributions to this project.

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CHAPTER 1. INTRODUCTION

Pollination and Pollen Limitation

Most angiosperms (flowering plants) rely on the movement of pollen from male (i.e. anthers) to female reproductive structures (i.e. stigma) by abiotic or biotic vectors for successful fruit and seed production (Wilcock and Neiland 2002). Biotic vectors (i.e. animal pollinators) are considered the most effective, diverse, and efficient form of pollination (Faegri and Van Der Pijl 2013), and approximately 87.5% of all flowering plant species rely on pollinators for successful seed production (Ollerton et al. 2011). However, pollination failure is common and can occur at all steps in the pollination process (Wilcock and Neiland 2002), resulting in inadequate quantity or quality of pollen delivered to stigmas. Low quantity and/or quality of pollen can in turn limit plant reproductive success, a process known as ‘pollen limitation of plant reproduction’ (hereafter; pollen limitation). In fact, more than 60% of plant populations have been reported to experience some degree of pollen limitation (Burd 1994, Ashman et al. 2004, Knight et al. 2005). Thus, it is key to advance our knowledge of the causes and consequences of pollen limitation in natural plant communities to increase our understanding of how these will respond in the face of increasing human disturbances.

Pollen limitation is divided into two main components, pollen quantity and quality, both of which can limit seed production (Ashman et al. 2004, Aizen and Harder 2007, Alonso et al. 2013). Pollen quantity limitation, specifically, occurs due to low pollinator availability, which can lead to low flower visitation rates and small pollen loads delivered to stigmas. In this case, the small quantity of pollen received limits seed production (Knight et al. 2005, Azen and Harder 2007), particularly compared to what plants would have produced with adequate pollen receipt (Knight et al. 2005). For instance, in *Arisaema triphyllurm,* an herbaceous forest perennial, hand pollinated plants (full pollen receipt) produced 70% more seeds compared to naturally pollinated plants, indicating that seed production was being limited by pollinator availability and the amount of pollen received (Bierzychudek 1981).

Low pollen quality can also limit seed production. This typically occurs when pollen grains fail to germinate or successfully reach and fertilize the ovules (i.e., low quality), even when large pollen loads are received. (Toms and Lesperance 2013, Arceo-Gómez et al. 2016, Waser and Price 1991). For instance, in self-incompatible species (i.e., plants that reject genetically identical pollen), self-pollen would be consider of low quality as it cannot achieve ovule fertilization when deposited on stigmas of the same or related plants (Waser and Price 1991, Tehrani and Brown 1992, Fernández et al., 2012). Mechanisms of self-incompatibility are widespread and present in at least 50% of species of angiosperms (Richard 1986). Self-pollen can also be considered of low quality even in self-compatible plants, as it has been shown to have slower germination and pollen tube growth rates compared to outcross pollen (i.e., pollen from distantly related plants), which is typically considered the highest ‘quality’ pollen. (Ashman et al., 2004; Aizen and Harder, 2007, Ashman 2020). Finally, heterospecific pollen (i.e., pollen from a different plant species), is considered of the ‘lowest’ quality, as it invariably results in unsuccessful ovule fertilization when deposited on stigmas (Nettancourt 1977, Ashman et al. 2011, Ashman 2014, Briggs et al. 2016, Gomez et al. 2016). Overall, an increase in self-pollen or heterospecific pollen deposition can reduce pollen load quality an increase pollen limitation in plants. However, to date, is still not fully known what the relative contribution of pollen quality versus quantity limitation is in limiting seed and fruit production in natural plant populations.

Co-Flowering

Co-flowering species (i.e., multiple plant species flowering at the same time), commonly share flower visitors and have the potential to influence each other's pollination success via changes in the amount and quality of pollen they receive (Knight et al. 2005, Moeller et al.. 2005). For instance, pollinator facilitation can occur if co-flowering of multiple plant species increases pollinator visitation and reproductive success of one or multiple species (Rathcke 1983, Braun and Lortie 2019). This increase in pollinator availability due to an increasing number of flowering resources would in turn lead to an increase in pollen deposition and seed production (Fig. 1A), and hence decrease the degree of pollen quantity limitation. For instance, Mesgaran et al. (2017) found evidence that co-flowering of multiple species increased pollinator attraction and decrease pollen quantity limitation. In another study, Yang et al. (2013) found that *P. densispica* was facilitated when co-flowering with *A. pastorius*. In this case honeybee visitation and seed production increased by 70 % when both species co-flower together compared to when *P. densispica* flowered alone (Yang et al. 2013). It is also possible, however, that competition among co-flowering species for the attraction of a limited pollinator pool could reduce pollinator visitation and the amount of pollen deposited on individual stigmas, hence increasing the degree of pollen quantity limitation (Fig. 1B; Baker and Baker 1975, Seymore et al. 2003, Wright and Schiestl 2009 ,Grossenbacher and Whittall 2011;). In a recent 2022 study, Johnson et al. found that in a community of five species, one which was autonomously self-pollinated, three experienced competition and received less pollen, which increased pollen quantity limitation when growing with a different plant species. Interestingly, the self-compatible species did not experience pollen limitation. Showing that self-compatibility could prevent pollen limitation in certain species.

Co-flowering species also have the potential to affect the degree of pollen quality limitation. For instance, a low amount of pollen delivered as a result of pollinator competition could increase the need for self-pollination (i.e. low quality pollen; Bennett, et al. 2005, Moeller, et al. 2005), hence increasing pollen quality limitation (Larson 2000, Lázaro 2009; Fig. 1C). For example, in a study done by Kehrberger and Holzschuh (2019) on *Pulsatilla vulgaris* as the number of co-flowering plant species increased, the number of flower visitors declined, resulting higher self-pollination rates in this species (also see Larson and Barrett 1999, Ashman et al., 2004; Aizen and Harder, 2007). This could increase overall pollen limitation due low pollen quality. Finally, an increase in the diversity of the co-flowering community could also lead to an increase in heterospecific pollen transfer (exchange of pollen between different plant species), potentially increasing the degree of pollen quality limitation (Fig. 1C) (Arceo-Gomez and Ashman 2011, Ashman et al. 2020, Arceo-Gomez et al. 2020). Heterospecific pollen transfer occurs when different plant species exchange pollen via shared pollinators (Ashman and Arceo-Gomez 2013), causing a decrease in pollen tube formation and seed formation. For example, a study done by Peuker et al. (2020), found that of 1117 stigmas examined in a diverse co-flowering community, heterospecific pollen was present on 42% of these, although its potential impact on pollen tube formation and seed set was not evaluated. In a study done by Ashman and Arceo-Gomez 2013, found 2-100% of flowers in their study received some heterospecific pollen in a co-flowering community and over half of the flowers species studied received >%50 of heterospecific pollen (Ashman and Arceo-Gomez 2013). And in another study by Arceo-Gomez et al. 2018, found that depositing *Zea mays* pollen grains onto *Mimulus guttatus* acting as heterospecific pollen transfer significantly decreased reproductive success and decreased amount of produced pollen tubes (Arceo-Gomez et al. 2018). Co-flowering plant species have the potential to positively and/or negatively affect the reproduction of neighboring plant species, our study is aimed at evaluating how changes in co-flowering diversity (i.e., number of co-flowering species) impact the degree of pollen quantity and quality limitation of plant reproductive success.

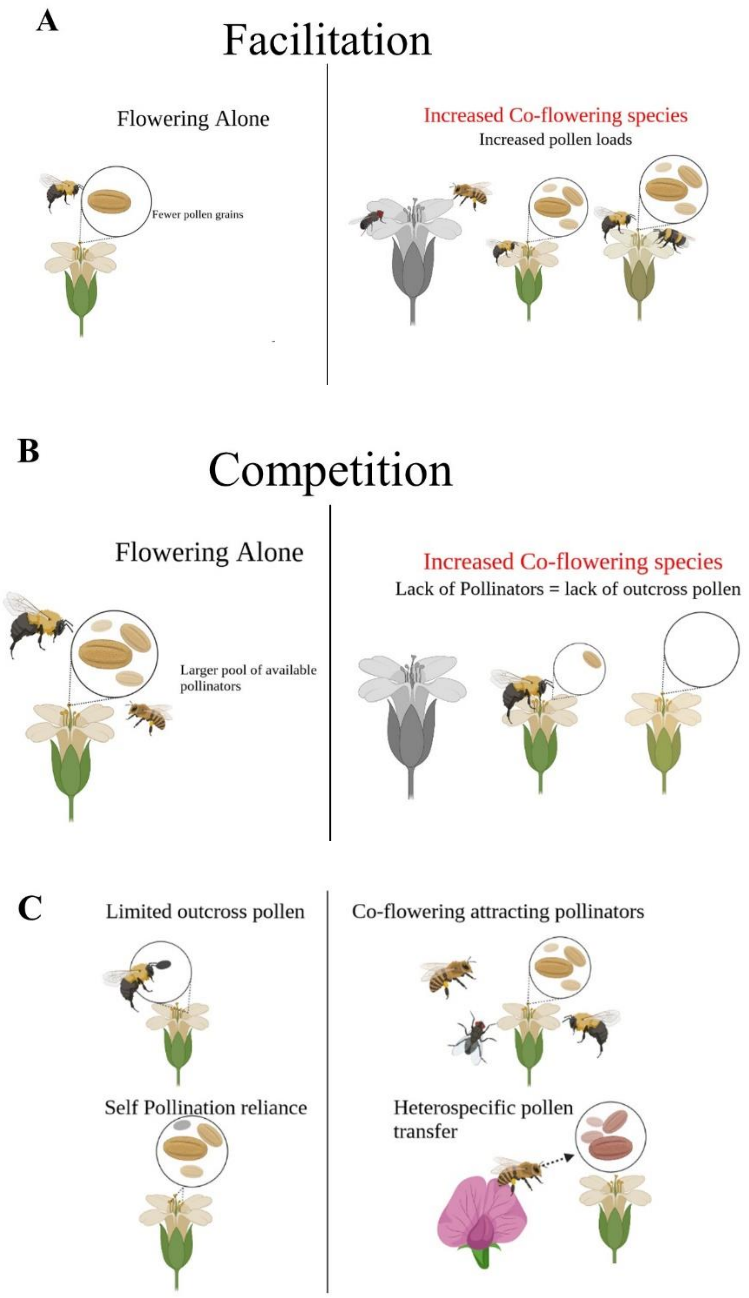


Fig. 1A) Facilitation, where flowering alone will attract a limited pollinator pool, a higher amount of co-flowering species may in fact attract a higher number of pollinators. B) competition, flowering alone the plant species has no competition for a limited pollinator resource, however co-flowering with other species may not attract a larger number of pollinators. 1C. A limited amount of pollen deposition could lead to self-pollination and transfer of pollen grains from different species could result in heterospecific pollen becoming a higher probability or, reduced pollen quality.

Study Species-Clarkia

Clarkia (Onagraceae) species growing in the foothills of the Sierra Nevada in California are an ideal system to evaluate how an increase in the number of co-flowering species can affect the degree of pollen quantity and quality limitation of plant reproduction. In this system, four species of *Clarkia* can co-exist, *Clarkia cylindrica*, *Clarkia speciosa, Clarkia unguiculata* and *Clarkia xantiana*. These *Clarkia* species typically bloom in closed communities (no other plant species in flower are present) and can be found in communities of one to four species flowering simultaneously (Geber et al. 2021). These *Clarkia* species can also rely on self-pollination (i.e., self-compatible) and thus may be susceptible to pollen quality limitation if pollinators are unavailable (Lewis 1959, Bloom 1976, Geber, and Jonas 1999, Runions and Geber 2000, Fausto et al. 2001). In this study we ask the following questions: 1) What is the relative importance of pollen quantity versus quality in limiting plant reproductive success? 2) Does the contribution of pollen quality versus quantity limitation to plant reproduction vary by *Clarkia* species? And 3) does the relative importance of quality versus quality limitation change with increasing number of co-flowering species in the community?

CHAPTER 2. METHODS

Study System

Four species of *Clarkia* including *Clarkia speciosa, Clarkia cylindrica, Clarkia unguiculata* and *Clarkia xantiana* were studied. All four *Clarkia* species are similar in flower color and size and are endemic to California (Fig. 2) (Gould 2016). The studied *Clarkia* species grows in the southern California Sierra Nevada, alongside its foothills and the adjacent Transverse Ranges. This area has a Mediterranean climate with hot dry summers and cold wet winters (Jepson Manual. 1993, Geber 1999, Geber 2005, Eisen and Geber, 2019). The typical vegetation in this area consists of foothill oak and pine woodlands, grasslands, roadside habitats, and steep slopes. All species flower in Late April/Early May until early June usually in response to winter rain (Lewis 1953, Eisen et al. 2019). All species of *Clarkia* are annuals, and these communities can persist in the same location for long periods—30 to 40 years or more (Lewis 1953; Eisen and Geber, 2019) The studied *Clarkias* are not known to hybridize in the wild (Geber 1999). All *Clarkias* are self-compatible and can autonomously self-pollinate (Lewis 1953; Ruane et al. 2020), however the degree of self-pollination can vary greatly among species and populations (Geber 1999, Gould 2016, Eisen et al. 2019). Fertilization in these species typically occurs after 38- 40 hours of pollen deposition. *Clarkia* pollen grains can remain viable for at least 48 hours after being shed from anthers (Smith-Huerta and Vasek 1987).

Clarkia species rely on a shared group of pollinators over the flowering season (Anderson et al. 2021). These include *Clarkia* specialist bee species such as *Hesperapis regularis* or *Lasioglossum pullilabre*, *Megachile gravita* and *Megachile pascoensis* (MacSwain et al. 1973; Fausto et al. 2001; Moeller 2005). Other bees like *Lasioglossum incompletum*, a generalist bee species, have also been found to pollinate *Clarkia* in these communities (Wang and Dudareva et al. 1997). In *C. cylindrica*, each open flower is a bowl of four fan-shaped petals up to about 3.5 centimeters long in intergrading shades of lavender, white, and magenta. *C. Speciosa* has fan-shaped petals 2.5 centimeters long and may be lavender to pink to deep red, sometimes fading to white or yellowish at the base. *C. unguiculata* has showy flowers, fused sepals forming a cup beneath the corolla, and four petals each one to 2.5 centimeters long. *C. xantiana* each petal is up to 2 centimeters long and light to medium purple in color, sometimes with a dark, ringed spot on the petals of the upper whorl (Fig. 2; Lewis & Lewis 1955, Small et al. 1971, Jepson Floral Project 2012).

Clarkia cylindrica **Clarkia speciosa**

Clarkia unguiculata **Clarkia xantiana**

Figure 2: Example of the four *Clarkia* species. Images from Calflora.org

Sample Collection

Styles were collected from naturally pollinated flowers in all 25 total populations. Styles were collected randomly, one style per plant, and from spent/wilted flowers (i.e., flowers at the end of their lifetime), which assured that flowers had received the maximum amount of pollen. Styles were collected across the entire flowering season, approximately twice a week, from all four different *Clarkia* species. There were two sampling periods, one in the summer of 2014 and one in the summer of 2017. In total there were twenty-five sites, divided into locations with one to four co-flowering species (Fig. 3). Style collection ranged between 29 to 100 per site/species with an average of 60 per site. A total of 1,600 styles were collected, placed in microcentrifuges tubes containing 70% alcohol and processed for pollen grain and pollen tube counting.

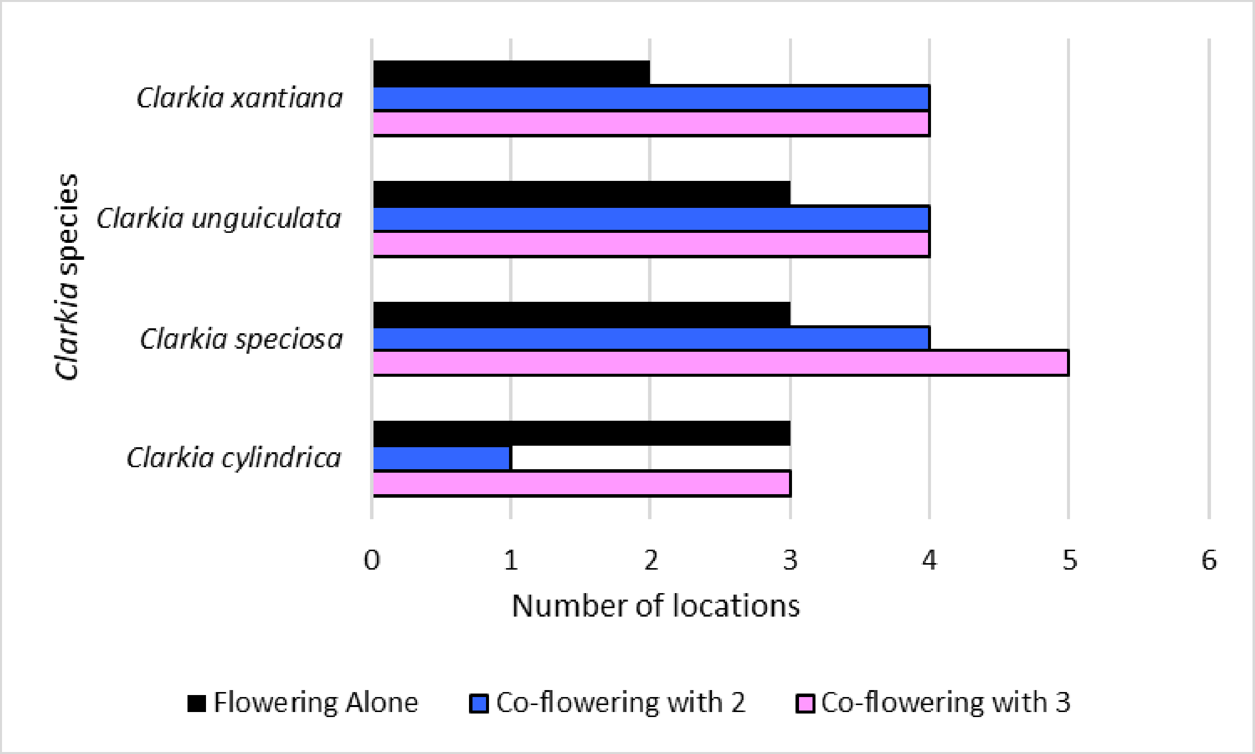


Figure 3: Graph showing number of locations with species and co-flowering species present. There were at minimum 1 site per co-flowering community.

Sample Processing

Styles were softened using 60 ml of KOH and placed into a 64℃-water bath for 10 minutes. KOH was removed using a pipette and then washed with deionized water. Decolorized alkaline blue dye was then added until the style was completely submerged and was placed back into 64°C water bath for 15 minutes. Styles were removed, placed onto a microscope slide with a cover glass and edges were sealed with clear nail polish. Slides were placed under a microscope and the total amount of *Clarkia* pollen grains was counted under 40X magnification (Fig. 4A). *Clarkia* has large and distinct pollen grains (Fig. 4A). Styles with no *Clarkia* pollen grains were rare (only 0.2% of stigmas) and were not considered in this study. Pollen tube counting was conducted under a fluorescent microscope at 40x magnification (Fig. 4B) and the total number of pollen tubes reaching the base of the style was recorded.

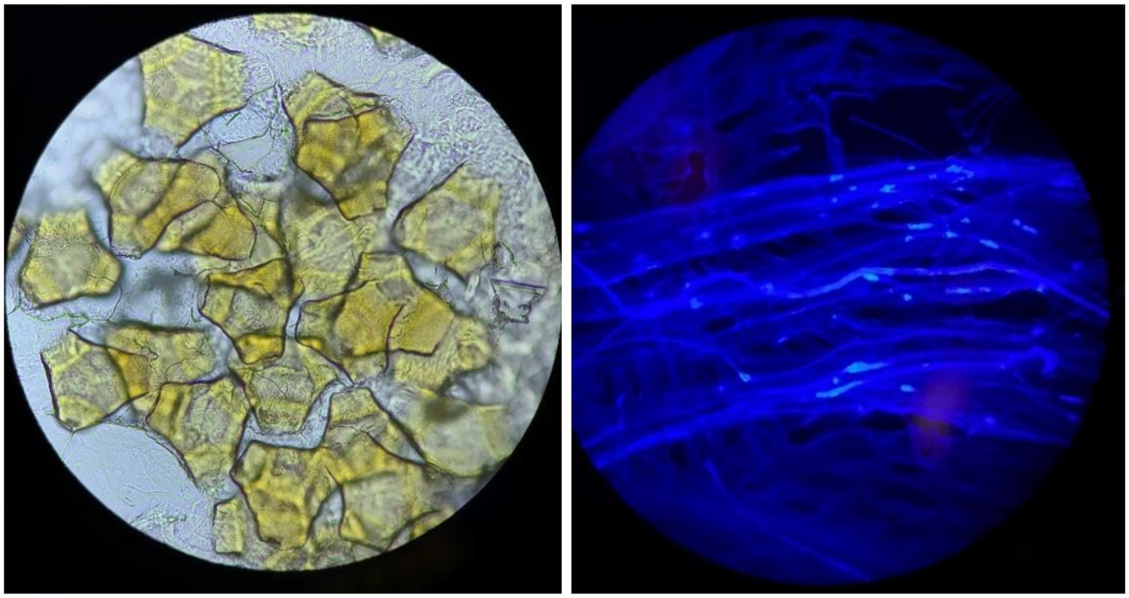


Figure 4A: Pollen grains under 40x power 4B. Pollen tube analysis under 40x power with fluorescent light.

Data Analysis

A mixed-effect model was conducted in SAS 9.4 to evaluate differences between sites, species, and their interaction on pollen load size and the proportion of successful pollen tubes (i.e. pollen tubes that reach the base of the style). Species identity (*C. cylindrica, C. unguiculata , C. speciosa, C. xantiana*) co-flowering richness at a site (alone, 1 or 3 other *Clarkia* species) and their interaction were considered fixed predictor variables. Year of collection and individual site ID were considered as random variables in the analyses. Differences in total pollen load size (pollen quantity) and the proportion of pollen tubes formed in relation to the total number of pollen grains received on a stigma (Pollen tubes/pollen grains) were evaluated. The latter was used as an estimator of pollen quality (i.e., proportion of pollen grains that form successful pollen tubes). For these models we used a lognormal and a beta-binomial distribution respectively, which are considered appropriate for count and proportion data. A partition of variance analyses (proc var comp in SAS 9.4) was conducted to evaluate how variance in the two response variables evaluated (i.e. pollen quantity and quality) was distributed across individual populations, species, and individuals within a population. (Arceo-Gómez et al. 2016, Arceo-Gómez et al. 2019)

CHAPTER 3. RESULTS

Pollen Quantity

Clarkia species varied significantly in the average amount of pollen they received per stigma across all sites (F= 30.96, P <.0001). Specifically, *Clarkia speciosa* received the highest amount of pollen with 400 ± SD 7.5 pollen grains and *C. xantiana* received the least amount of pollen with 160 ± SD 4.2 pollen grains on average per stigma (Fig. 5).

The interaction between 'co-flowering richness' and species identity had a significant effect on pollen quantity (F = 3.96, P = 0.0006). This suggests that the number of other co-flowering *Clarkia* species present at a given site can affect the total pollen load received on stigmas. However, this effect depended on focal *Clarkia* species identity. Specifically, we only observed a statistically significant increase in total pollen load with increasing number of co-flowering species in *C. unguiculata* (P = 0.01, F=30.96). *C. unguiculata* alone received 145 ± 7.04 pollen grains, however when all 4 species were present it received 262 ± 15.3 pollen grains on average (Fig. 6C). Although *Clarkia xantiana* also seemed to receive more pollen grains when co-flowering with other species (Fig. 6D), this difference was not significant.

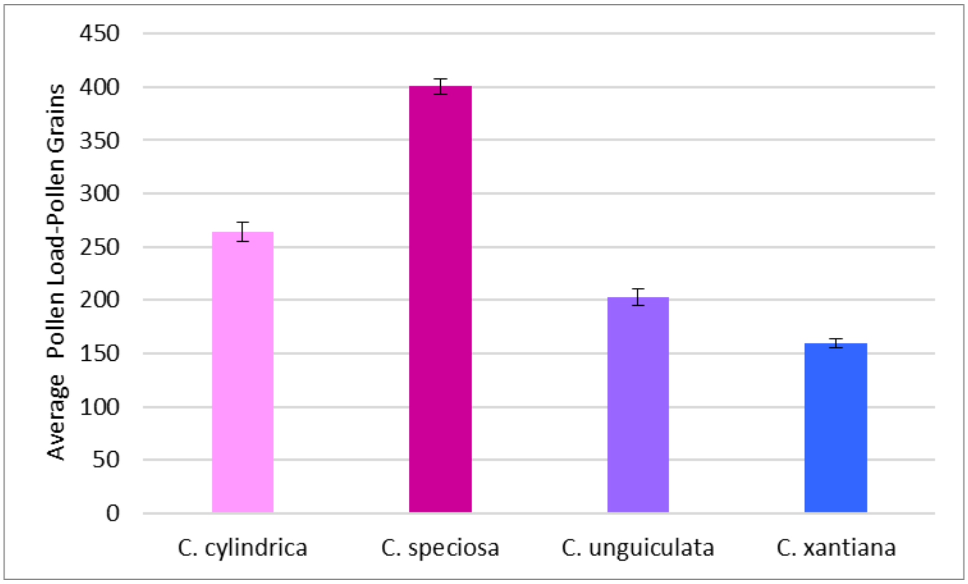
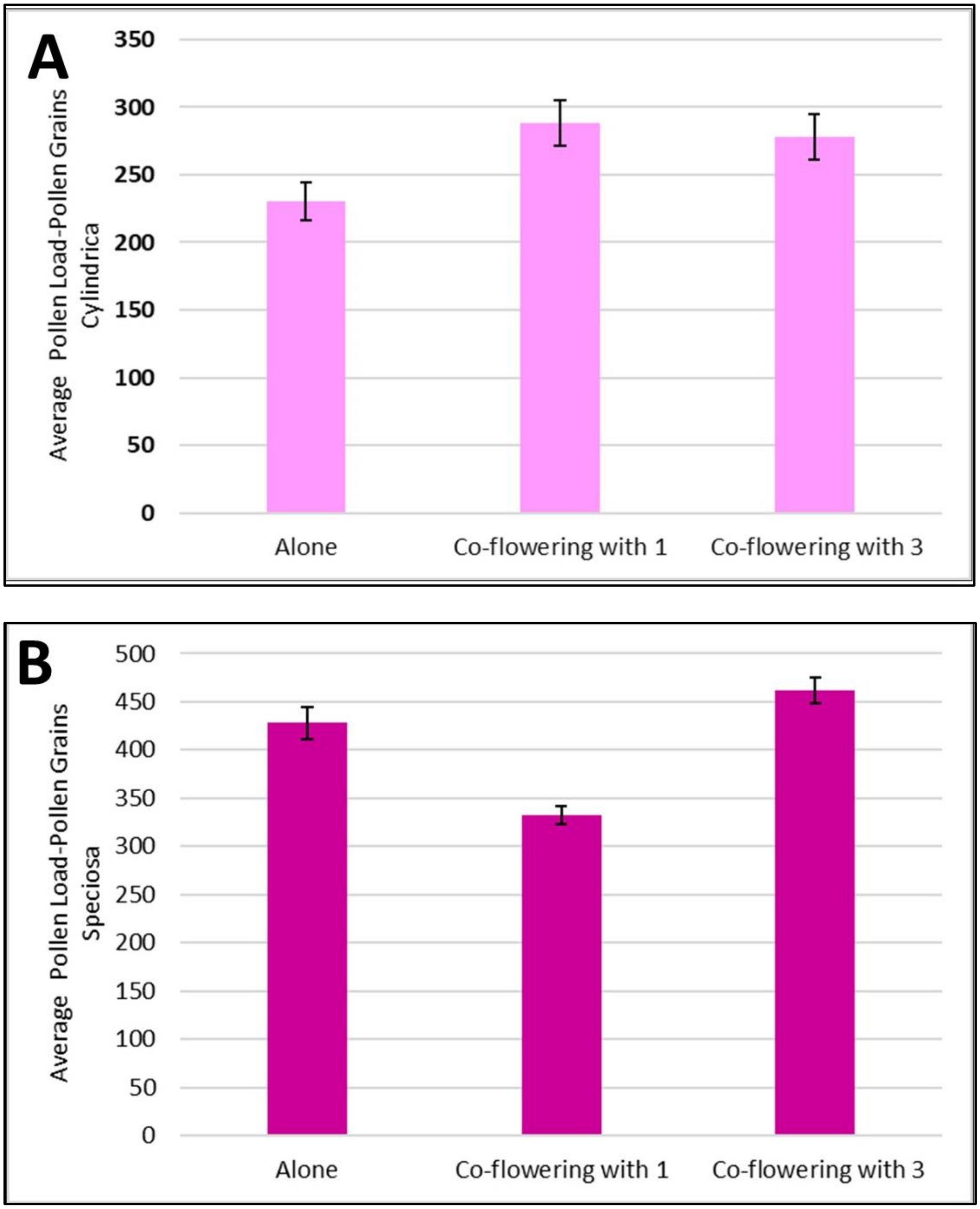
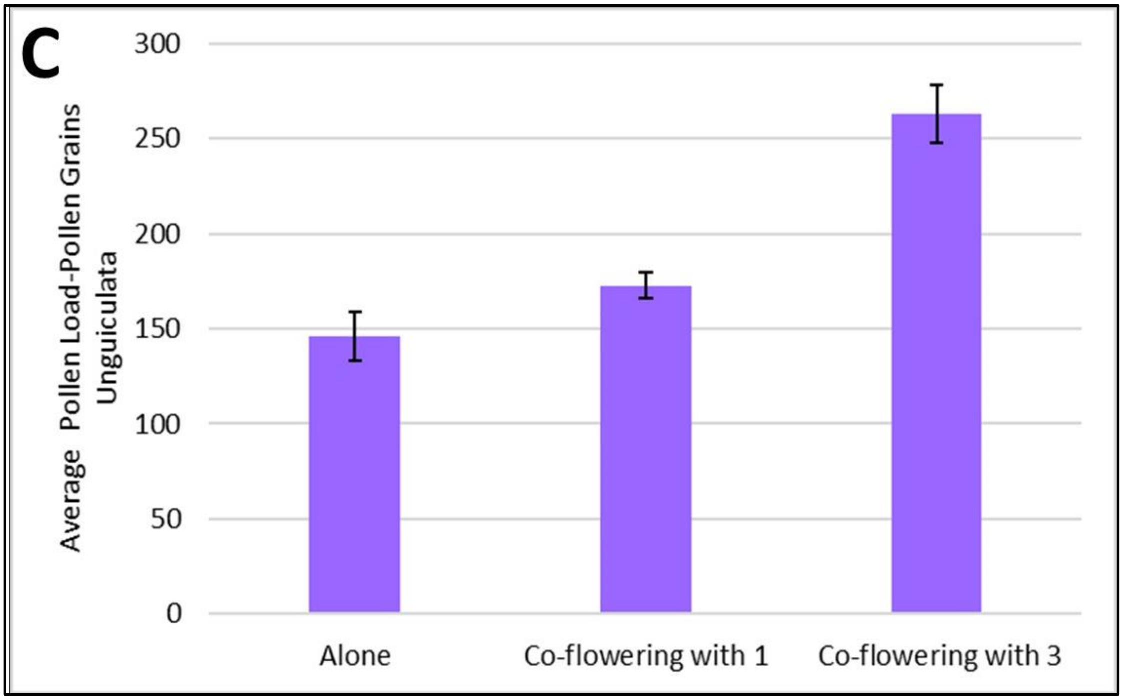


Figure 5: Average pollen load of *Clarkia* pollen grains received by species overall sites, depending on amount of diversity present for all 4 *Clarkia* species.





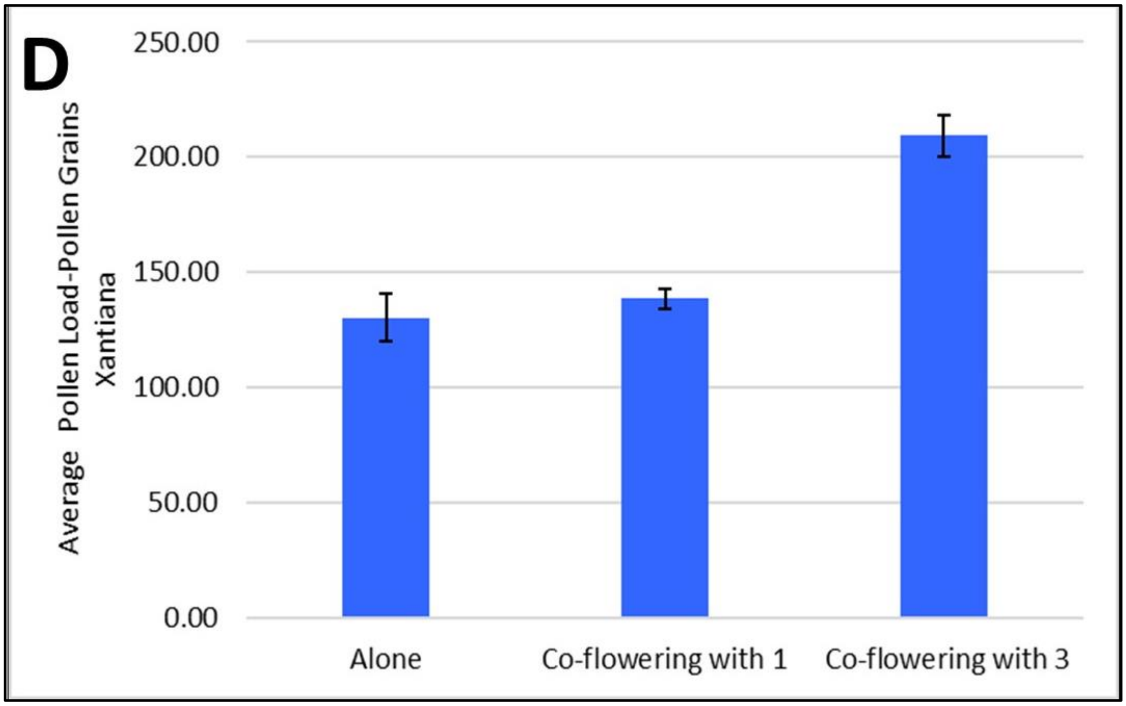


Figure 6.A) Comparison of average *C. cylindrica* pollen grains when flowering alone versus all four present. B) Comparison of average *C. speciosa* pollen grains when flowering alone, with 2 species present and all 4 present. C) Comparison of averages pollen grain load on *C. unguiculata*, when flowering alone, with 2 species present and all 4 species present. No data was avaible for ungucilata

flowering with 3 other species D) Comparison of the average of pollen grain load for *C. xantiana* when flowering alone versus when all 4 species are present.

Pollen Quality

Pollen quality (i.e., proportion of pollen tubes produced) was significantly different among *Clarkia* species (P<.0001, F=14.28). For instance, *C. xantiana*, despite receiving the least amount of pollen (Fig. 5), it produced the highest amount of pollen tubes compared to the other three species (Fig. 7). On the contrary, *C. speciosa* received the highest amount of pollen grains (Fig. 5) yet produced the lowest amount of pollen tubes compared to the other *Clarkia* species (Fig. 7). The number of co-flowering species at a site however did not significantly affect pollen quality (P = 0.74, F =0.30) neither its interaction with species identity (P = 0.48, F = 0.92).

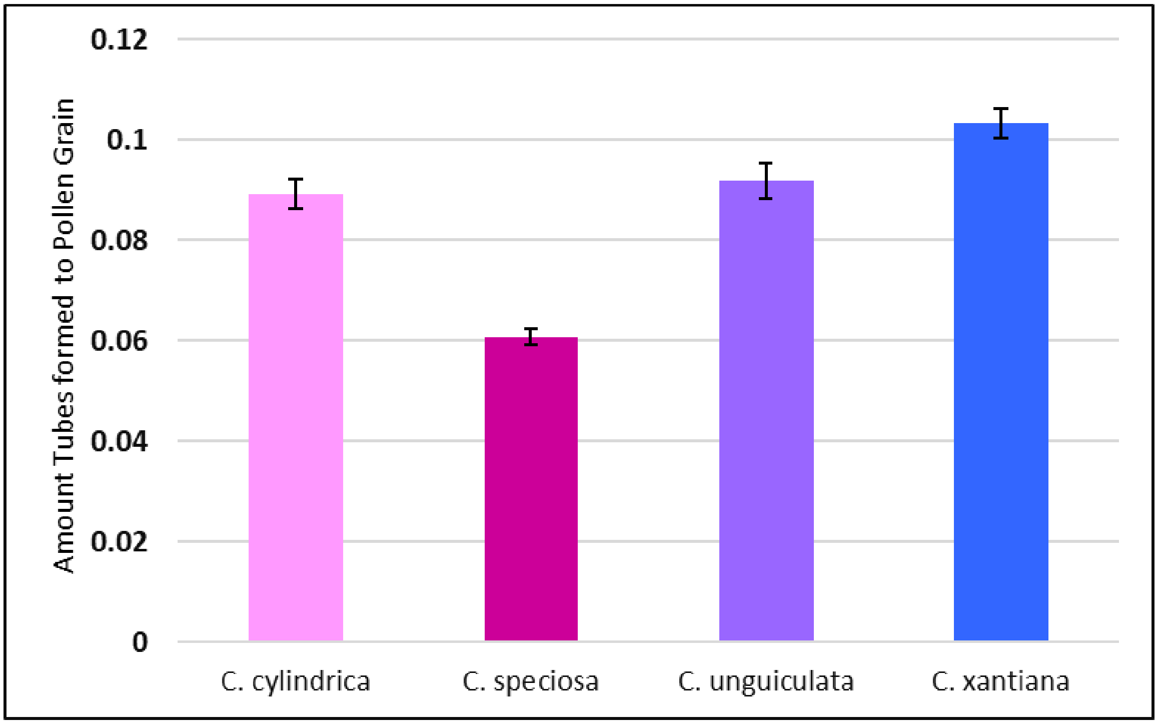
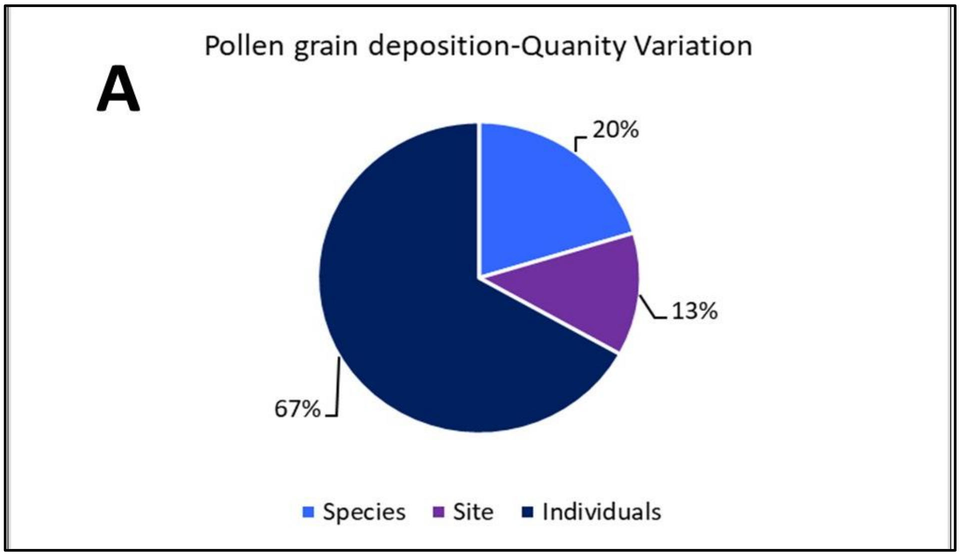


Figure 7: *Clarkia* species pollen tube formation success based on species, *Clarkia xantiana* showing the highest success.

Partition of Variance

Partition of variance analyses showed that the highest amount of variation in the total number of pollen grains received (i.e., pollen quantity) and pollen tubes produced (i.e., pollen quality) occurred among individuals within a population (Fig. 8A-B). Specifically, between-individual variation explained 67% of the total variation in pollen load size (pollen quantity) followed by variation among species (20%) and sites (13%; Fig. 8A). This result indicates that individuals within the same population vary more in total pollen received that the variation observed among different sites and even among different species. For pollen quality (proportion of pollen tubes formed) between-individual variation explained 82% of total variation followed by variation among sites (15%) and differences among species explained the least amount of variation (3%) (Fig. 8B).



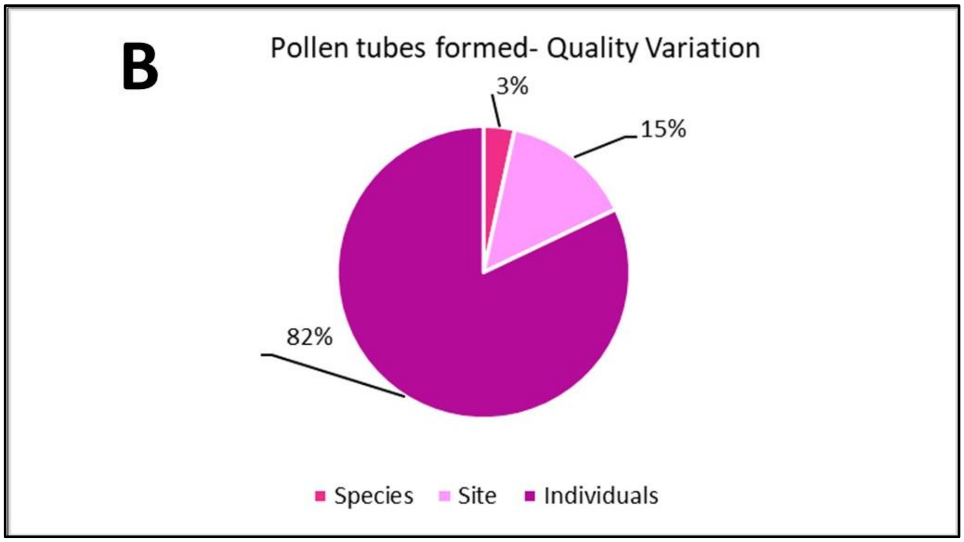


Figure 8A) Variation that occurs regarding quantity, by Site 13%, by species 20% and by Individuals 67%. B) Variation that occurs regarding quality, by species 3%, By site 15% and by individuals 82%

CHAPTER 4. DISSCUSSION

Overall, our results suggest that the diversity of co-flowering *Clarkia* species at any given location does not significantly affect the quantity and quality aspects of pollination success in three of our four studied species (Fig. 6). However, *Clarkia* species themselves do differ in the amount of total pollen they receive. For instance, *C. speciosa* (Fig. 7B) received the highest amount of pollen while *C. xantiana* received the lowest, and this did not depend on the specific site, or on the number of other co-flowering species at that location. (Fig. 7D). Thus, differences in the total amount of pollen received observed may depend on intrinsic reproductive characteristics or pollinator visitation relations associated with each species. For instance, pollen loads can vary depending on the degree to which species can autonomously self-pollinate (i.e., ability to self-deposit pollen on stigmas without the aid of a vector). They can also vary depending on the degree of pollinator specialization, as specialized pollination systems are more efficient than generalized ones (Larsson 2005, Sargent, and Kay 2009). In the case of *C. xantiana*, Moeller (2005) found that even though this species can be visited by 49 different species of pollinators, only a few of them act as efficient pollinators, and specialist *Clarkia* bees are the most effective (Moeller 2005). Thus, the low amount of pollen deposited in *C. xantiana* in our study may be due to low visitation rates by the specialist bees that efficiently pollinate this species, as these may have to compete with a large diversity of other less efficient pollinators. *C.speciosa*, on the other hand, received the highest amount of pollen compared to all other species (Fig. 6). This could be due to high pollinator visitation rates; however, this species also typically relies in a less diverse but highly efficient pollinator community that may deposit large amount of pollen with each visit (MacSwain et al. 1973; Wang, and Dudareva et al. 1997; Fausto et al. 2001; Moeller 2006, Eisen et al. 2019). Based on these results, it could be inferred that the overall degree of pollen quantity limitation is the most severe in *C. xantiana* and the least severe in *C. speciosa* (Lewis 1955, Moller, and Geber 2005). These results thus emphasize the importance of evaluating both, quantity and quality aspects of pollen limitation, as these can differentially impact plant reproductive success even in closely related species growing in the same geographic region.

Other studies have found that an increasing number of co-flowering species can increase pollinator visitation rate and pollen load size, which decreases pollen quantity limitation (i.e. facilitation; Mesgaran et al. 2017). In this study we did observe evidence of pollinator facilitation via an increase in pollen quantity, but only for 1 species, *C. unguiculata. C. unguiculata* is a bee pollinated species mainly through generalist pollinators such as *Bombas* (Moller and Geber 2005) and 10 specialist solitary bee species, but also are most found in areas with other *Clarkais* present, mostly *C. xantiana* and *C. speciosa* (MacSwain et al. 1973, Moller 2005, Peach et al. 2020). These species have seemingly low attraction rates, mainly observed in their low UV areas of attraction. (Peach et al. 2020). This low attraction rate could indicate that *C. unguiculata* is relying on outcross visitation rates for pollen deposition, but the pollen grains deposited are low quality due to other species flowering in the area. In a study done by Goodwille et al., found that flowers diameter has a positive correlation to outcrossing rates, and as these flowers are smaller than others in this study, this could lead to smaller attraction (Goodwille 2009). In a study not related to *Clarkia* but similar in experimental design and flowers morphology, researchers Mačukanović-Jocić et al. 2011 compared florally attraction among 5 study species and found that the largest attraction rate were flower scent and color, followed by the shape and size (Mačukanović-Jocić et al. 2011). They also found that the study plant species *S. sclarea* had the least attraction; which appear to be smaller and have less color than the other species used in this study (Mačukanović-Jocić et al. 2011). We could hypothesis that since *C. unguiculata* has smaller areas of UV attraction, is smaller in size and whose shape (Fig. 2) may lead it to require to flower with other *Clarkias* to receive more pollen deposition, however leaving it open to receiving heterospecific pollen loads.

The proportion of pollen tubes formed in *C. xantiana* was the highest compared to all other *Clarkia* species (Fig. 7), even though the amount of pollen received was the lowest. This implies that the quality of the pollen received in this species is the highest, which may compensate for the low amounts of pollen received and can increase reproductive success in this species. *C. xantiana* has a small abundance of reliable visitation rates, however the species that tend to act as efficient pollinators are specialist *Clarkia* species pollinators. 61% of *Clarkia xantiana* pollinators were *Clarkia* specialist, dominated by a single bee species, *L. pullilabre* (Eckhart et al. 2006). These specialists while small in abundance are efficient in pollination, resulting in high quality pollen deposition, depositing only *C. xantiana* to these species' stigmas, reducing pollen quality limitation.

Pollen quality (proportion of pollen tubes produced) in *C. speciosa* on the other hand is the lowest (Fig. 7) even though this species received the highest amount of pollen grain of all *Clarkia* species (Fig. 5). While *C. speciosa* is visited by large amounts of observed specialist pollinators that deliver high amounts of pollen, such as *Hesperapis regularis*, *Megachile gravita* and *Megachile pascoensis* (MacSwain et al. 1973; Wang, and Dudareva et al. 1997; Fausto et al. 2001; Moeller 2006, Eisen et al. 2019). These species are often shared between other *Clarkia* species, mainly *C. xantiana* and *C. unguiculata* (Moeller 2005, Arceo-Gomez et al. 2016) and the 'quality' of the pollen is low (i.e. self-pollen or heterospecific pollen). As *Clarkia* species can have high levels of pollinator sharing (Moeller 2005, Arceo-Gomez et al. 2016), that may lead to heterospecific pollen transfer (Arceo-Gomez et al. 2016, Fang et al. 2023) resulting in incomplete pollen fertilization (Ashmen et al. 2020, Arceo-Gomez 2021).

This could be the case in *C. speciosa* if its specialized pollinators also visit other *Clarkia* species in the community. *Clarkia* pollen grains are morphologically identical and are therefore difficult to differentiate on the stigma when counting (self-observation). Heterospecific pollen grains would fail to germinate on the stigma since hybridization between different *Clarkia* species is not known to occur in natural populations (Gerber 1999, Arceo-Gomez et al. 2016). Shared pollinators transfer pollen from different species, different plants or different flowers have been noticed (Fausto et al. 2001, Moeller 2005, Eisen and Geber 2019). *C. speciosa* cup shape and larger showy flowers could have been a large attractor or landing area for the flowers, increasing Heterospecific pollen deposition. *C. speciosa* is 97% primarily an outcrossing species and does not hybridize (Lewis 1955, Moeller, and Geber 2005) and hence deposition of large quantities of self- or closely related pollen can lead to low pollen tube growth and ovule fertilization (Bloom 1977, Eisen et al. 2019). A previous study strongly suggested that Heterospecific pollen traits are mediated by style length, stigma type and stigma area, and the flowers bowl shape may in fact lead to a higher frequency in heterospecific pollen transfer than *C. xantiana* (Arceo-Gomez et al. 2016).

Overall, our results suggest that the reproductive success of *Clarkia* species can be differentially limited by quantity and quality components of the pollination process, even when co-existing in the same community. Furthermore, the relative importance of pollen quality and quantity in limiting the reproductive success of these species seems to be independent of the surrounding floral neighborhood and the number of other co-flowering *Clarkia* species in the community, except for *C. unguiculata*. Supporting these results we also observed that the largest amount of variation in pollen quantity (total pollen received) and quality (proportion of pollen tubes formed) occurs between individual plants within the same population. This suggests that large-scale population differences (biotic and/or abiotic), such as the number of co-flowering species, play a minor role in mediating pollination success in most of these *Clarkia* species. On the contrary, small-scale differences within the same site such as differences in micro-environmental conditions or plant phenotypic differences (e.g., flower size, nectar, color, scent) play a large role in affecting pollinator foraging preferences and hence pollination success within a given site (Fig. 8). Future work employing experimental hand pollination treatments would further help confirm the degree of overall pollen limitation in these *Clarkia* species. We expected process such as pollinator competition and facilitation to be important in this system and to influence patterns of pollen deposition as the number of co-flowering *Clarkia* species increased. However, intrinsic reproductive features associated to each individual species, such as its mating system and pollinator community seem to play a much larger role in mediating quantity and quality aspects of pollination success. Therefore, it is imperative that we fully evaluate all aspects of pollen limitation, and how these can affect pollination and plant reproductive success.

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