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ORIGINAL ARTICLE

Co-flowering richness has variable effects on pollen quantity and quality limitation in four *Clarkia* species

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- **Background and Aims** Pollination failure occurs from insufficient pollen quantity or quality. However, the relative contributions of pollen quantity vs. quality to overall pollen limitation, and how this is affected by the co-flowering context, remain unknown for most plant populations. Here, we studied patterns of pollen deposition and pollen tube formation across populations of four predominately outcrossing species in the genus *Clarkia* to evaluate how the richness of co-flowering congeners affects the contribution of pollen quantity and quality to pollen limitation.
- **Methods** We partition variation in pollen deposition and pollen tube production across individuals, populations and species to identify the main sources of variation in components of reproductive success. We further quantify the relative contribution of pollen quantity and quality limitation to the reproductive success of the four *Clarkia* species using piecewise regression analyses. Finally, we evaluate how variation in the number of co-flowering *Clarkia* species in the community affects the strength of pollen quality and quality limitation.
- **Results** Across all contexts, pollen deposition and the proportion of pollen tubes produced varied greatly among individuals, populations and species, and these were not always correlated. For instance, *C. xantiana* received the smallest pollen loads yet produced the highest proportion of pollen tubes, while *C. speciosa* exhibited the opposite pattern. Yet, co-flowering richness had variable effects on the strength of pollen quantity and quality limitation among populations. Specifically, breakpoint values, which are an indicator of overall pollen limitation, were two-fold higher in the four-species community compared with one- and two-species communities for two *Clarkia* species, suggesting that pollen limitation can increase with increasing richness of co-flowering congeners.
- **Conclusions** Our results reveal a complex interplay between the quantity and quality of pollen limitation and co-flowering context that may have different evolutionary outcomes across species and populations.

Key words: *Clarkia*, facilitation, piecewise regression, pollination, pollen tubes, pollen deposition.

INTRODUCTION

Angiosperms often rely on biotic vectors to move pollen from male (anthers) to female reproductive structures (stigmas) for successful reproduction (Waser *et al.*, 1996; Wilcock and Neiland, 2002; Ollerton *et al.*, 2011). However, pollination failure is common, and occurs when plants receive an inadequate quantity and/or quality of pollen on stigmas, a phenomenon known as ‘pollen limitation’ (Burd, 1994; Larson and Barrett, 2000; Ashman *et al.*, 2004; Knight *et al.*, 2005). In fact, global estimates suggest that reproduction is limited by pollen receipt in more than 60 % of plant populations (Ashman *et al.*, 2004; Knight *et al.*, 2005; Vamosi *et al.*, 2006; Bennett *et al.*, 2020), and that half of all flowering species could experience an 80 % decline in seed production without pollinators (Rodger *et al.*, 2021). Thus, advancing our knowledge of the causes and consequences of pollen limitation is central to understanding the factors that limit plant reproductive success and to safeguarding natural plant populations and communities, particularly considering increasing human-mediated disturbances.

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Decreases in both pollen quantity and quality can limit seed production but these can result from different ecological processes (Ashman *et al.*, 2004; Aizen and Harder, 2007; Alonso *et al.*, 2013). Pollen quantity limitation can occur when a decrease in pollinator availability leads to insufficient pollen deposition on stigmas (Larson and Barrett, 2000; Knight *et al.*, 2005; Gómez *et al.*, 2010), thus decreasing seed production (Ashman *et al.*, 2004; Knight *et al.*, 2005). On the other hand, pollen quality limitation occurs when pollen grains fail to germinate on the stigma or fail to successfully fertilize the ovules (Waser *et al.*, 1996; Toms and Lesperance, 2003; Aizen and Harder, 2007; Arceo-Gómez *et al.*, 2016*a*). Pollen quality limitation can occur due to the deposition of self-pollen in self-incompatible species (Waser and Price, 1991; Tehrani and Brown, 1992; Eckert *et al.*, 2010), which represent at least 50 % of all angiosperms (Goodwillie *et al.*, 2005; Eckert *et al.*, 2010). Even in self-compatible plants, self-pollen can have slower germination and pollen tube growth rates compared with outcross pollen (i.e. cryptic self-incompatibility), and thus be of lower quality (Bateman, 1956; Weller and Ornduff, 1977; Bowman, 1987; Eckert *et al.*, 2010). Pollen quality limitation may also result from heterospecific pollen – pollen from a different plant species – as it almost invariably results in unsuccessful ovule fertilization when deposited on stigmas (Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013; Ashman *et al.*, 2020), although effects may vary with pollen donor identity (Arceo-Gomez *et al.*, 2019*b*; Streher *et al.*, 2020). Despite the overall importance of understanding the mechanisms driving pollen limitation, to date, the relative contributions of pollen quality vs. quantity to pollen limitation is only known for a few plant populations (e.g. Aizen and Harder, 2007; Harder *et al.*, 2016*a*, 2016*b*).

One component of the environment that can influence quantity and quality aspects of pollination is the diversity and composition of the co-flowering community (Moeller, 2004; Ghazoul, 2006; Mitchell *et al.*, 2009; Arceo-Gómez and Ashman, 2014). Co-flowering species can either increase or decrease pollen quantity limitation. For instance, if co-flowering species provision a larger pollinator community, then the increase in pollinator availability can increase conspecific pollen deposition and seed production, hence decreasing pollen quantity limitation (Rathcke, 1983; Moeller, 2004; Ghazoul, 2006). However, competition among co-flowering plants has been more commonly observed (Mitchell *et al.*, 2009). In these systems, co-flowering species compete to attract a limited pollinator pool, reducing pollinator visitation and thus increasing pollen quantity limitation (Waser, 1978; Campbell, 1985; Mitchell *et al.*, 2009; Runquist and Stanton, 2013; Johnson *et al.*, 2022).

Co-flowering plant species also have the potential to affect the quality of pollen received. For instance, low amounts of pollen delivered as a result of pollinator competition can lead to an increase in self-pollination (Moeller and Geber, 2005; Eckert *et al.*, 2010), reducing pollen quality (Larson and Barrett, 2000; Aizen and Harder, 2007; Lázaro *et al.*, 2009). An increase in the number of co-flowering species can also lead to higher levels of heterospecific pollen transfer if pollinators visit more than one species in a single foraging bout (inconstant foraging; e.g. McLernon *et al.*, 1996; Arceo-Gomez and Ashman, 2011; Fang and Huang, 2013; Ashman *et al.*, 2020). Heterospecific pollen transfer is widespread in diverse co-flowering communities with some plant species receiving >50 % of heterospecific pollen and experiencing negative consequences for reproductive success (Morales and Traveset, 2008; Ashman and Arceo-Gómez, 2013). However, plants may evolve traits that minimize the effects or promote the tolerance of heterospecific pollen (Hopkins and Rausher, 2012; Ashman and Arceo-Gómez, 2013). While the influence of co-flowering species on pollinator visitation and reproduction of neighbouring plants is well-documented (Ghazoul, 2006; Mitchell *et al.*, 2009; Braun and Lortie, 2019), the effect of co-flowering species richness on pollen quantity vs. quality limitation to plant reproductive success is not well understood.

Our ability to quantify the relative magnitude of pollen quantity and quality limitation has recently improved via the use field-collected styles from spent flowers to characterize the relationship between pollen grains deposited on stigmas and pollen tubes growing in styles (Fig. 1) (Alonso *et al.*, 2012; Arceo-Gómez and Ashman, 2014; Cisternas-Fuentes and Koski, 2024). In contrast to traditional hand-pollination experiments, estimating pollen limitation from field-collected styles is more tractable across large biological and spatial scales, including across multiple species and populations (Arceo-Gómez and Ashman, 2014; Cisternas-Fuentes and Koski, 2024). Specifically, the piecewise approach developed by Alonso *et al.* (2012) distinguishes between quantity and quality limitation by estimating differences in the number and quality of pollen grains required to reach a saturation point in the number of pollen tubes that reach the base of the style (Fig. 1). This approach has the advantage that it avoids the confounding effects of resource availability and re-allocation which can influence estimates of pollen limitation based on hand-pollination experiments (Ashman *et al.*, 2004; Knight *et al.*, 2005; Alonso *et al.*, 2012). Additionally, traditional hand-pollination approaches rely on manual supplementation of outcross pollen (high-quality pollen), potentially confounding quality and quantity aspects of pollen limitation (Ashman *et al.*, 2020). The piecewise approach thus provides a unique opportunity to evaluate both aspects of pollen limitation (quantity and quality) across large spatial scales that encompass a range of biotic and abiotic contexts.

Uncovering patterns of natural variation in pollen deposition and pollen tube formation and evaluating how this variation is structured at different levels of biological organization (i.e. populations, plants within populations) can provide further insights into the factors determining plant reproductive success in nature (Herrera *et al.*, 2002; Arceo-Gomez *et al.*, 2016*b*). For instance, large variance among populations would indicate that community attributes, such as co-flowering richness (e.g. Schuett and Vamosi, 2010; Sargent *et al.*, 2011; Arceo-Gómez and Ashman, 2014), are important drivers of pollination success. On the other hand, greater variance among plants within a population would indicate that plant differences in traits that influence pollinator attraction such as flower size and floral display (e.g. Fishman and Willis, 2008; Sandring and Ågren, 2009) may play an important role. Understanding the main sources of variation in pollen receipt and pollen tube formation is hence important for determining the forces that mediate plant

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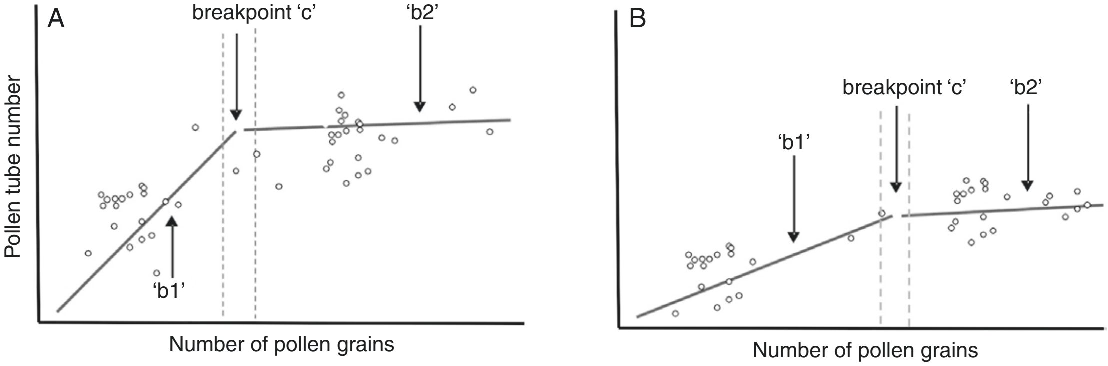

FIG. 1. Conceptual framework for the use of piecewise regression to evaluate patterns of pollen quantity and quality limitation by evaluating the relationship between the number of pollen grains deposited on stigmas and the number of pollen tubes at the base of the style (Alonso *et al.*, 2012). The pollen grain–tube relationship is shown for two hypothetical populations (A and B) where each dot represents data from individual senesced flowers collected in the field. Each relationship is characterized by three parameters: the first slope (b1) represents the 'rate' at which pollen tube saturation is reached, the breakpoint (c) represents the number of pollen grains required to achieve a saturation point (±95 % confidence interval) and the second slope (b2) estimates the degree to which pollen quality alone increases pollen tube production after an asymptote has been reached. (A) A population with a lower breakpoint and a steeper first slope (b1), which are indicative of higher pollen quality because a smaller number of pollen grains (compared to the population in B) are necessary to reach saturation in pollen tube production. In species where the quality of pollen is high, pollen quantity is expected to be the main factor limiting plant reproductive success. (B) A population where pollen quality is lower (smaller b1 value and a larger breakpoint). In this population, pollen quality is expected to be the main factor limiting reproductive success because increases in pollen deposition have little effect on pollen tube production relative to increases in pollen quality.

reproductive success in nature (e.g. Herrera *et al.*, 2002; Arceo-Gómez *et al.*, 2016*b*).

In this study, we quantify pollen deposition and pollen tube formation to evaluate how their variance is structured across individuals and populations of four *Clarkia* species. We further use piecewise regression to estimate quantity and quality components of pollen limitation of the four *Clarkia* species across 25 sites with varying richness of co-flowering *Clarkia* species. We ask the following specific questions: (1) What are the major sources of variation (species, populations or individual plants) in pollen deposition and proportion of pollen tubes produced in *Clarkia*? (2) What is the relative contribution of pollen quantity and quality limitation to plant reproductive success and does it vary by *Clarkia* species? (3) Does an increase in the number of co-flowering *Clarkia* species affect the strength of pollen quality and quality limitation and does the effect depend on *Clarkia* species identity?

MATERIALS AND METHODS

Study system

Four common species of *Clarkia* were studied in Kern Canyon of the southern California Sierra Nevada: *Clarkia speciosa* subsp. *polyantha* H. Lewis and M. Lewis, *Clarkia cylindrica* subsp. *clavicarpa* W. Davis, *Clarkia unguiculata* Lindley and *Clarkia xantiana* subsp. *xantiana* A. Gray (Fig. 2). In this region, one to four of the *Clarkia* species are found at study sites (Appendix 1; Moeller, 2004; Eisen and Geber, 2018; Eisen *et al.*, 2019). This area has a Mediterranean climate with hot, dry summers and cold, wet winters (Jonas and Geber, 1999; Geber and Eckhart, 2005). The vegetation consists of oak and pine woodlands and grasslands (see Appendix 2 for common plant species). *Clarkia* are among the last species to flower from late April/early May to mid-June, and therefore overlap little in flowering time with co-occurring forbs. Additionally, the most frequent flower visitors to the four *Clarkia* species are a guild of largely solitary bee species that specialize on the genus *Clarkia* (MacSwain, 1973; Moeller and Geber, 2005; Singh, 2014; James *et al.*, 2022; Appendix 3). These specialist bees carry only *Clarkia* pollen and thus do not transfer pollen from non-*Clarkia* species (Moeller and Geber, 2005; James *et al.*, 2022), and they carry a lot more *Clarkia* pollen than generalist bee visitors (Moeller and Geber, 2005). The main specialist bee pollinators are *Hesperapis regularis*, *Lasioglossum pullilabre*, *Megachile gravita*, *Megachile pascoensis* and *Diadasia angusticeps* (Appendix 3; MacSwain, 1973; Moeller, 2004; Moeller and Geber, 2005; Eckhart *et al.*, 2006; Singh, 2014; James *et al.*, 2022). These attributes of *Clarkia*'s pollination biology mean that pollinator-mediated interactions are largely limited to interactions among *Clarkia* species rather than between *Clarkia* and co-occurring non-*Clarkia* species (James and Geber, in review).

Patterns of pollen limitation may vary among *Clarkia* species because differences in their degree of pollinator specialization can lead to variation in the size and composition of pollen loads they receive (e.g. Lázaro *et al.*, 2015), particularly of congeneric *Clarkia* pollen. Congeneric pollen is the lowest quality pollen delivered to stigmas because *Clarkia* pollen does not germinate and pollen tubes do not grow on heterospecific stigmas and styles (Lewis and Lewis, 1955; Arceo-Gómez *et al.*, 2015). *Clarkia* species differ in the frequency of visitation by generalist bees and in the degree of pollinator sharing (Appendix 3; Singh, 2014; James, 2020; James *et al.*, 2022). For instance, *C. unguicuata* receives the lowest fraction of visits by specialist bees (~40 %), followed by *C. xantiana* (~55 %), *C. speciosa* and *C. cylindrica* (~80 %) (Appendix 3; Singh, 2014). Some of the common specialists (e.g. *Hesperapis*, *Megachile* sp., *Lasioglossum pullilabre*) are broadly shared by two or more *Clarkia* species and are likely to transfer heterospecific *Clarkia* pollen (Appendix 3; James *et al.*, 2022). By contrast, the specialist *Diadasia angusticeps* visits *C. speciosa* almost exclusively, is *C. speciosa*'s most frequent visitor and carries little

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FIG. 2. Four studied co-flowering *Clarkia* species. All *Clarkia* species occur in the southern California Sierra Nevada foothills and can be found in populations of one to four species flowering simultaneously (modified from Eisen and Geber, 2018).

pollen of other *Clarkia* species (Appendix 3; Singh, 2014; James *et al.*, 2022).

The four *Clarkia* species are annuals, and populations persist in the same location for long periods of time (e.g. 30–40 years; Lewis, 1953; Arceo-Gómez *et al.*, 2015; Eisen and Geber, 2018). The studied *Clarkia* species are not known to hybridize in the wild (Lewis and Lewis, 1955; Jonas and Geber, 1999). All four species are self-compatible but rely on pollinators for reproduction because autonomous selfing is limited by pronounced protandry and herkogamy (Lewis, 1953). Outcrossing rates are generally high but variable across populations (Hove *et al.*, 2016; Ivey *et al.*, 2016). *Clarkia* species have similar numbers of ovules per flower, though the average ovule is slightly higher in *C. cylindrica* (James and Geber, in review; ovule number per flower: *C. cylindrica* mean = 62, range = 8–143; *C. speciosa* mean = 56.1, range = 2–199; *C. unguiculata* mean = 50.1, range = 1–106; *C. xantiana* mean = 53.6, range = 3–105). The species typically produce 20–30 seeds per flower but vary in flower number per plant under natural conditions, with *C. speciosa* having the fewest flowers per plant (Németh and Smith-Huerta, 2003, James and Geber, in review; *C. cylindrica* mean = 10.8, range = 1–115; *C. speciosa* mean = 5.2, range = 1–50; *C. unguiculata* mean = 11.2, range = 1–135; *C. xantiana* mean = 19.2, range = 1–200). Flowers open sequentially, with only a subset of flowers, from as few as one to ~20 flowers open at the same time depending on the size of the plant. Sequential flower opening limits opportunities for geitonogamous selfing on small plants.

Sample collection and processing

To evaluate how different components of pollination success change with increasing number of co-flowering *Clarkia* species, styles were collected randomly from wilted, naturally pollinated flowers at 24 different sites and 46 total species/site combinations (Appendix 1). Sites were selected to represent replicates of different combinations of one-species, two-species and four-species sites across Kern Canyon. *Clarkia* flowers typically wilt at the end of the day (M. Geber, pers. obs.) and pollen tubes reach the ovary 8 h after pollen deposition (Briscoe Runquist *et al.*, 2014; Arceo-Gómez *et al.*, 2015). Thus, by collecting wilted flowers in the morning/afternoon (i.e. wilted from the previous day) we ensured that all flowers had reached maximum pollen loads and pollen tubes and that no additional pollen had accumulated for at least 12 h. The number of *Clarkia* species at a site varied from a single *Clarkia* species (one-species sites; $n = 12$), to two *Clarkia* species (two-species sites; $n = 17$), to all four *Clarkia* species (four-species sites; $n = 17$). One style per plant was collected randomly from 30–118 individuals per species per site across the entire flowering season (approx. every 4–5 d) for a total of 3776 styles. Styles were sampled from 31 populations (species by site combinations) in 2014 and 15 populations in 2017 (Appendix 1). Each sampling year was considered an independent sampling event but variation between years was accounted for in the analyses (see below). Styles were stored in microcentrifuge tubes containing 90 % ethanol and processed in the lab for pollen grain and pollen tube counting. Styles were softened with 1 M KOH and then stained using decolorized aniline blue (Arceo-Gómez *et al.*, 2016*b*). Styles were mounted onto microscope slides and the amount of *Clarkia* pollen grains (i.e. pollen quantity) was counted with a compound microscope under 40× magnification (Arceo-Gómez *et al.*, 2016*b*). Pollen grains from different *Clarkia* species cannot be distinguished morphologically (pers. obs.; Ha and Ivey, 2017), and counts of pollen grains on stigmas are therefore counts of both conspecific and heterospecific pollen. The number of pollen tubes at the base of the style (i.e. pollen quality) was counted under an epifluorescence microscope (Nikon Eclipse 80i/Leica DM 3000) at 40× magnification (Arceo-Gómez *et al.*, 2016*b*).

Data analysis

Variation in pollen deposition and proportion of pollen tubes. We estimated the relative contributions of *Clarkia* species identity, the co-flowering context (one-species, two-species and four-species sites) and of intrinsic individual plant characteristics (residual variance) to the overall variance in *Clarkia* pollen deposition (pollen quantity) and proportion of pollen tubes produced (pollen tubes at the base of the style/total *Clarkia* pollen load on stigmas). We use the proportion of pollen tubes for this analysis as it reflects the proportion of pollen grains that are successful (pollen quality) and is a measure of reproductive success that standardizes for variation in the number of pollen grains received on stigmas across individuals and plant species. Specifically, we compared the variance explained by focal species identity, co-flowering richness, the focal species identity × co-flowering richness interaction, as well as variance among individual sampling sites and years (2014 and 2017). For this, we fitted a generalized linear mixed

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model (GLMM) for each response variable (pollen deposition and proportion of pollen tubes) with focal species identity, co-flowering context and their interaction as fixed effects, and individual site ID and year as random intercepts. The pollen deposition model was adjusted using a Poisson error distribution and a log-link function, as suggested by Bolker *et al.* (2009) for count data associated with ecological processes. The proportion of pollen tubes was modelled using a Gaussian (normal) distribution and an identity link function. GLMMs with significant interaction effects between fixed variables were analysed with a post-hoc Tukey's test to determine sources of significant differences. No evidence of overdispersion was observed in any model ($P > 0.05$ for all cases; 'testDispersion' in the R package DHARMa; Hartig, 2021). We used the coefficient of determination R^2 to determine the proportion of explained variance in each GLMM that can be attributed to the fixed and random variables. Specifically, we employed semi-partial coefficients of determination, also known as semi-partial R^2, which break down the variance of R^2 into components that are uniquely explained by individual predictors (i.e. fixed and random variables), while accounting for covariances among predictors. One advantage of semi-partial R^2 is that it utilizes the covariance structure of GLMMs instead of using analyses with independent structures and interpretations. GLMMs were fitted using the R package lme4 (Bates, 2010). Additionally, we ran Wald chi-square tests (Type III) from the R package car (Fox and Weisberg, 2019) to determine the overall significance of the predictors' effects. Semi-partial R^2 values for fixed and random effects were calculated using the partR2 package (Stoffel *et al.*, 2021). All analyses were conducted in R (v.4.05; Crawley, 2012).

Pollen quantity and quality limitation. We estimated the contribution of pollen quantity and quality limitation to plant reproductive success for every species and co-flowering context (one-species, two-species and four-species sites) combination ($n = 46$ species/site combinations) using the piecewise regression approach proposed by Alonso *et al.* (2012) (Fig. 1). The piecewise regression approach quantifies differences in the 'rate' at which pollen tube saturation is reached (slope of the dose–response relationship; b1 in Fig. 1), and in the number of pollen grains that are required to reach the saturation point (breakpoint in Fig. 1; Alonso *et al.*, 2012; Arceo-Gómez and Ashman, 2014) at the end of flower life. The number of pollen tubes at the base of the style has been shown to be a strong predictor of seed production in the studied *Clarkia* species (Arceo-Gómez *et al.*, 2015), and thus probably reflects pollen limitation at post-zygotic stages. To conduct the piecewise regression, we first fit a separate linear model for each species × co-flowering richness 'treatment' combination ($n = 46$) to approximate the relationship between stigmatic pollen loads (i.e. pollen deposition) and the number of pollen tubes reaching the base of the style (Alonso *et al.*, 2012; Arceo-Gómez and Ashman, 2014). Because this relationship is rarely monotonic, and instead exhibits a diminishing/shallower slope at high pollen load, the linear model was then partitioned into two separate regressions using the 'segmented' package in R (Muggeo, 2008) to determine the linear relationship's inflection point (i.e. the breakpoint), between the steeper initial slope at lower pollen loads and the shallower slope at higher pollen loads (Fig. 1; also see Alonso *et al.*, 2012). This piecewise model describes the pollen grain–tube relationship based on three parameters, the first (b1) and second regression slopes (b2), and the breakpoint (c) in the relationship (Fig. 1; Toms and Lesperance, 2003; Alonso *et al.*, 2012). Each parameter (b1, c and b2) was estimated using 1000 bootstraps (Toms and Lesperance, 2003; Alonso *et al.*, 2012).

Ecologically, a lower breakpoint and a steeper first slope (b1) are indicative of overall higher pollen quality because a smaller number of pollen grains are necessary to reach saturation in the number of pollen tubes that reach the base of the style (Fig. 1A; Arceo-Gómez and Ashman, 2014). In species where the quality of pollen received is high (high b1 and low breakpoint values), we expect pollen quantity to be the main limitation to plant reproductive success because additional pollen makes the greatest contribution to future pollen tube production (Fig. 1A; Alonso *et al.*, 2012, 2013; Arceo-Gómez and Ashman, 2014). The opposite is true for species that receive low-quality pollen (smaller b1 values and larger breakpoints; Fig. 1B), because increases in pollen deposition have little effect on pollen tube and seed production relative to increases in pollen quality (i.e. pollen quality limitation is strongest). The second slope (b2) measures the degree to which pollen quality alone increases pollen tube/seed production after an asymptote has been reached in the relationship between pollen grains and tubes (Fig. 1; Alonso *et al.*, 2012; Arceo-Gómez and Ashman, 2014). It is also possible that after saturation of the pollen grain–pollen tube relationship additional pollen deposition reduces reproductive success (i.e. negative b2 values; Alonso *et al.*, 2012). For instance, seed production may decrease if overcrowding of pollen on the stigma interferes with pollen germination (stigma clogging) or overcrowding of tubes in styles interferes with tube growth (Cruzan, 1986). It is important to note that in contrast to Alonso *et al.* (2012), here low-quality pollen can result not only from low-quality conspecific (self) pollen (as in Alonso *et al.*, 2012) but also from congeneric *Clarkia* pollen which does not lead to ovule fertilization (i.e. lowest quality pollen) and these are indistinguishable on the stigma. For instance, a small b1 value and a large breakpoint value (as in Fig. 1B) may result from deposition of low-quality heterospecific pollen, while large b1 values and small breakpoint values (as in Fig. 1A) would result only from high-quality conspecific pollen.

Clarkia species richness effects on pollen quantity and quality limitation. After estimating the two regression slopes (b1, b2) and breakpoint (c) value for each *Clarkia* species and site combination ($n = 46$), we used linear mixed models (LMMs) to analyse variation in each parameter (b1, c and b2), and assess differences in the relative contribution of pollen quantity and quality to plant reproductive success in the four *Clarkia* species across the three co-flowering contexts (i.e. one-, two- and four-species sites). The models included focal species identity, co-flowering species richness and their interaction as fixed effects. Sampling site and year were included as random effects (random intercepts) to account for intrinsic site characteristics and yearly differences that may influence pollen deposition and pollen tube production. Site ID also controls for clustered data structures such as when multiple species occur within a site (Schielzeth and Nakagawa, 2013). Residuals for all models were normally distributed (Shapiro–Wilks test, $P > 0.05$). We

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performed post-hoc Tukey's tests when significant main and interaction effects were observed. LMMs were fitted using the R package lme4 (Bates, 2010).

RESULTS

Variation in pollen deposition and proportion of pollen tubes

Variation among individual plants within a site (e.g. residual variation) was the main source of variation in pollen deposition and proportion of pollen tubes (Table 1). This was followed by variation due to the interaction between species focal identity and co-flowering context (22 and 8 % for pollen deposition and proportion of pollen tubes respectively) and variation among *Clarkia* species (15 and 3 % for pollen deposition and proportion of pollen tubes, respectively) (Table 1; Appendix 4). Co-flowering context (one, two or four *Clarkia* species), individual site and year contributed the least to variation in both response variables (Table 1; Appendix 4).

Pollen load size and proportion of pollen tubes differed among *Clarkia* species (Table 1). Average pollen grain receipt was lowest in *C. xantiana* (159 ± 4.2) followed by *C. unguiculata* (202 ± 7.6), *C. cylindrica* (263 ± 9.0) and *C. speciosa* (400 ± 7.5) (all pairwise comparisons were significant $P < 0.01$; Supplementary Data Table S1). However, the proportion of pollen tubes was lowest in *C. speciosa* (0.059 ± 0.001) followed by *C. unguiculata* (0.089 ± 0.003), *C. cylindrica* (0.092 ± 0.003) and *C. xantiana* (0.099 ± 0.002). The only significant difference in the proportion of pollen tubes was between *C. speciosa* and all the other *Clarkia* species ($P < 0.01$; Table S1).

There was no effect of co-flowering species richness on pollen deposition or proportion of pollen tubes (Table 1; Appendix 4). However, there was a significant two-way interaction indicating that the effect of co-flowering species richness on pollen deposition and proportion of pollen tubes depended on *Clarkia* species identity (Table 1; Figs 3 and 4). For instance, pollen deposition was higher at four-species sites for three out of the four focal species, with *C. speciosa* being the exception (Fig. 3; Supplementary Data Table S2). Similarly, the proportion of pollen tubes increased with co-flowering species richness in *C. unguiculata* and *C. cylindrica* (Fig. 4; Table S2) but not in *C. speciosa* or *C. xantiana* (Fig. 4; Table S2).

Table 1. *Fixed and mixed-effects model partitioning of variance in pollen deposition and proportion of pollen tubes.*

	Pollen deposition	Proportion of pollen tubes
Fixed effects	**0.397**	**0.114**
Focal species ID	0.155***	0.028***
Richness	$0.023^{\text{n.s.}}$	$0.001^{\text{n.s.}}$
Focal species ID × Richness	0.219***	0.085***
Random effects	**0.046**	**0.022**
Site	0.035	0.021
Year	0.011	0.001
Residual variation (among-individual variation)	**0.557**	**0.864**

Semi-partial R^2 estimate using GLMMs with a Poisson distribution. $^{***}P < 0.001$, $^{**}P < 0.01$, n.s. = not significant. $N = 3776$.

Pollen quantity and quality limitation

The initial slope of the pollen tube–pollen deposition dose response curve (b1) differed among *Clarkia* species (Table 2; Appendix 4), with the value of b1 being two times higher for *C. unguiculata* (0.112 ± 0.01) than for *C. speciosa* (0.074 ± 0.01; $P = 0.03$). The b1 values for *C. xantiana* (0.096 ± 0.01) and *C. cylindrica* (0.083 ± 0.01) did not differ from those of any other species ($P > 0.05$). We did observe differences in breakpoint (c) values among *Clarkia* species (Table 2; Appendix 4). Breakpoint values ranged from 199 (±62.7) pollen grains in *C. unguiculata* to 214 (±54.9) in *C. xantiana*, 250 (±33.1) in *C. cylindrica* and 350 (±88.3) in *C. speciosa*. However, only *C. speciosa*'s breakpoint value was significantly different from that of all other *Clarkia* species ($P < 0.005$ for all; Supplementary Data Table S3). We did not observe significant differences in the second slope (b2) among *Clarkia* species (focal species identity; Table 2; Appendix 4).

Clarkia species richness effects on pollen quantity and quality limitation

We did not find differences in b1 values due to co-flowering species richness or its interaction with *Clarkia* species identity (Table 2; also see Supplementary Data Fig. S1). However, breakpoint values were affected by co-flowering species richness, and the effect varied depending on *Clarkia* species identity (Table 2 and Fig. 5; Table S4). While breakpoint values were significantly higher for populations at the four-species sites compared to one- and two-species sites in *C. xantiana* and *C. unguiculata*, this was not the case for the other two *Clarkia* species (Fig. 5; Table S4; also see Fig. S1). Finally, b2 values were not affected by the co-flowering richness context or their interaction with focal species identity (Table 2).

DISCUSSION

We observed substantial variation in pollen load size on flower stigmas and in the proportion of pollen tubes in flower styles, with most of the variation attributable to differences among individual plants within a population. This result points to the importance of evaluating multiple sources of variation in pollination processes: variation across species and variation among individuals within a population (Gomez and Perfectti, 2012; Arroyo-Correa *et al.*, 2021, 2024; Soares *et al.*, 2021). We also found that closely related co-flowering species with similar floral phenotypes and overlapping pollinator communities (MacSwain, 1973; Moeller, 2004; Moeller and Geber, 2005) vary in the amount and quality of pollen received. For instance, per ovule, *Clarkia* species received between 2.9 and 7.1 pollen grains and produced between 0.29 and 0.42 pollen tubes. We further found that the co-flowering community modified patterns of pollen quality and/or quantity limitation within a species. Here we discuss the potential drivers of variation in

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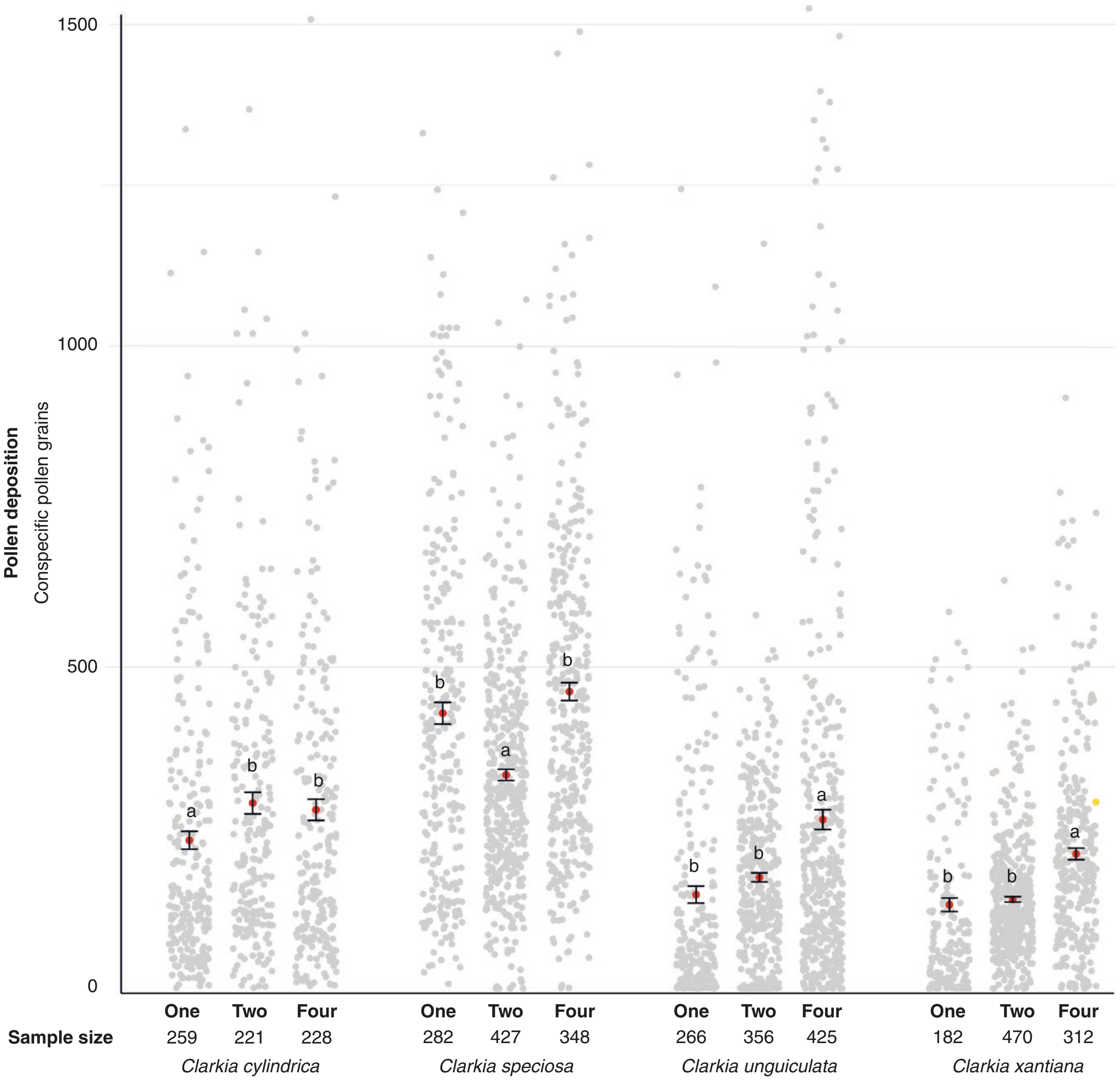

FIG. 3. Mean (±s.e.) pollen deposition on flowers for each *Clarkia* species at sites where it flowers alone (one-species sites), with one other (two-species sites) and with three other *Clarkia* species (four-species sites). Different letters represent significant differences ($P < 0.05$) between sites with different co-flowering richness within each *Clarkia* species.

patterns of pollen limitation among co-occurring species and across communities with varying *Clarkia* species richness.

The substantive variation in the quantity and quality of pollen received among individuals within a population suggests that small-scale differences in the biotic and/or abiotic environment within sites, and/or across time (sampling days), play a large role in mediating pollination success in these species. Although the importance of within-population variation in generating spatial mosaics of reproductive success and floral evolution has been previously acknowledged, it remains largely understudied (Herrera, 1995; Herrera *et al.*, 2002; Arceo-Gómez *et al.*, 2016*b*). Within-population variation in pollination success can result from differences in micro-environmental conditions (e.g. solar radiation and wind exposure; Hennessy *et al.*, 2020; Watson *et al.*, 2022; Plos *et al.*, 2023), intrinsic differences in pollination-related traits among individual plants (e.g. flower size, nectar, colour, scent; Herrera, 1995; Herrera *et al.*, 2002), or the spatial distribution of conspecifics and heterospecifics within a community (Dupont *et al.*, 2014; Arroyo-Correa *et al.*, 2021). While we have information on the average density of each *Clarkia* species at a subset of sites (see below), we lack data on the local density of conspecifics and heterospecifics surrounding focal plants to evaluate local neighbourhood effects on pollen receipt and pollen tube growth of individual flowers.

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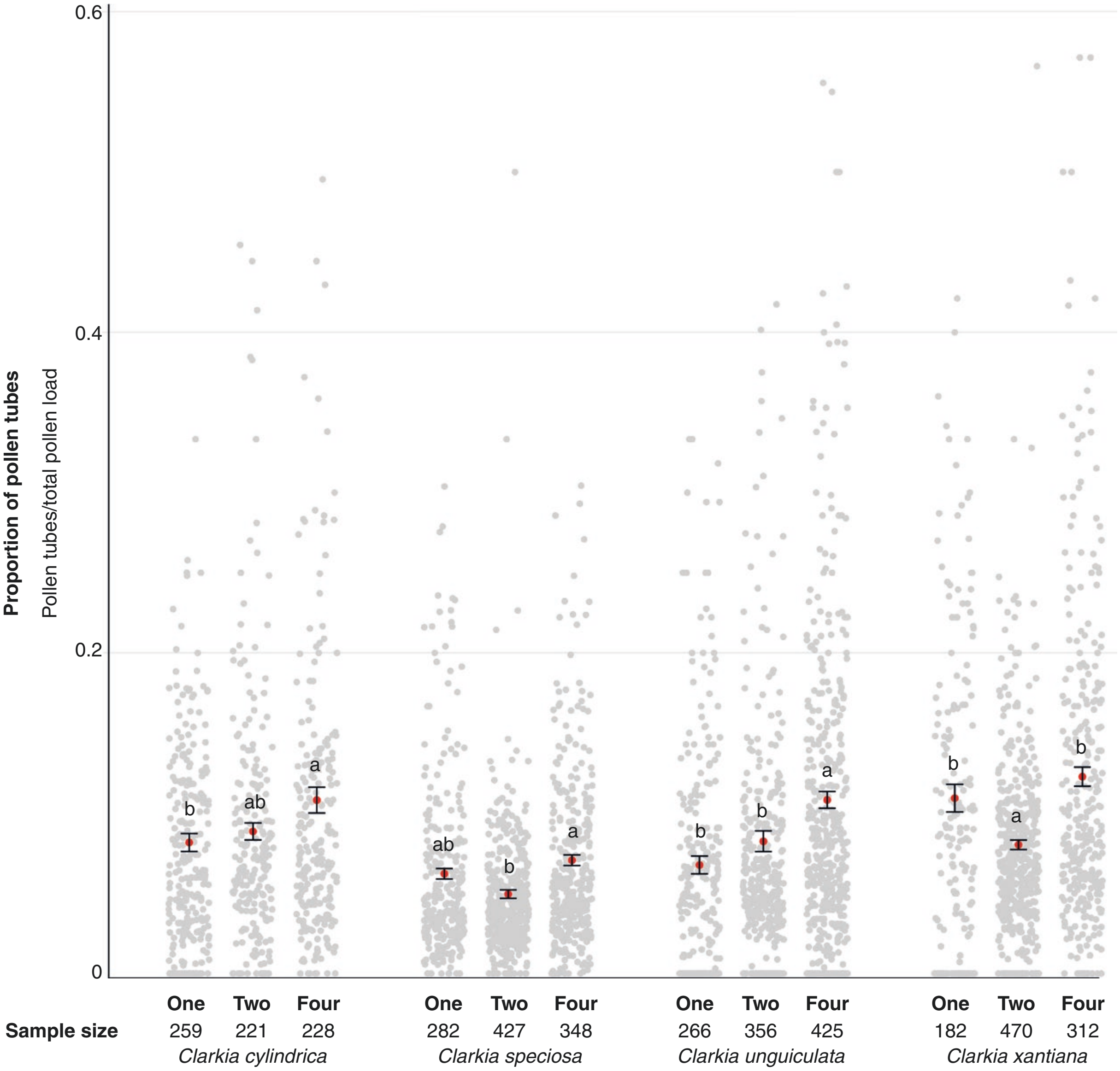

Fig. 4. Mean (±s.e.) proportion of pollen tubes (pollen tubes/total pollen load) in flowers for each *Clarkia* species at sites where they occur alone (one-species sites), with one other (two-species sites) and with three other *Clarkia* species (four-species sites). Different letters represent significant differences ($P < 0.05$) between sites with different co-flowering richness within each *Clarkia* species.

Table 2. *Linear mixed-effects models to assess the effect of focal species identity and co-flowering species richness on estimators of pollen quantity and quality limitation. The first regression slope (b1), breakpoint (c) and second regression slope (b2) were estimated using the piecewise method of Alonso et al. (2012).*

	Species ID		Richness		Species ID × Richness	
	χ^2	*P*-value	χ^2	*P*-value	χ^2	*P*-value
Slope 1	8.23	**0.04***	0.14	0.92	10.31	0.11
Breakpoint	52.75	**<0.001*****	2.08	0.035	20.94	**0.0018****
Slope 2	4.75	0.19	2.29	0.31	4.62	0.59

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Our findings nevertheless underscore the importance of considering these fine-scale sources of variation in pollination success (Herrera, 1995; Herrera *et al.*, 2002; Arceo-Gómez *et al.*, 2016*b*). Evaluating how variation in pollen receipt is structured at different spatial scales (i.e. populations, plants within populations) is key to advancing our understanding of the factors influencing plant reproductive success in nature (e.g. Aizen *et al.*, 1990; Dudash and Fenster, 1997; Kunin, 1997; Herrera *et al.*, 2002) and their role in the evolution of plant reproductive strategies (e.g. Dudash, 1993; Burd, 1994; Gomulkiewicz *et al.*, 2000).

We observed differences in patterns of pollen quantity and quality limitation using our proxies across species. Specifically, pollen quality limitation appeared to be stronger in *C. speciosa*, as it had the smallest first slope (b1) and the largest breakpoint (c) value of the four *Clarkia* species. In other words, *C.*

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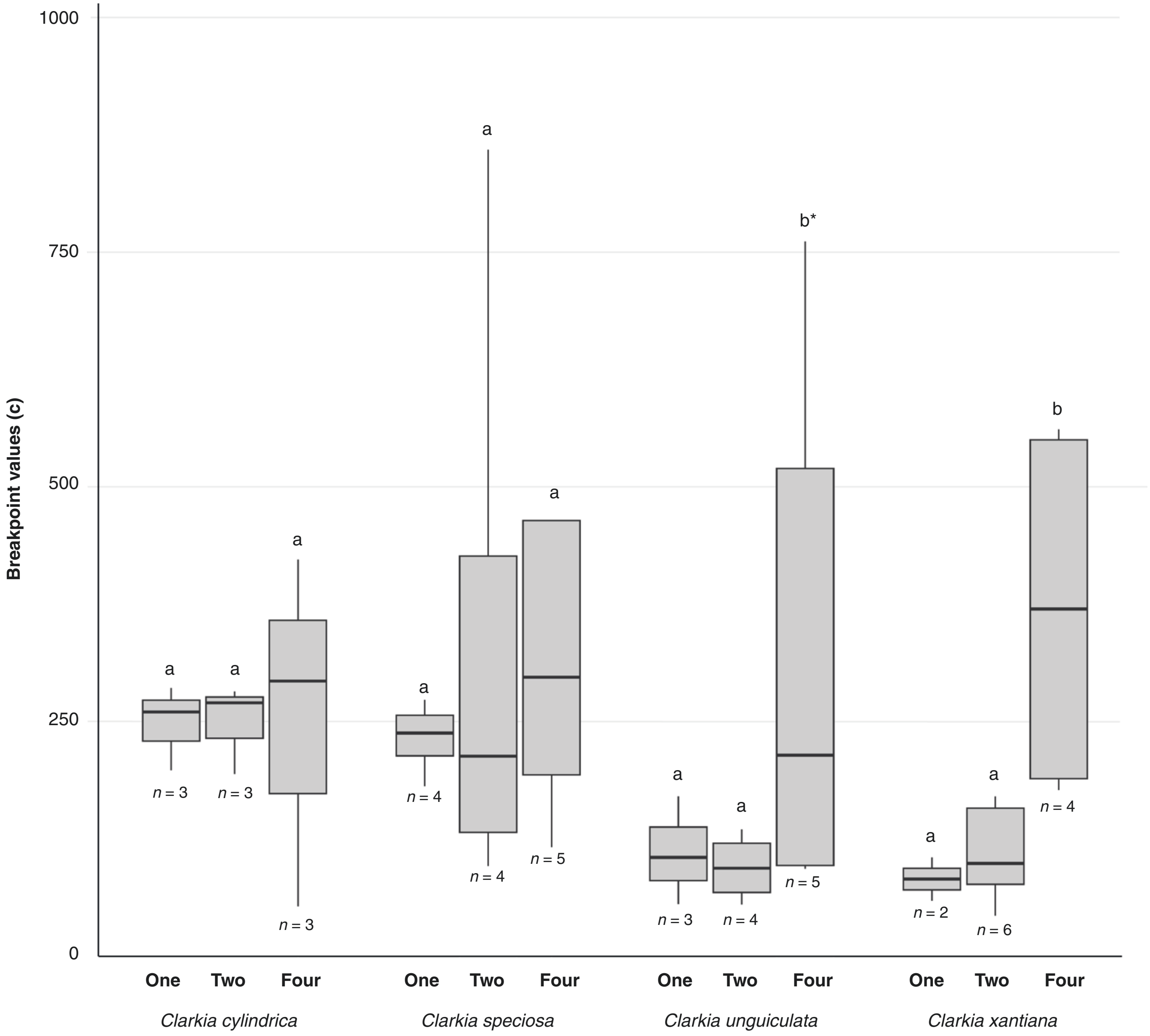

FIG. 5. Differences in average piecewise breakpoint values (c) for each *Clarkia* species at sites where it flowers alone (one-species sites), with one other (two-species sites) and with three other *Clarkia* species (four-species sites). The bottom and top limits of each box are the lower and upper quartiles respectively. The horizontal black lines within each box represent the median. Different letters show significant differences ($P < 0.05$) between sites with different co-flowering richness within each *Clarkia* species. *Marginal pairwise differences ($P = 0.08$; Supplementary Data Table S4).

speciosa required more pollen grains to reach saturation in the number of pollen tubes in styles compared to other species (e.g. Fig. 1B), which is consistent with it having the lowest proportion of pollen tubes (Fig. 2). This result is surprising, since a large fraction of visits to *C. speciosa* are by *D. angusticeps*, a single highly specialized bee (Appendix 3; Singh, 2014; James *et al.*, 2022), and thus we expected *C. speciosa* to be more strongly limited by pollen quantity than quality. Although the exact source of low-quality pollen is unknown (but see below on potential sources of low pollen quality), this finding suggests the potential for pollen quality to limit reproductive success even in highly specialized pollination systems (e.g. Fenster and Marten-Rodriguez, 2007; Armbruster, 2017). Equally unexpected was the finding that pollen quantity limitation appeared to be stronger in *C. unguiculata*, as its first slope value (b1) was two times higher than that of *C. speciosa*, and it had the lowest breakpoint value, suggesting higher pollen quality (e.g. Fig. 1A). The high pollen quantity limitation in *C. unguiculata* may also be due to the fact that, in spite of being visited by common generalist bees, such as honeybees and bumblebees (Appendix 3; Singh, 2014), these generalists typically carry very little *Clarkia* pollen (Moeller and Geber, 2005; James *et al.*, 2022). Given the highly specialized pollination biology of *C. speciosa* and the relatively generalized

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pollination biology of *C. unguiculata*, it seems unlikely that the difference in pollen quality received by the two species is the result of differences in the amount of heterospecific pollen. Rather, differences in pollen quality may be driven by differences in low-quality conspecific pollen deposition. While *C. speciosa* and *C. unguiculata* are both self-compatible species, rates of self-pollen deposition could differ between them. For instance, *C. speciosa* has a bowl-shaped, upward-facing flower which may facilitate self-pollen deposition as bees enter and forage on flowers from above, compared to the forward-facing flowers in *C. unguiculata* that bees approach from the front (Fig. 2; M. Geber, pers. obs.). Floral orientation has been found to help direct how pollinators approach flowers with various outcomes (Fenster *et al.*, 2009). However, *C. speciosa* produces the lowest number of flowers with few of them open at the same time and thus geitonogamous pollination is unlikely in this species. *Clarkia unguiculata*, on the other hand, has high levels of protandry (Dudley *et al.*, 2007) as has been noted in multiple herbaceous species (e.g. Darwin, 1871; Dudash, 1990), which could increase outcross pollen deposition (i.e. high pollen quality; but see Hove *et al.*, 2016; Ivey *et al.*, 2016). The exact mechanisms driving differences in the patterns of pollen quantity and quality limitation deserve further study. For instance, a recent study has shown that the degree of quality and quantity of pollen limitation is impacted by both the genetic diversity within a population and its genetic effective population size (Cisternas-Fuentes and Koski, 2024). Our findings further underscore the importance of evaluating inter- and intraspecific variation in pollen quality and quantity limitation and the potential for micro-evolutionary processes to shape the patterns observed. Our results also indicate that divergent strategies in terms of pollen quality and quantity receipt may exist despite strong similarities among *Clarkia* species in their ecological context and evolutionary history and thus highlight the importance of evaluating quantity and quality aspects of pollen limitation across multiple coexisting plant species.

Community context, i.e. the number of the co-flowering congeners, had significant but variable effects on pollen quantity and quality limitation across our focal species. Specifically, breakpoint values were two times higher in the four-species sites compared to one- and two-species communities for *C. xantiana* and *C. unguiculata*, but did not vary across community contexts for *C. speciosa* or *C. cylindrica*. The higher breakpoint values for *C. xantiana* and *C. unguiculata* at four-species sites suggest that overall pollen limitation increases in these species with increasing co-flowering species richness because a larger number of pollen grains are needed to reach pollen tube saturation at four-species sites compared to one- or two-species sites. Interestingly, total pollen load size also increased with increasing number of co-flowering *Clarkia* species in both species (Fig. 2), suggesting that they receive higher amounts of low-quality pollen (e.g. *Clarkia* heterospecific pollen) with increasing co-flowering richness. Pollinator inconstancy (i.e. switches between *Clarkia* species in successive visits by a pollinator) has also been observed to increase as local *Clarkia* diversity increases, probably leading to greater transfer of heterospecific and lower quality pollen (James, 2020). Other studies have also observed increases in heterospecific pollen deposition with increasing co-flowering richness in species with a large number of floral visitors (e.g. Arceo-Gómez and Ashman, 2014). However, the role of heterospecific pollen in mediating overall patterns of pollen limitation in natural populations is still largely underappreciated (Arceo-Gomez *et al.*, 2019*a*; Ashman *et al.*, 2020). Furthermore, in species-rich communities, the relative densities of individual species can be lower than in species-poor communities, and this could affect pollinator behaviour (e.g. constancy), and levels of con- and heterospecific pollen transfer. However, using data on mean density of each *Clarkia* species at a subset of populations ($n = 14$), we found no relationship between average focal *Clarkia* density, congeneric *Clarkia* density or total plant density on population estimates of pollen quantity and quality limitation (b1, c, b2; $P > 0.5$; Supplementary Data Table S5), nor on average pollen load size or pollen tube production ($P > 0.5$; Table S6). However, our finding of increased pollen limitation with increasing co-flowering species richness in *C. xantiana* is unexpected, and inconsistent with previous studies showing that this species experiences pollinator-mediated facilitation in the presence of other co-flowering *Clarkia* species (Moeller, 2004). It is also important to note that the influence of other non-*Clarkia* species in this system (Appendix 2), although possible, is expected to be minimal. This is mainly because most non-*Clarkia* species flower earlier, and *Clarkia* species show a strong reliance on specialist bees. Furthermore, a recent experimental study examining the effect non-*Clarkia* forb neighbours on seed set in focal *Clarkia* plants suggests the effects of non-*Clarkia* species are resource-based rather than pollinator-mediated (James and Geber, in review). Specifically, seed set decreased with increasing numbers of non-*Clarkia* neighbours and the reduction was equal in control flowers subject to natural levels of pollination and in flowers that received supplemental hand-pollination (James and Geber, in review). By comparison, the effect of *Clarkia* neighbours on focal individual seed set was almost entirely pollinator-mediated, affecting seed set in control flowers only (James and Geber, in review). Overall, our results suggest a complex interplay between pollen quantity and quality that mediates reproductive success across *Clarkia* species and communities. These dynamics have also been observed in other systems (e.g. Aizen and Harder, 2007; Chacoff *et al*, 2008; Alonso *et al.*, 2013, Arceo-Gómez and Ashman, 2014). For instance, Chacoff *et al.* (2008) found pollen quality as the main factor limiting reproductive success in *Crataegus monogyna* via post-zygotic embryo selection favouring outcross progeny. However, Alonso *et al.* (2013) found that the importance of pollen quantity vs. quality in limiting reproductive success varied strongly among species (endemic vs. non-endemic) and across geographical regions globally. The different mechanisms (e.g. differences in pollinator attraction vs. degree of inbreeding depression/heterospecific pollen transfer) underlying this interplay across species and communities probably can have different evolutionary outcomes.

It is important to note that our results showed no evidence of competition for pollinators among co-flowering *Clarkia* species, since we did not see a decrease in total pollen load size with increasing co-flowering species richness for any species. In fact, three species showed an increase in total pollen load received with increasing co-flowering richness (*C. cylindrica*, *C. unguiculata* and *C. xantiana*; Fig. 3), suggesting that pollinator visitation increases in more diverse communities (also

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see Moeller, 2004; Moeller and Geber, 2005). Thus, negative effects of co-flowering may only result from changes in the quality of pollen delivered to stigmas (conspecific related vs. unrelated or heterospecific) with potential consequences for the evolution of tolerance or avoidance strategies that minimize low-quality pollen deposition in species-rich communities (e.g. Ashman and Arceo-Gómez, 2013; Arceo-Gomez *et al.*, 2015). Overall, our results emphasize the importance of conducting large-scale studies that evaluate drivers of pollination success across multiple species and populations (e.g. Aizen and Harder, 2007; Chacoff *et al*., 2008; Alonso *et al*., 2013; Arceo-Gómez and Ashman, 2014), evaluating quality and quantity aspects of pollination success to elucidate the mechanisms driving pollen limitation.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Figure S1. Representative example of piecewise regressions slopes and breakpoints (red dot) based on the pollen grain–tube dose–response relationship for two *Clarkia* species at one-, two- and four species sites. **Table S1.** Pairwise Tukey post-hoc comparisons of pollen deposition and proportion of pollen tubes (pollen tubes/total pollen load) among *Clarkia* species. **Table S2.** Pairwise Tukey post-hoc comparisons of pollen deposition and proportion of pollen tubes (pollen tubes/total pollen load) among *Clarkia* species in response to co-flowering species richness. **Table S3.** Pairwise Tukey post-hoc comparisons of the breakpoint values (c) among *Clarkia* species. **Table S4.** Pairwise Tukey post-hoc breakpoint values (c) among *Clarkia* species in response to co-flowering species richness. **Table S5.** Linear models to assess the effect of population ($N = 14$)-level (A) congeneric *Clarkia* floral density, (B) focal *Clarkia* species density and (C) total density (conspecific + heterospecific) on estimators of pollen quantity and quality limitation [first regression slope b1, breakpoint (c) and second regression slope b2]. **Table S6.** Linear models to assess the effect of the total *Clarkia* species' focal floral density, congeneric *Clarkia* floral density, and total floral density (*Clarkia* species' focal floral density + congeneric *Clarkia* floral density) on average pollen deposition (conspecific pollen grains) and average proportion of pollen tubes (pollen tubes/pollen deposition). $N = 14$ species × site combinations.

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APPENDIX 1

List and location (latitude, longitude) of sites where flower styles were collected, year(s) of collection, species richness and composition of sites, and species (x) whose styles were collected at each site (C: *Clarkia cylindrica*; S: *C. speciosa*; U: *C. unguiculata*; X; *C. xantiana*).

						Species collected			
Site name	Latitude (DD)	Longitude (DD)	Year(s)	Co-flowering species richness	Species composition	C	S	U	X
S_Flat	35.5801	−118.5222	2014, 2017	1	C	x			
S45	35.5881	−118.5209	2014	1	C	x			
SquirrelTank	35.6102	−118.4121	2017	1	S		x		
Black_Gulch	35.5934	−118.5241	2014	1	S		x		
S41	35.5949	−118.5242	2014	1	S		x		
S60	35.5893	−118.5045	2014	1	S		x		
SiteFifty	35.4890	−118.7102	2014, 2017	1	U				x
S47	35.5773	−118.5393	2014	1	U			x	
LCWW	35.4803	−118.7092	2014	1	X				x
TCF	35.5233	−118.6620	2014	1	X				x
OKR_30.4	35.5765	−118.5544	2014	2	C, S	x	x		
Live_Oak	35.4835	−118.7330	2014	2	C, U	x			
S30	35.4933	−118.7055	2014	2	C, U			x	
S34	35.4742	−118.7282	2014	2	C, U			x	
ChinaGarden	35.5379	−118.6489	2017	2	C, X	x			x
BLGW	35.5930	−118.5282	2014	2	S, X		x		x

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Site name	Latitude (DD)	Longitude (DD)	Year(s)	Co-flowering species richness	Species composition	Species collected			
						C	S	U	X
GRE	35.5989	−118.5056	2014	2	S, X		x		x
S8	35.5908	−118.5120	2014	2	S, X		x		x
DEMJr.	35.5306	−118.6237	2017	2	U, X			x	x
S31	35.4808	−118.7473	2014	2	U, X			x	x
DELfour	35.5464	−118.6170	2017	4	C, S, U, X	x	x	x	x
MillCreek	35.5363	−118.6142	2014, 2017	4	C, S, U, X	x	x	x	x
CHG	35.5371	−118.6487	2014	4	C, S, U, X		x	x	x
Democrat	35.5289	−118.6266	2014	4	C, S, U, X		x	x	

APPENDIX 2

Common species (and family) of trees, shrubs, forbs and grasses in the Kern River region where collection sites are located. Not all plant species occur at a given site.

Species	Family
Trees	
Quercus chrysolepis	Fagaceae
Quercus douglasii	Fagaceae
Quercus wislizeni	Fagaceae
Pinus sabiniana	Pinaceae
Aesculus californica	Sapindaceae
Juniperus californica	Cupressaceae
Shrubs	
Sambucus cerulea	Adoxaceae
Toxicodendron diversilobum	Anacardiaceae
Hesperoyucca whipplei	Asparagaceae
Artemisia douglasiana	Asteraceae
Artemisia tridentata	Asteraceae
Baccharis salicifolia	Asteraceae
Encelia actoni	Asteraceae
Ericameria linearifolia	Asteraceae
Ericameria nauseosa	Asteraceae
Eriodictyon californicum	Boraginaceae
Ribes quercetorum	Grossulariaceae
Diplacus aurantiacus	Phrymaceae
Eriogonum fasciculatum	Polygonaceae
Eriogonum wrightii	Polygonaceae
Ceanothus cuneatus	Rhamnaceae
Cercocarpus betuloides	Rosaceae
Forbs	
Asclepias californica	Apocynaceae
Triteleia laxa	Asparagaceae
Achillea millefolium	Asteraceae
Chaenactis glabriuscula	Asteraceae
Eriophyllum ambiguum	Asteraceae
Eriophyllum confertiflorum	Asteraceae
Lasthenia californica	Asteraceae

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Species	Family
Lasthenia gracilis	Asteraceae
Layia pentachaeta	Asteraceae
Madia elegans	Asteraceae
Senecio sp.	Asteraceae
Uropappus lindleyi	Asteraceae
Amsinckia eastwoodiae	Boraginaceae
Amsinckia menziesii	Boraginaceae
Amsinckia menziesii	Boraginaceae
Cryptantha sp.	Boraginaceae
Hydrophyllum occidentale	Boraginaceae
Nemophila menziesii	Boraginaceae
Phacelia cicutaria	Boraginaceae
Phacelia distans	Boraginaceae
Phacelia ramosissima	Boraginaceae
Phacelia tanacetifolia	Boraginaceae
Pholistoma auritum	Boraginaceae
Pholistoma mambranaceum	Boraginaceae
Plagiobothrys sp.	Boraginaceae
Erysimum captitatum	Brassicaceae
Lepidium sp.	Brassicaceae
Calystegia longipes	Convolvulaceae
Croton setiger	Euphorbiaceae
Acmispon glaber	Fabaceae
Lotus micranthus	Fabaceae
Lupinus albifrons	Fabaceae
Lupinus benthamii	Fabaceae
Lupinus bicolor	Fabaceae
Lupinus microcarpus	Fabaceae
Erodium cicutarium	Geraniaceae
Salvia columbariae	Lamiaceae
Calochortus venustus	Liliaceae
Claytonia perfoliata	Montiaceae
Camissonia campestris	Onagraceae
Clarkia exilis	Onagraceae
Castilleja exserta	Orobanchaceae
Castilleja subinclusa	Orobanchaceae
Eschscholzia californica	Papaveraceae
Papaver heterophyllum	Papaveraceae
Platystemon californicus	Papaveraceae
Collinsia heterophylla	Plantaginaceae
Gilia capitata	Polemoniaceae
Gilia tricolor	Polemoniaceae
Leptosiphon bicolor	Polemoniaceae
Leptosiphon montanus	Polemoniaceae
Linanthus dichotomus	Polemoniaceae
Naverretia capillaris	Polemoniaceae
Eriogonum inflatum	Polygonaceae

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Species	Family
Eriogonum nudum	Polygonaceae
Datura wrightii	Solanaceae
Dichelostemma congestum	Themidaceae
Grasses	
Avena barbata	Poaceae
Bromus diandrus	Poaceae
Bromus rubens	Poaceae
Bromus tectorum	Poaceae
Festuca microstachys	Poaceae
Hordeum murinum	Poaceae
Polypogon monspeliensis	Poaceae

APPENDIX 3

Common specialist and generalist bee species that visit the four *Clarkia* species based on surveys at multiple sites in the Kern River region in 2010 (20 sites) and 2011 (17 sites), as reported in Singh, 2014 (chapter 1, tables 1 and 2). Sites varied in *Clarkia* species composition (2–4 *Clarkia* species) and were surveyed for bees 2–4 times during the flowering season, with each survey including morning and afternoon censuses. Bee species are listed in decreasing order of abundance (visit number). The proportion of visits by each bee species to each of the four *Clarkia* species across all sampling sites is shown where the visit total is ≥15 (C: *Clarkia cylindrica*; S: *C. speciosa*; U: *C. unguiculata*; X; *C. xantiana*; 0.00 0.50 1.00). Also shown are the total and proportion of all visits by specialists and the total and proportion of all visits by generalists to each *Clarkia* species, as well the proportion of all bee visits to each *Clarkia* species that are by specialists.[¶]

Species	Family	Visit number	% visits to C	% visits to S	% visits to U	% visits to X
Specialist visitors						
Hesparapis regularis	Melittidae	1053	0.27	0.20	0.22	0.30
Diadasia angusticeps	Apidae	274	0.01	0.99	0.00	0.00
Megachile gravita and *M. pascoensis*[†]	Megachilidae	199	0.07	0.59	0.06	0.29
Lassioglossum pullilabre[§]	Halictidae	128	0.52	0.27	0.14	0.07
Melilisodes clarkiae	Apidae	20	0.05	0.20	0.00	0.75
Andrena lewisorum	Andrenidae	17	0.88	0.00	0.12	0.00
Ceratina sequoiae	Apidae	15	0.00	0.33	0.00	0.67
Total visits by specialists		1889	466	857	766	830
% of all specialist visits			0.20	0.36	0.16	0.25
Generalist visitors						
Apis mellifera	Apidae	439	0.00	0.02	0.67	0.31
Halictus farinosus	Halictidae	210	0.13	0.39	0.11	0.38
Lassioglossum sp.[‡]	Halictidae	183	0.24	0.23	0.23	0.30
Bombus vandykei and *B. vosnesenski*[†]	Apidae	126	0.01	0.01	0.51	0.48
Anthophora urbana	Apidae	50	0.02	0.76	0.14	0.08
Hoplitus albifrons	Megachilidae	26	0.19	0.00	0.19	0.62
Xylocopa tabaniformis	Apidae	9				
Bombus crotchii	Apidae	9				
Coelioxys sericaudata	Megachilidae	8				
Osmia sp. 1	Megachilidae	6				
Osmia sp. 2	Megachilidae	5				
Agapostemon texanus	Halictidae	4				
Total visits by generalists		1075	83	172	457	362
% of all generalist visits			0.08	0.16	0.43	0.34

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Species	Family	Visit number	% visits to C	% visits to S	% visits to U	% visits to X
% of all visits			0.16	0.29	0.26	0.28
% of all visits that are by specialists			0.82	0.80	0.40	0.56

[¶] Visits by unidentified bees (<3 % of all visits) in Singh (2014) are omitted from the table.

[†] *Megachile gravita* and *M. pascoensis* are lumped together because they are difficult to distinguish in flight. The same applies for *Bombus vandykei* and *B. vosnesenskii*.

[§] *Lasioglossum pullilabre* is a putative specialist because bees carry large amounts of *Clarkia* pollen (Moeller and Geber, 2005).

[‡] *Lasioglossum* sp. consists of multiple species from several subgenera. These *Lasioglossum* carry little *Clarkia* pollen (Moeller and Geber, 2005) and are considered generalists, though they were categorized as specialists in Singh (2014).

APPENDIX 4

Pollen deposition, pollen tubes, and estimates of pollen quantity and quality limitation for each *Clarkia* species at each collection site.

Species	Site	Year	*N*	Co-flowering richness	Average pollen deposition	Average pollen tubes	Average proportion pollen tube	First regression slope (b1)	Break-point (c)	Second regression slope (b2)
Clarkia cylindrica	S_Flat	2017	60	1	498.383	26.667	0.068	0.093	260.000	0.004
Clarkia cylindrica	S_Flat	2014	100	1	150.900	16.180	0.112	0.112*	198.000	0.061*
Clarkia cylindrica	S45	2014	99	1	148.747	3.939	0.059	0.021*	285.393	-0.006
Clarkia speciosa	Black_Gulch	2014	100	1	414.620	23.370	0.082	0.131*	180.986	0.012
Clarkia speciosa	S41	2014	60	1	323.567	9.500	0.035	0.044	224.000	–0.002
Clarkia speciosa	S60	2014	72	1	412.486	19.028	0.056	0.061	273.000	0.014
Clarkia speciosa	SquirrelTank	2017	50	1	603.820	31.080	0.065	0.109	251.001	0.013
Clarkia unguiculata	S47	2014	106	1	73.179	4.981	0.075	0.103*	55.382	0.036*
Clarkia unguiculata	S50	2014	100	1	31.310	2.770	0.063	0.066*	105.000	0.153*
Clarkia unguiculata	SiteFifty	2017	60	1	465.683	23.200	0.064	0.147	170.034	-0.006
Clarkia xantiana	LCWW	2014	82	1	41.500	1.829	0.046	0.055*	59.000	0.008
Clarkia xantiana	TCF	2014	100	1	203.020	25.340	0.161	0.182*	105.000	0.065*
Clarkia cylindrica	ChinaGarden	2017	60	2	506.917	43.667	0.115	0.104	282.001	0.014
Clarkia cylindrica	Live_Oak	2014	106	2	246.349	17.613	0.085	0.083*	194.000	0.036*
Clarkia cylindrica	OKR_30.4	2014	55	2	131.073	7.255	0.066	0.041*	269.800	0.005
Clarkia speciosa	BLGW	2014	108	2	450.519	20.907	0.050	0.038*	858.950	0.002
Clarkia speciosa	GRE	2014	102	2	315.098	9.196	0.036	0.085	95.850	0.007*
Clarkia speciosa	OKR_30.4	2014	110	2	225.218	10.200	0.062	0.071*	143.850	0.002
Clarkia speciosa	S8	2014	107	2	339.766	13.607	0.049	0.042*	282.000	–0.002
Clarkia unguiculata	DEMJr.	2017	50	2	283.480	37.640	0.216	0.260	71.998	0.032
Clarkia unguiculata	S30	2014	116	2	139.155	6.828	0.061	0.068*	135.000	0.009
Clarkia unguiculata	S31	2014	111	2	184.027	9.108	0.053	0.080*	115.000	0.020*
Clarkia unguiculata	S34	2014	79	2	136.203	9.101	0.071	0.189*	55.000	0.015
Clarkia xantiana	BLGW	2014	108	2	100.213	7.704	0.085	0.099*	77.387	0.014
Clarkia xantiana	ChinaGarden	2017	50	2	256.520	17.000	0.083	0.020	444.001	0.069
Clarkia xantiana	DEMJr.	2017	30	2	251.167	18.900	0.103	0.103	170.000	–0.001
Clarkia xantiana	GRE	2014	61	2	121.525	10.230	0.094	0.130*	76.000	0.016
Clarkia xantiana	S31	2014	103	2	129.049	8.757	0.071	0.091*	120.000	0.010
Clarkia xantiana	S8	2014	118	2	112.263	6.771	0.070	0.159*	43.000	0.017*
Clarkia cylindrica	DELfour	2017	60	4	479.933	41.817	0.124	0.106*	422.296	–0.009
Clarkia cylindrica	MillCreek	2017	60	4	372.600	44.783	0.171	0.091	293.000	–0.009

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Species	Site	Year	*N*	Co-flowering richness	Average pollen deposition	Average pollen tubes	Average proportion pollen tube	First regression slope (b1)	Break-point (c)	Second regression slope (b2)
Clarkia cylindrica	MillCreek	2014	108	4	113.204	5.648	0.064	0.096*	53.000	0.013*
Clarkia speciosa	CHG	2014	100	4	464.650	24.770	0.066	0.119	193.000	0.006
Clarkia speciosa	DELfour	2017	38	4	647.263	34.553	0.065	0.025*	1182.100	−0.150
Clarkia speciosa	Democrat	2014	77	4	261.506	13.429	0.061	0.090	116.000	−0.001
Clarkia speciosa	MillCreek	2017	51	4	537.824	43.314	0.101	0.049	463.999	0.030*
Clarkia speciosa	MillCreek	2014	82	4	513.329	28.220	0.069	0.109*	297.000	−0.009
Clarkia unguiculata	CHG	2014	106	4	104.236	10.368	0.110	0.146*	93.000	0.033*
Clarkia unguiculata	DELfour	2017	60	4	840.700	36.417	0.073	0.048	519.374	0.001
Clarkia unguiculata	Democrat	2014	99	4	160.465	7.354	0.051	0.049*	214.000	−0.018
Clarkia unguiculata	MillCreek	2017	60	4	374.600	38.333	0.126	0.045*	761.450	−0.170
Clarkia unguiculata	MillCreek	2014	100	4	117.990	16.920	0.175	0.153*	96.530	0.073*
Clarkia xantiana	CHG	2014	109	4	165.294	9.844	0.062	0.068*	177.000	0.000
Clarkia xantiana	DELfour	2017	43	4	407.837	31.419	0.092	0.056	546.452	−0.057
Clarkia xantiana	MillCreek	2017	50	4	266.820	29.660	0.146	0.037*	561.000	0.228
Clarkia xantiana	MillCreek	2014	110	4	149.464	22.836	0.184	0.156*	193.000	0.052*

N = sample size. *Values of b1 and b2 that are different from zero, considering a 95 % confidence interval obtained from 1000 randomizations during the piecewise model fitting.

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