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Floral density and co-occurring congeners alter patterns of selection in annual plant communities

Katherine E. Eisen,[1,2] **Amy C. Wruck,**[1] **and Monica A. Geber**[1]

[1] *Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853*

[2] *E-mail: kee39@cornell.edu*

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Although the evolution and diversification of flowers is often attributed to pollinator-mediated selection, interactions between co-occurring plant species can alter patterns of selection mediated by pollinators and other agents. The extent to which both floral density and congeneric species richness affect patterns of net and pollinator-mediated selection on multiple co-occurring species in a community is unknown and is likely to depend on whether co-occurring plants experience competition or facilitation for reproduction. We conducted an observational study of selection on four species of *Clarkia* (Onagraceae) and tested for pollinator-mediated selection on two *Clarkia* species in communities differing in congeneric species richness and local floral density. When selection varied with community context, selection was generally stronger in communities with fewer species, where local conspecific floral density was higher, and where local heterospecific floral density was lower. These patterns suggest that intraspecific competition at high densities and interspecific competition at low densities may affect the evolution of floral traits. However, selection on floral traits was not pollinator mediated in *Clarkia cylindrica* or *Clarkia xantiana*, despite variation in pollinator visitation and the extent of pollen limitation across communities for *C. cylindrica*. As such, interactions between co-occurring species may alter patterns of selection mediated by abiotic agents of selection.

KEY WORDS: **Coevolution, competition, morphological evolution, plant-insect interaction, reproductive strategies, selection—natural.**

Although pollinator-mediated selection is the most commonly invoked explanation for the evolution and diversification of flowers, floral traits are frequently shaped by selection from other abiotic and biotic factors (reviewed in Strauss and Whittall 2006; Caruso et al. 2019). One potential factor contributing to variation in selection through space and time is the interaction between co-occurring plants in a community. Because species interactions can alter the availability of abiotic (e.g., soil water availability) and biotic (e.g., pollinators) resources, community context factors including conspecific and heterospecific density and the presence of co-flowering species in a community affect pollinator visitation and plant fitness in many systems (studies in plain text in Table 1). These interactions between co-occurring species should modify the opportunity for selection (Benkman 2013; Vanhoenacker et al. 2013), and have variable effects on patterns of selection, given that interactions can range from facilitative to competitive both within species and among species (Rathcke 1983; Moragues and Traveset 2005; Muñoz and Cavieres 2008; Seifan et al. 2014).

For both intraspecific and interspecific interactions, selection will be stronger at low densities if there is facilitation, or at high densities if there is competition (Table 1). These predictions stem from the expectation that positive interactions will not be strong enough to reduce variation in reproductive success at low densities, whereas strong competitive interactions at high densities will increase variation in reproductive success (Rathcke 1983). In both cases, the existence of variation in reproductive success is expected to create a greater opportunity for selection and lead to stronger selection at these densities (Benkman 2013; Vanhoenacker et al. 2013). In communities that contain more

Table 1. Expectations for how variation in plant density and co-occurring species in a community will affect selection on floral traits. For each community property, the different potential conditions and their expected effects on pollinator visitation and selection are presented alongside theoretical (^{T}), empirical (^{E}), and review (^{R}) papers that generated or tested these expectations. References in bold estimated patterns of selection using Lande and Arnold (1983) selection gradient analysis methods. References in plain text measured the effects of these factors on pollinator visitation rates and/or plant fitness but did not estimate selection. Full citations for references are given in Supplement 2.

Community property	Conditions	Effects on pollinator visitation	Effects on selection	References
Conspecific plant density	Intraspecific facilitation	Higher visitation at higher densities	Stronger selection at low densities	Thomson 1978^{E}, 1981^{E}, Kunin 1993^{E}, 1997^{R}; Steven et al. 2003^{E}; Knight 2003^{E}; Moeller 2004^{E}; Ghazoul 2005^{R}; Gunton and Kunin 2009^{E}; Hegland et al. 2009^{E}; Dauber et al. 2010^{E}; Sun et al. 2010^{E}; Lázaro and Totland 2010^{E}; Essenberg 2012 $^{E, T}$
	Intraspecific competition	Higher visitation at low densities	Stronger selection at high densities	Rathcke 1983^{T}; Goulson 2000^{T}; Steven et al. 2003^{E}; Campbell and Husband 2007^{E}; Gunton and Kunin 2009^{E}; Spigler and Chang 2009^{E}; Wirth et al. 2011^{E}
			No effect	Schmitt 1983^{E}; Feinsinger et al. 1986^{E}; Caruso 1999^{E}, **2002**^{E}; **Donohue et al. 2000**^{E}; **Stanton et al. 2004**^{E}; Sun et al. 2010^{E}; **Weber and Kolb 2013**^{E}
Hetero-specific plant density	Interspecific facilitation	Higher visitation to focal species at high heterospecific densities	Stronger selection at low densities	Schemske 1981^{E}; Thomson 1982^{E}; Kunin 1993^{E}; Moeller 2004^{E}; Muñoz and Cavieres 2008^{E}; Lázaro and Totland 2010^{E}; Liao et al. 2011^{E}; Yang et al. 2013^{E}; Seifan et al. 2014^{E}
	Interspecific competition	Higher visitation to focal species at low heterospecific densities	Stronger selection at low densities	Thomson 1982^{E}; Feinsinger 1987^{T}; Feinsinger et al. 1991^{E}; Lázaro and Totland 2010^{E}; Vesprini and Pacini Ettore 2010^{E}
			No effect	Feinsinger et al. 1986^{E}; Caruso 1999^{E}, **2002**^{E}; Sieber et al. 2011^{E}; Wirth et al. 2011^{E}
Effects of co-flowering species	Facilitation or relaxed competition	Increased visitation	Weaker selection with more species	Thomson 1978^{E}; Laverty 1992^{E}; Kunin 1997^{R}; Johnson et al. 2003^{E}; Moeller 2004^{E}; **Moeller and Geber 2005**^{E}; **Lau et al. 2010**^{E}; **Wassink and Caruso 2013**^{E}; TerHorst et al. 2015^{T}
	Conflicting indirect/higher order interactions	Visitation from pollinators with different preferences		
	Increased competition and pollen limitation	Decreased visitation	Stronger selection with more species	Waser 1986^{E,T}; Stout et al. 1998^{E}; **Caruso 2000**^{E}, **2001**^{E}; Bell et al. 2005^{E}; Gegear and Laverty 2005^{E}; Vamosi et al. 2006^{R}; Hersch and Roy 2007^{E}; **Smith and Rausher 2008**^{E}; Flanagan et al. 2010^{E}, 2011^{E}; Arceo-Gómez and Ashman 2014^{E}; TerHorst et al. 2015^{T}; **Kooyers et al. 2017**^{E}
	Analogous indirect/higher order interactions	Visitation from pollinators with similar preferences		
			No effect	Feldman 2008^{E}; **Lau et al. 2010**^{E}; **Simonsen and Stinchcombe 2010**^{E}; **Chaney and Baucom 2014**^{E}; **Parachnowitsch et al. 2014**^{E}

species, selection may be weaker if there is facilitation that decreases variance in reproductive success and thus decreases the opportunity for selection (Cardinale et al. 2007; Benkman 2013) (Table 1). Alternatively, selection may be stronger if there is competition that increases variance in reproductive success and thus increases the opportunity for selection (Vamosi et al. 2006; Benkman 2013) (Table 1). Because facilitation or competition between co-occurring plants could be driven by pollinator sharing or by interactions with the abiotic environment, experimental tests are needed to confirm that variation in selection is pollinator mediated (Wade and Kalisz 1990).

Of the small number of published studies that have investigated how community context may affect selection on floral traits (bolded entries in Table 1), most have manipulated the density of plants or presence of competitors in field or greenhouse experiments (but see Caruso 2000, 2001, 2002; Weber and Kolb 2013). As a result, there are three key gaps in our understanding of how natural variation in community context may affect selection on floral traits. First, although the density and species richness of a community jointly determine community context (Lazaro et al. 2009; Lau et al. 2010; Dietzsch et al. 2011; Essenberg 2012; but see Feldman 2008), most studies have not investigated the effects of variation in multiple aspects of community context simultaneously (see Table 1). Second, despite accumulating evidence that species interactions in more complex communities cannot be predicted from pairwise interactions (Strauss and Irwin 2004; Walsh 2013; Mayfield and Stouffer 2017; Eisen and Geber 2018; TerHorst et al. 2018), most examinations of the effects of co-flowering species on selection have estimated selection on a focal species in the presence or absence of one additional species ("effects of co-flowering species" studies in Table 1; but see Arceo-Gómez and Ashman 2014; Parachnowitsch et al. 2014). Third, most studies have estimated phenotypic selection on the floral traits of only one focal species in a community (Caruso et al. 2019). Estimating selection on more focal species within the same communities can indicate how variation in traits or evolutionary history may contribute to how species respond to the same set of community conditions. Because species' traits may not be adaptations to the contemporary environment (e.g., Aigner 2006), these comparisons will help to determine whether different phenotypes (e.g., rewarding and deceptive orchids [Trunschke et al. 2017]; early vs. late phenology [Giménez-Benavides et al. 2010]) experience different patterns of selection in the same environment. Given that even closely related species often differ in ecological strategies in some ways, we expect co-occurring species to experience different magnitudes or directions of selection in the same communities, such that similarities in patterns of selection would provide evidence for a strong effect of community context.

Species in the genus *Clarkia* (Onagraceae) provide an ideal system for investigating how community context affects net and pollinator-mediated selection on floral traits. *Clarkia* generally share solitary bee pollinators that specialize on the genus (MacSwain et al. 1973), and the composition and richness of communities are generally stable over time (Lewis 1953; authors pers. obs.). These dynamics can create an opportunity for species interactions to affect the selective environment (Thompson 2005). In the southern foothills of the Sierra Nevada (Kern County, CA), four outcrossing species frequently co-occur; communities that contain a single species of *Clarkia* are equally as common as communities that contain multiple species (Eisen and Geber 2018). This natural variation in the congeneric species richness of communities creates the opportunity to estimate selection on four different species in a range of community contexts. Because *Clarkia* are the latest species to flower in this region (Lewis and Lewis 1955; MacSwain et al. 1973; Moeller 2004), we expect that intraspecific interactions or interspecific interactions with congeners have significant effects on the selective environment. Previous research in the system has generated predictions as to how community context factors may affect patterns of selection. *Clarkia xantiana* experiences facilitation due to joint pollinator attraction (Moeller 2004), which may have led to weaker selection on floral traits in experimental populations that contained congeners (Moeller and Geber 2005). Additionally, *C. xantiana* has higher tolerance of heterospecific pollen transfer in these communities (Arceo-Gómez et al. 2016). Together, these results suggested that selection may be weaker in more species-rich *Clarkia* communities. In addition, we predicted that selection may be stronger at low conspecific or high heterospecific floral densities, given that pollen deposition is positively correlated with conspecific floral density in *C. xantiana* populations (Moeller 2004) and that high heterospecific densities can generate competition for pollination (e.g., Seifan et al. 2014). Given that pollinator visitation to and pollen limitation of *C. xantiana* varies across communities (Moeller 2004, 2005), the strength of pollinator-mediated selection is also likely to vary across communities.

In this study, we investigated how the congeneric species richness of a community and the floral density of a local neighborhood affect patterns of pollinator-mediated and net selection on floral traits of four species of *Clarkia*. Specifically, we conducted an observational study of selection in 2015 and an experimental study of pollinator-mediated selection in 2017 to test four questions. To determine if net selection on floral traits is weaker in communities with more congeneric species (Q1), we measured selection on four *Clarkia* species in 2015 and two *Clarkia* species in 2017 in six to 10 communities (per species and year) that differed in *Clarkia* species richness. To assess whether net selection is stronger for plants surrounded by low conspecific or high heterospecific floral density (Q2), we analyzed how relationships between traits and fitness changed with local density in the communities where selection was estimated in 2015 and 2017. To determine if pollinator-mediated selection on floral traits varies across the above-described communities (Q3), we conducted a supplemental pollination experiment on two species of *Clarkia* in 2017. Lastly, we compared all results from both years of the study across the focal species to assess whether co-flowering congeneric plants experience similar patterns of selection (Q4).

Material and Methods

STUDY SYSTEM

Out of the approximately 40 *Clarkia* species that are endemic to the Western United States, many species have partially overlapping ranges, such that multiple species often co-occur, with communities containing up to six *Clarkia* species (Lewis 1953). Co-occurring species generally share pollinators, which are primarily solitary bees that specialize on the genus (MacSwain et al. 1973), but vary in floral traits including flowering time (Lewis 1961; Jonas and Geber 1999; Moeller 2004; Singh 2014), floral orientation (Lewis 1961), petal coloration (Lewis and Lewis 1955), flower size (Eisen and Geber 2018), and floral scent (Miller et al. 2014).

In the Southern Sierra Nevada (Kern River Canyon, Kern County, CA), communities contain up to four outcrossing *Clarkia* species: *C. unguiculata* Lindley, *C. cylindrica* ssp. *clavicarpa* W. Davis, *C. xantiana* ssp. *xantiana* A. Gray, and *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis. Hereafter, we refer to each species without its subspecies designation (e.g., *C. xantiana* for *C. x. xantiana*). The species provide pollen and nectar rewards for bee visitors, and are not known to hybridize in the field (MacSwain et al. 1973). These species are all primarily outcrossing because flowers are protandrous and herkogamous: eight anthers mature over 2–3 days before the stigma becomes receptive (Lewis 1953). As such, any selfing would need to be mediated by pollinators, and outcrossing rates in *C. unguiculata* range from 0.79 to 1.0 (Vasek 1965; Ivey et al. 2016), and from 0.59 to 0.85 in *C. xantiana* (Moeller et al. 2012; Ivey et al. 2016). The species vary in flowering period and floral orientation: *C. cylindrica* (bowl-shaped flower) and *C. unguiculata* (open-faced flower) flower in early-mid May, whereas *C. speciosa* (bowl-shaped flower) and *C. xantiana* (open-faced flower) flower in early-mid June (Moeller 2004; Singh 2014). Previous work has confirmed that *Clarkia* in the Kern River Canyon share pollinators (MacSwain et al. 1973; Moeller 2006; Singh 2014), which include generalist (polylectic) bees and 10 pollen specialists (oligolectic bees) that represent four bee families (Andrenidae, Apidae, Megachilidae, and Melittidae; MacSwain et al. 1973; Moeller 2005).

STUDY OVERVIEW

To determine if net selection is weaker in communities with more congeneric species (Q1), we estimated selection on three floral traits (anther-stigma separation, floral diameter, and flower number) of all four species of *Clarkia* in 2015 and of two species of *Clarkia* in 2017. For each species, we estimated selection at six to 10 communities in each year that contained between one and four species of *Clarkia* (see Tables S1 and S2). To determine if net selection is stronger on plants surrounded by low conspecific or high heterospecific density (Q2), we analyzed how relationships between traits and fitness changed with local density in the communities where selection was estimated in 2015 and 2017. To determine if selection was pollinator mediated (Q3), we conducted a supplemental pollination experiment on two species of *Clarkia* in 2017. For each of the two focal species, we estimated selection on the same three traits on open-pollinated control fruits and supplemental-pollinated fruits at nine or 10 communities; the communities contained one, two, or four species of *Clarkia*. Weather during the 2015 growing season was very dry; locations throughout the range of *C. xantiana* accumulated between 160 and 200 mm of rainfall from November to June (unpublished data). Weather during the 2017 growing season was two to three times wetter than 2015, with locations throughout the range of *C. xantiana* accumulating between 400 and 600 mm of rainfall from November to June (unpublished data).

FIELD METHODS

Q1: Is net selection on floral traits weaker in communities with more congeneric species?

To investigate how the congeneric species richness of a community affects selection, we estimated selection on all four *Clarkia* species in 2015 at 18 communities that contain one to four *Clarkia* species; there were two or three replicate communities of each community type (Table S1). In 2017, we estimated selection on two *Clarkia* species at 16 communities that contain one, two, or four *Clarkia* species; there were three or four replicate communities of each community type (Table S2). When possible, we worked at the same communities studied in 2015 (Tables S1 and S2). We defined the boundary of a community as the point at which *Clarkia* density became scarce (fewer than five plants per square meter). Although some of our communities are relatively close to each other (e.g., less than 1-km apart), we consider these locations to be distinct communities because *Clarkia* are primarily pollinated by ground-nesting solitary bees that are central place foragers (MacSwain et al. 1973; Moeller 2005). The exact foraging distances of these bees are unknown, but foraging appears to occur primarily within the immediate vicinity of the nest, which matches short-distance foraging patterns that have been documented for other solitary bee species (Zurbuchen et al. 2010). In 2015, we did not include *C. unguiculata* alone communities because peak flowering at these communities occurred prior to the beginning of the study period. Across both years we chose to work at two-species communities that contain *C. cylindrica* and *C. unguiculata* and *C. speciosa* and *C. xantiana* because these pairs of species flower concurrently but have different floral orientations.

At each community, we haphazardly selected 50 focal plants per species in 2015 and between 60 and 80 focal plants per species in 2017. Focal plants were 2-m apart at minimum. Two floral traits were measured in situ using calipers: anther-stigma separation and floral diameter. Anther-stigma separation can affect pollination efficiency and the degree of outcrossing by determining where pollen is placed on pollinators and how pollinators make contact with a flower's reproductive organs (Armbruster et al. 1994; Mazer et al. 2016). If higher plant densities or the presence of co-flowering species in a community increase competition for pollination, a species may adapt by evolving less anther-stigma separation, which could lead to greater reproductive assurance via selfing (Levin 1972; Moeller and Geber 2005). Flower size traits (Strauss et al. 1996; Parachnowitsch and Kessler 2010) can increase pollinator attraction, and can also affect pollination efficiency (Galen and Newport 1987). In addition, we counted the number of open flowers.

To estimate fitness, we returned to our focal plants when the plants had set fruit, approximately three weeks after measuring plant and floral traits. We collected 1–2 fruits per focal plant; these fruits were collected from the same flowers on which we measured floral traits, or from adjacent flowers if the measured fruits had been damaged by herbivores. Fruits were only collected when the length of the fruit was intact. Fruits were stored in separate coin envelopes that were given unique ID numbers cross-referenced to the focal plant. We then counted the number of mature seeds in each fruit.

Q2: Is net selection stronger for plants surrounded by low conspecific or high heterospecific floral density?

In 2015 and in 2017, we recorded the number of open flowers of any *Clarkia* sp. within a 1-m radius of the focal individual at the time of trait measurement. We assessed density on a local scale because pollen deposition to individual flowers of *C. xantiana* increased with the density of local (within a 1-m radius) conspecific neighbors (Moeller 2004), which suggests that interactions at a fine spatial scale can have a significant effect on reproductive success in these communities.

Q3: Does pollinator-mediated selection vary across communities?

To determine if selection is pollinator mediated, we manipulated pollen limitation and compared patterns of selection on fruits of supplementally-pollinated flowers to selection on fruits of open-pollinated control flowers (see methods for Q1) for two species of *Clarkia* (*C. cylindrica* and *C. xantiana*) in 2017. The three pollination traits (anther-stigma separation, floral diameter, and flower number) were measured as in 2015. On each of the 60–80 focal plants per species per community, we selected two female-phase flowers. Although natural selection operates on the level of the individual plant, not on the level of the individual flower, supplemental and control pollination treatments were applied to different flowers on the same plant to control for any plant-level effects on reproductive success. In addition, previous work in *Clarkia* indicates that estimates of pollen limitation from partial plant manipulations are not significantly different from estimates of pollen limitation for whole plant manipulations (Runquist and Moeller 2013), such that resource re-allocation is unlikely to bias our results. One flower was marked with a dark green sticker on the plant's stem just below the flower but not manipulated (control), whereas the other flower was marked with a lighter green sticker and also received a supplemental pollination treatment. The position of the control and supplemental flowers on the plant was recorded in the event that stickers did not remain on the plant. Supplemental pollen was collected from conspecific individuals >5-m away from the focal plant and was applied using a toothpick and/or by brushing the anthers of a flower across the stigma. One person performed all supplemental pollinations to control for any possible variation in the quantity of pollen applied. When fruits were collected (see Q1 methods above), supplemental and control fruits were stored in separate coin envelopes that were given unique ID numbers cross-referenced to the focal plant. The number of focal plants per species per community with undamaged supplemental and control fruits ranged from 39 to 65 (average: 55 plants; Table S2).

We conducted pollinator observations within two days of measuring the majority of focal plants at a community. Pollinator observations were conducted in four 1-m^2 circular plots at each community. Each plot contained one focal individual and the locations of the four plots were selected to represent the range of floral densities present at the community. One observer recorded the number of visits to the open flowers of the focal individual during a 15-minute observation period in the morning (0830h-1130h) and in the afternoon (1200h-1500h). The number of open flowers on the focal individual and on all other *Clarkia* individuals within the 1-m^2 plot at the time of observation was recorded. This yielded a total of four observation periods conducted in the morning and four observation periods conducted in the afternoon for each species at each community.

STATISTICAL ANALYSIS

Most analyses (see exceptions below) were performed using linear mixed effects in R (lme4 package; Bates et al. 2015), and separate analyses were conducted on each *Clarkia* species.

Models were assessed to ensure normally distributed residuals with homogenous variance. The fixed effects and dependent variables analyzed to address each question are described below. Because selection acts at the level of the population, we fit models that allowed for variation in the slopes of the relationship between fitness and traits among the replicate populations from a given community type. However, these models did not represent a significant improvement over models that allow for variation in the intercepts only among replicate communities, due to very few differences in the direction of selection across communities and similar ranges in trait and fitness values both across and within communities. As such we present the simpler (variation in intercepts only) models, where community nested within community type was included as a random effect in all models. Estimates of selection within each community and summary statistics on the raw trait and fitness values for each community are presented in Appendix 1.

Trait values were standardized by subtracting the species mean from each individual trait value, and dividing this difference by the species standard deviation. Fitness values (the average number of seeds per fruit) were standardized by dividing individual values by the species mean. We assessed the significance of fixed effects in these models by using the anova function in the lmerTest package version 2.0-29 (Kuznetsova et al. 2015) to perform type III F tests using the Satterthwaite approximation for the denominator degrees of freedom. All data are available via Dryad (https://doi.org/10.5061/dryad.mkkwh70w4).

Q1

To determine if net selection on a trait varied across community types, we used an ANCOVA approach. This type of approach has been used to compare selection across populations (e.g., Caruso 2000, 2002), and here we adapt it to compare patterns of selection across community types because replicate communities generally exhibited similar patterns of selection (see STATISTICAL ANALYSIS above). We subset the entire dataset by species, and we standardized trait values and fitness values (described above) by species. Relative fitness was modeled as a function of three fixed effects: a single plant or floral trait (anther-stigma separation, floral diameter, or flower number), community type (the number of species at a community), and the interaction between the trait and community type. To calculate observed power values for each F test, we used the powerSim function in the simr package (Green and MacLeod 2016). We used the emmeans function in the emmeans package in R (Lenth 2019) to estimate net selection for each trait at each community type, and we used nonparametric bootstrapping to estimate 95% confidence intervals on the estimates of net selection (1000 iterations, boot function; Canty and Ripley 2017). We used the bias-corrected and accelerated (BCa) method, which adjusts for bias and skewness in the bootstrap distribution (Efron 1987). In general, the three traits measured were not highly correlated (Table S3).

Q2

To determine if conspecific and heterospecific floral densities affected patterns of selection, relative fitness was modeled as a function of three fixed effects: a single plant or floral trait (anther-stigma separation, floral diameter, or flower number), a density measure, and the interaction between the trait and the density measure. The effects of two density measures (absolute conspecific floral density and absolute heterospecific floral density) were tested in two separate sets of models. Models including absolute conspecific density were run using the complete dataset, whereas models including absolute heterospecific density were run using the subset of focal plants that were found at multi-species communities.

When the interaction between the trait and the density metric was significant, we conducted a simple slopes analysis using the sim_slopes function from the jtools package in R (Long 2019). This analysis can indicate what dynamics are driving a significant trait × density interaction by testing whether the slope of the trait-fitness relationship is different from zero at low, average, and high floral densities. These analyses were visualized using the interact_plot function from the jtools package.

Q3

To determine if pollinator visitation differed across community types, we used a Tweedie generalized linear mixed effects model with a Poisson power variance function and a log-link power variance function. This model was fit using the glmer function in the lme4 package in R (Bates et al. 2015) and the tweedie model family specification from the statmod package in R (Giner and Smyth 2016). This type of model was used because our dataset was zero-inflated, and the response variable of interest was visitation rate, which could not be modeled as a count. The response of the average number of visits per flower per 15 minutes was modeled as a function of community type (fixed effect) and plot ID nested within community (random effects). We used the emmeans function in the emmeans package to estimate the mean pollinator visitation rate for each community type; model estimated means and 95% confidence interval limits were back-transformed into the original units by exponentiating the output from emmeans. Additionally, we used a pairwise contrast in emmeans to determine if visitation differed across the community types.

To determine if there were trait (anther-stigma separation, floral diameter, or flower number) or fitness (seed set per fruit) differences between the hand-pollination and the open-pollination treatments, we used paired t-tests. A significant positive difference in seed set indicates that the hand-pollination fruits set more seeds than the open-pollination fruits, which provides evidence for pollen limitation. A significant negative difference in seed set indicates that the open-pollination fruits set more seeds than the hand-pollinated fruits, which could occur if the hand-pollination process was detrimental to seed set.

To determine if selection was pollinator-mediated in 2017, we used an ANCOVA approach (Sandring and Ågren 2009; Sletvold and Ågren 2010). Trait and fitness values were standardized by species and by community type (see above). Relative fitness was modeled as a function of three fixed effects: one of our three pollination-related traits (anther-stigma separation, floral diameter, or flower number), treatment (hand- or open-pollination), and their interaction. A significant interaction would indicate a difference in selection on the trait between the two pollination treatments. Stronger selection in the open-pollination treatment relative to the hand-pollination treatment indicates pollinator-mediated selection (Sandring and Ågren 2009; Sletvold and Ågren 2010).

Results

Q1: IS NET SELECTION WEAKER IN COMMUNITIES WITH MORE SPECIES?

In 2015, there was a significant interaction between community type and flower number in predicting relative fitness for all four species (Fig. 1; Tables S4 and S5). For each species, selection was strongest in the least diverse communities (two-species communities for *C. unguiculata*; one-species communities for all other species). In other words, the number of flowers per plant had a stronger positive relationship with seed set per fruit in less diverse communities.

Net selection on floral diameter in 2015 varied across community types in two species (Fig. 2; Tables S4 and S5). In *C. speciosa*, net selection was strongest at one species communities compared to all other community types (Fig. 2B; Table S5), whereas net selection on floral diameter of *C. cylindrica* was stronger at three-species communities than it was at four-species communities, where it was nonsignificant (Fig. 2A; Table S5). Net selection on floral diameter for *C. unguiculata* (Fig. 2C; Table S5) or *C. xantiana* (Fig. 2D; Table S5) did not differ across community types; there were no differences in net selection on anther-stigma separation across community types in any of the four species (Tables S4 and S5), although these inferences may be limited by low power (Tables S4 and S5).

In 2017, net selection on anther-stigma separation varied across community types in *C. cylindrica* (Tables S4 and S6). Net selection was stronger at two-species communities than at one-species communities or four-species communities (Table S6). There were no differences in net selection on anther-stigma separation across community types for *C. xantiana* (Table S6). Net selection on floral diameter and on flower number did not differ across community types for either species in 2017 (Tables S4 and S6).

Q2: IS NET SELECTION STRONGER FOR PLANTS SURROUNDED BY LOW CONSPECIFIC OR HIGH HETEROSPECIFIC FLORAL DENSITIES?

Across all community types and for all species, average conspecific and heterospecific floral densities were less than 10 flowers per square meter in 2015 (Table S7). In contrast, densities were generally higher in 2017: conspecific floral densities ranged from 11 to 200 flowers per square meter and heterospecific floral densities ranged from 2 to 68 flowers per square meter (Table S7).

Conspecific floral density affected patterns of positive directional net selection of four traits across all of the species and both years of the study (Fig. 3; Tables S8 and S9). There was stronger net selection at higher conspecific floral densities on anther-stigma separation in *C. xantiana* in 2015 (Fig. 3A), on flower number in *C. speciosa* in 2015 (Fig. 3B), and on flower number of *C. cylindrica* in 2017 (Fig. 3D). Floral diameter of *C. unguiculata* in 2015 displayed a similar pattern (Fig. 3C), with marginally significant negative directional net selection at low floral densities, compared to positive but nonsignificant directional net selection at average and higher floral densities.

Net selection varied with heterospecific floral density on two traits in 2015 and on one trait in 2017 (Fig. 4; Tables S8 and S9). Net selection was strongest at low floral densities, weaker at average floral densities, and insignificant or marginally significant at high floral densities on floral diameter of *C. cylindrica* in 2015 (Fig. 4A) and in 2017 (Fig. 4C), and on flower number of *C. unguiculata* in 2015 (Fig. 4B).

Q3: DO POLLINATORS IMPOSE MEASURABLE SELECTION ON FLORAL TRAITS IN THE ABOVE-DESCRIBED COMMUNITIES?

Floral traits (anther-stigma separation and floral diameter) were measured at nine communities for *C. cylindrica* and at 10 communities for *C. xantiana*. In general, trait values did not differ between the hand- and open-pollination treatment groups at each community (Table S10).

Pollinator visitation to *C. cylindrica* varied across community types, with lower visitation (average visitation per flower per 15 minutes: 0.011) at two-species communities relative to one- and four-species communities, where the average visitation per flower per 15 minutes was 0.17 visits (Table S11). Visitation to *C. xantiana* did not vary across the community types and ranged from 0.178 to 0.354 visits per flower per 15 minutes.

Comparisons of seed set between the hand- and open-pollinated flowers were used to determine the extent of pollen limitation (Table S10). Flowers in the hand-pollination treatment

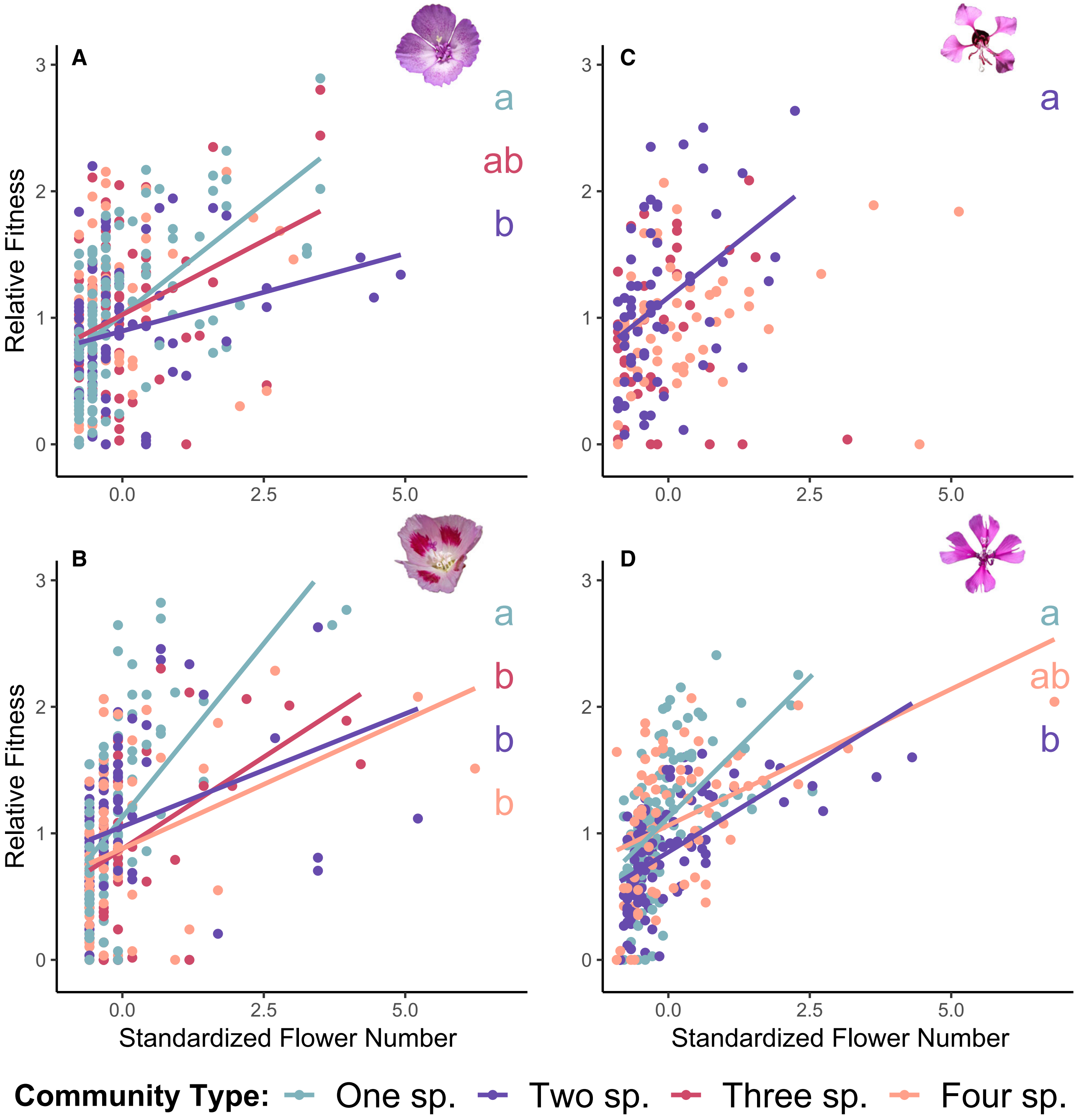

Figure 1. Net selection (S) on flower number varies across community types for each species in 2015: (A) *C. cylindrica*, (B) *C. speciosa*, (C) *C. unguiculata*, and (D) *C. xantiana*. Colors correspond to the number of species present at a given site (community type): one-species communities (blue gray), two-species communities (purple), three-species communities (raspberry), and four-species communities (orange). Trend lines are drawn through colored points when the slope at that community type was significantly different from zero. Within each species, slopes that are significantly across community types are labeled with different letters.

set more seeds than the open-pollinated control flowers at four out of the nine *C. cylindrica* communities. Of these four communities that were pollen limited, two were two-species communities and two were four-species communities (Table S10); supplemental pollination increased seed set by 15–29% at these communities. At all 10 *C. xantiana* communities, flowers in the hand-pollination treatment did not set more seeds than flowers in the open-pollination treatment (Table S10).

Although a few traits at a number of different community types were under selection in 2017 (Table S6), there was only

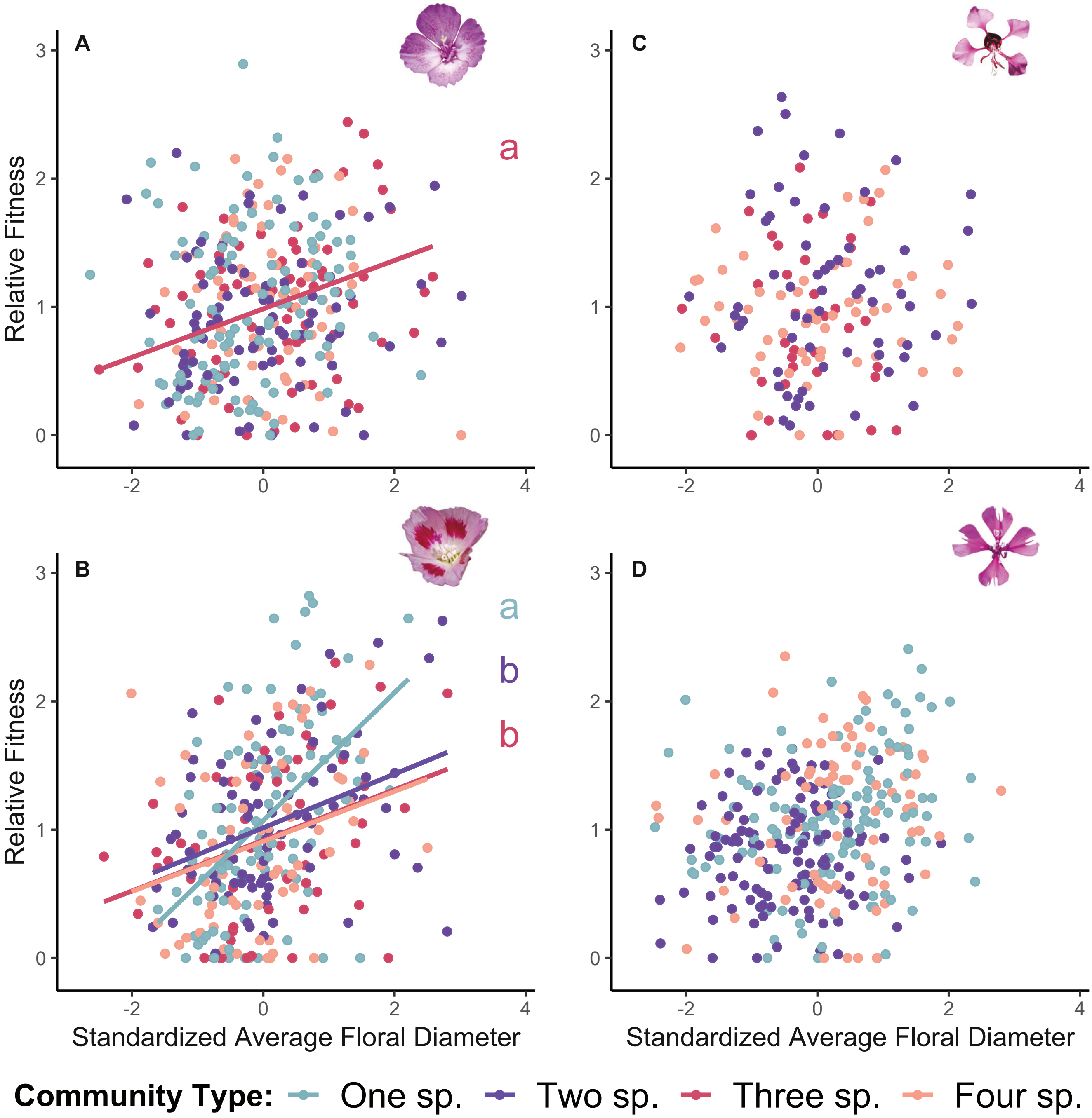

Figure 2. Net selection (S) on average floral diameter varies across community types for some species in 2015: (A) *C. cylindrica*, (B) *C. speciosa*, (C) *C. unguiculata*, and (D) *C. xantiana*. Colors correspond to the number of species present at a given community (community type): one-species communities (blue gray), two-species communities (purple), three-species communities (raspberry), and four-species communities (orange). Trend lines are drawn through colored points when the slope at that community type was significantly different from zero. Within each species, slopes that are significantly across community types are labeled with different letters.

a difference in selection between the two treatments for anther-stigma separation at *C. xantiana* one-species communities (Table S6). The hand- and open-pollinated flowers were not under selection for this trait at this community type, but the estimates of selection were opposite in sign, such that the difference between them was significant. Other traits that were under selection in the open-pollinated control group were under similar patterns of selection in the hand-pollination treatment group (Table S6).

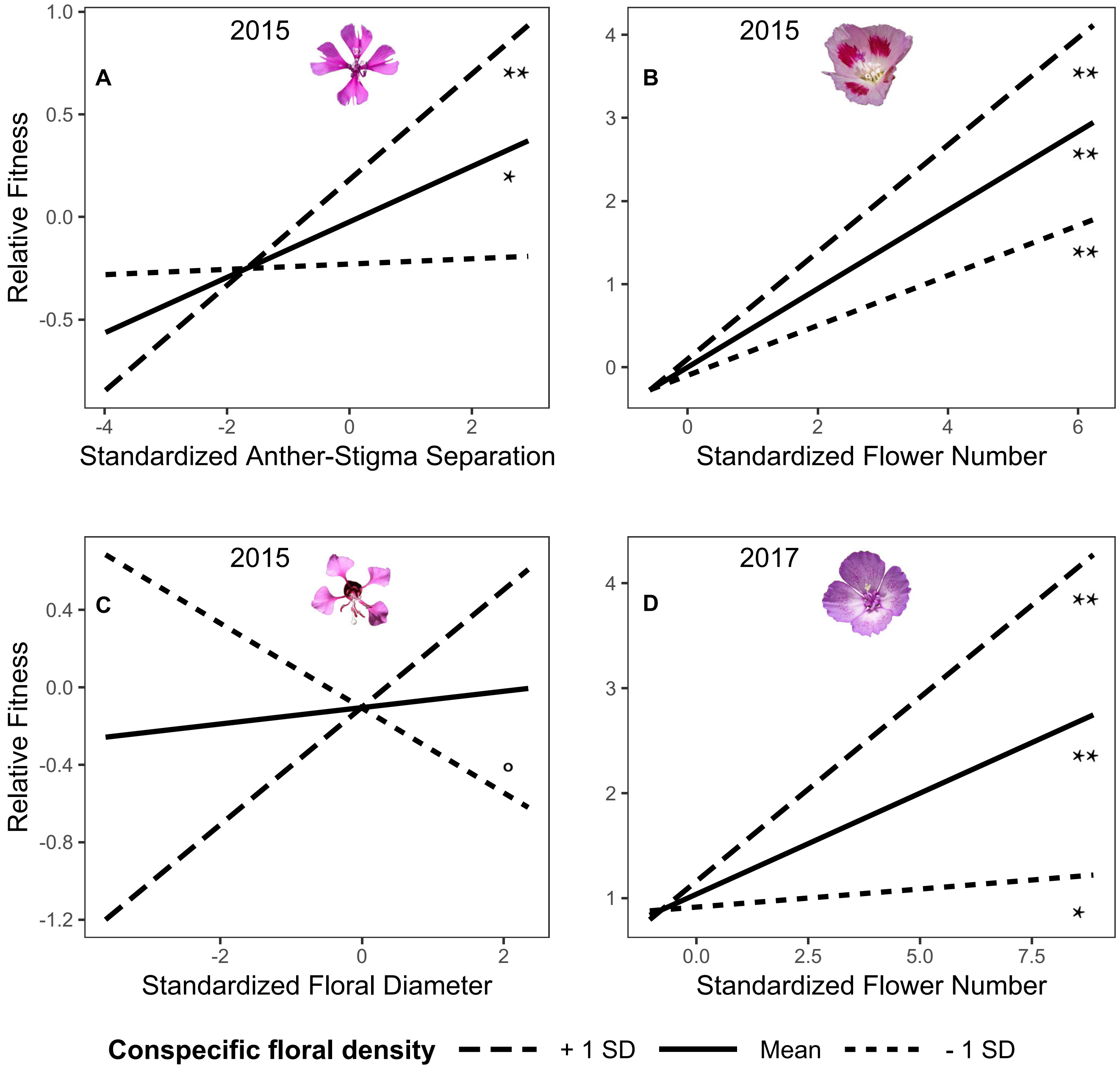

Figure 3. Visualizations of simple slope analysis for traits where conspecific floral density had a significant effect on patterns of selection for a given species in a given year (a significant density × trait interaction; exact results of simple slope analysis in Table S6). Selection was generally stronger at high conspecific densities for anther-stigma separation of *C. xantiana* in 2015 (A), flower number of *C. speciosa* in 2015 (B), floral diameter of *C. unguiculata* in 2015 (C), and flower number of *C. cylindrica* in 2017 (D). The relationship between the trait of interest and relative fitness is plotted at three conspecific floral densities: mean density + 1 SD (dashed line), mean density (solid line), and mean density – 1 SD (dotted line). Significance levels of the slopes of the lines: °$P < 0.1$; *$P < 0.05$; **$P < 0.01$.

Q4: DO CO-FLOWERING SPECIES EXPERIENCE SIMILAR PATTERNS OF SELECTION?

In addition to the pattern of stronger selection on flower number at communities with fewer species, which was observed for all species in 2015, two pairs of species had some similarities in patterns of selection across communities or floral densities. Floral density affected patterns of selection on floral diameter for the co-flowering species *C. cylindrica* and *C. unguiculata* (Table 2); *C. cylindrica* experienced stronger selection on floral diameter at low heterospecific floral densities, whereas *C. unguiculata* experienced stronger selection on floral diameter at high conspecific floral densities. The two bowl-shaped species *C. cylindrica* and *C. speciosa* both experienced an effect of congeneric species richness on selection on floral diameter in 2015 (Table 2).

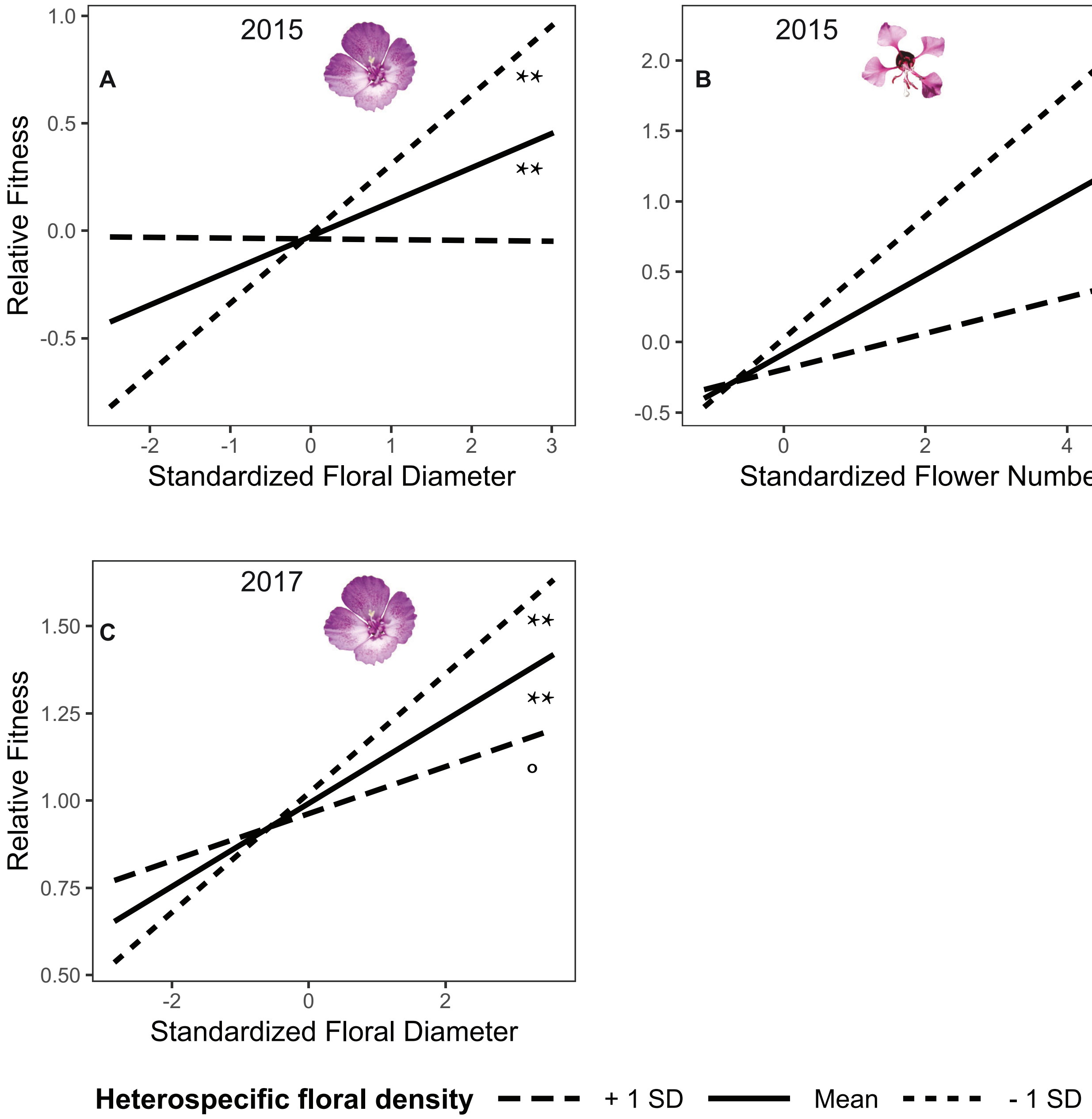

Figure 4. Visualizations of simple slope analysis for traits where heterospecific floral density had a significant effect on patterns of selection for a given species in a given year (a significant density × trait interaction; exact results of simple slope analysis in Table S6). Selection was generally stronger at low heterospecific densities for floral diameter of *C. cylindrica* in 2015 (A) and in 2017 (C), and for flower number of *C. unguiculata* in 2017 (B). The relationship between the trait of interest and relative fitness is plotted at three heterospecific floral densities: mean density + 1 SD (dashed line), mean density (solid line), and mean density − 1 SD (dotted line). Significance levels of the slopes of the lines: °$P < 0.1$; *$P < 0.05$; **$P < 0.01$.

Discussion

Our study sought to determine whether patterns of net and pollinator-mediated selection on floral traits vary with the congeneric species richness of a community and the floral density of a local neighborhood for four co-occurring congeners. Across two years in which plant and floral density varied by roughly 200%, we observed more effects of congeneric species richness on patterns of net selection in the low-density year, whereas the effects of floral density were fairly consistent in both years. These results suggest that the evolutionary consequences of

Table 2. A summary of the effects of community and neighborhood properties on estimates of selection on three floral traits of four *Clarkia* species in 2015 and two *Clarkia* species in 2017. A total of 18 estimates of selection were made: 12 in 2015 and six in 2017 (cells with thick borders, each of which is subdivided into a species composition and density subcell). Blank subcells indicate that species composition or density did not affect selection on that trait of that species in that year. A plus sign (+) indicates a positive relationship between the property and the strength of selection (e.g., stronger selection at higher densities or in communities with more species); an underlined plus sign (±) indicates a marginally significant relationship. In contrast, a minus sign (–) indicates a negative relationship between the property and the strength of selection (e.g., weaker selection at higher densities or in communities with more species). A bullet indicates a property that had significant effects on patterns of selection but that the effects were not in a consistent direction. For density, all positive relationships (+) refer to conspecific density and all negative relationships (–) refer to heterospecific density.

Year	Species	Anther-stigma separation		Floral diameter		Flower number	
		Species richness	Density	Species richness	Density	Species richness	Density
2015	*C. cylindrica*			•	–	–	
	C. unguiculata				±	–	–
	C. speciosa			–		–	+
	C. xantiana		+			–	
2017	*C. cylindrica*	•			–		+
	C. xantiana						

species co-occurrence at the community level may depend on overall community density, whereas the local density surrounding a plant may have consistent effects on the selective environment regardless of overall community density. Additionally, we observed variation in patterns of selection on floral traits that appeared unrelated to variation in pollinator visitation and pollen limitation. This lack of concordance demonstrates the importance of continuing to evaluate the conditions under which we expect pollinator-mediated selection on floral traits versus selection mediated by other agents (e.g., Sletvold and Ågren 2014; Sapir 2017; Caruso et al. 2019). Lastly, we found similar patterns of selection among two pairs of species—one pair has the same flowering time and the other has the same floral orientation—which indicate that co-occurring species may be subject to similar patterns of selection. Together these results contribute to our understanding of the context dependency of natural selection in ecological communities, and the potential for non-pollinator-mediated selection to shape the evolution of floral traits.

THE EFFECTS OF CONGENERIC SPECIES RICHNESS AND FLORAL DENSITY ON PATTERNS OF SELECTION

In our two-year study of three traits of two to four focal species, we generated 18 distinct estimates of net selection. One or more community context factor(s) modified the relationship between a trait and fitness in 11 of these 18 estimates of selection: floral density had an effect on four of 11 estimates, community type had an effect on four of 11 estimates, and both factors had an effect on three of 11 estimates (summarized in Table 2). Together with a small number of previous studies (see bolded references in Table 1), these results demonstrate that biotic interactions both at the local- and community-level can affect the strength of selection (e.g., Sletvold et al. 2013; Sletvold and Ågren 2014). Because few studies have tested if selection on floral traits varies with floral density (Donohue et al. 2000; Caruso 2002; Stanton et al. 2004; Weber and Kolb 2013), our ability to predict how density will affect patterns of selection from the ecological and evolutionary context of a community is currently limited. In contrast, a larger body of literature has examined whether the presence of co-flowering species affects patterns of selection (see bolded references in Table 1). Our results generally follow the expected pattern of weaker selection when co-occurring species are facilitative (Moeller and Geber 2005) or when competition is weak and asymmetric (Wassink and Caruso 2013). Below we discuss what types of interactions between plants and local community conditions may drive these patterns, and what additional data would be needed to attribute these patterns to specific drivers.

THE EFFECTS OF CONSPECIFIC AND HETEROSPECIFIC FLORAL DENSITY WERE CONSISTENT ACROSS YEARS

Across both years of our study, we generally observed stronger selection on floral traits at high conspecific floral densities (Fig. 3; Table 2). This result was contrary to our expectation of stronger selection at low conspecific densities, which was based on the positive relationship between conspecific floral density and pollen deposition observed in *C. xantiana* (Moeller 2004) and many other systems (reviewed in Ghazoul 2005). This result was also contrary to findings from other systems where the strength of selection on a number of floral traits did not vary with the abundance of conspecifics or competitors (Donohue et al. 2000; Caruso 2002; Stanton et al. 2004; Weber and Kolb 2013). Stronger selection at high local conspecific floral densities indicates that a change in a trait value has a larger effect on fitness when a focal plant is surrounded by more neighboring plants, which could be due to intraspecific competition for pollinators or resources at high densities. Experimentally manipulating both abiotic resources and access to pollinators would indicate if and under what circumstances the abiotic environment may be a significant agent of selection on floral traits in this system (Caruso et al. 2005; Sletvold et al. 2017).

We also observed stronger selection for larger flowers in *C. cylindrica* and for more flowers in *C. unguiculata* at low heterospecific floral densities (Fig. 4; Table 2). These two species co-occur more frequently than expected by chance (Eisen and Geber 2018), which suggests these species may experience interspecific facilitation where they co-occur at low densities (Rathcke 1983; Muñoz and Cavieres 2008; Seifan et al. 2014). In general, stronger selective pressure is expected under high heterospecific densities that lead to interspecific competition for pollination (e.g., Feinsinger 1987). However, facilitation can generate selection on different traits that would promote pollinator constancy (Armbruster et al. 1994; Grant 1994; Gumbert et al. 1999; Moeller 2004; Sargent and Ackerly 2008; Waelti et al. 2008).

THE EFFECTS OF CONGENERIC SPECIES RICHNESS WERE MORE PREVALENT IN THE LOW-DENSITY YEAR (2015)

Of the seven significant interactions between the congeneric species richness of a community and selection on a trait (summarized in Table 2), six occurred during 2015, when density was considerably lower relative to 2017. Within this pattern of more congeneric species richness effects during the low-density year, a striking result of our study was that all four species generally experienced weaker selection on flower number in communities with more species in 2015 (Fig. 2). Because reduced intraspecific competition can lead to higher productivity in more diverse communities (Johnson et al. 2006; Cardinale et al. 2007, Cardinale et al. 2011), facilitation or the relaxation of competition is expected to lead to weaker patterns of selection in more species-rich communities (Moeller and Geber 2005; Wassink and Caruso 2013; Parachnowitsch et al. 2014). In our study, this pattern was not present in the year with higher density (2017), which suggests that intraspecific competition may have predominated and generated the same effects on focal species regardless of the community context (e.g., Wirth et al. 2011). Interactions between plant species that range from facilitative to competitive have been observed in a number of systems (e.g., Moragues and Traveset 2005; Muñoz and Cavieres 2008; Seifan et al. 2014) and are generally attributed to the presence of an additional species facilitating joint pollinator attraction at low densities but becoming a competitor for pollination at high densities.

POLLINATORS AS AN AGENT OF SELECTION

Because selection on floral traits may be driven by a number of abiotic or biotic agents of selection, we conducted an experimental test of pollinator-mediated selection in 2017. We found little evidence for pollinator-mediated selection but different patterns between pollinator visitation and pollen limitation in the two focal species. In *C. xantiana*, pollinator visitation rates did not differ across community types, there was no evidence of pollen limitation, and no differences in selection between the hand- and open-pollinated treatments at any community type (Tables S6, S10, and S11). These patterns indicate that any selection on floral traits of *C. xantiana* was likely not mediated by pollinators in 2017, which could result from high floral densities decreasing the opportunity for selection (Richards et al. 2009; Benkman 2013; Trunschke et al. 2017), or from post-pollination processes that strongly modify the signal of pollinator-mediated selection (Totland 2004; Caruso et al. 2005). Given that floral traits of *C. xantiana* have been under selection in previous studies (Moeller and Geber 2005; Runquist et al. 2017), we suspect that the lack of pollinator-mediated selection in 2017 was the result of high mate availability and a low opportunity for selection, rather than the effects of postpollination processes. In addition, we estimated selection exclusively via a component of female fitness, seed set, but both the direction and magnitude of selection can vary between male and female function in a number of systems (O'Connell and Johnston 1998; Sahli and Conner 2011; Kulbaba and Worley 2012), including *Clarkia* (Runquist et al. 2017).

The patterns of visitation, pollen limitation, and pollinator-mediated selection observed for *C. cylindrica* join a growing body of literature that does not support two key expectations about these dynamics. First, in contrast to the expectation that pollinator availability will determine reproductive success (Knight et al. 2005; Benkman 2013), *C. cylindrica* experienced low pollinator visitation at two species communities and higher visitation at four species communities, but was pollen limited at both types of communities. Variation in pollinator visitation that does not correspond to variation in pollen limitation has also been observed in other systems, including *Crocus vernus* (Totland et al. 1998) and *Asclepias syriaca* (Caruso et al. 2005). Together with our results from *C. cylindrica*, these studies suggest that additional factors beyond pollinator visitation are likely important determinants of fitness, such as pollinator efficiency or effectiveness (Campbell et al. 1991; Eckhart et al. 2006; Koski et al. 2018), abiotic resource limitation (Campbell and Halama 1993; Ashman and Morgan 2004; Sapir 2017), and herbivory

(Gómez 2003; Bartkowska and Johnston 2015). Second, although theoretical (Reynolds et al. 2009; Benkman 2013) and some empirical studies (Sletvold and Ågren 2016; Trunschke et al. 2017) have demonstrated a positive correlation between the strength of pollinator-mediated selection and the degree of pollen limitation, our data suggest that selection was not pollinator mediated, even in populations that were pollen limited (no difference between the strength of selection in the hand- and open-pollinated treatments; Table S6). Patterns of selection that do not follow from patterns of pollen limitation have occurred in other systems due to correlational selection (Campbell and Bischoff 2013) and variation in selection that was driven more by a change in the functional significance of traits than by variation in interaction intensity (Sletvold and Ågren 2014). Taken together, these results reflect the complex processes that shape patterns of selection on floral traits (reviewed in Caruso et al. 2019) and reinforce the idea that pollinator-mediated selection may be nonexistent or difficult to detect if selection is primarily mediated by other agents or if current phenotypes represent a response to past episodes of pollinator-mediated selection (e.g., Aigner 2006).

VARIATION IN PATTERNS OF SELECTION ACROSS CLOSELY RELATED SPECIES IN A COMMUNITY

Given limited previous research into variation in selection on co-occurring species, a goal of our study was to compare patterns of selection on multiple *Clarkia* species that frequently co-occur in the Kern River Canyon (Kern County, CA). Across two pairs of species, one that has the same flowering time and one that has the same floral orientation, similarities in patterns of selection reinforce our previously discussed finding of intraspecific competition at high floral densities and interspecific facilitation at low floral densities. The co-flowering species *C. cylindrica* and *C. unguiculata* both experienced an effect of floral density on selection on floral diameter—*C. unguiculata* was under stronger selection at higher conspecific floral densities, whereas *C. cylindrica* was under stronger selection at lower heterospecific floral densities. These patterns could result from intraspecific competition at high floral densities and interspecific facilitation at low floral densities (see above). In addition, the two bowl shaped species *C. cylindrica* and *C. speciosa* both experienced an effect of floral density on selection on flower size in 2015, which also suggests that these species may experience facilitation in more species-rich communities (Parachnowitsch et al. 2014). The results of our two-year study suggest that community context had similar effects on patterns of selection on a number of co-occurring species (Irwin 2000). More studies of natural selection on multiple co-occurring congeners are needed to provide insight into the aspects of community context that tend to facilitate similarities or differences in patterns of selection.

Conclusions

Our study contributes to the growing body of literature that suggests the evolution of floral traits is not only determined by pollinator-mediated selection but also by other agents of selection (Strauss and Whittall 2006; Caruso et al. 2019). Conspecific and heterospecific floral density and congeneric species richness are properties of *Clarkia* communities that affected patterns of net selection on floral traits, which could be non-pollinator mediated. In particular, our results are consistent with facilitation in species-rich communities leading to weaker patterns of selection, which could be further tested by comparing patterns of selection in communities along a competition to facilitation gradient. Although the strength of the effects of floral density and congeneric species richness varied across species and with the conditions of a community in a given year, our results suggest that in general, intraspecific competition at high floral densities and interspecific competition at low floral densities may be key species interactions among co-occurring plants that affect selection on floral traits. Because the effects of these interactions occurred in the absence of pollinator-mediated selection, we speculate that community context likely affects selection mediated by resource competition in many systems.

AUTHOR CONTRIBUTIONS

KEE and MAG designed the study. KEE and ACW conducted the fieldwork and data processing. KEE analyzed the data with input from MAG. KEE wrote the manuscript, with input from ACW and MAG.

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DATA ARCHIVING

Data are available via Dryad: https://doi.org/10.5061/dryad.mkkwh70w4.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. 2015 Sites. *N* indicates the number of focal plants of each species that survived to fruiting at each site.
Table S2. 2017 Sites. For each of the two focal species (*C. cylindrica* and *C. xantiana*), there were three or four replicates of each of the three types of communities included in the study: one species communities, two species communities, and four species communities.
Table S3. Pearson correlation coefficients for correlations between the three traits measured in this study, for each species in each year.
Table S4. *F* values for the effect of the interaction between a trait and community type (number of species present at a site) on phenotypic selection of four *Clarkia* species in two years.
Table S5. Net selection (S ± 1 SE) on three floral traits by community type in 2015.
Table S6. Net selection (S) on three pollination-related traits and across two pollination treatments at three types of communities for two *Clarkia* species in 2017.
Table S7. Density of *Clarkia* flowers (open flowers/m^2) in 2015 and 2017.
Table S8. *F* values for the effect of floral density on phenotypic selection of three floral traits of four *Clarkia* species in two years.
Table S9. Simple slopes analysis for the traits where density had a significant effect on patterns of selection for a given species in a given year (a significant density x trait interaction).
Table S10. Trait and fitness means for open- and hand-pollinated flowers of *C. cylindrica* and *C. xantiana* studied in 2017.
Table S11. Back-transformed model estimated means and 95% confidence intervals for pollinator visitation data.