Pollinator-mediated interactions in experimental arrays vary with neighbor identity[1]

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PREMISE OF THE STUDY: Local ecological conditions influence the impact of species interactions on evolution and community structure. We investigated whether pollinator-mediated interactions between coflowering plants vary with plant density, coflowering neighbor identity, and flowering season.

METHODS: We conducted a field experiment in which flowering time and floral neighborhood were manipulated in a factorial design. Early- and late-flowering *Clarkia unguiculata* plants were placed into arrays with *C. biloba* neighbors, noncongeneric neighbors, additional conspecific plants, or no additional plants as a density control. We compared whole-plant pollen limitation of seed set, pollinator behavior, and pollen deposition among treatments.

KEY RESULTS: Interactions mediated by shared pollinators depended on the identity of the neighbor and possibly changed through time, although flowering-season comparisons were compromised by low early-season plant survival. Interactions with conspecific neighbors were likely competitive late in the season. Interactions with *C. biloba* appeared to involve facilitation or neutral interactions. Interactions with noncongeners were more consistently competitive. The community composition of pollinators varied among treatment combinations.

CONCLUSIONS: Pollinator-mediated interactions involved competition and likely facilitation, depending on coflowering neighbor. Experimental manipulation helped to reveal context-dependent variation in indirect biotic interactions.

KEY WORDS *Clarkia unguiculata*; context-dependency; facilitation; indirect interactions; interspecific competition; intraspecific competition; pollination; pollinator-mediated interactions; specialist pollinators

Flowering-plant reproductive success is often limited by pollen availability (Knight et al., 2005), and under such circumstances, pollinators may indirectly mediate facilitation or competition between coflowering plants. Sharing mutualists such as pollinators often results in exploitation competition (Addicott, 1985). In particular, pollinator sharing can lead to competition if pollinators preferentially visit one species (Mosquin, 1971) or if sharing reduces pollination quality (Campbell and Motten, 1985; Arceo-Gómez and Ashman, 2014). For example, the native *Lythrum alatum* experienced fewer pollinator visits and reduced reproductive success when grown in arrays with the invasive congener *L. salicaria* (Brown et al., 2002). Alternatively, pollinator sharing can be neutral (Moragues and Traveset, 2005) or beneficial. In *Clarkia xantiana*

the presence of co-occurring congeners facilitated pollination likely due to the joint maintenance of specialist pollinators (Moeller, 2004). The consequences of these interactions may influence the evolution of floral traits or mating systems (Sargent and Ackerly, 2008; Mitchell et al., 2009) as well as population and community dynamics (Pauw, 2013; Underwood et al., 2014).

The consequences of pollinator-mediated interactions can depend on the species of pollinators involved (Gyan and Woodell, 1987), which other pollinators are present in the community (Thomson and Thomson, 1992; Ivey et al., 2003), as well as their effectiveness, all of which can vary with ecological context (Ivey et al., 2003; Sahli and Conner, 2007). The outcome of pollinator sharing, for example, may depend on whether the shared pollinator is a generalist or specialist since these pollinators can differ in effectiveness (Larsson, 2005; Moeller, 2005). Pollinator behaviors that influence effectiveness, such as flower handling time (Conner et al., 1995), can also vary (Strickler, 1982; Ivey et al., 2003), as can pollinator abundance. When pollinators are rare and plants are pollen limited, neighboring plants may have particularly strong

effects on pollination. Shifts in the dynamics of plant–pollinator interactions, as well as interactions among plants involving pollinators, are consequential in that they can influence the maintenance and assembly of plant communities (Sargent and Ackerly, 2008; Pauw, 2013).

We studied the effects of pollinator-mediated interactions between *Clarkia unguiculata* Lindl (Onagraceae) and its coflowering neighbors, with particular attention to effects of neighbor identity on whole-plant pollen limitation. Using experimental arrays, we addressed the following questions:

(1) Do other *Clarkia* species facilitate or compete for pollination with *C. unguiculata* by sharing specialist pollinators?

(2) Is the effect of a congeneric neighbor on pollination success of *C. unguiculata* different than that of a neighbor from a different genus?

In addition, our study was designed to address a third question, although unforeseen complications limited our ability to explore this question fully (see Results and Discussion):

(3) Do pollinator-mediated interactions between *C. unguiculata* and coflowering neighbors change between early and late spring?

The potential for plants to interact via shared pollinators has inspired decades of study (Robertson, 1895; Lewis, 1961; Levin and Anderson, 1970). Plants occur in mixed communities, and thus these interactions may change with the identity or density of coflowering neighbors; attention to such context-dependency is increasingly advocated (Mitchell et al., 2009). Through manipulative experimentation, our study clarifies that interactions between coflowering plants can be sensitive to local ecological conditions.

MATERIALS AND METHODS

Study system—*Clarkia unguiculata* is a widely distributed California annual that occurs at elevations of <1500 m a.s.l. (Lewis, 2012). It flowers from late April to July (Oswald, 2002). Plants produce an erect stem <1 m tall, bearing one to several showy, pink-purple flowers 2–5 cm in diameter (Lewis, 2012). Each flower holds about 80–100 ovules (Delesalle et al., 2007), 60–70% of which mature into seeds (Hove et al., 2016). The species is capable of low levels of autogamy (Mazer et al., 2010), but pronounced protandry and herkogamy (Dudley et al., 2007) lead to predominant outcrossing in nature (Hove et al., 2016; Ivey et al., 2016). *Clarkia unguiculata* routinely co-occurs and coflowers with other species of *Clarkia* as well as wildflowers of other genera, and it shares with these other taxa a variety of specialist and generalist pollinators, potentially leading to facilitation or competition (Lewis, 1961; MacSwain et al., 1973; Moeller 2004).

We chose *C. biloba* subsp. *biloba* A. Nelson & J.F. Macbr as a congener for our study because it co-occurs and coflowers with *C. unguiculata* in natural populations (personal observation), with which it shares several specialist and generalist pollinators (MacSwain et al., 1973). We chose *Raphanus sativus* L. ("wild radish"; Brassicaceae) and *Erysimum linifolium* J. Gay (Brassicaceae) as coflowering neighbors from a different genus (hereafter referred to as "heterogeners") because they were expected to share only generalist pollinators with *C. unguiculata*. We used purple morphs of *R. sativus* for Early arrays (see below), and *E. linifolium*, a cultivar native to Spain (Bailey, 1949), for Late arrays as neither species was flowering in sufficient abundance to use during both periods. Like *C. unguiculata*,

R. sativus also occurs adventively at low elevations (<1500 m a.s.l.) in disturbed areas (Al-Shehbaz, 2012). The purple flowers of *R. sativus* and *E. linifolium* are similar in shape and color to the red-purple flowers of *C. unguiculata*.

Experimental design—To study temporal variation in pollinator-mediated interactions between coflowering neighbors, we placed arrays of 12 potted *C. unguiculata* plants in the field (Appendix S1; see Supplementary Data with this article). Arrays were arranged factorially with two flowering times and four neighborhood treatments. Early-flowering plants ("Early") were sown indoors at the end of January and flowered from April to mid-May, whereas late-flowering plants ("Late") were sown at the end of March and flowered from late May to July. Early plants were flowering before the peak flowering time of *Clarkia* in nature (Oswald, 2002), which was intended to allow us to examine pollinator-mediated interactions when specialist *Clarkia* pollinators were absent, in contrast to Late arrays.

Floral neighborhood was manipulated by changing the density and identity of coflowering plants. Arrays contained either no additional plants (low-density control, "LDControl") or 15 additional *C. unguiculata* (high-density control, "HDControl"), congener *C. biloba* ("Congener"), or heterogener ("Heterogener") plants (Appendix S1). The experiment had three replicate blocks (replicate field locations within the study site) during the early season and four during the late season. Each neighborhood treatment was replicated once in each block for a total of 144 (12 plants * 4 neighborhood treatments * 3 blocks) and 192 (12 plants * 4 neighborhood treatments * 4 blocks) focal plants in the early and late seasons, respectively. Because of inclement weather and disturbance from bears, our final sample size (plants that survived, post-flowering, to set seeds) was 43 Early focal plants and 170 Late plants. One entire replicate array was lost for one of the Early neighborhood treatments (Appendix S2), which compromised the scope of inference for flowering-time treatments (see Discussion). Although we retain these data for analyses, we caution here and below (Results and Discussion) that interpretation of flowering-time treatment effects should not be considered definitive, due to survival differences between flowering seasons. Damaged neighbor plants were replaced using similar plants from the same sources. Arrays within blocks were ≥38 m apart to reduce pollinator-mediated interactions between arrays. Deer were excluded from the arrays by green Safety Fence with 5 × 5 cm openings (Uline, Pleasant Prairie, Wisconsin, USA).

Study site—The experiment was conducted in a savanna grassland at 270 m a.s.l. within the Big Chico Creek Ecological Reserve ("BCCER") in Butte County, California, USA (Appendix S3). Of the species chosen for experimental arrays, only *R. sativus* occurred naturally at the reserve, although none grew within our study sites. Close proximity of *Clarkia purpurea* and *C. rhomboidea* to our experimental arrays suggested that specialist bees would be available to visit experimental arrays. These plant taxa were not noticeably more common near any one of the treatments, and random placement of arrays within blocks minimized any bias.

Responses—We measured pollen limitation, pollinator visitation and behavior, and pollen deposition to evaluate the potential for interactions between focal *C. unguiculata* and other plants in experimental arrays. Unless otherwise mentioned, these responses

were measured on each plant, and individual plants were the statistical subject. Analyses were conducted using R version 3.1.0 (R Development Core Team, 2014) or SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). Copies of code are available upon request.

Pollen limitation—We estimated whole-plant pollen limitation of female fitness (seed production) in focal plants by hand-pollinating all flowers on six of the 12 focal *C. unguiculata* plants in each array ("supplemented") using supplemental pollen from 50 plants reserved as pollen sources. Every morning, freshly dehisced pollen was collected from all available anthers on pollen-source plants and spread evenly across the surfaces of all receptive stigmas on experimental plants using a plastic brush toothpick (The Doctor's Brushpicks, Prestige Brands, Tarrytown, New York, USA) within 4 h of collection. The remaining six focal plants in each array ("unsupplemented"), as well as supplemented plants, were available for open pollination.

More seeds per capsule in supplemented versus unsupplemented plants was considered evidence of pollen limitation. We counted seeds per plant using ImageJ 1.45s (Rasband, 2011). Seed number per capsule was estimated for each plant by dividing total seeds by total capsule number, including capsules that did not produce seeds.

To test the effect of supplementation on seed number per capsule, we used a generalized linear mixed model (GLMM) with a Poisson error distribution. Pollen supplementation, neighborhood treatment, flowering time, and all two- and three-way interactions were fixed factors. Whole-plant flower number was a covariate, and array identity nested within block (replicate field) and flowering time*block were included as random factors.

Within each floral-neighborhood × flowering-time combination, we compared seed number per capsule between supplemented and unsupplemented plants using Bonferroni-adjusted contrast comparisons. Plants were considered to be pollen limited if the difference in seed number per capsule between pollen-supplemented and unsupplemented plants was significantly greater than zero.

If focal *C. unguiculata* plants in mixed-species arrays were pollen limited but control arrays were not, this was interpreted as evidence of competition for pollinators, and the reverse was considered evidence of facilitation. Comparison with HDControl arrays allowed us to distinguish effects of floral density from neighbor identity. Similarly, comparison of LDControl and HDControl arrays identified intraspecific competition or facilitation. Comparison of pollen limitation in Congener and control arrays provided insight toward answering our first question regarding interactions between *C. unguiculata* and other species of *Clarkia* (see Introduction). Similarly, comparison of pollen limitation in Heterogener and Congener arrays, or between Early and Late flowering times, provided an understanding of the extent to which these interactions differed between *Clarkia* and other taxa, or through time (see Introduction: questions 2 and 3).

Pollinator visits—Pollen limitation in focal plants could result from infrequent pollinator visitation. We observed experimental arrays in multiple 30 min observation periods (total = 41 h), during which we recorded the number of pollinators arriving to visit plants. We also recorded the total number of flowers that pollinators visited on each plant. Any insect that contacted anthers or stigma of focal *C. unguiculata* flowers was considered a pollinator.

We compared the number of pollinator visits flower^{-1} h^{-1} among treatment combinations using a mixed model. The response was log transformed prior to analysis to improve model fit. Neighborhood treatment, flowering time, and their interaction were fixed factors; array identity nested within block and block × flowering time were included as random effects. We also compared the number of flowers visited among treatments using a mixed model. The response (total flower number visited for each plant) was log transformed prior to analysis to improve model fit. Neighborhood treatment, flowering time, and the interaction of neighborhood and flowering time were fixed factors, and average flower number per plant across the number of times observed was included as a covariate. Array identity nested within block and block × flowering time were random effects, and number of observation periods was included as an offset.

Pollinator community composition—The proportion of pollinators in each taxonomic category, as well as the frequency of number of flowers visited by each type of pollinator, was compared between flowering times, among neighborhood treatments, and among three replicate fields that were used in both flowering times with separate contingency tables. We included in these counts some visits to focal plants for which the individual plant number was not recorded. Pollinators were classified as Apidae, Halictidae, Megachilidae, or "other taxa." When comparing flowering times, Halictidae and Megachilidae were collapsed into a single category to meet minimum requirements for expected counts. In addition, bee pollinators were identified to genus and classified as specialists or generalists (Appendix S4). The proportion of visits by *Clarkia* specialists (as listed in MacSwain et al., 1973) and the proportion of flowers visited by specialists were compared among neighborhood treatments using a Fisher's exact test (see Results).

Pollinator constancy—Pollinator movements between species may contribute to pollen limitation via heterospecific pollen deposition. We scored movements of pollinators foraging on focal plants as constant (i.e., between focal plants) or inconstant (from a focal plant to a neighbor). Due to seasonal differences in sample size (see Results), we assessed pollinator constancy for late-season data only.

To assess pollinator constancy, we used a GLMM with binomial errors to analyze whether the likelihood of pollinator constancy differed among HDControl, Congener, and Heterogener neighborhood treatments. Each pollinator movement between plants that we observed, including multiple movements by the same pollinator, was included. Individual array identity nested within block and individual pollinator identity was included as random factors. The proportion of focal flowers out of the total flowers in the array was initially included as a covariate but was removed from the final model because it was not significant.

Geitonogamy potential—Geitonogamy, or self-pollination between flowers of a plant, can lead to pollen limitation (Vaughton and Ramsey, 2010). To estimate the potential for geitonogamous pollination, each pollinator movement between *C. unguiculata* flowers was categorized as either between-plant or within-plant. Due to seasonal differences in sample size (see Results), the potential for geitonogamous pollination was estimated only for Late plants.

To compare potential geitonogamy among the Late HDControl, Congener, and Heterogener arrays, we used a GLMM model with binomial errors. The likelihood of within-plant movement between

flowers was the response variable, and neighborhood treatment was the fixed factor. Array identity nested within block and the identity of the individual pollinator were random factors. The number of flowers that each pollinator had previously visited on the current plant, the number of flowers that each pollinator had previously visited within the entire array, the total number of flowers open on the current plant, and the total number of flowers in the array were initially included in the model as covariates but were subsequently removed because they were not significant.

Pollen deposition—We counted pollen grains on *C. unguiculata* stigmas to verify the effects of supplementation and to compare open-pollen deposition by pollinators between flowering times and among neighborhood treatments. A single stigma from up to two supplemented and two unsupplemented plants in each array was dried and soaked in 8M NaOH for 24–48 h. To ensure that stigma collection did not damage developing capsules, we collected senescent stigmas. Some stigmas fell after senescence; thus, only 75 of the 112 (4 plants * 28 arrays) possible stigmas were collected, 39 of which were unsupplemented. The total number of *Clarkia* pollen grains adhered to each stigma was counted twice at 100× and averaged for analysis. Though *C. unguiculata* and *C. biloba* pollen grains reportedly differ slightly in size and shape (Small et al., 1971), the *Clarkia* pollen grains observed, including those from Congener arrays, could not be classified into two species. Thus, stigmas sampled may have lacked *C. biloba* pollen, or the two species' pollen may have been too similar for us to distinguish.

Pollen deposition was compared across experimental treatments using two GLMMs with Poisson distributions. In the first model, flowering time, pollen supplementation, and their interaction were fixed factors, and array identity nested within block and array identity × flowering time were random factors. In the second model, only unsupplemented stigmas were analyzed. In this model, flowering time, neighborhood treatment, and their interaction were fixed factors, and array identity nested within block and array identity × flowering time were random factors. The number of flowers produced and petal length of the first flower were included as covariates for both models, but petal length was ultimately removed because it was not significant.

Pollinators in natural populations—To evaluate whether any pollen limitation observed in experimental arrays was associated with unusual patterns of pollinator visitation, we surveyed two natural populations. The first population ("Burma Rd."; 37.6 km from BCCER) lacked *Clarkia* congeners, whereas *C. unguiculata* at second population ("Hogan Dam Rd."; 190.5 km from BCCER) grew among *C. biloba* and *C. purpurea* (Appendix S3).

We counted the number of *C. unguiculata* flowers visited by pollinators in plots that were the same size as experimental arrays and where *C. unguiculata* density most closely resembled that of experimental arrays (13–30 plants plot^{-1}). We also recorded the taxa visiting flowers as described above. Nine plots within which *C. unguiculata* was the only insect-pollinated plant were observed at Burma Rd., and thirteen plots of intermixed *C. unguiculata*, *C. biloba*, and *C. purpurea* were observed at Hogan Dam Rd. We observed some plots twice, resulting in a total observation time within the natural populations of 13 h.

The proportion of pollinators by taxon, as well as the proportion of number of flowers visited by taxon, was compared among the three sites (BCCER, Burma Rd., and Hogan Dam Rd.) using

contingency tables. Pollinators were classified as Apidae or non-Apidae to meet requirements for minimum expected counts. The proportion of visits by *Clarkia* specialists and the proportion of flowers visited by specialists, was compared among sites using a Fisher's exact test.

We compared number of flowers visited per plot (or experimental array) among sites using a GLMM with a Poisson probability distribution. Because individual plants were not labeled in natural populations, we assessed visits at the array level rather than the plant level in this analysis. In this case, field site (BCCER, Burma Rd., or Hogan Dam Rd.) was the fixed factor. The average number of open flowers within observation plots (Burma Rd. and Hogan Dam Rd.) or experimental arrays (BCCER) was included as a covariate in the final model, but observation length was not included because it was not significant. Factor significance was evaluated using Wald chi-square tests. We then compared number of flowers visited among flowering time × neighborhood treatment combinations and among sites using post hoc Benjamini-Yekutieli pairwise comparisons using the "testInteractions" function in the package phia (De Rosario-Martinez et al., 2015). Least-squares means were calculated using the "lsmeans" function in the lsmeans package (Lenth, 2015) and are reported ± SE.

RESULTS

Pollen limitation—Mean seed number per capsule depended on the combination of neighborhood treatment, flowering time, and pollen supplementation (i.e., significant three-way interaction; Fig. 1 and Table 1). Pollen-supplemented plants produced significantly more seeds per capsule than unsupplemented plants (indicating pollen limitation) in Late HDControl and Heterogener arrays (Fig. 1), suggesting intraspecific and interspecific competitive interactions, respectively, between focal *C. unguiculata* and neighbor plants. Focal *C. unguiculata* in Early LDControl and Early Heterogener arrays were also pollen limited (Fig. 1); and, in Early Congener arrays, unsupplemented plants produced more seeds per capsule than pollen-supplemented plants (Fig. 1; "negative" pollen limitation—see Young and Young, 1992), but early-season results were based on relatively small sample sizes (Appendix S2).

Pollinator visits—We observed a total of 233 pollinators arrive to forage on focal *C. unguiculata* plants in the experimental arrays, and pollinators visited a total of 466 flowers. Back-transformed per flower rate of visitation to plants ranged among treatment combinations from a low of lsmean = 1.06 ± 0.14 visits flower^{-1} hr^{-1} for Early LDControl to a high of lsmean = 1.32 ± 0.10 for Late LDControl, and it did not vary significantly among experimental neighborhood or flowering-time treatments (Table 2). Number of flowers visited per plant was nearly three times higher in Late arrays than in Early arrays but did not differ among neighborhood treatments (Table 2 and Fig. 2).

Pollinator community composition—Bee pollinators of experimental arrays were primarily generalists from Apidae, Halictidae, and Megachilidae, although at least two specialist taxa were observed (Table 3 and Appendix S4). The proportion of flowers visited by bees from Apidae (largely *Bombus* spp.) was over five times greater in Early arrays than in Late arrays (Table 3; Pearson χ^2 = 114.3, df = 2, *P* < 0.001), largely due to a dramatic increase in the number of flowers visited by other taxonomic categories during the
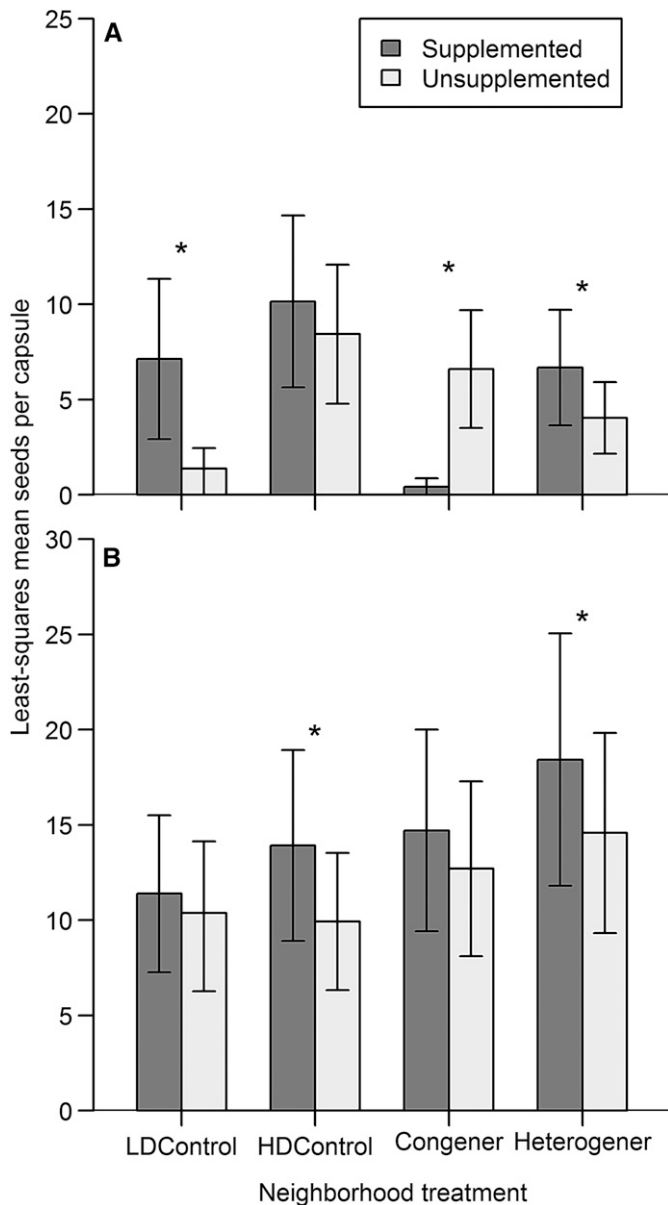
FIGURE 1 Least-squares mean (± SE) seed number per capsule of focal *Clarkia unguiculata* plants growing in experimental arrays from four neighborhood treatments and experimentally manipulated (A) early or (B) late flowering times (for abbreviations of neighborhood treatments, see Materials and Methods). All focal plants were open-pollinated, and all flowers on half of the focal plants were also hand-pollinated (supplemented). Neighbor–flowering time combinations are marked with an asterisk if pollen-supplemented and unsupplemented plants differ at *P* < 0.05 by Bonferroni-corrected contrast comparisons. See Table 1 for statistical analysis.

Late treatment (Table 3). A similar pattern occurred with the proportion of pollinators from the different taxonomic categories (Table 3; Pearson χ^2 = 15.1, df = 2, *P* < 0.001). The proportion of flowers visited by bees from Apidae that were in HDControl arrays was at least eight times greater than that of any other treatment (Table 3; Pearson χ^2 = 161.6, df = 9, *P* < 0.001). This pattern was less pronounced for the proportion of pollinators (Table 3; Pearson χ^2 = 30.8, df = 9, *P* < 0.001). Proportion of flowers visited by other

taxa (mostly nonbees) was ≥57% higher in Heterogener arrays than in the other treatments (Table 3). There was no difference among replicate fields in either the proportion of flowers visited by different taxa (Pearson χ^2 = 5.1, df = 6, *P* = 0.6) or the proportion of pollinators from different taxa (Pearson χ^2 = 4.0, df = 6, *P* = 0.7).

As expected, no specialist bees were observed visiting Early arrays. Within Late arrays, the proportion of flowers that were visited by specialists differed among neighborhood treatments (Fisher's exact test, *P* = 0.02), but the number of specialist visitors did not (Fisher's exact test, *P* = 0.24). The most pronounced difference was that no specialists were observed visiting LDControl arrays, but Congener, HDControl, and Heterogener arrays were visited by specialists (Table 3).

Pollinator constancy—We observed 84 movements of pollinators between plants during the late season, 25 of which were movements between focal plants (i.e., constant) within an array. Constancy did not differ among neighborhood treatments ($F_{2, 46}$ = 1.26, *P* = 0.3; lsmean probability of constancy = 0.33 ± 0.16, 0.31 ± 0.18, and 0.10 ± 0.07, respectively, for HDControl, Congener, and Heterogener).

Geitonogamy potential—During the late season, 122 of 260 observed intraspecific pollinator movements were between flowers of the same plant, thus potentially leading to geitonogamous self-pollination. The probability of within-plant pollinator movements did not differ among neighborhood treatments ($F_{3, 201}$ = 0.80, *P* = 0.5). The lsmean probability that a pollinator movement between flowers would occur within a plant (i.e., geitonogamously) was 0.51 ± 0.10 for LDControl, 0.56 ± 0.12 for HDControl, 0.57 ± 0.14 for Congener, and 0.34 ± 0.11 for Heterogener arrays.

Pollen deposition—We confirmed that pollen-supplemented plants received more pollen than unsupplemented plants (lsmean = 129.1 ± 26.6 and 46.2 ± 9.5 grains stigma^{-1}, respectively), but pollen deposition did not differ between flowering times (Table 1). Among unsupplemented stigmas, pollen deposition did not differ among neighborhood-treatment × flowering-time combinations (Table 1).

Pollinators in natural populations—Number of flowers visited per plot differed among the three field sites (Wald χ^2 = 411.2, df = 2, *P* < 0.001). More flowers were visited in plots at Burma Rd., followed by Hogan Dam Rd. and BCCER (lsmean flowers visited per plot = 4.0 ± 0.07, 3.5 ± 0.06, and 2.4 ± 0.05, respectively, after adjusting for flower number and number of observations). The proportion of flowers visited by different taxonomic groups also differed among field sites (Pearson χ^2 = 667.4, df = 2, *P* < 0.001), as did the proportion of pollinators in the different taxonomic categories (Pearson χ^2 = 138.1, df = 2, *P* < 0.001). Ten percent of flowers visited in Late arrays at BCCER were by bees from Apidae, compared to 97% (Burma Rd.) and 90% (Hogan Dam Rd.) in natural populations (Table 3). Similar trends were observed in comparing the proportions of pollinators among sites (Table 3). The proportion of flowers visited by specialist bees, however, did not differ among field sites, nor did the proportion of specialist bees visiting (Fisher's exact test; *P* > 0.2 for both).

DISCUSSION

Pollinator-mediated indirect interactions between *Clarkia unguiculata* and coflowering neighbors varied with the identity of the neighboring plant (Fig. 1 and Table 1). Interactions involved

TABLE 1. Effects of experimental hand-pollination (pollen supplementation), manipulation of flowering time, and floral neighborhood on seed number per capsule and pollen deposition onto stigmas in experimental arrays of *Clarkia unguiculata*. Flower number per plant was included as a covariate. S + U included pollen counts on stigmas of supplemented and unsupplemented plants; U included only unsupplemented plants.

Source of variation	df	$F_{ndf, ddf}$		
		Seeds capsule^{-1}	Pollen deposition	
			S + U	U
Pollen supplementation	1	$0.08_{1,190}$	$1890_{1,69}$ ****	
Flowering time	1	$5.27_{1,5.3}$†	$0.02_{1,21.9}$	$0.03_{1,4.8}$
Pollen supplementation × flowering time	1	$0.93_{1,190}$	$0.1_{1,69}$	
Neighborhood	3	$2.64_{3,9.8}$		$1.47_{3,12.9}$
Pollen supplementation × neighborhood	3	$4.21_{3,190}$ **		
Flowering time × neighborhood	3	$3.33_{3,9.9}$†		$2.64_{3,12.9}$†
Pollen supplementation × flowering time × neighborhood	3	$4.92_{3,190}$ **		
Flower number	1	$73.05_{1,190}$ ****	$107.4_{1,69}$ ****	$479.3_{1,65}$ ****

†$P < 0.1$, ** $P < 0.01$, **** $P < 0.0001$.

intraspecific competition (Fig. 1; Late HDControl), interspecific competition (Fig. 1; Late Heterogener), and possibly congeneric facilitation (Fig. 1; Late Congener, see below). Evidence for seasonal effects was more tenuous due to early-season problems in the field that reduced sample size (Appendix S2). Overall, these results suggest that the nature of interactions between coflowering plants can be fluid and depend on the ecological context in which a plant occurs, at least with respect to pollinator community and floral neighborhood, and possibly with flowering time (Ramsey, 1995; Ishii and Higashi, 2001; Moeller, 2004, 2005; Bell et al., 2005; Flanagan et al., 2009). Changes in local ecological conditions apparently induced changes in the behavior or abundance of pollinators that ameliorated or magnified pollen limitation, depending on the treatment combination. The ecological conditions that varied among treatments did not involve density of the focal taxon; we manipulated only the identity of coflowering neighbors and thus were able to avoid some common pitfalls involved in studies of species interactions (Connolly, 1988; Underwood et al., 2014). In addition, by measuring whole-plant pollen limitation of seed set, we were able to reveal that facilitation or competition can occur even when per flower visitation rate is unaffected by changes in plant density (in contrast to Thomson, 1978; Feldman et al., 2004) or when pollen deposition is constant (Johnson et al., 2003; Bell et al., 2005; Flanagan et al., 2009). Instead, our results suggest that these interactions can be mediated by other aspects of pollinator quality (see below; Mitchell et al., 2009). No estimate of male fitness was measured in our study, however, and thus a comprehensive evaluation of the extent and consequences of these interactions (*sensu* Mitchell et al., 2009) awaits further study.

TABLE 2. Effects of floral neighborhood and flowering time on pollinator visitation rate (number of visits flower^{-1} h^{-1}) and number of flowers visited in experimental arrays of *Clarkia unguiculata* at the Big Chico Creek Ecological Reserve in Chico, California, USA. Experimental arrays were observed for a total of 41 h.

Dependent variable	Source	$F_{ndf, ddf}$
Visitation rate	Flowering time	$1.95_{1,4}$
	Neighborhood treatment	$0.27_{3,25.3}$
	Flowering time × neighborhood treatment	$0.85_{3,21.7}$
Number of flowers visited	Flowering time	$14.3_{1,4.9}$ **
	Neighborhood treatment	$1.04_{3,26.7}$
	Flowering time × neighborhood treatment	$1.17_{3,22.5}$
	Number of open flowers	$4.11_{1,204.4}$ *

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

***Question 1: Do other* Clarkia *species facilitate or compete for pollination with* C. unguiculata *by sharing specialist pollinators?*—Pollinator-mediated interactions between *C. unguiculata* and a coflowering congener (*C. biloba*) appeared to be neutral or involve facilitation (reduced pollen limitation; Fig. 1), but it is unlikely that these interactions were mediated by specialist pollinators. Previous studies have reported neighboring *C. unguiculata*, *C. speciosa*, and *C. cylindrica* to facilitate the pollination of *C. xantiana* in the southern Sierra Nevada through the joint maintenance of specialist pollinators (Moeller, 2004, 2005). Competitive interactions between neighboring *Clarkia* have also been reported previously, although the role of specialist visitors was unclear. Lewis (1961) reported "pollinators" (no taxa noted) to move indiscriminately between *C. biloba* and *C. lingulata* in experimental sympatric populations, suggesting competition, given that their hybrid offspring are infertile. The visits we observed from specialists were rare and were not associated with higher pollen deposition or per capsule seed set. The likelihood of visitation by a specialist bee appeared to be more strongly influenced by overall plant density than by the identity of coflowering species, in that we observed specialists visiting all neighborhood treatments except LDControl. This may indicate that specialists benefit from the presence of additional flowers, even those of heterogeners, perhaps while foraging as generalists for nectar. Some *Clarkia* specialists are known to forage for nectar on plants other than *Clarkia* (MacSwain et al., 1973), and *Lasioglossum pullilabre*, a *Clarkia* specialist, has been seen foraging for pollen on other genera (MacSwain et al., 1973). In any case, we were unable to detect whether the specialists we observed preferred a plant species, given the small number of specialist visits observed.

***Question 2: Is the effect of a congeneric neighbor on pollination success of* C. unguiculata *different than that of a neighbor from a different genus?*—Interactions between *C. unguiculata* and heterogeners were likely competitive, given that seed-set was pollen limited for plants in Heterogener arrays during both seasons (Fig. 1). In contrast, pollen limitation was absent among *C. unguiculata* plants in Late Congener arrays (Fig. 1), which could indicate that indirect interactions with coflowering congeners involve facilitation (compared to pollen-limited plants in Late HDControl arrays) or that such interactions are neutral (compared to Late LDControl plants, which were not pollen limited). In the early season, *C. unguiculata* in arrays with *C. biloba* experienced low (negative) pollen limitation, whereas pollen limitation was either absent or positive in other treatments (Fig. 1), but we caution that early-season results were based on a small sample size (Appendix S2). Overall, our observations are
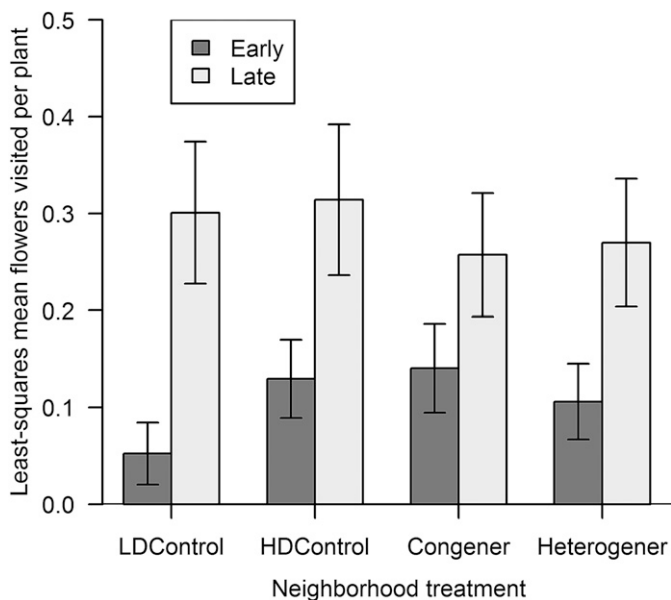
FIGURE 2 Least-squares mean (± SE) flowers visited per plant on focal *Clarkia unguiculata* plants growing in experimental arrays in which flowering time (Early or Late) and floral neighborhood were manipulated (for abbreviations of neighborhood treatments, see Materials and Methods).

consistent with the idea that indirect interactions between *C. unguiculata* and its coflowering neighbors differ between congeners and heterogeners.

The patterns of pollinator behavior or quality driving differences in interactions between Congener and Heterogener treatments were not immediately apparent. One dimension of quality that can drive pollinator-mediated indirect interactions involves pollen transfer between species; pollinator movements between species, for example, could cause pollen limitation by blocking stigmas with heterospecific pollen (Feinsinger, 1978; Campbell and Motten, 1985). This seems unlikely to explain the patterns we observed, however, because interspecific pollinator movements were observed in Late Congener arrays (which were not pollen limited) but

not in Late Heterogener arrays (which were pollen limited). Alternatively, pollinator movements between congeners might decrease pollen limitation compared to heterogeners if geitonogamous self-pollination is reduced (Liao et al., 2011); in our study, however, the probability of within- versus between-plant conspecific pollinator movements did not differ between Congener and Heterogener arrays. Another element of pollinator visit quality involves the quantity of pollen deposited (e.g., Herrera, 1987), but we found no difference among arrays in pollen deposition. Other studies have reported facilitation between coflowering *Clarkia* to be mediated by pollinator visits (Moeller, 2005), but we found no significant differences among neighborhood treatments in the per flower rate of visitation or number of flowers visited (Table 2 and Fig. 2). Thus, the contrasting interactions we observed between *C. unguiculata* and heterogeners versus congeners were not readily explained by the measurements we collected.

Question 3: Do pollinator-mediated interactions between* C. unguiculata *and coflowering neighbors change between early and late spring?—Unfortunately, interpretation of Early treatment effects was compromised by low survivorship and capsule production. About 30% of Early plants survived to produce seeds, whereas ~90% of Late plants survived; some of the early-season mortality might be attributed to the cold and rainy spring, as well as to meddlesome bears (see Methods and Appendix S2). The small sample size likely limited statistical power to detect neighborhood treatment effects in the early season as well as seasonal differences.

The patterns we observed, however, suggest that intraspecific and possibly congeneric pollinator-mediated interactions may differ between seasons, whereas those involving heterogeners appeared to be more consistent in both seasons. Plants in Early LDControl arrays were pollen limited, for example, but those in Early HDControl arrays were not (Fig. 1), suggesting some intraspecific facilitation with increased density. This pattern is reminiscent of the Allee effects reported in other *Clarkia* taxa (Groom, 1998; Moeller and Geber, 2005). During the late season, on the other hand, plants in HDControl arrays were pollen limited, whereas those in LDControl arrays were not, which is consistent with intraspecific competition for pollination. Other studies have

TABLE 3. Number of specialist and generalist pollinators visiting *Clarkia unguiculata* flowers in experimental and natural populations in California, USA (with total number of flowers visited by each category of pollinator in parentheses). Among the 27 experimental arrays, visits were observed in four different neighborhood treatments and during two flowering times at the Big Chico Creek Ecological Reserve.

Neighborhood treatment/ Location	Flowering time	*Clarkia* specialists	Generalists Api	Hal	Meg	Oth	Total pollinator observation time (hr)
Experimental Arrays							
LDControl	Early	0	0	0	0	0	3.5
HDControl	Early	0	4 (26)	2 (2)	0	0	3.8
Congener	Early	0	2 (2)	1 (1)	0	2 (2)	5.3
Heterogener	Early	0	1 (1)	3 (5)	0	7 (14)	5.5
LDControl	Late	0	1 (9)	13 (119)	6 (26)	11 (21)	6.1
HDControl	Late	1 (3)	7 (36)	10 (41)	20 (40)	4 (4)	5.3
Congener	Late	2 (5)	1 (1)	12 (69)	3 (6)	4 (11)	5.5
Heterogener	Late	2 (3)	1 (1)	15 (62)	4 (10)	4 (5)	5.9
Natural populations							
Burma Rd.		1 (1)	50 (212)	1 (2)	0	2 (2)	4.5
Hogan Dam Rd.		2 (4)	49 (247)	0	3 (8)	12 (14)	8.0

Notes: Apidae (Api), Halictidae (Hal), Megachilidae (Meg), and other taxa (Oth). Arrays had either no additional plants (LDControl) or 15 additional neighboring *C. unguiculata* (HDControl), *C. biloba* (Congener), or *Raphanus sativus* (Early) or *Erysimum linifolium* (Late; Heterogener). See Materials and Methods for neighborhood treatment abbreviations. See Appendix S3 for location details.

also reported pollinator-mediated intraspecific competition to be higher later in the flowering season (Zimmerman, 1980, 1984). Pollen limitation was low (negative) for *C. unguiculata* placed within Congener arrays, whereas it was absent or positive for other treatments during the early season (Fig. 1), which might indicate facilitation or simply be a complication of the small sample size (see also Young and Young, 1992). During the late season, the lack of pollen limitation in Congener arrays could indicate facilitation (in comparison to HDControl arrays, which were pollen limited) or neutral interactions (compared to LDControl; Fig. 1). Plants in Heterogener arrays were pollen limited during both flowering periods (Fig. 1), which is consistent with competition. Different species of experimental heterogeners were used during the two flowering seasons (see Methods), however, so direct comparisons of the Heterogener treatment across seasons were not possible.

Patterns in natural populations—Experimental arrays at BCCER received fewer pollinator visits than either natural population (Burma Rd. or Hogan Dam Rd.), and pollinator community composition differed the three field sites. Pollinators at BCCER may have been less likely to visit *C. unguiculata* because it does not occur there (although other species of *Clarkia* are common). The lower visitation may be a consequence of geographical variation in pollinator communities or some characteristic of our experimental design. Even so, we were able to discern neighborhood treatment effects in responses likely to reflect pollinator behavior, such as pollen limitation.

The low abundance of specialist bee visits in both natural populations and experimental arrays was somewhat unexpected. Over 60% of visitors collected by Moeller (2005) in Southern California populations of *C. xantiana xantiana* were specialists. Comparable data from our study populations of *C. unguiculata* were unavailable, but MacSwain et al. (1973) visited 29 natural populations and collected multiple individuals of each of the 10 *Clarkia* specialist species they observed visiting *C. unguiculata* flowers. In fact, they collected more bees (*Lassioglossum [Evylaeus] pullilabris*) on *C. unguiculata* than on any other *Clarkia* species. The low frequency of specialist visitation we observed may have been atypical; visitation rates of pollinators often vary substantially from year to year (Ivey et al., 2003). Additionally, our study site may have lacked habitat for ground nesting (Pascarella et al., 1999) or sufficient *Clarkia* pollen to support specialist populations. While *C. purpurea quadrivulnera* and *C. rhomboidea* occurred at the study site, they are primarily self-fertilizing (MacSwain et al., 1973). Specialists are known to forage on selfing species, where they co-occur with outcrossing *Clarkia* species (MacSwain et al., 1973), but the selfing species alone may not have provided sufficient pollen to maintain robust specialist populations. Alternatively, *Clarkia* specialist bees may be experiencing declines similar to those reported for other native bees (Cameron et al., 2011; Goulson et al., 2015).

CONCLUSIONS

Our work highlights the context-dependent nature of indirect interactions between coflowering plants within a community and shows that they may shift from facilitation to competition, depending on local conditions. Although previous work has shown that such interactions can vary (Campbell, 1985; Mitchell et al., 2009; Hegland and Totland, 2012; Runquist, 2013; Arceo-Gómez and Ashman, 2014), ours may be the first study to experimentally examine

the interacting effects of plant density, coflowering neighbor identity, and flowering season on whole-plant pollen limitation. Future studies that use experiments to study indirect interactions between coflowering plants will help unravel the complex influences of pollinators on plant community structure and coexistence.

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