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RESEARCH ARTICLE

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Direct tracking of pollen with quantum dots reveals surprising uniformity in dispersal distance across 11 populations of an annual plant

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Abstract

Premise: Pollen movement is a crucial component of dispersal in seed plants. Although pollen dispersal is well studied, methodological constraints have made it challenging to directly track pollen flow within multiple populations across landscapes. We labeled pollen with quantum dots, a new technique that overcomes past limitations, to evaluate the spatial scale of pollen dispersal and its relationship with conspecific density within 11 populations of *Clarkia xantiana* subsp. *xantiana*, a bee-pollinated annual plant.

Methods: We used experimental arrays in two years to track pollen movement across distances of 5–35 m within nine populations and across distances of 10–70 m within two additional populations. We tested for distance decay of pollen dispersal, whether conspecific density modulated dispersal distance, and whether dispersal kernels varied among populations across an environmentally complex landscape.

Results: Labeled pollen receipt did not decline with distance over 35 m within eight of nine populations or over 70 m within either of two populations. Pollen receipt increased with conspecific density. Overall, dispersal kernels were consistent across populations.

Conclusions: The surprising uniformity in dispersal distance within different populations was likely influenced by low precipitation and plant density in our study years. This suggests that spatiotemporal variation in the abiotic environment substantially influences the extent of gene flow within and among populations.

KEYWORDS

bee pollination, *Clarkia xantiana* (Onagraceae), gene flow, pollen flow, pollen tracking, pollination, quantum dots

The movement of pollen is a key component of plants' dispersal (Ghazoul, 2005; Auffret et al., 2017). Dispersal plays a critical role in determining the extent of genetic connectivity within and among populations, the spatial scale of local adaptation, and the prevalence of genetic drift (Ronce, 2007; Auffret et al., 2017; Bonte and Dahirel, 2017). Considerable past work has aimed to understand the spatial scale of pollen movement and how it varies among species and across landscapes (Schaal, 1980; Schmitt, 1983; Kunin, 1993; Hardy et al., 2004; van Rossum et al., 2011; Santa-Martinez et al., 2021; Lewis et al., 2023). While this work has provided valuable insights on pollen dispersal, scaling up direct studies of pollen dispersal to examine multiple populations or large numbers of individuals has largely been infeasible. As such, our understanding of how patterns of pollen dispersal within populations vary across broad spatial scales or in relation to environmental variation remains incomplete.

Historically, most studies of pollen dispersal have used one or more of three common approaches: (1) tracking pollinators, (2) tracking pollen analogs, and (3) using molecular tools to identify parentage (e.g., Schmitt, 1983; Campbell, 1991; Adler and Irwin, 2006; Broquet and Petit, 2009). Each of these methodologies has important constraints that limit the accuracy and precision with which pollen dispersal can be estimated and/or the number of individuals and populations that can be studied. Observations of pollinators can be unreliable because they do not

directly track pollen movement and therefore cannot determine how quickly pollen is removed from the pollinator's body and where in the pollinator's foraging path it is deposited (Schaal, 1980; King et al., 2013). Pollen analogs, such as fluorescent dye powders, improve upon pollinator observations because analogs can be tracked by researchers, omitting the need for animal proxies. However, analogs may not move in the same fashion as pollen because the dye is not directly attached to pollen grains. For example, Waser (1988) found that stigmas received 2–3 times more pollen than dye on average, and flowers that received pollen did not always receive dye. Consequently, dye experiments can underestimate maximum dispersal distances (Campbell, 1991; Adler and Irwin, 2006; van Rossum et al., 2011).

As molecular tools have become increasingly feasible to apply in wild plant populations, paternity analyses have been used more often because they unambiguously determine dispersal distances (e.g., Campbell, 1991; Butcher et al., 2011; van Rossum et al., 2011; Matter et al., 2013; Rhodes et al., 2017). Studies of paternity have frequently found that long-distance dispersal is more common than expected, indicating that other approaches tend to miss such events (Campbell, 1991; Massey and Hamrick, 1999; Ashley, 2010; Matter et al., 2013). Although accurate, paternity analyses require the genotyping of most possible pollen donors, limiting their application to plants with small population sizes or to controlled experiments outside of natural populations (reviewed by Ashley, 2010). Advances in molecular techniques have also prompted the development of indirect measures, which use the genetic structure of male gametes from sets of offspring (Smouse et al., 2001; Robledo-Arnuncio et al., 2006; Broquet and Petit, 2009). Although these approaches can be particularly valuable for landscape-level studies, they may not be accurate when inbreeding is common or when genetic structure is not at equilibrium (Smouse et al., 2001; Broquet and Petit, 2009). Because these violations of model assumptions are common in plant populations, indirect approaches are not ideal for many systems.

Despite some limitations, previous work has elucidated important general trends. In animal-pollinated species, dispersal kernels are usually leptokurtic, with pollen receipt declining sharply with distance from the pollen donor. The distances over which this sharp decline occurs typically range from 1 to 50 m in herbaceous plants (Schaal, 1980; Handel, 1983; Waser, 1988; Hardy et al., 2004; van Rossum et al., 2011; Santa-Martinez et al., 2021). The shape of the dispersal kernel can be explained by pollinator movement patterns and how pollen is removed from pollinators' bodies. Pollinators tend to make mostly short trips between flowers, and most pollen is deposited at the first flower visited, with the amount of deposited pollen decreasing as more flowers are visited (Thomson and Plowright, 1980; Morris et al., 1994; Richards et al., 2009; Harder et al., 2021).

The distance that pollen travels is often sensitive to conspecific plant density. Lower density populations tend to have increased pollen dispersal distance at a local scale relative to higher density populations (Levin, 1979; Schmitt, 1983; Karron et al., 1995; Richards, 1997; Côrtes et al., 2013; Castilla et al., 2017; Diaz-Martin et al., 2023). This density dependence arises because short flights between flowers in higher density patches minimize search costs. However, at a landscape scale, the density dependence of pollen dispersal can be reversed, with dispersal distance decreasing at lower densities (Diaz-Martin et al., 2023). In addition to modulating dispersal distance, density impacts pollen receipt directly, with plants in lower density patches generally receiving less visitation and less pollen than plants in higher density microsites (Schmitt, 1983; Kunin, 1993; Richards, 1997; Bosch and Waser, 1999; Ghazoul, 2005; Hendrickson et al., 2018; Santos et al., 2021). However, very high densities can cause pollinators to leave a patch without visiting every flower, reducing mean per-flower pollen receipt (Rathcke, 1983). Therefore, dispersal kernels may depend upon ecological context, but few studies have examined enough populations to evaluate such broad-scale variation.

Recently, a new technique was developed for labeling pollen grains with nanoparticles called quantum dots (hereafter q-dots), which bind to the lipids in the pollen coat (Minnaar and Anderson, 2019). By labeling pollen directly, this method eliminates the need to track proxies, increasing the accuracy and precision of dispersal estimates. Furthermore, q-dots are inexpensive and simple to implement, unlike paternity analyses, allowing for pollen dispersal to be tracked for many individuals and populations.

In this study, we used q-dots to quantify the spatial scale of pollen dispersal in *Clarkia xantiana* subsp. *xantiana*, a primarily outcrossing, bee-pollinated plant. We characterized pollen dispersal kernels within 11 populations spanning ca. 43 km of the subspecies' geographic range. Specifically, we asked:

1. What is the spatial scale of pollen dispersal within populations?
2. To what extent does conspecific density modulate pollen dispersal distance?
3. To what extent do dispersal kernels vary among populations?

MATERIALS AND METHODS

Study species

Clarkia xantiana subsp. *xantiana* A. Gray (Onagraceae, hereafter *C. x. xantiana*) is a winter annual endemic to southern California, United States (Eckhart and Geber, 1999). Bees are the primary pollinators of *C. x. xantiana*, many of which are solitary bees specialized on the genus *Clarkia* (Moeller, 2005; 2006). *Clarkia* specialists forage for both pollen and nectar, with females primarily collecting pollen and males collecting nectar (MacSwain

et al., 1973). Generalist visitors vary in their foraging behaviors on *C. x. xantiana*; for example, honey bees primarily forage for nectar and do not carry substantial pollen loads, whereas bumble bees are often effective pollinators (Moeller, 2005; Eckhart et al., 2006). Although *C. x. xantiana* is self-compatible, protandry and herkogamy strongly promote outcrossing (Moeller, 2006; Moeller et al., 2012). *Clarkia xantiana* subsp. *xantiana* typically occurs in large, discrete populations, with population size and density varying substantially across populations and years in response to fluctuations in precipitation (Eckhart et al., 2011). In both years of this study (2021 and 2022), population densities were low due to an ongoing megadrought affecting the Southwest of the United States (Williams et al., 2020; 2022).

Experimental arrays

In the first study year (2021), we tracked labeled pollen movement in nine populations of *C. x. xantiana*. These populations are distinct from one another, typically separated by gaps of several kilometers; the two closest populations in our study were 0.8 km from each other (Figure 1A). At each population, we constructed one array of flowering stems; each array was in the field for three days. We placed recipient plants at distances ranging from 5–35 m away from the labeled pollen donors (hereafter "mid-range arrays"). Since we found no decline in pollen dispersal with distance (see Results), we constructed larger arrays in two populations in the second year (2022) that doubled the tested distance to 70 m (hereafter "long-range arrays"). We also added arrays that examined pollen dispersal at <5 m ("short-range arrays") in the second year, since this very fine spatial scale was not included in our original mid-range arrays. The short-range arrays did not provide additional insights about pollen movement, and we have presented them in Appendix S1.

All focal plants used in our experiments (pollen donors and recipients) were cut stems placed in falcon tubes filled with water and camouflaged with tan paint to mimic soils in our populations. We used cut stems so that we could precisely manipulate the distances between pollen donors and recipients. We collected focal plants near, but not within, each population to ensure that we did not alter density within the study area. Focal plants were kept indoors overnight to allow anthers to dehisce and stigmas to open without any pollinator visits. We labeled pollen of the donor plants with q-dots (described in the next section), and flowers on recipient plants were unmanipulated.

In all arrays, we tracked pollen movement for four days when the local population was at or slightly past the peak of flowering. To test for local density effects on pollen receipt, we counted the number of naturally occurring *C. x. xantiana* flowers within a 0.5-m radius of each recipient plant on the first day of each array. In both years, we deployed arrays primarily in populations that do not co-occur with other *Clarkia* species. In one population, BG, one congener species is typically present but was rare due to drought. In addition, there were few, if any, other genera flowering at the same time.

On days 2 and 3 after the placement of each experimental array, we replaced all donor plants with new cut stems with freshly labeled pollen. On days 2, 3, and 4, we collected all mature stigmas from the recipient plants and preserved them in microcentrifuge tubes in a −20°C freezer for later pollen counting. Stigmas were considered mature if they had reflexed enough to be flat. On each of days 2–4, we also replaced recipient plants with new cut stems if they had no flowers remaining with potentially receptive stigmas.

Pollen labeling

Before placing plants in the experimental array, we labeled donor plants with $CuInS_3Zn$ quantum dots (Strem Chemicals, Newburyport, MA, USA; Jaiswal and Simon, 2004; Xu et al., 2016; Minnaar and Anderson, 2019; Färkkilä et al., 2021). We pipetted 2 µL of q-dots suspended in hexane (≥95% hexane, 5 mg/mL q-dot concentration) onto dehisced anthers of donor plants to label the pollen (Minnaar and Anderson, 2019). The hexane quickly evaporates, leaving the q-dots bound to the pollen exine. Quantum dots adhere to the majority of individual pollen grains in an anther, do not affect pollinator behavior or patterns of pollen deposition (Minnaar and Anderson, 2019), and are nontoxic (Xu et al., 2016), so they can be used safely in field settings.

Mid-range arrays

We deployed mid-range arrays in nine populations in the first year of study (2021). In each population, we placed 10 donor plants, each with eight labeled anthers, in a 1-m-diameter circle in the center of the array. The density of plants in our donor patches was within the range of natural densities in our focal populations (Appendix S2, Table S2). We placed six recipients at each of 5, 10, 15, 25, and 35 m from the center of the array (Figure 1C). The recipients were staggered to avoid creating straight rows of plants that bees might travel along.

Long-range arrays

We deployed long-range arrays in two populations in the second year of study (2022). In each long-range array, we placed eight donor plants, each with eight labeled anthers, in a 1-m-diameter circle. We arranged 26 recipient plants in a triangular shape at distances of 10, 20, 30, 40, 50, 60, and 70 m from the donor plants, which were at one vertex

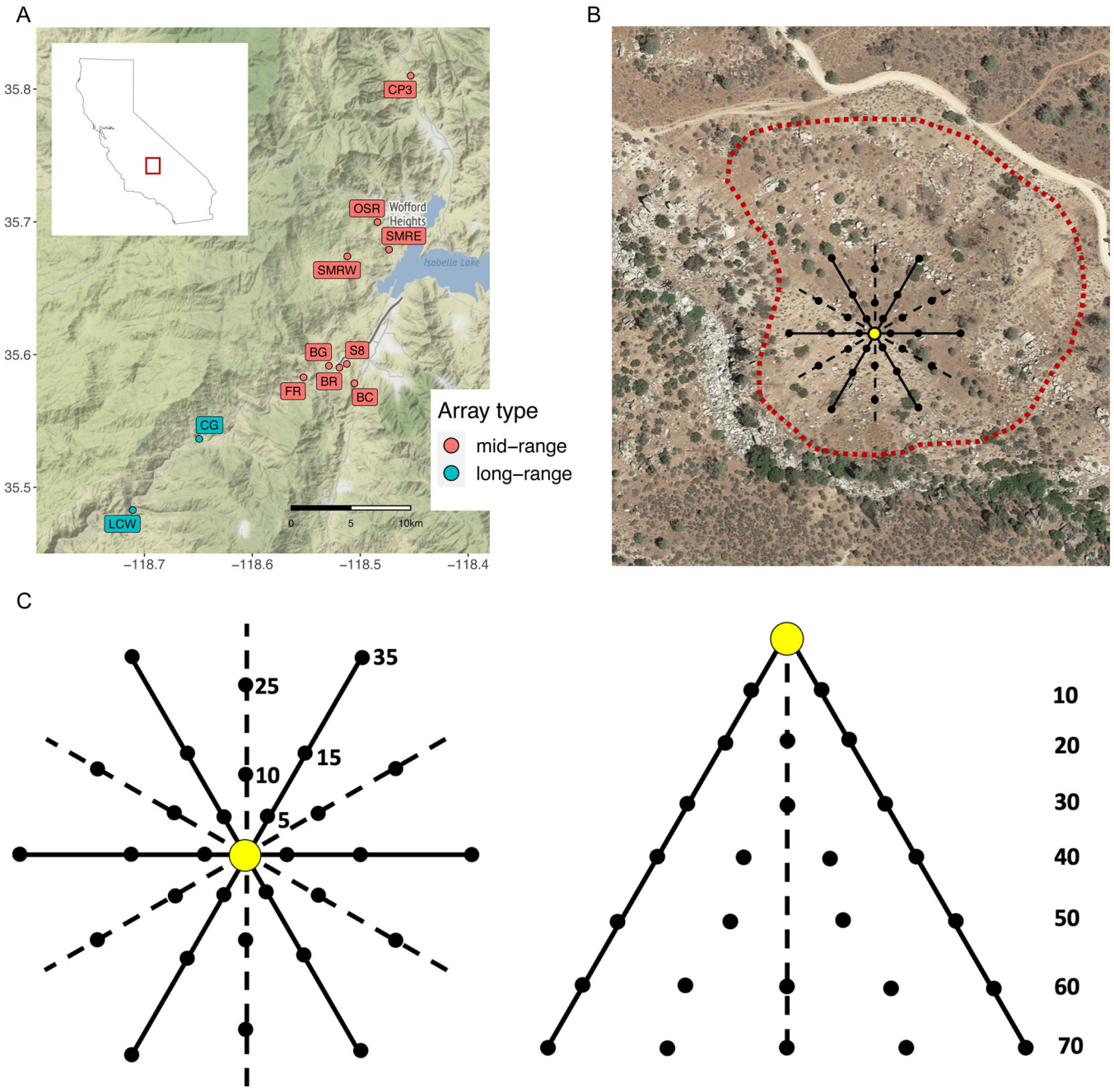

FIGURE 1 (A) Map showing sites where the mid- and long-range experimental arrays were set up in *Clarkia xantiana* subsp. *xantiana* populations in California, United States. (B) The mid-range arrays spanned large portions of the populations. The approximate boundaries of the example population (OSR) are marked by the red line. (C) Layout of the mid-range (left) and long-range (right) experimental arrays. Each array had pollen donors whose pollen grains were labeled with q-dots (yellow circles) and pollen recipients (black circles); all distances in the diagrams are in meters.

of the triangle (Figure 1C). Each recipient distance was replicated two to five times within an array to maintain similar spacing between recipients. The ratio of recipients to donors (3.25:1) was kept similar to that of the original mid-range arrays (3:1). We set up the long-range arrays in a slightly different part of the species' range than the mid-range arrays because in year 2, plants were extremely rare in the region where we had set up mid-range arrays in year 1.

Detecting labeled pollen

We mounted all collected stigmas ($N = 963$ for mid-range arrays, $N = 251$ for long-range) on microscope slides in glycerin jelly (Kearns and Inouye, 1993) and viewed each stigma with 365-nm UV light at 100× magnification using a Zeiss Axioskop 50 microscope. We counted the number of q-dot-labeled pollen grains on each stigma, identified by their fluorescence in UV light.

Statistical analyses

We conducted all analyses using R version 4.2.1 (R Core Team, 2022). Repeated stigma collections at a given location within an array were not independent, but we could not fit a model with recipient location as a random effect due to collinearity with density and distance. Therefore, we calculated the mean number of labeled pollen grains per stigma from the same location in the array (e.g., all stigmas from the 25-m position on one spoke of the array; see Figure 1C for array layouts) and used the mean as the response variable in all analyses. We built a separate linear model for each of the array types using the lm function. We modeled the mean number of labeled pollen grains per stigma (hereafter pollen deposition) at a location as dependent on population, distance from donors, conspecific flower density, the interaction of population and distance, and the interaction of distance and density, all of which were fixed effects. We treated population as a fixed effect because we were interested in testing whether dispersal kernels differed among populations. We were unable to include the interaction of population and density in the model because there was no variation in local density (all zero) in four populations. We $\log_e$-transformed pollen counts and distance under the assumption that pollen dispersal often exhibits exponential decay across distance; therefore, we could test the relationship between dispersal and distance using a linear model. The distribution of density was strongly right-skewed, so we also $\log_e$-transformed this variable.

We checked generalized variance inflation factors (GVIF) to determine whether multicollinearity affected the outcome of our models. In the mid-range array model, correlation between density and population resulted in GVIF > 10, so we removed the population by distance interaction, which resulted in a model with GVIF < 2 for all terms. This adjustment still allowed us to test for a distance by density interaction. We also examined diagnostic plots for the final models, which indicated that the assumptions of normality and homoscedasticity of residuals were met.

We tested the significance of effects using ANOVAs with Type II sums of squares (car package version 3.1-0; Fox and Weisberg, 2019) because we were interested in the effects of both distance and conspecific density separately, as well as their interaction (Hector et al., 2010). However, ANOVAs using Type II or Type III sums of squares produced qualitatively identical results. To test whether the relationship between pollen deposition and distance differed among populations, we fit separate linear models for each population individually, which included distance, density, and the interaction (all variables $\log_e$-transformed).

We also fit a linear model of mean dispersal distance for a population versus mean population density. Due to the small number of data points ($N = 9$ populations) and the bimodal distribution of mean densities (<0.5 or >1.5 flowers per 0.8 m^2; see Appendix S3, Figure S2), this analysis may be unreliable, but we have included the results in Appendix S3.

RESULTS

Mid-range arrays

Across all mid-range arrays, the median number of labeled pollen grains per stigma was 6 (range: 4–17 across populations), and the fraction of stigmas with labeled pollen was 84% (range: 77–97% across populations; $N = 963$ stigmas; see Appendix S4, Figure S3). The mean density of *C. x. xantiana* flowers within 0.5 m of recipients was 0.58 (range: 0–2.3 across populations) but varied substantially among plots within some populations (e.g., BR; mean: 1.82; range: 0–28), while other populations had no recipients with neighbors within 0.5 m (Appendix S2, Table S2).

When modeling all nine populations together, there was no evidence that pollen deposition varied with distance from pollen donors ($\beta = -0.08 \pm 0.07$ log Pollen deposition per log Distance [m]; $F_{1,251} = 2.0$, $P = 0.15$; Figure 2). However, pollen deposition differed among populations ($F_{8,251} = 8.8$, $P < 0.001$). When considering separate linear models for each population, we found a slight decline in deposition with distance in one population, FR ($\beta = -0.46 \pm 0.20$ log Pollen deposition per log Distance [m], $R^2 = 0.16$, $F_{1,28} = 5.3$, $P = 0.03$; all other populations: $P > 0.05$; Appendix S5).

Conspecific density had a strong positive effect on labeled pollen deposition ($\beta = 0.61 \pm 0.35$ log Pollen deposition per log Number of flowers, $F_{1,251} = 3.9$, $P = 0.05$). Density did not significantly modify the effect of distance on pollen deposition ($\beta = -0.17 \pm 0.14$ log Pollen deposition per log Number of flowers × log Distance [m], $F_{1,251} = 1.5$, $P = 0.22$). Although conspecific density varied to some extent within our populations, most recipients were surrounded by few, if any, conspecifics (Appendix S2, Table S2).

Long-range arrays

For the long-range arrays in the two populations, LCW and CG, the median number of labeled pollen grains per stigma was 2 and 18, and the fraction of stigmas with labeled pollen was 64% and 89% respectively ($N = 251$ stigmas; Appendix S4, Figure S3). The mean density of flowers within 0.5 m of recipients was 0.31 and 0.02, respectively.

As in the mid-range arrays, pollen deposition did not decline with distance from pollen donors, even though we doubled the distance over which we quantified pollen dispersal ($\beta = -0.06 \pm 0.15$ log Pollen deposition per log Distance [m]; $F_{1,46} = 0.8$, $P = 0.37$). Mean pollen deposition differed between the two populations ($F_{1,46} = 138.6$, $P < 0.001$), but there was no evidence that dispersal kernels differed between them (population × distance interaction: $F_{1,46} = 1.6$, $P = 0.22$). Unlike in the mid-range arrays, conspecific density did not affect pollen deposition ($\beta = -0.84 \pm 1.24$ log Pollen deposition per log Number of flowers; $F_{1,46} = 1.3$, $P = 0.26$). Dispersal distance was not dependent upon density ($\beta = 0.26 \pm 0.32$ log Pollen deposition per log Number of flowers × log Distance [m];

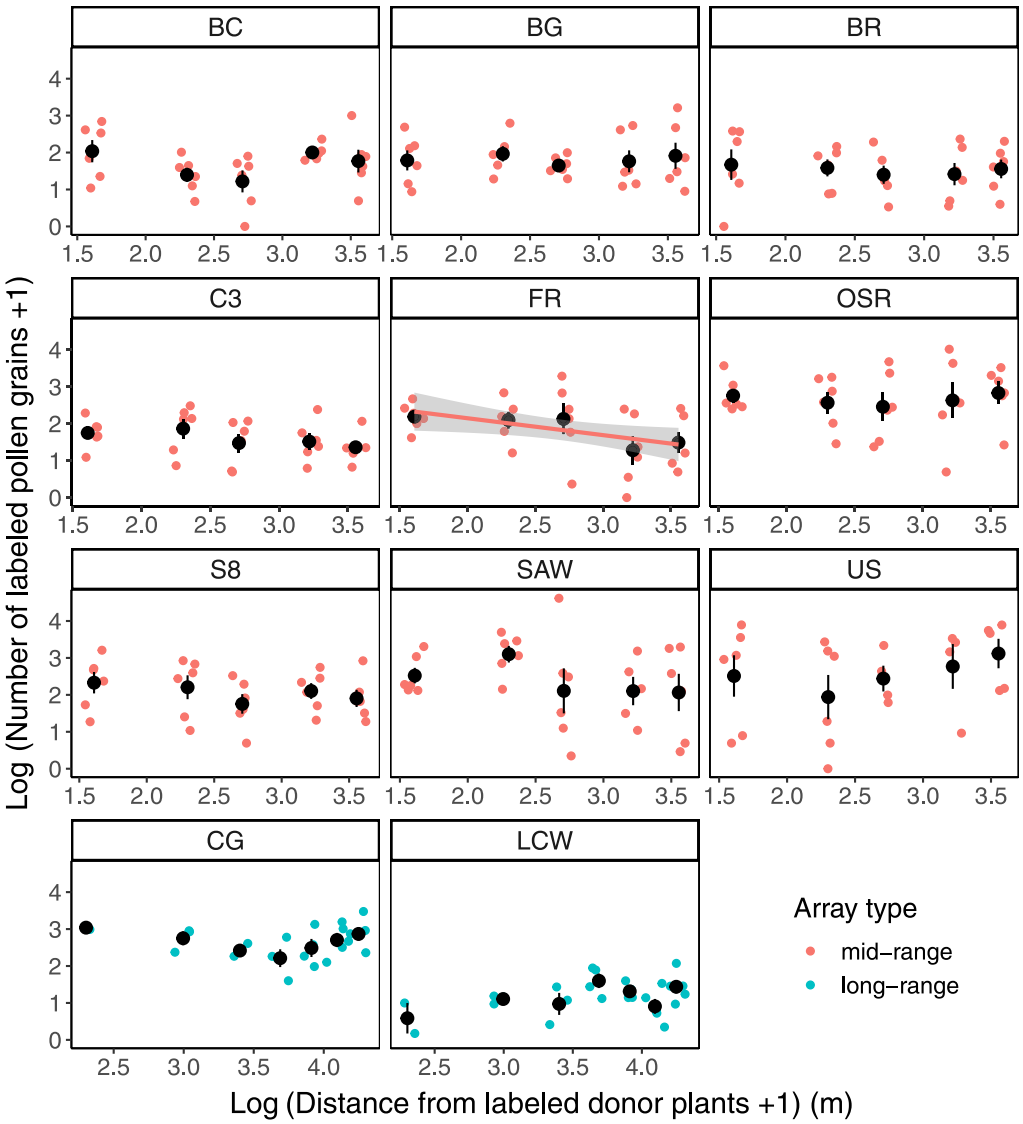

FIGURE 2 The mean count of labeled pollen deposited did not decline with distance from the pollen donor in 10 of the 11 populations. In population FR, pollen deposition declined slightly with distance, as shown by a simple regression (shading represents a 95% confidence interval). The colored points on the plot represent mean pollen deposition at a given location, with black points showing mean and standard error for each distance. Points are jittered, and both distance and pollen receipt were $\log_e$-transformed.

$F_{1,46} = 0.7$, $P = 0.41$). Average local density around recipient plants was low in both long-range arrays (Appendix S2, Table S2); therefore, it is possible that there was inadequate variation in density to detect an effect on pollen deposition.

DISCUSSION

We tracked pollen dispersal within floral arrays set up in 11 populations of *Clarkia xantiana* subsp. *xantiana* using quantum dots to determine the spatial scale of dispersal, how it is affected by conspecific density, and whether dispersal kernels differed among populations. In both the mid-range and long-range arrays, we found that pollen dispersal was largely uniform across distances up to 70 m, which contrasts with most prior studies of herbaceous plants. In the mid-range arrays, pollen receipt increased in higher density contexts. Despite variations in abiotic and biotic conditions across populations, our results suggest that pollen is dispersed over broad-enough spatial scales in all populations to minimize within-population genetic structure.

The lack of observed decline in labeled pollen deposition with distance from donors was unexpected. Previous work on bee-mediated pollen dispersal in herbaceous plants has often described dispersal kernels that decline sharply across distances of as little as 5 m (Schaal, 1980; Handel, 1983; Fenster, 1991; Llaurens et al., 2008; Matter et al., 2013; Butcher et al., 2020; Santa-Martinez et al., 2021) or across somewhat greater distances (e.g., 20–50 m) (Godt and Hamrick, 1993; Hardy et al., 2004; van Rossum et al., 2011; De Lucas et al., 2012; Scheepens et al., 2012).

The unusual dispersal pattern we found could result from differences in pollen tracking methods. One possibility is that our use of q-dots allowed us to capture dispersal events over longer distances better than the pollen analogs used in many past studies (Waser, 1988; Campbell, 1991; Adler and Irwin, 2006). However, paternity analyses, which are also effective at detecting long-distance dispersal, have found sharp declines in pollen dispersal over shorter distances than those in our study (Godt and Hamrick, 1993; Hardy et al., 2004; Llaurens et al., 2008; Butcher et al., 2011, 2020; De Lucas et al., 2012; Matter et al., 2013; Santa-Martinez et al., 2021). Moreover, there is no evidence that pollen labeled with q-dots disperses farther than unlabeled pollen (Minnaar and Anderson, 2019). As such, methodology alone is unlikely to explain the deviation of our results from more commonly observed patterns.

Another possibility is that the relationship between *Clarkia* and its specialist bees results in more uniform patterns of dispersal than is typical in more generalized pollination systems. Specialists are more effective pollinators than most generalists, as they exhibit higher floral constancy and have adaptations to efficiently forage on their host plants (Strickler, 1979; Müller, 1996; Goulson, 1999; Page et al., 2019; but see Parker et al., 2016). Specialist bees only forage on a subset of coflowering species, which is likely to increase the spatial scale over which they search for their host plant compared to generalists. However, the only study that we are aware of that has compared pollen dispersal distance via specialist versus generalist bees did not find any difference, although the specialists were more effective pollinators (Page et al., 2019). The arrangement of pollen on the bees' bodies could also be a factor in dispersal distance. In at least some plants, pollen competes for space on pollinators' bodies, causing the pollen that is collected earlier or later in a foraging bout to be deposited differentially on stigmas (Cocucci et al., 2014; Moir and Anderson, 2023). Grooming behavior could also potentially affect dispersal distance when pollen that has been covered by subsequent collection is uncovered by grooming.

Variation in dispersal kernels among populations could arise due to variation in pollinator community composition. Beyond differences in the foraging behavior of specialists versus generalists, bee species vary considerably in their foraging ranges, which could contribute to variation in dispersal distance (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007). In *C. x. xantiana*, the relative abundance of pollinators varies considerably among populations, and some pollinators are more effective at moving pollen than others (Moeller, 2005). In particular, small bees (e.g., *Lasioglossum* spp.) are far more common in western than eastern populations (Moeller, 2005), which leads to the prediction that dispersal distance increases from west to east. However, we observed virtually no variation in dispersal kernels despite characterizing populations over this gradient in pollinator community composition. We detected a decline in pollen receipt with distance in only one population (FR), although the magnitude of this effect was small. It is unclear why such a decline, albeit modest, would be present in this population but not others.

Previous work has shown that dispersal distance is often greater when flowering plant densities are lower (Levin, 1979; Schmitt, 1983; Richards, 1997; Castilla et al., 2017). In our study, we found no interaction of dispersal distance with density; however, the power to detect such an interaction was limited by the fact that densities were zero or low near most of our recipients in both years. The consistently low densities observed in our study populations were driven by a historic megadrought affecting the Southwest of the United States (Williams et al., 2020, 2022). The first year of our study (2021) was the second driest year in California in the past three centuries (Williams et al., 2022), and the density of *C. x. xantiana* populations is much lower in dry years (see Appendix S2 for a comparison of density in 2006–2017 and in this study). The number of bees that visit *C. x. xantiana* is influenced strongly by precipitation in the prior year, likely because the availability of pollen for nest provisioning was determined by the plant population size during this previous year (Moeller et al., 2012). Spring precipitation of the current year also affects bee abundance (Moeller et al., 2012), possibly because some bee species can remain dormant through a drought rather than emerging (Danforth, 1999). The different dynamics of drought and population size in bees and plants leads to years where there are many bees but few plants, or vice versa. However, the two years of our experiment and the preceding year were all dry (Williams et al., 2022), so it is unlikely that such a discrepancy played a large role in the pollen dispersal patterns we observed.

Another possibility is that the pattern of dispersal we observed has not been typical throughout much of the evolutionary history of *C. x. xantiana* but arises in unusually dry years such as 2021 and 2022. Pollen dispersal might decline with distance in years with more rainfall. In wetter years, rather than pollen receipt increasing consistently with density, the shape of the relationship could be parabolic, with extremely high densities leading to a decline in pollen receipt because pollinators are unable to visit every flower in very dense patches, causing plants to compete for pollinators (Rathcke, 1983; Geber and Moeller, 2006). Finally, we might also expect to see more variation in dispersal among populations in a wetter year when densities are not uniformly low due to extreme drought (Eckhart et al., 2011). Future work comparing patterns of pollen dispersal among populations and years spanning a larger range of plant densities will enable explicit testing of these hypotheses.

Over time, links between drought, density, and pollen movement may have consequences for subpopulation genetic structure. Spatial structure has been documented frequently within plant populations, particularly in herbaceous, insect-pollinated species (Linhart and Grant, 1996; Vekemans and Hardy, 2004; Gamba and Muchhala, 2022; but see Lewis et al., 2023). Because of density dependence to pollen dispersal, several consecutive years with drought may

diminish subpopulation structure, while sequences of wetter years with higher densities may promote it. Previous work has found that in high density populations, genetic structure is higher and genetic diversity is lower at the neighborhood level (Diaz-Martin et al., 2023). Under climate change, southern California is predicted to experience an increased frequency of extremely dry years (Diffenbaugh et al., 2015; Swain et al., 2018), which could lead to longer distances of dispersal on average. This alteration to spatial structure could limit the capacity for microscale adaptation across environmental gradients within populations.

Population genetic studies in *C. x. xantiana* have shown that there is genetic connectivity among populations across the landscape despite significant population structure (Moeller et al., 2011; Pettengill et al., 2016). Gene flow between populations may be elevated in drier years if the patterns of pollen dispersal that we found within populations can be extrapolated to greater spatial scales. However, the dynamics of between-population pollinator movement can be quite different than within-population movement (Gamba and Muchhala, 2022; Diaz-Martin et al., 2023). This difference may be especially pronounced for *Clarkia* specialist bees, which nest in *Clarkia* populations and do not appear to forage long distances across terrain that lacks *Clarkia* (Stage, 1966; MacSwain et al., 1973). Generalists such as bumble bees are also important pollinators (Moeller, 2005) and might move between populations more frequently because they are larger bodied and typically forage across greater distances (Greenleaf et al., 2007). Further work is needed to assess the frequency of between-population dispersal and whether it follows density-mediated patterns similar to those we observed within populations.

CONCLUSIONS

Pollen dispersal in *Clarkia xantiana* subsp. *xantiana* varied minimally with distance in each of 11 populations. Low plant densities in our two study years may have contributed to the high frequency of long-distance dispersal; however, more data are needed from years in which there is greater variation in density within and among populations. Data like these that directly quantify variation in dispersal kernels among populations and across environmental gradients are essential for understanding mechanistic links between climate change and dispersal and their consequences for the spatial scale of adaptation and geographic range shifts.

AUTHOR CONTRIBUTIONS

All authors contributed to the study design, fieldwork, plan for analyses, and editing of the manuscript. B.R.K. processed samples to identify labeled pollen, analyzed the data, and drafted the initial version of the manuscript.

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DATA AVAILABILITY STATEMENT

All data described in this study are archived with the Data Repository for the University of Minnesota and can be found at https://hdl.handle.net/11299/254562.

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SUPPORTING INFORMATION

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

APPENDIX S1. Full methods and results for short-range (0.5–5 m) experimental arrays.

APPENDIX S2. Comparison of *C. x. xantiana* population densities in 2006–2017 and in the experimental populations in 2021–2022.

APPENDIX S3. Model of mean pollen-dispersal distance vs. mean population density.

APPENDIX S4. Histograms of number of labeled pollen grains pollen deposited on stigmas for each array type.

APPENDIX S5. Full results of individual linear models for the mid-range arrays at each population.

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