Ecology, 93(5), 2012, pp. 1036–1048

Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant

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Abstract. Mutualisms are well known to influence individual fitness and the population dynamics of partner species, but little is known about whether they influence species distributions and the location of geographic range limits. Here, we examine the contribution of plant–pollinator interactions to the geographic range limit of the California endemic plant *Clarkia xantiana* ssp. *xantiana*. We show that pollinator availability declined from the center to the margin of the geographic range consistently across four years of study. This decline in pollinator availability was caused to a greater extent by variation in the abundance of generalist rather than specialist bee pollinators. Climate data suggest that patterns of precipitation in the current and previous year drove variation in bee abundance because of its effects on cues for bee emergence in the current year and the abundance of floral resources in the previous year. Experimental floral manipulations showed that marginal populations had greater outcross pollen limitation of reproduction, in parallel with the decline in pollinator abundance. Although plants are self-compatible, we found no evidence that autonomous selfing contributes to reproduction, and thus no evidence that it alleviates outcross pollen limitation in marginal populations. Furthermore, we found no association between the distance to the range edge and selfing rate, as estimated from sequence and microsatellite variation, indicating that the mating system has not evolved in response to the pollination environment at the range periphery. Overall, our results suggest that dependence on pollinators for reproduction may be an important constraint limiting range expansion in this system.

Key words: adaptation; California endemic plant; Clarkia xantiana *ssp.* xantiana*; climate; evolutionary constraint; mating system; mutualsim; pollen limitation; pollination; reproductive assurance; self-fertilization; species border and distribution.*

Introduction

Determining the causes of geographic range limits requires an understanding of how both abiotic and biotic factors limit population growth and expansion in marginal environments. Empirical studies of range limits using experiments (e.g., Geber and Eckhart 2005, Griffith and Watson 2006, Angert et al. 2008, Kellermann et al. 2009) and species distribution models (in the context of climate change [Thomas et al. 2004, Lenoir et al. 2008, Jiguet et al. 2010]) have mainly evaluated putative limiting abiotic factors (but see Samis and Eckert 2009). Theory suggests that species interactions, particularly antagonistic interactions such as predation, parasitism, and competition, may have similarly strong effects on distributional limits (Case and Taper 2000, Case et al. 2005, Holt and Barfield 2009). Surprisingly little is understood about the role of mutualists in limiting range expansion (Simberloff and Von Holle 1999; but see Chalcoff et al. 2011), despite abundant evidence that mutualists have important consequences for the fitness and population dynamics of their partners (Boucher et al. 1982, Morris et al. 2007).

Studies of invasions have been particularly influential in suggesting that mutualisms might influence species distributions. In plants, growth in newly occupied habitats has been shown to be limited by a lack of soil-dwelling symbionts, including rhizobia and mycorrhizal fungi (Parker et al. 2006, Nuñez et al. 2009). Outside of a native plant species' range, there is also evidence that the supply of rhizobial symbionts can be limited (Stanton-Geddes and Anderson 2011). These results suggest that mutualists that mediate fundamental processes such as nutrient uptake might influence the likelihood of population establishment and the rate of range expansion (Parker 2001) unless reduced dependence of plants on symbionts evolves during range expansion (Seifert et al. 2009).

Less is understood about the frequency or predictability of more diffuse mutualistic interactions, such as those involving plant dispersal (pollen and seeds) and plant protection (e.g., ant–plant associations) at and

Manuscript received 11 August 2011; revised 6 December 2011; accepted 8 December 2011. Corresponding Editor: N. Underwood.

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beyond the boundaries of geographic distributions (Richardson et al. 2000). Plant–pollinator interactions are among the best-studied mutualisms and have been the focus of increasing attention in the study of biological invasions. Recent work has suggested that invasion rate may be influenced by pollen limitation of reproduction (e.g., Parker 1997, Parker and Haubensak 2002), that reproduction of invasive plants is facilitated by the presence of nonnative pollinators (Barthell et al. 2001, Stout et al. 2002, Gross et al. 2010), and that plant invasions have failed to occur until nonnative pollinators were introduced (e.g., McKey and Kaufmann 1991, Nadel et al. 1992). Although some plants have highly generalized pollination systems and can rely on native pollinators in their introduced range, it is clear that reproduction in some systems may be limited by a lack of effective pollinators in novel environments.

For native distributions, the unreliability of pollinators at geographic range limits may be an important factor driving transitions in floral form. In particular, transitions between dominant pollinators (shifts in pollination syndrome [Grant and Grant 1965]) and shifts from outcrossing to self-fertilization frequently occur in marginal populations (Lewis and Lewis 1955, Lloyd 1965, Rick et al. 1977, Lloyd 1980, Guerrant 1989, Busch 2005). These patterns imply that plant access to effective pollinators may be limited as populations colonize unoccupied territory, and that transitions in reproductive mode facilitate range expansion. The transition to selfing has been considered a form of "evolutionary rescue," whereby adaptation prevents extinction due to reproductive failure (Lynch and Lande 1993, Gomulkiewicz and Holt 1995, Bodbyl Roels and Kelly 2011). Although a number of studies have documented shifts in floral form at range margins, there have been few substantial efforts to examine the frequency of interactions between plants and pollinators from the center to the margin of species' native ranges, and the consequences for reproduction in marginal populations.

This study examines the contribution of plant–pollinator interactions to the eastern geographic range limit of *Clarkia xantiana* ssp. *xantiana*, which has been the subject of ongoing research on the ecological and evolutionary causes of native geographic range limits. Multiyear studies of demography have shown that population growth rates decline from the range center to edge (Eckhart et al. 2011). The decline in growth rates is likely affected by both abiotic factors (such as climate and topography) and biotic factors (such as natural enemies and mutualists). In this study, we examined plant–pollinator interactions and plant reproductive ecology in populations arrayed along a geographic transect from the center to the eastern edge of *C. xantiana* ssp. *xantiana*'s distribution.

We combined observations of pollinator visitation, climate monitoring, experimental floral manipulations, and population genetic variation to test hypotheses about contributions of plant–pollinator interactions to the geographic range limit of *Clarkia xantiana* ssp. *xantiana*. First, we tested the prediction that plant–pollinator interactions are less frequent (i.e., that pollinator abundances and visitation rates are lower) in marginal populations than in central populations, through observations and collections of bee pollinators. Our results indicate substantial temporal and spatial variation, with fewer pollinators and lower visitation rates in marginal populations. To evaluate possible causes of this variation, we tested whether precipitation in the current and previous year was associated with bee abundance, because precipitation is a primary driver of plant population dynamics in this system (Eckhart et al. 2011) and of bee emergence dynamics in arid regions (Danforth 1999). Second, we used experimental floral manipulations to test the hypothesis that reduced pollinator availability results in greater pollen limitation of outcross reproduction in marginal populations, and that autonomous selfing provides reproductive assurance where plants are most outcross pollen limited. Third, we used DNA sequence and microsatellite data to estimate selfing rates, to test the hypothesis that a shift in mating system has occurred as a response to reduced pollinator availability in marginal populations.

Study system

Clarkia xantiana is composed of two parapatric subspecies, *Clarkia xantiana* ssp. *xantiana* and ssp. *parviflora*; both are winter annuals occurring mainly on steep, open slopes (Eckhart and Geber 1999). The former is primarily outcrossing and the latter is primarily selfing. The two subspecies are distinct in phenotype and infrequently hybridize where they co-occur in sympatry, and phylogenetic studies indicate that they exhibit a high degree of genealogical exclusivity (Pettengill and Moeller, *in press*). This study examines the eastern (parapatric) range margin of the outcrosser, subspecies *xantiana,* which we have located through extensive geographic surveys over the last 15 years (Fig. 1). Populations in this study occur along a geographic transect from the core of the geographic range to the most peripheral populations.

Flowers have two whorls of four anthers, which gradually release pollen over the course of several days (see Plate 1). During this period, the style grows to a variable distance beyond the dehiscing anthers, and the stigma generally does not become receptive until anthers have fully dehisced (Runions and Geber 2000). All populations of *C. xantiana* ssp. *xantiana* are self-compatible, but autonomous self-fertilization is strongly minimized by protandry and herkogamy (Eckhart and Geber 1999). As a result, plants set few fruits and seeds in pollinator-free environments (Moeller 2006). Bees that collect both pollen and nectar from flowers effect outcross pollination (see Plate 1). Although our taxon-wide surveys of pollinator communities have discovered >60 bee species visiting *C. xantiana* ssp. *xantiana* flowers, a

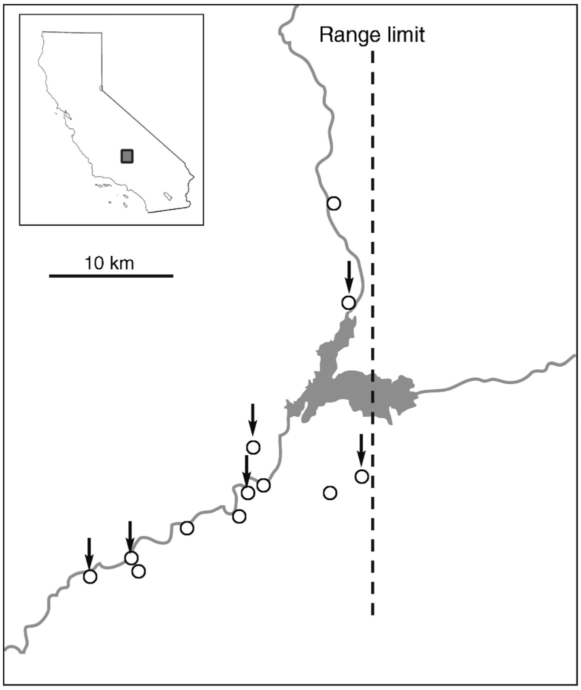

FIG. 1. Map showing the location of the 12 populations of the California endemic plant *Clarkia xantiana* ssp. *xantiana* in which floral manipulations, bee collections, and observations were conducted in California, USA. The dashed line shows the approximate location of the eastern range limit. The arrows point to populations for which selfing rates were estimated.

subset of specialist solitary bees (about five species) and several large-bodied generalist bees are the most effective at pollen transfer (Moeller 2005, Eckhart et al. 2006).

We chose populations for this study that differ in geographic position but not in population size (regression of population size on distance to range edge, $F_{1,17} = 0.0$, $P = 0.917$); or population density (regression of population density on distance to range edge, $F_{1,17} = 0.0$, $P = 0.835$). Previous work has shown that precipitation is a dominant abiotic factor affecting fitness (Eckhart et al. 2010, Mazer et al. 2010) and population dynamics (Eckhart et al. 2011). Precipitation is lower and more variable at the eastern range limit compared to the range center (Eckhart et al. 2011). Eastern range limit populations occur at somewhat higher elevation, on average, than range center populations (Appendix A). Precipitation begins in late fall to early winter, during which time plants germinate and slowly grow. Plant growth accelerates during the spring and flowering occurs between mid-May and late June. Variation in precipitation may also affect the population dynamics of *C. xantiana*'s solitary bee pollinators through its effects on plant resources and because precipitation can be a cue for emergence (Danforth 1999). Floral morphology differs somewhat between range center and edge populations, primarily due to lower herkogamy in range edge populations (Moeller 2006).

METHODS

Spatial and temporal variation in plant–pollinator interactions

We examined pollinator visitation rates to flowers along a geographic transect from the center to the periphery of *C. xantiana* ssp. *xantiana*'s range over four years (Fig. 1; Appendix A). In our analyses, we used longitude as an index for distance to the range limit. In each population, one researcher (Moeller) made standardized collections of bee pollinators along transects of flowering plants, and two observers recorded visitation rates by bee pollinators to plots of flowering plants in 10, 5, and 10 populations in 2006, 2007, and 2008, respectively. Collections and observations occurred only on sunny days and when winds were calm. In 2006, each site was visited twice (approximately one week apart) near the peak of flowering. In 2007 and 2008, each site was visited once near the peak of flowering. Standardized collections of bees were also made in 2001 along the same geographic transect and using the same protocol (Moeller 2006); data from sites that overlapped with those studied in 2006–2008 (six populations) were included in the analyses presented here. In total we analyzed pollinator collection rates from 31 population × year combinations from four years of study.

One researcher made standardized net collections of bees at each site during eight observation periods from 0830 to 1530 hours. Collections occurred along four 35 × 2 m belt transects that spanned most of each study population; these four transects were sampled from 08:30 to 11:30 during 30-minute observation periods, and this procedure was repeated from 12:30 to 15:30. This protocol ensured that sampling includes potential spatial and diurnal variation in bee pollinators within study populations. During each sampling period, the researcher walked a given transect repeatedly, collecting as many visitors as possible that contacted flowers, and afterwards, estimated the number of flowers within 1 m on either side of the transect (see Moeller 2006 for more detailed methods). These data were used to calculate bee collection rates (bees collected per flower per observation period). In total, collections took place in 296 observation periods covering 106 hours over four years. Bees from net collections were pinned, identified to species, and categorized as specialists vs. generalists according to MacSwain et al. (1973). We deposited voucher specimens in the University of Minnesota Insect Collection.

Bee visitation rates were also estimated by watching patches of plants for 10-minute intervals from ~08:30 to 15:30 on each observation day. Eight to 10 plots were observed by each of two researchers between 08:30 and 11:30, and those same plots were watched again between 12:30 and 15:30. Plots varied in size depending on the density of plants; when plants were less dense we used larger plots in order to observe as many plants as possible. For each plot, the number of open flowers was recorded. During each 10-minute period, researchers recorded the number of visits to flowers in the observed plot. In total, we observed visitation rates during 726 10-minute observation periods over three years. We could not reliably determine bee species identity for plot observations. In total we analyzed pollinator observation rates from 25 population × year combinations from three years of study. For our analyses, we have treated observations of plant populations in different years as independent assays of the pollination environment across regions of the species' range. Although a repeated-measures analysis would be ideal, we were not able to sample all populations in all years. If population effects are not independent across years, this could inflate the degrees of freedom in the analysis; therefore we have interpreted our results conservatively.

Climate variation and plant–pollinator interactions

As part of a broader study, we used a network of automated weather stations to document precipitation from October 2005 through June 2009, the interval of time during which plant–pollinator interactions were studied. Nine of the 21 weather stations in the network were in the same sites where plant–pollinator interactions were studied. For sites that lacked weather stations, spatial interpolation was used to estimate precipitation based on information from weather stations nearby (kriging in ArcGIS 9.3 Spatial Analyst, ESRI, Redlands, California, USA, with default parameters and with grid cells of 1 ha). Our analyses examined the association of bee collection rates with precipitation in the year of bee collection and the previous year. Most bee visitors encountered in this study are univoltine, where adults produce clutches of offspring that emerge in the following year. Therefore, precipitation in the year prior to bee collection could influence the abundance of flower resources for bees and affect bee reproductive success.

We also parsed precipitation into two phases: winter rains, which occur from November through January, and spring rains, which occur from February through June. Winter rains primarily affect seed germination and seedling establishment. Spring rains fall during the period when most plant growth occurs and preceding the period when bees emerge. For bee collections from 2006, we lacked information on precipitation in the previous year, whereas such information was available for collections in 2007 and 2008. No information on weather was available for collections from 2001.

Pollen limitation and reproductive assurance

In each of the three years, we conducted floral manipulations at most sites studied for plant–pollinator interactions. We conducted our studies at seven sites in 2006, five sites in 2007, and nine sites in 2008 ($n = 21$ population × year combinations). At each site, we haphazardly selected ∼75–100 sets of four nearby focal plants matched for size. One flower on each plant received a treatment (approximately the middle flower of the inflorescence). The four treatments were: (1) unmanipulated; (2) supplemental outcross treatment in which stigmas were supplied with supplemental outcross pollen from an individual several meters away; (3) supplemental selfing treatment in which stigmas were supplied with supplemental self pollen from a different flower on the same plant; and (4) emasculation treatment in which all anthers were removed prior to stigma receptivity. (Emasculation does not otherwise damage flowers or inhibit seed set [Moeller 2006].) Supplemental pollination was accomplished by brushing freshly dehisced pollen onto receptive stigmas. Pilot studies have indicated that reallocation of resources is not an important contributor to variation in pollen limitation, and that manipulation of one flower per plant does not appear to bias estimates of pollen limitation. Progressive supplemental pollination of flowers does not reduce subsequent flower production (M. A. Geber and V. M. Eckhart, *unpublished data*), and plants where few flowers receive supplemental pollen do not produce more seeds per fruit than those where many flowers per plant receive supplemental pollen (D. A. Moeller, *unpublished data*).

We recovered 3126 fruits from our floral manipulations, which produced 96 519 seeds (mean = 30.8 seeds per fruit). Based on the seed set from flowers in the four treatments we calculated three statistics: outcross pollen limitation of reproduction (OPL), self-pollen limitation (SPL), and reproductive assurance (RA) (Eckert et al. 2010). Pollen limitation was calculated as PL = (supplemented − unmanipulated)/supplemented. OPL measures the extent to which supplemental outcross pollen elevates seed production, mitigating limited pollinator or mate availability. Similarly, SPL measures the extent to which supplemental self-pollen elevates seed production over unmanipulated flowers; this treatment can determine whether competing or delayed selfing can reduce pollen limitation of reproduction. Reproductive assurance was calculated as RA = (unmanipulated − emasculated)/ unmanipulated. RA measures the extent to which autonomous selfing contributes to seed production by comparing flowers where autonomous selfing is possible (unmanipulated) to those where pollinator-mediated outcrossing or geitonogamy is required for fertilization. We excluded fruits damaged by insect herbivores from our analyses. In the most extreme cases, more than half of fruits in a population were damaged, but the probability of receiving damage was independent of pollination treatment ($P = 0.753$). We calculated OPL, SPL, and RA based on means for each treatment (for each population × year combination) because pairs of plants receiving different treatments were often incomplete due to fruit herbivory. As with pollinator observations, we have considered population × year combinations to be

TABLE 1. Estimates of selfing rates of *Clarkia xantiana* in its range in California, USA, from INSTRUCT based on sequence and microsatellite data (see *Methods: Mating system variation*).

Population	Longitude easting (km)†	Selfing rate ($K = 1$)	SE	Selfing rate (variable K)	SE
Cow Flat	350.0	0.328	0.019	0.444	0.034
Delonegha	350.5	0.351	0.019	0.465	0.039
Borel Road	362.5	0.273	0.017	0.376	0.017
Keyesville	362.9	0.379	0.019	0.377	0.019
Golf Course	370.6	0.471	0.026	0.455	0.036
Squirrel Mountain	371.7	0.275	0.019	0.378	0.011

Notes: Populations are ordered from most central to marginal in the species' range. K is the number of demes (see *Methods*). Additional details on population locations can be found in Appendix A.

† Easting (km) is the distance east of the reference longitude for UTM zone 11, using NAD 1927.

independent assessments of plant–pollinator interactions across the geographic range over three years. If population effects are not independent across years, the degrees of freedom for this analysis could be somewhat inflated.

Statistical analyses

We examined the effect of the distance to the range limit (which was estimated using longitude), year, and their interaction on each metric of bee pollinator availability using ANOVA. Bee collection rates were square-root transformed prior to analysis to improve the homoscedasticity of residuals. We dropped interaction terms from statistical models when $P > 0.2$. We examined the relationship between bee collection rates and precipitation using multiple regression with current-year and previous-year precipitation as independent variables. Separate analyses were conducted for all bees, specialists, and generalists. Because both current and previous-year precipitation significantly influenced bee collection rates, we then used multiple regression to examine the contribution of winter vs. spring precipitation (in the current and previous year separately) to the associations observed with cumulative precipitation.

We used one-tailed t tests to test for differences in seed set between specific combinations of floral treatments for which we made a priori predictions: supplemental outcross > unmanipulated; supplemental self > unmanipulated; supplemental outcross > supplemental self; and unmanipulated > emasculated. We conducted tests across all populations pooled and for each specific population × year combination. Using data from across all populations and years, we examined the effect of distance to range limit and year, and their interaction, on levels of OPL, SPL, and RA. Finally, we tested for an association between bee collection rates and levels of OPL, SPL, and RA using ANCOVA, with bee collection rate and year as independent variables. All analyses were performed in JMP, version 9.0.0. (SAS Institute 2010).

Mating system variation

We estimated rates of self-fertilization based on data from six of the populations where we examined plant–pollinator interactions and conducted floral manipulations. Two populations were central to the range, two were intermediate in position, and two occurred within 1 km of the range limit (Fig. 1, Table 1). We collected sequence data from nine nuclear loci (1867 sequences, 621 haplotypes) and four microsatellite loci (55 alleles) from between 15 and 25 individuals per population, which were collected in 2005. These data were previously used to examine demographic history and population structure in *C. xantiana* ssp. *xantiana*, and a detailed description of data collection can be found in Moeller et al. (2011). Here, we conducted new analyses to estimate selfing rates using INSTRUCT (Gao et al. 2007). INSTRUCT simultaneously infers population structure (the number of demes $= K$) and selfing rates, both of which can cause deviations from Hardy-Weinberg equilibrium. In this analysis, estimates of selfing rates are affected by multiple generations of selfing and outcrossing, rather than a single generation as in progeny array analyses (Ritland 2002). Indirect estimates of selfing rates from INSTRUCT analyses can be affected by biparental inbreeding in addition to autonomous and geitonogamous selfing. These analyses also assume that molecular variation is neutral, which is a reasonable assumption for these data (Moeller et al. 2011). We allowed K to vary from 1 to 10 and INSTRUCT selected the best model according to the Deviance Information Criterion (DIC). Separate selfing rates were estimated for each deme (K) inferred from the data set. To obtain selfing rates for each field population, we used the weighted average of selfing rates across demes present in a field population. We also examined selfing rates for each field population separately with K fixed at 1, an analysis that assumes no subdivision within populations.

RESULTS

Plant–pollinator interactions

We collected 1846 bees in four years (2001, 2006–2008) and observed 12 171 bee visits to 49 902 flowers in three years (2006–2008). We found a significant positive correlation between the two measures of pollinator availability: collection rate of all bees and the visitation rate to flowers observed in plots (pairwise $r = 0.60$, $P < 0.01$). Analyses of geographic patterns were conducted with ANCOVAs, including distance to the range edge and year as independent variables. The rate of bee collection (bees per flower per 30-minute observation

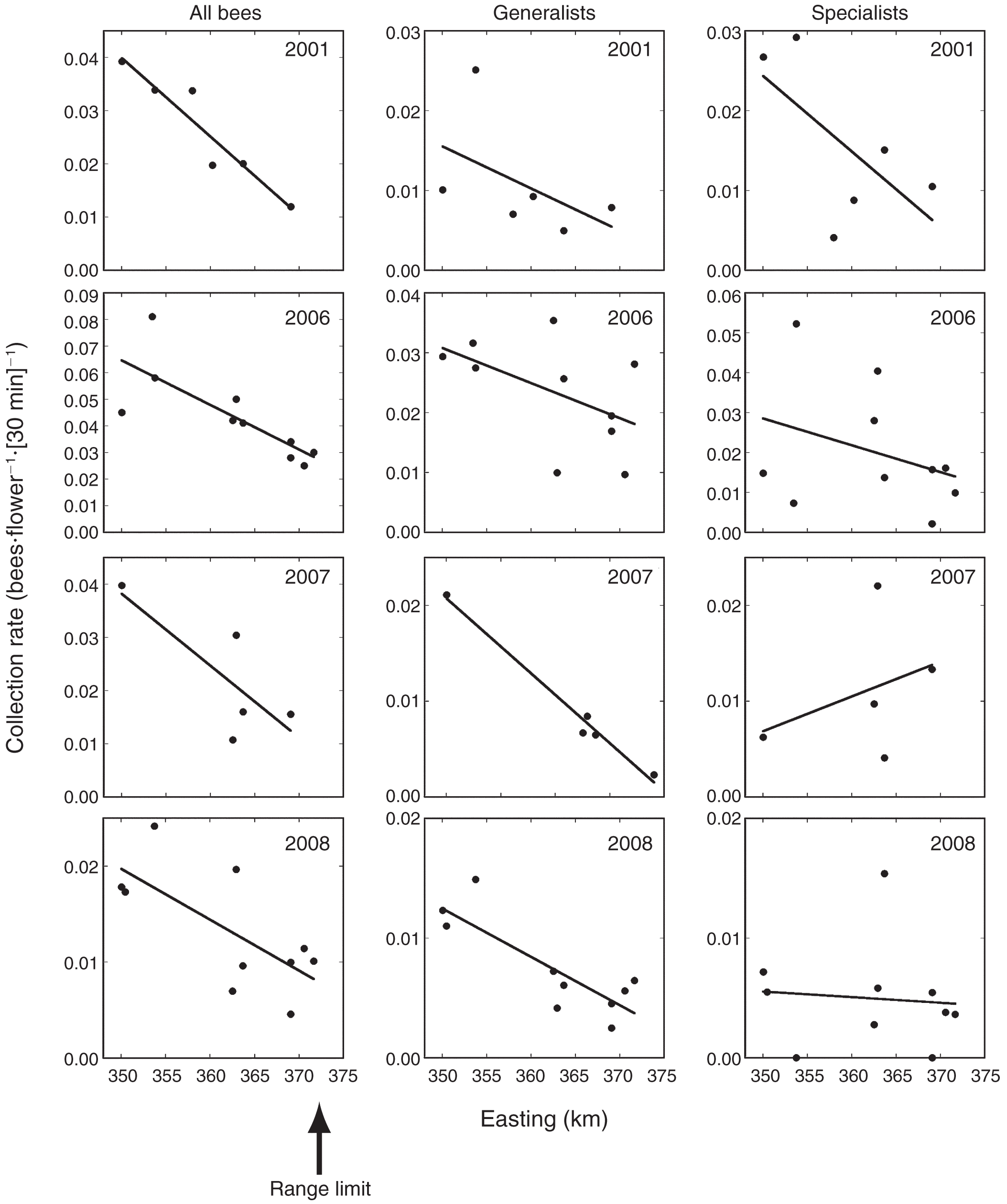

FIG. 2. The relationship, in four sample years, between bee pollinator availability and longitude (UTM easting), which describes proximity to the range limit of *Clarkia xantiana*, for the collection rate of all bees, generalists, and specialists. Data points represent population means. For a definition of easting, see Table 1.

period) declined significantly from range center to edge ($F_{1,26} = 38.6$, $P < 0.0001$; Fig. 2). Bee collection rates were over twice as high, on average, in central compared to edge populations. Generalist visitors were more consistently of lower abundance near the range limit ($F_{1,26} = 15.7$, $P < 0.001$; Fig. 2). Specialists exhibited a somewhat weaker and marginally significant decline in frequency approaching the range limit, which was largely driven by data from 2007 ($F_{1,26} = 3.5$, $P = 0.071$; Fig. 2). We did not find a significant relationship between bee visitation rates (from observing plots of flowering plants) and distance to range limit, although the slopes were similarly negative ($F_{1,21} = 1.1$, $P = 0.314$).

All three metrics of pollinator availability differed strongly among years, with bee collection rates and observation rates varying approximately twofold among years (all bee collection rates, $F_{3,21} = 30.4$, $P < 0.0001$; generalists, $F_{3,21} = 16.7$, $P < 0.0001$; specialists, $F_{3,26} = 5.1$, $P < 0.01$; observation rates, $F_{2,21} = 8.6$, $P < 0.01$). Because of strong year-to-year variability in pollinator abundance, we asked whether the coefficient of variation in bee availability to flowers was greater in peripheral

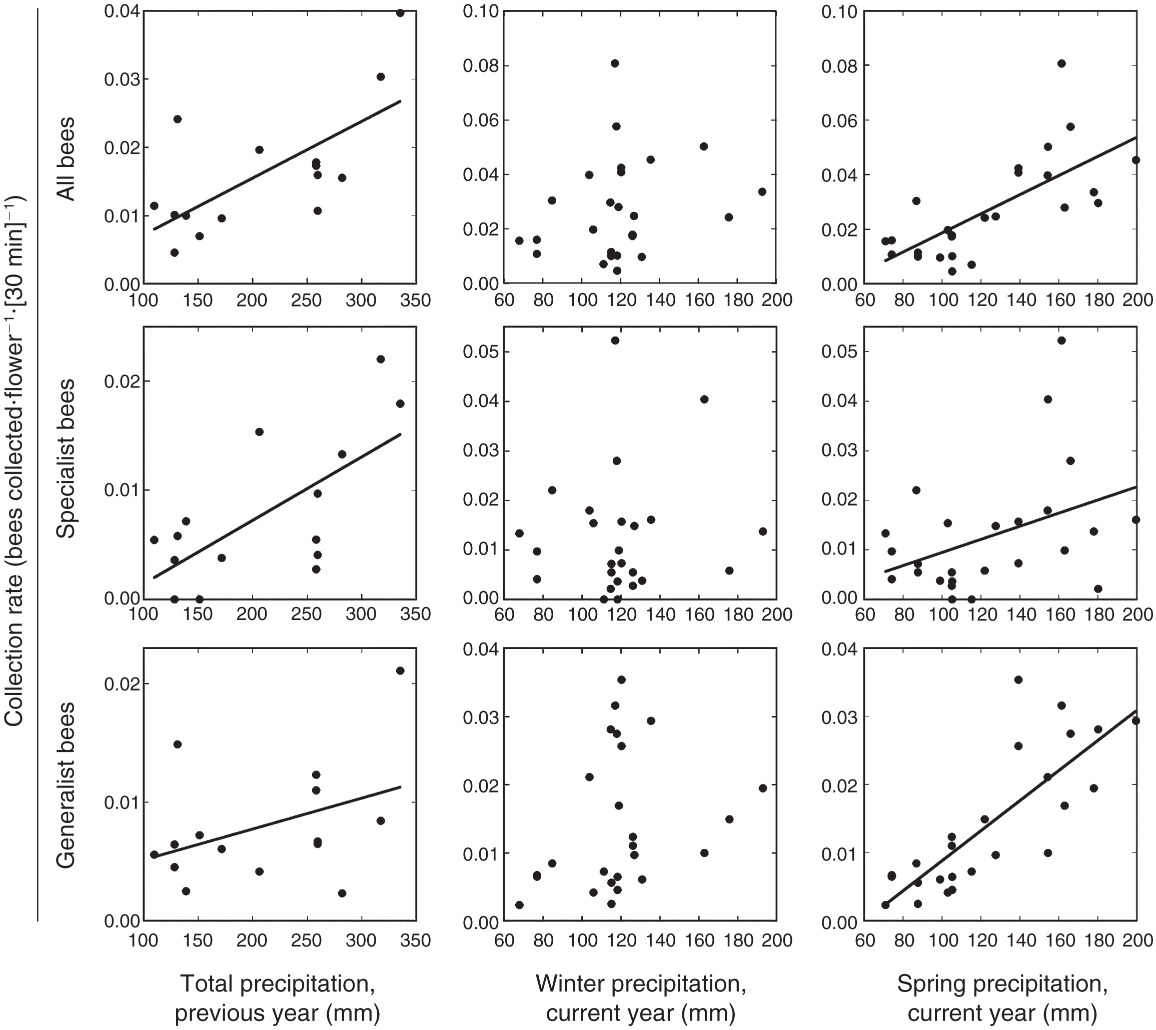

FIG. 3. The association of bee collection rate with variation in precipitation (mm) in the previous and current year. Total precipitation in the previous year includes only precipitation during the growing season (November–June). Precipitation in the current year is parsed into winter (November–January) and spring precipitation (February–June) because these phases of time had differing effects on bee availability.

compared to central populations, but found no evidence for it from any of the metrics of pollinator availability ($P > 0.21$).

Climatic control of plant–pollinator interactions

Total bee collection rate was significantly and positively associated with precipitation in both the current and previous year, with the previous year being the stronger predictor of total bee abundance (Fig. 3; Appendix B). The positive association was more pronounced when generalist visitors were examined separately, but for generalists, precipitation in the current year was a stronger predictor of abundance. For *Clarkia* specialist bees, by contrast, collection rates were affected positively only by precipitation in the previous year (Fig. 3; Appendix B).

When total precipitation for the current year was parsed into winter vs. spring precipitation, our data suggest that only spring precipitation was positively associated with bee collection rates. This positive association of bee abundance with spring precipitation was detected in all data sets: all bees, generalists, and specialists (Fig. 3; Appendix B). When total precipitation for the previous year was parsed into winter vs. spring precipitation, we found no significant association of precipitation with bee collection rates; therefore, only cumulative precipitation in the previous year was a predictor of bee abundance in the current year (Fig. 3; Appendix B).

Pollen limitation and reproductive assurance

Across all sites combined, we found significantly greater seed set in the supplemental outcross pollen treatment compared to the open pollination treatment, indicating outcross pollen limitation (OPL: 7.1% overall; Appendix C). OPL significantly increased toward the geographic range limit (ANCOVA; distance to range limit, $F_{1,17} = 4.5$, $P = 0.049$; year, $F_{2,17} = 0.4$, $P = 0.651$; Fig. 4) and was significantly negatively associated with the availability of pollinators, as one might predict given that bee availability declined and OPL increased toward the range limit (ANCOVA, bee collection rate, $F_{1,17} = 4.3$, $P = 0.054$; year, $F_{2,17} = 3.84$, $P = 0.042$; Appendix D). At the range periphery, the mean OPL across population × year combinations was 9.7%, whereas the mean for intermediate and central populations was 5.9% and 1.8%, respectively.

No tests for differences between the SO (supplemental outcross) and U (unmanipulated) treatment were significant for individual populations (though five comparisons were marginally significant [$0.05, P < 0.10$]; Appendix C); however, reduced sample sizes due to herbivory may have limited power in many cases. When samples within regions were pooled, we found evidence for regional differences in pollen limitation. In particular, the strongest evidence for pollen limitation was found in the range periphery (significantly greater seed set in SO than U, $P = 0.004$). We found significant, but somewhat weaker, pollen limitation in intermediate populations (significantly greater seed set in SO than U, $P = 0.025$); we did not detect a significant difference between treatments for central populations (nonsignificant difference in seed set between SO and U, $P = 0.131$; Appendix C).

Across all sites combined, flowers receiving supplemental self-pollen also produced significantly more seeds than unmanipulated flowers (Appendix C), but we found no association between population variation in SPL and distance to the range limit (ANCOVA; distance to range limit, $F_{1,11} = 0.1$, $P = 0.728$; year, $F_{2,11} = 0.0$, $P = 0.989$; Fig. 4).

Floral manipulations also suggest that autonomous selfing does not occur at an appreciable frequency and therefore does not appear to provide reproductive assurance (RA). Across all sites combined, with >1000 manipulated flowers in each treatment, we found no significant difference between emasculated and unmanipulated flowers (Appendix C). We also did not find a significant effect of distance to range limit or year on population variation in RA (ANCOVA, distance to range limit, $F_{1,17} = 2.4$, $P = 0.143$; year, $F_{2,17} = 0.3$, $P = 0.715$; Fig. 4) or an association between RA and the availability of bees (ANCOVA; bee collection rate, $F_{1,17} = 0.3$, $P = 0.596$; year, $F_{2,17} = 0.2$, $P = 0.844$). Our tests for treatment differences within regions also did not uncover significantly greater seed set in unmanipulated compared to emasculated flowers; the trend was in the direction of slightly greater seed set in emasculated flowers (Appendix C).

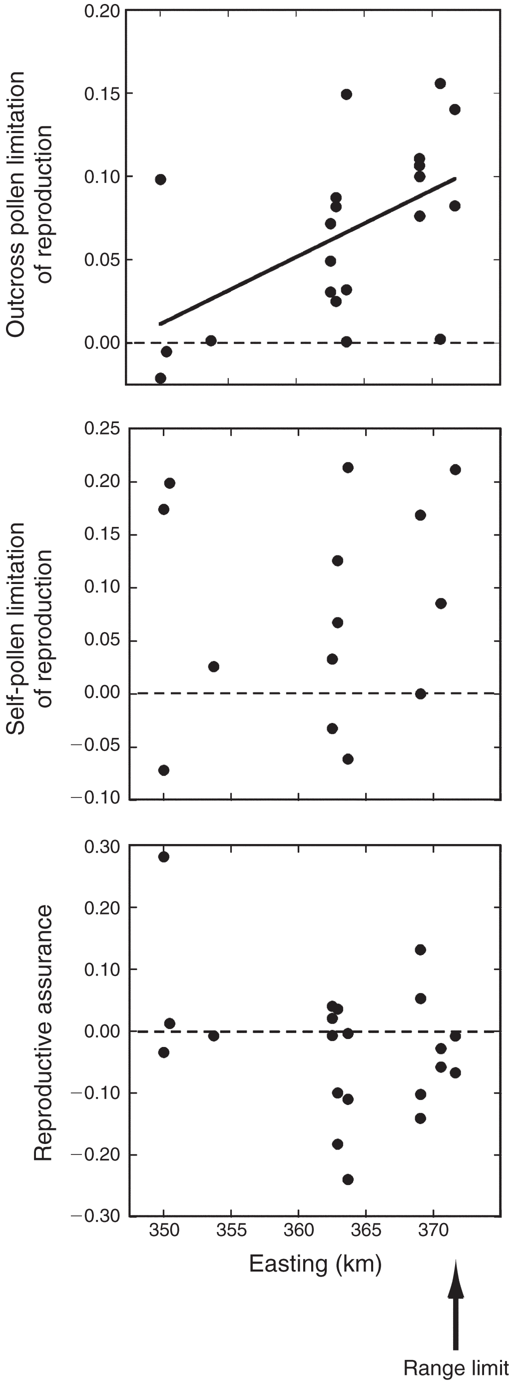

FIG. 4. Outcross pollen limitation, self-pollen limitation, and reproductive assurance from the center to the margin of *Clarkia xantiana*'s geographic range. All three metrics are based on seeds per fruit. Calculations of these metrics are described in the *Methods*. For a description of easting, see Table 1.

Mating system

Analysis of sequence and microsatellite data revealed no evidence that mating system differed across the geographic range. There was evidence for genetic differentiation among field populations (Appendix E) but no increase in selfing rates from the range center to edge ($F_{1,4} = 1.0$, $P = 0.375$; Table 1). Similarly, we found no relationship between range position and selfing rate when K was fixed at 1 for each population ($F_{1,4} = 0.1$, $P = 0.735$). The former analysis produced similar, but slightly higher, estimates of selfing rate (mean = 0.41) compared to the latter (mean = 0.35).

Discussion

The interactions between plants and pollinators at the margins of geographic ranges have been proposed to drive transitions in pollination syndrome or between outcrossing and autonomous selfing as dominant modes of reproduction (Grant and Grant 1965, Jain 1976, Wyatt 1988). These transitions are thought to occur because effective pollinators or mates are unreliable and pollen limitation is strong at the range margin. If evolutionary changes in floral form have not occurred to alleviate pollen limitation, reduced reproductive success could be an important factor limiting population growth in marginal populations and the propensity for range expansion. Here we show that over four years of study, pollinator availability to *C. xantiana* ssp. *xantiana* was consistently lower at the range margin compared to the range center, which resulted in greater outcross pollen limitation of reproduction. However, we found no evidence that autonomous selfing was possible as a fail-safe mechanism of reproduction, or that selfing rates were higher at the range margin. These results suggest that mating system has not evolved in response to selection imposed by pollen limitation near range margins. As such, dependency upon pollinators may be an important constraint limiting range expansion in this system.

The gradient in plant–pollinator interactions from range center to edge was consistent across four years despite considerable fluctuations in bee abundance among years (see also Herrera 1988, Horvitz and Schemske 1990, Price et al. 2005). Rates of collection and visitation varied approximately twofold over this period, but those fluctuations appear to have similarly influenced populations such that the geographic pattern of variation was largely similar through time. Temporal and spatial fluctuations in the frequency of plant–pollinator interactions were associated with climatic variation during this period. In particular, bee abundance appears to be influenced by precipitation during the previous year. The effect of the previous-year precipitation is likely indirect; greater precipitation tends to increase flower production in *Clarkia* (Eckhart et al. 2010, 2011), which in turn may increase the food supply and reproductive success of its specialist pollinators. Because the specialist solitary bees of *Clarkia* are univoltine, elevated levels of reproductive success will not be apparent until the subsequent year when offspring emerge. Minckley et al. (1994) observed the same sort of population dynamics in the oligolectic bee, *Dieunomia triangulifera*, in response to year-to-year variation in floral resources of *Helianthus annuus*. This time lag in the population dynamics of bees in response to their food plants may explain in part the dramatic fluctuations we observed in the visitation rates of bees to flowers. We also found a strong association between current-year spring precipitation (occurring from February to June) and the abundance of all bees. These results are consistent with the observation that precipitation cues bee emergence in arid regions, and that bees may remain dormant through periods of drought (Danforth 1999).

The decreased availability of pollinators near the range edge was more pronounced for generalist than specialist bees. While generalists gradually declined in abundance from range center to edge, there was considerable year-to-year variation in patterns of specialist abundance. Our previous work has shown that specialist bees are entirely absent beyond the eastern range limit (Fausto et al. 2001, Moeller 2006), suggesting that their geographic distribution is truncated where *C. xantiana* ssp. *xantiana* reaches its range limit. How do *Clarkia* specialists persist in marginal plant populations despite having limited foraging ranges and dispersal (MacSwain et al. 1973, Gathmann and Tscharntke 2002, Greenleaf et al. 2007)? Molecular population genetic analyses of *C. xantiana* ssp. *xantiana*'s demographic history have indicated that marginal populations have large effective population sizes (inbreeding N_e) and no signature of historical demographic changes, suggesting that populations have been large and stable over an appreciable period of time (Moeller et al. 2011). Seed dormancy (and seed banks) may be important to population persistence in marginal *C. xantiana* ssp. *xantiana* populations (Eckhart et al. 2011). This stability of floral resources at the range margin may have allowed for the long-term persistence of specialist bees. In cases where marginal plant populations exhibit high rates of population extinction and recolonization, the persistence of specialized mutualists may be considerably less likely.

In contrast to specialists, we observed a gradual decline in the availability of generalist visitors toward the range edge. Although specialists are clearly important pollinators in this system, our past work has suggested that in some years and in some plant populations generalist visitors can be the most common visitors and effective pollinators of *Clarkia* (Moeller 2005, Eckhart et al. 2006). Indeed, our floral manipulations suggest that pollen limitation is greatest in marginal populations where generalist visitors are least common. Pollen limitation may have important effects on population persistence in this system given that marginal populations have population growth rates near or slightly below 1 (stochastic λ), the threshold for population persistence (Eckhart et al. 2011). Increased fecundity could tip population growth rates above 1 and buffer marginal populations against local extinction. We did not detect severe pollen limitation in this study (~9–10% at the range limit), so it is unclear as to whether this effect is large enough to affect population growth. When *C. xantiana* ssp. *xantiana* is transplanted beyond its eastern border, reproduction is more severely pollen limited and fruit set low (~33% fruit set [Geber and Eckhart 2005]), which suggests that pollen limitation is more likely to threaten population persistence beyond the range boundary.

In many plant species, pollen limitation of outcross reproduction is compensated for by higher rates of self-fertilization (Eckert et al. 2006). For example, following a period of time where selfing is minimized in flowers through dichogamy and herkogamy, some species exhibit "delayed selfing" (Lloyd 1979, Dole 1990, Kalisz et al. 1999, Elle et al. 2010). This floral strategy tends to elevate seed production during periods when pollinators or mates are unreliable and may facilitate population persistence (Morgan et al. 2005). In this study, we have shown that autonomous selfing does not occur at any appreciable frequency, and does not appear to compensate for reduced levels of outcrossing. Despite >1000 flowers in each treatment, the difference in seed set between emasculated and unmanipulated flowers did not approach significance overall. In flowers of *C. xantiana* ssp. *xantiana*, anthers tend to migrate away from the stigma as the flower ages and senesces, the opposite of what occurs in most delayed selfers. More importantly, protandry can be substantial, typically 2–4 days, which increases the probability that pollen has been removed by bees or fallen from the dehisced anthers by the time the stigma is receptive. Therefore, our studies to date indicate that reproduction in *C. xantiana* ssp. *xantiana* requires pollinators whether it results in outcrossing or pollinator-mediated selfing.

In addition to our experimental data, we found that selfing rates, as inferred from molecular data, do not differ between central and marginal populations. Estimates of selfing rates were >25% for each population, suggesting that geitonogamous selfing and biparental inbreeding are likely common. This is not surprising given that inflorescences typically have multiple flowers open at a given time, and that individuals may often grow near siblings due to passive seed dispersal. Geitonogamous selfing is common in bee-pollinated plants (Harder and Barrett 1995, Mitchell et al. 2004), particularly for later flowers that are visited after bees have visited earlier flowers (Karron et al. 2009). The pattern of similar selfing rates across the geographic range is also interesting in light of a previous common garden study showing that herkogamy is somewhat reduced in marginal relative to central populations (Moeller 2006). Reduced herkogamy could be an adaptation to reduced pollinator availability at the range limit, but our data suggest that this change in floral form has no apparent functional consequences for the mating system. These results differ from a study of *Clarkia tembloriensis* by Holtsford and Ellstrand (1992) where outcrossing rates were correlated with variation in herkogamy among populations; however, it is important to point out that variation in herkogamy was broader in the study of *C. tembloriensis* compared to that observed here. Our results are similar to a study of *Aquilegia canadensis* where marginal populations had reduced herkogamy but with no parallel change in outcrossing rate (Herlihy and Eckert 2005). It is unclear whether reduced herkogamy evolved as a correlated response to selection on other characters, or whether a past selective environment (that no longer exists) favored such a change (Schueller 2004).

PLATE 1. Flowers of *Clarkia xantiana* ssp. *xantiana* visited by a specialist solitary bee, *Hesperapis regularis*. Photo credit: D. A. Moeller.

Chronic pollen limitation caused by reduced pollinator availability is expected to result in strong selection on floral traits that promote visits from alternative pollinators or traits that increase the likelihood of self-fertilization (Ashman et al. 2004, Knight et al. 2005, Morgan and Wilson 2005). The results of our studies within and beyond *C. xantiana* ssp. *xantiana*'s range suggest that selection caused by reduced pollinator availability and elevated pollen limitation can be substantial, but that no evolutionary response has occurred in marginal populations to facilitate greater reproductive success. Phylogenetic studies have indicated that the divergence of the primarily selfing *C. xantiana* ssp. *parviflora* from ssp. *xantiana* likely occurred 10 000–65 000 years ago, and that populations of ssp. *parviflora* have since come into secondary contact with ssp. *xantiana* (Pettengill and Moeller, *in press*). This suggests that a shift to self-fertilization might be possible at ssp. *xantiana*'s current eastern border, and that the apparent failure of marginal populations to respond to selection may represent an evolutionary constraint contributing to the stable range limit (Futuyma 2010). It is important to note that range expansion beyond the eastern distributional limit is likely to require not only changes in reproductive strategy in response to a gradient in pollinator availability, but also simultaneous changes in physiology and life history in response to gradients in precipitation and temperature (Eckhart et al. 2004, Geber and Eckhart 2005, Dudley et al. 2007, Eckhart et al. 2010, Mazer et al. 2010). The suite of genetic changes required to adapt along complex multivariable environmental gradients is considerably less likely than adaptation involving single traits along single-variable gradients, and may represent an important constraint on adaptive evolution and range expansion (Antonovics 1976).

Acknowledgments

We thank Raven Bier, Eric Fabio, Emily Looney, and Indrani Singh for assistance with field work and Chelsea Jones and Alex Wang for assistance with processing bee collections. We are grateful to the U.S. Department of Agriculture Forest Service (Fletcher Linton, Sequoia National Forest) and the Bureau of Land Management (Denis Kearns, Caliente Resource District) for access to field sites and to the U.S. National Science Foundation (DEB 0515466 to P. Tiffin and D. Moeller, DEB 051466 to M. Geber, and DEB 0515409 to V. Eckhart) and the University of Minnesota for funding.

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Supplemental Material

Appendix A

The names and attributes of *Clarkia xantiana* ssp. *xantiana* populations in this study (*Ecological Archives* E093-090-A1).

Appendix B

The results of regression analyses of bee collection rate in relation to precipitation (*Ecological Archives* E093-090-A2).

Appendix C

Means (±SE) of seed set from floral manipulations for each population × year combination along with tests for differences between pairs of treatments (*Ecological Archives* E093-090-A3).

Appendix D

The relationship between outcross pollen limitation of reproduction and the collection rate of bees from three years of study (*Ecological Archives* E093-090-A4).

Appendix E

Deme assignments from the INSTRUCT analysis for six *Clarkia xantiana* ssp. *xantiana* populations (*Ecological Archives* E093-090-A5).