doi:10.1111/evo.13836

SSE SOCIETY for the STUDY of EVOLUTION

EVOLUTION INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Maladaptation beyond a geographic range limit driven by antagonistic and mutualistic biotic interactions across an abiotic gradient

John W. Benning[1,2] and **David A. Moeller**[1]

[1] *Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, Minnesota 55108*

[2] *E-mail: jbenning@umn.edu*

Received March 17, 2019

Accepted July 24, 2019

Species' geographic range limits often result from maladaptation to the novel environments beyond the range margin. However, we rarely know which aspects of the *n*-dimensional environment are driving this maladaptation. Especially of interest is the influence of abiotic versus biotic factors in delimiting species' distributions. We conducted a 2-year reciprocal transplant experiment involving manipulations of the biotic environment to explore how spatiotemporal gradients in precipitation, fatal mammalian herbivory, and pollination affected lifetime fitness within and beyond the range of the California annual plant, *Clarkia xantiana* ssp. *xantiana*. In the first, drier year of the experiment, fitness outside the range edge was limited mainly by low precipitation, and there was some evidence for local adaptation within the range. In the second, wetter year, we did not observe abiotic limitations to plant fitness outside the range; instead biotic interactions, especially herbivory, limited fitness outside the range. Together, protection from herbivory and supplementation of pollen resulted in three- to sevenfold increases in lifetime fitness outside the range margin in the abiotically benign year. Overall, our work demonstrates the importance of biotic interactions, particularly as they interact with the abiotic environment, in determining fitness beyond geographic range boundaries.

KEY WORDS: Biotic interactions, *Clarkia xantiana* ssp. *xantiana*, geographic range limit, herbivory, pollination, reciprocal transplant, species distributions.

> "It is undoubtedly always a combination of factors which accounts for an animal's geographic range in all parts of the periphery of that range. It is most certainly never one factor alone."
>
> Joseph Grinnell (1917)

The distributions of species are determined largely by their environmental tolerances. Although historical contingencies and dispersal dynamics certainly influence these distributions, it is generally proposed that, for most species at broad scales, populations occur where the environment is suitable for them to persist (Darwin 1859; Grinnell 1917; MacArthur 1972). In these cases, somewhat tautologically, species are restricted to their realized niches (*sensu* Hutchinson 1957)—populations do not persist beyond their niche limits, which in physical space are realized as geographic range limits. This environmental filtering of organisms leads to spatial patterns of occurrence that form the basis of many ecological and evolutionary questions. Recent studies suggest that for many species, geographic range limits do reflect niche limits (Hargreaves et al. 2014; Lee-Yaw et al. 2016), whereas for others range limits are influenced by dispersal lag (e.g., Svenning et al. 2008; Alexander et al. 2018). However, the hyperdimensionality of these niches (Hutchinson 1957) means that, for most organisms, we do not know what aspects of the environment are important in setting these range limits (Grinnell 1917; Gaston 2009).

By far the most examined niche axes in regard to range limits are climatic variables such as temperature and precipitation. Partly due to their ease of measurement, and partly due to anticipated climatic changes, these abiotic variables have been at the forefront of most research on contemporary and future range limits (Sexton et al. 2009; Nadeau et al. 2017). The literature is rife with predictions for future species distributions based on shifting temperature isotherms, and indeed, there is good evidence that many species have already shifted up in latitude or elevation with recent warming (Parmesan and Yohe 2003; Chen et al. 2011; Rumpf et al. 2018). But important to note is the large variation in responses among taxa. For example, Chen et al (2011) found that 22% of the species they examined underwent range shifts in the direction opposite that expected from climatic trends (e.g., downslope in spite of warming). Similarly, in a survey of plants in the European Alps, Rumpf et al (2018) found that nearly half of the species had at least one range attribute (range center, leading edge, or rear edge) that shifted downslope. Clearly climatic niches are not the whole story.

And we should not expect them to be, given the myriad interactions every organism has with other species, and the wide-ranging consequences these biotic interactions have on the ecology and evolution of populations (Bridle and Vines 2007; Louthan et al. 2015; Urban et al. 2016). As far back as Darwin (1859), it was proposed that biotic interactions could set geographic range limits, and this idea is well supported by theory (Hochberg and Ives 1999; Case and Taper 2000; Gravel et al. 2011). Empirical evidence, especially experimental tests, is relatively scarce, but studies have supported the notion that mutualists (Moeller et al. 2012; Afkhami et al. 2014), competitors (Bullock et al. 2000; Ettinger and HilleRisLambers 2017), and predators (Bruelheide and Scheidel 1999; deRivera et al. 2005; Baer and Maron 2018; Benning et al. 2019) can influence the location of geographic range limits.

Despite its long history, the majority of work on range limits has been correlational—that is, relating species occurrences to environmental variables (usually climatic) across the landscape. This approach is the foundation of modern descriptive and predictive species distribution models. However, correlational approaches are confounded by the fact that many aspects of the environment covary across the landscape, and that the spatial autocorrelation of species distributions will rarely fail to correlate with some similarly spatially autocorrelated environmental variable (see Fourcade et al. 2018). By contrast, transplant experiments can offer much deeper insights into the causes of both local and/or elevational (e.g., Bruelheide and Scheidel 1999; Angert and Schemske 2005; Angert et al. 2008; Emery et al. 2009; O'Brien et al. 2017) and geographic range limits (e.g., Levin and Clay 1984; Geber and Eckhart 2005; Griffith and Watson 2006; Samis and Eckert 2009). They can directly test whether a range is limited by maladaptation, as opposed to failure to disperse. If paired with experimental manipulations of putatively important environmental variables, these experiments can also isolate factors constraining range expansion (e.g., Griffith and Watson 2006; Anderson et al. 2015). Due to logistical complexity, manipulative transplant experiments are rare, but they are essential to untangle covarying environmental gradients and determine their relative importance in setting distributions. Range limit experiments ideally (1) incorporate multiple sites within and outside the range limit, (2) estimate lifetime fitness of the focal species, and (3) manipulate putatively important environmental factors (Hargreaves et al. 2014). These experimental approaches are also ideally coupled with a historical perspective on range dynamics offered by population genetic approaches (Moeller et al. 2011).

When researchers do examine biotic constraints on species' distributions, they most often find evidence supporting the role of such interactions in contributing to geographic range limits. However, most of these studies are, again, correlational (Sexton et al. 2009; Louthan et al. 2015), finding negative correlations between the density of a focal species and some putative competitor or predator. Of course, if the distributions of these two species are also associated with adaptation to particular abiotic conditions that vary across the landscape, the emergent patterns could look very much like those predicted by a limiting effect of biotic interactions (but see Aragón and Sánchez-Fernández 2013). Lack of evidence for abiotic controls is also sometimes presented as evidence for biotic controls on range limits (e.g., Ettinger et al. 2011; O'Brien et al. 2017). Manipulative experiments are the most direct tests of causal relationships between distributions and species interactions (facilitation, competition, etc.). There are very few studies that both manipulate a biotic interaction in a transplant experiment within and beyond a geographic range limit, and calculate the interaction's direct effect on components of lifetime fitness (but see Stanton-Geddes et al. 2012; Anderson et al. 2015; Katz and Ibáñez 2017).

We investigated the influence of two biotic interactions on lifetime fitness in a California annual plant, *Clarkia xantiana* ssp. *xantiana* (Onagraceae), within and outside its geographic range limit. As is likely to be the case with most species, *C. x. xantiana* is distributed across a complex environmental gradient comprising many covarying abiotic and biotic environmental variables. Both probability of herbivory and pollinator availability change across the subspecies' range (increase and decrease, respectively), and prior work has demonstrated that spatial variation in these interactions can have large fitness consequences for *xantiana* (Anderson et al. 2015; Benning et al. 2019). We used a manipulative transplant experiment across two years and at multiple sites within and outside the range to address three sets of questions:

1. How does lifetime fitness vary from the center to edge of the range and in multiple sites beyond the edge?
2. To what extent is there local adaptation in different parts of the range? Do source populations differ in lifetime fitness outside the range edge?
3. How do interactions with mammalian herbivores vary across the range and beyond? To what extent does amelioration of herbivory and pollen limitation of reproduction increase

fitness, alter patterns of local adaptation, and affect the likelihood of population persistence beyond the range edge?

Methods

STUDY SYSTEM

Clarkia xantiana ssp. *xantiana* A. Gray (hereafter, *xantiana*) is a winter annual native to the southern Sierra Nevada foothills of California (USA; Eckhart and Geber 1999). *Xantiana* is distributed across a complex west-to-east environmental gradient, with western and central *xantiana* populations found primarily on steep, sandy slopes in relatively mesic oak woodlands of the Kern River Canyon, and eastern edge populations found in drier pine woodlands (Fig. 1A; Eckhart et al. 2011; Gould et al. 2014). Most populations, including all used in this study, occur on sandy, fast-draining soils derived from granitic rock (Eckhart et al. 2010). The eastern range edge is stark (Fig. 1A) and extensive searching over the past 20+ years has uncovered no *xantiana* populations beyond this limit.

Xantiana is distributed across an aridity gradient (with precipitation lower and more variable toward and outside its eastern range edge) that contributes to reduced performance at the range edge and beyond (Eckhart et al. 2010, 2011). In contrast to most study systems, the influence of biotic interactions on *xantiana*'s distribution has received a relatively large amount of attention in several within and beyond-range experiments. These studies have shown that mutualistic interactions with pollinators are weaker at and beyond the range limit, resulting in greater pollen limitation (PL) of reproduction (Moeller et al. 2012; Anderson et al. 2015). By contrast, antagonistic interactions with mammalian herbivores (primarily lagomorphs: *Sylvilagus audubonii* and *Lepus californicus*) are stronger at and beyond the range limit (Benning et al. 2019). The current study builds on these prior efforts in three important ways. First, prior transplant studies occurred at relatively few sites, whereas the current study involves three sites within the range, and three beyond. Second, whereas Benning et al. (2019) used a post hoc simulation approach to estimate the effect of mammal herbivory on fitness in a previous transplant experiment, here we experimentally manipulate the presence versus absence of herbivores *in situ*. Third, the joint effects of gradients in the two key biotic interactions (pollinators and herbivores) on lifetime fitness, and their interactions with abiotic gradients, have not been estimated before this study.

RECIPROCAL TRANSPLANT

To estimate the effects of multiple biotic interactions, geography, and source population on *xantiana* lifetime fitness, we planted seeds from three *xantiana* populations into six sites spanning from the center to 22 km outside the natural distribution of *xantiana*.

The six sites were at *xantiana*'s range center (Center), between the center and range edge (Intermediate), eastern range edge (Edge), 5 km outside the eastern range edge (Just Beyond), 14 km outside the range edge (Beyond), and 22 km outside the range edge (Far Beyond; Fig. 1; Supporting Information 1). The three sites within the range contain natural *xantiana* populations, and the three sites outside contain its sister subspecies, *parviflora*. Sites containing *C. x. parviflora* are a good approximation of "suitable" habitat outside the eastern range edge of *C. x. xantiana*, given that both subspecies occupy similar niches and sites in the region where they are sympatric. The experiment was conducted across two growing seasons (2015–2016 and 2016–2017; hereafter, year 1 and year 2).

Xantiana seeds were sourced from the Center and Edge sites in year 1, and Center, Edge, and Intermediate sites in year 2. We did not include the Intermediate site in year 1 due to a seed collection error. In year 1, seeds used for planting were all collected from the field (30–70 maternal families per population). Due to drought and thus low site productivity in 2016, we generated seed for year 2 planting in the greenhouse by crossing among 26–30 maternal families per population. No crosses occurred within maternal families. Within source populations, we pooled seeds from all maternal families before planting.

At each of the six sites, we installed 120 plastic grids arranged into six blocks (20 grids per block) set into the natural vegetation matrix ($N = 720$ grids total). Grids were cut from white plastic diffusion screens (used for fluorescent light fixtures) and set onto the soil surface after scraping away the top ca. 1 cm of soil underneath to avoid contamination from the local *C. xantiana* seed bank, and filled with soil dug from ca. 20 cm below the surface (Fig. 1B). Grids comprised a 7 × 7 matrix of 3 cm × 3 cm cells (with ca. 2 cm high walls); only the inner 36 cells were planted to avoid potential edge effects. These grids allowed us to follow individual seeds while maintaining a natural growing environment for the experimental plants. We did not weed or otherwise alter natural vegetation around the grids. Because the grids are enmeshed in the surrounding matrix of soil and vegetation, and are almost level with the soil surface, they are unlikely to influence rates of herbivory. Incidental germination (i.e., *xantiana* germination in empty cells) was very low—for example, at the Center site, where the *xantiana* seed bank is likely largest, eight out of 1920 empty cells had a germinant in year 1 (0.4%); any incidental germinants were removed from grids.

The source populations were randomly assigned to cells within grids using three randomized planting schemes (five cells per source population per grid in year 1 and four cells per source population per grid in year 2); each grid was randomly assigned to a scheme. Two seeds were planted per cell in October of each year (in year 2, all seeds were planted into empty cells that were not planted into in year 1). Thus, in year 2, the experiment

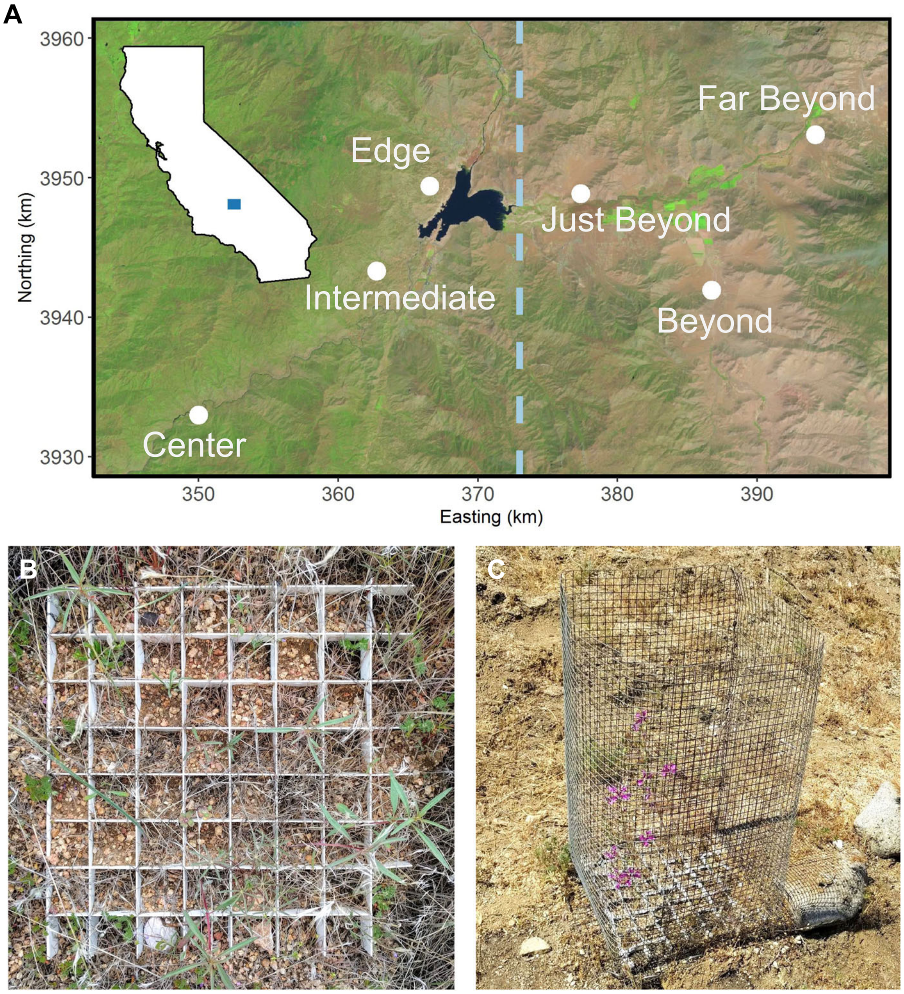

Figure 1. Overview of study area and implementation of the experiment. (A) Study area in Southern California and the locations of transplant sites (circles). The dashed blue line marks *xantiana*'s eastern range limit. Background image is 19 April 2016 LANDSAT imagery of study area. Axes are UTM coordinates; Zone 11 S. (B) Planting grid installed in the ground, with *xantiana* seedlings visible at top left and bottom right. (C) Herbivore exclusion cage around flowering *xantiana* individuals.

included newly planted seeds as well as any seeds that were planted in year 1 and did not germinate. We account for these different seed cohorts in our analyses.

We visited sites in February and March to score germination and early season survival, respectively, and monitored late season survival, mammal herbivory, and total seed set May through June in each year. If there were two germinants in one cell, we randomly culled one germinant to maintain natural plant densities. We estimated seed set in each collected fruit using a linear model that predicted fruit seed set as a function of individual fruit weight (Supporting Information 2). A plant's lifetime fitness is equal to the sum total seeds contained in all of its fruits.

We had access to precipitation monitoring stations (HOBO Onset) at four of the six transplant sites (Center, Intermediate, Just Beyond, and Beyond), which are part of a long-term study of *C. xantiana* population dynamics. For the Edge and Far Beyond sites, we used nearby weather stations (4 and 13 km from site, respectively) to estimate precipitation during transplant years. To interpret annual precipitation patterns relative to long-term trends, we used the PRISM climate dataset (PRISM Climate Group, 2004) to obtain interpolated estimates of monthly precipitation data for each site for years 1990–2017, at 4-km grid cell resolution (Table S1).

HERBIVORE EXCLUSION

In March of 2016 and 2017, half of the grids in each block were surrounded by open-topped 0.6-m high herbivore exclosures made from 1.3-cm galvanized steel mesh (Fig. 1C), unless the grid contained no plants. Grids were randomly assigned exclosure treatments, and we alternated exclosure grids between the two years (i.e., no grid was caged or uncaged for both years). Toward the end of the growing season in each year, we also attached tops to these exclosures to prevent rodents from breaching the cages.

The experiment also included a soil manipulation treatment that was fully crossed with the caging treatment, where we filled grids with soil from one of four source sites (local, Center, Intermediate, or Beyond) prior to planting. Results of these soil manipulations will be reported in a later manuscript; for the current analyses, all effects of biotic treatments, site, and source population are averaged over these soil microbial manipulations.

We found no evidence that caging itself affected plant growth (Supporting Information 3, Table S2). We do note that, simply by chance, there were differences in germination among grids in caged and uncaged treatments at some sites (Table 3), even though grids were not caged until months after germination. The only instance where this could potentially confound interpretation of our results is at the Far Beyond site, where caged seeds showed slightly higher germination rates than uncaged seeds (0.11 vs. 0.09, respectively). Thus, the effect of caging at the Far Beyond site should be interpreted with a modicum of reserve.

POLLEN LIMITATION OF REPRODUCTION

In year 2, we supplemented pollen on a subset of plants at all sites to estimate PL of reproduction across and beyond *xantiana*'s range. We performed the supplementation experiment on all plants that had at least two flowers, including one with a stigma that was receptive during our site visits. For each plant, we selected two flowers to serve as supplemented and control flowers (on some large plants, we selected an additional pair of supplemented and control flowers, but 94% of plants had only one supplemental/control pair). The control flower was either directly above or below the supplemented flower; relative orientation of the pair was haphazardly rotated among plants. Prior research on *C. xantiana* has shown that resource reallocation does not occur among neighboring fruits and therefore does not bias estimates of PL (Briscoe Runquist and Moeller 2013). If multiple flowers had receptive stigmas, we haphazardly chose one for supplementation, avoiding flowers at the ends of branches. We collected pollen from a natural *xantiana* population near the Edge site and applied pollen across the entire receptive stigmatic surface of each supplemented flower. Fruits were collected at maturity and all seeds counted.

We also quantified pollinator visitation by conducting pollinator observations at all sites during the flowering period. All flower visitors were bees, particularly *Clarkia* specialists inside the range (Moeller 2005, 2006). Flowers on subsets of experimental plants were watched for 10-min periods and all bee visitors were recorded (average of 43 observation periods in each region; average of 13 flowers watched per observation period; total of 860 min of observations). All observations were conducted between 0800h and 1630h on sunny days with minimal cloud cover and wind.

ANALYSES

Transplant experiment

All analyses were conducted in R (R Core Team 2013). We used *aster* life history models (Geyer et al. 2007; Shaw et al. 2008) in R (R Core Team 2013) to evaluate the effects of site, source population, and caging treatment on lifetime fitness, analyzing years 1 and 2 separately. *Aster* models use a graphical approach that links sequential components of lifetime fitness, each modeled with its appropriate statistical distribution. Our *aster* model incorporated six components of lifetime fitness (*nodes* in the graphical model) for this experiment: germination, early survival (March), late survival (May), survival to flowering, fruit production (i.e., did the plant produce any seed-bearing fruits), and total seed set. The first five components were modeled as Bernoulli variables (0,1), and total seed set was modeled as a zero-truncated Poisson variable:

$$1 \rightarrow \underset{(0,1)\ \text{Bernoulli}}{\textbf{germination}} \rightarrow \underset{(0,1)\ \text{Bernoulli}}{\textbf{early survival}} \rightarrow \underset{(0,1)\ \text{Bernoulli}}{\textbf{late survival}} \rightarrow$$

$$\underset{(0,1)\ \text{Bernoulli}}{\textbf{survival to flowering}} \rightarrow \underset{(0,1)\ \text{Bernoulli}}{\textbf{fruit production}} \rightarrow \underset{\text{zero} - \text{truncated Poisson}}{\textbf{seeds produced}}$$

For year 1, we built an *aster* model with site, source population, caging treatment, and all interactions as predictors; response variables are those associated with each component of lifetime fitness. To estimate the effects of each predictor on lifetime fitness, each predictor was fit at the level of total seed set in the model (Shaw et al. 2008). We did not include block in these models to avoid overfitting at latter life history stages, when the total number of surviving individuals at a site was sometimes very low. We used likelihood ratio tests (LRTs) comparing submodels to fuller models to test each predictor of interest.

Because very few plants survived at two sites outside the range in year 1, we could not model lifetime fitness at all sites simultaneously. This is due to inherent limitations of maximum likelihood parameter estimation (Geyer 2009) that arise when all records in one category of a predictor have the same response at one node of the *aster* graph (e.g., every plant in Site A that is alive in March is dead in May). To circumvent this issue, we added one additional record, producing one seed, to each site/block/source population/caging treatment combination. This allowed us to use one model to estimate lifetime fitness at all sites. These "pseudorecords" made only miniscule differences to overall average seed production at each site (max difference between average fitness calculated from dataset with only observed records and dataset including pseudorecords = 0.02 seeds). For year 2, we built *aster* models as above but also included a planting year term to account for differences in seeds planted in 2015 and 2016. We did not need to include pseudorecords for year 2.

We tested preplanned contrasts of lifetime fitness for caged and uncaged plants at each site by using LRTs to compare an *aster* model with all main effects, and site × source population and site × caging interactions, to a submodel that did not include the specific coefficient of interest. For example, to test whether lifetime fitness of caged and uncaged plants was significantly different at the Center site, we compared the large *aster* model to a model where caged and uncaged plants at the Center site were combined into one category, which removes the *Center*:*Caging* coefficient from the model. We adjusted test *P*-values with a Bonferroni correction. We also examined potential local adaptation within *xantiana*'s range. Local adaptation of *xantiana* populations would be evidenced by either (1) local populations having higher lifetime fitness than foreign populations at a site ("local vs. foreign" criterion) or (2) populations having highest lifetime fitness at their home site ("home vs. away" criterion). We tested "local vs. foreign" and "home vs. away" pairwise contrasts using LRTs.

Components of lifetime fitness

Because site, source population, and caging factors likely influence lifetime fitness through different components of lifetime fitness, we also tested the influence of these predictors at each life history stage separately. We used logistic regressions to test the effects of site, source population, caging, site × caging, and site × source population on our Bernoulli life history components (germination through fruit production). Using the same model structure, we used linear regression for seed production, with a log transform of the response. For germination, we included a site × planting year interaction to account for seed age (planted in year 1 or year 2), but because there was no influence of seed planting year on the following stage (early survival), we dropped this term for stages after germination. Because there was no support in either year for a significant source population × caging term in the *aster* models of lifetime fitness, we did not include that interaction in our fitness component models. These separate fitness component analyses are "conditional" in the sense that, for each component after germination, we only used the subset of records that survived the previous life history stage (e.g., analysis of the probability of survival to May only included those plants that survived to March).

Predicting mean lifetime fitness

The fitness metric of most interest was mean seeds produced per planted seed. Because we planted two seeds per cell, and culled extra germinants, fitness predictions from our *aster* model would be inflated relative to this metric. Thus, we obtained predicted values for lifetime fitness and their associated standard errors by taking the product of germination probabilities (see above) and unconditional parameter estimates from a full *aster* model that did not include a germination node. Standard errors for these products were calculated using the Delta method (Buehler 1957).

Pollen limitation

We analyzed PL in three ways. We first calculated a PL metric for each control/supplemental pair by dividing the difference in control and supplemental seed set (supplemental−control) by the larger of those two values (in some cases, supplemented flowers will produce fewer seeds than control flowers for reasons beyond the control of the researcher). The resulting metric is thus the percent difference in seed set between supplemented and control flowers (e.g., $PL = 0.1$ means that control flower seed set was 90% of supplemented seed set). We tested whether PL differed within and outside the range using a linear model with region and site (nested within region) as predictors, and PL (calculated from pairs of flowers) as the response. We deemed PL significant in a region if the 95% confidence interval for that region's PL estimate did not overlap zero. Second, we used paired, one-sided *t*-tests to ask whether supplemented flowers set more seed than control flowers within and beyond the range. Finally, we used logistic regression to test whether the probability of a flower pair exhibiting PL (i.e., having a positive PL metric) differed among regions, with site nested within region.

Pollinator visitation rates for each observation period were calculated as the number of visits divided by the number of flowers watched during that period. To test whether pollinator visitation differed inside and outside the range, we used a Mann-Whitney *U*-test, as residuals from a linear regression model were highly nonnormal and transformations did not appreciably improve their distribution.

Joint effect of biotic interactions

We estimated the joint effect of herbivory and PL on mean lifetime fitness outside the range in year 2 using a simulation (we did not perform pollen supplementations in year 1). We used estimates of pollen supplementation effects for each site (see *Pollen limitation*, above) to inflate seed counts of caged plants in that site by that PL estimate (for the Just Beyond site estimated PL was –0.02, so we did not simulate any change in seed set). Then, using this simulated dataset, we obtained mean lifetime fitness predictions, using the procedure described above. Comparison of lifetime fitness estimates for uncaged plants without simulated pollen supplementation and caged plants with simulated pollen supplementation estimates the joint effect of these biotic interactions on fitness outside the range. Data and code for all analyses are archived in the Dryad Digital Repository.

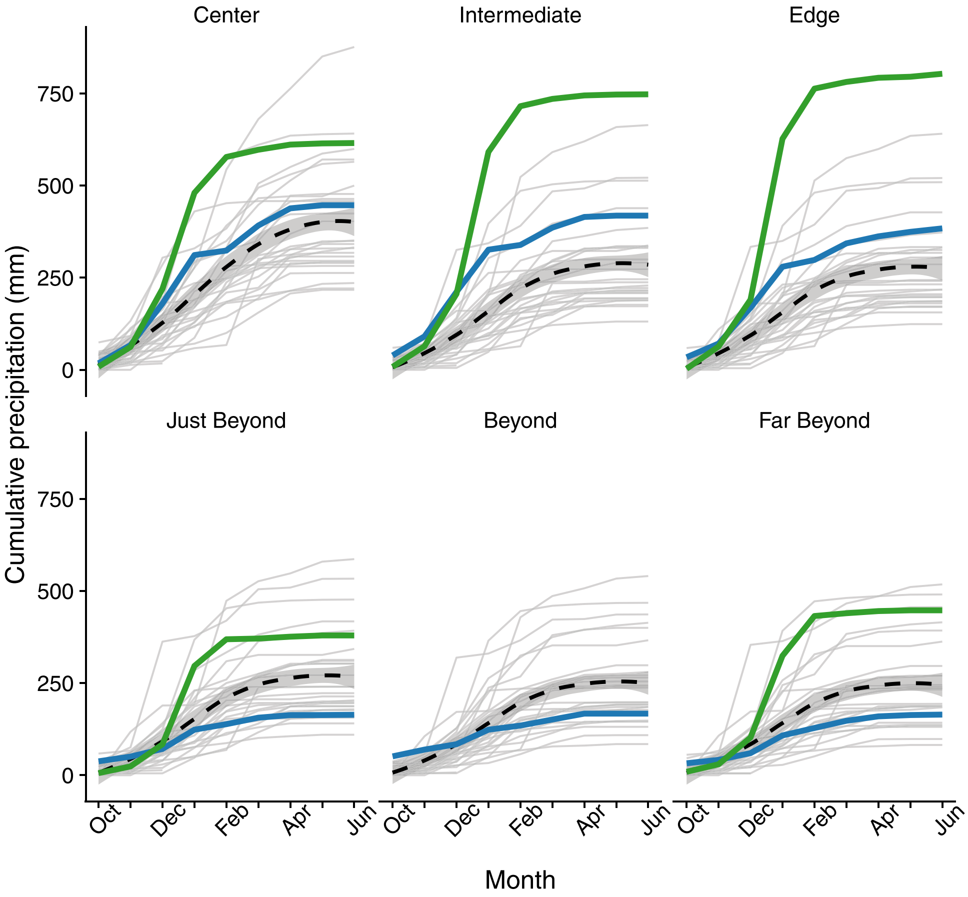

Figure 2. Cumulative precipitation across the growing season (October–June) within the study area. Shown are precipitation patterns during the transplant experiment (year 1: blue lines; year 2: green lines), using data from weather stations at or near the sites. We also plotted precipitation for the years 1990–2017 at each site location (thin gray lines), using interpolated estimates from PRISM, to help interpret study year precipitation patterns in the context of long term trends (dashed black line shows long-term trend with 95% confidence band). Precipitation data for the Beyond site in year 2 were unavailable due to a wildfire destroying our weather station.

Results

PRECIPITATION

Year 1 was much drier than year 2 with ca. 40–170% more growing season (October through June) precipitation in year 2 (Fig. 2; Table S1). Mean precipitation for sites outside the range was 39% that of sites inside the range in year 1 (164 and 416 mm, respectively) and 57% that of sites inside the range in year 2 (413 and 722 mm, respectively). Compared to long-term trends at each site, precipitation in year 1 was above average inside the range but below average outside the range, resulting in a steep gradient across the range margin, whereas precipitation in year 2 was considerably above average at all sites.

PATTERNS OF LIFETIME FITNESS

Year 1

In year 1, mean predicted lifetime fitness for *xantiana* was 150 times higher inside the range than outside the range (12.0 vs. 0.1 seeds per planted seed, respectively; Fig. 3; Table 1). However, this difference was disproportionately driven by extremely high fitness estimates at the Center site, where mean fitness estimates were 12 times higher than those at the next highest site, Edge (32.5 vs. 2.7, respectively). Relatively high fitness at Center was in large part driven by high fecundity (Fig. 3G; Table 2). Outside the range edge, mean fitness was low (<0.3) at all sites.

Site, source population, and caging treatment were all significant predictors of lifetime fitness, and the effect of source population differed across sites (Table 1). The highest rates of herbivory were recorded at the Edge and Beyond sites (20% and 28% of uncaged plants alive at March census eaten, respectively), with low herbivory (<6%) at the two most interior sites. No herbivory was recorded at the other two sites outside the range limit, likely because so few plants survived (<6 uncaged plants at either site in May). There was support for a positive, but modest main effect of caging on lifetime fitness (LR = 7.3; P = 0.007), but tests of caging effects within individual sites uncovered

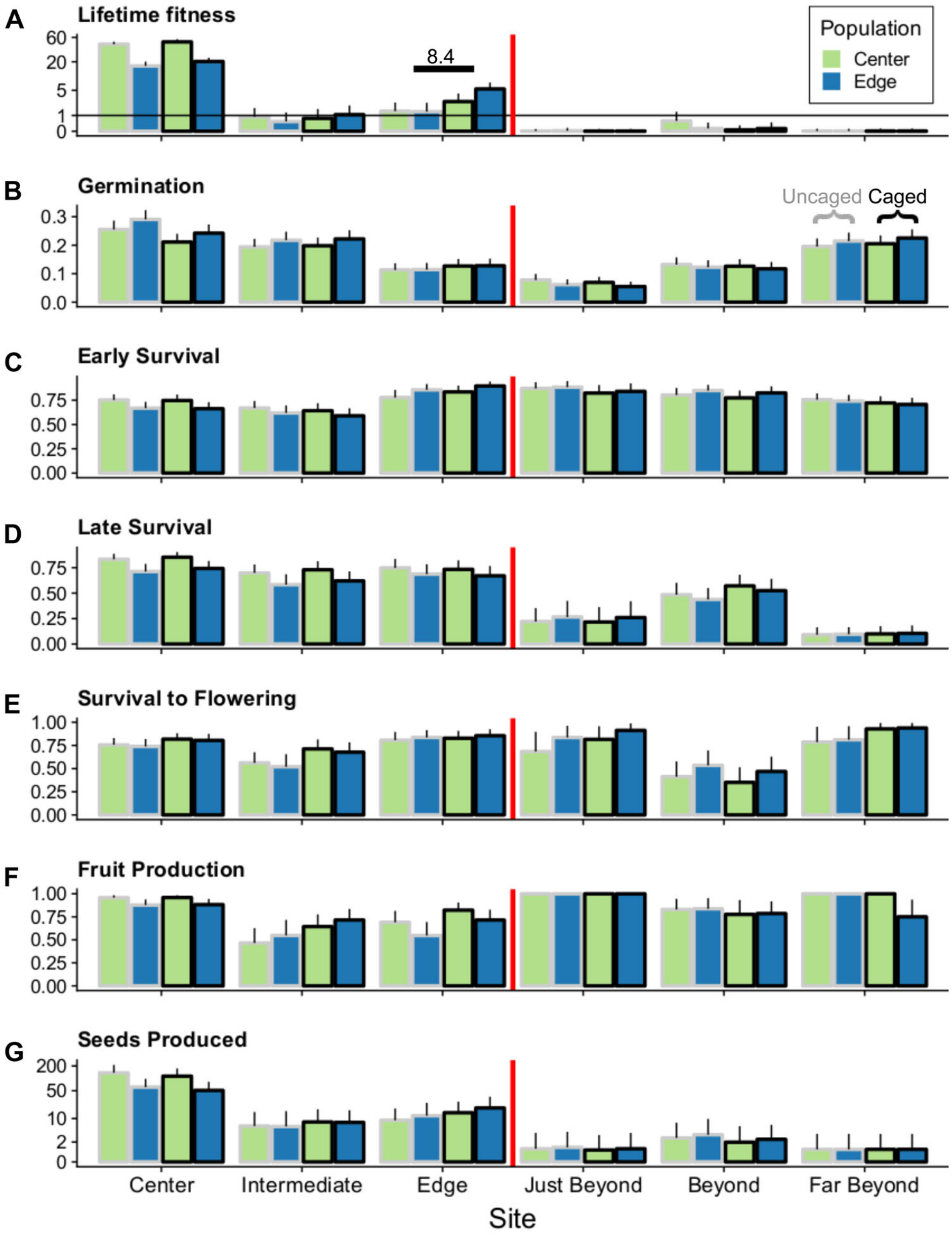

Figure 3. *Xantiana* mean lifetime fitness in year 1 estimated from the full *aster* model (panel A) and least squares means for components of lifetime fitness (panels B–G), by site, population, and caging treatment. Caged treatment is indicated by a black border around the bar. Significant differences in lifetime fitness among caging treatments are indicated with horizontal black bars; numbers above bars are deviance values from LRT test of the effect of caging at that site. The red line demarcates sites within the range (left) and outside the range (right). Lifetime fitness (panel A) and seeds (panel G) are on the log scale to aid visualization. All line ranges are 95% confidence intervals. Following germination, fitness component analyses are "conditional"—that is, only those plants that had nonzero values for the preceding fitness component are analyzed. Thus, early survival shows the probability of a germinated seed surviving until March.

significant differences between caging treatments at only the Edge site, where caged plants had mean lifetime fitness ca. three times higher than uncaged plants (4.0 vs. 1.4 seeds per planted seed, respectively; adjusted $P = 0.02$).

There was evidence for local adaptation (*local* > *foreign* fitness and *home* > *away* fitness) of the Center population in year 1. At the Center site, predicted lifetime fitness of the Center population was more than double that of the Edge population (46.6 vs. 18.4 seeds per planted seed, respectively; LR = 119.6; $P < 0.0001$; Fig. 3A). Center genotypes also performed best at their home site, Center (all $P < 0.0001$). Overall, there was no significant difference in performance of Edge

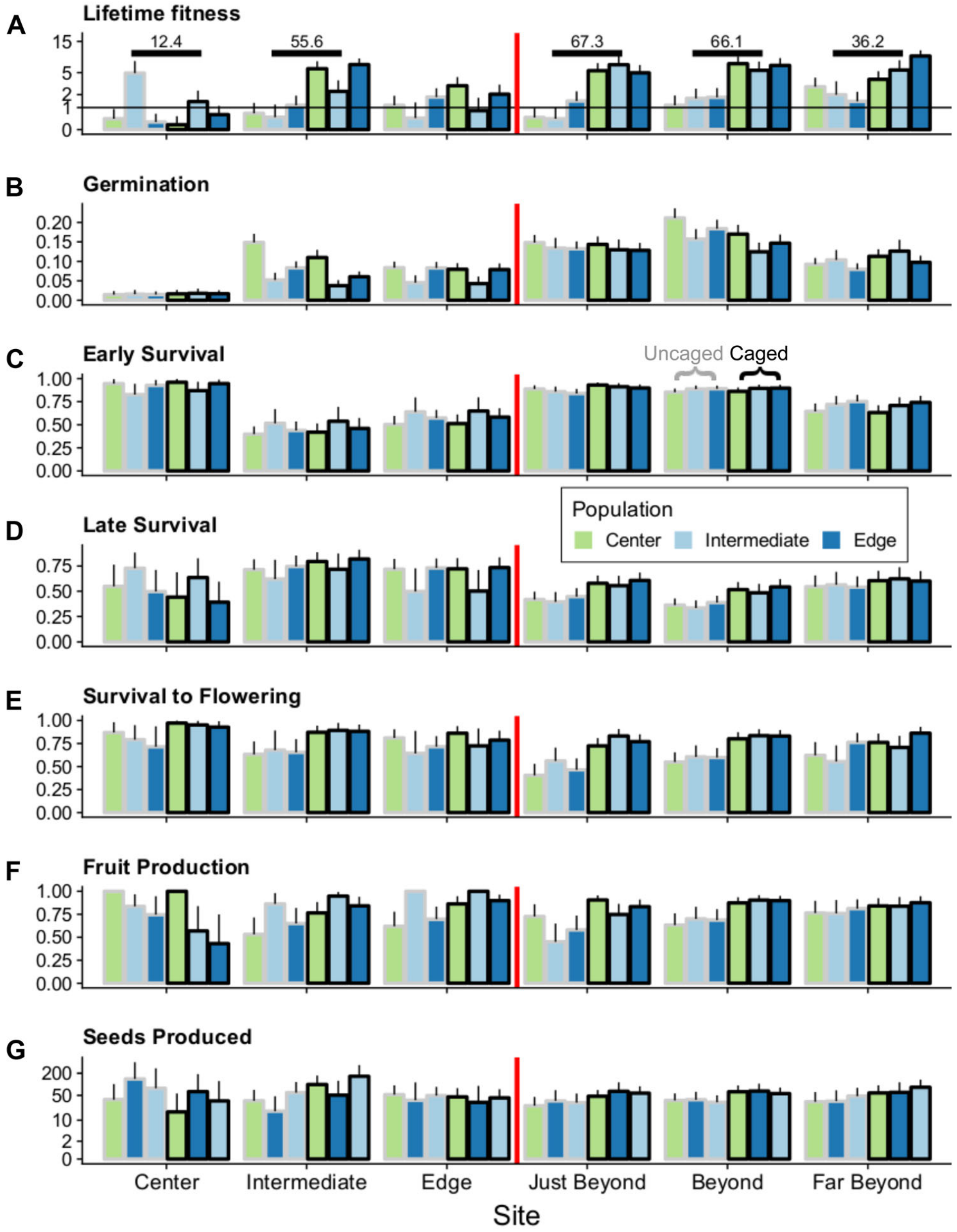

Figure 4. *Xantiana* mean lifetime fitness in year 2 estimated from the full *aster* model (panel A) and least squares means for components of lifetime fitness (panels B–G), by site, population, and caging treatment. Caged treatment is indicated by a black border around the bar. Significant differences in lifetime fitness among caging treatments are indicated with horizontal black bars; numbers above bars are deviance values from LRT test of the effect of caging at that site. The red line demarcates sites within the range (left) and outside the range (right). Lifetime fitness (panel A) and seeds (panel G) are on the log scale to aid visualization. All line ranges are 95% confidence intervals. Following germination, fitness component analyses are "conditional"—that is, only those plants that had nonzero values for the preceding fitness component are analyzed. Thus, early survival shows the probability of a germinated seed surviving until March.

and Center populations at the Edge site (LR = 2.4; P = 0.12). However, when protected from herbivory, mean fitness of the Edge population was about twice that of the Center population at the Edge site, although this contrast was only marginally significant (LR = 3.5; P = 0.06; Fig. 3A). Mean lifetime fitness of Center and Edge populations did not vary significantly outside the range limit.

In analyses of individual life history components, site was a strong predictor of performance at each stage (Table 2), although geographic patterns varied through *xantiana*'s life history (Fig. 3). Probability of germination decreased from the center to edge, then increased further east beyond the range margin (Fig. 3B). Late season survival was generally low outside the range, especially at Just Beyond and Far Beyond sites, likely due to water scarcity

Table 1. Summary of results from *aster* model comparisons testing effects of site, population (Pop), caging treatment (Caged), and their interactions, on lifetime fitness in *xantiana*, in both years of the experiment.

	Year 1				Year 2			
Term	Resid. df	Test df	Dev	*P*-value	Resid. df	Test df	Dev	*P*-value
Full	29				42			
Planting Year					41	1	30.29	<0.001
Site × Caged × Pop	24	5	1.52	0.91	32	10	30.63	<0.001
First Order Interactions	24				32			
Caged × Pop	23	1	0.98	0.32	30	2	0.84	0.66
Site × Caged	18	5	5.81	0.32	25	5	80.71	<0.001
Site × Pop	13	5	29.25	<0.001	20	10	104.28	<0.001
Main effects only	13				15			
Caged	12	1	7.32	0.007	14	1	159.57	<0.001
Pop	12	1	93.34	<0.001	13	2	5.07	0.08
Site	8	5	1366.5	<0.001	8	5	80.02	<0.001

Table 2. Summary of type II analysis of deviance for logistic regressions (germination through fruit production) and linear regression (seeds produced) testing effects of site, population, caging treatment, and their interactions, on sequential components of *xantiana* lifetime fitness in year 1.

	Year 1						
Term	df	Germination χ^2	Early survival χ^2	Late survival χ^2	Survival to flowering χ^2	Fruit production χ^2	Seeds produced (*F*)
Site	5	463.45***	62.87***	455.70***	66.30***	72.61***	1092.00***
Population	1	2.16	0.96	7.17**	0.20	2.65	8.85
Caged	1	0.76	0.62	1.14	3.93*	3.48$^{\hat{}}$	0.00
Site × Population	5	7.77	8.28	5.62	2.81	7.76	25.45
Site × Caged	5	8.54	2.50	1.27	4.33	6.05	8.38

$^{\hat{}}P < 0.1$
$^{*}P < 0.05$
$^{**}P < 0.001$
$^{***}P < 0.0001$.

as the season progressed (Fig. 3D). Fruiting plants at Center produced far more seeds than did plants at other sites (Fig. 3G). Neither *source population* nor the *source population* × *site* interaction was identified as significant predictors in germination or early survival models (Table 2; Fig. 3), suggesting that maternal environmental effects did not influence early life stages (where they are most likely to be realized) in a way that would confound overall results.

Year 2

In year 2, mean predicted lifetime fitness (across treatments) was relatively high at all sites (1.3–4.1 seeds produced per planted seed). Site and caging treatment were significant predictors of lifetime fitness, but there was no support for a main effect of source population; however, there were significant interactions of site × source population, site × caging treatment, and site × caging × source population (Table 1).

Overall, herbivory rates were higher in year 2 (Table S1). Twenty percent of uncaged plants were eaten at the Intermediate site, and approximately 37% were eaten at Just Beyond and Beyond sites. The Far Beyond site had 12% of plants eaten. At the Center and Edge sites, plants were subject to herbivory at rates of ca. 4%. Comparisons of caging treatments within individual sites showed that caging was associated with three- to sevenfold increases in lifetime fitness at the four sites with high herbivory rates (all $P < 0.0001$; range of LR: 36.2–67.6; Fig. 4A). There was no difference in fitness between caging treatments at the Edge site, where herbivory was very low. At Center, caging treatment

Table 3. Summary of type ii analysis of deviance for logistic regressions (germination through fruit production) and linear regression (seeds produced) testing effects of site, population, caging treatment, and their interactions, on sequential components of *xantiana* lifetime fitness in year 2.

	Year 2						
Term	df	Germination χ^2	Early survival χ^2	Late survival χ^2	Survival to flowering χ^2	Fruit production χ^2	Seeds produced (F)
Site	5	1293.67^{***}	371.73^{***}	83.19^{***}	22.36^{***}	4.50	1.15
Population	2	41.14^{**}	$5.09^{\wedge}$	1.84	1.10	0.09	0.65
Caged	1	5.90^{*}	1.20	24.33^{***}	55.46^{***}	30.28^{***}	13.04^{***}
Plant Year	1	353.84^{***}					
Site × Population	10	56.56^{***}	10.21	8.57	9.65	19.23^{*}	1.10
Site × Caged	5	22.93^{***}	3.21	8.09	5.37	$9.93^{\wedge}$	2.23
Site × Plant Year	5	391.32^{***}					

$^{\wedge}P < 0.1$

$^{*}P < 0.05$

$^{**}P < 0.001$

$^{***}P < 0.0001$.

was associated with a modest reduction in fitness (LR = 12.43; $P = 0.002$), although this should be interpreted with caution given the disproportionate influence of one plant in the uncaged treatment, which produced almost four times the seeds as the next most fit plant.

The effect of source population on lifetime fitness varied across sites (Table 1; Fig. 4A). However, there was no evidence of local adaptation for any source population at any site, regardless of caging treatment. Seed planting year was highly significant due to higher germination rates of seeds planted in year 2 (Table 3).

In analyses of individual life history components, site, caging, source population, and their interactions were all significant predictors at some stage(s) (Fig. 4; Table 2). Germination generally increased going from the center to outside of the range (Fig. 4B). The effects of caging were mostly realized in the latter life history stages, especially late survival to seed production (Fig. 4; Table 3), which was when the majority of herbivory occurred in the field.

Pollen limitation

PL differed significantly within versus outside the range ($F_{1, 161} = 4.43$; $P = 0.04$) in year 2. There was evidence for significant PL outside the range (mean: 0.08; 95% CI: 0.001–0.15), but not inside the range (mean: –0.07; 95% CI: –0.18–0.03). Paired, one-sided t-tests gave no evidence for supplemented fruits setting more seed than control fruits inside the range ($t_{57} = 1.82$, $P = 0.96$), but indicated supplementation increased seed set outside the range ($t_{113} = -2.36$, $P = 0.01$). Logistic regression also showed that the probability of PL was 77% higher outside the range than inside the range (probability within: 0.32; probability beyond: 0.55; $P = 0.008$). PL increased with distance from the range limit (Just Beyond, mean PL = –0.02; Beyond, PL = 0.07; Far Beyond, PL = 0.19).

Pollinator visitation rates differed strongly between regions ($W = 428$, $P < 0.0001$). Visitation was significantly higher inside the range (visits per flower per 10 min: mean = 0.80; median = 0.33) than outside the range (mean = 0.06; median = 0.00).

Joint effects of biotic interactions outside the range limit

Amelioration of herbivory (*in situ*) and PL (*in silico* based on field estimates) together increased predicted mean lifetime fitness three- to sevenfold outside the range edge (Just Beyond: 630% increase relative to uncaged plants with no simulated pollen supplementation; Beyond: 342%; Far Beyond: 251%). At all sites, this fitness increase was primarily due to prevention of herbivory, but pollen supplementation in silico further increased lifetime fitness at the Beyond and Far Beyond sites by 7% and 19%, respectively.

Discussion

Evolutionary and ecological studies of species distributions often make assumptions about climatic factors primarily driving range limits, but there is no *a priori* reason to think biotic interactions are of any less import for determining geographic range boundaries. Given their demonstrated effects on local adaptation and population dynamics, the potential for biotic interactions to influence large-scale distributions is increasingly discussed in the literature (e.g., Araújo and Luoto 2007; Van der Putten et al. 2010; HilleRisLambers et al. 2013; Wisz et al. 2013; Hargreaves et al. 2014; Godsoe et al. 2015; O'Brien et al. 2017; Bridle et al. 2019). However, there is a paucity of studies that move beyond correlational approaches to examine the influence of species interactions on range limits (Louthan et al. 2015). In the present study, we found that biotic interactions contribute significantly to maladaptation beyond the range limit of *xantiana*, where abiotic conditions become increasingly stressful. There was also a strong temporal abiotic × biotic interaction—the effects of fatal herbivory were strongest when high precipitation led to relatively benign abiotic conditions beyond the range limit. Together these results illustrate how the interplay of abiotic and biotic factors across complex environmental gradients can limit species' geographic distributions.

FITNESS VARIATION IN THE ABSENCE OF PLANT-ANIMAL INTERACTIONS

Variability in precipitation drove both temporal and spatial variation in *xantiana* fitness. During year 1, sites within the range received near or above average precipitation, whereas sites outside the range received below average rainfall. Those sites outside the range limit received less than half the precipitation than did sites inside the range, which led to relatively high *xantiana* fitness at all sites inside the range limit, and mean fitness near zero outside of it (Supporting Information 5; Fig. S1). At Center, relatively strong performance at all life history stages led to high fitness, but the site was most differentiated from other within-range sites by high fecundity (seed set), which resulted from the large size of plants at this site (on average, fruiting plants at Center produced ca. four times the number of fruits as plants elsewhere within the range). Outside the range edge, low fitness mainly resulted from a combination of low germination rates, low late season survival, and low fecundity.

In year 2, all sites received above average growing season rainfall, which led to high mean lifetime fitness at all sites. In contrast to year 1, for uncaged plants, mean fitness was relatively equal within and beyond the range edge. Sites inside the range tended to have lower germination but higher late survival than sites outside the range. This relative parity of performance between regions was realized, even though the two regions still received substantially different amounts of rainfall in year 2 (70% more rainfall within range). Supplementary analyses support the idea that lifetime fitness tended to decrease with increasing precipitation in year 2, when water availability was not likely limiting (Supporting Information 5; Fig. S1). This pattern suggests that plants outside the range edge were able to better capitalize on the adequate water resources of year 2 than plants inside the range, potentially because of less severe competition with other forbs and grasses. For example, the site where fitness was highest in year 1, Center, had much lower mean fitness in year 2, largely due to a reduction in germination rates. Observations lead us to believe that this was likely due to vigorous growth of annual grasses at the site (i.e., plant-plant interactions). These grasses germinate early and grow quickly after the first winter rains, and in year 2 could have either prevented germination or caused early death of young *xantiana* germinants (which we could not observe and thus scored as a lack of germination).

For a species distributed across a continuous environmental gradient, it is generally thought that dispersal and subsequent local adaptation of peripheral populations to that gradient will enable range expansion (Mayr 1963; Kirkpatrick and Barton 1997; Bridle and Vines 2007; Polechová 2018). We found evidence of strong local adaptation for the Center population in year 1, but not for the Edge population, and no indication that Edge populations fared better outside the range limit. Interestingly, there was a trend of the Edge population outperforming the Center population at the Edge site when protected from herbivory ($P = 0.06$). This suggests that the Edge population may be locally adapted to other aspects of the Edge site, such as lower precipitation, but not to increased fatal herbivory at the range edge. Adaptation to local conditions at the range edge, and the associated increase in population size, has important demographic (e.g., export of colonists) and genetic (e.g., increased variation, reduced influence of drift, potential "preadaptation") consequences for range expansion (Kawecki 2008). Geber and Eckhart (2005) also found no evidence of regional adaptation of edge *xantiana* populations; this lack of a signal of adaptation to the local environment at the range edge merits consideration. Theory predicts that lack of local adaptation in edge populations could arise from maladaptive gene flow from more abundant central populations (Haldane 1956; Antonovics 1976; Kirkpatrick and Barton 1997), or from the fact that smaller edge populations simply harbor less genetic variation upon which selection can act or are more prone to the effects of genetic drift (Whitlock 2004; Sexton et al. 2011). For *xantiana*, despite very high effective population sizes at the range edge, edge populations do exhibit modest reductions in private alleles (Moeller et al. 2011), although this may not be a good analog for quantitative genetic variation in ecologically important traits (Reed and Frankham 2001). Moeller et al. (2011) also found some evidence of asymmetric gene flow from central to edge populations, which could potentially "swamp" locally adaptive alleles over long time scales. Furthermore, recent theory has highlighted how the demographic cost of such maladaptive gene flow can enable genetic drift to overpower selection in range edge populations, leading to the formation of a range limit (Polechová and Barton 2015; Polechová 2018). These hypotheses deserve further attention in *xantiana*.

In year 2, there was no evidence for local adaptation of any population. Although we certainly expect local adaptation of many populations (Antonovics 1987), we should not expect it to be temporally consistent if the environment is not (O'Brien et al. 2017; Brady et al. 2019). Homing in on an optimal phenotype is made more difficult by temporal variation in selection (Milner et al. 1999; Kirkpatrick and Peischl 2013; Hao et al. 2015), and it is unlikely for a phenotype to arise anywhere that is optimally adapted to all possible conditions at that site across years. Rather, theory predicts that fluctuating environments will often select for "intermediate" phenotypes that perform best in the "average" environment (Sæther and Engen 2015).

BIOTIC INTERACTIONS LIMIT FITNESS BEYOND THE RANGE EDGE

One major goal of this study was to estimate the effect of an antagonistic biotic interaction, fatal mammal herbivory, on lifetime fitness in *xantiana* using experimental field manipulations within and outside the subspecies' range. Previous work has identified two lagomorph species, the desert cottontail (*Sylvilagus audubonii*) and the black-tailed jackrabbit (*Lepus californicus*), as the primary herbivores causing mortality of *xantiana* inside and outside its range (Geber and Eckhart 2005; Benning et al. 2019). In the first year of our experiment, herbivory had little effect on mean lifetime fitness except at one site with high rates of herbivory (Edge), and geographic patterns in fitness largely reflected spatial variation in rainfall. In year 2, when experimental populations were partially "released" from abiotic limitations due to increased rainfall, protection from herbivores had large effects on mean fitness at all sites outside the range, and one site inside the range. For a *xantiana* population to persist outside its current range limit, low mean fitness in low precipitation years would have to be offset by high mean fitness in high precipitation years. As noted in Geber and Eckhart (2005), *xantiana* population persistence in hypervariable environments like the southern Sierra Nevada likely depends on both seed dormancy to temper the effects of "poor" years and large inputs into a seed bank in years of favorable climate (e.g., Templeton and Levin 1979; Pake and Venable 1996). In this study, uncaged plants outside the range had the capacity to capitalize on adequate water resources in the high precipitation year 2 and, despite herbivory, achieved mean fitness levels roughly on par with sites inside the range. However, protection from herbivory outside the range led to three- to sevenfold increases in mean fitness of caged plants, resulting in fitness estimates even higher than those inside the range.

Demographically, this large fitness increase due to release from herbivore pressure would provide an additional buffer against years of poor abiotic conditions outside the range limit. For annual plants like *xantiana*, population mean lifetime fitness approximates population growth rate. It is interesting to note that at sites with high herbivory in year 2, only caged plants had mean lifetime fitness values whose 95% confidence intervals exceeded one (i.e., demographic replacement). In terms of adaptive evolution, any increase in effective population size would also make it more likely that colonizing populations of *xantiana* would adapt to the novel environment outside the current range edge, and decrease the influence of drift in colonizing populations (Kawecki 2008). Not only do herbivores have the potential to reduce *xantiana* population sizes, in this experiment they also preferentially ate larger plants (Supporting Information 4). Given that a large *xantiana* individual can produce thousands of seeds, herbivore preference for large plants could result in an outsized effect on mean population fitness.

The results of our PL experiment showed that although PL was both more likely and significant outside the range limit, the magnitude of PL was somewhat modest—supplemented flowers set, on average, 8% more seeds than unsupplemented controls outside the range limit. Estimates of PL increased with increasing distance beyond the range edge, with the strongest effects of supplementation (PL = 0.19) at the Far Beyond site. This likely reflects the parallel geographic trend of decreasing pollinator abundance and diversity that we found previously (Moeller 2006; Moeller et al. 2012). The results of this experiment suggest that PL is unlikely to be high enough to, on its own, prevent *xantiana* populations from establishing outside the subspecies' eastern range limit. It is important to note, however, that a colonizing population of *xantiana* would be significantly smaller than those in the current experiment, and past evidence indicates that reproduction can be strongly pollen limited in small populations (Moeller 2004; Moeller and Geber 2005).

GRADIENTS IN BIOTIC INTERACTIONS

Recent evolutionary models suggest that range limits can be mainly explained by the influence of genetic drift on leading-edge populations, and the fitness cost of migration beyond the current range limit (Polechová and Barton 2015; Polechová 2018). The fitness cost of migration is determined by the steepness of the relevant environmental gradient, which determines the rate at which the optimal phenotype shifts. One of the most interesting patterns to emerge from these and another recent theoretical inquiry (Bridle et al. 2019) is how nonlinear environmental gradients seem to be essential for the collapse of local adaptation and the formation of an abrupt range edge. Both this transplant experiment and our previous work mapping spatial gradients in herbivory (Benning et al. 2019) demonstrate that the probability of fatal herbivory increases sharply near *xantiana*'s eastern range limit. Our previous work also provided evidence that this steep, nonlinear gradient in herbivory is tied to a steep cline in optimal phenology, with faster development enabling escape from herbivory and therefore favored outside the range (Benning et al. 2019). Other work has shown that the abundance of pollinators declines sharply along this same spatial gradient, with *Clarkia* specialist bees dropping out completely beyond the range limit (Moeller 2006), which may result in a similarly steep gradient in optimal values of floral traits like herkogamy (Moeller and Geber 2005; Moeller 2006). These steep gradients in optimal phenotype may be too great an adaptive hurdle for colonizing *xantiana* populations to overcome.

ABIOTIC × BIOTIC INTERACTIONS

In a recent paper, Louthan et al. (2015) reviewed the "Species Interactions—Abiotic Stress Hypothesis" (SIASH), which posits that biotic interactions will dominate in abiotically benign environments, whereas abiotic conditions will largely control population growth in abiotically stressful environments (*sensu* Darwin 1859; Dobzhansky 1950; MacArthur 1972). The results of the current experiments do not align with the SIASH, at least interpreted in a spatial context as it classically is—most measures of "stress" on population growth rate tend to vary collinearly for *xantiana*. As one moves from the center of the range to the eastern edge, rainfall decreases and becomes more variable, and mammal herbivory and PL increase. However, our results do align with SIASH in a *temporal* context—biotic interactions were relatively more important, in terms of their effects on mean fitness, in year 2 when the abiotic conditions outside the range edge were more benign. Thus, the negative effects of herbivory were realized most strongly in years that would be the most important for promoting long-term persistence outside the range limit, a finding consistent with a meta-analysis of the effects of invertebrate herbivory on plant population growth (Katz 2016).

Conclusion

The conclusion that "range limits are complex" perhaps should not surprise us, given the demonstrated power of natural selection to produce adaptations to novel environments. The results above highlight what Joseph Grinnell observed in 1917—geographic range margins are multifaceted phenomena that will most often result from multiple interacting factors. In addition, the relative importance of these factors can vary temporally, requiring multiple year studies to detect. Although correlative approaches can provide initial insights into the environmental variables associated with species' distributional limits, manipulative experiments are necessary for robust tests of specific hypotheses. For *xantiana*, transplant experiments have suggested that limited precipitation contributes to maladaptation beyond the range limit. As in many systems, this was simple to predict given that the range limit falls along an obvious abiotic gradient. Much less obvious was the spatial gradient in fatal herbivory that drove severe fitness losses beyond the range boundary, particularly in the abiotically favorable year when population growth rates could potentially be high. Generally, gradients in biotic interactions are easy to overlook given that many are difficult to measure without significant field efforts, and/or vary collinearly with abiotic gradients. However, these interactions may be pivotal in explaining the sharp geographic gradients in fitness that theory predicts are most likely to prevent range expansion (Polechová 2018; Bridle et al. 2019).

AUTHOR CONTRIBUTIONS

JWB and DAM designed and conducted the experiments. JWB performed all analyses with substantial input from DAM. JWB wrote the manuscript with DAM contributing substantially to revisions.

ACKNOWLEDGMENTS

The authors thank Lana Bolin, Haley Branch, Alexai Faulkner, Adam Kostanecki, Sarah Tran, and Anna Peschel for assistance with field and lab work. The authors appreciate insightful comments from Peter Kennedy, Ruth Shaw, and Peter Tiffin on experimental design, analyses, and interpretation of results. Our work was generously supported by grants from the National Science Foundation (DEB-1701072 to JWB and DAM and DEB-1255141 to DAM), the Society for the Study of Evolution (JWB), and the Bell Museum at the University of Minnesota (JWB). Any opinions, findings, and conclusions expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

DATA ARCHIVING

Pending acceptance, all data and code will be archived in the Dryad Digital Repository. The doi for data is https://doi.org/10.5061/dryad.8mb0c27.

LITERATURE CITED

Afkhami, M. E., P. J. McIntyre, and S. Y. Strauss. 2014. Mutualist-mediated effects on species' range limits across large geographic scales. Ecol. Lett. 17:1265–1273.

Alexander, J. M., L. Chalmandrier, J. Lenoir, T. I. Burgess, F. Essl, S. Haider, C. Kueffer, K. McDougall, A. Milbau, M. A. Nuñez, et al. 2018. Lags in the response of mountain plant communities to climate change. Glob. Chang. Biol. 24:563–579.

Anderson, J. T., V. M. Eckhart, and M. A. Geber. 2015. Experimental studies of adaptation in *Clarkia xantiana*. III. Phenotypic selection across a subspecies border. Evolution 69:2249–2261.

Angert, A. L., and D. W. Schemske. 2005. The evolution of species' distributions: reciprocal transplants across the elevation ranges of *Mimulus cardinalis* and *M. lewisii*. Evolution 59:1671–1684.

Angert, A. L., H. D. Bradshaw Jr., and D. W. Schemske. 2008. Using experimental evolution to investigate geographic range limits in monkeyflowers. Evolution 62:2660–2675.

Antonovics, J. 1976. The nature of limits to natural selection. Ann. Mo. Bot. Gard. 63:224–247.

———. 1987. The evolutionary dys-synthesis: which bottles for which wine? Am. Nat. 129:321–331.

Aragón, P., and D. Sánchez-Fernández. 2013. Can we disentangle predator–prey interactions from species distributions at a macro-scale? A case study with a raptor species. Oikos 122:64–72.

Araújo, M. B., and M. Luoto. 2007. The importance of biotic interactions for modelling species distributions under climate change. Glob. Ecol. Biogeogr. 16:743–753.

Baer, K. C., and J. L. Maron. 2018. Pre-dispersal seed predation and pollen limitation constrain population growth across the geographic distribution of *Astragalus utahensis*. J. Ecol. 106:1646–1659.

Benning, J. W., V. M. Eckhart, M. A. Geber, and D. A. Moeller. 2019. Biotic interactions contribute to the geographic range limit of an annual plant:

herbivory and phenology mediate fitness beyond a range margin. Am. Nat. 193:786–797

Brady, S., D. I. Bolnick, R. D. H. Barrett, L. J. Chapman, E. Crispo, A. M. Derry, C. G. Eckert, D. J. Fraser, G. F. Fussmann, A. Gonzalez, et al. 2019. Understanding maladaptation by uniting ecological and evolutionary perspectives. Am. Nat. https://doi.org/10.1086/705020

Bridle, J. R., and T. H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends Ecol. Evol. 22:140–147.

Bridle, J. R., M. Kawata, and R. K. Butlin. 2019. Local adaptation stops where ecological gradients steepen or are interrupted. Evol. App. 12:1449–1462.

Briscoe Runquist, R. D. and D. A. Moeller. 2013. Resource reallocation does not influence estimates of pollen limitation or reproductive assurance in Clarkia xantiana subsp. parviflora (Onagraceae). Am. J. Bot. 100:1916–1921.

Bruelheide, H., and U. Scheidel. 1999. Slug herbivory as a limiting factor for the geographical range of *Arnica montana*. J. Ecol. 87:839–848.

Bullock, J. M., R. J. Edwards, P. D. Carey, and R. J. Rose. 2000. Geographical separation of two *Ulex* species at three spatial scales: does competition limit species' ranges? Ecography 23:257–271.

Buehler, R.J. 1957. Confidence intervals for the product of two binomial parameters. J. of the Amer. Stat. Assoc. 52:482–493.

Case, T. J., and M. L. Taper. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. Am. Nat. 155:583–605.

Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science 333:1024–1026.

Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London, UK.

deRivera, C. E., G. M. Ruiz, A. H. Hines, and P. Jivoff. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 86:3364–3376.

Dobzhansky, T. 1950. Evolution in the tropics. Am. Sci. 38:209–221.

Eckhart, V. M., and M. A. Geber. 1999. Character variation and geographic distribution of *Clarkia xantiana* A. Gray (Onagraceae): flowers and phenology distinguish two subspecies. Madroño 46:117–125.

Eckhart, V. M., I. Singh, A. M. Louthan, A. J. Keledjian, A. Chu, D. A. Moeller, and M. A. Geber. 2010. Plant-soil water relations and species border of *Clarkia xantiana* ssp. *xantiana* (Onagraceae). Int. J. Plant Sci. 171:749–760.

Eckhart, V. M., M. A. Geber, W. F. Morris, E. S. Fabio, P. Tiffin, and D. A. Moeller. 2011. The geography of demography: long-term demographic studies and species distribution models reveal a species border limited by adaptation. Am. Nat. 178:S26–43.

Emery, N. C., M. L. Stanton, and K. J. Rice. 2009. Factors driving distribution limits in an annual plant community. New Phytol. 181:734–747.

Ettinger, A., and J. HilleRisLambers. 2017. Competition and facilitation may lead to asymmetric range shift dynamics with climate change. Glob. Chang. Biol. 23:3921–3933.

Ettinger, A. K., K. R. Ford, and J. HilleRisLambers. 2011. Climate determines upper, but not lower, altitudinal range limits of Pacific Northwest conifers. Ecology 92:1323–1331.

Fourcade, Y., A. G. Besnard, and J. Secondi. 2018. Paintings predict the distribution of species, or the challenge of selecting environmental predictors and evaluation statistics. Glob. Ecol. Biogeogr. 27:245–256.

Gaston, K. J. 2009. Geographic range limits: achieving synthesis. Proc. R. Soc. Lond. B Biol. Sci. 276:1395–1406.

Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in *Clarkia xantiana*. II. Fitness variation across a subspecies border. Evolution 59:521–531.

Geyer, C. J. 2009. Likelihood inference in exponential families and directions of recession. Electron. J. Stat. 3:259–289.

Geyer, C. J., S. Wagenius, and R. G. Shaw. 2007. Aster models for life history analysis. Biometrika 94:415–426.

Godsoe, W., R. Murray, and M. J. Plank. 2015. Information on biotic interactions improves transferability of distribution models. Am. Nat. 185:281–290.

Gould, B., D. A. Moeller, V. M. Eckhart, and P. Tiffin. 2014. Local adaptation and range boundary formation in response to complex environmental gradients across the geographical range of *Clarkia xantiana* ssp. *xantiana*. J. Ecol. 102:95–107.

Gravel, D., F. Massol, E. Canard, D. Mouillot, and N. Mouquet. 2011. Trophic theory of island biogeography. Ecol. Lett. 14:1010–1016.

Griffith, T. M., and M. A. Watson. 2006. Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. Am. Nat. 167:153–164.

Grinnell, J. 1917. Field tests of theories concerning distributional control. Am. Nat. 51:115–128.

Haldane, J. B. 1956. The relation between density regulation and natural selection. Proc. R. Soc. Lond. B Biol. Sci. 145:306–308.

Hao, Y.-Q., M. A. Brockhurst, O. L. Petchey, and Q.-G. Zhang. 2015. Evolutionary rescue can be impeded by temporary environmental amelioration. Ecol. Lett. 18:892–898.

Hargreaves, A. L., K. E. Samis, and C. G. Eckert. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. Am. Nat. 183:157–173.

HilleRisLambers, J., M. A. Harsch, A. K. Ettinger, K. R. Ford, and E. J. Theobald. 2013. How will biotic interactions influence climate change-induced range shifts? Ann. N. Y. Acad. Sci. 1297:112–125.

Hochberg, M. E., and A. R. Ives. 1999. Can natural enemies enforce geographical range limits? Ecography 22:268–276.

Hutchinson, G. E. 1957. Concluding remarks. Cold. Spring. Harb. Symp. Quant. Biol. 22:415–427.

Katz, D. S. W. 2016. The effects of invertebrate herbivores on plant population growth: a meta-regression analysis. Oecologia 182:43–53.

Katz, D. S. W., and I. Ibáñez. 2017. Differences in biotic interactions across range edges have only minor effects on plant performance. J. Ecol. 105:321–331.

Kawecki, T. J. 2008. Adaptation to marginal habitats. Annu. Rev. Ecol. Evol. Syst. 39:321–342.

Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a species' range. Am. Nat. 150:1–23.

Kirkpatrick, M., and S. Peischl. 2013. Evolutionary rescue by beneficial mutations in environments that change in space and time. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20120082.

Lee-Yaw, J. A., H. M. Kharouba, M. Bontrager, C. Mahony, A. M. Csergő, A. M. E. Noreen, Q. Li, R. Schuster, and A. L. Angert. 2016. A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. Ecol. Lett. 19:710–722.

Levin, D. A., and K. Clay. 1984. Dynamics of synthetic *Phlox drummondii* populations at the species margin. Am. J. Bot. 71:1040–1050.

Louthan, A. M., D. F. Doak, and A. L. Angert. 2015. Where and when do species interactions set range limits? Trends Ecol. Evol. 30:780–792.

MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. Harper and Row, New York.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press. Cambridge, MA.

Milner, J. M., S. D. Albon, A. W. Illius, J. M. Pemberton, and T. H. Clutton-Brock. 1999. Repeated selection of morphometric traits in the Soay sheep on St Kilda. J. Anim. Ecol. 68:472–488.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology 85:3289–3301.

Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant-pollinator interactions in Clarkia xantiana ssp. xantiana. Oecologia 142:28–37.

Moeller, D. A. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. Ecology 87:1510–1522.

Moeller, D. A., and M. A. Geber. 2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. Evolution 59:786–799.

Moeller, D. A., M. A. Geber, and P. Tiffin. 2011. Population genetics and the evolution of geographic range limits in an annual plant. Am. Nat. 178:S44–57.

Moeller, D. A., M. A. Geber, V. M. Eckhart, and P. Tiffin. 2012. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. Ecology 93:1036–1048.

Nadeau, C. P., M. C. Urban, and J. R. Bridle. 2017. Climates past, present, and yet-to-come shape climate change vulnerabilities. Trends Ecol. Evol. 32:786–800.

O'Brien, E. K., M. Higgie, A. Reynolds, A. A. Hoffmann, and J. R. Bridle. 2017. Testing for local adaptation and evolutionary potential along altitudinal gradients in rainforest Drosophila: beyond laboratory estimates. Glob. Chang. Biol. 23:1847–1860.

Pake, C. E., and D. L. Venable. 1996. Seed banks in desert annuals: implications for persistence and coexistence in variable environments. Ecology 77:1427–1435.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

Polechová, J. 2018. Is the sky the limit? On the expansion threshold of a species' range. PLoS Biol. 16:e2005372.

Polechová, J., and N. H. Barton. 2015. Limits to adaptation along environmental gradients. Proc. Natl. Acad. Sci. USA 112:6401–6406.

PRISM Climate Group. 2004. PRISM climate data. Oregon State University, Corvallis, OR. http://prism.oregonstate.edu

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.

Rumpf, S. B., K. Hülber, G. Klonner, D. Moser, M. Schütz, J. Wessely, W. Willner, N. E. Zimmermann, and S. Dullinger. 2018. Range dynamics of mountain plants decrease with elevation. Proc. Natl. Acad. Sci. USA 115:1848–1853.

Runquist, R. D., E. Chu, J. L. Iverson, J. C. Kopp, and D. A. Moeller. 2014. Rapid evolution of reproductive isolation between incipient outcrossing and selfing *Clarkia* species. Evolution 68:2885–2900.

Sæther, B.-E., and S. Engen. 2015. The concept of fitness in fluctuating environments. Trends Ecol. Evol. 30:273–281.

Samis, K. E., and C. G. Eckert. 2009. Ecological correlates of fitness across the northern geographic range limit of a Pacific Coast dune plant. Ecology 90:3051–3061.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annu. Rev. Ecol. Evol. Syst. 40:415–436.

Sexton, J. P., S. Y. Strauss, and K. J. Rice. 2011. Gene flow increases fitness at the warm edge of a species' range. Proc. Natl. Acad. Sci. USA 108:11704–11709.

Shaw, R. G., C. J. Geyer, S. Wagenius, H. H. Hangelbroek, and J. R. Etterson. 2008. Unifying life-history analyses for inference of fitness and population growth. Am. Nat. 172:E35–E47.

Stanton-Geddes, J., P. Tiffin, and R. G. Shaw. 2012. Role of climate and competitors in limiting fitness across range edges of an annual plant. Ecology 93:1604–1613.

Svenning, J.-C., S. Normand, and F. Skov. 2008. Postglacial dispersal limitation of widespread forest plant species in nemoral Europe. Ecography 31:316–326.

Templeton, A. R., and D. A. Levin. 1979. Evolutionary consequences of seed pools. Am. Nat. 114:232–249.

Urban, M. C., G. Bocedi, A. P. Hendry, J.-B. Mihoub, G. Pe'er, A. Singer, J. R. Bridle, L. G. Crozier, L. De Meester, W. Godsoe, et al. 2016. Improving the forecast for biodiversity under climate change. Science 353:aad8466.

Van der Putten, W. H., M. Macel, and M. E. Visser. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365:2025–2034.

Whitlock, M. C. 2004. Selection and drift in metapopulations. Pp. 153–174 *in* I. Hanski and O. Gaggiotti, eds. Ecology, genetics and evolution of metapopulations. Elsevier Academic Press, Burlington, MA.

Wisz, M. S., J. Pottier, W. D. Kissling, L. Pellissier, J. Lenoir, C. F. Damgaard, C. F. Dormann, et al. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. Biol. Rev. Camb. Philos. Soc. 88:15–30.

Associate Editor: M. Cardillo
Handling Editor: D. W. Hall

Supporting Information

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

Table S1. Site locations, growing season precipitation, and fatal herbivory during experiment years.

Table S2. Average plant size of caged and uncaged plants at each site in year 1 after ca. 2 months of caging.

Figure S1. Mean lifetime fitness plotted against growing season precipitation for years 1 (a) and 2 (b); data points are site means of plants in the caged treatment. Linear regressions with 95% confidence bands are overlaid on both plots; slope is not significantly different from zero in plot b.