

Biotic Interactions Contribute to the Geographic Range Limit of an Annual Plant: Herbivory and Phenology Mediate Fitness beyond a Range Margin

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ABSTRACT: Species' geographic distributions have already shifted during the Anthropocene. However, we often do not know what aspects of the environment drive range dynamics, much less which traits mediate organisms' responses to these environmental gradients. Most studies focus on possible climatic limits to species' distributions and have ignored the role of biotic interactions, despite theoretical support for their importance in setting distributional limits. We used field experiments and simulations to estimate contributions of mammalian herbivory to a range boundary in the Californian annual plant *Clarkia xantiana* ssp. *xantiana*. A steep gradient of increasing probability of herbivory occurred across the boundary, and a reanalysis of prior transplant experiments revealed that herbivory drove severalfold declines in lifetime fitness at and beyond the boundary. Simulations showed that populations could potentially persist beyond the range margin in the absence of herbivory. Using data from a narrowly sympatric subspecies, *Clarkia xantiana* parviflora, we also showed that delayed phenology is strongly associated with *C. xantiana* ssp. *xantiana*'s susceptibility to herbivory and low fitness beyond its border. Overall, our results provide some of the most comprehensive evidence to date of how the interplay of demography, traits, and spatial gradients in species interactions can produce a geographic range limit, and they lend empirical support to recent developments in range limits theory.

Keywords: geographic range limit, herbivory, phenology, biotic interactions, adaptation, *Clarkia xantiana* ssp. *xantiana*.

Introduction

Understanding the causes of species' geographic range limits is a fundamental problem in ecology and evolution. For the vast majority of species, however, we still cannot answer

why an organism occurs on one side of its range boundary and not the other (Gaston 2009). Pinpointing the underlying environmental drivers and demographic and genetic mechanisms restricting species distributions is of utmost importance for understanding species' responses to global change (Alexander et al. 2015; Ettinger and HilleRisLambers 2017), the spread of invasive species (Colautti et al. 2010), and the limits to natural selection (Antonovics 1976; Kawecki 2008).

Some species have simply not had time to colonize environmentally suitable areas (dispersal lag; Svenning et al. 2008; Alexander et al. 2017), and in other cases, abrupt dispersal barriers can prevent range expansion (Chardon et al. 2015; Weir et al. 2015). However, most species' borders occur along seemingly gradual environmental gradients (Kirkpatrick and Barton 1997; Sexton et al. 2009) and likely reflect underlying variation in the environment across the landscape and corresponding variation in adaptation. Species may be restricted to their current distribution simply because they are maladapted to the environment beyond their range boundary.

Several theoretical models address the apparent failure of natural selection to result in adaptation to novel environments outside a species' range (e.g., Kirkpatrick and Barton 1997; Case and Taper 2000; Polechová and Barton 2015). Population dynamics in these models are based on the difference between a population's realized value of some important trait and the optimal trait value dictated by the environment; this difference determines the degree of population maladaptation and population growth (Kirkpatrick and Barton 1997). A key factor in these models of range limits is the steepness of the environmental gradient along which populations must adapt. As gradients become steeper, adaptation to areas outside the current range becomes less likely due to high levels of maladaptation in colonists dispersing from the range edge; with shallow gradients, adaptation and expansion of the range limit can proceed (Kirkpatrick and Barton 1997; Polechová

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and Barton 2015). Most models assume linear gradients in environmental variables, but nonlinear gradients can be especially important in generating distributional limits due to rapid change in the optimal phenotype across space (Case and Taper 2000; Polechová and Barton 2015).

Given the central role of environmental gradients in structuring species' distributions, identifying important gradients is usually a first goal of range limit studies, with climatic variables being likely candidates. While climatic niche limits often do explain species' distributions (Lee-Yaw et al. 2016), it is increasingly recognized that biotic interactions can contribute to large-scale distributional limits (Bruehlheide and Scheidel 1999; Hochberg and Ives 1999; Case and Taper 2000; Briers 2003; Case et al. 2005; deRivera et al. 2005; Araújo and Luoto 2007; Holt and Barfield 2009; Gravel et al. 2011; Stanton-Geddes and Anderson 2011; Moeller et al. 2012; HilleRisLambers et al. 2013; Afkhami et al. 2014; Hargreaves et al. 2014; Louthan et al. 2015; Baer and Maron 2018). However, most evidence for biotic interactions influencing range limits is correlational, and there is a paucity of empirical studies that connect spatial gradients in biotic interactions to population demography and the geographic range limits of native species (Louthan et al. 2015).

Though correlative approaches such as species distribution models lend first insights into potential drivers of range limits, transplant experiments including sites outside the range limit are the only way to test range-boundary hypotheses directly (Hargreaves et al. 2014). When paired with field measurements of potentially important traits, transplant experiments can reveal trait-environment relationships that underlie geographic variation in performance (Hoffmann and Blows 1994; Angert et al. 2008; Sexton et al. 2009; Hargreaves et al. 2014). These trait-environment correlations can then be investigated further through direct manipulation of traits (e.g., production of a segregating F_2 generation to generate phenotypic variation in traits of interest [e.g., Angert et al. 2008] or directly manipulating traits such as phenology [e.g., Griffith and Watson 2006]).

Investigating ecological causes of a species' distributional limit thus has three main components: characterizing environmental gradients, linking gradients to individual and population fitness, and determining the trait(s) mediating fitness responses. Studies rarely tackle these three points in concert (but see Angert et al. 2008), especially in regard to biotic interactions. Here we investigate the role of an antagonistic interaction, fatal mammalian herbivory, in limiting the range of an annual plant, *Clarkia xantiana* ssp. *xantiana*. With 2 years of stem translocation experiments, we showed that herbivory exhibits a steep, nonlinear gradient across a major range boundary. Based on a 2-year reciprocal transplant experiment across the same boundary (Geber and Eckhart 2005), we calculated the magnitude of mammalian herbivory over

C. x. xantiana's full life span and used those estimates in simulations of herbivory's effects on population mean fitness. These simulations revealed large reductions in population growth rates due to herbivory at the range margin and outside the range and showed that, in the absence of herbivory, populations could potentially persist outside the range margin. Finally, we showed that susceptibility to herbivory is strongly associated with a specific plant trait, phenology, beyond the range margin.

Material and Methods

Study System

Clarkia xantiana comprises two annual subspecies, *Clarkia xantiana* ssp. *xantiana* A. Gray and *Clarkia xantiana* ssp. *parviflora* (Eastw.) Harlan Lewis and P.H. Raven (hereafter, *xantiana* and *parviflora*). Their combined range in the southern Sierra Nevada foothills spans a complex west-to-east environmental gradient, with *xantiana* found in the wetter western region in oak woodlands and *parviflora* found in the eastern region in arid scrub and pinyon-juniper woodland (fig. 1; Eckhart and Geber 1999).

The two taxa are in secondary contact (in a narrow zone of sympatry) after diverging ca. 65,000 years ago (Pettengill and Moeller 2012a, 2012b) and have differentiated most strongly in mating system and phenology (Eckhart and Geber 1999). *Parviflora* completes its life cycle more quickly than *xantiana*, which contributes to the near-complete reproductive isolation between the subspecies (Briscoe Runquist et al. 2014). A reciprocal transplant experiment showed each subspecies to be strongly locally adapted to its own home range, and there was preliminary observation that herbivory by small mammals influenced *xantiana*'s performance beyond its range edge (Geber and Eckhart 2005). Mammalian herbivory occurs most often from two lagomorphs, the desert cottontail (*Sylvilagus audubonii*) and the black-tailed jackrabbit (*Lepus californicus*), and less often from smaller rodents. These herbivores typically cause fatal herbivory, where the entire aboveground portion of a plant is removed (e.g., clipped at the stem base) and the plant does not resprout to set any seed.

We used two data sets in the analyses presented below. The more recent (2015–2016) uses stem translocation experiments to model fine-scale geographic trends in the probability of fatal herbivory for *xantiana*, which allows us to link these results to new range limits theory on geographic gradients in trait optima. To provide a uniquely comprehensive picture of how this biotic interaction affects fitness within and outside a range limit, we also analyze a previously published data set (transplant experiment years 1997–1999; Geber and Eckhart 2005) that includes information on lifetime fitness and herbivory at three sites: at

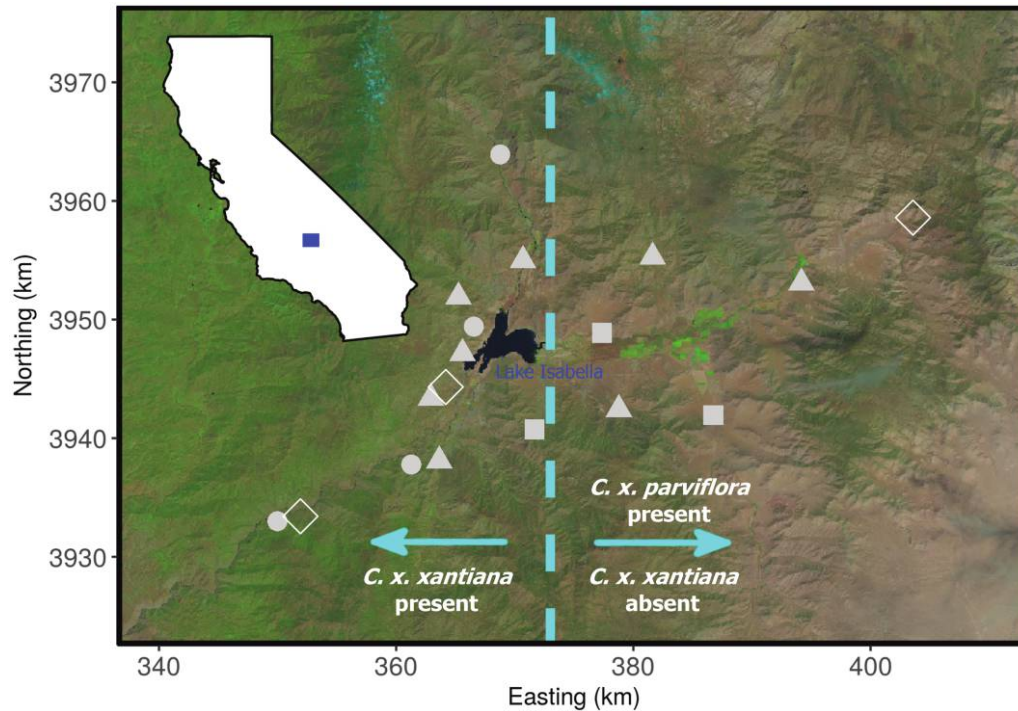


Figure 1: Geographic distribution of *Clarkia xantiana*, where the dashed blue line marks ssp. *xantiana*'s eastern range limit. The bulk of ssp. *parviflora*'s distribution lies east of this limit, though the two taxa share a narrow zone of sympatry around Lake Isabella. *Parviflora*'s western range edge is located near 360 km easting. Points mark locations of stem translocation sites (circles: 2015; triangles: 2016; squares: both years) and reciprocal transplant sites (diamonds). The background image is Landsat imagery of the study area on April 19, 2016.

xantiana's range center, at the range edge, and beyond the range.

Quantifying Gradients in Herbivory across and beyond the Range

To identify fine-scale spatial and temporal variation in plant-herbivore interactions, we performed a stem translocation experiment across 2 years at 15 sites inside and outside *xantiana*'s range. Clipping living adult stems from natural populations to establish experimental arrays, we quantified herbivory while avoiding confounding spatial variation in genotype, plant size, or phenology found among *xantiana* populations. Experiments were conducted in or near natural *xantiana* and *parviflora* populations.

In 2015, we quantified broadscale variation in herbivory across most of the west-to-east extent of *xantiana*'s range and beyond the range limit. We sourced *xantiana* stems from the center of the range and within 6 km of the eastern edge; stems were collected from plants that were still green (i.e., with buds, flowers, and/or immature fruits). We placed stems at seven sites (two at range center, three at range edge, two that were 5 and 14 km beyond the eastern range limit; fig. 1). At each site, we installed two transects of 24 stems, alternating

central and edge genotypes, with stems placed 1 m apart. Plant stems were maintained in 13-cm florist picks filled with water and secured with an attached metal rod sunk into the ground (fig. A1a, A1b; figs. A1–A3, B1–B3, C1–C3 are available online). Plants maintained in this way continued to open new flowers and set fruits after pollination (J. W. Benning, personal observation). To explore temporal variation in herbivory, we installed four temporal replicates of stems in May and June (approximately once per week from May 24 to June 19) at each site. For each temporal replicate, we scored stems for fatal herbivory (having no buds, flowers, or fruits remaining, usually because most of the stem was completely removed) 5 days after installation (fig. A1d). At the five sites within *xantiana*'s range, we also followed naturally occurring plants near experimental arrays to determine whether geographic patterns of herbivory on experimental plants mimicked those on natural plants (app. sec. A1; apps. A–C are available online).

Our 2015 experiment showed that herbivory was low in the range center and much stronger at the range edge and beyond; however, the coarse geographic scale covered did not allow for a fine-scale characterization of the environmental gradient at the range limit. In 2016, we established experimental arrays in six sites near to or at the range limit and five

sites outside the range limit (fig. 1). As the 2015 experiment showed no effect of population source (central vs. edge genotypes), plants used in 2016 were a mixture of genotypes from across the range. At each site, we installed three transects of 10 stems placed 1 m apart. In an attempt to further mimic natural plant conditions, we placed 2016 stems in 50-mL conical tubes sunk completely into the ground (fig. A1*b*). We installed three temporal replicates of stems at each site and scored herbivory 5 days after installation. In 2016, wildfires destroyed the third round of experimental stems at three sites.

We used logistic regression to test the effects of easting (i.e., longitude), time (temporal replicate), and, in 2015, genotype source (central vs. edge), and all interactions, on the probability of herbivory. For both years, transect was included as a term nested within census date and easting position. Models were constructed using the *glm* function in R (R Core Development Team 2017), with binomial error distribution and logit link. We used Bayesian information criterion (BIC) scores to compare models with linear, linear plus quadratic, and linear plus quadratic plus cubic easting terms. We tested the significance of each term using type II ANOVAs with likelihood ratio tests (car package; Fox and Weisberg 2011).

Quantifying the Effects of Herbivory on Population Fitness

We used data from a 2-year reciprocal transplant experiment to ask how herbivory affects population fitness and the likelihood of population persistence across and beyond the range limit of *xantiana*. We compared our results for *xantiana* to those of *parviflora* as a means of identifying how trait differences between the two taxa may result in differing performance and susceptibility to herbivory. The majority (86%) of plants that suffered mammalian herbivory in the experiment set no seed (i.e., herbivory was fatal and lifetime fitness was zero); “herbivory” below refers only to these cases of fatal herbivory.

Reciprocal Transplant. In 1997–1999, we conducted a reciprocal transplant experiment to examine variation in phenotypic traits and lifetime fitness of both subspecies planted within and outside their respective ranges. In each year of this experiment, we planted six populations of *xantiana* and 12 populations of *parviflora* at one site within *xantiana*’s range center (but outside *parviflora*’s western range limit; “center”), one site at *xantiana*’s range edge where it narrowly overlaps with *parviflora*’s range (“edge”), and one site beyond the eastern *xantiana* range limit (but within *parviflora*’s distribution; “beyond edge”; fig. 1). We planted seeds into 8,488 planting positions (eight seeds per position) arranged in 10 blocks per site in October and scored germination and survival monthly from January through July, culling to one seedling per position after germination. The experiment included a supple-

mental pollination treatment in a subset of blocks; the fitness analyses below exclude these blocks (as in Geber and Eckhart 2005). The 2 years of the experiment markedly differed in precipitation, and this led to strong differences in lifetime fitness estimates between years; hereafter, we refer to the 2 years of the experiment as “wet” (1997–1998) and “dry” (1998–1999). Full experimental details can be found elsewhere (Eckhart et al. 2004; Geber and Eckhart 2005).

Simulation of Fitness in the Absence of Herbivory. We took a post hoc simulation approach to estimate mean population fitness at each site under two scenarios—no fatal herbivory and reduced fatal herbivory—and compared these fitness estimates to those derived from the observed data set. We first simulated a scenario where there was no fatal mammalian herbivory during the 2-year field experiment. In essence, we took the original experiment’s data set and, for each plant that suffered fatal herbivory, estimated how many seeds it would have produced had it not been eaten. Predictive models were evaluated using R^2 statistics, Kolmogorov-Smirnov tests, and comparisons of predicted versus observed values (for details on model construction and evaluation, see app. B). We used these predictive models built with field data to produce lifetime fitness estimates for eaten plants that reflected all other environmental aspects of the sites, while “removing” herbivory. We simulated these data 100 times to allow for stochastic fluctuations in predicted fitness for these eaten plants (app. sec. B2). As in Geber and Eckhart (2005), average lifetime fitness through female function (i.e., seeds produced per seed planted) for each planting position was calculated as the number of germinants multiplied by the product of predicted seed set and probability of reproduction (zero or one).

After calculating predicted fitness values for eaten plants, we examined the extent to which average lifetime fitness would change at each site if there were no fatal mammalian herbivory. We estimated average lifetime fitness through female function (seeds produced per planted seed) for each subspecies at each site in both years. We used linear mixed models of lifetime fitness with site, year, and subspecies as fixed factors and block (nested within site and year) and population (nested within subspecies) as random factors (as in Geber and Eckhart 2005). We built these models for each of the 100 simulated data sets; overall fitness estimates were averaged over the 100 model estimates. Comparison of least square means from models based on the original data (with herbivory) and this simulation (no herbivory) estimated the influence of herbivory on average lifetime fitness for each subspecies at each site. Data, simulation code, and code for all other analyses have been deposited in the Dryad Digital Repository: <https://dx.doi.org/10.5061/dryad.b7k2791> (Benning et al. 2019).

Simulation of Fitness beyond the Range Edge with Reduced Herbivory. In the transplant experiment, herbivory rates be-

yond the range edge were ca. 100% higher than at the center and edge of the range (see “Results”). Thus, we were also interested in simulating a more “moderate” scenario where herbivory was not completely absent but, rather, herbivory rates beyond the range edge were similar to rates within the range. Thus, we used the same fitness simulations for eaten plants as above, but estimated mean fitness for both subspecies under the scenario where herbivory rates in the beyond-edge site were the same as in the edge site (i.e., a reduction instead of complete removal of herbivory; details in app. sec. B3). The lifetime fitness estimates for each subspecies in the beyond-edge site for both years were averaged over the 100 simulations. Comparison of least square means from models based on the original data and this reduced-herbivory simulation estimated the effect of increased herbivory rates outside the range limit on *xantiana* population persistence.

To What Extent Does Plant Phenology Mediate Susceptibility to Herbivory?

We predicted that differences in development rate between *parviflora* and *xantiana* contributed to the former’s escape from late-season mammalian herbivory at the edge and beyond-edge sites during the transplant experiment (table B1; fig. B1; tables A1, B1–B4, C1–C5 are available online), given observations that *parviflora* individuals are often dry and senescent when *xantiana* is still green and likely attractive to herbivores. Thus, we tested whether plant phenology (as measured by flowering date) influenced a plant’s probability of late-season herbivory using data from the transplant experiment. We were not interested in the date of flowering per se but rather in using this as a proxy for a plant’s developmental speed. Thus, we predicted the date of flowering for plants that died before flowering (from herbivory or other factors), enabling us to “recover” this missing phenological information and make more robust estimates of model parameters (app. sec. C1).

Due to the very low survivorship and low incidence of herbivory in the dry year, the analyses below are only for the wet year. We tested the effect of date of flowering, with plant size and block as covariates, on a plant’s probability of fatal herbivory at each site using logistic regression with binomial error distribution and logit link. Because phenology is positively correlated with size in *C. xantiana* (Pearson’s r of $\log(\text{size})$ and date of flowering = 0.47), we included size (here the largest size a plant achieved) as a covariate in the models to isolate the effects of phenology. Plant size was calculated as the product of plant leaf number and average leaf length. Since we were interested in the relationship between phenology and late-season herbivory only, these analyses were restricted to plants that survived early-season herbivory (i.e., were alive at the March census); analyses including early-season herbivory produced qualitatively similar results (app. sec. C4). Since

some plants for which we predicted flowering date died from factors other than herbivory (thereby precluding any later herbivory), these tests are somewhat conservative (i.e., some plants with predicted flowering dates were not eaten simply because they died before herbivores had the chance to eat them); in plots below, we differentiate those plants that died from factors other than herbivory to assist in interpretation. We tested the significance of each term using type II ANOVAs with likelihood ratio tests (car package; Fox and Weisberg 2011). We also ran these same models including subspecies as a term to address potential confounding of phenology with other subspecies’ differences (app. sec. C3).

We estimated optimal flowering dates at each site by fitting a loess smoother to the function $\log(\text{fitness}) \sim \text{flowering date}$ to find the flowering date at which fitness was maximized. We included both subspecies to increase the phenological range over which we could evaluate fitness and included all plants that were alive at the March census (details in app. sec. C7).

Results

Stem Translocation Experiment

Herbivore Pressure Increases at and beyond the Range Limit. In 2015, the probability of fatal herbivory on translocated *xantiana* was close to zero at the range center and increased sharply near the range limit, exceeding 0.75 beyond the range limit (fig. 2a). The pattern of herbivory was best fit with the logistic model including longitude (easting) as a linear term (BIC: 1,324; $N = 1,278$; Nagelkerke’s $R^2 = 0.49$; table A1). Overall, the odds of a plant being eaten increased 9% for every kilometer eastward ($\chi^2 = 498.2$, $P < .001$), with the gradient in probability of herbivory becoming very steep near the range limit. For example, in the last census round, the probability of herbivory increased from 0.01 at the most central site to 0.13 at 10 km east of that site but over the next 10 km eastward increased to 0.7 approximately at the range limit. There was also a significant interaction of longitude with time ($\chi^2 = 41.5$, $P < .001$), with probability of herbivory increasing as the season progressed at the range-edge and beyond-range sites but not in the range center (fig. 2a). Genotype (plants sourced from the center vs. the edge of the range) had no effect on probability of herbivory ($\chi^2 = 0.36$, $P = .5$). Within *xantiana*’s range, herbivory on translocated stems generally matched that on natural plants, with rates at four of five sites differing by less than 5%; translocated stems experienced much more herbivory at one near-edge site, but removing this site did not qualitatively affect the modeled gradient in probability of herbivory (see app. sec. A1).

In 2016, the pattern of herbivory was best fit with a logistic model including longitude as linear and quadratic terms

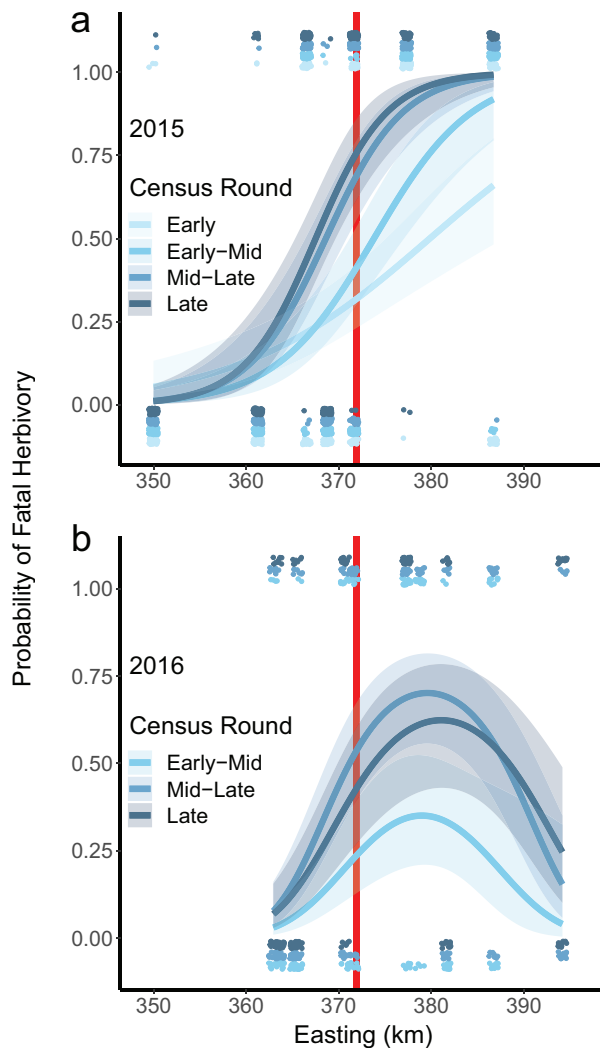


Figure 2: Spatiotemporal variation in probability of herbivory across and beyond *xantiana*'s range. The red line shows the location of *xantiana*'s eastern range limit. Plots show the relationship of probability of herbivory with easting and time (census round) from logistic regression for 2015 (a) and 2016 (b). For each plot, conditional effects of easting and time are shown, holding other model factors constant. Colors correspond to temporal replicates (ca. one replicate per week in June). Jittered points show individual plants, which either did or did not experience herbivory. Ribbons show 95% confidence bands for predictions.

(BIC: 696; $N = 561$; Nagelkerke's $R^2 = 0.33$; table A1). Probability of herbivory was low 10 km inside the range limit (ca. 0.07), increased toward the range limit to a maximum of ca. 0.62 at 8 km beyond the limit, and decreased farther east (fig. 2b). Probability of herbivory also increased from the first census round to later rounds ($\chi^2 = 86.3$, $P = .002$), though there was no significant interaction of time with easting as in 2015.

Transplant Experiment

Herbivory Threatens Population Persistence beyond the Range Limit. In the first, wetter year of the experiment, *xantiana* and *parviflora* suffered equal rates of herbivory (15% of germinated plants eaten) at the center site, but *xantiana* experienced higher herbivory farther east (34% for *xantiana* and 8% for *parviflora* at the edge site; 54% for *xantiana* and 19% for *parviflora* at the beyond-edge site; table B1). In the second, dry year, herbivory was very low throughout (1%–5%; table B1).

When we simulated a scenario with no fatal herbivory, effects on fitness were observed in the wet year but not the dry year, when plant survival and performance were low and herbivory rare. In the wet year, removal of herbivory had the largest effect on lifetime fitness for *xantiana* in the edge and beyond-edge sites, increasing lifetime fitness two- and sixfold, respectively, but *xantiana* mean fitness increased only 40% at the center site (fig. 3; table B4). Interestingly, removing herbivory beyond the range edge brought estimates of *xantiana* average lifetime fitness to one (i.e., population replacement). Removing herbivory also increased estimates of *parviflora* fitness at the edge and beyond-edge sites, but the effects were much smaller (24% and 107% increases, respectively; table B4; fig. B3).

When we simulated a scenario where herbivory was reduced in the beyond-edge site to levels observed at the edge site, *parviflora* and *xantiana* experienced increases in lifetime fitness estimates in the wet year but not in the dry year (fig. 3; table B4). In the wet year, average lifetime fitness for *parviflora* increased 50% to 3.63 (fig. B3) and for *xantiana* increased 300% to 0.60 (fig. 3).

Delayed Phenology Is Associated with Fatal Herbivory. Logistic regression showed that phenology was associated with probability of herbivory on *xantiana* and *parviflora* at all sites and especially strongly at the edge and beyond-edge sites (fig. 4). For each day delay in flowering, a plant's odds of herbivory in the range center, edge, and beyond-edge sites increased significantly by 2% ($\chi^2 = 3.9$, $P < .05$), 5% ($\chi^2 = 53.8$, $P < .001$), and 14% ($\chi^2 = 118.0$, $P < .001$), respectively (table C3). At the edge and beyond-edge sites, larger plants were more likely to be eaten ($P < .002$), whereas in the center site, smaller plants were more likely to be eaten ($P < .001$). Block effects at all sites ($P < .001$) indicated fine-scale spatial heterogeneity in herbivory. Differentiation in phenology between the subspecies is illustrated in figure 4, where *parviflora*'s earlier phenology is apparent. This difference is associated with a marked subspecies difference in susceptibility to fatal herbivory at the edge and beyond-edge sites. When we included subspecies as a term in the models to account for potential confounding of phenology with some other subspecies' difference, flowering date was still highly

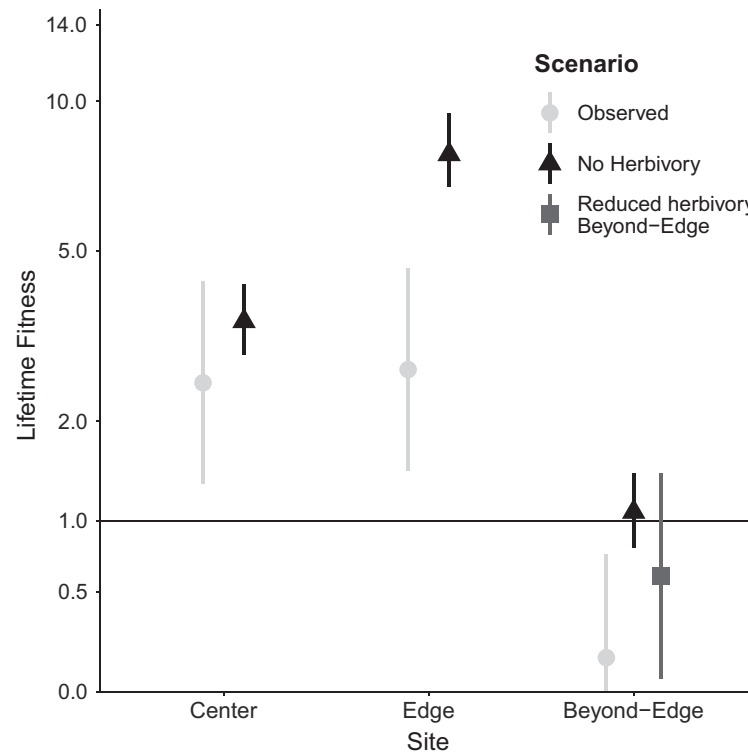


Figure 3: Lifetime fitness estimates (and 95% confidence intervals) for *xantiana* in the wet year with observed (i.e., unsimulated) values (light gray circles) and under two simulated scenarios: no herbivory at any site (black triangles), where we predicted fitness values for all plants eaten during the field experiment as if they had not been eaten, and reduced herbivory beyond the edge (dark gray square), where we simulated lowered herbivory rates outside *xantiana*'s range limit. Note that the Y-axis is on a log scale. $N = 4,185$ planting positions. Upper and lower confidence limits for simulation estimates are the 97.5% and 2.5% quantiles of the set of 100 estimated means.

significant ($P < .001$) beyond the range edge but not at the center or edge sites (app. sec. C3). Comparing optimal versus realized mean flowering dates showed that *xantiana* was far from the phenological optimum (ca. 18 days later) outside its range but was within ca. 4 days of optima at the center and edge sites (fig. 4).

Discussion

Recent reviews of transplant experiments support the idea that species' geographic range limits often reflect niche limits (Hargreaves et al. 2014; Lee-Yaw et al. 2016). But given the demonstrated power of natural selection to produce adaptations to novel environments, what prevents range expansion via sequential adaptation of marginal populations? The vast majority of work on geographic range limits has focused on gradients in abiotic variables, mainly temperature and precipitation. However, the field is increasingly calling for tests of how biotic interactions can modulate range boundaries, given experimental (e.g., Moeller et al. 2012; HilleRisLambers et al. 2013; Afkhami et al. 2014), theoretical (e.g., Hochberg and Ives 1999; Case and Taper 2000; Case et al. 2005; Gravel et al.

2011), and indirect or correlational (e.g., Araújo and Luoto 2007; Ettinger et al. 2011; Pigot and Tobias 2013; Scully et al. 2018) evidence for the influence of species' interactions on large-scale distributions. Here we showed that an antagonistic biotic interaction, mammalian herbivory, has large effects on population mean lifetime fitness at and beyond the subspecies' geographic range limit and that probability of herbivory exhibits a steep gradient across the range of *Clarkia xantiana*. We then showed that a specific plant trait, phenology, is strongly associated with probability of herbivory at and outside the range limit. Together, this set of results provides some of the strongest evidence to date that biotic interactions can play a pivotal role in determining the location of a geographic range limit.

Transplant and Translocation Experiments

Our simulations using the transplant data set showed that at range center, removal of herbivory had minor effects on *xantiana* lifetime fitness, but at and beyond the range edge, a complete absence of herbivory increased *xantiana* lifetime fitness two- and sixfold, respectively. For annual

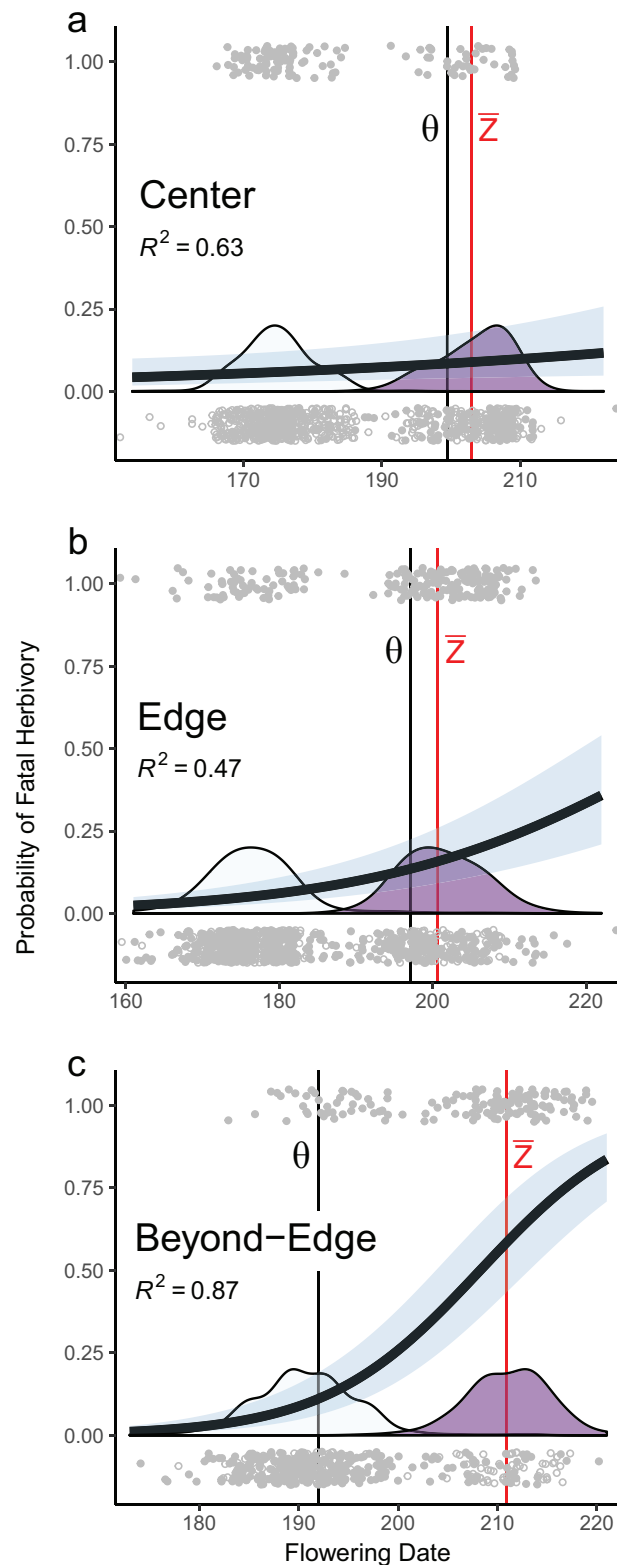


Figure 4: Conditional effects of phenology on probability of fatal herbivory (with 95% confidence interval bands) as modeled by logistic re-

gression, holding size and block constant, at center (a), edge (b), and beyond-edge (c) sites in the wet year. Kernel density estimates (smoothed histograms) indicate distribution of flowering date for each subspecies (white = *parviflora*; purple = *xantiana*). Jittered points are individual plants that either did or did not experience herbivory. Open points indicate plants that died due to factors other than herbivory. Optimal flowering date, where fitness was maximized, is marked by the black line labeled θ . The mean *xantiana* flowering date is marked by the red line labeled $\bar{Z}$. $N = 8,488$ planting positions.

plants such as *xantiana*, population mean lifetime fitness approximates population growth rate (λ). Interestingly, these simulations imply that in the absence of herbivory, *xantiana* population growth at the range edge could be double that at range center and that populations beyond the range edge could potentially replace themselves. This highlights how a biotic interaction can influence population demography at a species' range edge and potentially emigration and colonization outside the range limit.

When we simulated reduced herbivory outside the range (instead of complete removal), *xantiana* mean lifetime fitness increased 300% relative to field data in the wet year, to $\lambda = 0.60$. Though this is still below levels needed for population replacement, adaptive evolution beyond the range margin could potentially raise population mean fitness above replacement, given adequate heritable variation in ecologically important traits. There is evidence of substantial genetic variance for fitness in *xantiana* planted beyond its range limit (D. Moeller, unpublished data), which could allow population mean fitness to evolve and populations to “escape” extirpation (Fisher 1930; Gomulkiewicz and Shaw 2013).

The most direct test of the influence of herbivory on population fitness would be to manipulate access by herbivores with caging in the field. Here we took an alternative, post hoc simulation approach that allowed us to estimate mean population fitness at each site under two scenarios—no fatal herbivory and reduced herbivory—and compare these fitness estimates to those derived from the observed data set. Of course, our fitness predictions for eaten plants cannot be perfect reflections of what would have happened sans herbivory in the field. However, simulating fitness values across multiple instantiations of the experiment in silico allowed for stochasticity in the prediction process (see app. B) and provided a conceptually rigorous approximation of population fitness under different scenarios.

Our stem translocation experiments showed that herbivory exhibits a steep gradient across and beyond *xantiana*'s range, with a sharp increase in probability of herbivory near the eastern range margin. For example, during the last stem census in 2015, *xantiana* at the center of the range had a less than 5% chance of fatal herbivory, while only 8 km outside its range limit the probability of herbivory was over 15-fold higher (95%). This spatial pattern is in accord with predic-

gression, holding size and block constant, at center (a), edge (b), and beyond-edge (c) sites in the wet year. Kernel density estimates (smoothed histograms) indicate distribution of flowering date for each subspecies (white = *parviflora*; purple = *xantiana*). Jittered points are individual plants that either did or did not experience herbivory. Open points indicate plants that died due to factors other than herbivory. Optimal flowering date, where fitness was maximized, is marked by the black line labeled θ . The mean *xantiana* flowering date is marked by the red line labeled $\bar{Z}$. $N = 8,488$ planting positions.

tions from range limit models that the steepness of relevant environmental gradients is key to generating species' distributional boundaries (Kirkpatrick and Barton 1997; Polechová and Barton 2015).

Phenology and Herbivory

These above findings speak to the proximate, ecological causes of *xantiana*'s range limit, but the ultimate cause of a range limited by adaptation is genetic limits on trait evolution. We rarely know which traits would need to evolve to allow range expansion (but see Hoffmann et al. 2003; Griffith and Watson 2006; Angert et al. 2008; Colautti et al. 2010). In this study, we were able to use differentiated sister taxa to ask how a specific trait, phenology, influenced probability of herbivory at multiple sites. While phenology had little effect at range center, the difference in phenology between the two subspecies beyond the range limit was associated with large differences in susceptibility to fatal herbivory. It is certainly possible that other, unknown traits differing between the subspecies (e.g., defensive compounds) could contribute to *xantiana*'s increased probability of herbivory, though even when we include subspecies as a term in our models of herbivory given phenology, phenology remains a significant predictor outside the range edge (see app. sec. C3). The link between phenology and probability of herbivory is additionally supported by the significant effect of time (i.e., early to late growing season) in our statistical models of the stem translocation results—plants were more likely to be eaten as the season progressed (except for sites near the range center, where probability of herbivory was consistently near zero). This approach eliminated potential confounding of phenology with other subspecies' traits, as the translocation experiment used only *xantiana* and allowed us to ask how the probability of herbivory on green, nonsenescent plants varied across the growing seasons of both subspecies (i.e., early: *parviflora*; late: *xantiana*).

Phenology has been shown to be a key range-limiting trait in other plant species, though usually in the context of abiotic latitudinal range limits (Griffith and Watson 2006; Colautti et al. 2010). For *xantiana*, it seems phenology would have to evolve to enable eastward range expansion. Indeed, phenology did evolve in ancestral *xantiana* populations that diverged in allopatry to become *parviflora*, which later expanded in range such that it is now in secondary contact with *xantiana* (Pettengill and Moeller 2012a). Thus, the question becomes, what is now preventing adaptive evolution at *xantiana*'s range limit?

Linking to Theory

Recent theoretical work (Polechová and Barton 2015; Polechová 2018) showed that in models including genetic drift,

a range margin can form via two (non-mutually exclusive) mechanisms: a steepening (i.e., nonlinear) environmental gradient driving increasing maladaptation or a decrease in carrying capacity across space, leading to an increased influence of drift on population genetic variance. Both of these factors could be at play for *xantiana*. In these models, a steepening environmental gradient creates a sharp range margin near the environmental "inflection point." This is due to drift eroding genetic variance needed to adapt to a quickly changing trait optimum as small, colonizing populations encounter new environments to which they are very poorly adapted. The result is that population trait means closely track trait optima along most of the environmental gradient but fail to do so when this gradient suddenly steepens, like the gradient in probability of herbivory does near *xantiana*'s range limit. This increased mismatch between optimal and observed trait values drives demographically unsustainable declines in population mean fitness, which is in agreement with our empirical estimates of the difference between observed and optimal flowering dates outside the range margin (ca. 18 days), compared to within *xantiana*'s natural range (ca. 4 days). Increased herbivore pressure could also impose an extrinsic limit on *xantiana*'s carrying capacity outside its range edge, depressing population sizes so as to make any populations able to colonize outside the range limit more susceptible to drift eroding potentially adaptive genetic variance. The concordance of observed patterns in environmental variation and *xantiana*'s distribution with model predictions provides empirical support for recent range limit models (Polechová 2018).

Why Does Herbivory Vary across Space?

Geographic variation in herbivory across *xantiana*'s range could be explained by two phenomena. First, the herbivore community likely changes across *xantiana*'s range. Our field observations and surveys using motion-triggered cameras (2015 and 2016) suggest that two lagomorph herbivores often eat plants outside the range (desert cottontail and black-tailed jackrabbit), whereas only the desert cottontail is common in the center of *xantiana*'s range (fig. A1c). Habitat descriptions support these observations, reporting that the black-tailed jackrabbit is more common in arid, open scrubland typical of sites at and outside *xantiana*'s eastern range boundary (Arias-Del Razo et al. 2012). If there is increased herbivore pressure near *xantiana*'s range limit due to an additional lagomorph species, this could translate into higher herbivory rates on *xantiana* planted at and outside its range limit.

A second, non-mutually exclusive hypothesis is based on decreases in primary productivity, especially of herbaceous plants, across the west-to-east gradient (fig. 1). The availability of more forage at *xantiana*'s range center may dilute herbivore pressure on *xantiana*. In contrast, in the

more arid east where *parviflora*'s distribution is centered, *xantiana* may be increasingly attractive to herbivores due to limited forage and its late completion of development compared to co-occurring forbs. Field observations suggest that this pattern arises because *parviflora* is less palatable forage by the peak of late-season herbivory, whereas *xantiana* is still green and flowering. For example, during transplant experiments, *xantiana* was often the only herbaceous vegetation still green by early June, when surrounding ephemerals had already senesced.

Temporal Variability and Abiotic $\times$ Biotic Interactions

Another important takeaway from this study is that environmental constraints on species' ranges need not be static across time. In the dry year, fitness was limited outside the range (and everywhere) by low precipitation. In the wet year, the geographic gradient in aridity led to relatively fewer germinants in the beyond-edge site, but our simulations showed that the population may have been able to persist in the absence of herbivory. This sort of temporal variation in selection could prevent or slow changes in the frequency of beneficial alleles (Kirkpatrick and Peischl 2013). This highlights how temporal variability can alter selective environments and create "moving targets" for evolution at range edges (Hao et al. 2015) and echoes the recommendation of Hargreaves et al. (2014) that transplant experiments should occur over multiple years to capture as much temporal variation as possible.

In the wet year of the transplant experiment, the number of plants eaten by herbivores was 25% higher in the beyond-edge site than in the center site (251 and 203, respectively). However, the proportion of plants eaten, given the number of germinants, was double beyond the edge (31% vs. 15%), due to the lower number of germinants beyond the range edge. Thus, the effect of herbivory on population growth was compounded via other, likely abiotic factors (precipitation). This demonstrates how multiple environmental factors can interact to influence the distribution of a species.

The Multivariate Nature of Range Expansion

Thus far we have considered phenology in isolation, but range-edge *xantiana* populations will likely have to evolve multiple traits to colonize outside their range boundary (Antonovics 1976). To colonize areas outside its eastern range limit, where its sister taxon occurs, *xantiana* would likely need to adapt to not only increased herbivore pressure but lower and increased variation in precipitation and less abundant pollinator communities. For example, due to the low abundance of pollinators (especially *Clarkia* specialist bees; Moeller 2005) and higher pollen limitation at and beyond its range edge (Moeller et al. 2012), *xantiana* would need to evolve a higher selfing rate for reproductive assurance. Similarly, given the increased tem-

poral variation in rainfall in the east, increased seed dormancy would likely be advantageous outside the range limit (Eckhart et al. 2011). Thus, colonization of habitat beyond *xantiana*'s current range margin would likely require evolution of multiple ecologically important traits involving many genetic loci, which could slow or prevent adaptive evolution at the range edge (Antonovics 1976; Duputié et al. 2012). The original divergence of *parviflora* from *xantiana* may have been aided by relatively shallow environmental gradients (see "Linking to Theory" above) or the opportunity for sequential adaptation in relevant traits, as opposed to a sudden, concurrent shift in optima for multiple traits.

Generality of a Generalist Predator Enforcing Range Limits

Given the strong effects of herbivory on individual plant fitness, population growth, and local and elevational distributions (Louda 1982; Quinn 1986; Bruelheide and Scheidel 1999; Fine et al. 2004), it is surprising that only one recent study has examined herbivory's role in modulating plant species' geographic ranges (Baer and Maron 2018). To our knowledge, ours is the first study to explore the effects of herbivory on a geographic range limit using experimental transplants beyond the range boundary, which is optimal for the testing of range limit hypotheses. Case et al. (2005) pointed out that, theoretically, polyphagous predators can easily enforce geographic range limits of prey species, especially when two prey species are differentially susceptible to predation over a spatial gradient. This is the pattern we see in *C. xantiana*, but should we expect that generalist herbivores often regulate geographic distributions of plant species? Rapid phenology is commonly observed in arid systems, and this has long been presumed to be due to selection to escape the late-season drought and unpredictable hydric environments of arid areas (Aronson et al. 1992; Thuiller et al. 2004; Levin 2006; Volis 2007). "Phenological escape" from insect herbivory has been shown for multiple plant taxa (Pilson 2000; Krimmel and Pearse 2016; Mlynarek et al. 2017), but mammalian herbivore control on plant phenology and distributions in arid environments remains relatively unexplored.

Studies often focus on climatic control of geographic range limits, but given the intricate web of interspecific interactions in which every organism participates, we cannot ignore the potential role of biotic factors in structuring large-scale distributions. Combining multiple lines of evidence to link environmental variation, traits, and fitness, our study demonstrates how biotic interactions can generate adaptive hurdles for important traits and contribute to the formation of species' range limits.

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