VOL. 174, NO. 2 THE AMERICAN NATURALIST AUGUST 2009

Ecological Limits and Fitness Consequences of Cross-Gradient Pollen Movement in *Lasthenia fremontii*

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Submitted October 4, 2008; Accepted March 9, 2009; Electronically published June 22, 2009

ABSTRACT: The interaction between gene flow and environmental heterogeneity plays a key role in shaping the distribution patterns that we observe in natural populations. Although a growing body of theoretical work is exploring the effects of gene flow on the evolution of range limits and ecological specialization, explicit empirical tests of model assumptions and predictions in natural populations are almost entirely lacking. This study examines the potential for center-to-edge gene flow to occur and estimates the fitness consequences of cross-gradient gene flow in an annual plant species restricted to California vernal pool wetlands. Phenological differences and highly focused foraging patterns of pollinators reduce the potential for center-to-edge gene flow across populations within pools. Furthermore, controlled crosses simulating different patterns of gene flow across the environmental gradient reveal that center-to-edge gene flow does not reduce plant fitness at the edge but instead yields an increase in emergence rates and a trend toward overall higher fitness.

Keywords: Gene flow, pollen limitation, plant distributions, ecological specialization, range limits, *Lasthenia fremontii.*

Introduction

The scale and direction of gene flow relative to patterns of selection can largely influence the distribution patterns we observe in natural populations, ranging from geographic range limits (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Case and Taper 2000; Gaston 2003; Holt 2003) to ecological specialization within landscapes at local spatial scales (Holt and Gaines 1992; Holt 1996; Ronce and Kirkpatrick 2001). When a population is distributed across a heterogeneous environment, theory predicts that gene flow will proceed by simple diffusion processes from the center of the population, where population density and mean fitness are highest, to the edge of the population, where density and mean fitness are relatively low (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Case and Taper 2000; Lenormand 2002). This directional pattern of immigration can have various consequences for the individuals at the edge of a population. Center-to-edge gene flow can have a positive effect if it boosts population size at an edge where Allee effects are operating (Holt et al. 2004; Antonovics et al. 2006) or adds beneficial genetic variation where low densities have caused high levels of selfing, biparental inbreeding, or the loss of genetic variation by drift (Lennon et al. 1997; Alleaume-Benharira et al. 2006). Alternatively, center-to-edge gene flow may have negative consequences for the population edge if it prevents local adaptation by swamping locally favored alleles (Haldane 1956; Antonovics 1976; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997).

The "gene-swamping" hypothesis has received considerable attention as a mechanism for the evolution of stable range boundaries but has not been empirically tested in natural populations. A fundamental assumption of the hypothesis is that differences in population density and mean fitness across an environmental gradient drive a net flux of gene flow from the center to the edge of the population. However, the environmental gradient may also influence the life history and reproductive patterns of a population, which can influence patterns of gene flow. In flowering plant species, gene flow can occur through pollen transfer or seed dispersal, both of which can be sensitive to ecological conditions (Linhart and Grant 1996). Flowering phenology (Schemske 1977; Schmitt 1983; Rathcke and Lacey 1985), pollination mechanism (Eckert 2002; Moeller and Geber 2005; Hersch and Roy 2007), and pollinator behavior (Schmitt 1980; Turner et al. 1982) can all vary with environmental context. Environment-dependent seed dispersal and postdispersal offspring success can either reinforce or diminish any genetic structure generated by nonrandom pollen movement (Stanton and Galen 1997).

The theory underlying the gene-swamping hypothesis has largely been developed in the context of understanding the evolution of geographic ranges (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Case and Taper 2000; Gaston 2003; Holt 2003), and most of the

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Am. Nat. 2009. Vol. 174, pp. 221–235. © 2009 by The University of Chicago. 0003-0147/2009/17402-50783$15.00. All rights reserved.
DOI: 10.1086/600089

empirical work exploring the evolution of range limits has focused on large-scale distribution limits, including latitudinal climate (Etterson and Shaw 2001; Etterson 2004*a*, 2004*b*), elevation (Angert and Schemske 2005), and precipitation gradients (Eckhart et al. 2004; Geber and Eckhart 2005). However, this body of theory fundamentally examines population-level processes and can therefore be applied toward understanding the drivers of distribution limits at local scales. Furthermore, when dispersal among populations is rare compared to that within populations, processes operating in "local evolutionary arenas" may drive the evolution of large-scale distributions (Holt 2003). Because a species' geographic range usually consists of local populations that collectively span larger spatial scales, studies exploring the processes operating at the local population level can provide a valuable contribution toward understanding of the evolutionary limits of species' distributions in general.

This study explores the ecological effects of a small-scale environmental gradient on the ecological limits and fitness consequences of gene flow in *Lasthenia fremontii*, an annual plant species restricted to California vernal pools. Vernal pools develop each year in natural depressions underlain by an impervious substrate that prevents the downward percolation of water (Sawyer and Keeler-Wolf 1995), forming distinct patches in the (typically grassland) landscape. Within these patches, *L. fremontii* is often a dominant species (Barbour et al. 2003) and exhibits sharp population boundaries near the pool edge (Emery et al. 2009). Previous research has demonstrated that competition and physical stress interact to prevent *L. fremontii* from expanding its distribution into the uplands surrounding vernal pools and that it can maintain relatively stable borders even across years that vary in precipitation patterns and pool hydrology (Emery et al. 2009). By limiting populations to certain depths within vernal pools, these ecological processes also limit the larger-scale distribution of the species, as *L. fremontii* is not found outside ephemeral wetland habitats (Hickman 1993) nor even in particularly shallow vernal pools (N. C. Emery, personal observations).

The goals of this study were (1) to examine the potential for gene flow to occur from the center to the edge of a population distributed across an environmental gradient, as is commonly predicted, and (2) to test the hypothesis that center-to-edge gene flow by pollen movement reduces fitness at the edge of the population. Although typically spanning only a few meters, the vernal pool environmental gradient is complex: the annual cycles of accumulation and recession of water establish a gradient in a variety of abiotic variables, including soil moisture content and predictability, temperature, depth, texture, and pH (Linhart 1974, 1976; Lathrop 1976; Holland and Dains 1990). The biotic environment varies across the gradient as well; aboveground vegetative biomass can be twice as high at the pool edge than at the pool center (Emery et al. 2009). Previous studies indicate that patterns of selection (e.g., on inundation tolerance and inter- vs. intraspecific competitive ability) change across the inundation gradient within pools and are strong enough to lead to genetic differentiation between center and edge lineages in a primarily selfing species (Linhart and Baker 1973; Linhart 1974, 1988). In *L. fremontii*, gene flow across the gradient is likely regulated by a complex set of ecological factors. Within pools, center-to-edge gene flow may be facilitated by the close proximity of center and edge individuals (often only a few meters), the species' obligately outcrossing mating system (Ornduff 1966; through sporophytic self-incompatibility), and the steep gradient in population density. On the other hand, flowering occurs with the recession of water each spring, leading to gradient-specific variation in flowering phenology and, possibly, in pollinator behavior.

The following specific questions were addressed: (1) How does a small-scale environmental gradient influence phenological structure in *L. fremontii* populations? (2) How does flowering phenology interact with pollinator behavior to influence the probability of a plant receiving local versus nonlocal pollen, and does the nature of this interaction vary with position along the gradient? (3) Does center-to-edge gene flow by pollen or seed dispersal reduce offspring fitness in the edge environment compared to within-edge patterns of gene flow?

Methods

Study System

Lasthenia fremontii (A. Gray) is an annual plant in the family *Asteraceae* that is endemic to California vernal pools, seasonal wetlands that accumulate water during the winter rainy season and dry out in the late spring (Solomeshch et al. 2007). *Lasthenia fremontii* populations are generally distributed across the deeper microtopographic positions of vernal pools but can be restricted to a band at intermediate depths around the circumference of very large, deep pools (Barbour et al. 2003). Populations in the focal pools reach their highest densities at the pool bottoms and gradually decline in abundance with increasing microelevation. In the *L. fremontii* populations studied here, the population "center" corresponds to the deepest locations in the vernal pools, and the population "edge" occurs at higher microelevation positions near the periphery of pools (see below). Transplant experiments have demonstrated that individuals near the pool edge are ecologically marginal, experiencing more physical stress—and, in some years, more competition—than more centrally located individuals at the bottom of the pool (Emery et al. 2009).

The life-history cycle of *L. fremontii* is tightly coordinated with the precipitation and inundation regime in the vernal pool habitat. Seeds germinate in late fall or early winter, after the first heavy winter rains but before the accumulation of water in pools, so that seed movement by water transport is unlikely. Individuals spend the submerged period as seedlings in the cotyledon or two-leaf stage. Seeds possess a small, stiff pappus that is reduced compared with many other composites, so seed dispersal occurs primarily by gravity and is generally in the immediate vicinity of the parental plant (N. C. Emery, personal observations). Together, these attributes make it likely that, on average, gene flow by pollen movement occurs over distances larger than those for gene flow by seed dispersal. When the rain subsides and temperatures warm in the spring, individuals grow rapidly and flower as the pools dry. *Lasthenia fremontii* is pollinated by several types of insects, including specialist solitary bees and bee flies (Ornduff 1966; Thorp and Leong 1996).

Flowering Phenology across the Vernal Pool Inundation Gradient

The relationship between flowering phenology and plant position along the vernal pool inundation gradient was examined in the spring of 2003 in eight different pools containing *L. fremontii* at Mather Regional Park, Sacramento County, California. Because of constraints involving site access and the ability to sample all eight pools each sample day, pools could not be randomly selected from among all pools at the site. Pools spanning a range of depths, sizes, and distances from one another were nonrandomly selected; consequently, pool was treated as a fixed effect in all analyses.

In March 2003, before the flowering of *L. fremontii* at this site, a 1-m^2 grid was established over each vernal pool. One-third of the intersecting grid points were randomly chosen as sample points. Points that did not include any *L. fremontii* individuals within a 0.25 × 0.25-m^2 area were discarded. The total number of sample points per pool ranged from 28 points in the smallest pool to 101 points in the largest population, for a grand total of 477 sample points across all pools. Percent cover of flowering individuals was estimated at each sample point every 3–4 days during the entire flowering period at the site (March 31–June 2), for a total of 15 censuses. At each census, a 0.25 × 0.25-m quadrat was placed over each sample point in a consistent orientation, and the percent cover of flowering *L. fremontii* was visually estimated. When flowering subsided, the elevation of each sample point (relative to the bottom of its respective pool) was measured to the nearest centimeter with an auto level.

Two sets of analyses were conducted to examine the relationship between elevation and flowering phenology among pools. SAS software version 9.1 (SAS Institute 2003) was used for all statistical tests. First, an ANCOVA was used to test the effects of pool and relative elevation on the date that peak flowering was first observed at each sample point. Pool was treated as a categorical fixed effect in the model, and elevation was a covariate; the pool × elevation interaction was also included. Given the significant pool × elevation interaction found in the overall ANCOVA, a series of simple regressions was conducted to examine the effects of elevation on peak flowering date within each individual pool. In these analyses, elevation was the only effect in the model. Second, the effects of elevation and pool on the entire flowering distributions of the eight populations were examined using a repeated-measures ANCOVA. Relative elevation, pool, and the elevation × pool interaction were treated as between-subject effects, while census date and its interactions with elevation and pool were the within-subject effects. The response variable was the fraction of flowers open in each plot on each census date.

Subsequent experiments (see below) involved comparisons between the center and the edge of one population of *L. fremontii*; consequently, it was of interest to compare the degree of phenological overlap between the centermost and edgemost individuals in each population in the 2003 data set. To do this, the gradient spanned by each population was divided into thirds. Plots that fell within the upper third of the gradient were assigned to the pool edge (population edge) zone, while plots that fell within the lower third were categorized as the pool bottom (population center) zone. Plots falling between the pool bottom and edge zones were grouped into the middle gradient zone. This proportional designation provides a standardized method of selecting center and edge plots across pools that vary in total depth and provides a conservative estimate of phenological overlap by casting a rather broad slice over the extremes of the inundation gradient. The flowering distributions—that is, the proportion of plants in each plot that were flowering over time—were plotted for each zone in each pool. Hurlbert's (1978) general overlap index was used to estimate the proportion of overlap between the population center and population edge phenological curves. The overlap index is calculated as $\sum (C_i E_i / a_i)$, where C_i is the proportion of center individuals flowering at time i, E_i is the proportion of edge individuals flowering at time i, and a_i is the total proportion of individuals flowering at time i. An index value of 1.0 represents complete overlap of the phenological curves, and 0.0 represents no overlap.

Pollinator Behavior and Pollen Limitation

The effects of pollinator behavior, flowering time, and plant position on plant seed production in one *L. fremontii* population (hereafter "experimental pool") were examined in a field experiment conducted during the spring of 2005. On April 9, when the edgemost *L. fremontii* individuals were just beginning to flower, 50 numbered pin flags were inserted approximately every 1.5 m around the edge of the experimental vernal pool. This process was repeated in the center of the population on April 21, when the water was shallow enough to enter the deepest portions of the pool but before any individuals in the center of the population began to flower.

After pin flag locations were established, five unique pin flags were randomly selected every 2–3 days in each population zone (i.e., center and edge). At each selected pin flag location, the nearest *L. fremontii* individual with a single flower opening on its first inflorescence was identified and marked with a small bird band. A similar-sized second plant, also in the first stage of flowering, was found within 0.25 m of the first plant and was also marked with a bird band. Within each pair of plants, one individual was randomly selected to receive a pollen addition treatment, and the other was designated as a control. This procedure was repeated every 2–3 days until it was no longer possible to find individuals just starting to flower in each zone.

Eight 0.5 × 0.5-m pollinator exclosure cages were placed throughout the population in areas of high flower density to provide a source of pollen donors with high pollen loads. Exclosures were always more than 1 m from experimental plants and were moved to new locations when flowering subsided in a given area. Hand-pollinations involved collecting donor inflorescences with visible pollen loads from all exclosure cages that had flowering plants, randomly assigning donors to plants marked for pollen supplementation, gently rubbing the donor inflorescence against a recipient inflorescence, and then discarding the donor.

Pollen additions and phenological monitoring were done on experimental plants every 2–3 days, beginning the first day a focal plant was identified and ending when the plant finished flowering and set seed. If a plant had multiple inflorescences, all inflorescences received pollen additions. On both pollen-addition and control plants, the dates of various phenological stages (e.g., first flower opening, all flowers open, flowering complete) were recorded for each inflorescence. Inflorescences were collected from experimental plants when seeds were mature; the total numbers of viable and inviable seeds were counted in the lab. Viable seeds were easily recognized as dark brown, filled fruits, while inviable seeds were white, thin, and very small.

Throughout the course of the pollination experiment, ten 0.25 × 0.25-m reference plots were monitored to document the natural progression of flowering at the center and edge of the focal population. These plots were censused for percent cover of flowering *L. fremontii* every 2–4 days throughout the experiment. Within each zone, values were summed across plots to yield an estimate of the total fraction of the subpopulation in each zone that was flowering on each census day.

The effects of first flower date, pollination treatment, and population zone (center or edge) on lifetime seed production were examined with an ANCOVA using SAS software version 9.1 (SAS Institute 2003). If a focal plant produced more than one inflorescence, means were calculated across all inflorescences on the plant for the date of first flower, the number of viable seeds, and the number of ovules (viable seeds + inviable seeds) per inflorescence. The mean number of viable seeds per inflorescence was the response variable in the ANCOVA. Treatment, zone, and their interaction were treated as fixed effects, and the mean date of first flower was a covariate. All interactions among the day of first flower, treatment, and zone were tested in the model. The mean number of ovules per inflorescence was also included as a covariate to control for the effects of plant size and vigor on the number of viable seeds produced (Scheiner et al. 2000). To understand a significant three-way interaction observed in this ANCOVA, I performed a regression within each population zone, testing the effects of pollination treatment, the mean date of first flower, and their interaction on the mean number of viable seeds produced. The total number of ovules was again included as a covariate. A marginally significant interaction between pollination treatment and mean date of first flower in the edge population prompted a final series of regressions within each zone and treatment, with flowering time as the only factor in the model, the mean total number of ovules as a covariate, and the mean number of viable seeds produced as the response variable.

To gain insight into how pollinator behavior could influence patterns of pollen limitation in the experimental *L. fremontii* population, a small number of direct observations of pollinator movement were conducted on May 12, 2005. The 2005 sampling date was chosen because it fell roughly midway through the flowering period (when the overlap in flowering between center and edge plants was greatest), making it possible to document the maximum potential movement of pollinators across the inundation gradient. On this date, flowering plants were documented in reference plots at microelevations ranging from 3.0 to 20.5 cm above the pool bottom. Five different pollinators were monitored while pollinating *L. fremontii* between 1400 and 1530 hours, which roughly corresponded to the time of peak pollinator activity that day. For each observation, a pollinator was identified at a random position in the pool and was followed until the pollinator was lost. During each sequence, a numbered pin flag was inserted into the ground next to every fifth inflorescence visited by each pollinator. A "visit" consisted of any contact with an inflorescence that could result in pollen transfer. The number of visits observed ranged from 21 to 53 inflorescences, and the corresponding observation periods lasted between 4 and 12.5 min. None of the tracked pollinators contacted any plants other than *L. fremontii* during the observation periods. Although solitary bees that specialize on *Lasthenia* species are known to occur in some vernal pool communities (Thorp and Leong 1996), the only pollinators seen visiting *L. fremontii* during the observation period were *Bombylius* species (Diptera: Bombyliidae; specimens identified by R. Thorp), commonly known as bee flies. (Subsequent observations have confirmed that bee flies are by far the most common visitor of *L. fremontii* inflorescences at this site, with oligolectic bees on *Lasthenia* rare to absent.) After five separate visitation sequences had been marked, the horizontal distance between each pin flag was measured to the nearest centimeter, and the microelevation position of each pin flag was measured to the nearest 0.5 cm with an auto level.

Additional observations were conducted in a nearby pool with no ongoing experimental work (hereafter "pollinator observation pool") on May 12 and 19, 2006. These additional observations were conducted in the context of a separate study that is rigorously testing the hypothesis that pollinators move nonrandomly with respect to the inundation gradient (N. C. Emery, R. Runquist, and M. Stanton, unpublished manuscript), but relevant observation data are presented here. The entire flowering period of the resident *L. fremontii* population lasted approximately 5–6 weeks, so 2 days of observations encompassed approximately 5% of the entire flowering period. While this still represents a limited sampling of pollinator behavior, these data are presented simply to help interpret results from the pollen addition experiment (above) rather than to exhaustively describe pollinator behavior. Twenty pollinators were tracked on May 12, and 16 were followed on May 19. On both days, observations were conducted between approximately 1130 and 1430 hours, corresponding to the duration of pollinator activity on those days. The starting locations for observations were randomly selected grid points from the 2003 phenology survey (see above). To begin each observation, an observer started at a randomly selected grid point and found the nearest pollinator on *L. fremontii.* Points with no flowering plants within a $0.25 \times 0.25\text{-m}^2$ area were discarded. On May 12, flowering plants spanned elevations between 15.5 and 32 cm, while the elevational range of the flowering populations on May 19 was 1.0–30 cm above the pool bottom. The methodology for tracking pollinators and flagging plant locations was the same as that implemented in the experimental pool in 2005 (see above). The number of pollinator visits during an observation period ranged from 7 to 110 inflorescences (average = 21) on May 12 and from 2 to 62 inflorescences (average = 18) on May 19. Pollinators were again followed until they were lost; the duration of observations ranged from 50 s to over 16 min on May 12 and between 45 s and 16 min on May 19. While *Bombylius* species were the only pollinators observed on May 12, a small number of *Syrphidae* and one *Bombus* species were observed and followed on May 19.

Consequences of Cross-Gradient Pollen and Seed Movement

The relative consequences of within-zone and cross-zone pollen and seed transfer were examined with a field experiment using seeds that were generated from controlled pollinations among center and edge individuals. In early April 2004, 75 *L. fremontii* individuals with unopened buds were collected from the center and the edge of the experimental pool. Each plant was transplanted into plastic growth tubes ("Cone-tainers"; Stuewe and Sons, Corvallis, OR) and immediately transported to a greenhouse on the University of California, Davis, campus. Racks containing growth tubes were placed in clear tubs filled with nutrient-enriched deionized water (150 g/L GrowMore 4-18-38 No Boron prepackaged fertilizer; 150 g/L magnesium sulfate; 300 g/L calcium nitrate) to maximize survival and growth. Plants were randomly assigned to pairs associated with one of three cross types (sire × dam, respectively): center × center, center × edge, and edge × edge. Center × center crosses and edge × edge crosses simulated local pollination within each microhabitat, while center × edge crosses simulated pollen flow from the high-density, central habitat to the low-density, marginal habitat. Pollinations were performed as plants flowered until enough seeds were collected or the parental plants died. At least 20 pairs for each cross type successfully yielded seeds, but the number of seeds produced varied among cross types. Only nine edge × edge pairs generated more than 30 seeds, while 21 center × center pairs and 17 center × edge pairs produced 30 or more seeds. Since 30 seeds per pair were needed for the subsequent field experiment (see below), we used seeds from the most productive 10 pairs of the center × center and center × edge cross types and from the nine pairs of the edge × edge cross type. Thus, the most fecund crosses (i.e., those that would likely have contributed most to the subsequent generation under natural conditions) from all cross types were represented in the experiment. While excluding the less vigorous crosses may reduce the variance observed in our experiment, it should not bias our comparisons among cross types, as the same criteria were applied to all cross types.

In the fall of 2004, seeds from each cross type were planted into known grid locations in blocks located at the center and edge of the population in the experimental pool. Individual seeds were glued to toothpicks using a tiny dot of water-soluble glue. Toothpicks were planted into blocks by inserting the seed into the ground just below the soil surface. Each 9 × 30-cm plot consisted of three rows of 10 toothpicks at 3-cm spacing, for a total of 30 seeds per plot (10 seeds per cross type). The long dimension of each plot was oriented perpendicular to the inundation gradient to minimize microelevation heterogeneity within plots. Ten plots were established in the center of the population, and 20 plots were placed at the edge of the population in anticipation of low emergence rates at the edge, which had been observed in previous experiments. Toothpick locations were censused throughout the fall for seedling emergence and in the spring for survival and flowering. When an inflorescence finished flowering and its seeds were visibly mature, it was harvested, dried, and weighed to the nearest 0.001 mg. Inflorescence weight is strongly correlated with seed number in these populations ($r = 0.96$, $N = 131$, $P < .0001$; N. C. Emery, unpublished data).

Performance of the different cross types in each transplant destination was analyzed with a series of general mixed models using the MIXED procedure in SAS software version 9.1 (SAS Institute 2003). For these analyses, the mean values for each cross type within each plot were calculated and used as response variables. This yielded three separate estimates (one for each cross type) per plot for each response variable. A separate analysis was performed on each of three multiplicative fitness components: probability of emergence, probability of survival (of emerged plants) to produce a bud, and the inflorescence weight of plants that survived to produce a bud. To examine the cumulative fitness of seeds over the entire growing season, a final analysis assigned a fitness value of 0 to plants that did not emerge or survive. The distribution of residuals was sufficiently normal to meet assumptions of ANOVA for emergence, survival, and inflorescence weight, so no transformations were performed on plot means before analysis. The distribution of residuals was less normal for the analysis of cumulative fitness (Shapiro-Wilks $W = 0.90$) and included a large number of 0's, so the results of this analysis must be interpreted with caution. Each analysis treated pool zone (center or edge), cross type, and zone × cross type interaction as fixed effects and block nested within zone as a random effect. Post hoc contrasts among cross types were performed using the Tukey-Kramer adjustment for multiple comparisons.

Using plot means instead of individual data points may have caused a loss of power to detect significant effects and also made it impossible to examine the effects of lineages on cross type effects. To see whether this was causing any artifacts in the results, analyses were also performed with generalized linear mixed models (PROC GLIMMIX with a binary error distribution specified) for emergence and survival and a linear mixed model (PROC MIXED) for cumulative fitness using individuals as the data points (Littell et al. 2006). For each variable, the model that best fitted the data was identified by comparing Akaike's information criterion corrected values, corrected for small sample size, among models with and without family and plot included (Littell et al. 2006). Patterns of significance were consistent with those produced by the analyses using plot means, so only the results of plot means are presented below.

Results

During the spring of 2003, microelevation within vernal pools strongly influenced peak flowering time (microelevation effect in ANCOVA: $F = 277.42$, $\mathrm{df} = 1,461$, $P < .0001$) and the overall flowering curves (microelevation effect in repeated-measures ANCOVA: $F = 35.57$, $\mathrm{df} = 1,460$, $P < .001$) for *Lasthenia fremontii* (fig. 1). The flowering curves differed across elevations (microelevation × census date interaction, repeated-measures ANCOVA: $F = 26.9$, $\mathrm{df} = 14,447$, $P < .0001$). There were differences among pools in mean flowering time (pool effect in ANCOVA: $F = 47.15$, $\mathrm{df} = 7,461$, $P < .0001$; in repeated-measures ANCOVA: $F = 13.67$, $\mathrm{df} = 7,460$, $P < .0001$) and the steepness of the phenological gradient (microelevation × pool interaction in ANCOVA: $F = 9.49$, $\mathrm{df} = 7,461$, $P < .0001$; in repeated-measures ANCOVA: $F = 10.13$, $\mathrm{df} = 7,460$, $P < .0001$). Although the slope of the relationship between elevation and peak flowering date varied among pools, regressions conducted within pools indicated that *L. fremontii* individuals near the edge of the population consistently reached peak flowering significantly earlier than those at deeper positions ($P < .01$ in all cases; fig. 1).

Even though flowering time is closely tied to elevation position along the inundation gradient, there can still be substantial overlap in flowering between late-flowering individuals at the edge and early flowering individuals at the center of the pool (figs. 2, 3). Flowering time overlap between plants at the centermost and edgemost thirds of the pool ranged from 0.03 to 0.99. The overlap values were strikingly bimodal: four pools had overlap values between 0.03 and 0.15, while the remaining four pools had values

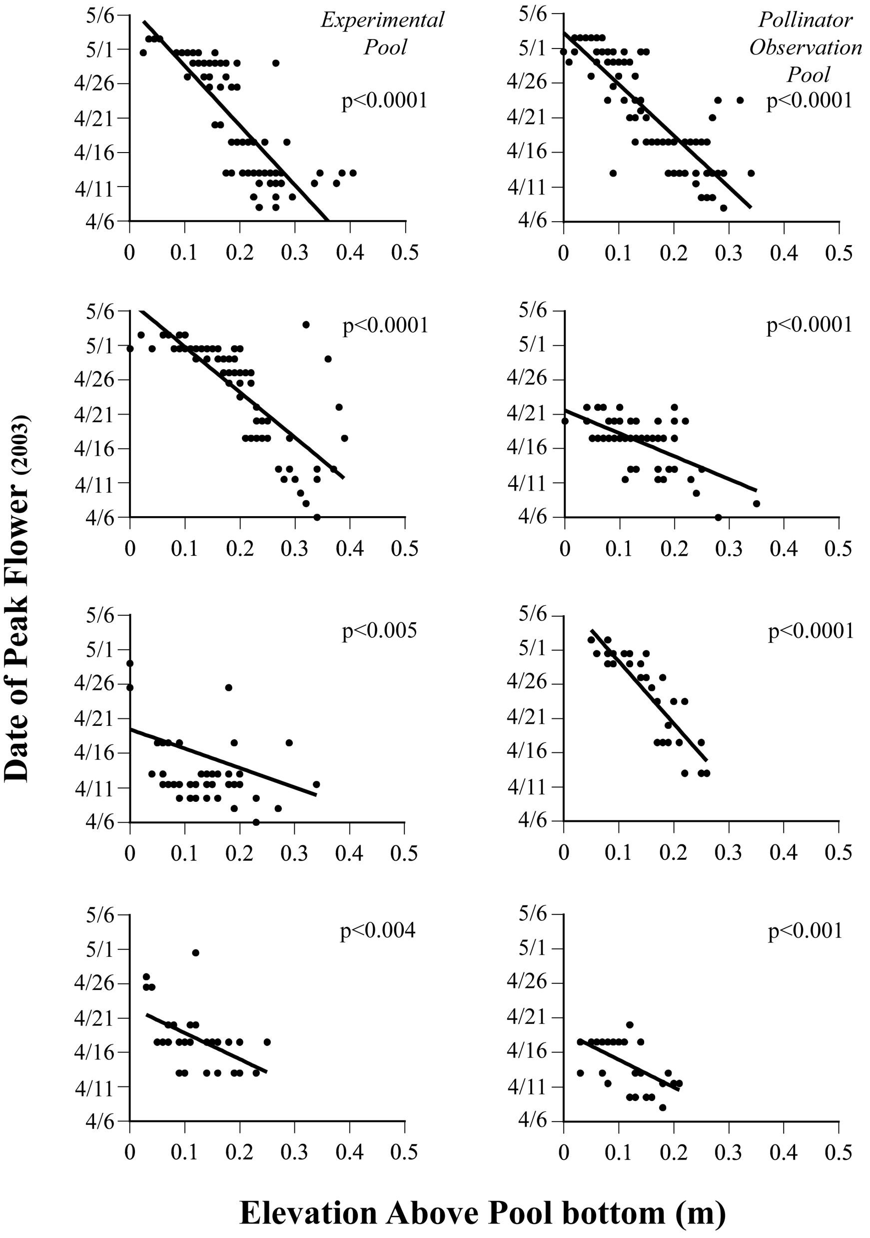


Figure 1: Relationship between elevation and flowering phenology in eight different *Lasthenia fremontii* populations at Mather Field in spring 2003. Elevation is presented relative to the bottom of each pool, with 0 representing the deepest point and the largest value representing the upper limit of the *L. fremontii* population. The *Y*-axis corresponds to the date at which sample plots reached peak flower. *P* values represent the significance of simple regressions performed on individual pools, with date of peak flower as the response variable and elevation as the predictor variable (see "Methods").

between 0.69 and 0.99. These differences may be at least partially explained by pool size, as there was a trend toward larger pools having lower overlap indexes than smaller pools, but largely because of one outlier and a small sample size, this correlation was not significant (correlation between pool size and proportion overlap: $r = -0.56$, $N = 8$, $P = .15$).

The degree of overlap can vary among years, depending on precipitation patterns. This pattern is illustrated in the experimental pool by comparing flowering data from sample plots falling within the center and edge microelevations in the 2003 census with those from reference plots that were monitored in the experimental pool during the pollination experiment in 2005 (fig. 3). In 2003, which was

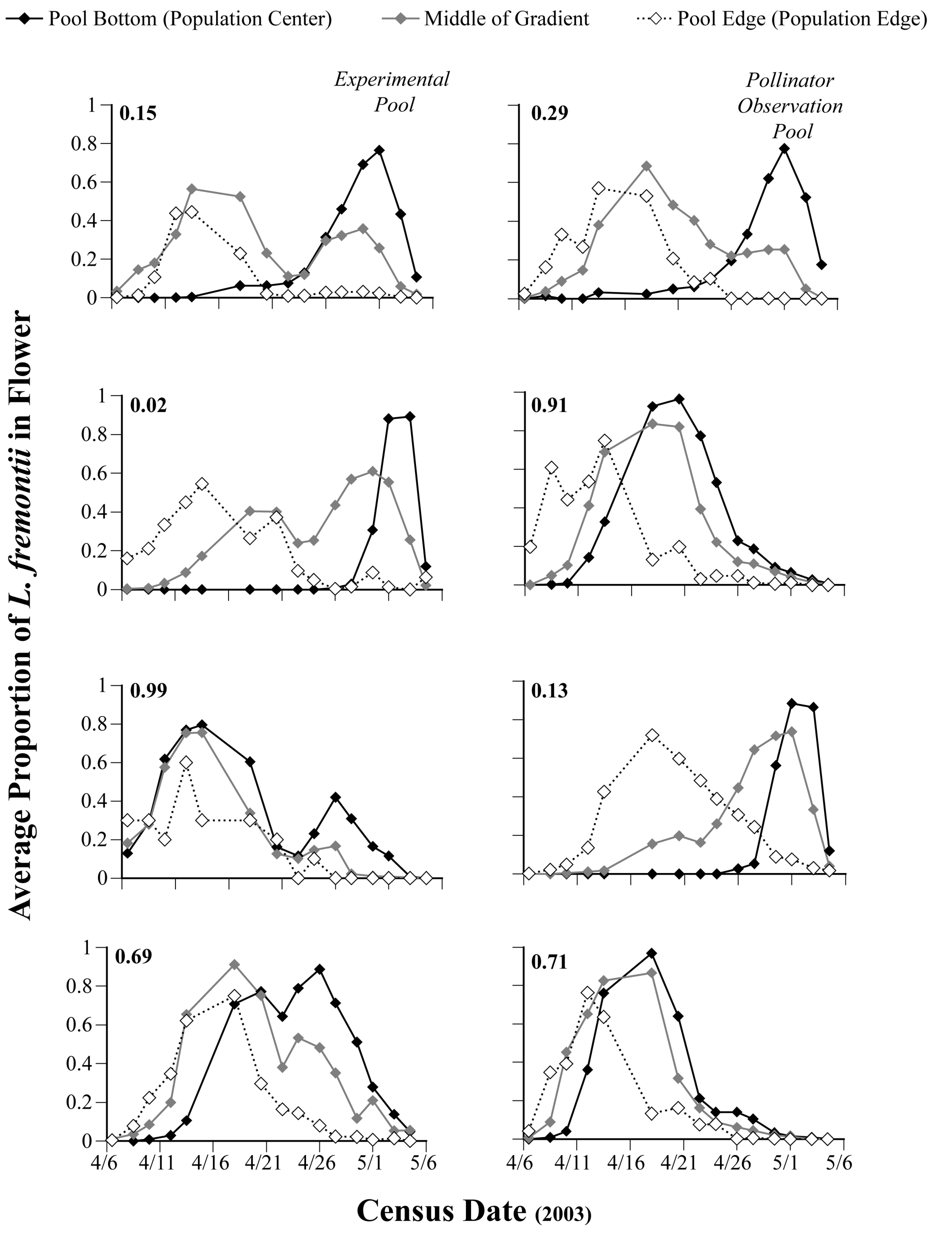


Figure 2: Flowering phenology and overlap of *Lasthenia fremontii* in plots at the center and edge of eight different pools in 2003. Within each pool, plots from the phenological census were grouped into zones based on their positions along the elevation gradient occupied by the population: plots falling within the lower third of the gradient were in the pool bottom zone, plots in the highest third of the plots were in the pool edge zone, and plots in between the pool bottom and pool edge were grouped into the middle gradient zone. The average proportion of plants flowering on each census date was calculated for each zone; a value of 1 would indicate that all individuals in all plots were flowering. The *Y*-axis represents the average proportion of individuals in each habitat that were in flower on a given day. The number in the upper left-hand corner of each panel represents Hurlbert's (1978) overlap index of pool bottom (population center) and pool edge (population edge) phenological curves.

characterized by a particularly wet spring, the edge of this population reached peak flowering approximately 23 days before the center of the population, and late spring rains enabled a mild second flush of flowering to occur at the edge. In 2005 (which had a relatively dry spring), the edge of the population reached peak flowering only 2 weeks before the center of the population, but again there was a great deal of overlap between flowering individuals in both population zones for a large portion of the flowering season.

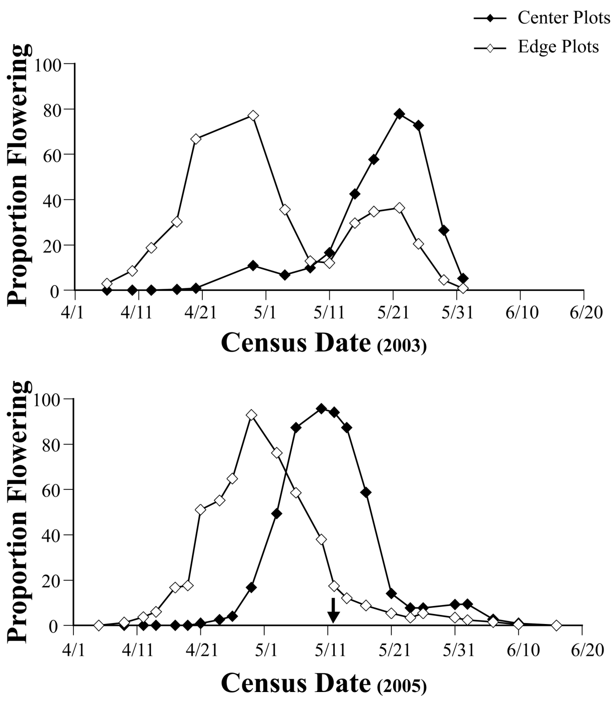


Figure 3: Flowering phenology of *Lasthenia fremontii* subpopulations at the center and edge of the experimental pool population in 2003 and 2005. Data from center and edge plots for 2003 were extracted from the larger multipool census, while the 2005 data were collected from reference plots placed at the center and edge of the same population in 2005 (full phenological curves for center and edge plots in all eight pools in 2003 are presented in fig. 2). Filled and open diamonds represent the average proportion of plants in center and edge plots, respectively, that were in flower on a given census date. The arrow in the bottom panel indicates the date on which pollinator observations were conducted in this population (see "Methods").

Results of the pollen addition experiment suggest that seed set is limited by pollen availability in late-flowering plants at the edge of the population. There was a general trend for early flowering plants to produce significantly more viable seeds than later-flowering individuals both in the center and at the edge of the population (table 1). Further analysis showed that this pattern was driven by control plants at the edge of the population, as reflected in the marginally significant three-way interaction in the full ANCOVA (table 1). In subsequent analyses within zones and treatments, flowering date did not have a significant effect on seed production in the center of the population, regardless of pollination treatment (two-way ANCOVA in center zone, flower date effect: $F = 0.31$; $\mathrm{df} = 1, 74$; $P = .5771$). In contrast, there was a marginally significant interaction between flower date and pollination treatment at the population edge ($F = 3.29$; $\mathrm{df} = 1, 91$; $P = .0729$). Flowering time did not determine viable seed production in the pollen addition treatment at the edge (one-way regression in edge plants with pollen addition treatment, flower date effect: $t = 1.31$, $\mathrm{df} = 46$, $P = .1940$). In contrast, the relationship between flower date and viable seed production was highly significant for control plants at the population edge (one-way regression in edge controls, flower date effect: $t = 1.73$, $\mathrm{df} = 44$, $P = .0004$).

Pollinator observations on May 12, 2005, suggest that pollinators forage within narrow elevation bounds of the flowering population. *Bombylius* pollinators moved within very narrow microelevational limits during the observation period, despite substantial horizontal movement within those boundaries (fig. 4). Although the distribution of flowering *L. fremontii* individuals spanned a vertical distance of 17.5 cm across the vernal pool gradient, the maximum net elevation change traveled by a single bee fly over an entire observation period was only 7.5 cm, and the average maximum elevation change per pollinator was 4.2 cm. The average elevation change by each fly between successive flags (placed at every fifth flower visited) ranged from 0 to 2.75 cm, and the average standard deviation of elevations visited by a pollinator was 1.3 cm. Although pollinators stayed within narrow microelevational bounds, they moved an average of 15–46 cm horizontally per five flowers visited. All pollinators observed remained between 7 and 17 cm above the pool bottom, even though flowering *L. fremontii* were documented at elevations ranging from 3 to 20.5 cm on this date.

The pollinator movements observed in the pollinator observation pool on May 12 and 19, 2006, reinforced that pollinator behavior is restricted to a narrower range of elevations than is occupied by flowering plants. On May 12, the flowering portion of the *L. fremontii* population spanned elevations from 15.5 to 32 cm above the pool bottom, but pollinators were observed only at elevations ranging from 19.5 to 32 cm. Furthermore, individual pollinators stayed within a narrow range of elevations, moving an average of 0.17 cm up or down the gradient while covering an average of 40.2 cm in horizontal distance between successive flags. The maximum elevation change observed for a single pollinator during an observation was 9 cm, and the average maximum elevation change was 4.3 cm. The average standard deviation for the elevations visited by a pollinator was 1.7 cm. On May 19, pollinators were observed across a broader range of the inundation gradient than the previous week, but individual pollinator sequences remained focused to narrow elevation ranges. On this day, flowering *L. fremontii* individuals were documented at elevations between 1.0 and 30.0 cm above the pool bottom, spanning almost the entire inundation gradient. Pollinators were observed at elevations ranging from 9.5 to 32.0 cm. The average elevation change between

Table 1: Results from an ANCOVA testing for effects of pollen addition treatment (control or pollen supplementation), population zone (center or edge), and the mean date of first flower on the mean number of viable seeds per inflorescence produced by *Lasthenia fremontii* individuals ($N = 50$ individuals/treatment/zone)

Source of variation	F	Pr > F
Treatment	.47	.4925
Zone	.13	.7140
Treatment × zone	3.57	.0605
Date of first flower	9.88	.0020
Date of first flower × treatment	.14	.7091
Date of first flower × zone	.67	.4155
Date of first flower × treatment × zone	3.45	.0651
Number of ovules	2,541.00	<.0001

Note: The mean number of ovules per inflorescence was included as a covariate in the model to account for differences in the effect of microenvironment quality and overall plant vigor on the number of viable seeds produced per inflorescence. For all tests, df = 1.

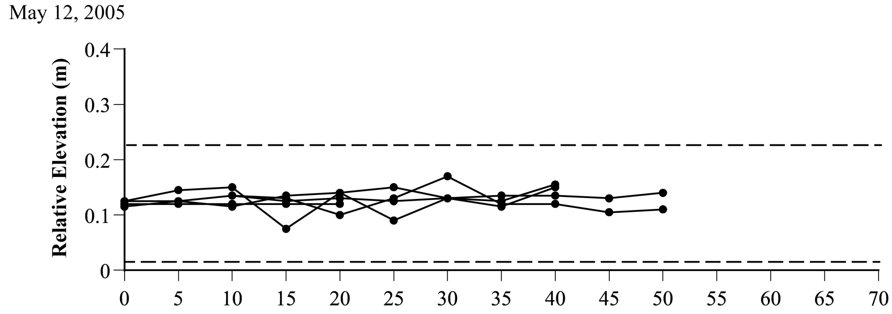
successive flags was only 0.14 cm, and the average standard deviation in elevations visited by a pollinator was 1.7 cm. The average maximum elevation change across an observation sequence was 3.3 cm, and the maximum change observed by a single pollinator was 9.0 cm. The average horizontal distance between successive flags decreased from the previous week to 28.2 cm every five visits.

The field experiment tested for effects of pollen movement and seed dispersal on offspring fitness and revealed differences among cross types in emergence rate and a trend toward differences in cumulative fitness (table 2; fig. 5). A posteriori comparisons showed that seeds from edge × edge crosses were less likely to emerge than either center × center crosses (Tukey-Kramer HSD, $P < .03$) or center × edge crosses (Tukey-Kramer HSD, $P < .006$). Seeds from all cross types were more likely to emerge as seedlings at the center of the pool than at the edge. After emergence, no significant effects of elevation zone or cross type were detected on the probability of survival or the mean inflorescence weight of surviving individuals, although both of these postemergence measures of performance varied among plots within elevation zones (table 2). In part because of high levels of variation among plots, the analysis of cumulative fitness (in which seeds that did not emerge were assigned a fitness of 0) revealed only a marginally significant trend for edge × edge plants to have lower cumulative fitness than the other cross types in both elevation zones ($P < .08$; table 2; fig. 5).

Discussion

This study challenges the assumption that center-to-edge pollen movement dominates the trajectory of gene flow in a population distributed across an environmental gradient and provides the first experimental test of the hypothesis that center-to-edge gene flow reduces fitness at population boundaries. In the *Lasthenia fremontii* populations followed in this study, population densities are generally highest at the bottoms of the pools and decline with increasing elevation toward pool edges, consistent with the density distributions predicted to yield center-to-edge gene flow dynamics in theoretical models (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Case and Taper 2000). However, results from observations and experiments present some clear deviations from the assumptions and predictions of the gene-swamping hypothesis. First, the environmental gradient, which drives the density differences observed between the center and the edge of a contiguous population, also reduces the potential for center-to-edge pollen transfer through its strong, direct effects on flowering phenology and indirect effects on pollinator visitation. Furthermore, cross-gradient gene flow did not lead to a reduction in fitness at the edge of the population in the year of the study, as would be expected if center-to-edge gene flow were “swamping” locally adapted alleles.

Flowering phenology and pollinator behavior restrict the potential for center-to-edge gene flow by pollen transfer in the *L. fremontii* populations examined in this study. In these populations, a wave of peak flowering tracks the recession of water in a pool, beginning at the edge of a pool in early spring and collapsing inward toward the pool bottom as temperatures rise and precipitation declines. This elevation-specific flowering time (fig. 1) restricts the potential for center-to-edge pollen transfer across the inundation gradient (figs. 2, 3). While some degree of overlap between the center and the edge of the population is common (fig. 2), the pollen addition experiment found that seed set is limited by pollen availability in late-flowering edge individuals. As a result, the late-flowering edge individuals—which are phenologically available to receive pollen from the center of the population—are less *a. Experimental Pool*



b. Pollinator Observation Pool

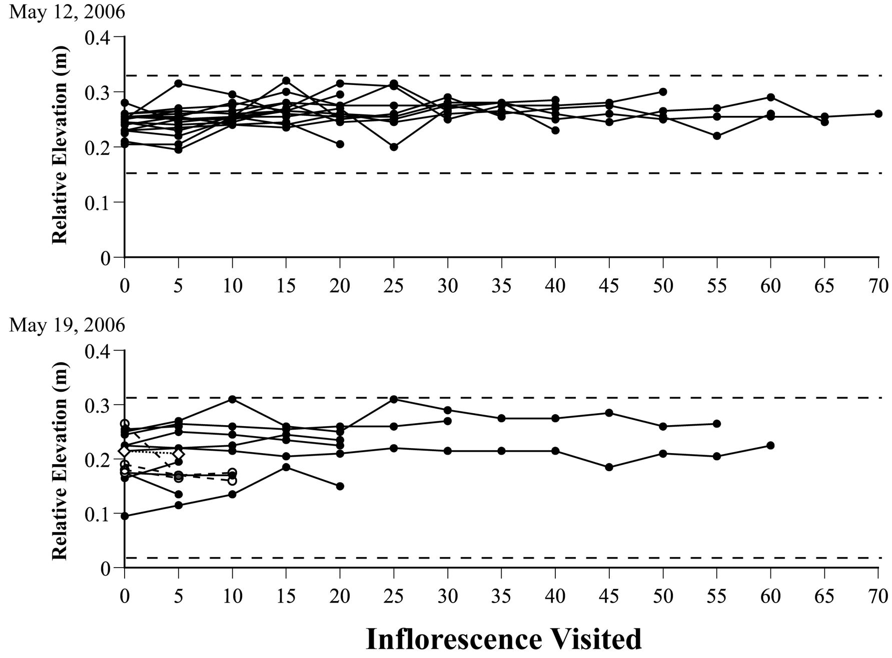


Figure 4: Pollinator movement patterns with respect to microelevation in the two different vernal pools. *a*, Experimental pool (as designated in fig. 1) during the middle of the flowering period in 2005. *b*, Pollinator observation pool on two separate days (early season and midseason) during the flowering period in 2006. During an observation, the position of every fifth inflorescence visited by each pollinator was flagged, and its elevation (relative to the bottom of the pool) was measured. The dashed lines indicate the upper and lower elevations of flowering *Lasthenia fremontii* on the day these observations were conducted. Symbols represent different genera of pollinators observed: *filled circles* = bee flies; *open circles* = syrphids; *open diamonds* = bees.

likely to be visited by pollinators, further restricting the potential for center-to-edge pollen movement to occur. This result is supported by limited observations of pollinator foraging behavior, which indicated that individual pollinators forage at remarkably consistent elevations within flowering *L. fremontii* bands (fig. 4). It is possible that pollinators are disproportionately foraging in areas of highest flower density, which moves downslope over the course of the spring. A separate study is rigorously testing the hypothesis that *L. fremontii* pollinators forage nonrandomly with respect to the inundation gradient and concentrate their activity to areas of peak flower density (N. C. Emery, R. Runquist, and M. Stanton, unpublished manuscript).

Results from the transplant experiment also did not provide clear evidence that swamping gene flow from the

Table 2: Results from a series of general mixed models testing for effects of transplant destination, cross type, and plot on the fitness components of experimental transplants

	Emergence	Survival	Inflorescence weight	Cumulative fitness
Fixed-effect analysis ($\Pr > F$):				
Destination zone	.0220	.6210	.2077	.5114
Cross type	.0058	.4428	.3193	.0830
Destination × cross type	.2975	.9572	.2148	.8819
Random-effect analysis ($\Pr(Z)$):				
Plot (destination)	.1329	.0471	.0102	.0081

Note: Seeds from three different cross types (center × center, center × edge, and edge × edge) were transplanted into destination plots at the center and the edge of the population. The following fitness components were analyzed: probability of emergence, probability of surviving to produce a bud (once emerged), inflorescence weight of plants that survived to produce a bud, and, finally, cumulative fitness per seed planted. Plot means for each cross type were used as the response variables. In the final analysis of cumulative fitness, individuals that did not emerge were assigned a fitness value of 0, and the distribution of residuals was only marginally normal (see "Methods").

center to the edge of the *L. fremontii* population is limiting adaptation at the edge. Significant lineage effects in emergence rate did indicate that patterns of gene flow influence offspring phenotypes. But contrary to model predictions, gene movement from the population center into the edge environment did not reduce mean fitness in edge individuals: center × center and center × edge lineages tended to outperform edge × edge lineages, even in the edge environment. These performance differences were driven by differences in emergence rates (fig. 5). One hypothesis explaining the observed differences among lineages is that higher variability in moisture conditions at the edge, compared with those at the center, has selected for higher dormancy rates in edge lineages. Environmental variability can favor lineages that spread their offspring across many years to reduce the risk of losing all offspring in a single unfavorable growing season (Cohen 1966; Venable and Brown 1988). If divergence in dormancy rates has occurred between center and edge lineages, then the fitness differences observed in the year of the transplant experiment may not be consistent across years; high germination rates in poor years might lead to the periodic extinction of central lineages that colonize the edge habitat and an overall fitness advantage of edge genotypes with more conservative germination rates. This overall fitness advantage would be detected only by following the fate of central and edge lineages across multiple years.

An alternative hypothesis explaining the observed differences between lineage types is that pure edge genotypes had lower seed viability and, thus, a trend toward lower overall fitness than the other lineage types. This hypothesis is supported by the observation that hand-pollinations between edge individuals resulted in fewer seeds than those involving center lineages under greenhouse conditions (see "Methods"). Low population densities may interact with phenological structure and local pollinator movement to generate inbreeding depression in edge individuals, which may be exacerbated by stressful conditions (Hoffman and Parson 1991). Low effective population sizes can also increase the strength of genetic drift at the edge compared with the center, driving a loss of genetic variation and the fixation of deleterious alleles. Genetic drift and inbreeding depression are expected to restrict range expansion in highly fragmented, marginal populations that are relatively isolated from more productive patches (Lennon et al. 1997; Alleaume-Benharira et al. 2006), but little is known about the potential for these processes to operate at the edges of contiguous populations. Future work aims to describe patterns of neutral molecular differentiation across *L. fremontii* populations to estimate patterns of genetic variation as a function of pool depth.

As in many natural populations, year-to-year and among-population environmental variability in *L. fremontii* has the potential to be quite high. The amount of annual precipitation received by a pool and the rate at which the water recedes from the pool in the spring largely determine the phenological structure across elevations in a given pool (figs. 1–3), ultimately influencing the potential for pollen to be transferred from the center to the edge of the population. Phenological structure also varies among pools (figs. 1, 2). The patterns of selection across the vernal pool gradient are understood to be driven largely by inundation patterns (Linhart and Baker 1973; Linhart 1974) and species composition (Linhart 1988), so the nature of the selection gradient itself may vary among pools and years. Even within pools, variation in the degree of temporal heterogeneity across the gradient may serve as an agent of divergent selection on traits such as dormancy rates or phenotypic plasticity. Adaptive divergence in edge lineages may transiently develop in years (or pools) with high phenological structure, only to be swamped out by years (or pools) with low phenological structure and high levels of

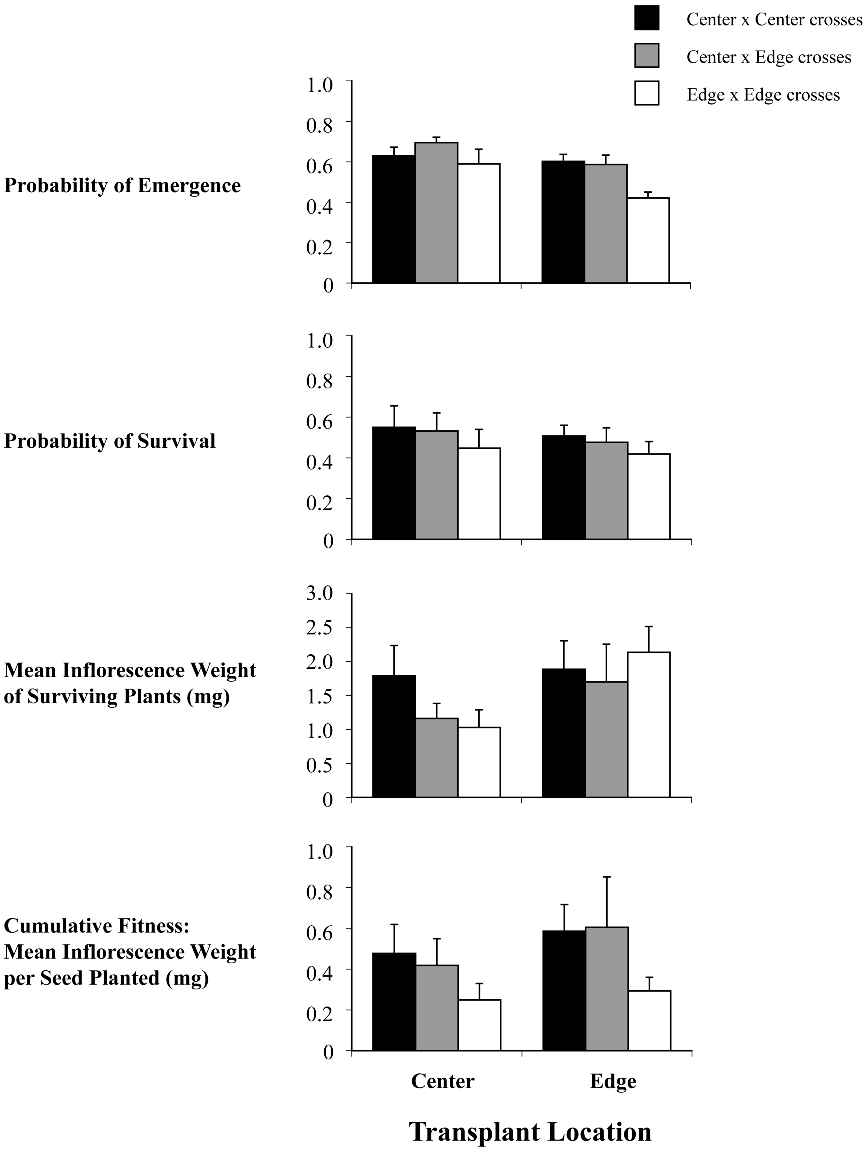


Figure 5: Effects of cross type and transplant destination zone on fitness components in *Lasthenia fremontii* transplants. Controlled crosses among field-collected plants were performed in the greenhouse to simulate within-zone (center × center, edge × edge) and between-zone (center sire × edge dam) pollen movement. Seeds generated from crosses were planted into plots located at the center and edge of the population. For each fitness component, plot means were calculated for each cross type, and means were used as the response variables for statistical analyses. The fitness components examined were the probability of emergence, the probability of survival of emerged seeds, and the mean inflorescence weight of survivors. Cumulative fitness estimates reflect the mean reproductive output per seed planted, with a fitness value of 0 assigned to seeds that did not emerge.

center-to-edge gene flow. In 2004–2005, the edge population was a "sink" in the experimental pool, but these results cannot be generalized across pools or even across years within the same pool. But while the environmental gradient can vary dramatically among years (Emery et al. 2009) and among pools, *L. fremontii* is consistently restricted to similar elevations within individual pools (Barbour et al. 2003; Emery et al. 2009). Perhaps this variability plays a key role in limits to adaptation at the edge in this species. Inconsistent demographic processes and selection pressures may be the primary challenge facing evolution of adaptation to the edge environment by edge lineages. Importantly, theoretical models of the evolution of range limits have largely considered scenarios where the underlying environmental heterogeneity is constant across generations, and thus the potential role of a gradient in environmental variation is unknown.

Empirical work on the evolution of distribution patterns (at all spatial scales) has generally lagged behind the development of theory in this area, and more experimental studies are greatly needed to test the assumptions and predictions of models. Results from this single study already suggest several routes that theory on the evolution of range limits and ecological specialization could explore. First, we know little about the complex effects that environmental heterogeneity can have on the potential for gene flow within natural populations, beyond the simple diffusion processes resulting from differences in population densities. Additionally, understanding the potentially negative effects of restricted gene flow may shed insight into the evolutionary processes restricting adaptation in marginal populations that are spatially or phenologically isolated from the primary population(s). Density differences across a distribution may lead to center-to-edge gene swamping, but they may also generate differences in the amount of genetic variation available, the relative strength of selection and drift, and the potential for inbreeding depression to develop. Genetic studies have shown that molecular and allozyme variation is lower near the edges of many species' ranges (Gaston 1998). This tension between the swamping effects of gene flow (i.e., "migration load") and the negative effects of restricted gene flow (i.e., "inbreeding load"; Lenormand 2002) may be important in determining whether the distributions of many species are conserved or expand over time. Finally, the conditions experienced by natural populations are often highly variable; the environmental heterogeneity that sets ecological limits to population boundaries can vary in time and across populations. Understanding how natural levels of variation influence the evolution of species' borders is an exciting and relatively unexplored avenue for both theoretical and empirical research.

Acknowledgments

I thank K. Rice, R. Sargent, D. Schemske, M. Stanton, and three anonymous reviewers for valuable comments on previous drafts of this article. B. Baythavong, O. Ervin, J. Hartman, M. Levine, K. Rice, R. Runquist, M. Stanton, and J. Wilcox-Wright all provided assistance with the design and implementation of this study. Funding was provided by a Doctoral Dissertation Improvement Grant (0309006) from the National Science Foundation.

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