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THE ECOLOGY AND EVOLUTION OF ELEVATION RANGE LIMITS IN MONKEYFLOWERS (*MIMULUS CARDINALIS* AND *M. LEWISII*)

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THE ECOLOGY AND EVOLUTION OF ELEVATION RANGE LIMITS IN MONKEYFLOWERS (*MIMULUS CARDINALIS* AND *M. LEWISII*)

By

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ABSTRACT

THE ECOLOGY AND EVOLUTON OF ELEVATION RANGE LIMITS IN MONKEYFLOWERS (*MIMULUS CARDINALIS* AND *M. LEWISII*)

By

Amy Lauren Angert

Living organisms inhabit an incredible array of environments across the planet, but any particular species occurs in only a subset of habitats and geographic areas, an observation so fundamental that its cause is rarely questioned. Nevertheless, the ecological and evolutionary forces that give rise to species' distribution limits remain poorly understood. To determine whether species are maladapted to the environment at and beyond the distribution boundary, I investigate how fitness changes across the elevation ranges of closely related species of monkeyflower, *Mimulus cardinalis* and *M. lewisii* (Phrymaceae) in the Sierra Nevada Mountains, California. I use transition matrix models to estimate asymptotic population growth rates and find that population growth rates of *M. lewisii* are highest at the range center and reduced at the range margin. Population growth rates of *M. cardinalis* are highest at the range margin and greatly reduced at the range center. Because observations of natural populations cannot determine fitness beyond a species' present distribution, I reciprocally transplanted *M. cardinalis* and *M. lewisii* within and beyond their present elevation ranges. For both species, I find the greatest average fitness at elevations central within the range, reduced fitness at the range margin, and zero or near-zero fitness when transplanted beyond the present elevation range limit.

To identify the underlying causes for changes in fitness versus elevation, I examine plant performance in growth chambers simulating low and high elevation temperature regimes and show that temperature alone generates patterns of differential survival and growth similar to those observed in reciprocal transplant gardens. *Mimulus lewisii* and *M. cardinalis* differ in photosynthetic physiology under temperature regimes characterizing their contrasting low and high elevation range centers, suggesting that the species' elevation range limits may arise, in part, due to metabolic limitations on growth that ultimately decrease survival and limit reproduction. To measure natural selection on physiological and phenological traits within and beyond elevation range limits, I transplanted interspecific hybrids to low and high elevation and find that selection favors early flowering at high elevation and increased leaf photosynthetic capacity in warm temperatures at low elevation. I also find that hybrids selected at high elevation display reduced biomass when grown in temperatures characteristic of low elevation, suggesting that adaptation to the environment within the range may entail a cost to adaptation in other environments that places evolutionary constraints on range expansion.

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CHAPTER 1

Demography of central and marginal populations of monkeyflowers (*Mimulus cardinalis* and *M. lewisii*)

Abstract—Every species occupies a limited geographic area, but how spatiotemporal environmental variation affects individual and population fitness to create range limits is not well understood. Because range boundaries arise where, on average, populations are more likely to go extinct than to persist, range limits are an inherently population-level problem that require a demographic framework. In this study, I compare demographic parameters and population dynamics between central and marginal populations of monkeyflowers, *Mimulus cardinalis* and *M. lewisii*, along an elevation gradient spanning both species' ranges. Central and marginal populations of both species differed marginally in survival and significantly in fecundity. For *M. lewisii*, these components of fitness were higher in central than in marginal populations, but for *M. cardinalis* the converse was true. To assess spatiotemporal variation in population dynamics, I used transition matrix models to estimate asymptotic population growth rates (λ) and found that population growth rates of *M. lewisii* were highest at the range center and reduced at the range margin. Population growth rates of *M. cardinalis* were highest at the range margin and greatly reduced at the range center. During the study period, temporal variation in λ was of smaller magnitude than spatial variation in λ. Using life table response analysis, I decomposed spatiotemporal variation in λ into contributions from each transition between life stages and found that transitions from large non-reproductive and reproductive plants to the seed class and stasis in the

reproductive class made the largest contributions to spatial differences in λ. These transitions had only low to moderate sensitivities, and sensitivity values were largely similar across all locations, indicating that differences in projected population growth rates resulted mainly from observed differences in transition matrix parameters and their underlying vital rates. Continued study of spatiotemporal variation in population dynamics, in combination with estimates of dispersal between central and marginal populations, will improve our understanding of the species' distribution limits.

Key words: population dynamics, range limit, matrix population models, life table response experiment

Every species occupies a limited geographic area. Sometimes ranges end at obvious environmental discontinuities, but more often ranges end at "seemingly arbitrary" points along gradual environmental gradients (Kirkpatrick and Barton 1997). Linking spatial and temporal variation in the environment to variation in both individual and population fitness is critical to understanding species' distribution limits (Holt and Keitt 2005). Because range boundaries arise where, on average, the probability of population extinction exceeds the probability of persistence, range limits are an inherently population-level problem for which a demographic framework is informative.

Range margins are often assumed to be coincident with ecological margins, such that species reach the limit of their environmental tolerance at a range boundary and are maladapted to conditions beyond the range (Antonovics 1976; Lesica and Allendorf 1995). Consistent with this characterization, species abundance (i.e., local population

density) often decreases with distance from the range center, presumably in response to an increasingly unfavorable environment (McClure and Price 1976; Svensson 1992; Telleria and Santos 1993; Brown et al. 1996; but see Sagarin and Gaines 2002 and references therein). Observations of individual performance across the range frequently find lower survival of certain life history stages or reduced fecundity at the range margin relative to the range center (Marshall 1968; Pigott and Huntley 1981; McKee and Richards 1996; Garcia et al. 2000; Jump and Woodward 2003). However, whether reductions in some fitness components impact population growth and persistence is not always evident. In some instances, reductions in individual performance alone, without consideration of its secondary effects on population dynamics, may be insufficient to explain the position of a range boundary (Prince and Carter 1985).

Carter and Prince (1988) suggested that the small reductions in fecundity observed across the range boundary of *Lactuca serriola* are insufficient to explain failure to occur beyond its present distribution. If populations are not seed limited, reductions in fecundity may not translate into reduced recruitment and population growth rates (Turnbull et al. 2000; Maron and Simms 2001). A decrease in one fitness component from the center to the edge of the range also may be mitigated by other differences. For example, a loss of migratory behavior may counterbalance lower juvenile fitness in southern marginal populations of the Iberian robin, *Erithacus rubecula* (Perez-Tris et al. 2000). In the aquatic plant *Decodon verticillatus,* vegetative reproduction may offset reductions in sexual reproduction in northern peripheral populations (Dorken and Eckert 2001), and conditional seed dormancy may ensure persistence of peripheral populations of wild barley, *Hordeum spontaneum* (Volis et al.

2004). To thoroughly understand geographic range limits, components of performance must be integrated into models of population growth across species' distributions (Pulliam 2000).

Temporal patterns of variation, as well as the interaction between spatial position and temporal dynamics, are also important to understanding the dynamics of populations across species' ranges (Ives and Klopfer 1997). Central populations might exhibit greater inter-annual variability if intrinsic rates of increase are high in optimal habitat or if regulation by biotic factors such as predation acts more strongly when population density is high (Williams et al. 2003). Alternatively, marginal populations may be at or near the limit of environmental tolerance, and consequently more vulnerable to environmental fluctuations that exceed mean tolerance levels in some years, causing marginal populations to vary in size or age structure more than central populations (Gaston 1990; Brown et al. 1995). Occasional climatic extremes have been observed to cause pulses of mortality or reproductive failure at northern range limits in populations of tropical trees (Olmsted et al. 1993), pool frogs (Sjögren 1991), pied flycatchers (Jarvinen and Vaisanen 1984), and North American birds (Mehlman 1997). Williams et al. (2003) found that marginal populations of three bird species were both less dense and experienced greater variability in density-independent population growth rates. If marginal populations are small and exhibit high variability, then they may be vulnerable to extinction (Curnutt et al. 1996; Nantel and Gagnon 1999; Maurer and Taper 2002; Vucetich and Waite 2003).

Several studies hypothesize that spatial gradients in extinction risk, colonization rates, and/or habitat availability can create stable range boundaries (Carter and Prince

1981; Lennon et al. 1997; Holt and Keitt 2000; Maurer and Taper 2002). Other models suggest that marginal populations may be demographic sinks, sustained only by immigration from source populations at the range center (Pulliam 1988; Kawecki 1995; Curnutt et al. 1996; Kirkpatrick and Barton 1997; Guo et al. 2005; Holt et al. 2005). Although time series and survey data have been used to examine spatiotemporal population variation across the range over long time periods and at broad spatial scales (Curnutt et al. 1996; Mehlman 1997; Doherty et al. 2003; Williams et al. 2003), very few investigations have compared the demography of central versus marginal populations (but see Nantel and Gagnon 1999; Stokes et al. 2004; Volis et al. 2004).

This paper presents a comparison of demographic parameters and population dynamics between central and marginal populations of sister species of monkeyflowers, *Mimulus cardinalis* and *M. lewisii*, along an elevation gradient spanning both species' ranges. To assess spatiotemporal variation in population dynamics, I used several analyses based on transition matrix models. I estimated the asymptotic population growth rate (λ) in central and marginal populations over three yearly transition intervals, and examined the sensitivity of λ to perturbations in matrix elements. I used life table response experiments (Caswell 2001) to decompose spatiotemporal variation in λ into contributions from each transition between life cycle stages. Specifically, this study investigated the following questions: 1) How do vital rates and population growth rates vary between central and marginal populations? and 2) Which life cycle transitions are responsible for observed differences in population growth rate between populations?

MATERIALS AND METHODS

Study System

Mimulus cardinalis and *M. lewisii* (Phrymaceae) are rhizomatous perennial herbs that grow along seeps and stream banks in western North America. Both species are self-compatible and animal pollinated (Hiesey et al. 1971; Schemske and Bradshaw 1999). The species occupy different latitudinal and altitudinal ranges. *Mimulus cardinalis* occurs from southern Oregon to northern Baja California and from coastal California to Arizona and Nevada. *Mimulus lewisii* is composed of two races, a northern form occurring from southern coastal Alaska to southern Oregon and eastward to the Rocky Mountains and a southern form occurring primarily in the Sierra Nevada Mountains of California (Hiesey et al. 1971; Hickman 1993; Beardsley et al. 2003). Here I study only the Sierran form of *M. lewisii*. In California, *M. cardinalis* occurs from sea level to 2400 m and *M. lewisii* occurs from 1200 m to 3100 m (Hickman 1993). In the Yosemite National Park region where this research was conducted, the species co-occur on larger watercourses between 1200 and 1500 m elevation (A. Angert, unpub. data). Repeated attempts to locate extant populations in the Yosemite region at the upper limits of the published Californian distributions were unsuccessful, so I consider 1200-1500 m to be the shared mid elevation range limit of both species.

Populations of each species were monitored along an elevation transect from 373 m to 2750 m within 37.464 and 38.098 ° N latitude in Yosemite National Park and the surrounding Stanislaus, Inyo and Sierra National Forests (Appendix A). Although this transect represents only a small fraction of each species' geographic range, it provides a gradient from elevation range center to elevation range margin for both species at a tractable scale. The Yosemite region of the Sierra Nevada Mountains offers a large area of undeveloped habitat in which to study species' natural distributions. Six

sites were selected for detailed demographic study based on elevation and habitat quality (Appendix A). Two sites were located at middle elevation (Wawona, 1208 m, and Carlon, 1320 m) where both species occur sympatrically at their range margin. The remaining four sites were located at the low (Buck Meadows, 830 m, and Rainbow Pool, 833 m) and high (May Lake, 2690 m, and Warren Fork, 2750 m) elevation range centers for *M. cardinalis* and *M. lewisii*, respectively. Additional sites were selected across a continuous range of elevations for estimates of plant density along 50-200 m transects (Appendix A).

Census Plots

During July-August 2000, multiple census plots were established within each of the six demographic sites. Plots varied in size and number across sites due to differences in habitat and plant density (average plot size 103.4 m^2, range 8 – 459 m^2; average total plot area per site 800.7 m^2, range 437.8 – 1160.5 m^2). The plots were chosen to span natural environmental variation present at each site and to encompass areas suitable for all life history transitions so that together they are representative of performance at the site as a whole. The corners of each plot were marked with rebar to facilitate relocation in subsequent years and to establish an (x, y)-coordinate system for mapping plant locations. Within each plot every *M. cardinalis* and/or *M. lewisii* individual was mapped to the nearest 5 cm on the (x, y)-coordinate grid and marked with a unique number using an aluminum write-on tag wrapped around a nail except when rocks prevented tag placement. When tag placement was impossible, (x, y)-coordinates were used to identify individuals. Individuals were defined as discrete clusters of stems separated from other stems by at least ten cm except when stems had

evidence of physical connection or were known to have arisen from multiple seedlings. Because both species are capable of clonal growth via rhizomes, a small number of stems marked as individuals, particularly at the beginning of the study, may have been ramets of the same genet.

Following plot establishment in 2000, censuses were conducted twice per year in early summer and autumn from 2001 - 2003. The date of censuses varied each year with the timing of snowmelt and spring floods. The early summer census captured over-winter survival of plants recorded in the previous year and spring seedling germination. The autumn census captured survival, growth and reproduction during the growing season. At the early summer census, new seedlings were mapped on the (x, y)-grid and given an impermanent colored marker. At the autumn census, permanent aluminum tags were given to all surviving recruits from the early summer census and to any additional recruits not present at the summer census. Over the four years of this study (2000 – 2003), the fates of a total of 16,849 plants were recorded (Buck Meadows, 569; Rainbow Pool, 2,157; Wawona, 128 *M. lewisii*, 4,557 *M. cardinalis*; Carlon, 1,537 *M. lewisii*, 3721 *M. cardinalis*; May Lake, 1,513; and Warren Fork, 2,667). Stem number, stem length, flowering status, and flower and fruit number of all plants was recorded each autumn. For each plant, up to 20 non-flowering and 20 flowering stems were measured from the ground to the base of the last pair of expanded leaves; all remaining stems were tallied and used to estimate total stem length based on the average stem length of the 40 measured non-flowering and flowering stems.

Plant fecundity was estimated by multiplying the number of mature fruits per flowering plant by the population mean seed number per fruit. Each fruit contains

approximately 500-2500 tiny seeds and flowering individuals may have hundreds of fruits. Each fall, two fruits were harvested from each of 10 individuals growing at least several hundred meters downstream of the census plots. Sampling downstream ensured that patterns of seedling emergence within the plots were not altered by seed removal. In the lab, samples of approximately 200 seeds per fruit were counted under a dissecting microscope and weighed to determine the relationship between seed mass and seed number. Seed number per fruit was then estimated from the total seed mass. Seed samples could not be obtained for *M. lewisii* at Carlon in 2002 or for *M. cardinalis* at Rainbow Pool in 2002 and Buck Meadows in 2003, so average seed number per fruit across all other years at the particular location was used to estimate fecundity.

Seed Dormancy

Mesh pouches. — Variation in population size and persistence may be influenced by recruitment from a seed bank (Volis et al. 2004). Air-dried seeds of *M. cardinalis* and *M. lewisii* remain viable for many years when stored at room temperature (pers. obs.). This suggests that a seed bank could play a role in seedling recruitment and population persistence. To ascertain whether significant seed dormancy exists in nature, a seed viability experiment was initiated at one central and one marginal site for each species in September 2001 (central: *M. cardinalis*, Rainbow Pool; *M. lewisii*, May Lake; marginal: both species, Carlon). Field-collected seeds were enclosed in 5 x 10 cm pouches made from fine mesh ("No Thrips", 150 x 150 μ opening size, Green-Tek, Inc., Edgerton, WI, USA), allowing the seeds exposure to air, water and light while preventing seed entry into or escape from the pouch. Approximately five hundred seeds were placed in each of four pouches per site. Each pouch was staked to the ground in

the vicinity of a reproductive plant to ensure that experimental seeds experienced environmental conditions similar to naturally dispersed seeds. No germination was observed while seeds were in the pouch. Two pouches were removed from each site in autumn 2002 and 2003, with the exception of the central *M. cardinalis* site where all pouches were destroyed by vandalism. Pouches were taken to Michigan State University where the contents were sieved to separate seeds from silt. Seeds were then placed on moistened soil and allowed to germinate in the greenhouse. After one year, germination was 4.9% (out of the initial 500) in *M. lewisii* seeds from May Lake, 15.2% in *M. lewisii* seeds from Carlon, and 7.8% in *M. cardinalis* seeds from Carlon. After two years, germination was 8.6% in *M. lewisii* seeds from May Lake, 3.6% in *M. lewisii* seeds from Carlon, and 0.2% in *M. cardinalis* seeds from Carlon, demonstrating that seeds of both species may remain dormant and viable for at least one year in the seed bank.

PVC stations. — To obtain parameter estimates for seed survival, detailed studies of seed dormancy were initiated at one central and one marginal site per species in September 2002 (central: *M. cardinalis*, Buck Meadows; *M. lewisii*, May Lake; marginal: both species, Carlon). At May Lake and Carlon, eight replicate 20 x 20 cm plots per species were excavated to a depth of 15 cm to remove any previously existing seed bank. In each plot, seed-free soil from above the floodplain was used to refill the excavated area. Four rings cut from sections of poly(vinyl chloride) pipe (10 cm diameter, 8 cm tall) were buried in each plot, leaving 1 cm of ring above ground level. Two rings per station were randomly assigned the seed treatment, in which approximately 2160 seeds by volume were added in September 2002, and the remaining

two rings served as "no-seed" controls. At Buck Meadows the design was identical except a seed shortage allowed only 1 seed treatment per station containing approximately 1250 seeds by volume. Each year seedlings were counted and removed with forceps from these rings, once in early summer after most emergence had occurred and again in the autumn to capture any additional germination. Numerous stations were lost to flooding, tree fall, and animal disturbance, including all stations at Carlon. Due to small remaining sample sizes, data were pooled within each site for species-specific estimates of seed survival parameters (Appendix B).

Stage Classification

Each population was classified into four stages present at the autumn census using biological criteria based on relationships between size, survival, and reproduction and examination of frequency distributions of stem lengths for different aged cohorts of plants. To facilitate comparisons among sites and years, classification criteria were developed using pooled data from all sites and years for each species. The boundary between small and large non-reproductive plants was defined as the midpoint between the median total stem length of first-year non-reproductives (i.e., seedlings) and the median total stem length of non-reproductives aged two and older (midpoint: 3 cm for *M. cardinalis*, 5 cm for *M. lewisii*). A seedling class based on age alone was not retained because rapid first-year growth frequently caused first-year plants to surpass older plants in size. Only one reproductive stage class was used because differences in the size distribution of reproductive plants between sites created small sample sizes in some reproductive classes when reproductive plants were subdivided by size. Also,

survival of reproductive plants within each site was not related to total stem length, indicating that subdivision of the reproductive class was not warranted.

Variation in Fates of Vegetative Plants

To examine variation in annual survival among locations and years, I modeled survival of each stage class as a function of position within the range (center or margin), population nested within range position, yearly transition interval (2000-2001, 2001-2002, or 2002-2003), and all interactions using a binomial distribution and a logit link function (PROC GLIMMIX, SAS, version 9, SAS Institute, Cary, NC, USA). Range position and year were considered as fixed effects and population within range position was considered as a random effect. To evaluate the significance of fixed effects, I used Type III estimable functions. To evaluate the significance of random effects, I tested whether the Z-value of each effect (its variance parameter divided by its approximate standard error) was different from zero (Juenger and Bergelson 2000). I report significance values for this and all other analyses with and without sequential Bonferroni adjustment to maintain a table-wide type I error rate of 0.05 for each species (Rice 1989, Moran 2003).

To examine variation in transition probabilities among locations and years, I performed log-linear analyses (Horvitz and Schemske 1995; Caswell 2001). All vegetative plants were classified into stage classes each year, including an extra class for dead plants. For each species, these analyses considered the following categorical variables: *state* (stage at time t: small non-reproductive, large non-reproductive, or reproductive), *year* (transition interval: 2000-2001, 2001-2002, or 2002-2003), *location* (*M. cardinalis*: Buck Meadows, Rainbow Pool, Wawona, or Carlon; *M. lewisii*: Wawona, Carlon, May Lake, or Warren Fork), and *fate* (stage at time t+1: small non-

reproductive, large non-reproductive, reproductive, or dead). The first set of analyses examined each state separately to ask whether the fate of a particular state varied among years and locations using three-way contingency tables defined by year, location, and fate for each initial state and the null hypothesis that fate was independent of year and location, given the predetermined distribution of plants into year and location categories (Horvitz and Schemske 1995). The second set of analyses examined whether the entire state by fate transition table varied between locations and years using the four-way contingency table defined by state, year, location, and fate and the null hypothesis that fate was affected by state but was independent of year and location (Caswell 2001). Likelihood ratio tests, obtained from the difference in goodness-of-fit G^2 values between two models that differed only in the factor being tested, were used to evaluate the significance of particular factors (Caswell 2001). Log-likelihood statistics for all three-way analyses were obtained with PROC CATMOD (SAS, version 8, SAS Institute, Cary, NC), using the LOGLIN option and adding 0.5 to all cell counts to avoid estimation problems caused by zeros (Horvitz and Schemske 1995). Log-likelihood statistics for four-way analyses were obtained by summing stage-specific G^2 values from three-way analyses across all stages (Horvitz and Schemske 1995).

Variation in Reproduction

To examine spatiotemporal variation in reproduction, I analyzed the effects of range position (center or margin), population nested within range position, and year on the reproductive variables total flower number (of flowering plants only), fruit set (the proportion of flowers maturing seeds), and seed number per fruit using PROC MIXED (SAS, version 8, SAS Institute, Cary, NC). Range position and year were considered as

fixed effects and population within range position was considered as a random effect. Missing seed counts per fruit at some locations in some years prevented the analysis of interactions with year for the dependent variable seed number per fruit. To meet the assumptions of traditional linear analysis, flower number was log-transformed and fruit set was arcsine square-root transformed. Analyses of transformed data produced qualitatively similar results to generalized linear analyses using Poisson (flower number) or binomial (fruit set) distributions; I present traditional linear analyses for simplicity. To evaluate the significance of fixed effects, I used Type III estimable functions, which tolerate unbalanced samples, with denominator degrees of freedom obtained by Satterthwaite's approximation. Likelihood-ratio tests, comparing each reduced model to the full model including all effects, were used to evaluate the significance of random effects.

Construction of Matrix Models

Transition matrix models of population dynamics were constructed using estimates of reproduction, seed dormancy, recruitment, and transition probabilities among the three vegetative stages. These calculations were performed for each location and transition year to generate a set of 12 location-year matrices per species. The calculations were also performed on data pooled across all years within each location to generate a set of 4 pooled location matrices per species. Due to small sample size (N=5) of large non-reproductive *M. lewisii* at Wawona during 2002-2003, estimates of transitions from the large non-reproductive stage class were obtained from average transition frequencies across all years at Wawona (as in Menges and Dolan 1998).

The projection matrix model for these analyses was a linear, time-invariant model of the form

$$\mathbf{n(t+1)} = \mathbf{A} \cdot \mathbf{n(t)},$$

where $\mathbf{n(t)}$ is a vector of stage-classified individuals in the population at time t, $\mathbf{n(t+1)}$ is the stage-classified vector of individuals at one time step in the future, and $\mathbf{A}$ is a 4 x 4 projection matrix of transition probabilities and stage-specific fecundities that shows how individuals in stage j at time t contribute to stage i at time t+1. The top left-hand corner, a_{11}, is seed dormancy; other cells in the top row, $a_{12} - a_{14}$, are fecundities (mean number of seeds produced by a reproductive plant at time t+1) weighted by the probability of an individual in class j at time t becoming reproductive at time t+1. Non-reproductive stages have a non-zero contribution to the seed class if they may become reproductive within one time step. Occasionally, rapid growth of spring germinants enabled them to reach the reproductive class by the autumn census, in which case the top left-hand corner is both seed dormancy and the seed contribution of newly germinated reproductive plants.

Matrix Analysis

The dominant eigenvalue of a projection matrix is the asymptotic population growth rate, λ (Caswell 2001). Although other interesting demographic parameters such as the stable stage distribution or reproductive values may be obtained from matrix projection analysis, I chose to focus on λ as a synthetic measure of demographic success in each environment.

A fixed-design life table response experiment (LTRE; Caswell 2001) was used to model λ of each species as a linear function of location, l, yearly transition interval, y, and their interaction, ly:

$$\lambda^{(ly)} = \lambda^{(\cdot\cdot)} + \alpha^{(l)} + \beta^{(y)} + (\alpha\beta)^{(ly)}$$

where $\alpha^{(l)}$ is the effect of the l^{th} level of the location treatment, $\beta^{(y)}$ is the effect of the y^{th} level of the year treatment, and $(\alpha\beta)^{(ly)}$ is the interaction of the l^{th} location and y^{th} year, measured relative to the projected growth rate of the reference matrix $(\cdot\cdot)$. The reference matrix can be obtained from an unmanipulated control or by combining data from all treatments into a mean (calculated by averaging transition frequencies) or pooled (calculated from pooled raw data) matrix (Miriti et al. 2001). I chose to use a pooled reference matrix, which weighted observed transitions by their frequency in the entire dataset (Horvitz and Schemske 1995) and better approximated observed lambdas than a mean reference matrix. Treatment effects were estimated as

$$\begin{aligned}
\alpha^{(l)} &= \lambda^{(l\cdot)} - \lambda^{(\cdot\cdot)} \\
&\approx \Sigma(a_{ij}^{(l\cdot)} - a_{ij}^{(\cdot\cdot)}) \cdot (\partial\lambda/\partial a_{ij}) \Big|_{(\mathbf{A}^{(l\cdot)} + \mathbf{A}^{(\cdot\cdot)})/2} \\
\beta^{(y)} &= \lambda^{(\cdot y)} - \lambda^{(\cdot\cdot)} \\
&\approx \Sigma(a_{ij}^{(\cdot y)} - a_{ij}^{(\cdot\cdot)}) \cdot (\partial\lambda/\partial a_{ij}) \Big|_{(\mathbf{A}^{(\cdot y)} + \mathbf{A}^{(\cdot\cdot)})/2} \\
\alpha\beta^{(ly)} &= \lambda^{(ly)} - \lambda^{(\cdot\cdot)} - \alpha^{(l)} - \beta^{(y)} \\
&\approx \Sigma(a_{ij}^{(ly)} - a_{ij}^{(\cdot\cdot)}) \cdot (\partial\lambda/\partial a_{ij}) \Big|_{(\mathbf{A}^{(ly)} + \mathbf{A}^{(\cdot\cdot)})/2} - \alpha^{(l)} - \beta^{(y)}
\end{aligned}$$

where the sensitivity of λ to changes in a matrix entry, $\partial\lambda/\partial a_{ij}$, was evaluated midway between the treatment and the reference matrices and obtained from the equation $\partial\lambda/\partial a_{ij} = v_i w_j / \langle \mathbf{w}, \mathbf{v} \rangle$, where $\mathbf{v}$ and $\mathbf{w}$ are the right and left eigenvectors of the matrix (Caswell 2001).

The above equations can be interpreted to mean that the effect of the treatments on population growth depends on both observed variation in matrix elements and the sensitivity of population growth to variation in those elements. The contribution of a particular matrix element a_{ij} to variation in λ may be low if a_{ij} did not vary between treatments and/or if λ is insensitive to variation in a_{ij}. A matrix element with high sensitivity may not contribute to variation in λ if the transition was unaltered by the treatments. Conversely, a matrix element with slight variation but high sensitivity may make a large contribution to variation in λ.

To assess uncertainty in population projections, I used bootstrapping to calculate bias-corrected 95% percentile confidence intervals around estimates of λ, sensitivities, and LTRE contributions (Caswell 2001). Bootstrap calculations were designed to mimic the data structure used to generate matrix parameters. For example, individuals were stored as columns in a data array, where rows represented fates and fruit numbers, and randomly selected with replacement to generate a bootstrapped dataset of size equal to the population sample size. For each bootstrapped dataset, transition probabilities among vegetative classes, total fruit number at times t and t-1, and fruit number per reproductive at time t+1 were calculated. For estimates involving seed number, a seed number per fruit was drawn at random from the empirical cumulative probability distribution of seed number for each time (t-1, t, and t+1) and then multiplied by fruit number within every bootstrap replicate. The empirical probability distribution for seed number was derived from the average seed count per fruit from ten individuals per species, location and year. An estimate of seed dormancy was drawn at random from the cumulative probability distribution of seed dormancy,

which was assumed to be normally distributed with mean and standard deviation derived from estimates across multiple seed stations for each species.

Non-parametric randomization tests based on random permutations of individuals between groups were used to test specific hypotheses about differences in λ among yearly transition intervals and between locations (Caswell 2001). To assess whether λ varied among yearly transition intervals within a location, individuals were randomly permuted among pairs of years, keeping sample sizes for each transition interval fixed (Fréville et al. 2004). Transition frequencies and fruit counts for each permuted dataset were calculated as described for each bootstrapped dataset above. Mean seed counts at times t-1, t, and t+1 were permuted independently, then combined with transition frequencies and fruit counts to generate matrices and calculate λ for each transition interval for each of 2000 permuted datasets. The significance of the observed standard deviation of λ among yearly transition intervals within each location was then compared to the randomized distribution of standard deviations with a one-tailed test (Caswell 2001; Fréville et al. 2004). To assess whether λ differed between locations, data were first pooled over all years within each location. Individuals, with their complete histories, and mean seed counts were randomly permuted between locations in a similar fashion to permutations among transition intervals as described above. The observed absolute value of the difference in λ was compared to the randomized distribution of absolute differences with a two-tailed test (Brys et al. 2004). Because six pairwise comparisons of locations were made for each species, significance levels were adjusted according to the sequential Bonferroni procedure (Rice 1989; Edgington

1995). All matrix calculations were performed in Matlab, version 6.1 (The MathWorks, Natick, MA, USA).

Transects

Fourteen (*M. cardinalis*) and sixteen (*M. lewisii*) additional census transects per species were established across a continuous range of elevations to examine spatiotemporal variation in local population density and to ensure that inferences about variation in population dynamics across the elevation ranges of *M. cardinalis* and *M. lewisii* were drawn from a representative sample of central and marginal sites (Appendix A). Along each 50 – 200 m transect, every small non-reproductive, large non-reproductive, and reproductive individual of each species was tallied in autumn 2001, 2002 and 2003. The area of suitable habitat along each transect was estimated to correct for variation across sites in habitat availability. Density of each stage class was expressed as the number of individuals per m^2. Linear regression models were used to examine variation in mean (over 2001-2003) stage class density versus elevation (PROC REG, SAS, version 8, SAS Institute, Cary, NC). To examine temporal variation in local population density, coefficients of variation in stage class density across years were calculated and regressed against elevation. The sequential Bonferroni procedure was used to maintain a table-wide type I error rate of $\alpha = 0.05$ for each species (Rice 1989).

RESULTS

Spatiotemporal Variation in Fates of Vegetative Plants

Small non-reproductive plants showed the lowest annual survival (*M. cardinalis*: 11 – 22%, *M. lewisii*: 7 – 26%), and reproductive plants showed the highest annual

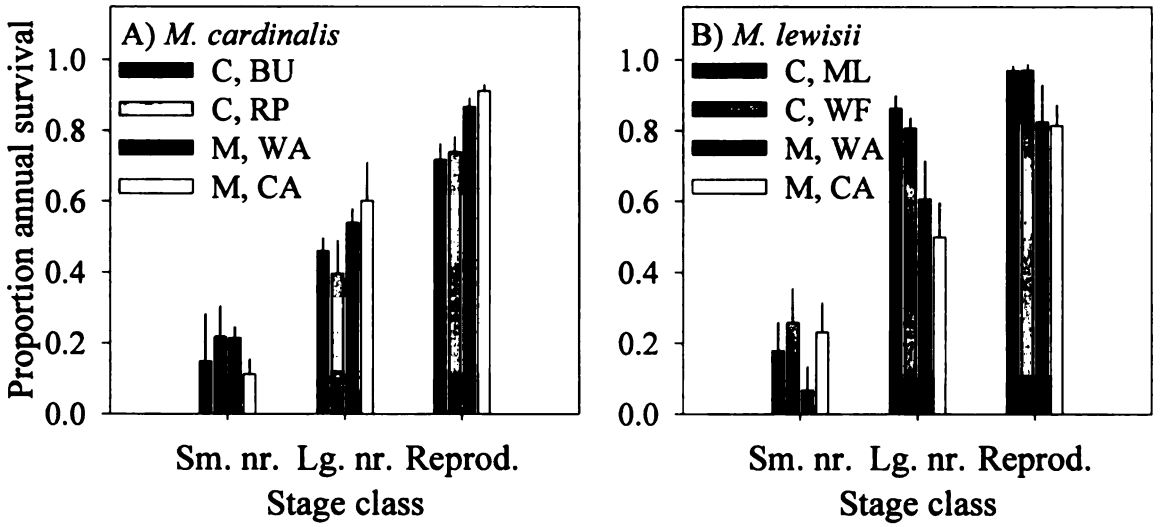

Figure 1. Spatial variation in proportion survival of each stage class. Data presented are means (over all yearly transition intervals) + SE. Stage class abbreviations as follows: Sm. non. = small non-reproductive, Lg. non. = large non-reproductive, Reprod. = reproductive. Location abbreviations as follows: C = central location, M = marginal location, BU = Buck Meadows (830 m), RP = Rainbow Pool (833 m), WA = Wawona (1208 m), CA = Carlon (1320 m), ML = May Lake (2690 m), WF = Warren Fork (2750 m).

survival (*M. cardinalis*: 72 – 91%, *M. lewisii*: 81 – 97%; Figure 1). Position within the elevation range affected survival of reproductive plants of both species and marginally affected survival of *M. lewisii* large non-reproductive plants, although these effects did not remain significant after sequential Bonferroni correction (Table 1). Survival of *M. cardinalis* reproductive plants was higher at the range margin than at the range center, whereas survival of *M. lewisii* reproductive plants was higher at the range center than at the range margin (Figure 1). Year did not affect annual survival, and the interaction of year and range position affected *M. lewisii* small non-reproductive plants only. The random effects of population within range position and the interaction of population and year were not related to annual survival of any stage class (Table 1).

Log-linear analyses of transition probabilities for each stage class revealed that, for both species, year significantly affected the fate of non-reproductive but not

Table 1. Generalized linear mixed models of the effects of range position, location within range position, and yearly transition interval on annual survival. Survival was modeled with a binomial distribution and a logit link function. F tests for fixed effects constructed by SAS GLIMMIX procedure. Z values for random effects obtained by dividing each variance estimate by its approximate standard error. No values remained significant after sequential Bonferroni adjustment to maintain table-wide type I error of 0.05 for each species (Rice 1989).

Species	Effects		Small non-reproductive		Large non-reproductive		Reproductive	
M. cardinalis	Fixed	df	F	P	F	P	F	P
	Position	1	0.00	0.9535	6.10	0.1322	20.20	0.0461
	Year	2	2.89	0.1672	4.39	0.0978	2.68	0.1827
	Position*year	2	1.17	0.3969	0.27	0.7741	0.06	0.9447
	Random		Estimate	P	Estimate	P	Estimate	P
	Location (position)		0.08 ± 0.33	0.4090	0		0.01 ± 0.08	0.4483
	Location*year (position)		0.59 ± 0.54	0.1335	0.13 ± 0.10	0.1056	0.00 ± 0.12	0.5000
M. lewisii	Fixed		F	P	F	P	F	P
	Position	1	0.47	0.5646	13.32	0.0675	19.53	0.0476
	Year	2	3.67	0.1245	0.91	0.4739	1.14	0.4069
	Position*year	2	17.06	0.0110	0.02	0.9819	0.66	0.5639
	Random		Estimate	P	Estimate	P	Estimate	P
	Location (position)		0.90 ± 1.20	0.2266	0.02 ± 0.21	0.4681	0	
	Location*year (position)		0		0.27 ± 0.44	0.2709	0.27 ± 0.40	0.2514

reproductive stages and location significantly affected the fate of all three stage classes (Table 2). Marginal and conditional tests of the effects of location and year produced very similar results. Log-linear analyses of the four-way contingency table of state by fate transitions across locations and years showed that the null model SLY, SF did not fit the data. Lack of fit of the null model indicates that initial state was not sufficient for predicting fate given the distribution of states over locations and years. Location, year and the interaction between location and year made significant contributions to explaining variation in state by fate transitions for both *M. cardinalis* and *M. lewisii* (Table 2).

Spatiotemporal Variation in Reproduction

Table 3 gives the results of mixed model analysis of variance tests of the effects of year, position within the range, and population nested within range position on reproduction. For *M. cardinalis*, position within the range affected flower number and marginally affected fruit set. For *M. lewisii*, position within the range affected fruit set and seed number per fruit. Year affected *M. cardinalis* seed number but did not affect *M. lewisii* reproduction. The effect of position within the range did not depend on year, as indicated by non-significant year by range position interactions for both species. Population and population by year interactions affected some reproductive variables for both species, but in general, between-population variation at a given range position did not overwhelm differences in reproduction between central and marginal areas of the elevation range. *Mimulus cardinalis* displayed reduced fecundity at the low elevation range center compared to the mid elevation range margin due primarily to reduced flower number per reproductive plant and reduced fruit set (Figure 2a, c, e). *Mimulus*

Table 2. Log-linear analyses of the effects of location, L, and year, Y, on fate, F, for each stage class, S. Summing the effects of each stage class gives the four-way model of the effects of location and year on the state-by-fate transition table, conditional on the differences in states among locations and years. Notation follows convention for hierarchical models, such that the presence of an interaction implies the presence of all lower-order interactions and single factor terms contained in the interaction. Values in boldface remain significant after sequential Bonferroni correction to maintain a table-wide type I error rate of 0.05 for each species (Rice 1989).

Species	Three-way Models	df	G^2 Small non-reprod.	Large non-reprod.	Reprod.	Sum (df) four-way model
M. cardinalis	Test 1: marginal effect of year on fate					
	LY, F‡	33	318.65	167.75	123.10	609.50 (99)
	LY, YF§	27	260.17	107.55	112.91	480.63 (81)
	YF	6	**58.48****	**60.20****	10.19^{NS}	**128.87** (18)****
	Test 2: marginal effect of location on fate					
	LY, F‡	33	318.65	167.75	123.10	609.50 (99)
	LY, LF¡	24	164.64	118.45	26.78	309.87 (72)
	LF	9	**154.01****	**49.30****	**96.32****	**299.63** (27)****
	Test 3: conditional effect of year on fate					
	LY, LF¡	24	164.64	118.45	26.78	309.87 (72)
	LY, YF, LF‖	18	127.42	44.78	19.10	191.30 (54)
	YF (given L)	6	**37.22****	**73.67****	7.68^{NS}	**118.57** (18)****
	Test 4: conditional effect of location on fate					
	LY, YF§	27	260.17	107.55	112.91	480.63 (81)

Table 2 (cont'd).

LY, YF, LF‖	18	127.42	44.78	19.10	191.30 (54)
LF (given Y)	9	**132.75******	**62.77******	**93.81******	**289.33** (27)****
Test 5: three-way interaction of year, location and fate					
LY, YF, LF	18	127.42	44.78	19.10	191.30 (54)
LYF	0	0	0	0	0
LYF (given all two-ways)	18	**127.42******	**44.78*****	19.10 NS	**191.30** (54)****
M. lewisii Test 1: marginal effect of year on fate					
LY, F‡	33	218.35	178.38	129.43	526.16 (99)
LY, YF§	27	109.83	165.54	120.43	395.80 (81)
YF	6	**108.52******	12.84*	9.00 NS	**130.36** (18)****
Test 2: marginal effect of location on fate					
LY, F‡	33	218.35	178.38	129.43	526.16 (99)
LY, LF¡	24	120.49	70.34	31.93	222.76 (72)
LF	9	**97.86******	**108.04******	**97.50******	**303.40** (27)****
Test 3: conditional effect of year on fate					
LY, LF¡	24	120.49	70.34	31.93	222.76 (72)
LY, YF, LF‖	18	54.97	38.83	20.32	114.12 (54)
YF (given L)	6	**65.52******	**31.51******	11.61†	**108.64** (18)****
Test 4: conditional effect of location on fate					
LY, YF§	27	109.83	165.54	120.43	395.80 (81)
LY, YF, LF‖	18	54.97	38.83	20.32	114.12 (54)
LF (given Y)	9	**54.86******	**126.71******	**100.11******	**281.68** (27)****

Table 2 (cont'd).

Test 5: three-way interaction of year, location and fate					
LY, YF, LF	18	54.97	38.83	20.32	114.12 (54)
LYF	0	0	0	0	0
LYF (given all two-ways)	18	**54.97****	**38.83****	20.32^{NS}	**114.12** (54)****

^{NS}P > 0.10, †0.05 < P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

‡ When summed over all stages, gives the four-way model SLY, SF.

§ When summed over all stages, gives the four-way model SLY, SYF.

¡ When summed over all stages, gives the four-way model SLY, SLF.

‖ When summed over all stages, gives the four-way model SLY, SYF, SLF.

Table 3. Results of mixed model analysis of variance testing the effects of range position, year, and population within range position on reproduction. Random effects denoted by '[R]'. Flower number log-transformed and fruit set (proportion of flowers maturing fruit) arcsine square-root transformed prior to analysis. F-tests for fixed effects constructed by SAS MIXED procedure, with denominator degrees of freedom obtained from the Satterthwaite approximation and indicated in parentheses below each F-value. χ^2 values for random effects from likelihood ratio tests. Values in bold remain significant after sequential Bonferroni adjustment to maintain table-wide type I error of 0.05 for each species (Rice 1989).

			Response variable		
Species	Source	*df*	Flowers / plant	Fruit set	Seeds / fruit
M. cardinalis	Position	1	**23.26***** (9.5)	15.89† (1.96)	2.34 (1.89)
	Year	3	2.71 (9.09)	1.22 (5.62)	**12.40****** (107)
	Year*Position	3	0.47 (9.09)	0.05 (5.62)	—
	Pop(Position) [R]	1	0.00	1.40	7.30**
	Pop*Year(Position) [R]	1	3.1†	**31.20******	—
M. lewisii	Position	1	4.06 (1.65)	**17.43**** (7.12)	**91.8****** (138)
	Year	3	0.04 (4.25)	2.44 (7.11)	1.72 (138)
	Year*Position	3	0.14 (4.25)	2.03 (7.11)	—
	Pop(Position) [R]	1	0.60	0.00	0.00
	Pop*Year(Position) [R]	1	5.20*	**15.70*****	—

† $0.05 < P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

lewisii displayed highest fecundity at the high elevation range center and reduced fecundity at the mid elevation range margin due to lower fruit set and an approximately two-fold reduction in seed number per fruit (Figure 2b, d, f).

Seed Dormancy

In 2003, 2291 seedlings emerged at May Lake from an initial total of 21,600 seeds placed at five seed stations in 2002, giving a germination percentage of 10.6%. At Buck Meadows, 38 seedlings emerged in 2003 out of an initial 2500 seeds at two stations, giving a germination percentage of 1.5%. Both germination estimates are corrected for seedlings emerging from "no seed" controls (May Lake: 17, Buck Meadows: 1). In 2004, 63 additional seedlings at May Lake and 7 seedlings at Buck Meadows emerged from dormant seed treatments, after correcting for seedlings in "no seed" controls (May Lake: 32, Buck Meadows: 0). Based on these observed germination rates and following the calculations of Horvitz and Schemske (1995), the estimated percentage survival of seeds was 19.9% for *M. cardinalis* and 13.4% for *M. lewisii* (Appendix B). Although both species displayed similar overall seed survival, *M. lewisii* seeds were more likely to germinate than to become dormant, whereas *M. cardinalis* seeds were more likely to become dormant than to germinate (Appendix B).

Projection Matrix Analyses

Lambda values ranged from 0.47 to 1.16 for *M. cardinalis* and from 0.68 to 1.33 for *M. lewisii* (Figure 3; Appendix C). For *M. cardinalis*, lambdas at the low elevation range center were significantly lower than lambdas at the mid elevation range margin (Figure 3). The 95% confidence intervals for *M. cardinalis* low elevation lambdas never overlapped one, the value for stable population size, except at Buck Meadows from

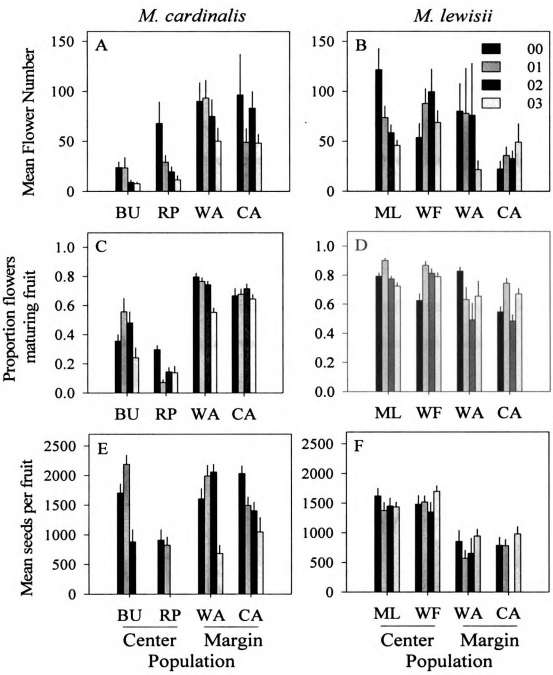

Figure 2. Spatiotemporal variation in reproduction. a) *M. cardinalis* flower number, b) *M. lewisii* flower number, c) *M. cardinalis* fruit set, d) *M. lewisii* fruit set, e) *M. cardinalis* seed number per fruit, and f) *M. lewisii* seed number per fruit. Data presented are means + SE. Location abbreviations as in Figure 1. Year abbreviations as follows: 00 = 2000, 01 = 2001, 02 = 2002, 03 = 2003.

2000-2001. In contrast, 95% confidence intervals for all *M. cardinalis* mid elevation lambdas overlapped or exceeded one except at Wawona from 2002-2003. For *M. lewisii*, lambdas at the high elevation range center were significantly higher than lambdas at the mid elevation range margin. However, most 95% confidence intervals at one marginal location (Carlon) overlapped one, whereas 95% confidence intervals at the second marginal location (Wawona) did not. At high elevation, the 95% confidence intervals for all lambdas overlapped one except at May Lake from 2002-2003. For *M. cardinalis*, significant temporal (among-year) variation in lambda was detected at one central (Rainbow Pool) and one marginal (Wawona) location (Figure 3). For *M. lewisii*, significant temporal variation in lambda was detected at all locations except for Wawona (Figure 3).

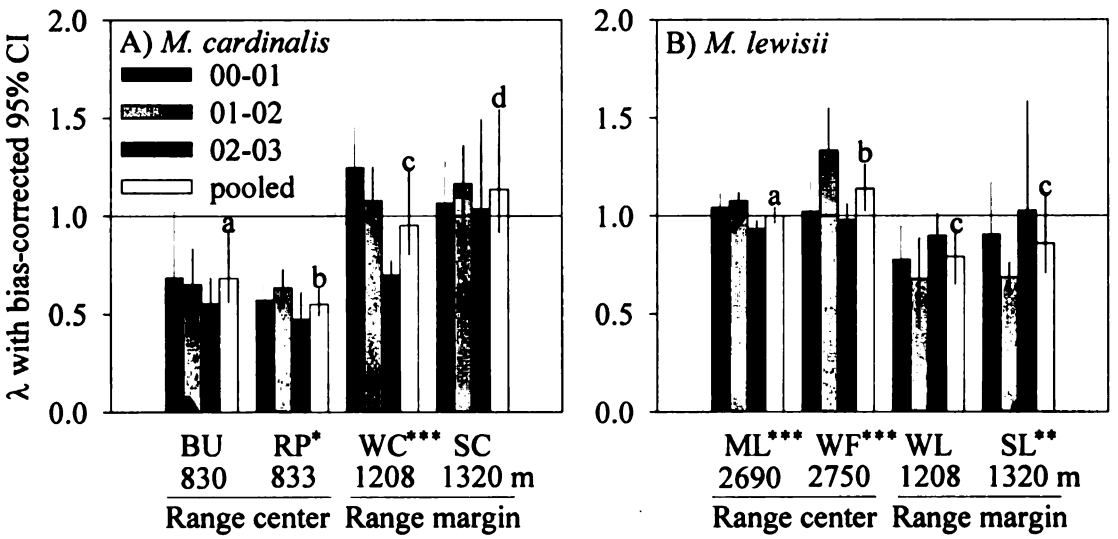

Figure 3. Asymptotic population growth rates (λ) for each location and transition interval and for pooled location matrices. Vertical bars indicate bias-corrected 95% confidence intervals (Caswell 2001). Asterisks indicate significant among-year variation within a location based on randomization tests (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). Pooled λ not sharing letters differ significantly from one another (after sequential Bonferroni adjustment) based on randomization tests. Abbreviations as in Figures 1 and 2.

Because separate estimates of dormancy from central and marginal populations were not available, I varied the dormancy component of the seed-to-seed transition by ± 50%. Decreasing seed dormancy by 50% decreased lambdas by 0.9 – 1.9% for *M. cardinalis* and by 0.03 – 0.2% for *M. lewisii*, and increasing seed dormancy by 50% increased lambdas by 1.3 – 3.0% for *M. cardinalis* and by 0.04 – 0.2% for *M. lewisii*. For both species, lambdas at all locations responded similarly to increases or decreases in seed dormancy, and the magnitude of change in lambda due to variation in the dormancy transition was not sufficient to erase differences in lambdas between central and marginal populations.

Transition matrices and sensitivity matrices are given in Appendix C. For both species at all locations and for all transition intervals, lambda was most sensitive to perturbations in transitions from seeds to vegetative stage classes, particularly from seeds to the reproductive stage class (Appendix C). Lambdas of both species were also sensitive to perturbations in transitions to the reproductive stage class from vegetative stages. Bias-corrected 95% confidence intervals were broadly overlapping among transition intervals and locations, indicating that all location-year matrices had similar sensitivity structure (data not shown).

Life Table Response Experiment

LTRE analysis confirmed that, for *M. cardinalis*, sites at the range center had a negative effect on lambda whereas sites at the range margin had a positive effect on lambda, where the overall effect of a particular treatment level is estimated by summing the contribution of each matrix element to variation in lambda (Figure 4a). For *M. lewisii*, on the other hand, sites at the range center had a positive effect on lambda and

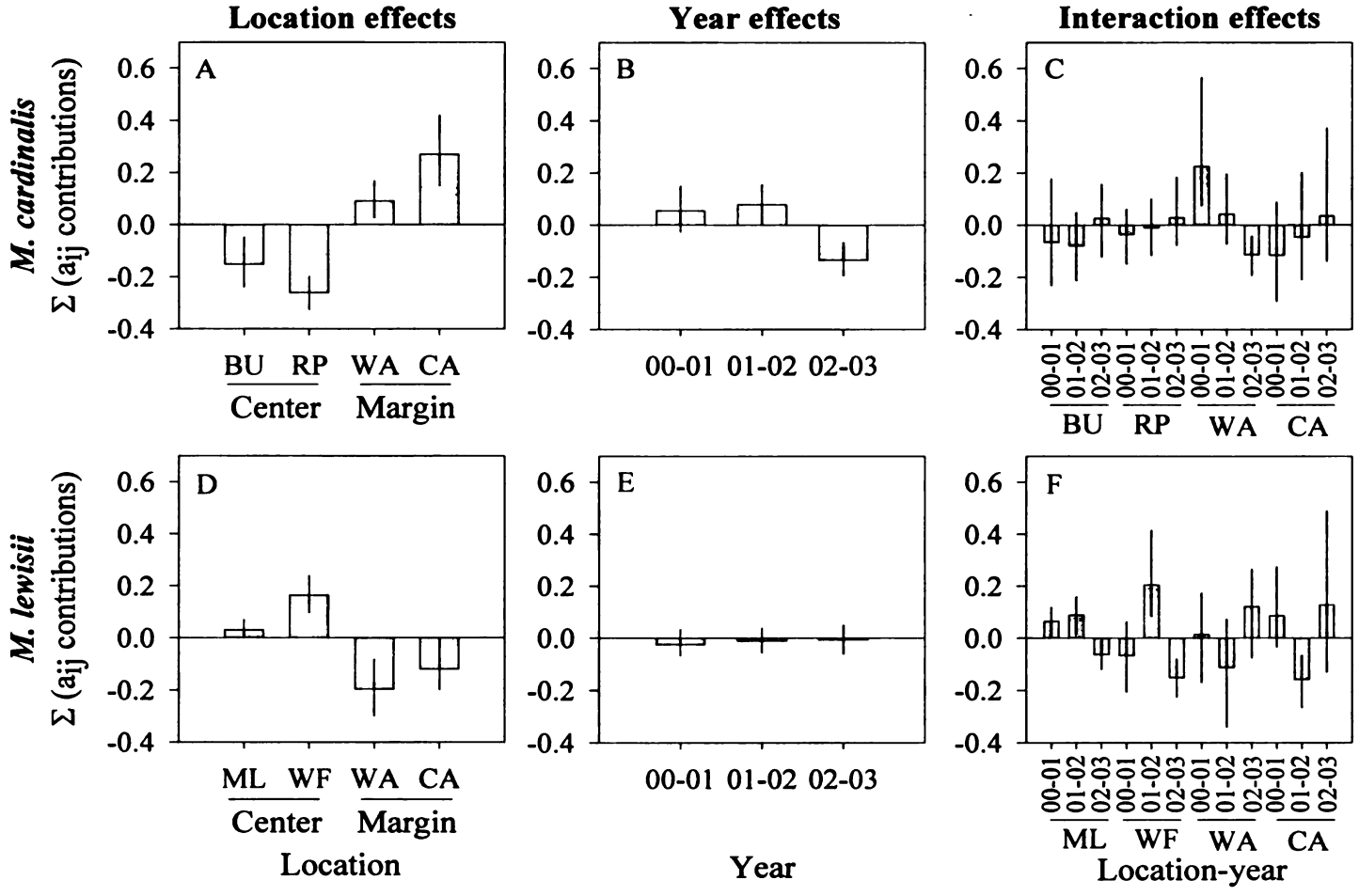

Figure 4. Life table response experiment (LTRE), with effects of location, yearly transition interval, and the interaction of location and year on λ. Effects obtained by summing the contribution of each transition matrix element to variation in lambda. Vertical bars indicate bias-corrected 95% confidence intervals (Caswell 2001). Abbreviations as in Figures 1 and 2.

sites at the range margin had a negative effect on lambda (Figure 4d). For both species, yearly transition interval had a much smaller effect on lambda than did location. For *M. cardinalis*, 2001-2002 had a positive effect on lambdas, and 2002-2003 had a negative effect on lambdas (Figure 4b). For *M. lewisii*, year effects did not differ from zero (Figure 4e). The interaction of location and year affected *M. cardinalis* lambdas at Wawona and *M. lewisii* lambdas at all sites except Wawona. At Wawona, *M. cardinalis* lambdas were significantly higher than expected based on the main effects of location and year in 2000-2001 and significantly lower than expected in 2002-2003 (Figure 4c). For *M. lewisii*, lambdas in 2001-2002 were higher than expected at the range center and lower than expected at the range margin. The converse was true in 2002-2003 (Figure 4f).

Several transitions made large contributions to spatial variation in lambda (Figure 5). For *M. cardinalis*, fecundity transitions from large non-reproductive and reproductive individuals to the seed class and stasis in the reproductive class had large negative effects on range center lambdas and large positive effects on range margin lambdas. In contrast, recruitment from seed to the large non-reproductive class made a positive contribution to lambda at the range center. Contrary to *M. cardinalis*, fecundity transitions from large non-reproductive and reproductive classes to seeds and stasis in the large non-reproductive and reproductive classes negatively affected *M. lewisii* range margin lambdas and positively affected range center lambdas. A large positive contribution of recruitment from seed to the large non-reproductive class also partially offset the negative contributions of other *M. lewisii* transitions at Carlon.

The *M. cardinalis* year and location by year interaction effects (Figure 4b, c)

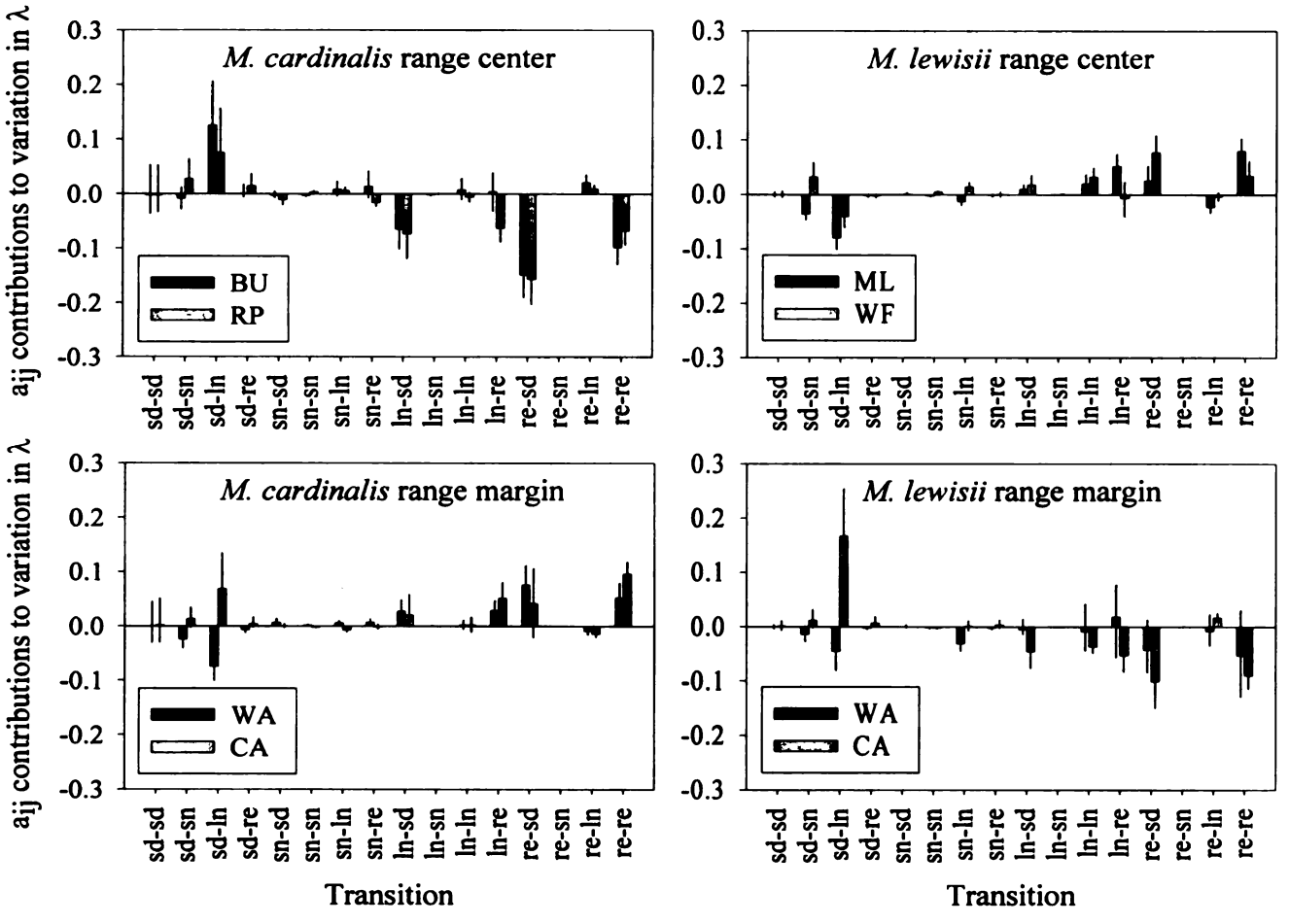

Figure 5. Transition matrix element contributions to spatial variation in λ. Vertical bars indicate bias-corrected 95% confidence intervals (Caswell 2001). Location abbreviations as in Figure 1. Stage class abbreviations as follows: sd = seed, sn = small non-reproductive, ln = large non-reproductive, re=reproductive.

were due to contributions from the same fecundity and stasis transitions that gave rise to the location effect (data not shown). For *M. lewisii*, however, location by year interaction effects arose primarily from spatiotemporal variation in the contribution of recruitment to the large non-reproductive stage class, a transition with high sensitivity. From 2000-2001 and 2001-2002, recruitment at the range center was high and recruitment at the range margin Carlon site was low; however, this difference was reversed from 2002-2003, when recruitment of large non-reproductive plants at Carlon was high and recruitment at the range center was low (data not shown).

Transects

All locations selected for detailed demographic study fell within the range of plant densities observed at similar elevations (Appendix A). Density of *M. cardinalis* small non-reproductive plants increased with elevation, although this difference did not remain significant after sequential Bonferroni adjustment (Table 4). Elevation did not predict density of other *M. cardinalis* stage classes or of any *M. lewisii* stage classes, nor did elevation predict temporal variation in stage class density of either species (Table 4).

DISCUSSION

Variation in Vital Rates

Observations of population vital rates demonstrate variation in performance across the elevation ranges of *M. cardinalis* and *M. lewisii*. For *M. cardinalis*, survival of reproductive plants was higher at the range margin than at the range center, whereas for *M. lewisii*, survival of this stage class was higher at the range center than the range margin. Log-linear analysis of vegetative stage class transitions revealed significant

Table 4. Linear regressions of stage class density (2001-2003 mean number of plants per m^2) and temporal variation in stage class density (coefficient of variation, CV, in 2001-2003 density) versus elevation along 50-200 m transects. After sequential Bonferroni correction to maintain a table-wide type I error rate of 0.05, no regression coefficients differed from zero.

Species	Dependent variable	N	b	SE(b)	t	P
M. cardinalis	Sm. non-repro.	18	0.00062	0.00023	2.68	0.02
	Lg. non-repro.	18	0.00001	0.00001	1.73	0.10
	Repro. density	18	0.00005	0.00006	0.84	0.41
	CV (sm. non-repro.)	18	-0.02212	0.04431	-0.50	0.63
	CV (lg. non-repro.)	18	-0.01918	0.05017	-0.38	0.71
	CV (repro.)	18	-0.00898	0.03624	-0.25	0.81
M. lewisii	Sm. non-reprod.	20	0.00003	0.00007	0.40	0.69
	Lg. non-reprod.	20	0.00000	0.00000	0.68	0.51
	Reprod.	20	0.00006	0.00003	1.73	0.10
	CV (sm. non-reprod.)	20	-0.00931	0.01723	-0.54	0.60
	CV (lg. non-reprod.)	20	0.02305	0.02070	1.11	0.28
	CV (reprod.)	20	0.00191	0.02035	0.09	0.93

temporal and spatial variation in the fates of vegetative plants. Components of plant fecundity also displayed significant variation between central and marginal populations of both species. Fecundity of *M. lewisii* was higher at its high elevation range center and lower at its mid elevation range margin. Reduction in plant fecundity at the range margin arose due to fewer flowers maturing fruit and an approximately two-fold reduction in seed number per fruit. It is unclear whether reduced fruit set and seed number per fruit resulted from physiological limitations on seed maturation or from

pollen limitation. Fecundity of *M. cardinalis* was higher at its mid elevation range margin and lower at its low elevation range center. Reproductive plants at the range center produced fewer flowers per stem and were of overall smaller size than reproductive plants at the range margin, resulting in fewer flowers per reproductive plant than at the range margin.

Variation in Population Growth Rates

The projection matrix summarizes how a particular environment affects the demographic parameters of a population. The asymptotic population growth rate, λ, is the rate at which the population would grow were the present environmental conditions to remain constant. Although the assumption of time invariance is almost certainly invalid, matrix projections remain extremely useful for summarizing the effects of different environmental conditions on projected population growth rates and population structure. Because $\ln \lambda = r$, the instantaneous growth rate, λ may also be interpreted as the average fitness of the population in the given environment (Fisher 1930; Charlesworth 1980; Caswell 2001). In this study, matrix projections revealed large differences in λ of central and marginal populations for both *M. cardinalis* and *M. lewisii*. Projected population growth rates of *M. lewisii* were highest at the high elevation range center and reduced at the mid elevation range margin. Projected population growth rates of *M. cardinalis* were highest at the mid elevation range margin and greatly reduced at the low elevation range center. Asymptotic projections were similar to observed year-to-year changes in population size. For example, the observed 2002-2003 population growth rate at Rainbow Pool was 0.4769, as compared to the asymptotic population growth rate of 0.4724.

Some temporal variation in population growth rates was also detected, but inspection of regional climate records does not reveal a clear relationship with variation in climatic variables such as precipitation or temperature. Temporal variation in λ observed during this four-year window may be due to within-site processes such as frequency of tree falls than climatic variation. Although statistically significant variation among years was detected at most locations with randomization tests, in general the magnitude of temporal variation was smaller than spatial variation during the study period, a finding supported by results from LTRE analysis. However, temporal environmental variation can play an important role in the population dynamics of riparian plant species (Menges 1990; Lytle and Merritt 2004), and it remains possible that temporal variation acting over a longer time scale or at irregular intervals has important consequences for *Mimulus* population dynamics.

Population growth rates of *M. lewisii* fit the expectation that central populations have high fitness and marginal populations have reduced fitness. Population growth rates of *M. cardinalis*, on the other hand, displayed the opposite pattern. The strikingly low λs observed for *M. cardinalis* at its range center contrast with results from a reciprocal transplant experiment in which *M. cardinalis* and *M. lewisii* were grown at 415, 1400, 2395 and 3010 m. In reciprocal transplant gardens, *M. cardinalis* and *M. lewisii* displayed the greatest average fitness at their respective low (415 m) and high (2395 m) elevation range centers, and reduced fitness at the mid elevation range margin (1400 m; Chapter 2). Reciprocal transplants and demographic observations have distinct advantages and disadvantages, and together, the two methods provide complementary information about how performance varies across species' ranges. By definition,

observations of extant populations cannot determine fitness levels beyond present range boundaries. Reciprocal transplant experiments are a powerful way to test for fitness variation both within and beyond present range limits, and the purpose of the reciprocal transplant experiment was to examine the effects of macroclimatic variables within and beyond the species' elevation ranges on components of fitness. To accomplish this, seedlings were grown in relatively uniform and favorable conditions (e.g., irrigated plots, minimal competition) to isolate the effects of climate on performance. However, because experimental gardens were established with seedlings, seed to seedling transitions were not observed. Observations of natural populations, on the other hand, integrate performance throughout the life cycle over all underlying, but often unknown, environmental variables.

One possible explanation for low λs at the *M. cardinalis* range center and the *M. lewisii* range margin is that downstream populations are demographic sinks maintained by immigration from upstream populations. Little is known about mechanisms of dispersal of *M. cardinalis* and *M. lewisii* seeds. Because both species occur in riparian habitats, it is possible that seed dispersal via downstream currents provides a mechanism for primarily unidirectional long-distance dispersal among populations, as has been demonstrated for *M. guttatus* (Waser et al. 1982).

Alternatively, temporal variation, particularly related to flood cycles, may operate over a longer time scale than the duration of this study and may have different effects on low versus mid elevation populations of *M. cardinalis*, leaving open the possibility that low elevation populations experience better "good" years than mid-elevation populations. Periodic floods may cause boom-bust cycles of mortality, bursts

of recruitment, and subsequent population attrition (Lytle and Merritt 2004). Low elevation populations may undergo greater variation following floods due to increased magnitude of floods on larger waterways at low elevation and/or to greater *potential* growth and fecundity of plants at low elevation in wet years. Examination of regional stream flow records (http://waterdata.usgs.gov/ca/nwis/nwis) confirms that flood magnitudes, both in absolute terms and in deviation from average peak flows, increase at lower elevations as catchment area increases. This hypothesis is consistent with the observation that, at low elevation, plants were recorded high on riverbanks and relatively distant from water at the beginning of the study, only three years after the largest recorded flood in the region (January 1997). Populations have since retreated to areas closer to water. This hypothesis is also consistent with plant performance in irrigated reciprocal transplant gardens, in which plant performance was measured under optimal conditions, and *M. cardinalis* exhibited greatest growth and reproduction at the low elevation range center (Chapter 2). A similar interaction between temporal variation and range position may also be possible for *M. lewisii*, although it is likely to be of limited extent due to smaller flood magnitude at mid and high elevations and limited growth potential of plants at mid elevations (Chapter 2). Further studies of both seed dispersal and spatiotemporal variation in population dynamics are necessary.

Only a handful of studies have examined the demography of geographically central and marginal native plant populations, each finding unique patterns of variation between central and marginal locations. Nantel and Gagnon (1999) studied two clonal plant species, *Helianthus divaricatus* and *Rhus aromatica*, and found that all populations exhibited high growth rates at least some of the time, but that northern

peripheral populations exhibited greater temporal variation in population growth rates than more centrally located populations. In a study of the annual grass *Hordeum spontaneum* along an aridity gradient from the center to the margin of its range, Volis et al. (2004) reported greater population growth rates in central populations in most years. However, local adaptation of seed dormancy traits in marginal desert populations ensured population persistence through drought periods. Finally, Stokes et al. (2004) examined congeneric shrubs, *Ulex gallii* and *U. minor*, whose parapatric distributions they hypothesized were limited by competition, but found that both species exhibited greatest population growth in marginal, sympatric areas.

The present study also examined the population dynamics of closely related congeners in marginal areas of sympatry, but it was not designed to estimate the effects of competition between *M. cardinalis* and *M. lewisii* on vital rates and population growth. It is interesting to note that marginal locations at mid elevation had negative effects on *M. lewisii* λs and positive effects on *M. cardinalis* λs, but from this study it is not clear to what extent this is due to competitive superiority of *M. cardinalis* versus adaptation of *M. lewisii* to high elevation environments. However, even with minimal competition, *M. lewisii* exhibits low fitness in reciprocal transplant gardens at middle and low elevations (Chapter 2) as well as in temperature regimes characteristic of low elevation (Chapter 3), suggesting that adaptation, or lack thereof, to the abiotic environment plays an important role in the performance of *M. lewisii* at its range margin.

Contribution of Life History Transitions to Variation in Population Growth Rates

Analysis of transition matrix data as a life table response experiment revealed several important life history transitions that contributed to differences in lambda between central and marginal populations. Transitions from large non-reproductive and reproductive plants to the seed class and stasis in the reproductive class made the largest contributions to spatial differences in lambda. These transitions had only low to moderate sensitivities, and sensitivity values were largely similar across all locations, indicating that differences in projected population growth rates resulted mainly from observed differences in transition matrix parameters. At the mid elevation range margin, *M. cardinalis* was more likely to become or remain reproductive and made more seeds per individual than at low elevations, and these differences in vital rates contributed to the observed differences in key transition matrix elements. Similar patterns of difference were observed for *M. lewisii* at the high elevation range center versus the mid elevation range margin.

Variation in local population density

Local population density is often used as an indicator of the degree to which a particular environment meets the niche requirements of a species (Brown et al. 1995). Many studies have concluded that abundance does in fact decrease towards range margins (McClure and Price 1976; Hengeveld and Haeck 1982; Huff and Wu 1992; Svensson 1992; Telleria and Santos 1993; Brown et al. 1995), but many others have not (Blackburn et al. 1999; Perez-Tris et al. 2000), and a recent review determined that fewer than half of all such studies found support for this generalization (Sagarin and Gaines 2002). The present study finds no clear relationship between population mean

fitness, as measured by λ, and local population density, despite differences in population growth rates between central and marginal areas of the elevation range.

In sum, this study demonstrates that central and marginal populations of both *M. cardinalis* and *M. lewisii* differ in vegetative stage class transitions and fecundity, and that these differences in vital rates contribute to substantial spatial variation in population growth rates. Continued study of spatiotemporal variation in population dynamics, in combination with estimates of dispersal between central and marginal populations, will improve our understanding of species' distribution limits.

CHAPTER 2

Variation in fitness within and beyond *Mimulus cardinalis* and *M. lewisii* elevation ranges

Abstract—Every species occupies a limited geographic area, but it remains unclear why traits that limit distribution do not evolve to allow range expansion. Hypotheses for the evolutionary stability of geographic ranges assume that species are maladapted at the range boundary and unfit beyond the current range, but this assumption has rarely been tested. To examine how fitness varies across species ranges, I reciprocally transplanted two species of monkeyflowers, *Mimulus cardinalis* and *M. lewisii*, within and beyond their present elevation ranges. I used individuals of known parentage from populations collected across the elevation ranges of both species to examine whether populations are adapted to position within the range. For both species I found the greatest average fitness at elevations central within the range, reduced fitness at the range margin, and zero or near-zero fitness when transplanted beyond their present elevation range limits. However, the underlying causes of fitness variation differed between the species. At high elevations beyond its range, *M. cardinalis* displayed reduced growth and fecundity, whereas at low elevations *M. lewisii* experienced high mortality. Weak differences in performance were observed among populations within each species and these were not related to elevation of origin. Low fitness of both species at their range margin and weak differentiation among populations within each species suggest that adaptation to the environment at and beyond the range margin is hindered, illustrating that range margins provide an interesting system in which to study limits to adaptation.

Key words: range limit, evolution of species' distributions, elevation gradient, reciprocal transplant, survivorship analysis

Every species occupies a restricted geographic area. In some cases, geographic ranges stop at an obvious barrier, such as a land – water interface. However, more frequently, ranges end at "seemingly arbitrary" points in space (Kirkpatrick and Barton 1997). Historically, ecologists and biogeographers have correlated range boundaries with climate to identify environmental determinants of range boundaries (Griggs 1914; Good 1931; Dahl 1951). Subsequent analyses have shown that range limits are associated with abiotic variables such as temperature or precipitation (Root 1988a; Cumming 2002), biotic factors such as competitors (Terborgh and Weske 1975; Bullock et al. 2000) or complex interactions between biotic and abiotic variables (Randall 1982; Taniguchi and Nakano 2000).

Even a mechanistic understanding of the relationship between environmental variables and distribution limits presents an evolutionary conundrum. Natural selection should continually improve adaptation at a range boundary and thus overcome current geographic limits, causing species' ranges to "grow by a process of annual accretion like the rings of a tree" (Mayr 1963). Several hypotheses for the evolutionary stability of range limits propose that populations at range boundaries do not have sufficient genetic variation to respond to natural selection (Bradshaw and McNeilly 1991; Hoffman and Blows 1994; Gaston 2003). Other hypotheses focus on other factors that may prevent populations from adapting to the environment at the range margin, such as genetic trade-offs among fitness-related traits in the marginal environment (Antonovics

1976), genetic trade-offs between fitness in central and border environments (Holt 2003), or gene flow from populations adapted to the range center (Haldane 1956; Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997). These hypotheses are not necessarily mutually exclusive, and may act synergistically to constrain range expansion.

All of the above hypotheses are united by the assumption that populations are maladapted at a range boundary and unfit beyond the current range. A corollary of this generalization is that concomitant environmental changes impose selection for local adaptation to the range edge. Surprisingly, these assumptions have rarely been directly tested.

Indirect evidence for a decline in fitness with distance from the range center is provided by the observation that, in some species, numerical abundance decreases with distance from the range center, presumably in response to an increasingly unfavorable environment (Brown 1984; Brown et al. 1996; Sagarin and Gaines 2002). Other indirect evidence for changes in fitness across species ranges comes from studies of fluctuating asymmetry. Developmental instability may increase when organisms are under genetic or environmental stress, as is predicted for individuals at range boundaries, and several studies of fluctuating asymmetry have found that populations at range boundaries do have higher levels of fluctuating asymmetry than central populations (Møller 1995; Carbonell and Telleria 1998; Gonzalez-Guzman and Mehlman 2001).

A more critical test for reduced fitness in marginal populations involves direct observation of fitness components across species ranges. Such studies have often found lower survival of certain life history stages or reduced fecundity at the range margin

relative to the range center (Marshall 1968; Pigott and Huntley 1981; McKee and Richards 1996; Garcia et al. 2000; Hennenberg and Bruelheide 2003). Unfortunately, the demographic consequences for such reductions in fitness are generally unclear. Perhaps the biggest stumbling block to observations of fitness variation, however, is that by definition, observations of extant populations cannot determine fitness levels beyond present range boundaries (Woodward 1990).

Reciprocal transplant experiments are a powerful way to test for fitness variation both within and beyond present range limits as well as the presence of genetically based local adaptation (e.g., Schemske 1984; Stanton and Galen 1997; Verhoeven et al. 2004). Although many classic studies used reciprocal transplants between areas within species ranges (Turesson 1922; Clausen et al. 1940), few have transplanted individuals beyond the range (Gaston 2003). I used reciprocal transplants to evaluate population and geographic variation in fitness for sister species of monkeyflower, *Mimulus cardinalis* and *M. lewisii* (Phrymaceae) across their elevation ranges in California, USA.

The study of closely related species with distinct distributions offers a conceptual advantage for the investigation of range limits. In a comparison of central versus border populations of a single species, one could never reject the possibility that border populations have not yet acquired the right mutation(s) to extend the border. In a comparison of parapatric sister species partitioning an environmental gradient, evolution from the common ancestor toward each species' native environment has already occurred, and the question of interest is what causes and constrains adaptation to different ends of the gradient.

Mimulus cardinalis and *M. lewisii* have been the subject of ecological and genetic studies for several decades and have many properties that make them ideal research subjects, including high seed number, high germination rates, and low transplant mortality (Vickery 1967; Hiesey et al. 1971; Vickery 1978; Bradshaw et al. 1998; Bradshaw and Schemske 2003; Ramsey et al. 2003). Pioneering studies of *M. cardinalis* and *M. lewisii* by Hiesey et al. (1971) revealed variation in performance across elevation, with *M. cardinalis* displaying low survival and reproduction at high elevation and *M. lewisii* displaying low survival and growth in a coastal climate. Unfortunately, several features of this study limit its usefulness for drawing definitive conclusions about variation in fitness versus elevation. First, populations were collected throughout the geographic ranges of both species from Washington to Baja California, but transplanted at only three sites (Stanford, elev. 30; Mather, elev. 1400; and Timberline, elev. 3050) along a narrow elevation transect in northern California. The wide latitudinal and longitudinal distances that separated most populations from the transplant sites are not easily separated from the effects of adaptation to elevation. Although the authors found significant population differentiation within each species (e.g., between coastal Californian and montane Arizonan *M. cardinalis*), regional and subspecies differences are not easily separated from differences related to elevation alone. Second, the use of vegetatively propagated clones eliminated information about the performance of early life history stages that may experience strong selection and be critical for population establishment (Travis 1994; Caswell et al. 2003; Davis et al. 2003; Lee et al. 2003; Zacherl et al. 2003). Finally, the low elevation transplant station

at Stanford (30 m) potentially conflated the effects of low elevation with a maritime climate.

I used reciprocal transplants within and beyond the elevation ranges of *M. cardinalis* and *M. lewisii* to examine how survival, growth and reproduction of each species change with elevation. I used individuals of known parentage from populations collected across the elevation ranges of both species to examine whether populations are adapted to their position within the range. Specifically, I asked 1) How do fitness components change from the center to the edge of ranges and beyond? and 2) Are populations locally adapted within their range?

MATERIALS AND METHODS

Study System

Mimulus cardinalis and *M. lewisii* (Phrymaceae) are rhizomatous perennial herbs that grow along seeps and stream banks in western North America. The species are self-compatible and animal pollinated (Hiesey et al. 1971; Schemske and Bradshaw 1999). *Mimulus cardinalis* occurs from southern Oregon to northern Baja California and from the coast of California inland to Arizona and Nevada. *Mimulus lewisii* is composed of two races, a northern form occurring from southern coastal Alaska to southern Oregon and eastward to the Rocky Mountains, and a southern form, occurring primarily in the Sierra Nevada Mountains of California (Hiesey et al. 1971; Hickman 1993; Beardsley et al. 2003). The two races are partially incompatible, and recent phylogenetic analysis suggests that the two races are sister to one another and together are sister to *M. cardinalis* (Beardsley et al. 2003). Here I study only the Sierran form of *M. lewisii*.

Mimulus cardinalis and *M. lewisii* segregate by elevation, with *M. cardinalis* occurring from sea level to 2400 m and *M. lewisii* occurring from 1200 m to 3100 m (Hiesey et al. 1971; Hickman 1993). In the Yosemite National Park region where this research was conducted, the species co-occur on larger watercourses between 1200 and 1500 m elevation (A. Angert, unpub. data). Although the published Californian distributions of *M. cardinalis* and *M. lewisii* extend to 2400 and 3100 m, respectively, repeated attempts to locate extant *M. cardinalis* populations above 1500 m and *M. lewisii* populations above 2900 m in the Yosemite region were unsuccessful. Therefore, I consider 1200 – 1500 m to be the shared mid-elevation distribution limit for both species and the western longitudinal distribution limit for *M. lewisii*.

Genetic Material: Population Collection and Crossing Design

Seeds from eight plants from each of six populations per species were collected in September 1999 along an elevation gradient from 590 m to 2750 m between 37.49 and 37.96° N latitude (Appendix A). One plant from each field-collected family was grown to flowering in the University of Washington greenhouse under standard greenhouse conditions. The eight plants from each population were crossed with one another in a partial diallel mating design (one per population, for a total of 12 partial diallels), where each plant served as sire and dam twice with no self- or reciprocal pollinations. Pollinations were performed by collecting all of the pollen from one flower with a flat toothpick and fully saturating the stigma of one flower. Seeds from four pollinations per full-sib family were pooled. This crossing design was intended to provide a genetically variable, outcrossed seed pool for reciprocal transplants rather than to accurately estimate genetic variance components. Sire and dam effects were

included in statistical models to account for the possible correlation of error and non-independence of individual measurements due to their family structure.

Reciprocal Transplant Methods

Garden locations.— To examine how species' performance varies across elevation ranges, I established experimental gardens along an elevation transect on the western slope of the Sierra Nevada Mountains. In June-July 2001, gardens were planted near Jamestown, California (37.917°N, 120.421°W; elev. 415 m), at Carnegie Institution of Washington field stations at Mather (37.886°N, 119.855°W; elev. 1400 m) and Timberline (37.962°N, 119.281°W; elev. 3010 m) and at the White Wolf Ranger Station in Yosemite National Park (37.872°N, 119.651°W; elev. 2395 m). These gardens were chosen to represent elevations for each species that are central within the elevation range (415 m for *M. cardinalis*, 2395 m for *M. lewisii*), at the range boundary (1400 m for both species, 3010 m for *M. lewisii*), and beyond the range boundary (2395 and 3010 m for *M. cardinalis*, 415 m for *M. lewisii*) in the Yosemite region (Figure 6).

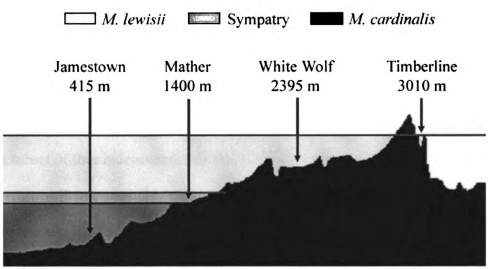

Figure 6. Schematic transect of the central Sierra Nevada Mountains, California, showing *M. lewisii* and *M. cardinalis* elevation ranges and placement of reciprocal transplant gardens, after Clausen et al. (1948).

Garden conditions.— Due to the tiny seed size and particular microhabitat requirements for germination of *M. cardinalis and M. lewisii*, experimental gardens were established with seedlings. Seeds from partial diallel crosses were sown in flats in the University of Washington greenhouse five weeks prior to transport to garden sites. The average age of transplanted seedlings was approximately three weeks after germination, corresponding closely to the size of plants observed in natural populations at the time of planting. Two seedlings from each full-sib family were planted at 10-cm intervals in a randomized block design for a total of 384 seedlings per block (2 seedlings / family x 16 full-sib families / population x 6 populations / species x 2 species). During June-July 2001, seedlings were planted in 3 blocks at 415 m (N=1152), 4 blocks at 1400 m (N=1536), 4 blocks at 2395 m (N=1536), and 3 blocks at 3010 m (N=1152), for a total of 5376 seedlings across all four transplant sites. Garden plots were covered in landscape fabric and irrigated daily to mimic conditions in the species' native riparian habitat and to standardize water treatments across environments.

Soils assay.— I collected soil samples from each garden site and grew plants in these soils under uniformly favorable greenhouse conditions to determine if site differences in performance were due to the effects of soils as opposed to other environmental factors. I measured the performance of four populations per species, using a subset of four independent full-sib families per population from the partial diallel crosses. Plants were able to flower on all soil types in the greenhouse environment and there was no evidence of local adaptation to soil type, therefore, I conclude that differences in soil properties are not primarily responsible for differences in fitness across elevation and I do not consider soil type further.

Measurements.— To assess fitness within each garden, I measured survival, growth and reproduction. Plants grew at vastly different rates among gardens. At 1400, 2395 and 3010 m, plants grew slowly and rarely attained a size where larger plants spread via rhizomes into neighboring plants' space. However, at 415 m, *M. cardinalis* plants began to spread via rhizomes into neighbors' space after one growing season, making it difficult to separate individuals and track identity. For this reason, I truncated observations at 415 m after one year, when all *M. lewisii* individuals were dead and surviving *M. cardinalis* were very large. Individuals transplanted in a large preliminary study at 415 m displayed very low mortality and continued rapid growth during the second growing season, indicating that truncation after one year does not bias the results (A. Angert, unpub. data).

Survival was monitored from 2001 – 2002 at 415 m and from 2001 – 2003 at 1400, 2395 and 3010 m. Survival was recorded at approximately two-week intervals throughout each growing season. Growth and reproduction were measured for one growing season at 415 m and for two growing seasons at 1400, 2395 and 3010 m. To measure plant growth, I recorded the total stem number and length of all stems. Stem number and total stem length were strongly correlated (*M. cardinalis*: R^2=0.73, N=2065, P=<0.0001; *M. lewisii*: R^2=0.72, N=1790, P=<0.0001). I present stem length data because they better describe overall plant size at high elevations, where plants often have only one stem but differ in stem length. Because permit restrictions prevented seed set at two transplant sites, I use flower number rather than seed number as a proxy for reproductive fitness. Flower number and fruit number measured from 2000 – 2004 in demographic census plots within natural central and border populations

are highly correlated (*M. cardinalis*: R^2=0.97, N=1132, P<0.0001; *M. lewisii*: R^2=0.98, N=1064, P<0.0001), suggesting that cumulative flower number is a good approximation of total fitness.

I estimated overall plant fitness, retaining zeros for plants that failed to flower or failed to survive, as the cumulative flower number over two growing seasons. I also summed year one and year two total stem length to estimate cumulative growth. For *M. cardinalis* grown at 415 m, only first year measurements of stem length and flower number were available. To keep measures comparable across all sites, I annualized measures of growth and fitness and compared average annual stem length and average annual fitness. Comparisons of first year growth and fitness at all sites as well as cumulative growth and fitness with the 415 m site excluded produced similar results; I present comparisons of annual averages for brevity.

Statistical Analysis

To examine fitness variation across species' elevation ranges, I analyzed the relationships between transplant site and the fitness components of survival and growth and between transplant site and average annual fitness. Too few individuals remained alive and flowering beyond their ranges to allow analysis of flower number for surviving plants. To determine whether populations are adapted to range position, I analyzed the relationships between population origin and performance within each transplant site. For all variables, I conducted separate analyses for each species. All analyses were performed in SAS, version 8.2 (SAS Institute, Inc., Cary, NC).

Survivorship.— I used accelerated failure time models to test for differences among sites and populations in patterns of survivorship. Accelerated failure time

models assume that factors affect failure time (e.g., time to mortality) multiplicatively, shifting the time periods when failures occur (see Fox 2001 for a general discussion of failure time analyses). For this study, accelerated failure time models were biologically appropriate because environmental differences among transplant treatments were expected to shift the distribution of time to failure (Jones and Sharitz 1998; Keith 2002; Denham and Auld 2004). To apply the accelerated failure time model, I used PROC LIFEREG with an underlying Weibull distribution of failure time (measured in days after transplantation). Survivorship was described using the function:

$$S(t) = e^{-(\lambda t)^p},$$

where the scale parameter λ scales the model to a baseline rate of mortality, t is the time since transplantation, and p is a dimensionless shape parameter that describes change in failure hazard over time, such that when $p < 1$ hazard monotonically decreases with time and when $p > 1$ hazard monotonically increases with time (Dudycha and Tessier 1999; Fox 2001; Keith 2002). I also ran models using an alternative plausible distribution, the exponential, which is a special case of the Weibull with the shape parameter $p = 1$, indicating a constant risk of mortality (Fox 2001). The exponential distribution gave a significantly poorer fit to the data than the Weibull according to likelihood ratio tests (*M. cardinalis*: χ^2=288.9, P<0.0001, *M. lewisii*, χ^2=31.8, P<0.0001) but yielded qualitatively similar results, indicating that the results are robust to the underlying distribution. For each species, I fit models with fixed effects of site, population and their interaction. For each categorical variable, one level was arbitrarily chosen as the reference level and its regression coefficient was set to zero. Regression coefficients and significance of all other levels were determined relative to the

reference, but this did not reveal whether differences among non-reference levels existed. Multiple comparisons were necessary to examine differences among levels other than the reference. I constructed Z-tests for multiple comparisons from estimated regression coefficients and the asymptotic covariance matrix according to the methods of Fox (2001). Because effects act multiplicatively on failure time, regression coefficients less than zero can be interpreted as shrinking the time to failure relative to the reference level, whereas positive regression coefficients expand the expected time to failure relative to the reference (Dudycha and Tessier 1999). Standard statistical packages do not incorporate random effects in survival time analyses, so for these analyses I was not able to include sire, dam or block effects. Observations were right censored if the individual remained alive at the end of the observation period.

Growth.— To examine the relationship between growth and transplant site, I performed mixed model analysis of variance on log-transformed data with PROC MIXED, which uses the restricted maximum-likelihood method (REML) to estimate variance components. I tested for variation in average annual stem length with respect to transplant site, population of origin, sire within population of origin, dam within each population of origin, and all interactions. Models including random block effects failed to converge, so I excluded block from the analyses. For this and all subsequent models, I considered transplant site and population of origin as fixed effects and sire and dam as random effects. To evaluate the significance of fixed effects, I used Type III estimable functions, which tolerate unbalanced samples, with denominator degrees of freedom obtained by Satterthwaite's approximation. Differences among levels of fixed effects were evaluated with Tukey-Kramer adjusted comparisons of least square means. I used

the PDMIX800 macro to convert pairwise differences between least square means to letter groupings, where means sharing the same letter code are not significantly different (Saxton 1998). I used likelihood-ratio tests (comparing each reduced model to the full model including all effects) to evaluate the significance of all random effects. Only two *M. lewisii* individuals remained alive for stem length measurements at 415 m, causing the full model containing all sites to contain many non-estimable parameters. To remedy this, I excluded the 415 m site from the *M. lewisii* stem length analysis.

Fitness.— I used mixed linear models to test for variation in average annual fitness with respect to transplant site, population of origin, sire within each population of origin, dam within each population of origin, and all interactions. The distribution of fitness was highly non-normal due to an excess of zeros and a long right tail. Examination of residuals in preliminary analyses revealed significant departures from parametric assumptions. Transformations only slightly improved the distribution of residuals. Therefore, I used two approaches to model annual fitness. First, I performed mixed model analysis of variance on log-transformed data with PROC MIXED as described for stem length above, with the exception that I first added 1/6 to each observation before log transformation (Kuehl 2000). Second, I used the GLIMMIX macro of PROC MIXED to fit generalized linear models, which are appropriate for a wider range of error structures than traditional linear models (Kuehl 2000). Generalized linear models extend traditional linear models in two key ways. First, they allow the distribution of the response variable to be any member of the exponential family of distributions (e.g., gamma, Poisson, binomial). Second, they relate the response variable to a set of linear predictor variables through a nonlinear link function (SAS

SASInstitute 1999). The GLIMMIX macro uses restricted/residual pseudo likelihood (REPL) estimation to fit a generalized linear model with random effects. I modeled variation in average annual fitness using a gamma distribution with a log link function, which is appropriate for positive, continuous data (SAS SASInstitute 1999; Juenger and Bergelson 2000). Observations were first transformed by adding one to each observation. I used Type III functions with denominator degrees of freedom obtained by Satterthwaite's approximation to test the significance of fixed effects. To evaluate the significance of random effects, I used the covtest option to obtain Z-tests, which tested whether the Z-value of each effect (its variance parameter divided by its approximate standard error) was different from zero (Juenger and Bergelson 2000). Because results obtained from PROC MIXED and GLIMMIX did not differ qualitatively and because the data violated the assumptions of traditional linear analysis, I present only the results from GLIMMIX.

Population variation.— To evaluate whether populations are adapted to their elevation of origin, I used two approaches. First, I examined population by site interactions in the analyses described above. A significant population by site effect indicates that populations differ in their response to elevation. If a significant population by site effect was found for failure time, I compared the confidence intervals of regression coefficient estimates to determine which population and site combinations were significantly different from one another. If a significant population by site effect was found for growth or fitness, I used Tukey-Kramer adjusted comparisons of least square means to determine which population and site combinations were significantly different from one another. Second, if populations are locally adapted to their elevation

of origin, then fitness should decrease as the difference between elevation of origin and transplant site elevation increases. For each transplant site, I examined the rank correlations of population average annual fitness with the absolute value of the difference between origin and transplant elevations using PROC CORR.

Table 5. Analysis of accelerated failure-time models for survival time, using 1339 uncensored values and 1273 right-censored values for *M. cardinalis*, 1339 uncensored values and 1073 right-censored values for *M. lewisii*, and a Weibull distribution.

Species	Variable	df	Estimate	SE	χ^2	P
M. cardinalis	Site	3			350.17	<0.0001
	(Jamestown, 415 m)	1	-0.6896	0.1298	28.24	<0.0001
	(Mather, 1400 m)	1	0.4662	0.1143	16.63	<0.0001
	(White Wolf, 2395 m)	0	0	0		
	(Timberline, 3010 m)	1	0.2233	0.1047	4.55	0.0330
	Population	5			6.16	0.2913
	(Mariposa, 590 m)	1	-0.1108	0.0874	1.61	0.2051
	(Moore, 830 m)	1	-0.0060	0.0898	0	0.9466
	(Bear, 860 m)	1	-0.0013	0.0896	0	0.9888
	(Snow, 950 m)	1	-0.0429	0.0889	0.23	0.6298
	(Tenaya, 1210 m)	1	0	0		
	(Tuolumne, 1320 m)	1	0.0977	0.0919	1.13	0.2879
	Site by Population	15			30.43	0.0105
	(Levels not shown)					
	Shape parameter	1	1.5715	0.0384		
M. lewisii	Site	3			4964.46	<0.0001
	(Jamestown, 415 m)	1	-5.0646	0.2199	530.68	<0.0001
	(Mather, 1400 m)	1	-2.0826	0.2182	91.11	<0.0001
	(White Wolf, 2395 m)	0	0	0		
	(Timberline, 3010 m)	1	-0.6668	0.2529	6.95	0.0084
	Population	5			3.06	0.6902
	(Tuolumne, 1320 m)	1	0.1323	0.2852	0.22	0.6426
	(Tamarack, 1910 m)	1	-0.3765	0.2527	2.22	0.1363
	(Porcupine, 2400 m)	1	0.1583	0.2852	0.31	0.5789
	(Tioga, 2580 m)	1	0.0476	0.2774	0.03	0.8636
	(Snow, 2690 m)	1	-0.0463	0.2709	0.03	0.8643
	(Warren, 2750 m)	0	0	0		
	Site by Population	15			16.05	0.3784
	(Levels not shown)					
	Shape parameter	1	1.1263	0.0228		

RESULTS

Survivorship

Table 5 gives the results of failure-time analyses. For both species, transplant site had a highly significant effect on survival time. All sites (Jamestown, 415 m, Mather, 1400 m, and Timberline, 3010 m) were significantly different from the reference site, White Wolf (2395 m), as indicated by regression coefficients different from zero. To examine differences among non-reference sites, I constructed Z-tests for comparisons of regression coefficients and found that all pairwise differences among non-reference sites were also significant, although for *M. cardinalis* the difference between Mather (1400 m) and Timberline (3010 m) was only marginally significant after correcting for multiple comparisons (Table 6). Population did not affect survival time for either species. For *M. cardinalis*, the population by site effect was significant, indicating that populations differ in their response to elevation. There was no population by site interaction for *M. lewisii* survivorship. For both species, the Weibull shape parameter was significantly greater than 1, indicating that the risk of mortality increased monotonically with time.

Table 6. Pairwise differences of transplant site regression coefficients from accelerated failure-time analyses. After correcting for multiple comparisons, only Z-scores > 2.12 remain significant at the 0.05 level.

	M. cardinalis		*M. lewisii*	
	Z	P	Z	P
Jamestown (415 m) vs. Mather (1400 m)	7.89	<0.0001	22.40	<0.0001
Jamestown (415 m) vs. Timberline (3010 m)	6.53	<0.0001	23.16	<0.0001
Mather (1400 m) vs. Timberline (3010 m)	1.93	0.0265	7.50	<0.0001

Mimulus cardinalis survival during the first year was highest at the 1400 m range border, intermediate at high elevations beyond the range, and lowest at the 415 m

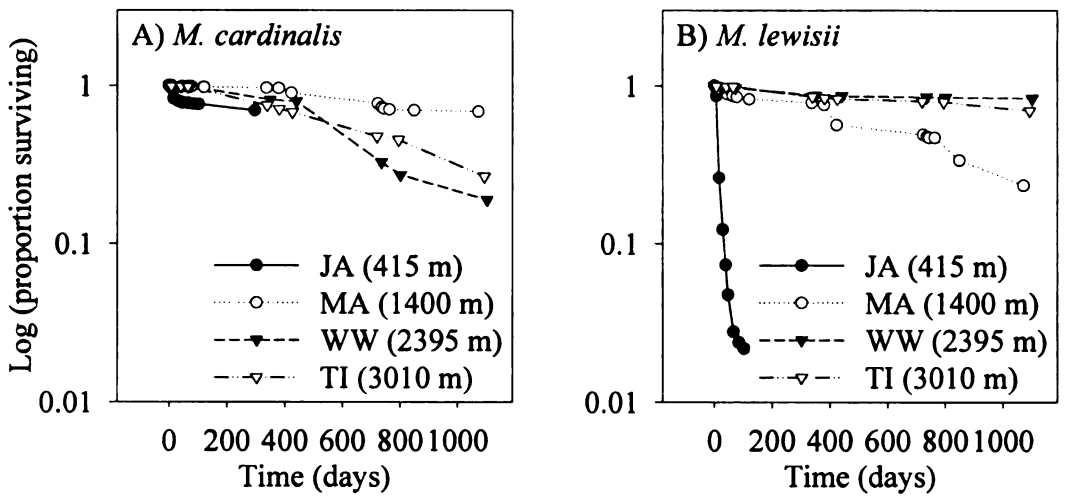

Figure 7. Survivorship at each transplant site. A) *M. cardinalis*. B) *M. lewisii*. Transplant site abbreviations as follows: JA = Jamestown, MA = Mather, WW = White Wolf, TI = Timberline.

range center (Figure 7a). There was an early decrease in survival during the first growing season at 415 m, whereas survival at 2395 and 3010 m was high during the first growing season and declined over the first winter. During subsequent years, survivorship remained highest at 1400 m and was reduced at 2395 and 3010 m. Examination of regression coefficient confidence limits for each site and population indicated that the *M. cardinalis* population by site interaction arose because of differences in elevation response between the low elevation Mariposa Creek population (590 m) and the mid elevation Tenaya Creek population (1210 m; data not shown). At 1400 m, the Mariposa Creek population survived longer than the Tenaya Creek population, and the converse was true at 3010 m.

Mimulus lewisii survival was highest at 2395 and 3010 m and intermediate at 1400 m (Figure 7b). At 415 m, *M. lewisii* suffered high mortality during the first growing season. The few individuals surviving after one growing season at 415 m died over the winter, resulting in 100% mortality within one year. At 1400 m, *M. lewisii*

experienced pulses of mortality at the end of the second and third growing seasons. At high elevations, mortality rates were roughly constant and low.

Growth

For both species, site had a highly significant effect on growth, measured as log-transformed average annual stem length (Table 7). There were no significant population or population by site effects for *M. cardinalis* growth, but both population and population by site effects significantly affected growth for *M. lewisii*. Sire, dam and all interactions involving sire or dam were non-significant for both species.

Table 7. Linear mixed model analysis of variance summary for log-transformed average annual stem length. F-tests for fixed effects constructed by SAS MIXED procedure, with denominator degrees of freedom obtained from the Satterthwaite approximation and indicated in parentheses below each F-value. All random effects (sire, dam, and their interactions) were estimated to be zero or near-zero and were not significant.

	M. cardinalis			*M. lewisii*		
Fixed effects	df	F	P	df	F	P
Site	3	934.29 (1284)	<0.0001	2	575.82 (1264)	<0.0001
Pop	5	1.17 (1284)	0.3197	5	5.85 (70.4)	0.0001
Site*Pop	15	0.45 (1284)	0.9629	10	2.58 (1263)	0.0043

Mimulus cardinalis growth was greatest at 415 m, intermediate at 1400 m, and greatly reduced at higher elevations (Figure 8a). Growth of *M. lewisii* peaked at 1400 and 2395 m and was reduced at 3010 (Figure 8b). The difference in growth between the 1400 m range margin and the 2395 m range center was not statistically significant in Tukey- Kramer adjusted post-hoc contrasts. High mortality resulted in small sample

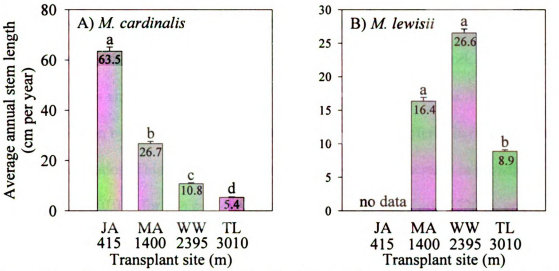

Figure 8. Species average annual stem length + SE at each transplant site (mean values given within each bar). A) *M. cardinalis* B) *M. lewisii*. Site means sharing the same letter are not significantly different. Note that species are graphed on different scales. Transplant site abbreviations as in Figure 7.

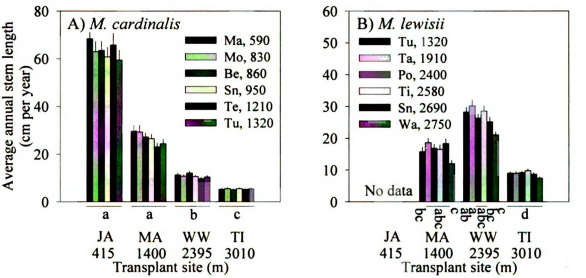

Figure 9. Population average annual stem length + SE versus transplant site. A) *M. cardinalis*. B) *M. lewisii*. Populations are arrayed in order of increasing elevation of origin. Population means sharing the same letter are not significantly different. Note that species are graphed on different scales. Transplant site abbreviations as in Figure 7. Population abbreviations as follows: Ma = Mariposa Ck., Mo = Moore Ck., Be = Bear Ck., Sn = Snow Ck., Te = Tenaya Ck., Tu = S. Fork Tuolumne R., Ta = Tamarack Ck., Po = Porcupine Ck., Ti = Tioga Rd., Sn = Snow Ck., Wa = Warren Fork Lee Vining R.

size for *M. lewisii* at 415 m (N=2). The *M. lewisii* population effect was due to the difference between the Warren Fork population (2750 m) and all other populations except for the South Fork population (1320 m; Figure 9). The Warren Fork population reached a smaller size than other *M. lewisii* populations regardless of site. The *M. lewisii* population by site interaction indicated that populations differed in their growth response to elevation. This difference was driven by the greater increase in growth at 2395 m versus 3010 m for two mid elevation populations (South Fork, 1320 m, and Tamarack Creek, 1900 m) relative to two high elevation populations (Snow Creek, 2690 m, and Warren Fork, 2750 m).

Fitness

Transplant site strongly affected average annual fitness of both species (Table 8). Population of origin and the interaction between population and transplant site had marginally significant effects on *M. cardinalis* fitness and highly significant effects on *M. lewisii* fitness. Sire and dam components of variance were not significant for either species. For *M. cardinalis*, the sire by site interaction was significant, and for *M. lewisii*, the dam by site interaction was significant. The existence of sire or dam by site interactions in these species is consistent with the presence of genetic variation for fitness across elevations. However, examination of sire and dam means revealed high variance and large heteroskedasticity of variance across sites (data not shown), making it more likely that the significance of these interaction effects is an artifact of variance (Juenger and Bergelson 2000).

Table 8. Generalized linear mixed model analyses of average annual fitness. F-tests for fixed effects constructed by SAS MIXED procedure, with denominator degrees of freedom obtained from the Satterthwaite approximation and indicated in parentheses below each F-value. Z-tests for random effects constructed by 'covtest' option.

		M. cardinalis		*M. lewisii*	
Fixed effects	df	F	P	F	P
Site	3	474.48	<0.0001	78.21	<0.0001
		(70)		(143)	
Pop	5	2.90	0.0722	3.98	0.0036
		(41.9)		(42)	
Site*Pop	15	1.82	0.0635	3.76	<0.0001
		(69.9)		(142)	
Random effects		Estimate	P	Estimate	P
Sire(Pop)		0.005	0.2231	0	
Dam(Pop)		0		0.0011	0.1407
Sire*Dam(Pop)		0		0	
Sire*Site(Pop)		0.055	<0.0001	0	
Dam*Site(Pop)		0.015	0.0535	0.006	0.0016
Sire*Dam*Site(Pop)		0		0.000	0.4750
Error		0.312		0.098	

Mimulus cardinalis fitness was highest at the 415 m range center, reduced at the 1400 m range border, and zero or near-zero at high elevations beyond its present range (Figure 10a). The population from the lowest elevation of origin, Mariposa Creek (590 m), was more fit than a population from middle elevation, Tenaya Creek (1210 m; Figure 11a). The population by site interaction was driven by populations differing in the degree of decrease in fitness from 1400 m to 2395 m. The Bear Creek population (860 m) displayed a greater decrease in fitness from 1400 to 2395 m than did the Tenaya Creek population (1210 m; Figure 11a).

Mimulus lewisii fitness was highest at the 2395 m range center, intermediate at the 1400 m and 3010 range borders, and lowest at 415 m, beyond its present range (Figure 10b). The Tioga Road population (2580 m) was more fit than the population

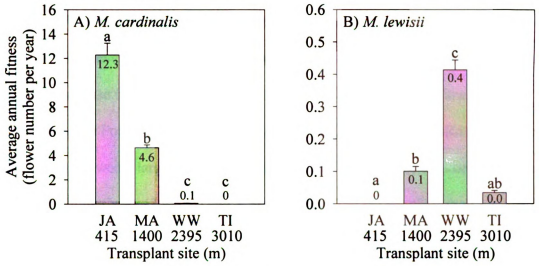

Figure 10. Species average annual fitness (in units of flowers per year) + SE versus transplant site (mean values given within each bar). A) *M. cardinalis* B) *M. lewisii*. Site means sharing the same letter are not significantly different. Note that species are graphed on different scales. Transplant site abbreviations as in Figure 7.

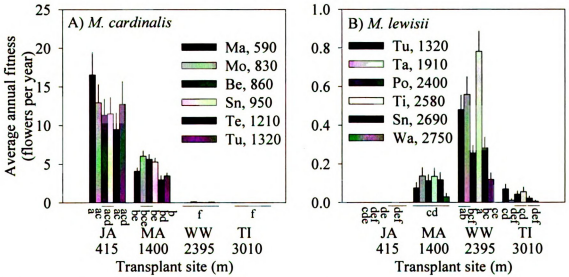

Figure 11. Population average annual fitness + SE versus transplant site. A) *M. cardinalis*. B) *M. lewisii*. Populations are arrayed in order of increasing elevation of origin. Population means sharing the same letter are not significantly different. Note that species are graphed on different scales. Abbreviations as in Figure 9.

from the highest elevation, Warren Fork (3010 m), across all sites (Figure 11b). The population by site effect indicated that populations differ in their reaction norms for fitness versus elevation. This interaction was the result of populations differing in the degree of increase in fitness at 2395 m, relative to the uniformly low fitness at other sites. The South Fork (1320 m), Tamarack Creek (1920 m) and Tioga Road (2580 m) populations showed a large increase in fitness at 2395 m, whereas the Porcupine Creek (2400 m), Snow Creek (2690 m) and Warren Fork (2750 m) populations did not show a statistically significant increase in fitness at 2395 m (Figure 11b).

To determine whether populations are adapted to their position within the elevation range, I also examined the rank correlation between average fitness and the difference in elevation between transplant site and population origin. If populations are adapted to position within the elevation range, then the correlation between fitness and the difference between origin and transplant elevations should be negative, indicating that fitness declines as the transplant environment becomes more different from the native environment. No correlations were statistically significant, suggesting that fitness variation among populations is not caused by differences in elevation of origin (Table 9).

Table 9. Rank correlation between population average annual fitness and $|$transplant elevation – population origin elevation$|$.

	M. cardinalis		*M. lewisii*	
Site	r	Prob > |r|	r	Prob > |r|
415	-0.0212	0.9024	0.0289	0.8669
1400	0.0683	0.6924	-0.0220	0.8988
2395	-0.0801	0.6425	0.0361	0.8344
3010	0.0084	0.9610	0.1076	0.5324

DISCUSSION

Geographic Variation in Fitness

The results of this reciprocal transplant experiment support the hypothesis that species are most fit at their range center and become increasingly maladapted as the distance from the range center increases. Both species exhibited the greatest average fitness at elevations central within their range (415 m for *M. cardinalis*, 2395 m for *M. lewisii*) and reduced fitness at elevations at the range margin (1400 m for both species, 3010 m for *M. lewisii*). Furthermore, both species exhibited zero or near-zero fitness when transplanted beyond their present elevation range limits (to higher elevations, 2395 and 3010 m, for *M. cardinalis*, or to lower elevation, 415 m, for *M. lewisii*).

However, the underlying causes of this fitness variation differed between the species. For *M. cardinalis*, first-year survival was relatively high across all elevations, but growth and fecundity were higher at the low elevation range center than at higher elevations. At higher elevations, few *M. cardinalis* individuals were able to reach reproductive maturity. Individuals that flowered at 2395 m did so in September, after most *M. lewisii* stopped flowering, and did not mature seeds before senescence. By contrast, *M. lewisii* confronted a strong survival barrier at its lower elevation range limit. Mortality during the first growing season at 415 m was rapid; most individuals died within one month of planting and all were dead within one year. Because experimental planting was timed to match the phenology of natural populations, transplanted seedlings were exposed to the climate they would have encountered if naturally dispersed to low elevation. A large preliminary study conducted at 415 m in June 2000 produced nearly identical results (*M. cardinalis* survival: 85.8% after four

months, 76.3% after 10 months, N=962; *M. lewisii* survival: 6.2% after four months, 0% after 10 months, N=953), indicating that observed patterns of mortality are not exaggerated by unusually harsh conditions in 2001.

These findings are largely congruent with the patterns of variation in performance across elevation in these species described by Hiesey et al. (1971) in their landmark reciprocal transplant study of *M. cardinalis* and *M. lewisii.* They demonstrated that low survival and reproductive capacity of *M. cardinalis* at high elevation and low survival and growth of *M. lewisii* in a coastal climate. However, in their study, *M. cardinalis* displayed the highest survivorship in the low elevation Stanford transplant garden (30 m), whereas I observed highest first-year survivorship in the mid elevation Mather garden. This difference highlights the important difference between the low elevation maritime environment and the low elevation foothills environment. A second difference between the present findings and the previous study is the relatively poor performance of *M. lewisii* that I observed at Timberline, where Hiesey et al. (1971) found that *M. lewisii* achieved its highest performance. This difference is likely due to several factors, including the addition of White Wolf as an intermediate transplant site between Mather and Timberline, exclusion of populations from the northern race of *M. lewisii*, and use of seedlings rather than vegetatively propagated clones. The use of seedlings provided important information about the performance of early life history stages, which may experience strong selection and be critical for population establishment (Lee et al. 2003; Zacherl et al. 2003). It is also important to note that none of the transplant sites used by Hiesey et al. (1971) were central within the elevation range of *M. lewisii.*

Several other experiments have demonstrated reduced growth, delayed phenology, and, as a result, reduced fecundity of plant species transplanted beyond their northern or high elevation range margins (Prince 1976; Davison 1977; Woodward 1990; Asselin et al. 2003). Analogous patterns of delayed development have also been reported for aphids (Gilbert 1980) and butterflies (Crozier 2004) transplanted beyond their latitudinal range limits. In these examples, fitness reductions generally are not due to a single environmental event such as a frost or to a single vulnerable life history stage, but rather result from the gradual accumulation and cascading effects of fitness reductions at many stages.

In contrast to expectations for northern or upland range limits, it is generally assumed that climate becomes more permissive for most organisms and that biotic interactions become relatively more important in setting southern or lowland distribution limits (MacArthur 1972; Woodward 1975; Sievert and Keith 1985; Hersteinsson and Macdonald 1992; Richter et al. 1997; Scheidel et al. 2003; Cleavitt 2004). Few studies of southern or lowland distributions limits find severe abiotic limitation as I have documented for *M. lewisii* at low elevations. Many plants showed signs of heat stress such as leaf scorching and reduced leaf size, and subsequent growth chamber studies have demonstrated strikingly similar patterns of mortality when *M. lewisii* are grown under the high temperatures characteristic of low elevation (Chapter 3).

Population Variation in Fitness

Although population and population by site effects were frequently statistically significant, they were of much smaller magnitude than site effects. I detected

differences among *M. cardinalis* populations, but not *M. lewisii* populations, in survivorship at different elevations. For *M. cardinalis*, the low elevation Mariposa Creek population (590 m) survived longer at middle elevation than the mid-elevation Tenaya Creek population (1210 m), but at high elevation the Tenaya Creek population had survived longer than the Mariposa Creek population. The direction of reversal in survivorship is consistent with adaptation of the range margin Tenaya Creek population to higher elevations, but it is not entirely consistent with adaptation to position within the range because of the poor relative performance of the mid-elevation population at middle elevation. No other differences among populations were significant, indicating that differentiation among populations for survivorship is low.

I detected differences among *M. lewisii* populations but not *M. cardinalis* populations in average annual stem length. Local adaptation of growth traits may take two possible forms. First, populations could exhibit genetically based clinal differences in growth in which populations originating from higher elevations display reduced growth rates or short stature across all environments (Clausen et al. 1940). Alternatively, populations could show decreasing growth with increasing distance from population origin. I find some slight evidence that the former scenario is true for *M. lewisii*. Differences among *M. lewisii* populations were consistent with a trend for genetically based clinal differences in average annual stem length, where populations from the mid elevation range margin reached larger size at 2395 m and the population from the highest elevation of origin was smallest at both 2395 and 1400 m. Hiesey et al. (1971) also found some evidence for genetically based clinal growth differences among *M. lewisii* populations. However, because in their study populations were collected from

throughout the geographic ranges of both species, the wide latitudinal and longitudinal distances that separated most populations from the transplant sites are not easily separated from the effects of adaptation to environmental variables that vary with elevation.

For both species, I detected variation among populations in reaction norms for average annual fitness versus transplant site, but these differences were not consistent with the hypothesis that populations are adapted to their elevation of origin. For example, at Mather (1400 m), the nearby South Fork populations of both species (1320 m) were not more fit than the distant *M. cardinalis* Mariposa Creek (590 m) or *M. lewisii* Warren Fork (2750 m) populations. The marginally significant *M. cardinalis* population by site interaction resulted solely from two populations differing in the degree of decrease in fitness from 1400 m to 2395. The significant *M. lewisii* population by site interaction arose because three populations (South Fork, 1320 m, Tamarack Creek, 1920 m, and Tioga Road seep, 2580 m) displayed significantly increased fitness at 2395 m versus other elevations and three did not (Porcupine Creek, 2400 m, Snow Creek, 2690 m, and Warren Fork, 2750 m). Reaction norms for fitness never crossed, but instead differed in the slope of decrease from the range center to range margins, suggesting that populations do not exhibit symmetrical "home" elevation advantages. This conclusion is supported by the lack of significant correlations between population mean fitness and the difference in elevation between population origin and transplant site.

Gene Flow and Selection

Range limits arise where populations are no longer able to adapt sufficiently to local environmental conditions. Low fitness of both species at their range margin suggests that adaptation to the marginal environment is hindered. Likewise, weak differentiation among populations within each species indicates that populations from the range margin have been unable to adapt to environmental conditions at the range boundary.

The lack of adaptation to elevation of origin that I observe is striking given the number of documented examples of adaptive differentiation both among populations at geographic scales (e.g., Clausen et al. 1940; Grant 1963) and within populations at extremely local spatial scales (e.g., Bradshaw 1960; Schemske 1984). Many species display ecotypic variation along altitudinal gradients (Clausen et al. 1940; Oleksyn et al. 1998; Jonas and Geber 1999). The populations used in this experiment were sampled along an elevation gradient that imposes variation in several important abiotic environmental variables, including length of growing season and temperature. Species may not be able to adapt to environmental conditions at the range margin if they lack appropriate genetic variation upon which selection can act or if differential natural selection is weak relative to the homogenizing effects of gene flow (Mayr 1963; Kirkpatrick and Barton 1997).

The interplay of gene flow and selection along environmental gradients or between discrete environments is important to several models of range or niche evolution (Holt and Gaines 1992; Kawecki 1995; Kirkpatrick and Barton 1997; Gomulkiewicz et al. 1999; Holt 2003). For example, Kirkpatrick and Barton (1997) modeled the evolution of a quantitative character determining fitness across a one-

dimensional environmental gradient. The character evolved under stabilizing selection toward an optimum phenotype that varied with the environmental gradient. Population density in their model depended on dispersal, density-dependent population regulation and the degree of mismatch between the optimum and population mean phenotypes. Stable range limits arose when gene flow imposed a strong constraint on local adaptation, as when dispersal was high or the environmental gradient was steep.

Although the focus of the Kirkpatrick and Barton model was on the swamping effects of gene flow, it also modeled adaptive trade-offs between environments because no single phenotype was optimal across the entire environmental gradient. Models of niche evolution explicitly consider the role of trade-offs between habitats in limiting species distributions, finding that selection to improve adaptation to environments outside of the niche may be weak due to the demographic asymmetry between habitats within versus outside of the niche (Kawecki 1995; Holt 1996; Gomulkiewicz et al. 1999). In a recent model of range evolution, Holt (2003) explicitly modeled the feedback between the evolution of dispersal and the evolution of habitat specialization (i.e., trade-offs) in a two-habitat model where neither habitat was initially outside of the niche. In this model the evolutionary dynamics of the geographic range depended on the shape of adaptive trade-offs between habitats and the initial habitat distribution of the population. For instance, a species initially specialized to one habitat may evolve habitat generalization if mutations that increase adaptation to a new habitat have little cost to fitness within the present habitat. Conversely, if a linear and symmetrical trade-off in fitness between two habitats exists, evolution will favor increased specialization to whichever habitat the species initially resides in. These models highlight the need to

understand the relative roles of dispersal, adaptive trade-offs and demographic asymmetries between habitats in range evolution. Further work is necessary to understand how these components interact to determine the elevation range limits of *Mimulus cardinalis* and *M. lewisii*.

Dispersal.— Elevation distributions offer a tractable experimental analog to latitudinal distributions at larger spatial scales, because both arise along continuous environmental gradients and encompass multiple populations. The environmental gradient from the center to the edge of elevation and latitude ranges is also similar, with temperature and length of growing season decreasing to the north and at higher elevations, although the rate of change in environmental parameters across space is greater for altitudinal than for latitudinal gradients. Indeed, a change of 100-200 m in elevation is roughly equivalent to a change of 1° in latitude (Criddle et al. 1994; Flebbe 1994). Due to the steepness of the environmental gradient across elevation, for a given dispersal distance, individuals encounter a more different environment than if dispersing across latitude, making it more likely that marginal populations may be swamped by centrally adapted phenotypes at altitudinal than at latitudinal range boundaries (Kirkpatrick and Barton 1997).

Little is known about mechanisms of dispersal of *M. cardinalis* and *M. lewisii* seeds. Because both species occur in riparian habitats, it is possible that seed dispersal via downstream currents provides a mechanism for primarily unidirectional long-distance dispersal among populations, setting up an interesting dichotomy between *M. cardinalis* and *M. lewisii* at their shared mid elevation range boundary. A net flux of migrants downstream would imply that the *M. lewisii* mid elevation range limit may be

subject to swamping gene flow from high elevation central populations, but that the *M. cardinalis* mid elevation range limit is not. However, gene flow via pollen may show the opposite pattern due to the greater flight distance of hummingbirds, the primary pollinator of *M. cardinalis*, compared to bumblebees, the primary pollinator of *M. lewisii*. Estimations of F_{st} among populations of each species are in progress to begin to identify patterns of gene flow among central and marginal populations of each species.

Adaptive trade-offs.— Because central and marginal populations of each species display few adaptive differences versus elevation, interspecific comparisons are necessary to understand adaptive trade-offs across the elevation gradient. Since their recent common ancestor, *M. cardinalis* and *M. lewisii* have evolved differences that restrict their distributions to different areas of the complex environmental gradient associated with elevation. Specialization to different elevation ranges suggests that different phenotypes are necessary for fitness at low versus high elevations. Estimation of the strength and direction of selection on phenotypic traits across the elevation gradient, in combination with genetic mapping of quantitative trait loci, will identify traits under selection at high versus low elevation and the underlying genetic architecture of those traits (Angert, Bradshaw, and Schemske, unpub. data). Experimental evolution of segregating hybrid populations at low and high elevation will also illuminate whether there are fitness costs of specialization to low versus high elevation (Angert, Bradshaw, and Schemske, unpub. data). Together, these studies will help elucidate mechanisms of adaptive trade-offs between low and high elevation environments. In conjunction with estimates of gene flow between central and marginal

populations, we can hope to understand what causes and constrains adaptation to different elevation ranges.

CHAPTER 3

Growth and leaf physiology of monkeyflowers (*Mimulus cardinalis* and *M. lewisii*) with different elevation ranges

Abstract—Every species is limited both geographically and ecologically to a subset of available habitats, yet for many species the causes of distribution limits are unknown. Temperature is thought to be one of the primary determinants of species distributions along latitudinal and altitudinal gradients. This study examined leaf physiology and plant performance under contrasting temperature regimes of sister species of monkeyflower, *Mimulus cardinalis* and *M. lewisii* (Phrymaceae), that differ in elevation distribution to test the hypothesis that temperature-dependent differences in growth are an important determinant of differences in fitness versus elevation. Each species attained greatest aboveground biomass, net photosynthetic rate, and effective quantum yield of photosystem II when grown under temperatures characteristic of the altitudinal range center. Although both species exhibited greater stem length, stomatal conductance, and intercellular CO_2 concentration in hot than in cold temperatures, these traits showed much greater reductions under cold temperature for *M. cardinalis* (native to low elevation) than for *M. lewisii* (native to high elevation). Survival of *M. lewisii* was also sensitive to temperature, showing a striking decrease in hot temperatures. Within each temperature regime, the species native to that temperature displayed greatest growth and leaf physiological capacity. Populations from the elevation range center and range margin of each species did not differ in most growth or leaf physiological responses to temperature. This study provides evidence that *M. cardinalis*

and *M. lewisii* differ in survival, growth, and leaf physiology under temperature regimes characterizing their contrasting low and high elevation range centers, and suggests that the species' elevation range limits may arise, in part, due to metabolic limitations on growth that ultimately decrease survival and limit reproduction.

Key words: range boundary, distribution limit, elevation, temperature, photosynthesis

No species occupies an unlimited area. Rather, every species is limited both geographically and ecologically to a subset of available habitats. Understanding the patterns and processes governing the distribution of species is a central goal of ecology, yet for many species the causes of distribution limits are unknown.

Identifying the causal mechanisms of distribution limits is challenging because environmental variables are often spatially correlated and dissecting organismal responses to even a single environmental variable is a complex task. However, temperature is thought to be one of the primary determinants of species distributions along latitudinal and altitudinal gradients. Evidence for the role of temperature in distribution limits comes from a diverse array of studies, including correlations between isotherms and distribution boundaries (e.g., McNab 1973; Grace 1987; Root 1988b), temperature tolerance and latitudinal or altitudinal distribution (e.g., Loik and Nobel 1993; Cunningham and Read 2002; Kimura 2004), extreme temperature events and periods of reproductive failure or high mortality at range boundaries (e.g., Silberbauer-Gottsberger et al. 1977; Jarvinen and Vaisanen 1984; Olmsted et al. 1993; Mehlman 1997), and studies of latitudinal and altitudinal changes in response to both historic and recent global warming trends (e.g., Huntley 1991; Parmesan et al. 1999; Hughes 2000;

Thomas et al. 2001). Further, temperature exerts a ubiquitous influence on many important cellular properties such as the rate of enzymatic reactions, protein conformations and membrane stability.

Temperature may influence species distributions in a multitude of ways, from imposing direct lethal limits to regulating processes of growth, development and reproduction (Cossins and Bowler 1987; Orfanidis 1993; Molenaar and Breeman 1994; Sewell and Young 1999). Study of the sensitivity of metabolic processes to temperature can elucidate the mechanisms underlying limitation at distribution boundaries (Heller and Gates 1971; McNab 1973; Criddle et al. 1994; Anthony and Connolly 2004). For plants, photosynthesis is a primary metabolic process and is the source of energy and substrates for all other biosyntheses.

Photosynthesis often exhibits a temperature optimum, deviations from which cause photosynthetic activity to decrease (Larcher 1995; Battaglia et al. 1996). Populations or species from contrasting temperature habitats often exhibit differences in photosynthetic optima and acclimation ability in response to temperature (Billings et al. 1971; Berry and Björkman 1980; Arntz and Delph 2001; Cunningham and Read 2003). Other gas exchange parameters are also sensitive to temperature. Without stomatal regulation, transpiration rises with rising temperature. However, extremes of temperature often elicit stomatal closure, which may decrease stomatal conductance and limit the availability of CO_2 for photosynthesis (Larcher 1995). Long-term acclimation to temperature may alter stomatal conductance as a result of changes in stomatal density or aperture (Ferris et al. 1996). Measurement of instantaneous leaf gas exchange parameters such as net photosynthetic rate and stomatal conductance offer a way to

detect functional limitations on plant metabolism imposed by environmental factors (Llorens et al. 2004).

Chlorophyll *a* fluorescence provides another non-destructive means to assess the functioning of the photosynthetic system. Light energy absorbed by a leaf can be used for photochemical reactions, dissipated as heat energy, or re-emitted as fluorescent light (Bolhar-Nordenkampf and Öquist 1993). The measured fluorescence signal from a leaf is determined by the rate constants of these competing reactions and the fraction of open reaction centers available for photochemistry and comes primarily from chlorophyll *a* of photosystem II (PS II; Krause and Weis 1984). Measurement of chlorophyll fluorescence of light-adapted leaves can determine the fraction of absorbed light energy used in electron transport, or the effective quantum yield of photosystem II (Φ_{PSII}). Thylakoid membranes are especially sensitive to heat and chilling, so disturbance of photosynthesis, particularly in PS II, is a first sign of temperature stress (Berry and Björkman 1980; Bolhar-Nordenkampf and Öquist 1993). Even when thylakoid membranes remain intact, temperature stress may decrease fluorescence yield due to down-regulation of PS II activity and increases in non-photochemical quenching resulting from the inhibition of carbon metabolism (Krause and Weis 1991; Owens 1994; Schreiber et al. 1994; Haldimann and Feller 2004). Thus, measurement of chlorophyll fluorescence can detect early stages of both low and high temperature stress.

This study examines leaf physiology and plant performance under contrasting temperature regimes of sister species of monkeyflower, *Mimulus cardinalis* and *M. lewisii* (Phrymaceae), that differ in elevation distribution. Reciprocal transplants

demonstrate that each species has high growth, survival and reproduction at its elevation range center and lower growth, survival and reproduction at its elevation range boundary and at elevations beyond its present elevation range (Chapter 2). Here I test the hypothesis that temperature is an important determinant of these differences in plant performance using temperature regimes measured in the field to simulate natural low and high elevation environments during the growing season. To examine adaptive differentiation among populations, populations from the elevation range center and range margin of each species were used as source material for the experiment. Specifically, this study asks 1) do *M. cardinalis* and *M. lewisii* differ in performance under temperature regimes characterizing their contrasting low and high elevation range centers? and 2) Do differences in leaf physiological traits underlie differences in performance under contrasting temperature regimes?

MATERIALS AND METHODS

Study System

Mimulus cardinalis and *M. lewisii* (Phrymaceae) are rhizomatous perennial herbs that grow along seeps and stream banks in western North America. Both species are self-compatible and animal pollinated (Hiesey et al. 1971; Schemske and Bradshaw 1999). *Mimulus cardinalis* occurs from southern Oregon to northern Baja California, Mexico and from the coast of California inland to Arizona and Nevada. *Mimulus lewisii* is composed of two races, a northern form occurring from southern coastal Alaska to southern Oregon and eastward to the Rocky Mountains, and a southern form, occurring primarily in the Sierra Nevada Mountains of California (Hiesey et al. 1971; Hickman 1993; Beardsley et al. 2003). The two races are partially incompatible, and recent

phylogenetic analysis suggests that the two races are sister to one another and together are sister to *M. cardinalis* (Beardsley et al. 2003). Here I study only the Sierran form of *M. lewisii*.

Mimulus cardinalis and *M. lewisii* segregate by elevation, with *M. cardinalis* occurring from sea level to 2400 m and *M. lewisii* occurring from 1200 m to 3100 m in California (Hickman 1993). In the Yosemite National Park region where this research was conducted, the species co-occur on larger watercourses between 1200 and 1500 m elevation (A. Angert, unpub. data). Although the published Californian distributions of *M. cardinalis* and *M. lewisii* extend to 2400 and 3100 m, respectively, repeated attempts to locate extant populations at these upper limits in the Yosemite region were unsuccessful. Experimental gardens planted at 415, 1400, 2395 and 3010 m on the western slope of the Sierra Nevada Mountains demonstrate that each species is most fit at its elevation range center, (415 m for *M. cardinalis*, 2395 m for *M. lewisii*), less fit at the mid-elevation range boundary, and unable to both survive and reproduce when transplanted to elevations beyond its current range (Chapter 2). For *M. lewisii*, reduced fitness at low elevations results primarily from high juvenile mortality within the first growing season. For *M. cardinalis*, reduced fitness at high elevations is due primarily to limited growth and reproduction (Chapter 2).

Genetic Material: Population Collection and Crossing Design

Seeds from eight plants in each of four populations per species were collected in September 1999 along an elevation gradient from 590 m to 2750 m between 37.49 and 37.95 ° N latitude (Appendix A). For each species, the chosen populations represent two locations from central within the range (low elevation for *M. cardinalis*, high elevation

for *M. lewisii*) and two locations from the range margin (mid elevation for both species). One plant from each field-collected family was grown to flowering in the University of Washington greenhouse under standard greenhouse conditions. The eight plants from each population were crossed with one another so that each plant served as sire or dam once with no self- or reciprocal pollinations, generating four independent full-sib families. Pollinations were performed by collecting all of the pollen from one flower with a flat toothpick and fully saturating the stigma of one flower. Seeds from four pollinations per full-sib family were pooled. These crosses generated outcrossed seeds from each population in a uniform environment to be used for controlled environment studies.

Chamber Conditions

Two incubators (Model I-36LL, Percival Scientific, Perry, IA, USA) were programmed to simulate low and high elevation temperature regimes for 60 days. To determine representative low and high elevation temperatures regimes during the growing season, data loggers (Hobo Pro Temp/External Temp, Onset Computer Corp., Bourne, MA, USA) recorded temperatures at low (415 m, near Jamestown, California) and high (2395 m, at the White Wolf Ranger Station in Yosemite National Park, California) elevation sites during June - September 2002. These sites have been used for reciprocal transplant gardens (Chapter 2) and are concordant with the range center of *M. cardinalis* and *M. lewisii*, respectively. Two data loggers at each site recorded air temperature every half hour. Loggers were mounted at plant height and shielded from direct sunlight with reflective covers.

Table 10. July 2002 mean temperatures recorded in reciprocal transplant gardens at 415 and 2395 m.

	Temperature (°C)					
Elev.(m)	Ave. daily max.	Max. daily max.	Days > 40	Ave. daily min.	Min. daily min.	Days < 0
415	34.48	41.67	4	14.80	11.67	0
2395	22.78	27.91	0	4.28	-1.97	2

Incubator temperature programs were set to reflect July average daily maximums and minimums at each elevation, with occasional temperature spikes or dips occurring at natural frequency (Table 10). July temperatures were used because plant growth is at its peak at both low and high elevation during this time. The cold, high elevation chamber was set for a 23 °C daytime maximum and 4 °C nighttime minimum, with one 0 °C freeze on night 15 and a second -2 °C freeze on night 36. Although few plants showed visible signs of tissue injury after exposure to 0° C, many plants were injured by the second, -2° C freeze. To quantify tissue damage, I estimated the percentage of total leaf tissue damaged on each plant. The hot, low elevation chamber was set for a 35 °C daytime maximum and 15 °C nighttime minimum, with 42 °C daytime maximums on days 18, 30, and 51. Daily maximum and minimum temperatures (including extremes) were held for four hours each with gradual ramps between maximum and minimum temperatures. Incubators were programmed for 14/10 hour day/night cycles with the maximum possible light output, 200 μmol photons m^{-2} s^{-1} during the daytime period. In natural environments *M. cardinalis* and *M. lewisii* grow in a range of light conditions from full sun on open gravel bars to full shade along riparian corridors (A. Angert, pers. obs.).

Four replicates of each full-sib family were sown in the Michigan State University greenhouse in January 2003. Five weeks after sowing, seedlings were transferred to either the hot or the cold incubator, for a total of 64 plants per temperature treatment (2 species x 4 populations / species x 4 families / population x 2 replicates per family). Seedlings were placed in random order within wire frames, and wire frames were placed in trays for sub-irrigation within the incubator. Frames were rotated several times per week to minimize position effects. Plants remained in each incubator for 60 days.

Leaf Physiological Trait Measurements

Simultaneous gas exchange and chlorophyll fluorescence measurements were performed following the last extreme temperature event for each treatment (day 53 hot, day 37 cold) with a portable open-flow gas exchange system equipped with leaf chamber fluorometer and CO_2 mixer (Li-Cor 6400, Li-Cor, Inc., Lincoln, NE, USA). The difference in time period preceding gas exchange measurements reflects natural differences in growing season length at low and high elevations. However, measurements made after the second extreme heat spike did not produce qualitatively different results, demonstrating that the patterns presented here are not unduly influenced by the length of exposure to low versus high temperatures. Measurements were made at midday during the 4-hour daily temperature maximum so that chamber temperature settings were not ramping throughout the course of the measurements. Because of sub-irrigation, plants were not water limited and gas exchange rates remained high at midday. This is realistic because *M. cardinalis* and *M. lewisii* normally inhabit stream banks or permanent seeps.

The youngest fully-expanded leaf (second or third node) was enclosed within the leaf chamber. Instantaneous net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}), and the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) were determined at the light intensity in which leaves developed, 200 μmol photons m^{-2} s^{-1}, a reference CO_2 concentration of 400 μmol mol^{-1}, a flow rate of 500 μmol s^{-1}, and block temperatures of 35 ° C (hot chamber) or 23° C (cold chamber). Stomatal conductance is an indicator of the degree of stomatal openness, which determines leaf loss of water and gain of carbon dioxide, and the ratio of intercellular to ambient CO_2 can indicate the degree to which stomatal closure limits the availability of CO_2 for photosynthesis. Calculations of stomatal conductance assumed a 0.5 ratio of conductances on the upper versus lower side of each leaf. Before statistical analysis, stomatal conductance at high temperatures were reduced by 2% per °C above 23 °C to normalize for decreased water viscosity with increased temperature (Tyree et al. 1995; Sack et al. 2002). Vapor pressure deficit and relative humidity within the leaf chamber were not controlled. Leaf temperature (°C) was measured with a fine wire thermocouple on the underside of each leaf. Steady-state fluorescence (F_s) and maximal light-adapted fluorescence during a saturating flash of light (F_m') were also measured simultaneously with gas exchange. These fluorescence parameters were used to calculate the effective quantum yield of photosystem II ($\Phi_{PSII} = (F_m' - F_s)/F_m'$), or the fraction of absorbed photons that a light-adapted leaf uses for photochemical reactions.

Measurement of Plant Performance

To quantify overall plant performance in each temperature environment, I measured final survival and growth. Traits were measured on day 60, at which time

plants were harvested to measure total stem length, number of nodes per stem, and aboveground biomass. Stem length and node number were highly correlated (*M. cardinalis*: Pearson's r=0.95, P<0.0001; *M. lewisii*: r=0.79, P<0.0001), whereas stem length and biomass were less so (*M. cardinalis*: r=0.78, P<0.0001; *M. lewisii*: r=0.21, P=0.11), thus I present only stem length and biomass data.

Statistical Analysis

I performed mixed model analysis of variance (ANOVA) for both temperatures and species combined to model variation in each trait (A, Φ_{PSII}, normalized g_s, C_i/C_a, aboveground biomass, and height) with respect to growth temperature, species, elevation of origin nested within species, population of origin nested within elevation, family nested within population, and all interactions. I also performed mixed model ANOVA within each temperature treatment to examine the effects of species, population, elevation of origin, and family on leaf temperature. Stomatal conductance and aboveground biomass were log-transformed to meet ANOVA assumptions. Temperature, species and elevation of origin were considered as fixed effects, whereas population and family were considered as random effects. To evaluate the significance of fixed effects, I used Type III estimable functions, which tolerate unbalanced samples, with denominator degrees of freedom obtained by Satterthwaite's approximation. Intraspecific differences between temperatures and interspecific differences within each temperature were evaluated by independent contrasts with a single degree of freedom. Likelihood-ratio tests (comparing each reduced model to the full model including all effects) were used to evaluate the significance of all random effects.

To examine variation in post-freeze tissue damage, I performed mixed model analysis of variance as described above, with the following exceptions. Differences in post-freeze tissue damage were examined within the cold temperature regime only, thus the model included only species, elevation, population, and family effects. For this model I also included position within the incubator as a covariate to account for an unexpected temperature gradient from the front to the back of the chamber during the freeze.

I did not model variation in survival with respect to growth temperature because no *M. cardinalis* died in the hot temperature treatment, causing model convergence problems. All analyses were implemented with PROC MIXED in SAS, version 8.2 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Leaf Physiological Traits

Table 11 gives the results of mixed model analysis of variance of instantaneous net photosynthesis, effective quantum yield, stomatal conductance (log-transformed and normalized to correct for temperature-induced changes in water viscosity), and C_i/C_a. The main effect of temperature affected stomatal conductance and C_i/C_a but not photosynthetic rate or effective quantum yield. The main effect of species only marginally affected effective quantum yield. However, species by temperature interactions affected all four parameters, indicating that the species differ in their leaf physiological response to temperature. Elevation of origin did not affect any leaf physiological trait, and the elevation by temperature interaction affected *M. cardinalis* stomatal conductance and C_i/C_a only, indicating that differentiation in leaf physiological

Table 11. Linear mixed model analysis of variance summary for four leaf physiological traits: instantaneous net photosynthetic rate (A), effective quantum yield (Φ_{PSII}), stomatal conductance (g_s), and the ratio of intercellular to ambient CO_2 (C_i/C_a). g_s was corrected for temperature-induced changes in water viscosity and log-transformed prior to analysis. F-tests for fixed effects constructed by SAS MIXED procedure, with denominator degrees of freedom obtained from the Satterthwaite approximation and indicated in parentheses below each F-value. All random effects (population nested within elevation of origin, family nested within population, and their interactions with temperature) were estimated to be zero or near-zero and were not significant. Abbreviations as follows: Temp. = temperature, Spp. = species, Elev. = elevation.

	F for fixed sources of variation				
Trait	Temp.	Spp.	Spp.*Temp.	Elev.(Spp.)	Elev.*Temp.(Spp.)
df	1	1	1	2	2
A	2.13	0.12	27.03**	0.08	0.78
	(3.71)	(3.78)	(3.71)	(3.78)	(3.69)
Φ_{PSII}	1.64	5.90†	44.20***	0.37	1.11
	(56.5)	(3.83)	(56.5)	(3.81)	(55.7)
g_s	94.30****	0.04	56.40****	1.35	4.30*
	(57.6)	(5.24)	(57.6)	(5.21)	(56.80)
C_i/C_a	66.59****	1.32	16.29***	2.99	6.91**
	(56.2)	(4.80)	(56.2)	(4.78)	(55.3)

†P<0.10;*P<0.05; **P<0.01;***P<0.001;****P<0.0001

Table 12. P-values from single degree of freedom independent contrasts of least square means testing the null hypotheses that interspecific differences in physiological parameters within a temperature regime and intraspecific differences between temperature regimes are equal to zero. g_s was corrected for temperature-induced changes in water viscosity and log-transformed prior to analysis.

	Intraspecific contrasts		Interspecific contrasts	
Trait	*cardinalis* hot vs. *cardinalis* cold	*lewisii* hot vs. *lewisii* cold	*cardinalis* hot vs. *lewisii* hot	*cardinalis* cold vs. *lewisii* cold
A	0.0123	0.0601	0.0201	0.0376
Φ_{PSII}	0.0002	<0.0001	0.0008	0.1309
g_s	<0.0001	0.1393	0.0004	0.0005
C_i/C_a	<0.0001	0.0065	0.1560	0.0080

traits between range margin and range center populations is low. The random effects of population, family, and their interactions with temperature did not affect leaf physiological traits (data not shown).

The species main effect in the model of effective quantum yield indicated that *M. cardinalis* had a marginally higher light-adapted photochemical efficiency than *M. lewisii*. Both species attained higher net photosynthetic rates and effective quantum yields when grown under the temperature regime of their elevation range center, although the difference was only marginally significant for *M. lewisii* net photosynthesis (Figure 12a, b, Table 12). The main effect of temperature indicated that stomatal conductance and C_i/C_a were higher in the hot temperature regime than in the cold temperature regime. Greater conductance and C_i/C_a were detected at high temperature despite greater vapor pressure deficit (VPD) and lower relative humidity (RH) (*M. cardinalis*: VPD_{hot}, 2.13±0.05, VPD_{cold}, 1.98±0.05; RH_{hot}, 19.95±0.32%, RH_{cold}, 31.39±0.46%; *M. lewisii*: VPD_{hot}, 3.06±0.15, VPD_{cold}, 1.80±0.05; RH_{hot}, 14.35±0.45%, RH_{cold}, 33.87±0.59%). *Mimulus cardinalis* conductance was much lower in cold temperatures than in hot, whereas *M. lewisii* conductance was not significantly different between temperature regimes (Figure 12c, Table 12). Both species displayed higher C_i/C_a in hot than in cold temperatures, but *M. cardinalis* showed a much larger decrease from hot to cold than *M. lewisii* (Figure 12d, Table 12). Within the hot temperature regime, *M. cardinalis* displayed greater photosynthetic rate, effective quantum yield, and stomatal conductance than *M. lewisii* (Figure 12, Table 12). Within the cold temperature regime, *M. lewisii* displayed greater photosynthetic rate, stomatal conductance, and C_i/C_a than *M. cardinalis* (Figure 12, Table 12).

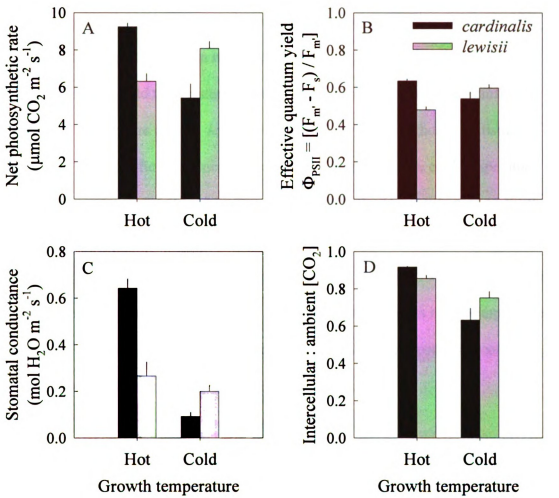

Figure 12. Comparisons of species mean + SE physiological responses to low elevation (hot) and high elevation (cold) temperature regimes: a) net photosynthetic rate (A, in μmol CO_2 m^{-2} s^{-1}), b) effective quantum yield [$\Phi_{PSII}=(F_{m'}-F_s)/F_{m'}$], c) stomatal conductance (g_s, in mol H_2O m^{-2} s^{-1}, corrected for increased water viscosity at high temperature), and d) the ratio of intercellular to ambient CO_2 (C_i/C_a).

Populations of *M. cardinalis* originating from the low elevation range center differed from populations originating from the mid elevation range boundary in the response of stomatal conductance and C_i/C_a to temperature. Low elevation populations showed greater decreases in conductance and C_i/C_a from hot to cold temperatures than mid elevation populations (data not shown), suggesting that mid elevation populations were more adversely affected by hot temperatures than low elevation populations or

were not as light-limited as low elevation populations in hot temperatures. However, no other *M. cardinalis* traits and no *M. lewisii* traits displayed a pattern consistent with adaptive differentiation between range center and range margin populations.

Within the cold temperature regime, *M. cardinalis* maintained a significantly higher leaf temperature than *M. lewisii* ($F_{1,17.5}$=4.67, P=0.04). Although statistically significant, interspecific differences in leaf temperature within the cold temperature regime averaged only 0.6 °C and leaf temperature of both species was near ambient temperature. Within the hot temperature regime, *M. cardinalis* maintained a significantly lower leaf temperature than *M. lewisii* ($F_{1,43}$=38.02, P<0.0001). At high temperatures, high conductance enabled *M. cardinalis* to maintain a leaf temperature approximately 10 °C below ambient, whereas *M. lewisii* leaf temperature was approximately 7 °C below ambient.

Post-Freeze Tissue Damage

Neither species was visibly damaged following the 0° C freeze. Individuals of both species were visibly injured by the -2° C freeze, but *M. cardinalis* exhibited an average of 68.1% visible leaf tissue damage, whereas *M. lewisii* exhibited an average of only 46.3% damage (mixed model ANOVA: species, $F_{1,55}$=14.77, P=0.0003).

Whole Plant Performance

Table 13 gives the results of linear mixed model analyses of stem length and log-transformed aboveground biomass. The main effect of temperature affected stem length but not aboveground biomass. The main effect of species and the interaction between species and growth temperature affected both traits. Elevation of origin,

Table 13. Linear mixed model analysis of variance summary for stem height and log-transformed aboveground biomass. F-tests for fixed effects constructed by SAS MIXED procedure, with denominator degrees of freedom obtained from the Satterthwaite approximation and indicated in parentheses below each F-value. All random effects (population nested within elevation of origin, family nested within population, and their interactions with temperature) were estimated to be zero or near-zero and were not significant. Symbols and abbreviations as in Table 11.

	F for fixed sources of variation				
Trait	Temp.	Spp.	Spp.*Temp.	Elev.(Spp.)	Elev.*Temp.(Spp.)
df	1	1	1	2	2
Length	489.41****	64.78****	219.02****	8.32	1.03
	(84.4)	(27.4)	(84.4)	(27.4)	(84.4)
Biomass	2.36	92.20****	38.31***	0.07	1.85
	(8)	(8)	(8)	(8)	(8)

population, family and their interactions with temperature did not affect either growth trait.

The main effect of temperature in the model of stem length indicated that plants were taller in the hot temperature regime than in the cold temperature regime. The main effect of species indicated that *M. cardinalis* had greater stem length and aboveground biomass than *M. lewisii*. However, the interaction between species and temperature indicated that the species differed in growth response to temperature. Both species achieved greater stem length in hot than in cold temperatures, but the magnitude of size difference was much greater for *M. cardinalis* than for *M. lewisii* (Figure 13a). Further, within the cold treatment, *M. lewisii* stem length was greater than *M. cardinalis* stem length (Table 14). Although *M. cardinalis* aboveground biomass was greater than *M. lewisii* biomass in both temperatures, *M. cardinalis* aboveground biomass was greater in hot than in cold temperatures, whereas *M. lewisii* biomass was greater in cold than in hot temperatures (Figure 13b, Table 14).

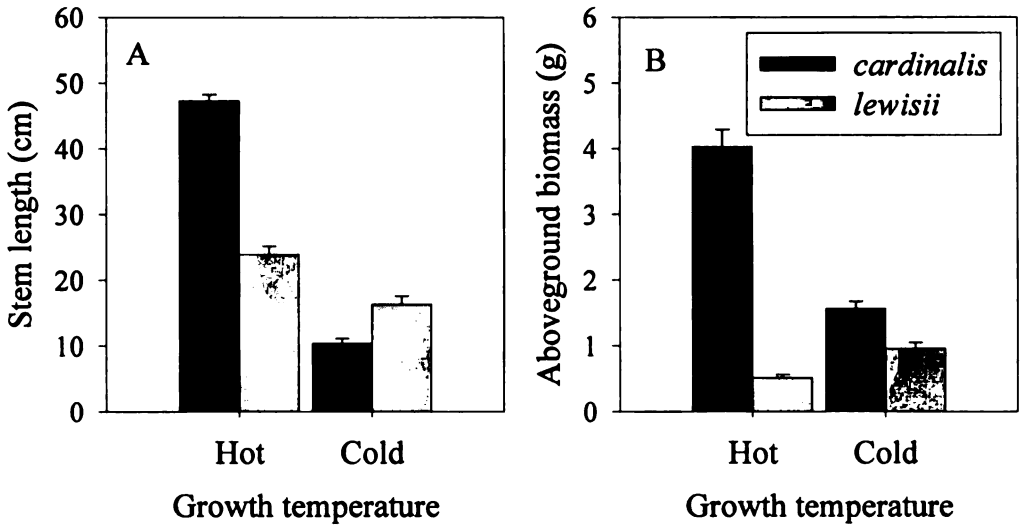

Figure 13. Species' mean + SE a) stem length (cm) and b) aboveground biomass (g).

Table 14. P-values from single degree of freedom independent contrasts of stem length and aboveground biomass least square means testing the null hypotheses that interspecific differences in growth parameters within a temperature regime and intraspecific differences between temperature regime are equal to zero.

	Intraspecific contrasts		Interspecific contrasts	
Trait	*cardinalis* hot vs. *cardinalis* cold	*lewisii* hot vs. *lewisii* cold	*cardinalis* hot vs. *lewisii* hot	*cardinalis* cold vs. *lewisii* cold
Length	<0.0001	<0.0001	<0.0001	<0.0001
Biomass	0.0006	0.0110	<0.0001	0.0423

Survival of both species was high in the cold temperature treatment (*M. cardinalis*: 96.9%; *M. lewisii*: 93.8%), and survival of *M. cardinalis* was 100% in the hot treatment. However, *M. lewisii* survival in the hot treatment was only 21.9%.

DISCUSSION

Interspecific Variation in Performance versus Temperature

Mimulus cardinalis and *M. lewisii* displayed clear differences in performance under contrasting temperature regimes. Each species attained its greatest aboveground biomass when grown under a temperature regime characteristic of its altitudinal range

center and displayed reduced mass when grown under a temperature regime beyond its present altitudinal range. Although both species exhibited greater stem lengths in hot than in cold temperatures, the stem length of *M. cardinalis* was more greatly reduced under cold temperatures than was that of *M. lewisii*. Survival of *M. lewisii* was also sensitive to temperature, showing a striking difference of 94% survival in cold temperatures and only 22% survival in hot temperatures. The low survival of *M. lewisii* in hot temperatures did not occur immediately upon exposure to high temperatures, but arose gradually throughout the experiment despite being well-watered. Plants appeared to waste away, implicating high respiration rates as the cause of reduced growth and survival (Hiesey et al. 1971). In hot temperatures, *M. cardinalis* displayed greater survival, aboveground biomass and stem length than *M. lewisii,* whereas in cold temperatures, *M. lewisii* displayed greater stem length and resistance to freezing damage than *M. cardinalis*.

Previous studies have also demonstrated that *M. cardinalis* and *M. lewisii* differ in growth response to temperature, although none have compared the species under natural temperature regimes (Cline and Agatep 1970; Hiesey et al. 1971). Hiesey et al. (1971) compared growth of *M. cardinalis* from the foothills of the Sierra Nevada Mountains in California and *M. lewisii* from subalpine habitat in the Rocky Mountains of Montana under constant warm (30° C) or cold (10° C) temperatures and found that *M. lewisii* grew poorly under hot temperatures whereas *M. cardinalis* was broadly tolerant of both hot and cold temperatures. Cline and Agatep (1970) grew Sierra Nevadan populations of each species (foothills *M. cardinalis*, subalpine *M. lewisii*) under constant day and night temperatures of 3, 7, 11, 15, 19, 23, or 27° C. Both species

attained maximum growth at 19° C. However, *M. lewisii* experienced high mortality under hot temperatures but grew twice as fast as *M. cardinalis* under cold temperatures.

The magnitude of the difference in growth and survival between *M. cardinalis* and *M. lewisii* was greater within the hot temperature regime than in the cold. Several factors may have played a role in producing the observed asymmetrical affect of temperature. First, in its natural habitat, particularly at mid elevations, *M. cardinalis* is likely to experience occasional freezes and cool daytime temperatures late in the growing season, whereas *M. lewisii* is unlikely to encounter the extreme high temperatures used here anywhere within its natural range. Second, high light levels in a natural high elevation environment may exacerbate the effects of cold temperature by inducing photoinhibition (Close and Beadle 2003; Sayed 2003), but in our experiment light levels were relatively low, potentially moderating the harmful effect of low temperatures. Finally, in reciprocal transplant gardens at high elevation, mortality of *M. cardinalis* is concentrated over the winter (Angert and Schemske, unpub. data). Because this experiment simulated conditions only during the growing season, it did not simulate the time period when *M. cardinalis* is most susceptible to mortality.

The patterns of differential growth and survival presented here are similar to those observed in reciprocal transplant gardens with these species at 415 m and 2395 m (Chapter 2), implying that temperature may be largely responsible for differences in growth and survival versus elevation. For example, after one growing season at 415 m, *M. cardinalis* survival was 77% whereas *M. lewisii* survival was only 2%. In contrast, at the high elevation site, 2395 m, survival of both species was greater than 95% after one

growing season. Also, at high elevation, *M. cardinalis* growth was reduced; seedlings were roughly two-thirds the size of *M. lewisii* after one growing season.

Interspecific differences in growth response to temperature have been reported for several other congeneric species pairs differing in elevation distribution (Woodward and Pigott 1975; Woodward 1979; Graves and Taylor 1986; Woodward 1990; Kao et al. 1998). For example, growth of the low elevation species *Sedum telephium, Dactylis glomerata,* and *Phleum bertolonii* increases with temperature but growth of high elevation *S. rosea, P. alpinum, and Sesleria albicans* is insensitive to temperature (Woodward 1975; 1979). The differential sensitivity of *S. telephium* and *S. rosea* growth to temperature results in a switch in competitive dominance between low and high elevations (Woodward and Pigott 1975). Similarly, Graves and Taylor (1986) found that growth of *Geum urbanum* in cool temperatures was more restricted than growth of *G. rivale*, which occurs at higher elevations. However, in field experiments, the species exhibited only slight differences in relative growth rates across elevation. The results of these studies differ from ours in that the effect of temperature was more pronounced in low elevation species, supporting the generalization that lower range limits of high elevation species result primarily from biotic interactions such as competition rather than physiological limitation (MacArthur 1972; Woodward 1975; Scheidel et al. 2003). Instead, this study suggests severe abiotic limitation for *M. lewisii* beyond its lower elevation range limit due to inability to survive and grow under hot temperatures.

Intraspecific Variation in Performance versus Temperature

To demonstrate that temperature limits species distributions requires the use of populations collected from range margins because marginal populations are often phenotypically or genetically divergent from more centrally located populations (Lesica and Allendorf 1995; Perez-Tris et al. 2000; Medail et al. 2002; Van Rossum et al. 2003; Faugeron et al. 2004) and may be differently adapted to temperature conditions at or beyond the range margin. However, in this study, populations from the range center and range margin of each species did not differ in growth or leaf physiological response to temperature, with the exception of *M. cardinalis* for g_s and C_i/C_a. In reciprocal transplants at 415 m and 2395 m, a similar lack of adaptive differentiation with respect to elevation of origin was observed among populations of *M. cardinalis* and *M. lewisii* (Chapter 2). Although finding no population differentiation in a controlled environment such as in the present study is consistent with results from the field, further experiments that simulate temperatures at the range margin are needed to investigate population variation in performance.

The likelihood of population differentiation depends on the amount of gene flow as well as the degree of environmental difference between populations. Graves and Taylor (1988) also found no difference in the temperature acclimation of photosynthesis between populations of *G. urbanum* and *G. rivale* separated by only several hundred meters. Conversely, Pitterman and Sage (2000) found that a cold-acclimated low elevation population of *Bouteloua gracilis* exhibited depressed rates of net photosynthesis at cold temperatures but that a population originating 1500 m higher exhibited enhanced rates of photosynthesis at cold temperatures. Patterns of ecotypic differentiation in temperature response have also been found for *Trifolium repens*

photosynthesis in populations from 600 and 2040 m (Mächler and Nösberger 1977), for *Eucalyptus pauciflora* photosynthesis in populations from 915 and 1770 m (Slatyer 1977), and for *Reynoutria japonica* growth in populations from 700 and 2420 m (Mariko et al. 1993). Greater altitudinal separation between populations implies not only greater environmental difference but also greater geographic isolation. Populations used in this experiment originated at the elevation range center and range margin of each species, a difference in elevation of 600 – 1200 m per species. Estimates of gene flow between range margin and range center populations of *M. cardinalis* and *M. lewisii* would help determine whether gene flow prevents the evolution of local adaptation to the temperature conditions at range margins (Kirkpatrick and Barton 1997).

Interspecific Variation in Leaf Physiology versus Temperature

Mimulus cardinalis and *M. lewisii* exhibit differences in leaf physiological response to temperature that are consistent with differences in growth response to temperature and with elevation distributions in nature. Each species attains the greatest net photosynthetic rate and effective quantum yield of PS II when grown under a temperature regime characteristic of its altitudinal range center and displays reduced photosynthetic rate and quantum yield when grown under a temperature regime beyond its present altitudinal range. Stomatal conductance and C_i/C_a are reduced under cold temperatures compared to hot temperatures, but *M. cardinalis* shows much greater reductions than does *M. lewisii*. Within each temperature regime, the species native to that temperature exhibits greatest leaf physiological capacity.

Differential sensitivity of *M. cardinalis* and *M. lewisii* net photosynthetic rates to growth temperature demonstrates that each species is limited in its ability to acquire

primary resources when grown under a temperature regime beyond its elevation range. Hiesey et al. (1971) also demonstrated that *M. cardinalis* and *M. lewisii* differ in photosynthetic response to temperature. When both species were grown at a constant temperature of 20° C, *M. lewisii* exhibited a light-saturated photosynthetic optimum that peaked at 25° C, but *M. cardinalis* photosynthesis did not decline until temperatures exceeded 30° C. Contrary to our results, Graves and Taylor (1988) found little difference in the temperature response of photosynthesis between two species of *Geum* with different elevation distributions. The authors suggested that growth differences between the species were driven by differences in the ability to utilize assimilated carbon for growth, rather than by differences in the ability to assimilate carbon.

Because photosynthesis is the primary source of energy and substrates for all other biosyntheses, when differences in photosynthetic rates are observed it is tempting to conclude that differences in carbon assimilation are directly related to differences in growth. However, although the observed differences in *M. cardinalis* and *M. lewisii* carbon assimilation rates are consistent with their growth responses to temperature, instantaneous net photosynthetic rate is often a poor indicator of growth (Nelson 1988; Arntz et al. 1998). To fully dissect differences in growth requires measurement of respiration rates, plant architecture, and patterns of allocation in addition to measurement of photosynthetic rate. Future studies of *M. cardinalis* and *M. lewisii* should identify how respiration, architecture and allocation vary between species and with temperature to further clarify growth limitations beyond the species' elevation ranges.

Interspecific differences in the response of light-adapted quantum yield to temperature indicate that each species is able to use a larger fraction of incoming light energy for photochemical reactions when grown under the temperature regime of its elevation range center. Effective quantum yield is determined by the efficiency of excitation energy capture by open reactions centers and by the number of open reaction centers available for photochemical reactions (Schreiber et al. 1994). Decreases in the effective quantum yield of PS II may result from temperature-induced damage to electron transport processes or from feedback inhibition of PS II activity resulting from temperature-induced reductions in carbon metabolism (Falk et al. 1996; Laisk et al. 1998). To distinguish between these alternatives requires additional data on the temperature sensitivity of particular fluorescence parameters (e.g., minimum fluorescence, variable fluorescence, and non-photochemical quenching) in addition to detailed study of gas exchange metabolism (Owens 1994; Laisk et al. 1998; Xiong et al. 1999; Haldimann and Feller 2004). Without such information, it is difficult to attribute changes in fluorescence yield to any particular process (Owens 1994). However, studies of depression of net photosynthesis in oaks (Haldimann and Feller 2004) and Antarctic plants (Xiong et al. 1999) have concluded that heat-induced damage to thylakoid membranes does not occur until temperatures well above those that depress photosynthesis, and thus that reduced enzymatic activity is the main cause of depressions in photosynthesis under high temperatures in the field. Likewise, low temperature may harm photosynthesis primarily through effects on carbon metabolism rather than effects on photochemistry (Leegood and Edwards 1996).

Patterns of variation in stomatal conductance and C_i/C_a differed from patterns for photosynthetic rate and effective quantum yield. In hot temperatures with high vapor pressure deficit and no water limitation, *M. cardinalis* showed high stomatal conductance, which allowed greater evaporative cooling of the leaf surface. Even with no water limitation, *M. lewisii* showed lower stomatal conductance under hot temperatures than *M. cardinalis*, higher leaf temperatures, and lower intercellular concentrations of CO_2. High C_i/C_a ratios in hot temperatures, particularly of *M. cardinalis*, indicate that photosynthesis at high temperatures was possibly light limited. However, subsequent experiments using higher light levels during growth and measurement find similar patterns of difference in photosynthetic rates between species and between temperature regimes (A. Angert, unpub. data), and it is unlikely that greater light levels would have eliminated the observed differences between *M. lewisii* and *M. cardinalis* in the hot temperature regime.

Without measurement of the CO_2 saturation point for photosynthesis, it is unclear whether lower stomatal conductance for both species, particularly *M. cardinalis*, in cold temperatures resulted in greater stomatal limitation to photosynthesis. However, it is likely that lower conductance resulted from, rather than caused, low photosynthesis. Long-term acclimation to growth temperature and light conditions during our study may have allowed changes in stomatal density or aperture that optimized conductance to reduce unnecessary transpiration in conditions of low CO_2 assimilation (Ferris et al. 1996). Several other studies support this hypothesis. Naidu and Long (2004) found that cold-acclimated *Zea mays* did not experience increased stomatal limitation to photosynthesis, despite greatly reduced stomatal

conductance. Similar results have been reported for tomato (Martin and Ort 1985), olive (Bongi and Long 1987), rye (Huner et al. 1986), wheat (Hurry and Huner 1991), and several C_4 grasses (Pitterman and Sage 2001; Naidu and Long 2004). As in these examples, it is likely that conductance decreased to match assimilatory use of CO_2 and that reduced intercellular concentrations of CO_2 resulted from, rather than caused, low photosynthetic rates.

Some of the observed physiological responses may be due to uncontrolled environmental variables that covaried with temperature, such as vapor pressure deficit or relative humidity, rather than temperature per se (Matzner and Comstock 2001). However, increased conductance was observed at high temperatures despite greater vapor pressure deficit and reduced humidity. Further, although these factors may be confounded in the present study, this represents a realistic natural scenario in temperate environments, where temperature and vapor pressure deficit often increase simultaneously (Iio et al. 2004).

This study provides evidence that *M. cardinalis* and *M. lewisii* differ in performance under temperature regimes characterizing their contrasting low and high elevation range centers. Differences in the species' leaf physiological responses under contrasting temperature regimes are consistent with differences in performance observed in both controlled and natural environments. Elevation range limits of *M. cardinalis* and *M. lewisii* may arise, in part, due to metabolic limitations on growth that ultimately decrease survival and limit reproduction.

CHAPTER 4

Natural selection within and beyond the elevation ranges of monkeyflowers (*Mimulus cardinalis* and *M. lewisii*)

Abstract.— Every species occupies a restricted geographic distribution, but why natural selection does not increase tolerance to limiting environmental variables and allow continual range expansion remains an evolutionary conundrum. At the heart of many of hypotheses for distribution limits is the idea that environments within and beyond the species range select for different phenotypes, and it is the difficulty of producing a phenotype adapted to environments both within and beyond the range that constrains range expansion. In this study, I examine natural selection in sister species of monkeyflower, *M. cardinalis* and *M. lewisii,* to identify traits that contribute to fitness within and beyond elevation range limits and to ask whether adaptation to environments beyond the range entails a cost to adaptation within the range. I transplanted interspecific hybrids to low and high elevation and found that selection favored early flowering at high elevation. Hybrids selected at low elevation displayed increased leaf photochemical efficiency in warm temperatures. Selection acted in the direction of the native parental species' trait value, supporting the hypothesis that *M. cardinalis* photosynthetic traits and *M. lewisii* flowering phenology are adaptive at their respective low and high elevation range centers. If adaptation to one environment entails a cost to adaptation in other environments, then selected hybrid populations should display reduced fitness, relative to an unselected control population, when grown in an environment in which they were not selected. One such tradeoff was observed in this

study, where hybrids selected at high elevation displayed reduced biomass when grown in temperatures characteristic of low elevation. Continued generations of experimental evolution and the reciprocal transplantation of selected populations to low and high elevation will allow definitive tests of the role of between-environment fitness tradeoffs in range limit evolution.

Key words: natural selection, experimental evolution, range limits, phenology, physiological adaptation

Species' distribution boundaries have long fascinated ecologists and biogeographers seeking explanations for why species fail to occur beyond their present limits (Griggs 1914; Grinnell 1917; Good 1931; Dahl 1951). Most studies of distribution limits have focused on identifying the proximate ecological factors that give rise to a distribution boundary. Such studies may examine populations, asking whether local abundance decreases towards the range margin (Brown et al. 1996; Sagarin and Gaines 2002) or whether marginal populations are demographic sinks or more prone to extinction than central populations (Carter and Prince 1981; Lennon et al. 1997; Mehlman 1997; Guo et al. 2005). Many other investigations of distribution limits focus on individuals, asking whether survival and reproduction decrease towards the range margin (Marshall 1968; Pigott and Huntley 1981; McKee and Richards 1996; Garcia et al. 2000; Hennenberg and Bruelheide 2003), and, if so, which environmental variables are responsible for variation in components of fitness (McNab 1973; Root 1988a; Cumming 2002). However, even when satisfactory answers to these questions are found, a central question remains: why does natural selection not continually improve

adaptation to limiting environmental variables and overcome current distribution limits? To answer this question, we must know which traits are under selection at and beyond the range boundary, and why they do not evolve to allow range expansion.

Many mechanisms have been proposed to limit the potential for adaptive evolution at and beyond range boundaries, including a lack of genetic variation in fitness-related traits (Antonovics 1976; Bradshaw and McNeilly 1991), an influx of maladapted genotypes from populations at the range center (Haldane 1956; Kirkpatrick and Barton 1997), and negative genetic correlations either among fitness-related traits or between fitness in environments within versus beyond the range (Antonovics 1976; Holt 2003). At the heart of many of these hypotheses is the idea that environments within and beyond the range select for different phenotypes, and it is the difficulty of producing a phenotype adapted to environments both within and beyond the range that constrains range expansion.

In this study, I examined natural selection in sister species of monkeyflower, *M. cardinalis* and *M. lewisii,* to identify traits that contribute to fitness within and beyond the elevation range limit and to ask whether adaptation to environments beyond the range entails a cost to adaptation within the range. Previous experiments have demonstrated that each species is most fit at its elevation range center (low elevation for *M. cardinalis*, high elevation for *M. lewisii*), less fit at the shared mid-elevation range boundary, and unable to survive and reproduce when transplanted to elevations beyond its current range (Chapter 2). For *M. lewisii*, reduced fitness at low elevation results primarily from high mortality within the first growing season. For *M. cardinalis*,

reduced fitness at high elevation is due primarily to limited growth and reproduction (Chapter 2).

Many features of the environment that affect plant survival, growth, and reproduction change with elevation, most prominently temperature and length of growing season. In growth chamber experiments, *Mimulus cardinalis* and *M. lewisii* display differences in survival, growth, leaf physiology, and freezing resistance under temperature regimes that mimic their contrasting low and high elevation range centers (Chapter 3). The species also differ in phenological traits that may contribute to differences in fitness versus elevation. When grown in a common environment, *M. lewisii* flowers earlier than *M. cardinalis* (Hiesey et al. 1971), suggesting that the ability to flower quickly and mature fruits rapidly may be favored in short growing seasons at high elevation. In this study, I measure natural selection on leaf physiology, freezing resistance, and flowering phenology. I hypothesize that these traits affect the ability to survive and reproduce at different elevations.

One major difficulty in measuring natural selection across species' ranges is that populations only exist above some threshold of fitness, limiting the environmental axis along which we can measure selection in natural populations. Also, trait variation within populations is likely to be minimized by stabilizing selection (Endler 1986) and influenced by factors other than natural selection, such as phylogenetic history (Harvey and Pagel 1991). These difficulties call for experimental approaches to detect selection. To increase the range of trait variation available to natural selection and to create trait combinations not found within either species, I created late-generation hybrids between *M. cardinalis* and *M. lewisii* and transplanted them to low and high elevation. After one

generation of evolution in each environment, I compared phenotypes between selected hybrid populations and an unselected greenhouse control population to identify traits that showed a shift in mean value after selection. I apply two criteria to assess trait evolution. First, if a particular trait is itself a target of natural selection or is genetically correlated with a trait that is the target of natural selection, then its mean value should differ significantly from the unselected control population. Second, if parental trait values are adaptive, then selected hybrid trait means should evolve in the direction of the parent native to that environment. Based on these criteria, I hypothesize that hybrids selected at high elevation will flower more rapidly, exhibit less tissue damage following freezes, and display greater leaf physiological capacity in cool temperatures characteristic of high elevation than the greenhouse control population. Likewise, I hypothesize that hybrids selected at low elevation will display greater leaf physiological capacity in warm temperatures characteristic of low elevation than the greenhouse control population.

To determine whether adaptation to low elevation entails a cost to adaptation at high elevation, and vice versa, I measured phenotypes on hybrids grown in two temperature regimes: one characteristic of low elevation and one characteristic of high elevation. If adaptation to one environment entails a cost to adaptation in another environment, then selected hybrid populations should display reduced fitness, relative to the control, when grown in the environment in which they were not selected. Alternatively, if reduced fitness in the unselected environment is not evident as a pleiotropic byproduct of evolution in the selected environment, then we can conclude

that fitness within each environment is able to evolve independently and between-environment fitness trade-offs are not present.

MATERIALS AND METHODS

Study system

Mimulus cardinalis and *M. lewisii* (Phrymaceae) are perennial herbs of riparian habitats in western North America. *Mimulus cardinalis* occurs from southern Oregon to northern Baja California, Mexico and from the coast of California inland to Arizona and Nevada. *Mimulus lewisii* is composed of two partially incompatible races, one occurring in the Pacific Northwest and the Rocky Mountains and one occurring primarily in the Sierra Nevada Mountains of California (Hiesey et al. 1971; Hickman 1993; Beardsley et al. 2003). In California, *M. cardinalis* and *M. lewisii* occupy different elevation ranges, with *M. cardinalis* occurring from sea level to 2400 m and *M. lewisii* occurring from 1200 m to 3100 m (Hickman 1993). In the Yosemite National Park region where this research was conducted, *M. cardinalis* is not found above 1500 m, *M. lewisii* is not commonly found above 2800 m, and the species may co-occur on larger watercourses between 1200 and 1500 m elevation.

Generation of hybrid populations for transplants

Seeds of *M. cardinalis* and *M. lewisii* were collected from a naturally occurring sympatric population along the South Fork of the Tuolumne River (Carlon Day Use Area, Tuolumne County, California, 1320 m) in September 1999. Two individuals of each species from distinct maternal plants were grown to flowering in the University of Washington greenhouse under standard greenhouse conditions and crossed to generate two independent F1 hybrid lines, using the same species (*M. lewisii*) as the maternal

parent in each cross. Two F1 individuals, one from each line, were grown to flowering and crossed to generate an F2 population. One thousand F2 individuals were grown to flowering and crossed to one another so that each plant served as pollen donor and recipient once (with no self- or reciprocal pollinations), generating 1000 hybrid seed lots with an additional round of recombination.

Transplant gardens

Experimental gardens were established near Jamestown, California (415 m) and at White Wolf Ranger Station in Yosemite National Park (2395 m; Figure 6). These locations were chosen to represent elevations for each species that are central within the elevation range (415 m for *M. cardinalis*, 2395 m for *M. lewisii*) and beyond the range boundary (2395 m for *M. cardinalis*, 415 m for *M. lewisii*). Seeds from 500 hybrid seed lots were sown in flats in the University of Washington greenhouse five weeks prior to transport to garden sites. The average age of transplanted seedlings was approximately three weeks after germination. In July 2001, 8110 seedlings (16-17 individuals from each of 500 seed lots) were transplanted in random order at 2395 m. To assess the strength of selection in each environment, 319 seedlings of each parental species were randomly interspersed among the hybrid individuals. In April 2003, seedlings were transplanted to 415 m following identical methods, except that space limitations allowed only 6000 individuals (11-12 from each of the same 500 hybrid seed lots plus 156 of each parent) to be transplanted. Garden plots were covered in landscape fabric and irrigated daily to approximate conditions in the species' native riparian habitat and to standardize water treatments across environments. Due to irrigation system failure in one area of the Jamestown garden, 27 *M. cardinalis*, 24 *M. lewisii*, and 933 hybrids

were excluded from analysis. Survival and day of first flowering were recorded from 2001-2003 at White Wolf (2395 m) and in 2003 at Jamestown (415 m). After only one growing season at Jamestown, most *M. lewisii* were dead and the majority of surviving plants had reached the flowering stage. Observations were conducted over a longer time period at White Wolf because of the length of time necessary for plants to reach reproductive maturity at high elevation. Data were recorded at approximately two-week intervals throughout each growing season.

Generation of selected and control hybrid populations

Because some phenotypes of interest were impractical to measure in the field, I generated a selected seed population at each elevation for trait measurement under controlled conditions. Selected seed populations were made by crossing subsets of individuals that were able to survive and flower within the transplant gardens. At White Wolf, pollinations were conducted at two-week intervals in 2003, beginning two weeks after flowering commenced and proceeding throughout the flowering period. Up to 80 individuals were crossed to one another within each pollination cohort, using only those individuals that began flowering within the interval. Buds were enclosed in mesh bags to prevent pollinator visitation. Each plant served as pollen donor and recipient only once. When more than 80 individuals began flowering within the two-week period, individuals were haphazardly selected from throughout the garden. Because this method of crossing potentially flattened the flowering time distribution of the offspring, for subsequent experiments I included fruits from each pollination cohort in proportion to the total number of individuals that began flowering within the interval. At Jamestown, pollinations during the growing season of 2003 were unsuccessful, so dormant rhizomes

of individuals that survived and flowered in 2003 were transported to the Michigan State University greenhouse in February 2004, where plants were regrown to flowering. Pollinations of Jamestown plants grown in the greenhouse were conducted following identical methods to those used at White Wolf, defining pollination cohorts by the flowering times recorded within the transplant garden. An additional population of hybrids from 250 of the original 500 hybrid seed lots was grown under favorable conditions in the greenhouse, where selection was assumed to be minimal (survival 100%, only 6 out of the initial 250 lines not included in crosses due to pollen sterility), and crossed to generate a control population of hybrid seeds.

Measurement of phenotypic traits

To examine the relationship between flowering time and seed set at White Wolf, seed set per fruit was quantified for hand pollinations conducted at two-week intervals (see *Generation of selected and control hybrid populations* above). In the lab, samples of approximately 150-200 seeds per fruit were counted under a dissecting microscope and weighed to determine the relationship between seed mass and seed number. Seed number per fruit was then estimated from the total seed mass.

All other phenotypic traits were measured in growth chambers on two sets of selected and control hybrids, one grown in a temperature regime characteristic of low elevation and a second grown in a temperature regime characteristic of high elevation. Low and high elevation temperature regimes were based on July temperatures recorded within the Jamestown and White Wolf transplant gardens (detailed in Chapter 3). The low elevation chamber was set for a 35 °C daytime maximum and 15 °C nighttime minimum, with 42 °C daytime maximums on days 50 and 64. The high elevation

chamber was set for a 23 °C daytime maximum and 4 °C nighttime minimum, with two -2 °C freezes on nights 50 and 64. Daily maximum and minimum temperatures were held for four hours each with gradual ramps between maximum and minimum temperatures. Two identical plant growth chambers were used for low and high elevation temperature regimes (Model GC-20BDAF-REFR404, Econair, Winnipeg, Canada) except during freezing events, when plants were transferred for a period of 24 hours to a chamber capable of holding sub-zero temperatures (Model GC-20BDAF-REFR-22, Econair, Winnipeg, Canada). Chambers were programmed for 14/10 hour day/night cycles. Light averaged 350 μmol photons m^{-2} s^{-1} at plant height during the daytime period.

In October 2004, seeds of selected and control hybrid populations were sown in either the low or the high elevation temperature regime. Pots were placed in random order within wire frames, and the frames were placed in trays for sub-irrigation within the growth chamber. Frames were rotated several times per week to minimize position effects. Approximately 10 seeds were sown per 10 cm pot and seedlings were randomly thinned to one seedling per pot three weeks after sowing so that each temperature regime contained 35 individuals from each hybrid population plus 15 individuals of each parent species. After thinning, the cotyledon diameter of each remaining seedling was measured to account for potential differences in performance between selected populations due to maternal growth environment (greenhouse or 2395 m garden). However, cotyledon diameter did not differ between selected populations (one-way analysis of variance, low elevation temperature regime: $F_{2,163}=0.12$, $P=0.89$; high

elevation temperature regime: $F_{2,155}=1.01$, $P=0.37$), indicating that seed quality did not measurably influence early seedling growth.

Simultaneous gas exchange and chlorophyll fluorescence measurements were performed following the last extreme temperature event for each treatment to characterize leaf photosynthetic function in low and high elevation temperature environments. Measurements were conducted as described in Chapter 3. Four leaf physiological traits were quantified: 1) instantaneous net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}); 2) effective quantum yield of photosystem II [$(F_{m'} - F_s)/ F_{m'}$], the fraction of absorbed photons that a light-adapted leaf uses for photochemical reactions, determined by chlorophyll fluorescence readings; 3) stomatal conductance (mol H_2O m^{-2} s^{-1}), an indicator of the degree of stomatal openness, which determines leaf loss of water and gain of carbon dioxide; and 4) the ratio of intercellular to ambient CO_2, which can indicate the degree to which stomatal closure limits the availability of CO_2 for photosynthesis. These variables may indicate particular processes that limit photosynthetic activity in suboptimal temperatures and, together, summarize leaf photosynthetic function. To quantify post-freeze tissue damage, I estimated the percentage of total leaf tissue damaged on each plant on the day following each freeze event within the low elevation temperature regime. The date of first flower was recorded for every flowering plant. After 87 days (low elevation temperature regime) and 127 days (high elevation temperature regime), plants were harvested for measurement of aboveground biomass, length of the main stem, and flower number. The difference in time period preceding harvest reflects large differences in growth rates between temperatures. Despite additional time in the growth chamber, very few

plants (1 *M. lewisii*, 7 hybrids) reached the flowering stage within the high elevation temperature regime. For this reason, flowering phenology and flower number were compared within the low elevation temperature regime only.

Data analysis

Performance and reproductive phenology in transplant gardens.— To examine differences among parents and hybrids in the probability of surviving and flowering at each elevation, I performed logistic regressions (PROC LOGISTIC, SAS Institute, Cary, NC, USA), using the 'contrast' statement to test for pairwise differences between parents and hybrids and the sequential Bonferroni procedure to control type I error rates. To examine differences among parents and hybrids in the day of first flower at each elevation, I performed analysis of variance (ANOVA) on log-transformed data (PROC GLM, SAS Institute, Cary, NC, USA). Pairwise differences between parents and hybrids were evaluated with Tukey-Kramer adjusted comparisons of least square means. To examine the relationship between flowering time and seed set at White Wolf, linear regressions of seed count per fruit versus pollination date were performed (PROC REG, SAS Institute, Cary, NC, USA). Separate linear regression analyses were conducted for *M. lewisii* and hybrids; small sample size of flowering *M. cardinalis* (N=2) prevented analysis of seed set data for this species.

Performance, physiology, and phenology in growth chambers.— Two sets of analyses were performed. I first analyzed data from parental species to verify interspecific differences and assess the effect of each temperature regime, and then analyzed hybrid populations to test for evolved differences after natural selection. Variables were analyzed as one-way designs to assess the effect of species (or hybrid

selection regime) within each temperature environment. One-way designs were chosen because different variables were analyzed in each temperature environment (e.g., freezing damage only in the high elevation temperature regime, reproductive variables only in the low elevation temperature regime), precluding two-way designs that included the effect of temperature. To examine differences in survival among hybrid populations, I used logistic regressions as described above; no mortality of *M. cardinalis* prevented analysis of interspecific differences in survival. For all other variables, univariate ANOVA tests were performed using PROC GLM (SAS, SAS Institute, Cary, NC, USA). Multivariate approaches were not chosen because the overall goal was to assess the effect of selection regime on each dependent variable singly, rather than to understand the effect of selection regime on the multivariate distribution of response variables (Huberty and Morris 1989). Care was taken to select independent or only weakly correlated variables and to control the type I error rate with sequential Bonferroni adjustments. To examine pairwise differences between hybrid populations, I used Tukey-Kramer adjusted comparisons of least square means.

RESULTS

Performance in reciprocal transplant gardens

At low elevation, survival of *M. cardinalis* was high (81%) and nearly every surviving plant flowered in the first growing season (Table 15). In contrast, survival of *M. lewisii* at low elevation was very low (17%), and fewer than half of all surviving plants flowered. At high elevation after three growing seasons, *M. cardinalis* survival was low (7%) whereas *M. lewisii* survival was much higher (41%). Only two *M. cardinalis* plants flowered at high elevation, whereas approximately two-thirds of

Table 15. Survival and flowering of parental species and interspecific hybrids at low elevation (Jamestown, 415 m) and high elevation (White Wolf, 2395 m) sites in the central Sierra Nevada Mountains, California. Data recorded at Jamestown after one growing season and at White Wolf after three growing seasons.

Garden	Genotype	N Planted	N Alive (flowering)	N Alive (vegetative)	% survival	% flowering
Jamestown	*M. cardinalis*	129	102	3	81	79
(1 year)	*M. lewisii*	132	6	8	17	5
	Hybrid	4755	2978	83	64	63
White Wolf	*M. cardinalis*	319	2	21	7	1
(3 years)	*M. lewisii*	319	88	42	41	28
	Hybrid	8001	814	1206	25	10

Table 16. Logistic regressions of the probability of survival and probability of flowering for *M. cardinalis*, *M. lewisii*, and interspecific hybrids grown at low (Jamestown, 415 m) and high (White Wolf, 2395 m) elevation. All differences remain significant after sequential Bonferroni adjustment.

Garden	Effect		Survival		Flowering	
	(Contrasts)	df	χ^2	P	χ^2	P
Jamestown	Genotype	2	107.7833	<0.0001	86.7512	<0.0001
	(*cardinalis* vs. hybrid)	1	15.0678	0.0001	13.8389	0.0002
	(*cardinalis* vs. *lewisii*)	1	99.2659	<0.0001	86.4138	<0.0001
	(*lewisii* vs. *hybrid*)	1	91.6439	<0.0001	72.2826	<0.0001
White Wolf	Genotype	2	84.7034	<0.0001	103.9157	<0.0001
	(*cardinalis* vs. hybrid)	1	45.4358	<0.0001	16.5353	<0.0001
	(*cardinalis* vs. *lewisii*)	1	79.4660	<0.0001	32.4326	<0.0001
	(*lewisii* vs. hybrid)	1	37.0755	<0.0001	86.2436	<0.0001

surviving *M. lewisii* flowered in the third growing season. Within each garden, survival and flowering of hybrids was intermediate to the parents. Logistic regressions of the probability of survival and flowering confirm that, within each garden, the species native to that elevation was more likely to survive and flower than either the non-native species or hybrids (Table 16). Relative fitness of parents and hybrids within each garden was calculated by dividing the proportion of plants surviving to flower by the proportion observed for the species native to that elevation (Figure 14). At low elevation, hybrid relative fitness was approximately 0.8, whereas at high elevation, hybrid relative fitness was approximately 0.4, indicating stronger selection against hybrids at high elevation than at low.

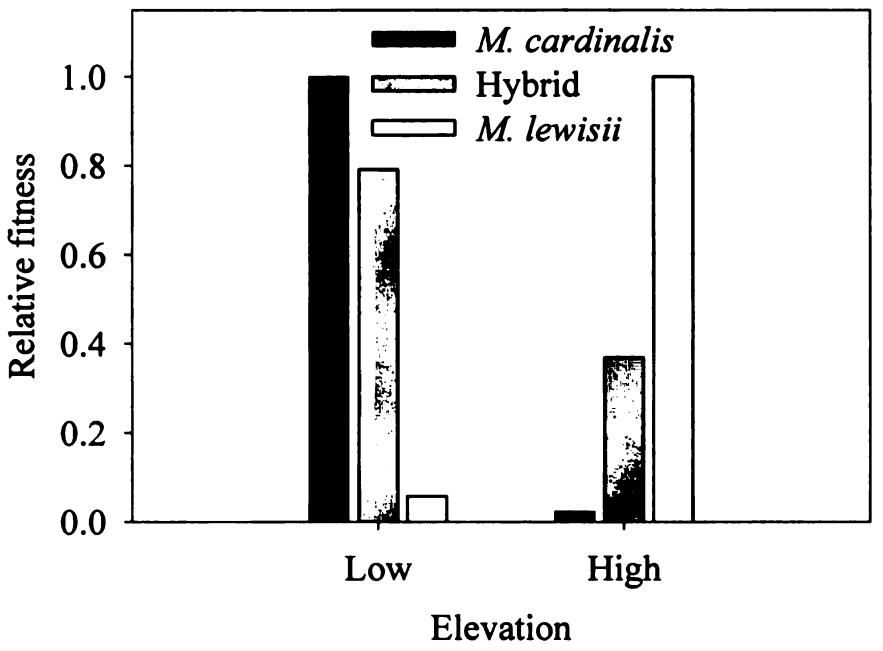

Figure 14. Relative fitness of parental species and hybrids transplanted to low (Jamestown, 415 m) and high (White Wolf, 2395 m) elevation.

Reproductive phenology

The day of first flower differed significantly among parents and hybrids at both low elevation (ANOVA, $F_{2,3083}$=6.54, P=0.0015) and high elevation (ANOVA, $F_{2,901}$=41.63, P<0.0001) in 2003. At low elevation, *M. cardinalis* flowered on average 4 days later than hybrids (t_{3083}=3.60, Tukey-Kramer adjusted P=0.0009). At high elevation, *M. cardinalis* and hybrids flowered significantly later than *M. lewisii*. On average, hybrids flowered approximately 13 days after *M. lewisii* (t_{901}=2.87, Tukey-Kramer adjusted P=0.0118), and the two *M. cardinalis* to flower did so approximately 35 days after *M. lewisii* (t_{901}=8.98, Tukey-Kramer adjusted P<0.0001). Although all plants at high elevation flowered approximately one week later in 2003 than in 2002, relative differences among hybrids and parents were similar in both years (data not shown). At high elevation, seed number per fruit declined linearly with pollination date

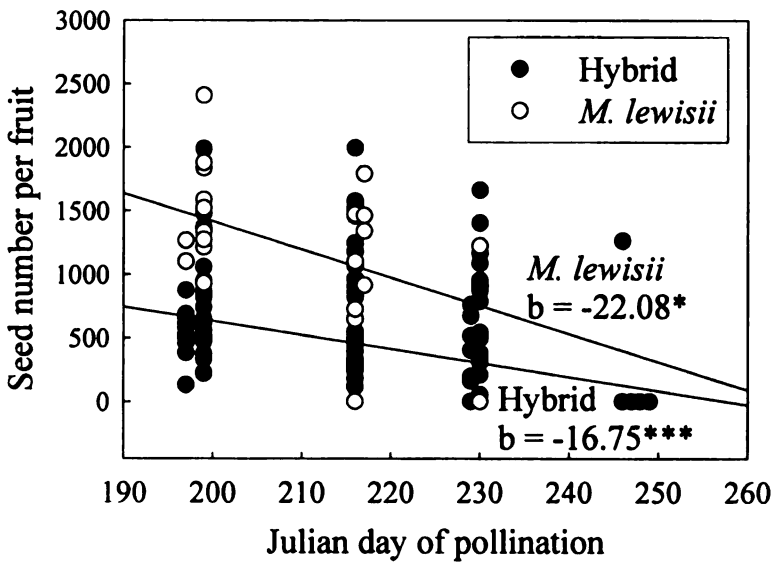

Figure 15. Seed number per fruit versus pollination date for *M. lewisii* and hybrids grown at high elevation (White Wolf, 2395 m). Regression coefficients (b) from linear regression analysis (* P < 0.05, *** P < 0.001).

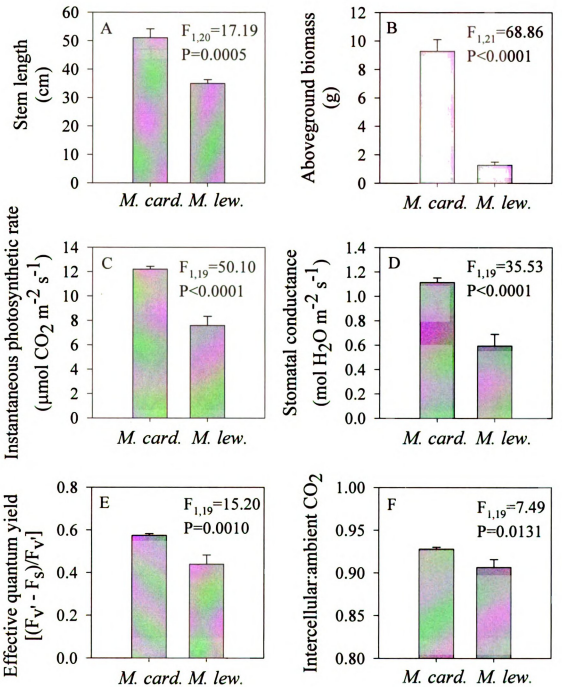

Figure 16. Mean (+ SE) trait values of *M. cardinalis* and *M. lewisii* in the low elevation temperature regime. a) stem length, b) aboveground biomass, C) instantaneous net photosynthetic rate, d) stomatal conductance, e) effective quantum yield, and f) intercellular: ambient CO_2. Results of one-way ANOVA testing the effect of species given for each trait. All values remain significant after sequential Bonferroni adjustment to maintain a type I error rate of 0.05.

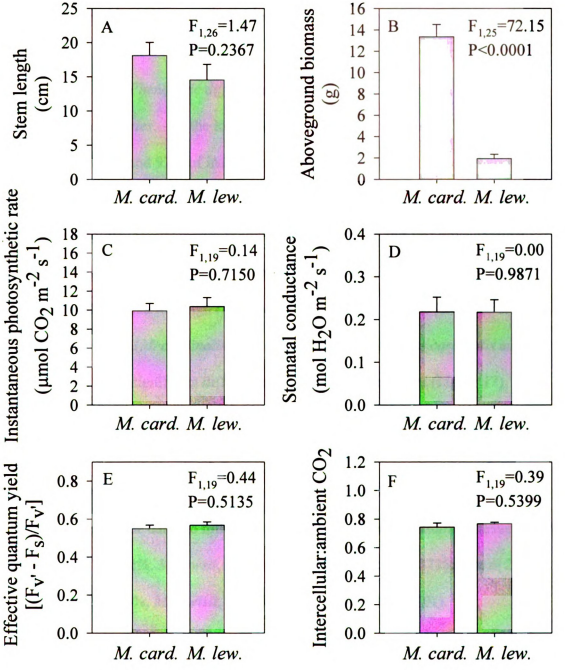

Figure 17. Mean (+ SE) trait values of *M. cardinalis* and *M. lewisii* in the high elevation temperature regime. A) Stem length, B) aboveground biomass, C) instantaneous net photosynthetic rate, D) stomatal conductance, E) effective quantum yield, and F) intercellular: ambient CO_2. Results of one-way ANOVA testing the effect of species given for each trait. The difference in biomass remains significant after sequential Bonferroni adjustment to maintain a type I error rate of 0.05.

for both *M. lewisii* (b = -22.08, N = 24, t=-2.30, P=0.0311) and hybrids (b = -16.75, N = 149, t = 3.41, P = 0.0008), indicating selection for early flowering at high elevation (Figure 15).

Phenotypic differences within growth chambers

Parental species.—In the low elevation temperature regime, *M. cardinalis* survival was 100%, and 92% of individuals reached the flowering stage, whereas *M. lewisii* survival was only 36% and no individuals flowered. Within the low elevation temperature regime, *M. cardinalis* exhibited greater growth and leaf physiological capacity than *M. lewisii* (Figure 16). Within the high elevation temperature regime, survival of both species was high (*M. cardinalis*, 100%; *M. lewisii*, 92%) and few individuals flowered (*M. cardinalis*, 0%; *M. lewisii*, 9%). *Mimulus cardinalis* and *M. lewisii* did not show significant differences in most measured traits (Figure 17), although *M. cardinalis* again attained greater biomass. Although not statistically significant, *M. lewisii* had slightly greater rates of photosynthesis, effective quantum yield, and intercellular CO_2 concentration than *M. cardinalis* (Figure 17) and numerically less tissue damage following freeze events than *M. cardinalis* (Freeze 1: *M. cardinalis*, 27.1 ± 8.2%, *M. lewsii*, 15.0 ± 6.8 %, $F_{1,25}$=0.82, P=0.3735; Freeze 2: *M. cardinalis* 19.2 ± 8.8%, *M. lewisii*, 9.2 ± 7.4%, $F_{1,23}$=1.03, P=0.3201).

Hybrid populations.— High correlations were detected for two pairs of leaf physiological traits: instantaneous photosynthetic rate and effective quantum yield, and stomatal conductance and the ratio of intercellular to ambient CO_2 (Table 17). For this reason, photosynthetic rate and the ratio of intercellular to ambient CO_2 were excluded from analyses examining the effects of selection regime on trait evolution. Effective

Table 17. Correlation matrices of traits measured on hybrids grown at low and high elevation temperature regimes in growth chambers. Correlations within the high elevation temperature regime given above the diagonal. Correlations within the low elevation temperature regime given below the diagonal. Flowering was not observed in the high elevation temperature regime and freeze damage was not observed in the low elevation temperature regime (indicated by "n/a" = not applicable). Asterisks indicate significant Pearson correlation coefficients (†P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). Sample size given in parentheses below each coefficient.

	Photosynthetic rate	Stomatal conductance	Intercell.: ambient CO_2	Quantum yield	Days to flower	% damage (freeze 1)	% damage (freeze 2)
Photosynthetic rate		0.68****	0.36***	0.87****	n/a	0.03	-0.09
		(89)	(89)	(89)		(87)	(88)
Stomatal conductance	0.54****		0.87****	0.44****	n/a	0.14	0.05
	(95)		(89)	(89)		(87)	(88)
Intercell.: ambient CO_2	0.11	0.72****		0.14	n/a	0.24*	0.14
	(95)	(95)		(89)		(87)	(88)
Quantum yield	0.74****	0.37***	0.08		n/a	-0.03	0.00
	(95)	(95)	(95)			(87)	(88)
Days to flower	0.34**	0.27*	0.14	0.35**		n/a	n/a
	(68)	(68)	(68)	(68)			
% damage (freeze 1)	n/a	n/a	n/a	n/a	n/a		0.06
							(91)
% damage (freeze 2)	n/a	n/a	n/a	n/a	n/a	n/a	

Table 18. Analysis of variance summary for physiological and phenological traits and fitness components of hybrids grown in a low elevation temperature regime. For each variable, the effect of selection regime (low elevation, high elevation, or greenhouse control) was tested with one-way ANOVA. Values in boldface remain significant after sequential Bonferroni correction (Rice 1989).

Category	Response	df num. (den.)	SS (SSE)	MS (MSE)	F	P
Trait	Conductance	2 (92)	0.6 (5.8)	0.3 (0.1)	4.44	0.0144
	Quantum yield	2 (92)	0.04 (0.2)	0.02 (0.002)	8.99	**0.0003**
	Days to flowering	2 (70)	418.9 (6854.1)	209.5 (97.9)	2.14	0.1254
Fitness component	Stem length	2 (98)	608.4 (23266.2)	304.2 (237.4)	1.28	0.2823
	Biomass	2 (98)	174.8 (1421.5)	87.4 (14.5)	6.03	**0.0034**
	Flower number	2 (98)	386.6 (97463.7)	193.3 (994.5)	0.19	0.8237

Table 19. Analysis of variance summary for traits and fitness components of hybrids grown in a high elevation temperature regime. For each trait, the effect of selection regime (low elevation, high elevation, or greenhouse control) was tested with one-way ANOVA. Stem length was log-transformed and percent tissue damage was arcsine square-root transformed prior to analysis. No values remain significant after sequential Bonferroni correction (Rice 1989).

Category	Response	df num. (den.)	SS (SSE)	MS (MSE)	F	P
Trait	Conductance	2 (83)	0.1 (1.1)	0.02 (0.01)	1.68	0.1922
	Quantum yield	2 (83)	0.01 (1.3)	0.002 (0.02)	0.19	0.8259
	% Damage (freeze 1)	2 (95)	1.6 (19.2)	0.8 (0.2)	3.93	0.0228
	% Damage (freeze 2)	2 (89)	0.16 (12.19)	0.1 (0.1)	0.57	0.5652
Fitness component	Stem length	2 (94)	0.8 (56.6)	0.4 (0.6)	0.69	0.5054
	Biomass	2 (93)	68.6 (3922.0)	34.3 (42.2)	0.81	0.4467

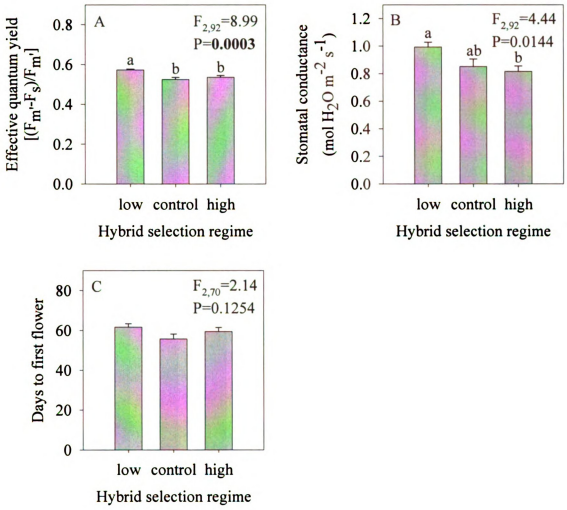

Figure 18. The effect of selection regime (low elevation, greenhouse control, or high elevation) on two leaf physiological traits and flowering phenology of hybrids grown in a temperature regime characteristic of low elevation. Values given are mean + SE a) effective quantum yield, b) stomatal conductance, and c) days to first flower. P-values in boldface remain significant after sequential Bonferroni correction. Hybrid means not sharing letters differ significantly based on Tukey-Kramer adjusted comparison of least square means.

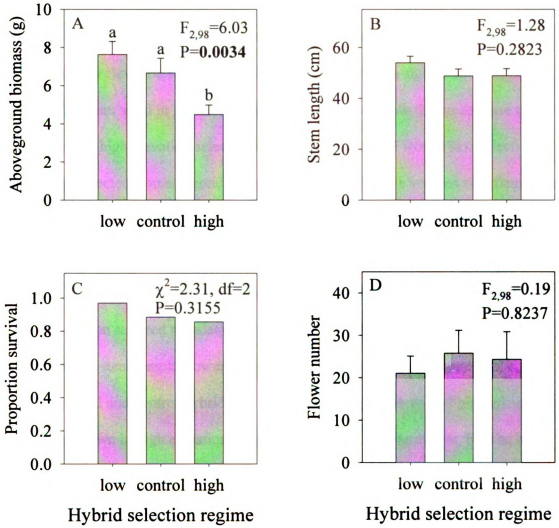

Figure 19. The effect of selection regime (low elevation, greenhouse control, or high elevation) on fitness components of hybrids grown in a temperature regime characteristic of low elevation. Values given are mean + SE a) aboveground biomass, b) stem length, c) proportion survival, and d) flower number. P-values in boldface remain significant after sequential Bonferroni correction. Hybrid means not sharing letters differ significantly based on Tukey-Kramer adjusted comparison of least square means.

quantum yield, a chlorophyll fluorescence parameter indicating the photochemical efficiency of light energy use, and stomatal conductance, a gas exchange parameter indicating stomatal openness to water vapor and carbon dioxide, were both retained.

The low elevation selected population of hybrids had a significantly higher effective quantum yield in low elevation temperatures than both the greenhouse control population and high elevation selected population (Table 18, Figure 18a). Selection regime also affected stomatal conductance, although difference was not significant after Bonferroni adjustment (Table 18, Figure 18b). Hybrid populations did not differ in the onset of flowering (Table 18, Figure 18c). The high elevation selected population attained significantly less aboveground biomass than either the greenhouse control or the low elevation selected population (Table 18, Figure 19a). Hybrids did not differ significantly in stem length, flower number, or survival (Table 18, Figure 19b, c, d).

Selected and control hybrids did not differ in the measured leaf physiological traits when grown in high elevation temperatures (Table 19, Figure 20a, b). The high elevation selected population showed less tissue damage after first exposure to freezing temperatures than the low elevation selected population, although this difference did not remain significant after Bonferroni adjustment (Table 19, Figure 20c, d). Within the high elevation temperature regime, hybrids did not differ in the fitness components of growth and survival (Table 19, Figure 21).

DISCUSSION

Selection on traits

In this study, I found evidence that selection favors early flowering at high elevation and increased leaf physiological capacity in hot temperatures at low elevation.

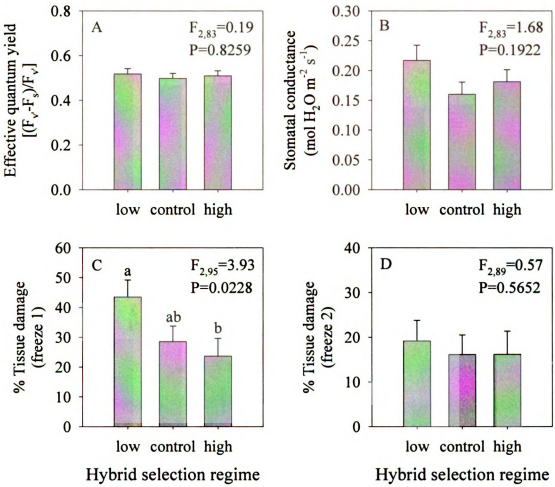

Figure 20. The effect of selection regime (low elevation, greenhouse control, or high elevation) on two leaf physiological traits and post-freeze tissue damage of hybrids grown in a temperature regime characteristic of high elevation. Values given are mean + SE a) effective quantum yield, b) stomatal conductance, c) % tissue damage following freeze 1, and d) % tissue damage following freeze 2. The effect of selection regime on tissue damage following freeze 1 does not remain significant after sequential Bonferroni correction.

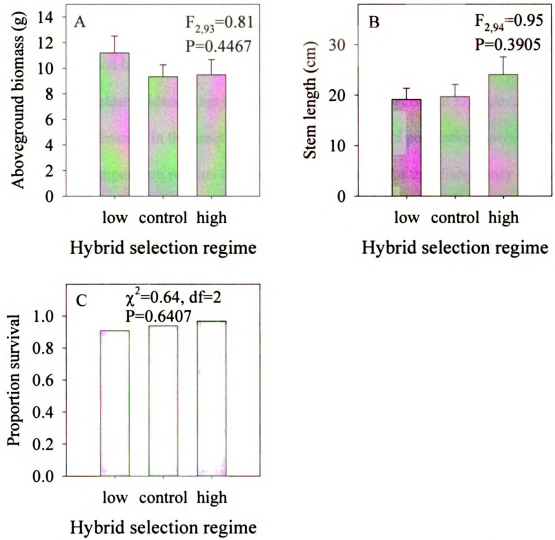

Figure 21. The effect of selection regime (low elevation, greenhouse control, or high elevation) on fitness components of hybrids grown in a temperature regime characteristic of high elevation. Values given are mean + SE a) aboveground biomass, b) stem length, and c) proportion survival.

When significant patterns of selection were observed, selection always acted in the direction of the native parental species' trait value, supporting the hypothesis that *M. cardinalis* photosynthetic traits and *M. lewisii* flowering phenology are adaptive at their respective low and high elevation range centers.

Despite clear evidence that selection favored early flowering at high elevation, I did not find differences in the onset of flowering among hybrid populations grown in a low elevation temperature regime. One possible explanation for this discrepancy is that flowering time has low heritability. However, this explanation seems unlikely because *M. lewisii* and *M. cardinalis* display genetic differentiation for flowering time which should be segregating in interspecific hybrid populations. A more likely explanation is that I could not observe flowering phenology in a high elevation temperature regime, where differences in the onset of flowering may have been more pronounced than in the low elevation temperature regime. Differences in flowering phenology between parental species are much greater at high elevation than at low. It remains possible that selected hybrid populations will display differences in the onset of flowering when grown in a high elevation environment. In this study, such differences could not be analyzed because most plants did not flower within the high elevation temperature regime.

Within the low elevation temperature regime, interspecific hybrid populations selected at low elevation displayed increased leaf physiological capacity relative to both an unselected control and to a population selected at high elevation. I did not detect selection on leaf physiological traits at high elevation, nor did the low elevation population experience a physiological cost when grown in a high elevation temperature regime. Photosynthetic acclimation to temperature is well documented (Billings et al.

1971; Berry and Björkman 1980), and to understand the evolution of photosynthetic traits requires characterization of photosynthetic physiology across a range of relevant environments, so that population or genotypic physiological breadth as well as mean values are quantified.

Other studies that have used segregating hybrid populations to measure natural selection on leaf morphology (e.g., Jordan 1991; Nagy 1997) and leaf physiology (e.g., Lexer et al. 2003; Ludwig et al. 2004) have also found selection operating in the direction of mean trait values in native populations or species. These studies have all used multivariate regression analysis to quantify within-generation phenotypic selection differentials and gradients on traits. In the present study, phenotypic selection differentials and gradients on physiological traits were not calculated. Instead, selection was evaluated as the difference in trait mean value between control and selected populations, an assessment of response to selection that could arise from direct phenotypic selection as well as underlying genetic correlations. Future studies combining within-generation multivariate selection analysis with measurement of between-generation selection responses will yield valuable information about the strength and direction of phenotypic selection, relationships among measured traits, and the trajectory of trait evolution.

Between-environment fitness trade-offs

A strength of the experimental evolution approach used here is the ability to examine not only patterns of trait evolution but also the fitness consequences of trait changes. If adaptation to one environment entails a cost to adaptation in another environment, then selected hybrid populations should display reduced fitness, relative to

an unselected control population, when grown in an environment in which they were not selected. One such tradeoff was observed in this study, where hybrids selected at high elevation displayed reduced biomass when grown in temperatures characteristic of low elevation. Other patterns of difference in fitness components between selected and control hybrid populations were suggestive of evolution of greater fitness within the selected environment at a cost to fitness within the unselected environment (e.g., survival of both selected populations was numerically higher than the control in the selected environment and lower than the control in the unselected environment; Figures 19c, 21c). However, no other fitness components exhibited significant between-environment tradeoffs, and in no case did measured fitness components significantly increase for populations grown in the environment in which they were selected, calling into question the effectiveness of selection and/or of the measurement conditions.

Low ability to detect differences in fitness among hybrid populations may be due to several factors. First, populations have only experienced one generation of evolution in each environment, perhaps leaving considerable segregating variation within each population. Second, selected and unselected environments were simulated in growth chambers. The measurement of fitness components within growth chambers is not ideal for several reasons, including small sample size, poor flowering within the high elevation temperature regime, and the inability to simulate overwinter conditions. The latter two limitations apply to the high elevation temperature regime in particular, in which expected differences between the parental species were not detected. Although the lack of significant interspecific differences for some traits may be due to low power (e.g., post-freeze tissue damage), other traits displayed very small differences that

cannot be attributed to lack of power alone, suggesting that measurement conditions were not sufficiently favorable for *M. lewisii* and high elevation selected hybrids. More definitive tests of the costs of adaptation to each environment will come from continued generations of experimental evolution and the reciprocal transplantation of selected populations to low and high elevation for a more thorough assessment of fitness.

A related approach that will yield further insight into the causes and consequences of adaptation to alternate environments will combine the identification of quantitative genetic loci underlying traits of interest with field studies of their ecological effects. Segregating hybrid populations transplanted to low and high elevation can be used to identify quantitative trait loci (QTL) for fitness in each environment. The effects of major QTL can then be assessed with near-isogenic lines (NIL), containing single QTL regions from one species introgressed by repeated backcrossing into the genetic background of another. In this manner the phenotypic effects and fitness consequences of changes in single genomic regions can be characterized in environments within and beyond the species' range, leading to greater understanding of evolutionary constraints on range expansion.

APPENDIX A

Population sampling

Table A1. Species present, location (county, nearest landmark), watercourse, latitude and longitude coordinates (°N, °W), elevation (m), and plant density (individuals per m^2) of populations chosen for detailed demographic study (D), transects of stage class abundance (T), reciprocal transplants (R), or incubator experiments (I). Abbreviations as follows: Species: *c* = *M. cardinalis*, *l* = *M. lewisii*; County: Ma = Mariposa, Tu = Tuolumne.

Species	County, landmark	Watercourse	°N, °W	Elev.	Type	Density
c	Ma, El Portal	Merced R.	37.642, 119.926	373	T	0.00
c	Ma, El Portal	Sweetwater Ck.	37.642, 119.925	378	T	0.26
c	Ma, El Portal	Moss Ck.	37.670, 119.819	510	T	0.30
c	Ma, Mariposa	Mariposa Ck.	37.487, 119.969	590	R, I	—
c	Ma, El Portal	Crane Ck.	37.677, 119.776	603	T	1.87
c	Tu, Jawbone Lava Flat	Clavey R.	37.899, 120.072	729	T	0.20
c	Tu, Buck Meadows	Moore Ck.	37.777, 120.064	830	D, R	0.21
c	Tu, Rainbow Pool	S. Fork Tuolumne R.	37.821, 120.011	833	D	0.72
c	Ma, Midpines County Park	Bear Ck.	37.526, 119.919	860	T, R, I*	0.10
c	Ma, Ponderosa Basin	E. Fork Chowchilla R.	37.464, 119.738	894	T	0.16
c	Ma, Triangle Rd.	Snow Ck.	37.517, 119.837	950	T, R	0.25
l	Ma, Yosemite Valley	Cascade Ck.	37.725, 119.712	1050	T*	0.10
c	Ma, Wawona	S. Fork Merced R.	37.539, 119.654	1208	D	2.04
l	Ma, Wawona	S. Fork Merced R.	37.539, 119.654	1208	D	0.12
c	Ma, Yosemite Valley	Tenaya Ck.	37.743, 119.562	1210	T, R, I	0.05
l	Ma, Yosemite Valley	Tenaya Ck.	37.743, 119.562	1210	T, R, I	0.30
c	Ma, Yosemite Valley	Sentinel Ck.	37.733, 119.603	1211	T	2.30
l	Ma, Yosemite Valley	Sentinel Ck.	37.733, 119.603	1211	T	0.01
c	Ma, Yosemite Valley	Illilouette Ck.	37.725, 119.558	1277	T	0.01
l	Ma, Yosemite Valley	Illilouette Ck.	37.725, 119.558	1277	T	0.59
c	Ma, Foresta	Crane Ck.	37.699, 119.756	1303	T	1.87
c	Tu, Carlon Day Use Area	S. Fork Tuolumne R.	37.815, 119.866	1320	D, R, I	0.50
l	Tu, Carlon Day Use Area	S. Fork Tuolumne R.	37.815, 119.866	1320	D, R, I	0.30
c	Tu, Middlefork Day Use Area	Middle Fork Tuolumne R.	37.857, 119.864	1335	T	0.90

Table A1 (cont'd).

l	Tu, Middlefork Day Use Area	Middle Fork Tuolumne R.	37.857, 119.864	1335	T	0.07
c	Tu, Longbarn	N. Fork Tuolumne R.	38.098, 120.106	1405	T	1.53
l	Ma, Wawona	Chilnualna Ck.	37.554, 119.631	1440	T	0.07
l	Tu	Clavey R.	38.082, 120.011	1590	T	0.12
l	Ma, Panorama Point	Illilouette Ck.	37.712, 119.561	1802	T	0.08
l	Ma, Tamarack Flat	Cascade Ck.	37.743, 119.707	1863	T	0.57
l	Ma, Tamarack Flat	Tamarack Ck.	37.757, 119.740	1920	T, R, I	2.06
l	Ma, Snow Creek Falls	Snow Ck.	37.774, 119.540	2052	T	0.48
l	Ma, Sentinel Dome	Sentinel Ck.	37.722, 119.592	2244	T	0.07
l	Ma, Yosemite Creek Picnic Area	Yosemite Ck.	37.855, 119.573	2288	T	0.18
l	Ma, Porcupine Flat	Porcupine Ck.	37.798, 119.547	2376	T, R	0.77
l	Ma, McGee Lake	Cathedral Ck.	37.893, 119.442	2411	T	0.01
l	Tu, Tenaya Lake	Murphy Ck.	37.841, 119.464	2535	T	0.29
l	Tu, Tioga Road	Unnamed seep	37.813, 119.504	2580	R	—
l	Tu, May Lake	Snow Ck.	37.837, 119.494	2690	D, R, I	0.18
l	Ma, Warren Fork	Lee Vining R.	37.952, 119.226	2750	D. R. I	0.49

*Population extinct during study period

APPENDIX B

Calculation of seed dormancy and recruitment parameters

Calculation of seed dormancy parameters, following the methods of Horvitz and Schemske (1995)

Mimulus lewisii, May Lake (range center, 2690 m)

a) Germination percentage from seed

= 2291 $seedlings_{2003}$/21,600 $seeds_{2002}$

= 10.6% seedlings/seed

b) Number of dormant seeds in 2003

= 63 $seedlings_{2004}$/0.106 seedlings/seed

= 594 seeds

c) Percentage of dormant seeds in 2003

= 594 dormant $seeds_{2003}$/21,600 $seeds_{2002}$

= 2.8%

d) Percentage survival of seeds in 2003

= percentage germinated + percentage dormant

= 10.6% + 2.8%

= 13.4%

Mimulus cardinalis, Buck Meadows (range center, 830 m)

a) Germination percentage from seed

= 38 seedlings in 2003/2,500 seeds in 2002

= 1.5% seedlings/seed

b) Dormant seeds in 2003

= 7 seedlings in 2004/0.015 seedlings /seed

= 460 seeds

c) Percent dormancy in 2003

= 460 dormant seeds/2500 seeds in 2002

= 18.4%

d) Percent survival of seeds from 2002 to 2003

= 1.5% + 18.4%

= 19.9%

Calculation of recruitment parameters

Estimates of seed germination from the seed stations could not be used for matrix calculations because the rate of seed germination in the stations far exceeded the rate of seedling recruitment. In other words, seedling germination observed in the seed stations was truncated before plants became fully established, causing the rate of germination to overestimate the rate of juvenile recruitment. Better estimates of transitions from seed to vegetative classes for each site and year were obtained by calculating the ratio of recruitment to seed production. Because seeds are known to live at least one year in the seed bank, seed production was estimated as the moving sum across a two-year window, yielding the following estimate of transitions from seed to a particular stage class:

a_{i1} = number of recruits in class i_{t+1}/(seed production$_t$ + seed production$_{t-1}$)

Because the dormant seed bank is of unknown size and age, this calculation may underestimate the true denominator. To assess the affects of uncertainty in estimates of recruitment on population growth, I increased the size of the seed bank by as much as 50% and found that lambda decreased by 1.4 – 6.7% for *M. cardinalis* and 0.7 – 5.7% for *M. lewisii*. Lambdas at all locations responded similarly to increases or decreases in

the size of the seed bank, and the magnitude of change in lambda due to variation in seed bank size was not sufficient to erase differences in lambdas between central and marginal populations.

APPENDIX C

Transition matrices, sensitivity matrices, and lambdas

Table C1. Transition matrices, sensitivity values, and λ for each species, location, and yearly transition interval. Stage class abbreviations as follows: "Sm. nr." = small non-reproductive (*M. cardinalis*: stem length $\leq$ 3 cm; *M. lewisii*: stem length $\leq$ 5 cm), "Lg. nr." = large non-reproductive (*M. cardinalis*: stem length > 3 cm; *M. lewisii*: stem length > 5 cm), and "Repro." = reproductive. Bias-corrected 95% confidence intervals for λ (Caswell 2001) given in parentheses below each value.

	Transition matrix					Sensitivity matrix				
	Seed	Sm. nr.	Lg. nr.	Repro.		Seed	Sm. nr.	Lg. nr.	Repro.	λ (95% CI)
***M. cardinalis*, Buck Meadows (830 m)**					**2000-2001**					
Seed	0.2128	0	2038	9624		0.28	0.00	0.00	0.00	0.6867
Sm. nr.	2.49e-5	0	0	0		0.00	0.00	0.00	0.00	(0.4939 - 1.0229)
Lg. nr.	3.74e-5	0	0.3529	0.5000		3644.30	0.12	0.49	0.08	
Repro.	8.31e-7	0	0.0588	0.2778		11017.98	0.37	1.48	0.23	
					2001-2002					
Seed	0.1840	376	720	1340		0.13	0.00	0.00	0.00	0.6486
Sm. nr.	2.14e-5	0.0345	0.0104	0		1150.63	0.05	0.30	0.23	(0.5050 - 0.8321)
Lg. nr.	2.14e-5	0.2759	0.3229	0.2632		1598.05	0.06	0.41	0.31	
Repro.	0	0.1034	0.1979	0.3684		2107.65	0.08	0.54	0.42	
					2002-2003					
Seed	0.1840	0	144	916		0.05	0.00	0.00	0.00	0.5527
Sm. nr.	3.93e-6	0	0	0		40.04	0.00	0.02	0.01	(0.4103 - 0.6839)
Lg. nr.	2.23e-5	0.0303	0.3662	0.2069		730.40	0.01	0.35	0.24	
Repro.	0	0	0.0704	0.4483		1842.39	0.01	0.89	0.60	
					pooled					

Table C1 (cont'd).

Seed	0.19	441	1262	3636		0.20	0.00	0.00	0.00	0.6819
Sm. nr.	2.25e-5	0.0139	0.0043	0		852.02	0.03	0.16	0.06	(0.5586 - 0.9197)
Lg. nr.	3.26e-5	0.1250	0.3447	0.3725		2348.15	0.08	0.44	0.17	
Repro.	3.46e-7	0.0417	0.1191	0.3431		4611.84	0.16	0.87	0.33	
***M. cardinalis*, Rainbow Pool (833 m)**					**2000-2001**					
Seed	0.1858	0	77	668		0.03	0.00	0.00	0.00	0.5720
Sm. nr.	5.76e-5	0.2606	0.0643	0.0076		112.57	0.05	0.14	0.05	(0.4870 - 0.6447)
Lg. nr.	1.57e-5	0.0904	0.4143	0.3106		387.57	0.17	0.47	0.17	
Repro.	1.16e-6	0	0.0500	0.4318		1025.48	0.45	1.24	0.45	
					2001-2002					
Seed	0.1859	8	280	1279		0.05	0.00	0.00	0.00	0.6329
Sm. nr.	2.39e-5	0.1152	0.0051	0		224.78	0.01	0.08	0.06	(0.5298 - 0.7264)
Lg. nr.	1.66e-5	0.1264	0.3299	0.3205		866.14	0.04	0.31	0.23	
Repro.	7.10e-7	0.0028	0.1066	0.4872		2325.09	0.12	0.84	0.63	
					2002-2003					
Seed	0.1840	0	28	1210		0.08	0.00	0.00	0.00	0.4724
Sm. nr.	1.82e-4	0.0143	0.0149	0		12.98	0.01	0.01	0.00	(0.3383 - 0.6118)
Lg. nr.	1.59e-4	0.0429	0.1940	0.2258		138.75	0.06	0.11	0.03	
Repro.	0	0	0.0100	0.4355		3626.63	1.54	2.95	0.80	
					pooled					
Seed	0.19	3	125	1003		0.06	0.00	0.00	0.00	0.5494
Sm. nr.	5.44e-5	0.1345	0.0242	0.0037		135.12	0.02	0.07	0.04	(0.4892 - 0.6293)
Lg. nr.	2.39e-5	0.0994	0.3011	0.2941		530.03	0.09	0.27	0.16	

Table C1 (cont'd).

Repro.	1.08e-6	0.0015	0.0558	0.4485		2163.43	0.35	1.11	0.64	
***M. cardinalis*, Wawona (1208 m)**					**2000-2001**					
Seed	0.2315	5612	38931	133773		0.26	0.00	0.00	0.00	1.2458
Sm. nr.	2.43e-5	0.0559	0.0125	0		5756.07	0.11	0.03	0.03	(1.0321 - 1.6302)
Lg. nr.	2.94e-6	0.0721	0.3625	0.0805		34402.93	0.69	0.18	0.18	
Repro.	2.90e-7	0.0342	0.2375	0.8161		85839.69	1.71	0.46	0.45	
					2001-2002					
Seed	0.2056	2778	26853	87287		0.22	0.00	0.00	0.00	1.0758
Sm. nr.	2.01e-5	0.0702	0.0511	0		4850.19	0.10	0.04	0.03	(0.9125 - 1.2492)
Lg. nr.	3.06e-6	0.1020	0.2482	0.1488		26185.17	0.56	0.20	0.18	
Repro.	1.82e-7	0.0234	0.2263	0.7355		68353.84	1.45	0.52	0.48	
					2002-2003					
Seed	0.1840	241	1739	14790		0.05	0.00	0.00	0.00	0.6997
Sm. nr.	1.10e-5	0.1437	0.0338	0		1597.38	0.03	0.05	0.05	(0.6249 - 0.7723)
Lg. nr.	1.68e-6	0.1106	0.3768	0.2055		5761.54	0.13	0.19	0.18	
Repro.	0	0.0101	0.0725	0.6164		23649.95	0.52	0.76	0.73	
					pooled					
Seed	0.20	1980	14162	65242		0.18	0.00	0.00	0.00	0.9516
Sm. nr.	2.05e-5	0.1018	0.0354	0		4134.11	0.09	0.04	0.04	(0.8004 - 1.2202)
Lg. nr.	2.90e-6	0.0954	0.3325	0.1554		19419.87	0.44	0.19	0.17	
Repro.	1.60e-7	0.0214	0.1533	0.7062		60660.41	1.36	0.60	0.53	

Table C1 (cont'd).

***M. cardinalis*, Carlon (1320 m)**					**2000-2001**					
Seed	0.2239	936	14496	41089		0.19	0.00	0.00	0.00	1.0657
Sm. nr.	3.77e-5	0.0339	0.0769	0		1542.89	0.06	0.02	0.02	(0.8718 - 1.2853)
Lg. nr.	3.70e-6	0.0435	0.4231	0.1316		18821.10	0.74	0.22	0.29	
Repro.	7.41e-7	0.0174	0.2692	0.7632		34177.90	1.34	0.40	0.53	
					2001-2002					
Seed	0.1840	1209	20127	67161		0.19	0.00	0.00	0.00	1.1629
Sm. nr.	3.14e-5	0.0615	0.0244	0		2911.97	0.10	0.03	0.03	(0.9368 - 1.3592)
Lg. nr.	3.46e-6	0.1154	0.3537	0.0909		19582.40	0.65	0.21	0.21	
Repro.	0	0.0154	0.2561	0.8545		46798.48	1.56	0.51	0.50	
					2002-2003					
Seed	0.2015	99	3396	24261		0.21	0.00	0.00	0.00	1.0345
Sm. nr.	3.92e-5	0.0062	0	0		227.58	0.01	0.01	0.01	(0.8351 - 1.4926)
Lg. nr.	3.94e-5	0.0372	0.2951	0.1343		3991.16	0.15	0.24	0.10	
Repro.	5.49e-7	0.0031	0.1066	0.7612		20896.41	0.80	1.25	0.54	
					pooled					
Seed	0.21	893	10987	48924		0.23	0.00	0.00	0.00	1.1365
Sm. nr.	3.98e-5	0.0349	0.0174	0		1093.43	0.04	0.03	0.02	(0.9153 - 1.5427)
Lg. nr.	1.68e-5	0.0574	0.3304	0.1188		9807.98	0.34	0.24	0.15	
Repro.	4.72e-7	0.0145	0.1783	0.7938		31924.84	1.10	0.78	0.49	
***M. lewisii*, Wawona (1208 m)**					**2000-2001**					
Seed	0.0280	0	0	23488		0.00	0.00	0.00	0.00	0.7778

Table C1 (cont'd).

Sm. nr.	1.39e-5	0	0	0	0.00	0.00	0.00	0.00	(0.5714 - 0.9474)
Lg. nr.	1.64e-6	0	0.5000	0.0556	0.00	0.00	0.00	0.00	
Repro.	0	0	0	0.7778	31326.54	0.52	0.37	1.00	
				2001-2002					
Seed	0.0280	0	9754	11379	0.02	0.00	0.00	0.00	0.6772
Sm. nr.	3.97e-6	0	0	0	0.00	0.00	0.00	0.00	(0.4286 - 0.8848)
Lg. nr.	7.95e-7	0	0.2857	0.1429	17874.01	0.11	0.31	0.75	
Repro.	0	0	0.4286	0.5000	15825.64	0.09	0.28	0.67	
				2002-2003					
Seed	0.0280	0	1782	9505	0.00	0.00	0.00	0.00	0.8953
Sm. nr.	6.34e-6	0.2000	0	0	0.00	0.00	0.00	0.00	(0.6315 - 1.0084)
Lg. nr.	1.59e-5	0	0.5500	0.2000	0.00	0.00	1.00	0.00	
Repro.	0	0	0.1500	0.8000	0.00	0.00	1.09	0.00	
				pooled					
Seed	0.03	0	423	19468	0.02	0.00	0.00	0.00	0.7892
Sm. nr.	8.80e-6	0.0303	0	0	0.00	0.00	0.00	0.00	(0.6464 - 0.9069)
Lg. nr.	1.35e-6	0	0.5500	0.1190	12146.64	0.15	0.29	0.44	
Repro.	0	0	0.1500	0.6905	18780.73	0.23	0.45	0.69	
***M. lewisii*, Carlon (1320 m)**				**2000-2001**					
Seed	0.0353	719	2810	13588	0.14	0.00	0.00	0.00	0.9056
Sm. nr.	4.32e-5	0.0909	0.0263	0	1777.84	0.10	0.17	0.07	(0.7854 - 1.1713)
Lg. nr.	1.05e-5	0.2727	0.5132	0.3248	3913.68	0.22	0.38	0.16	
Repro.	3.08e-7	0.0303	0.1184	0.5726	9355.43	0.51	0.92	0.38	

Table C1 (cont'd).

					2001-2002					
Seed	0.0280	0	1350	12680		0.03	0.00	0.00	0.00	0.6856
Sm. nr.	8.89e-6	0.0476	0	0		814.53	0.01	0.05	0.04	(0.6000 - 0.7597)
Lg. nr.	2.78e-6	0.1224	0.4528	0.2500		4243.66	0.06	0.29	0.19	
Repro.	0	0	0.0629	0.5909		15083.28	0.21	1.01	0.67	
					2002-2003					
Seed	0.0611	953	3540	21336		0.29	0.00	0.00	0.00	1.0242
Sm. nr.	4.63e-5	0.0769	0.0286	0		760.31	0.04	0.08	0.02	(0.7301 - 1.5829)
Lg. nr.	7.80e-5	0.0256	0.2000	0.1296		2933.13	0.15	0.29	0.08	
Repro.	8.90e-7	0.0256	0.0952	0.5741		14471.03	0.75	1.45	0.38	
					pooled					
Seed	0.04	238	2385	15124		0.17	0.00	0.00	0.00	0.8582
Sm. nr.	3.12e-5	0.0594	0.0168	0		753.57	0.03	0.08	0.03	(0.7044 - 1.0948)
Lg. nr.	2.92e-5	0.1279	0.4111	0.2587		3495.90	0.15	0.35	0.13	
Repro.	3.80e-7	0.0091	0.0913	0.5792		12508.15	0.54	1.26	0.45	
***M. lewisii*, May Lake (2690 m)**					**2000-2001**					
Seed	0.0326	0	24199	89430		0.06	0.00	0.00	0.00	1.0452
Sm. nr.	3.77e-6	0.0467	0.0625	0		1223.63	0.01	0.01	0.01	(0.9865 - 1.1135)
Lg. nr.	1.24e-6	0.0272	0.6094	0.0652		44857.24	0.19	0.21	0.45	
Repro.	4.77e-8	0	0.2500	0.9239		71653.11	0.30	0.34	0.72	
					2001-2002					
Seed	0.0280	749	17896	65717		0.08	0.00	0.00	0.00	1.0746

Table C1 (cont'd).

Sm. nr.	7.46e-6	0.0860	0	0		7444.59	0.07	0.05	0.10	(0.9655 - 1.1170)
Lg. nr.	2.91e-7	0.2366	0.6081	0.0286		28737.00	0.29	0.19	0.40	
Repro.	0	0.0108	0.2568	0.9429		46572.94	0.47	0.31	0.65	
					2002-2003					
Seed	0.0302	0	8543	34830		0.02	0.00	0.00	0.00	0.9335
Sm. nr.	4.88e-6	0.0566	0.0260	0		1690.77	0.01	0.02	0.04	(0.8824 - 0.9726)
Lg. nr.	5.90e-7	0.0755	0.5714	0.1017		19644.13	0.12	0.19	0.46	
Repro.	5.37e-8	0	0.2078	0.8475		33020.79	0.20	0.31	0.78	
					pooled					
Seed	0.03	124	16586	63041		0.05	0.00	0.00	0.00	0.9989
Sm. nr.	6.49e-6	0.0569	0.0279	0		2890.15	0.02	0.02	0.04	(0.9618 - 1.0427)
Lg. nr.	7.79e-7	0.0801	0.5953	0.0667		32769.37	0.23	0.19	0.47	
Repro.	3.46e-8	0.0018	0.2372	0.9016		52199.73	0.37	0.30	0.75	
***M. lewisii*, Warren Fork (2750 m)**					**2000-2001**					
Seed	0.0280	0	22612	99899		0.11	0.00	0.00	0.00	1.0238
Sm. nr.	5.55e-5	0.125	0.0909	0		0.00	0.00	0.00	0.00	(0.8922 - 1.1739)
Lg. nr.	3.51e-6	0	0.5152	0.1639		31787.47	2.01	0.30	0.25	
Repro.	0	0	0.1818	0.8033		74860.17	4.73	0.71	0.59	
					2001-2002					
Seed	0.0404	990	29380	112532		0.17	0.00	0.00	0.00	1.3319
Sm. nr.	2.36e-5	0.2358	0.0345	0		4890.49	0.11	0.06	0.04	(1.1712 - 1.5479)
Lg. nr.	3.88e-6	0.1992	0.5862	0.0755		23923.73	0.55	0.30	0.19	
Repro.	1.02e-7	0.0081	0.2414	0.9245		52172.25	1.19	0.64	0.42	

Table C1 (cont'd).

2002-2003

Seed	0.0280	0	8286	78268		0.08	0.00	0.00	0.00	0.9793
Sm. nr.	3.63e-5	0.1149	0.0230	0		1741.48	0.07	0.03	0.02	(0.8939 - 1.0597)
Lg. nr.	9.26e-7	0.0920	0.6609	0.1286		16370.48	0.70	0.32	0.17	
Repro.	0	0	0.0862	0.8143		52039.61	2.22	1.01	0.53	
					pooled					
Seed	0.03	528	15064	96077		0.14	0.00	0.00	0.00	1.1377
Sm. nr.	3.41e-5	0.1840	0.0340	0		3112.45	0.12	0.05	0.03	(1.0231 - 1.2597)
Lg. nr.	223e-6	0.1505	0.6241	0.1250		17649.46	0.66	0.31	0.15	
Repro.	2.69e-8	0.0046	0.1321	0.8424		51934.62	1.93	0.91	0.44	

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