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Local adaptation of two cryptic species, Lasthenia californica and Lasthenia gracilis, to distinct regions within a serpentine outcrop

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LOCAL ADAPTATION OF TWO CRYPTIC SPECIES, *LASTHENIA CALIFORNICA* AND *LASTHENIA GRACILIS*, TO DISTINCT REGIONS WITHIN A SERPENTINE OUTCROP

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ABSTRACT

LOCAL ADAPTATION OF TWO CRYPTIC SPECIES *LASTHENIA CALIFORNICA* AND *LASTHENIA GRACILIS* TO DISTINCT REGIONS WITHIN A SERPENTINE OUTCROP

by Teri Barry

Intraspecific variation providing tolerance to specific edaphic conditions may contribute to population differentiation, speciation, and species coexistence. This process is often examined using reciprocal transplant experiments of closely related species in contrasting edaphic conditions. The two cryptic species *Lasthenia californica* and *L. gracilis* occur on a serpentine outcrop in parapatry at Jasper Ridge Biological Preserve. I hypothesized that each species would demonstrate greater fitness in its home range. A reciprocal transplant experiment was conducted in the field to determine home site advantage. Seedlings from each species were planted in both home ranges and in the transition zone where both species occur. Soil was found to vary significantly by outcrop region, particularly with respect to the calcium-to-magnesium ratio. *Lasthenia californica* performed best in its home range, but *L. gracilis* demonstrated greater survival and fitness in the transition zone. These findings provided evidence of local adaptation of *L. californica* to the bottom of the slope where the soil calcium concentration is lower and magnesium concentration is higher, and local adaptation of *L. gracilis* to the transition zone and the drier top of the slope. Studies on local adaptation using reciprocal transplants are ideal tools for understanding plant evolution and provide valuable information for habitat restoration.

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INTRODUCTION

Plant evolution under extreme soil conditions---Extreme edaphic conditions such as serpentine, limestone, guano deposits, or mine tailings are ideal models for the study of plant evolution because these soil conditions can lead to adaptations that contribute to rapid speciation (Rajakaruna, 2004). In order for adaptation and ultimately speciation to occur, there must be intraspecific genetic variation (O'Dell and Rajakaruna, 2011), and contrasting soil types allow such variation within species to be maintained (Rajakaruna, 2004). Plants often follow a series of steps to become edaphically endemic species, as shown in Figure 1 (adapted from Kruckeberg, 1986). Plants first become tolerant to an edaphic condition and evolve in response to selection into an ecotype or race, which is unique in edaphic tolerance from the original species. The ecotypes or races become genetically distinct and reproductively isolated from the ancestral species (thus locally adapted), leading to speciation (Kruckeberg, 1986; O'Dell and Rajakaruna, 2011).

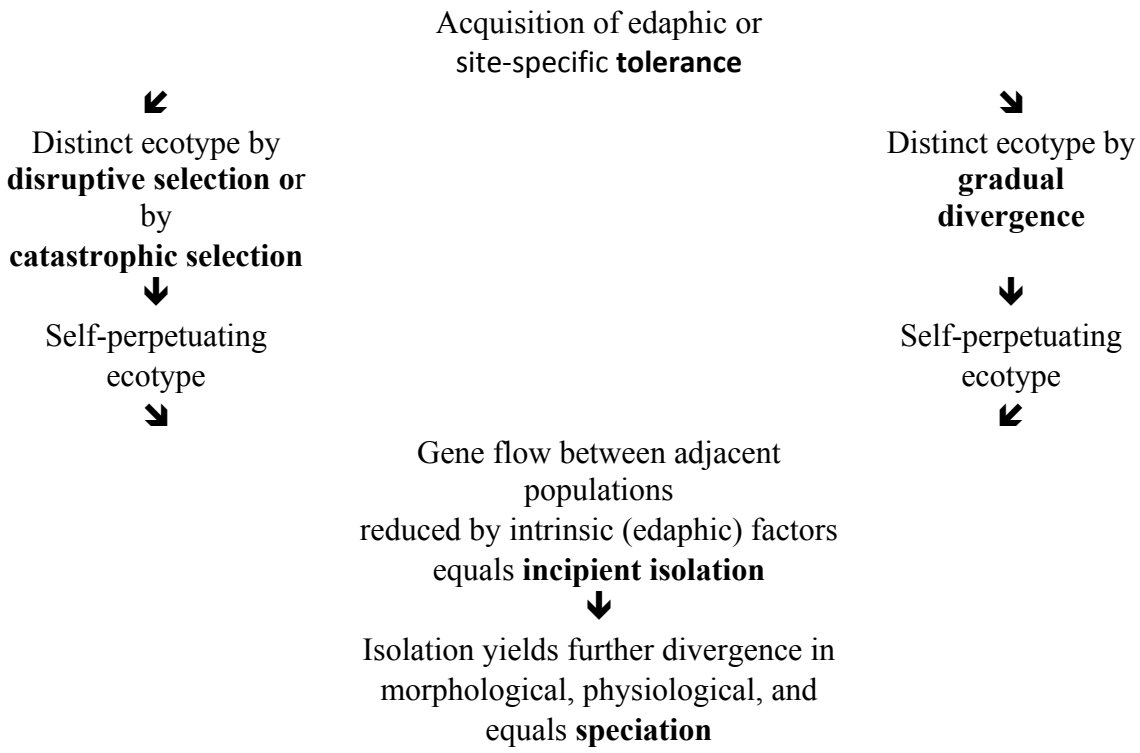

Figure 1. Possible speciation pathway redrawn from Kruckeberg (1986).

***Research on local adaptation*---**Studies on local adaptation in plants are of great value to conservation biologists and climate change researchers (Leimu and Fischer, 2008), and such studies are beneficial in examining how gene flow and other drivers of evolution impact natural selection (Kawecki and Ebert, 2004). Research on local adaptation provides valuable information for the planning of successful restoration projects. The source of plants used in restoration projects can be more carefully selected if we know how introduced plants will adapt to a new location. Plants most suitable for restoration are usually collected locally or from areas of similar habitats (McKay et al., 2005).

Studies on local adaptation in plants typically use reciprocal transplant experiments in the field and test fitness traits of two or more plant groups transplanted into their home site and away sites. Fitness can be estimated with floral, vegetative, and survival measurements. Ideally seed number or weight measures fitness, but in long-lived species fitness is often estimated from growth measurements (e.g., plant height) because larger plants probably produce more seeds (Wright and Stanton, 2011). Flowering time is also an important measure because differences in the maturation of reproductive structures can lead to changes in pollination, herbivory, and reproductive success (Levin, 2006). Reciprocal transplant studies have been widely used to determine local adaptation. A meta-analysis of 36 local adaptation studies revealed 71% of the plants studied overall showed greater fitness in their home site than in a foreign site but showed reciprocal adaptation (both plant types perform better in their home range and worse in away sites) only 45.3% of the time (Leimu and Fischer, 2008). Some experts believe adaptation does not always have to be reciprocal; hence, fitness reaction norms do not always have to cross to demonstrate local adaptation (Wright and Station, 2011).

Numerous studies have been published since Leimu and Fischer's 2008 meta-analysis and many other reciprocal transplant experiments were not included in their analysis. I will review some examples of local adaptation to serpentine soil, habitat types such as inland and coastal, and elevation differences. I will also address the importance to local adaptation of scale and how long-lived a species is. I will primarily discuss experiments utilizing annuals but will include a few studies on long-lived plant species to

show a broader picture of the importance of local adaptation for the ecology, evolution and conservation of plants.

Reciprocal transplants and serpentine soil---Serpentine soil provides an excellent model for the study of plant speciation that can answer questions on how adaptation may lead to speciation and how much geographic isolation is needed for population differentiation (Kay et al., 2011). Serpentine soil (weathered products of ultramafic rocks) provides a harsh environment for plants resulting in reduced fitness or the exclusion of many plants from the soil. Serpentine soil is generally low in nutrient levels such as nitrogen and calcium, but high in levels of magnesium, iron, and trace metals such as nickel and chromium (Safford et al., 2005). Low plant productivity and low soil moisture are also characteristic of serpentine soils (Brady et al., 2005). Plant ecologists take interest in serpentine soils because serpentine endemism is prominent worldwide (Brooks, 1987). Serpentine endemism is prevalent in North America, the Mediterranean region, Africa, Australia, New Zealand, Asia, New Caledonia, and Cuba (Rajakaruna et al., 2009). In North America, serpentine endemics are primarily found in California (Safford et al., 2005), but a few are also found in the Appalachian Mountains (Rajakaruna et al., 2009). Much of California's plant endemism is due to geoedaphics. Twelve and one half percent of all California endemic plant species have some association with serpentine soil (Safford et al., 2005).

Many reciprocal transplant experiments have been conducted on closely related plant populations from serpentine and non-serpentine soils. One example of local adaptation to serpentine soil concerns *Collinsia sparsiflora* Fisch. & C.A. Mey.

(Plantaginaceae; formally Scrophulariaceae) in the North Coast Range of California. In a two-year reciprocal transplant experiment on serpentine and non-serpentine ecotypes of *C. sparsiflora* there was a greater probability of flowering and fruiting in plants grown in their home site than a foreign site (Wright et al., 2006). Wright and Stanton (2007), however, found no significant difference in various estimates of fitness such as emergence date, cotyledon size, date of first flower, petal width, calyx length, corolla length, or petal color intensity between plants grown in serpentine and non-serpentine soils. The authors believed the traits measured in their latter study were not the traits driving local adaptation.

Alongside edaphic factors, other factors such as facilitation and competition can also play roles in driving local adaptation. When seeds are sown in non-native soil, fitness may be positively (facilitation) or negatively (competition) affected depending on planting density. Espelend and Rice (2007) examined intraspecific facilitation (positive interactions among species closely growing together) of *Plantago erecta* E. Morris (Plantaginaceae) in serpentine and non-serpentine soil. When seeds were planted at higher densities emergence was decreased, but there was no effect on mortality. Facilitation was, however, demonstrated with biomass when seeds from a non-serpentine source were planted in serpentine soil. Average above ground biomass of non-serpentine plants growing in serpentine soil increased as planting density increased, but no significant biomass increase was shown in serpentine plants growing in non-serpentine soil. Dense planting when competition occurs may also negatively impact plants. Sambatti and Rice (2006) found when competition of *Helianthus exilis* A. Gray

(Asteraceae) was prevented local adaptation occurred. Mortality was generally higher with competition. There was no significant difference in seed production of local and non-local plants with competition; however, with low competition serpentine plants produced more seeds than non-serpentine plants in serpentine sites. The authors found there was no differentiation between the serpentine and non-serpentine populations because of significant gene flow among the populations (demonstrated by microsatellite markers).

Reciprocal transplants in other contrasting habitats---Ecotypes or subspecies are often found in contrasting habitat types other than serpentine, such as inland sand hill and coastal dune locations. These different populations (e.g., races, varieties, and subspecies) of a species can be locally adapted to contrasting environments (e.g., Hall and Willis, 2006; Lowery et al. 2008; Nagy and Rice, 1997). While soil differences probably play a big role, other differences such as climate and moisture may also influence local adaptation. A few reciprocal transplant experiments conducted on inland and coastal populations of *Mimulus guttatus* DC. (Phrymaceae, formally Scrophulariaceae) provide evidence of local adaptation to habitat type. Hall and Willis (2006) provided evidence for divergent evolution with differences in flowering time between coastal and inland populations of *M. guttatus*. They also found that plants had greater fitness in their native sites but extremely poor fitness outside their native range. Morphological and genetic differences were found in a subsequent experiment on coastal and inland populations of *M. guttatus* (Lowry et al., 2008). Molecular markers distinguished the populations as genetically different, and the reciprocal transplant experiment conducted found

significantly greater survival to flowering and number of flowers when plants were transplanted into their home sites. In another study, inland populations of *Gilia capitata* Sims subsp. *capitata* (Polemoniaceae) and coastal populations of *G. capitata* Sims subsp. *chamissonis* (Greene) V E Grant were more likely to emerge and produce the greatest number of flowers when transplanted into their home site (Nagy and Rice, 1997). These reproductive traits demonstrated strong evidence of local adaptation, while leaf length did not. Vegetative traits are not as critical for measuring fitness, and thus studies of local adaptation often focus on estimating fitness via reproductive traits.

Local adaptation is demonstrated more often in contrasting habitats than in geographically similar habitats. For example, local adaptation is more likely to be observed between a coastal and inland population than between two different coastal populations (e.g., Herford and Winn, 2008). Six populations of *Diodia teres* Walt. (Rubiaceae) were examined in three types of habitat (coastal dunes, sand hill, and inland) and two sets of each population were transplanted into each of six areas (Herford and Winn, 2008). There was no significant difference in number of fruits produced by this self-compatible annual when populations from two similar habitat types (e.g., two different coastal dune habitats) were reciprocally transplanted, but when habitat type was different (e.g., a dune and a sand hill habitat) local adaptation was demonstrated by higher numbers of fruits produced at home sites.

Intraspecific variation or variation among closely related species is often observed along an elevational gradient. Plants may be locally adapted to a particular elevation or range of elevations. A classic paper by Clausen et al. (1941) found climatic races of

Potentilla glandulosa L. (now *Drymocallis glandulosa* (Lindl.) Rydb.) (Rosaceae), *Achillea millefolium* L. (Asteraceae), and *Artemisia vulgaris* L. (Asteraceae) varied in survival rate when transplanted into their home climatic (elevational) zone compared to two other climatic zones. The California Coast Range races and the Sierra Foothill races, for example, did not survive at timberline (10,000 ft). Local adaptation is often more evident in a plants' mid-range elevation than along the edges where more plant diversity often occurs. This preference for mid-range elevation was demonstrated by Angert and Schemske (2005) with a reciprocal transplant of *Mimulus cardinalis* Benth. (Phrymaceae, previously Scrophulariaceae), and *Mimulus lewisii* Pursh. Survivorship, growth, and fitness were greater in both species at the center of their natural range. Beyond their range plants' fitness was nearly zero. Another reciprocal transplant experiment demonstrated plants could be adapted to a specific range (often narrow) of elevations. Survivorship of the Australian subalpine grass *Poa hiemata* Vickery (Poaceae) was greater in plants transplanted in their altitude of origin (Byers et al., 2007). High altitude *P. hiemata* had smaller leaf length and larger circumference than lower altitude *P. hiemata*. These phenotypic expressions were noted in the reciprocal transplant even when seedlings from field collected seeds were transplanted out of their home range.

Scale of experiments---The scale of the experiment as well as soil, habitat, or elevation can impact whether local adaptation is detected. Adaptation of plants can be studied on a smaller local scale, or on a larger regional scale. *Aster amellus* L. (Asteraceae) seeds and seedlings from six diploid populations in two regions (moderate slopes of marl and rocky slopes of limestone) were reciprocally transplanted into each

region to determine local adaptation (Raabová et al., 2007). Local adaptation on a large scale was demonstrated; however, some but not all populations were locally adapted to their home site on a local scale. A possible explanation for this variability could be plots were not weeded prior to sowing seeds, thus naturally occurring areas of bare soil were variable among the plots. Another important point is ecologically similar habitats (climate and vegetation) may be better suited for transplant than a closer, but less similar habitat. Significant increases in fitness on a local scale (sub sites within larger regions in Europe) were not seen in the declining grassland perennial *Carlina vulgaris* L. (Asteraceae) (Ute et al., 2006). On a regional scale, fitness reduced as distance from the plants' origin increased. Plants grown in their home site had greater survival and fitness than plants grown in a foreign site. Similar results were found with *Hypochaeris radicata* L. (Asteraceae), a short-lived wide spread European perennial (Ute et al., 2008). The further away from its home site a plant was planted in general, the lower the survivorship and rosette size were. Region of transplant, however, did not impact fitness. On a small scale plants' fitness varied depending on the site.

Vernal pools offer a great model for examining the impact of spatial scale on local adaptation. Vernal pools are depressions over hardpan, and are common in the Central Valley grasslands of California. These pools fill during the wet season, dry up in spring, and remain dry until the next wet season. Plants are often locally adapted to a specific pool depth range. As the pool dries up in spring, rings of different plant species emerge throughout the season (Kruckeberg, 2006). Emery et al. (2009) examined *Lasthenia fremontii* (A. Gray), a species found in vernal pool bottoms, in a study of five common

vernal pool species. Seeds from species found in the bottom, the edges, and the transition between the two areas were planted in all three zones of three different pools. Lower fecundity was demonstrated at the pool edge than at the bottom or transition zones. In a later study, Emery et al. (2012) examined spatial scale and the components of niche (climate, habitat, and within habit) on members of the genus *Lasthenia* found in vernal pools and found the different axes of niche were often not correlated with each other. *Lasthenia* at the climate axis were more adaptable to change than at the within habitat (pool depth) axis, thus adaptability to one axis did not predict adaptability to another axis.

Experiment duration and long-lived species---The duration of an experiment and how long-lived a plant species is needs to be considered when determining local adaptation from reciprocal transplant experiments. Replication of more than one season is ideal; however, many reciprocal transplant experiments conducted on annuals demonstrate evidence of local adaptation in a matter of one or a few seasons (Nagy and Rice, 1997; Wright et al., 2006; Hereford and Winn, 2008). Long-lived plant species on the other hand may require very long duration experiments to demonstrate local adaptation. Fitness is hard to measure in long-lived species; therefore, vegetative measures such as biomass, plant height, diameter, and number of leaves must be used in the absence of reproductive structures. One greenhouse reciprocal transplant experiment on *Quercus ilex* subsp. *ballota* (Desf.) Samp. (Fagaceae) did not show better performance in seedlings planted in their native soil compared to non-native soil. Populations of *Q. ilex* subsp. *ballota* are bodenvag meaning they are found on and off serpentine soils. Non-serpentine seedlings grew taller and at a faster rate in both soils than serpentine

seedlings (Branco, 2009). Long-term experiments are needed to demonstrate local adaptation of *Q. ilex* subsp. *ballota*, but the results of this experiment of adaption at the seedling stage provide an important baseline for longer-term studies. Another experiment on oak seedlings found that local adaptation could be measured by insect damage (area of leaf damaged). Strong evidence of local adaptation caused by insect herbivores was demonstrated in three microhabitats of *Quercus rubra* L. seedlings in a one-year period (Sork et al., 1993). Total leaf area and leaf area damaged by insects were used to determine fitness. Less insect damage may allow a seedling a better chance at establishing, but it is unclear if lifelong fitness can be predicted at such an early stage in a plants' lifecycle. Long-term studies would give a better picture of local adaptation in long-lived plants such as trees. Wright (2007) found strong support for local adaptation of a population of *Pinus ponderosa* Douglas ex Lawson & C. Lawson (Pinaceae) to soil type. After 20 years local adaptation to serpentine or non-serpentine soil was supported by plant height, basal diameter, biomass, and allozyme data.

Shrubs, another long-lived plant group, may pose similar challenges in determining local adaptation as trees. Bieger et al. (2012) conducted a post fire reciprocal transplant on three species of shrub seedlings (*Adenostema fasciculatum* Hook. & Arn. Rosaceae, *Ceanothus cuneatus* Nutt. Rhamnaceae, and *Eriodictyon californicum* (Hook. & Arn.) Torr. Boraginaceae) found on serpentine and sandstone soils. All plants from both sources were planted on serpentine and sandstone, both in combination with northerly or southerly slopes, at Walker Ridge, California, USA. None of the three plants above exhibited greater survival at home than away at two years of age; although,

seedlings from all sources performed better on sandstone than serpentine soil. Slope effects (greater survival on north-facing slopes) were significant, but minimal compared to soil effects.

Lasthenia as a model for local adaptation---The genus *Lasthenia* (Asteraceae) is composed of 21 species and subspecies grouped into seven sections (Chan, 2001). *Lasthenia* are distributed mostly in the Californian Floristic Province and are found in a range of edaphically distinct habitats, including serpentine outcrops, salt flats, coastal bluffs, vernal pools, deserts, grasslands, open woodlands, and guano deposits (Rajakaruna, 2003). The two closely related species, *Lasthenia californica* DC. ex Lindl. and *Lasthenia gracilis* (DC.) Greene differ by an 11 base pair deletion in the internal transcribed spacer (ITS) region between 18S and 5.8S ribosomal genes (Chan et al., 2002). They can therefore serve as a model for research on local adaptation and edaphic differentiation (Rajakaruna, 2003; Bohm and Rajakaruna, 2006). *Lasthenia gracilis* is morphologically distinguishable by an ovate-lanceolate pappus, while *L. californica* has a more linear pappus. However, populations of both species sometimes do not possess a pappus, i.e. epappose (Chan et al., 2002). The populations at my study site in Jasper Ridge are not epappose; therefore, species can be identified by pappus morphology.

Different races of *L. californica* were found (Rajakaruna and Bohm, 1999) based on two main flavonoid profile types and allozyme banding differences (Bohm et al., 1989; Desrochers, 1992; Desrochers and Bohm, 1995). Race A is found primarily in ionically harsh, clay soils while race C is found in drier, less ionically harsh soils (Rajakaruna and Bohm, 1999). *Lasthenia californica* and *L. gracilis* consist of both race

A or C variants (Rajakaruna, 2003). In both *L. californica* and *L. gracilis* race A plants are more tolerant to Mg^{2+} and Na^{+} and accumulate more of the ions than race C plants. Parallel evolution of the edaphic races was suggested because ion accumulation and metal tolerance were similar in the two races found in both species (Rajakaruna et al., 2003a) and the races appear to be partially reproductively isolated both within and between species (Rajakaruna and Whitton, 2004). The populations at Jasper Ridge described below are race A/*L. californica* and race C/*L. gracilis*. From this point, I will refer to my study populations at Jasper Ridge as *L. californica* and *L. gracilis*, not as race A and C, to avoid confusion.

A strong boundary between *L. californica* and *L. gracilis* has been documented for over three decades on the serpentine outcrop at Jasper Ridge Biological Preserve in San Mateo County, California at about 37˚25'N and 122˚2.5' W (Bohm et al., 1989; Bohm and Rajakaruna, 2006). *Lasthenia gracilis* is mostly found at the upper reaches of the outcrop, while *L. californica* predominates at the bottom swale (Rajakaruna et al., 2003c). The soil at the swale where *L. californica* is found is higher in pH, clay, Mg^{2+}, Na^{+}, and organic acids than the soil where *L. gracilis* plants are located (Rajakaruna and Bohm, 1999). The upper reaches of the outcrop, where *L. gracilis* predominates, are chemically benign compared to the bottom swale; however, they appear to be water deficient due to the sandy texture of the soil (Rajakaruna and Bohm, 1999). *Lasthenia californica* and *L. gracilis*, both winter annuals and obligatory outcrossers (Rajakaruna and Bohm, 1999), are associated mostly with Poaceae (natives: *Elymus glaucus* Buckley, *Hordeum brachyantherum* Nevski, *Danthonia californica* Bol., *Stipa pulchra* Hitchc.,

and *Festuca microstachys* Nutt. non-natives: *Bromus hordeaceus* L.*, Festuca perennis* (L.) Columbus & J.P. Sm.*,* and *Polypogon monspeliensis* (L.) Desf.) at the study site. Annuals other than *Lasthenia* found at the site include *Plantago erecta* (Plantaginaceae)*, Centaurium* (Gentianaceae)*,* owl's clover and cream sacs in the genus *Castilleja* (Orobanchaceae), *Layia* (Asteraceae)*, Sisyrinchium bellum* (Iridaceae)*,* and *Eschscholzia californica* (Papaveraceae) (Barry, 2010 personal observation). The *L. californica – L. gracilis* complex at Jasper Ridge is pollinated by small insects such as Coleoptera (Melyridae), Hymenoptera, Lepidoptera (including Arctiidae), and Diptera (Barry, 2010 personal observation). Both species are reproductively isolated from each other by a seven to ten day lead in flowering time of *L. gracilis* (Rajakaruna, 2003). Rajakaruna and Whitton (2004) further demonstrated reproductive isolation in a preliminary test on seed set and pollen tube growth of seven populations including both species from Jasper Ridge (*L. californica* and *L. gracilis*). Lowest pollen tube growth and viable seed counts were found in inter-species crosses. This reduced viability of inter-species crosses supports the idea that the species are reproductively isolated under natural conditions. Other preliminary work conducted in spring 2009 on pollen-stigma compatibility also suggests reproductive isolation between the species at the Jasper Ridge may be enforced by pollen-stigma incompatibility (Kay, unpublished). The outcrop provides an ideal setting for research on local adaptation of the *L. californica - gracilis* complex to edaphically distinct regions within a serpentine outcrop (Bohm and Rajakaruna, 2006). The *L. californica - gracilis* complex is an ideal model for local adaptation research because both species are annuals so the entire life cycle can be examined in one year. A

reciprocal transplant experiment can help answer questions as to why these two closely related species could live in such close proximity, yet remain reproductively isolated. The small size of *L. californica* and *L. gracilis* also makes these plants ideal for a reciprocal transplant experiment with minimal environmental impact.

Three studies on the *Lasthenia* population at Jasper Ridge have tested the effects of soil moisture and chemistry on the fitness of the two species (then *L. californica* race A and C) in the greenhouse. First, a common garden study was conducted in a greenhouse to examine the influence of edaphic factors on fitness estimated by growth and measured by flower head production (Rajakaruna and Bohm, 1999). The results of greater flower head production of plants in their native soil demonstrated local adaptation. Rajakaruna et al. (2003b) examined water stress differences in the two species. They found when the two species were exposed to three different watering treatments in the greenhouse (high, medium and low) *L. gracilis* produced significantly more flower heads than *L. californica*, suggesting a greater number of viable seeds in *L. gracilis*. Finally, a correlation between soil chemistry and accumulation of elements of plants was observed in field collected plants and soil (Rajakaruna and Bohm, 1999). Discriminant Functional Analysis found calcium, sodium, and calcium-to-magnesium ratio to be highly correlated with species distribution at Jasper Ridge. Magnesium was only significantly correlated with *L. californica*, and potassium was only correlated with *L. gracilis*. In a later study at Jasper Ridge and other California populations NaCl and $MgSO_4$ tolerance as measured by percent germination, survivorship, and root length was greater in race A plants from both *L. californica* and *L. gracilis*. This study was

conducted under hydroponic conditions using ion concentrations similar to natural soil conditions where the plants grow (Rajakaruna et al., 2003a). These studies on the Jasper Ridge *Lasthenia* population provide groundwork for a field reciprocal transplant experiment because several distinct variables have shown in isolation to be involved in local adaptation of each species to its own area of the serpentine outcrop. A reciprocal transplant experiment in the field would better demonstrate how all the habitat variables were working in concert. For example, how soil moisture and soil chemistry change together and correlate with plant fitness can be determined only by a study done in the field. There are also many variables in the field such as fluctuating ambient temperature, wind, soil microbe activity, and herbivory which may affect the experimental results. While all variables were not measured in this study, it is important to verify if a plant is locally adapted to a location with all natural conditions present.

To my knowledge no reciprocal transplant studies have been conducted on the *L. californica - gracilis* complex, although the reciprocal transplant study conducted by Emery et al. (2009) previously discussed included *Lasthenia fremontii*. Her experiment however, compared five species not as closely related as *L. californica* and *L. gracilis*. The purpose of my study was to show *L. californica* and *L. gracilis* at Jasper Ridge are locally adapted to different soil conditions within the serpentine outcrop. I tested the hypothesis that both species will show higher fitness and survivorship in their home region within the outcrop by conducting a reciprocal transplant experiment and an accompanying soil analysis to determine how seasonal changes in soil chemistry contributes to natural selection over the growing season. I addressed the question of

whether the boundary previously described still exists with DNA analysis on plants at the bud stage and morphological differentiation based on pappus type (Chan, 2001) of mature flowers. Rainfall and ambient temperature play a major role in any seasonal distribution; therefore, I collected data on soil moisture in order to examine how it may impact plant fitness. Local adaptation at such a small scale would demonstrate the need for very specialized conservation efforts. If, for example, reintroduction of *Lasthenia* to the upper reaches of the serpentine outcrop (where the population has declined in recent years) was desired transplantation of morphologically similar *L. californica* located just meters away at the lower region might prove unsuccessful.

MATERIALS AND METHODS

Seasonal distribution study---In fall 2009 I established four transects on the serpentine outcrop at Jasper Ridge Biological Preserve parallel to trail 9, starting at the fire road and ending at the swale down-slope at the edge of a non-serpentine oak-grassland (similar to that of Rajakaruna and Bohm, 1999). Transects were selected based on where *Lasthenia* naturally occurred the previous season. I marked 1 X 1 meter quadrats at three intervals northwest of each transect. The intervals were at 5 m from the fire road where *L. gracilis* exclusively occurs, at 58 to 64 m where only *L. californica* is found, and at the transition zone (48 m) where both species occur based on preliminary sampling along the transects in spring 2009 and previous studies (Rajakaruna and Bohm, 1999).

I sampled four plants at each quadrat starting at the center and spiraling clockwise to form a 5 m radius (which included outside the quadrat) in late February and mid-

March during the bud stage for genotype analysis. I expanded the sampling areas because the *Lasthenia* density was low within many of the quadrats, and the primary purpose of the quadrats was for the reciprocal transplant portion of this study (discussed in *Local adaptation study* section below). Because the species are not distinguishable prior to flowering, I genotyped them for presence/absence of the 11 bp deletion in the ITS rDNA locus, identified by Chan et al. (2002). Approximately 50 mg (four leaves) were collected from each plant and stored at minus 80˚C for later DNA extraction. DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Valencia, California, USA) with modification. I disrupted the tissue using a BioSpec Products, Inc., Mini Bead Beater (model number 607), and placed about six 2.3 mm Chrome Steel Beads (BioSpec Products, Inc.) inside o-ring tubes prefilled with plant tissue. Next 400 µl of AP1 buffer and 40 µl of dilute RNase (36 µl nanopure water plus 4 µl RNase) were added to each sample. I disrupted the tissue in a bead beater for five 30 sec intervals, and incubated the samples for 30 min at 65˚C. Vials were inverted two times during the process. After incubation, I followed steps nine through 19 using the Qiagen handbook protocol. I stored the extracts in a -20˚C freezer until ready for amplification.

I amplified DNA extracts using a BiONEER AccuPower™ PCR PreMix kit (Alameda, California, USA) and using an Applied Bio Systems GeneAmp® PCR System 9700 thermal cycler (Foster City, California, USA). For each reaction, I added 1 µl of DNA template, 1 µl each of 10pmol/µl forward *L. californica* primer (aga acg acc cgt ctt gt) and reverse *L. californica* primer (ggt tgc cca aag gga agt), and 17 µl of nanopure water to the prepared 0.2 ml PCR tube provided by BiONEER. I repeated the above

process using *L. gracilis* 10 pmol/µl forward (ata gca gaa cga ccc gtg aa) and reverse primer (ctc atg gtt gcc cam gaa c). All primers (see Yost et al., 2012) used were designed by Dr. Kathleen Kay at UC Santa Cruz and provided by BiONEER. Following a one-minute hold at 95˚C, DNA was denatured at 95˚C for one minute, annealed at 55˚C for 30 seconds, and extended at 72˚C for 30 cycles. A final two holds at 72˚C for seven minutes was programed following the last cycle. I stored the PCR products in the refrigerator until ready for analysis.

I prepared a 0.8% TAE gel with 5 µl of GelRed™ Nucleic Acid Gel Stain 10,000X in water (Biotium; Hayward, California, USA). I ran the gel using a Fisher Scientific (Athens, Georgia, USA) electrophoresis machine (model number FB300). I loaded 4 µl of each PCR product into a well, and Fisher Bio Reagents exACT Gene 50 base pair mini DNA ladder (25bp – 650 bp) to the middle and each end well. The machine was run at 90 volts for one hour. Since the primers spanned the boundary of the indel, species identity was determined upon amplification of reverse and forward primers from one species but not the other (*L. californica* or *L. gracilis*).

Once full flowers developed species could be determined by pappus morphology so I did not do DNA analysis to identify later season plants. I removed a few disc flowers using forceps from 10 flowers from the same quadrat areas used for the DNA analysis collections, and collected samples at two-week intervals for the rest of the season yielding a total of three sets of collections. To determine species, I examined disc flowers under a dissecting microscope to determine pappus type.

Soil analysis---I collected soil samples every two weeks during the flowering season for chemical analysis. I collected the minimum amount of soil (100g), as recommended by A & L Western Laboratories, Inc. (Modesto, California, USA) for SN2 analysis (www.al-labs-west.com), just outside each quadrat sampled from the surface down to 10cm. I air dried the soil samples in paper bags, then crushed and filtered it through a 2mm sieve. Soil chemistry analyses by A & L Laboratories, Inc. were conducted following the procedures from the Soil and Plant Analytical Methods of the North American Proficiency Testing Program (http:www.naptprogram.org). Extractable K, Mg, Ca, Na, and sulfate sulfur were analyzed using the 1.0 N ammonium acetate at pH 7.0 method. Cation exchange capacity for K, Mg, Ca, and Na were determined using the ammonium replacement procedure. Soil pH was measured using the saturated paste method. Nitrate was determined by the 2.0 n KCl/Cadmium reduction procedure. Extractible phosphorus was tested with the sodium bicarbonate method for slightly acidic to alkaline pH soil, and the dilute acid-fluoride method for neutral pH soil. Organic matter was rated by loss on ignition at 360˚C. Soluble salts were determined by the saturated paste extract method. Volumetric water content was also measured just outside the quadrats once a week using a Spectrum Technologies TDR200 soil moisture meter with 6.35cm probes. The averages of three readings from different sides of each quadrat were recorded.

Local adaptation study---I arbitrarily collected seeds in April 2009 from 24 individual *L. californica* at the bottom of the outcrop about 50 to 60 meters below the fire road, and from 24 individual *L. gracilis* at the top of the outcrop about five to 20 meters

down slope of the fire road. Seeds were not collected randomly along a transect due to the heterogeneous distribution of the plants. I stored the seeds in coin envelopes at room temperature until they were ready for germination in winter 2009. Prior to germination seeds and filter paper were surface sterilized with 1% bleach then rinsed three times in deionized water then placed in petri dishes (up to 25 seeds per dish). Next, I placed the petri dishes in the refrigerator (for cold striation) for four days then placed them in a Conviron E7 Plant Growth Chamber (Winnipeg, Canada) to germinate. Simulated day conditions were 18°C and 12 hours of light from two fluorescent 115 watt bulbs (Sylvania Cool White, Canada), and for night 12°C with 12 hours of darkness. When the first true leaves appeared I transferred the seedlings to one-inch germination trays filled with Sunshine mix #3 (Canadian sphagnum moss, vermiculite, dolomitic limestone, gypsum, and wetting agent), a germination mix by Sun Gro Horticulture Canada Ltd. (Seba Beach, Alberta, Canada). Seedlings were watered as needed (about every other day) to keep the germination mix moist. Seedlings remained in the growth chamber until they were about the size of the ones in the field (20 to 50 mm tall). One week before the seedlings were transplanted in the field Conviron temperatures were reduced to 16˚C during the day and 6˚C at night so the plants could acclimate to field temperatures. Of the 24 individual flower heads collected from each species, 18 *L. gracilis* and 14 *L. californica* produced useable (close to height of field plants) seedlings for the transplant. In mid-February 2010 I transplanted the seedlings into the same quadrats in the serpentine outcrop used for the seasonal distribution study so established species zones could be further tested using a reciprocal transplant experiment. It is ideal to plant seeds

directly into the field because local adaptation may occur at the germination stage (Hereford and Winn, 2008) but *Lasthenia* seeds are very small and can have a less than 50% germination rate (preliminary germination trials, in fall 2009). I randomly selected eight *L. californica* and eight *L. gracilis* plants and placed in an alternating pattern within each quadrat (Figure 3.). Plants within each quadrat were from different individuals. An area large enough to accommodate each plant was cleared, but to minimize the environmental impact of this experiment I did not weed the remaining areas of the quadrats. Minimal weeding also allowed for natural competition. To determine if the plants are locally adapted to a specific region of the serpentine outcrop natural conditions should be altered as little as possible. I watered the seedlings once a day for the first three days, and replaced any plants that died within the first five days because transplant shock was assumed.

Once plants were established for five days I measured height from soil level to the tip of the plant as it stood in the field (plants were not manipulated during measurement) every two weeks. Once the first few transplants reached the flowering stage I recorded number of buds, flower heads, green leaves (more than 50% green), and brown leaves (less than 50% green) on the same day as height measurements. Measurements were increased to once a week because plants develop quickly during the flowering season. I collected flower heads upon seed set, and calculated percent viable seeds for each plant (all flowers from each plant were pooled). Dark fuller seeds were considered viable, while lighter flatter seeds were considered unviable (Rajakaruna, personal communication). I dug up senesced plants, rinsed in deionized water, air dried, and

placed them in white paper bags which I later further dried in an Industrial and Laboratory oven (National Appliance Company) at 70˚C for one week. Finally, I weighted the above ground biomass on a mettle Toledo AB54-S analytical balance with MonoBloc inside weighing technology (maximum 51g, minimum 10mg).

Data analysis---I performed principal components analyses on all the soil variables for each collection date separately to examine which variables accounted for most of the variation among the three regions of the outcrop throughout the growing season. I performed a series of repeated measures ANOVAs with deviation contrasts on soil variables examined in past studies (Rajakaruna et al., 2003 a & b), and with the highest PC1 loading scores (at least > 0.5) to tease out any significant variations in soil variables throughout the season. I tested data used for all ANOVAs for the assumption of constant variance by inspecting the residual plot of standardized residuals against predicted values if the data points fell within three standard deviations the assumption of constant variance was met.

To evaluate how both plant fitness and growth varied between each species native vs. transplanted habitat I performed multivariate analysis of variance (MANOVA) and follow up analysis of variance (ANOVA) for each species on floral (fitness) and vegetative (fitness estimates) data with zone, and transect used as fixed factors (found under general linear model- multivariate). The Tukey HSD test was used to determine if there were any significant differences among transects. I reran the MANOVAs with only zone as a fixed factor once the main effect of transect was found insignificant. Square root transformations were performed on variables violating the assumption of constant

variance. I analyzed survival for each species at each zone using the Kaplan-Meier test with compare pair-wise strata. Four plants were lost to animal disturbance and were treated as missing data in all analyses. In order to rule out the effect of herbivory on the results, follow up MANOVAs were performed with plants exhibiting any reduction in height (six plants with a reduction in height > 2mm from establishment date, and collected above ground biomass 0.0000g) as missing variables (censored).

Soil, survival, and floral data from my reciprocal transplant experiment were also analyzed using hierarchical modeling aster analysis in R (Shaw et al., 2008) and published in the American Journal of Botany (Yost et al., 2012). Analyses were performed using IBM SPSS 20.

RESULTS

Seasonal distribution---Species distribution in the top zone near my four plots was 100% *L. gracilis*, and in the bottom zone the distribution was 100% *L. californica*. There was some variation in species distribution in the middle (transition) zone, as *L. californica* distribution ranged from 30 to 55.6% and *L. gracilis* ranged from 44.4 to 70% (Figure 2).

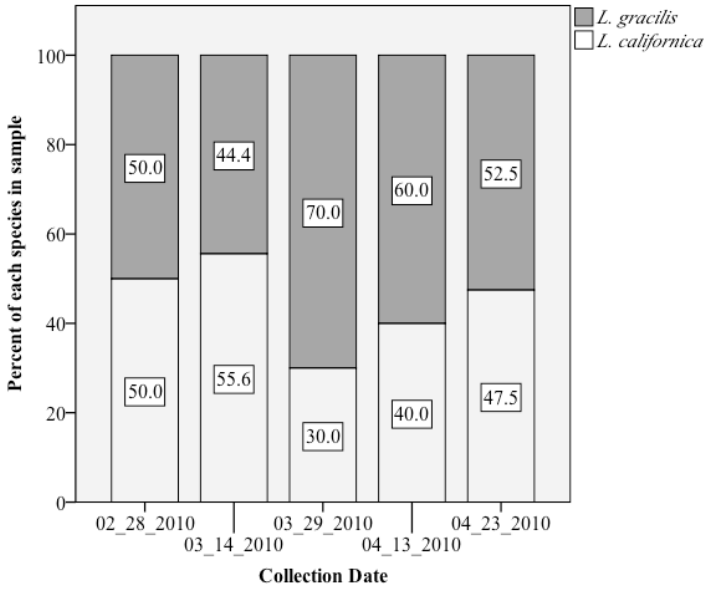

Figure 2. Species distribution throughout the season in the transition zone. Sample size was 12 on first two dates when DNA analysis was required for identification and 40 on the last three dates when flowers were present for identification.

Soil analysis---Principal components analysis revealed soil variables tested consistently varied between the top, middle, and bottom of the hillside throughout the season. PC1 accounted for 38.255 to 52.555 percent, and the PC2 accounted for 15.590 to 18.913 percent of the variation for the seven dates sampled (Table 1).

Table 1. Eigenvalues and total variance explained by components 1 and 2 by date.

Soil Collection Date	PC1 Eigenvalue	PC1 % of Variance	PC2 Eigenvalue	PC2 % of Variance
02-08-2010	7.730	45.469	3.400	19.998
02-28-2010	6.503	38.255	2.927	17.218
03-14-2010	8.561	50.357	3.215	18.913
03-29-2010	7.775	45.734	3.123	18.370
04-13-2010	7.214	42.433	2.744	16.142
04-23-2010	8.934	52.555	2.650	15.590
05-14-2010	6.910	40.645	2.987	17.569

Calcium to magnesium ratio (Ca:Mg) consistently loaded high on PC1 (accounted for most of the variability for all dates but the last, where it was the second highest). Potassium (K), organic matter (OM), and estimated nitrogen release (ENR, calculated by A&L Laboratories, Inc.) on average were the next highest loaders (Yost et al., 2012), but loading order varied by date. Volumetric water content (VWC) also loaded high on all soil collection dates and was often among the top four on PC1. Some variables such as pH and nitrogen loaded high on PC1 some dates and high on PC2 other dates (Table 2).

Table 2. Component matrix, extraction method: principal component analysis. Soil collections analyzed by date.

02-08-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.928	-0.29
Volumetric water content	0.901	0.239
Na:K	0.886	0.013
Estimated nitrogen release (lbs/acre)	0.82	-0.164
Organic matter (%)	0.819	-0.168
Calcium (ppm)	-0.799	0.143
Magnesium (ppm)	0.758	0.539
Potassium (ppm)	-0.726	-0.003
pH	0.713	-0.422
Cation exchange capacity (meq/100/ x g)	0.689	0.602
Sulfur as SO_4^{-2} (ppm)	-0.631	0.425
Hydrogen (meq/100 x g)	-0.575	0.547
Phosphorus (Bray-ppm)	-0.2	0.691
Soluble salts (mmhos/cm)	0.478	-0.668
Phosphorus (Olsen-ppm)	0.419	0.667
Nitrogen as NO_3^- (ppm)	-0.117	-0.513
Sodium (ppm)	0.139	0.489

Table 2. (Continued)

02-28-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.961	-0.01
Organic matter (%)	0.875	-0.276
Estimated nitrogen release (lbs/acre)	0.875	-0.27
Volumetric water content	0.785	0.428
Magnesium (ppm)	0.753	0.375
Potassium (ppm)	-0.702	0.408
Cation exchange capacity (meq/100/ x g)	0.685	0.433
Calcium (ppm)	-0.673	0.285
pH	0.629	-0.214
Hydrogen (meq/100 x g)	-0.604	0.253
Na:K	0.567	0.292
Sulfur as SO_4^{-2} (ppm)	0.437	0.029
Nitrogen as NO_3^- (ppm)	0.148	-0.724
Soluble salts (mmhos/cm)	-0.08	-0.71
Phosphorus (Olsen-ppm)	-0.114	0.562
Sodium (ppm)	0.274	0.531
Phosphorus (Bray-ppm)	0.246	0.443

Table 2. (Continued)

03-14-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.945	0.289
Organic matter (%)	0.942	0.122
Estimated nitrogen release (lbs/acre)	0.938	0.121
Sulfur as SO_4^{-2} (ppm)	0.905	0.364
Magnesium (ppm)	0.904	-0.125
Cation exchange capacity (meq/100/ x g)	0.861	-0.201
Calcium (ppm)	-0.86	0.233
Volumetric water content	0.846	-0.048
Nitrogen as NO_3^- (ppm)	0.71	-0.405
Sodium (ppm)	-0.631	-0.365
Phosphorus (Bray-ppm)	0.512	0.151
Soluble salts (mmhos/cm)	0.383	-0.002
Hydrogen (meq/100 x g)	-0.48	-0.793
pH	0.356	0.784
Phosphorus (Olsen-ppm)	0.135	0.734
Potassium (ppm)	-0.473	0.637
Na:K	0.395	-0.592

Table 2. (Continued)

03-29-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.966	-0.126
Volumetric water content	0.942	-0.154
Magnesium (ppm)	0.926	-0.219
Cation exchange capacity (meq/100/ x g)	0.92	-0.212
Sodium (ppm)	0.892	0.008
Na:K	0.892	0.256
Sulfur as SO_4^{-2} (ppm)	0.892	-0.126
Potassium (ppm)	-0.729	-0.336
Nitrogen as NO_3^- (ppm)	0.728	0.186
Soluble salts (mmhos/cm)	0.469	0.308
Organic matter (%)	0.333	-0.312
Estimated nitrogen release (lbs/acre)	0.33	-0.298
Phosphorus (Olsen-ppm)	-0.001	0.882
Phosphorus (Bray-ppm)	0.118	0.769
Hydrogen (meq/100 x g)	-0.065	0.717
pH	0.414	-0.624
Calcium (ppm)	-0.417	-0.457

Table 2. (Continued)

04-13-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.891	0.176
Volumetric water content	0.881	0.07
Magnesium (ppm)	0.825	0.37
Na:K	0.808	-0.491
Organic matter (%)	0.803	-0.062
Estimated nitrogen release (lbs/acre)	0.802	-0.059
Cation exchange capacity (meq/100/ x g)	0.755	0.416
pH	0.744	0.109
Sodium (ppm)	0.729	0.017
Potassium (ppm)	-0.635	0.415
Hydrogen (meq/100 x g)	-0.585	-0.09
Phosphorus (Olsen-ppm)	0.549	0.497
Nitrogen as NO_3^- (ppm)	0.072	-0.771
Calcium (ppm)	-0.477	0.65
Sulfur as SO_4^{-2} (ppm)	0.265	0.585
Soluble salts (mmhos/cm)	-0.102	-0.502
Phosphorus (Bray-ppm)	0.078	-0.315

Table 2. (Continued)

04-23-2010		
Soil variable	PC1	PC2
Ca:Mg	-0.958	0.08
Organic matter (%)	0.917	0.049
Estimated nitrogen release (lbs/acre)	0.913	0.046
Na:K	0.896	0.189
Calcium (ppm)	-0.871	0.13
Magnesium (ppm)	0.869	0.1
Volumetric water content	0.84	0
Cation exchange capacity (meq/100/ x g)	0.82	0.182
Potassium (ppm)	-0.736	0.271
Sulfur as SO_4^{-2} (ppm)	0.685	0.025
Soluble salts (mmhos/cm)	0.618	-0.007
Sodium (ppm)	0.56	0.492
Nitrogen as NO_3^- (ppm)	0.41	-0.022
Phosphorus (Olsen-ppm)	-0.158	-0.823
Hydrogen (meq/100 x g)	-0.442	0.775
pH	0.604	-0.738
Phosphorus (Bray-ppm)	-0.433	-0.636

Table 2. (Continued)

05-14-2010		
Soil variable	PC1	PC2
Calcium (ppm)	-0.944	-0.145
Ca:Mg	-0.937	-0.284
Potassium (ppm)	-0.935	-0.096
Na:K	0.917	-0.004
Volumetric water content	0.877	0.299
pH	0.683	-0.569
Hydrogen (meq/100 x g)	-0.627	0.555
Organic matter (%)	0.584	-0.491
Estimated nitrogen release (lbs/acre)	0.572	-0.495
Sodium (ppm)	0.388	-0.175
Sulfur as SO_4^{-2} (ppm)	0.25	0.108
Cation exchange capacity (meq/100/ x g)	0.445	0.781
Magnesium (ppm)	0.616	0.667
Phosphorus (Bray-ppm)	-0.375	0.413
Phosphorus (Olsen-ppm)	-0.35	0.384
Nitrogen as NO_3^- (ppm)	-0.114	-0.361
Soluble salts (mmhos/cm)	0.246	0.351

When PCA was performed with soil variables averaged over the season sodium, phosphorus, nitrogen, and soluble salts loaded high on PC2 (Yost et al., 2012). The principal components clearly vary by zone, and also appear to vary by date within each zone (Figure 3).

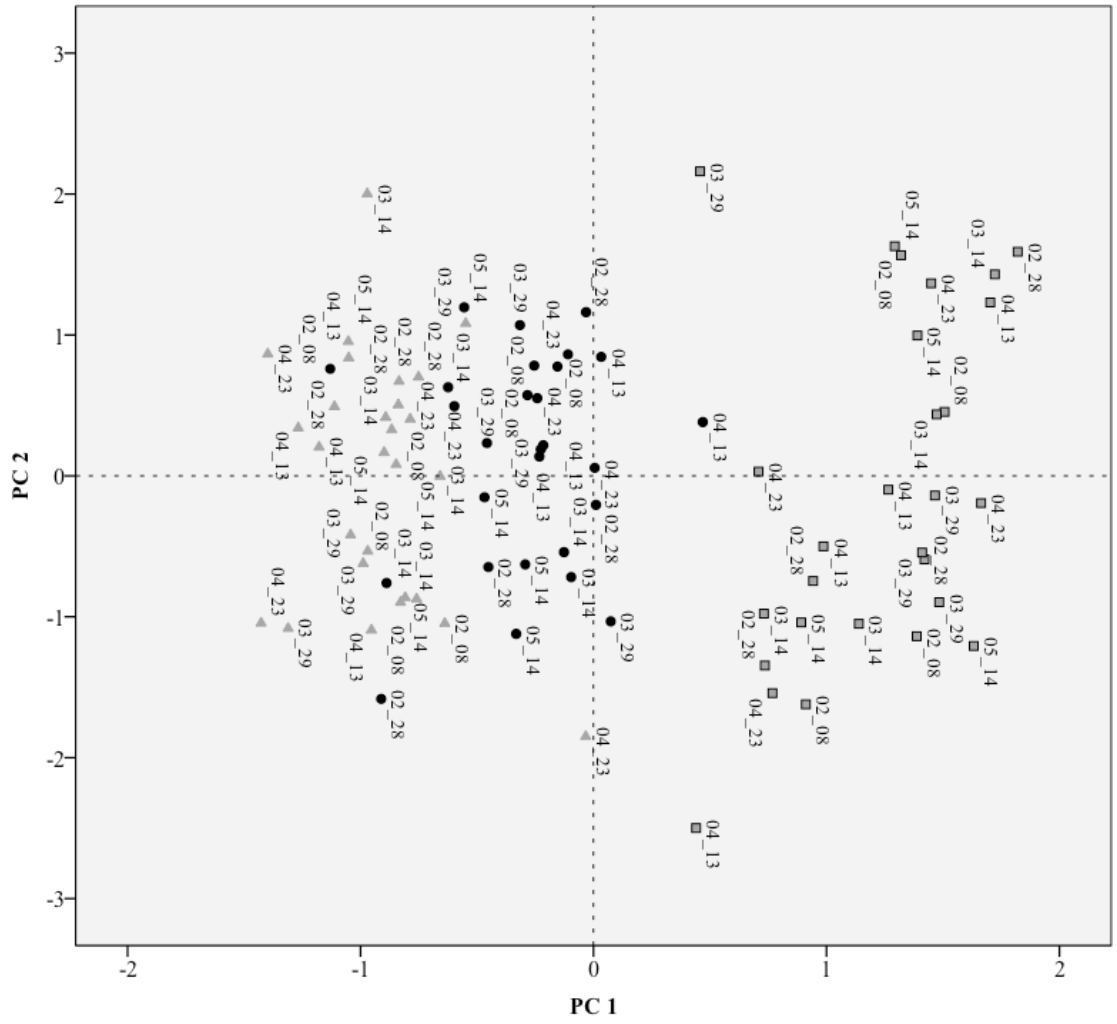

Figure 3. Variation by zone and date of loading scores from the first two principal components. Each data point represents a date and an outcrop zone. Zones are labeled as follows: square = bottom, circle = middle, and triangle = top.

Repeated measures ANOVAs performed on Ca:Mg, K, OM, and ENR revealed some variations in soil chemistry by date and zone (Figures 4-7). Ca:Mg varied significantly from the seasonal mean on the first collection date $F_{1,9} = 37.157$, $p<0.001$, and date four

$F_{1,9} = 5.893$, $P < 0.05$. All zone comparisons were significantly different (bottom vs. middle $P < 0.01$, bottom vs. top $P < 0.001$, and middle vs. top $P < 0.05$). For all dates mean Ca:Mg was highest at the top and lowest at the bottom. On dates four ($F_{1,9} = 19.305$, $P < 0.05$) and seven ($F_{1,9} = 54.148$, $P < 0.001$) K significantly varied from the seasonal mean. Tukey tests revealed top vs bottom and top vs middle of the outcrop were the only significant comparisons ($P < 0.05$). Mean K was highest at the top and lowest at the bottom for all dates. OM and ENR did not significantly vary by date, but all zone comparisons were significant ($P < 0.05$). Mean OM and ENR were lowest at the top and generally highest at the bottom of the outcrop ($P < 0.001$). The assumption of sphericity (Mauchy's test) was not violated for any of the above soil variables ($P > 0.05$).

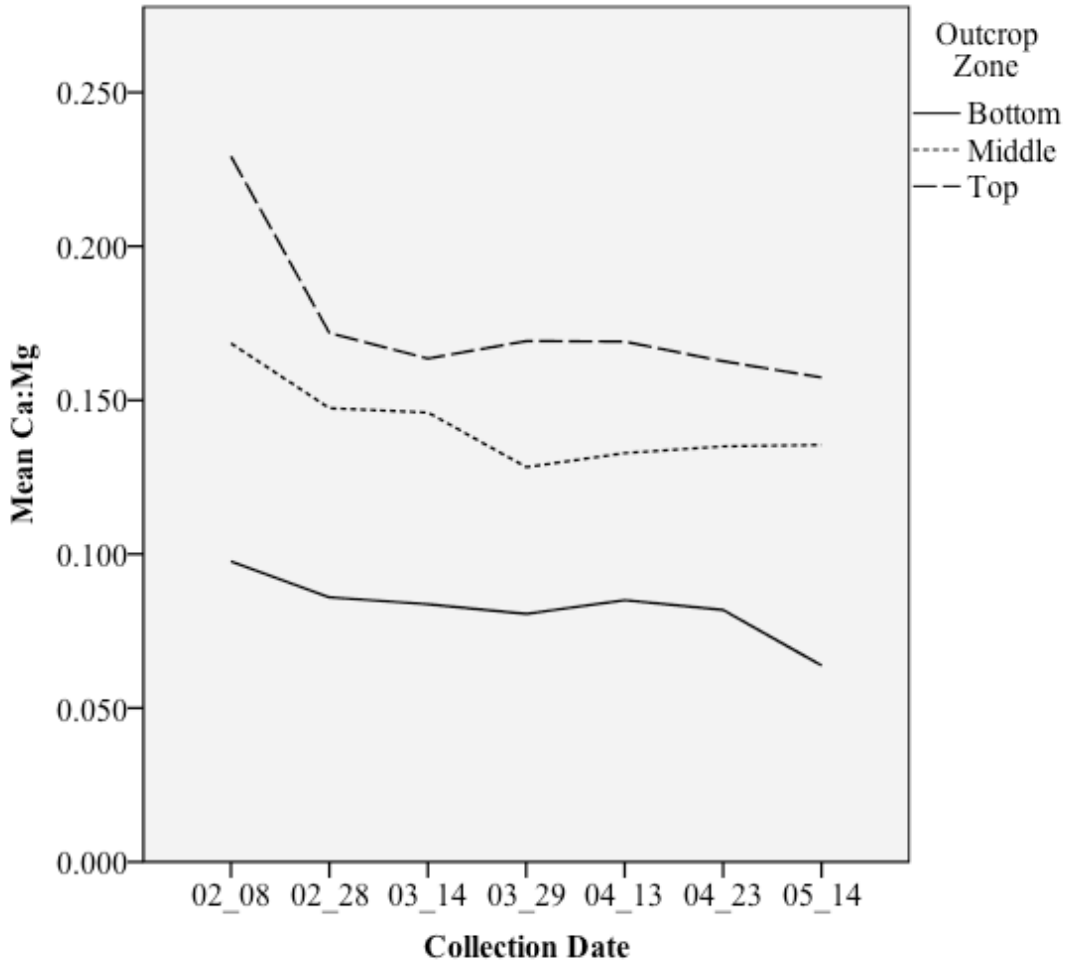

Figure 4. Variation of Ca:Mg throughout the season within each outcrop zone.

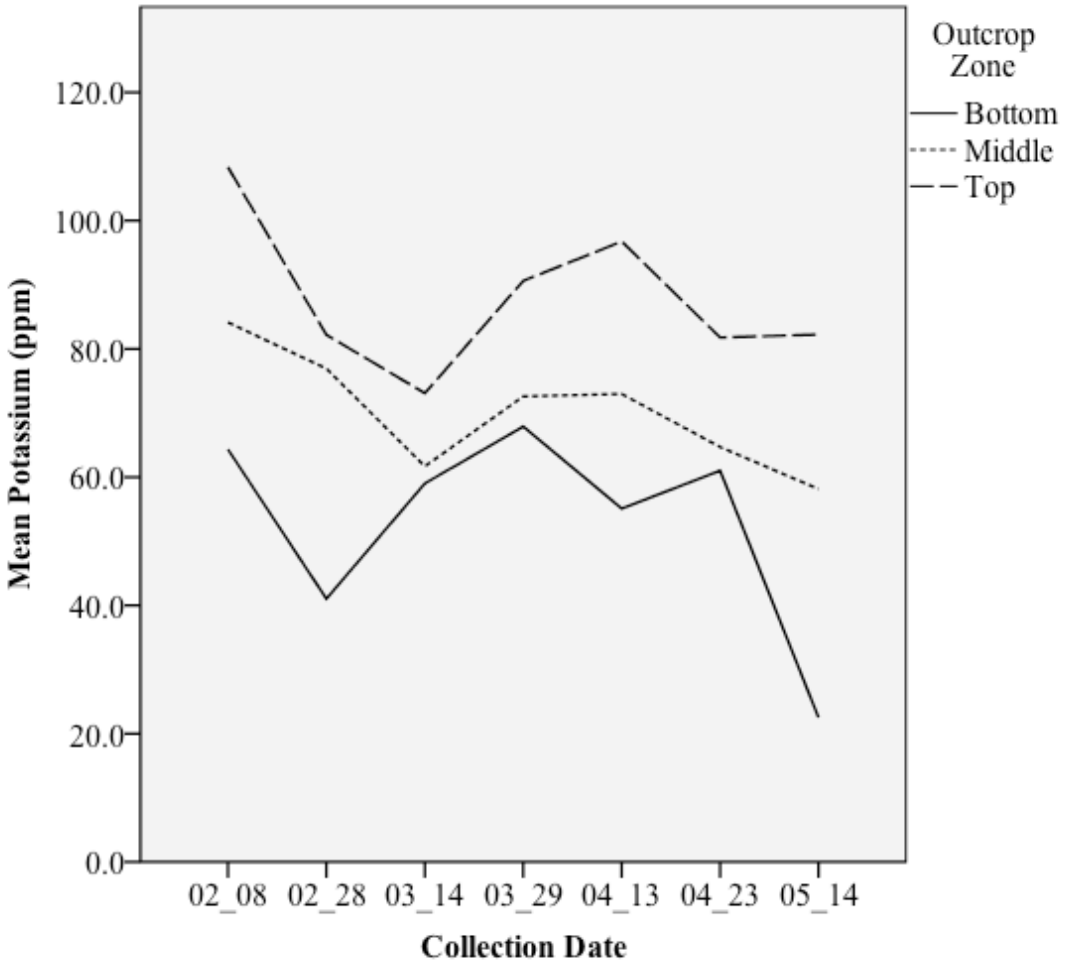

Figure 5. Mean Potassium (ppm) throughout the season for each outcrop zone.

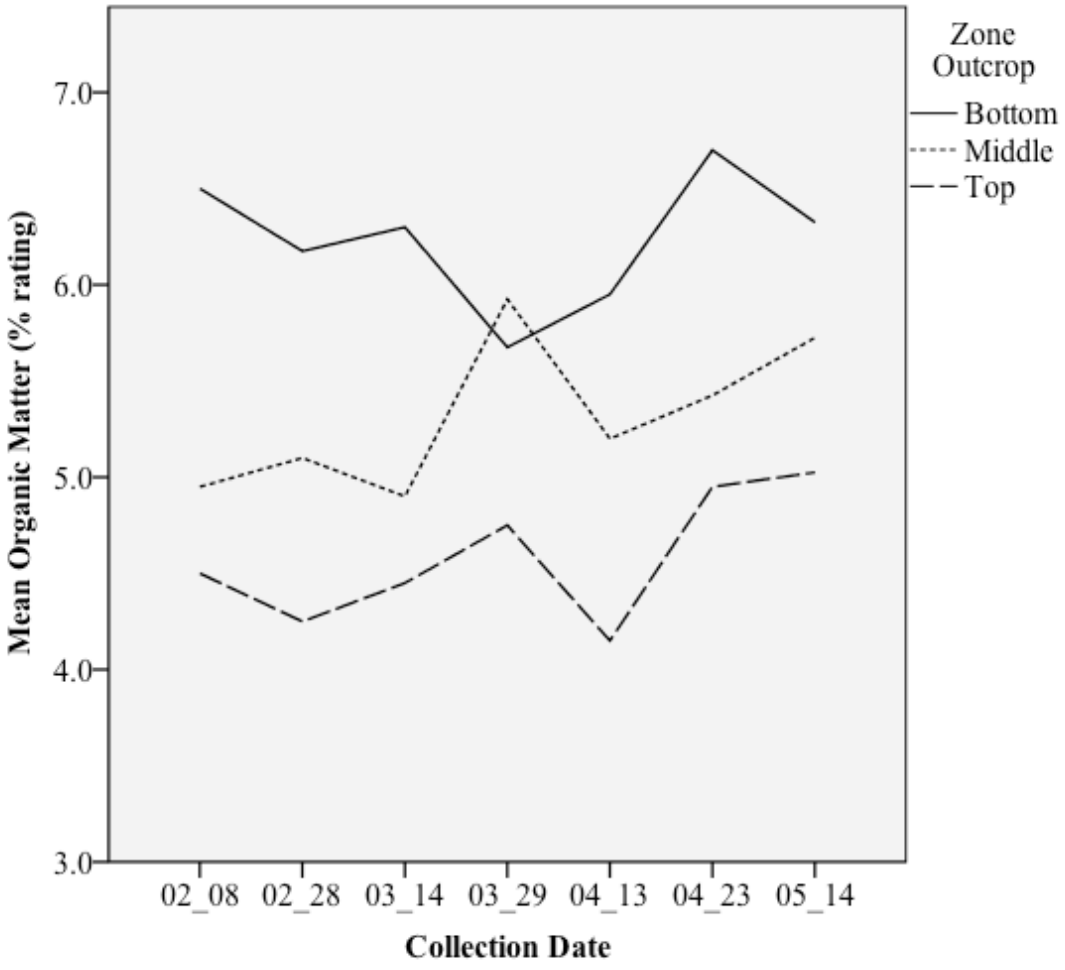

Figure 6. Variation of organic matter (%) throughout the season (not significant) for each outcrop zone.

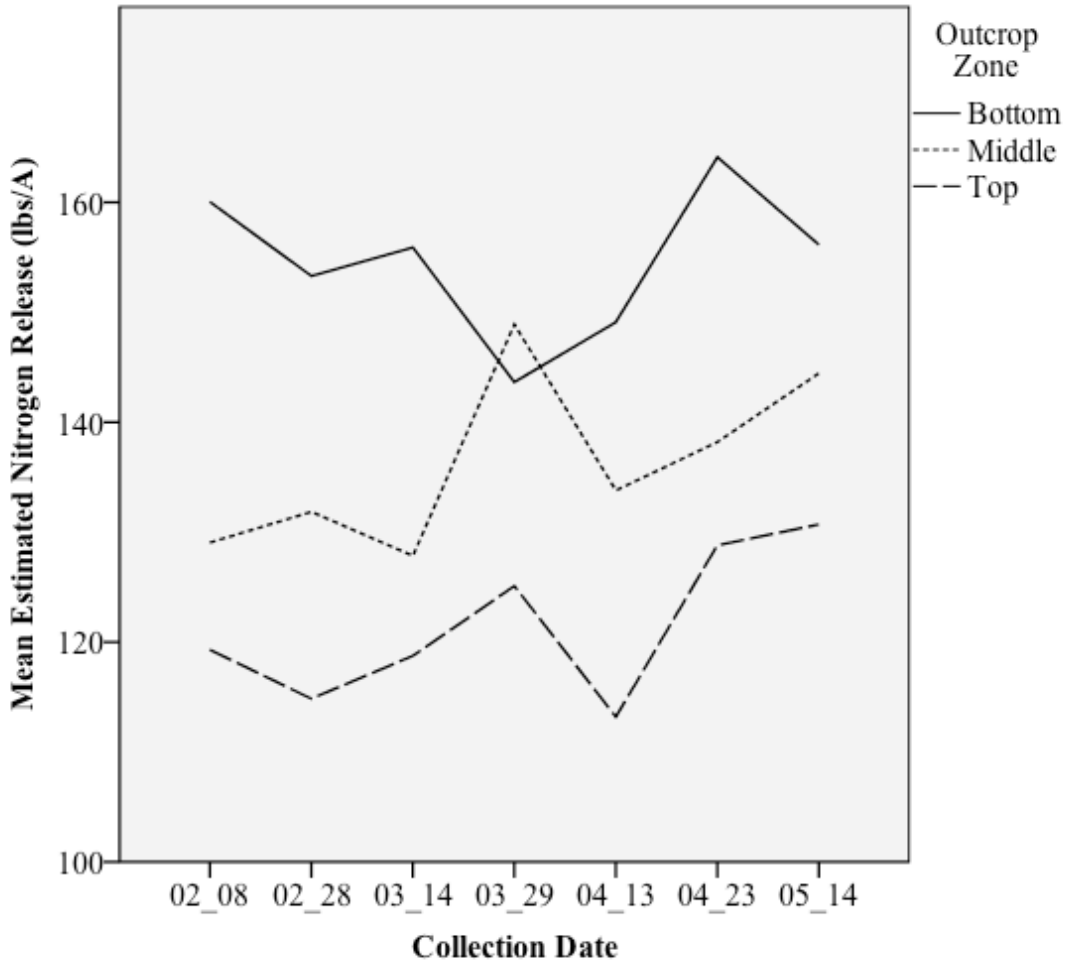

Figure 7. Variation in estimated nitrogen release (lbs/acre) throughout the season (not significant) for each outcrop zone.

Plant fitness and growth---MANOVA using Pillai's trace revealed a significant effect of outcrop region on floral and seed production (fitness), $V = 0.313$, $F_{6,168} = 5.202$, $P < 0.01$ but not for vegetative measurements $V = 0.087$, $F_{8,170} = 0.971$, $P > 0.05$ in *L. gracilis*. Both fitness $V = 0.496$, $F_{6,172} = 9.449$, $P < 0.01$ and vegetative $V = 0.461$, $F_{8,178} = 6.672$, $P < 0.01$ measures varied significantly by region in *L. californica*. Follow up ANOVA post hoc Tukey HSD tests revealed significant ($P < 0.05$) differences in *L.*

gracilis flower and seed production (viable and total) between the top and middle zones and between the middle and bottom zones for viable (not total) seed production. In *L. californica*, all three floral variables were significantly ($P < 0.001$) different between the top and the middle, and the top and the bottom only. There were no significant zone comparisons for change in height, number of leaves, or above ground biomass in *L. gracilis*, but all four vegetative measures varied significantly ($P < 0.05$) between the top and middle (except peak number of leaves), and the top and bottom of the outcrop in *L. californica*. All of the floral and vegetative variables were highest at the bottom and lowest at the top for *L. californica*, and all variables except for number of leaves were greatest at the middle and lowest at the top for *L. gracilis* (Table 3, and Figures 8-14). Transect was not significant for the model main effects of zone x transect for the floral MANOVA (*L. gracilis* $V = 0.129$, $F_{9,246} = 1.226$, $P > 0.05$, and *L. californica* $V = 0.075$, $F_{9,252} = 0.723$, $P > 0.05$) or the vegetative MANOVA (*L. gracilis* $V = 0.127$, $F_{12,249} = 0.920$, $P > 0.05$, and *L. californica* $V = 0.104$, $F_{12,261} = 0.783$, $P > 0.05$) using Pillai's Trace. Similar levels of significance resulted when MANOVAs were rerun with the six plants demonstrating evidence of herbivory (as discussed in the data analysis section) as missing data. The only significant finding was the difference between peak numbers of leaves at the top compared to the middle was no longer significant in *L. californica* (note that peak number of green leaves was still significant).

Table 3a. Means +/- standard error for *Lasthenia gracilis* floral and vegetative variables.

Outcrop Zone	Statistic	Number of flowers	Number of viable seeds	Total number of seeds	Above ground biomass (g)	Height change (mm)	Peak number of leaves	Peak number of green leaves
Top	Mean	1	27	65	0.0105	55.0	12	9
	Standard error +/-	0	7	16	0.0021	7.1	1	1
	Minimum	0	0	0	0.0000	-2.0	5	0
	Maximum	7	144	408	0.0612	135.0	33	27
Middle	Mean	2	68	117	0.0146	72.7	14	11
	Standard error +/-	0	10	19	0.0017	6.6	1	1
	Minimum	0	0	0	0.0002	4.0	8	4
	Maximum	5	209	508	0.0422	137.0	26	24
Bottom	Mean	1	23	77	0.0111	65.6	12	9
	Standard error +/-	0	6	19	0.0031	6.7	1	1
	Minimum	0	0	0	0.0000	-4.0	4	0
	Maximum	7	127	475	0.0919	135.0	34	30

Table 3b. Means +/- standard error for *Lasthenia californica* floral and vegeatative variables.

Outcrop Zone	Statistic	Number of flowers	Number of viable seeds	Total number of seeds	Above ground biomass (g)	Height change (mm)	Peak number of leaves	Peak number of green leaves
Top	Mean	1	3	26	0.0073	37.2	11	8
	Standard error +/-	0	1	8	0.0014	6.9	1	1
	Minimum	0	0	0	0.0000	-10.0	3	2
	Maximum	4	27	171	0.0359	115.0	19	16
Middle	Mean	2	84	160	0.0210	88.0	14	11
	Standard error +/-	0	17	30	0.0031	8.0	1	1
	Minimum	0	0	0	0.0008	5.0	5	4
	Maximum	6	274	490	0.0637	154.0	22	20
Bottom	Mean	3	124	187	0.0274	109.6	14	12
	Standard error +/-	0	20	30	0.0041	6.0	1	1
	Minimum	0	0	0	0.0000	5.0	8	6
	Maximum	10	404	672	0.1003	162.0	28	26

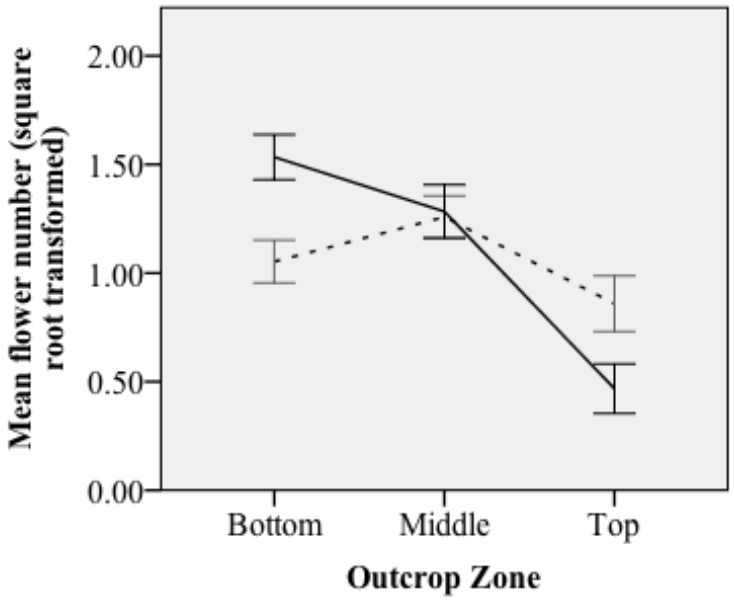

Figure 8. Variation of the mean number of *L. californica* (solid line) and *L. gracilis* (dashed line) for flower heads in each outcrop zone. Error bars are +/- 1 standard error.

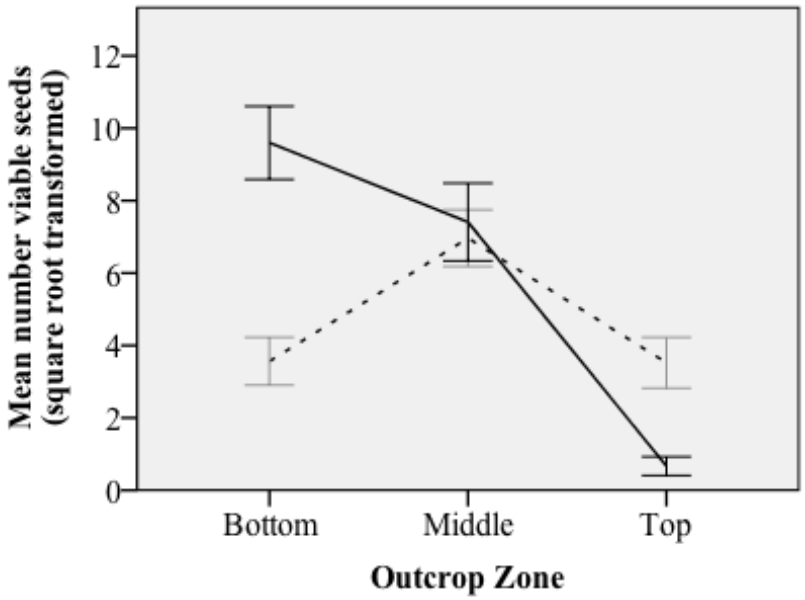

Figure 9. Variation of the mean number of *L. californica* (solid line) and *L. gracilis* (dashed line) for viable seeds in each outcrop zone. Error bars are +/- 1 standard error.

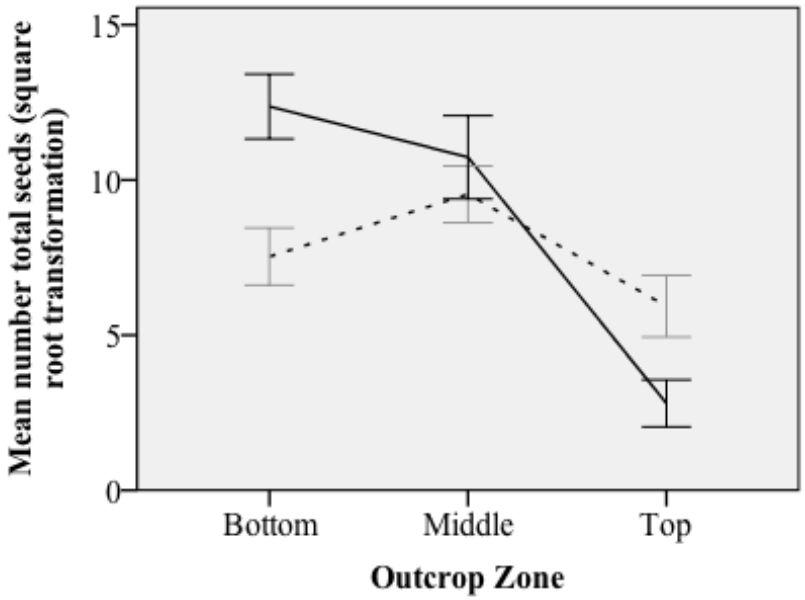

Figure 10. Variation of the mean number of *L. californica* (solid line) and *L. gracilis* (dashed line) for total seeds in each outcrop zone. Error bars are +/- 1 standard error.

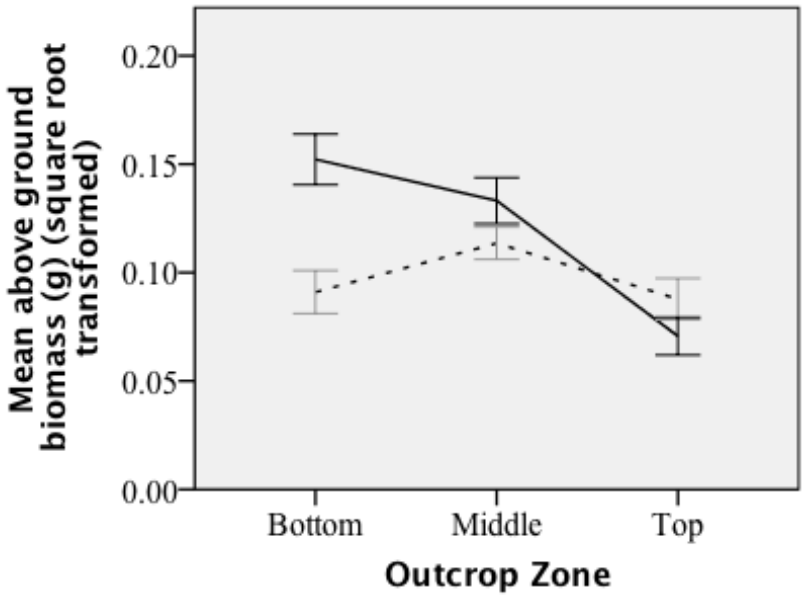

Figure 11. Variation of the mean number of *L. californica* (solid line) and *L. gracilis* (dashed line) for above ground biomass in each outcrop zone. Error bars are +/- 1 standard error.

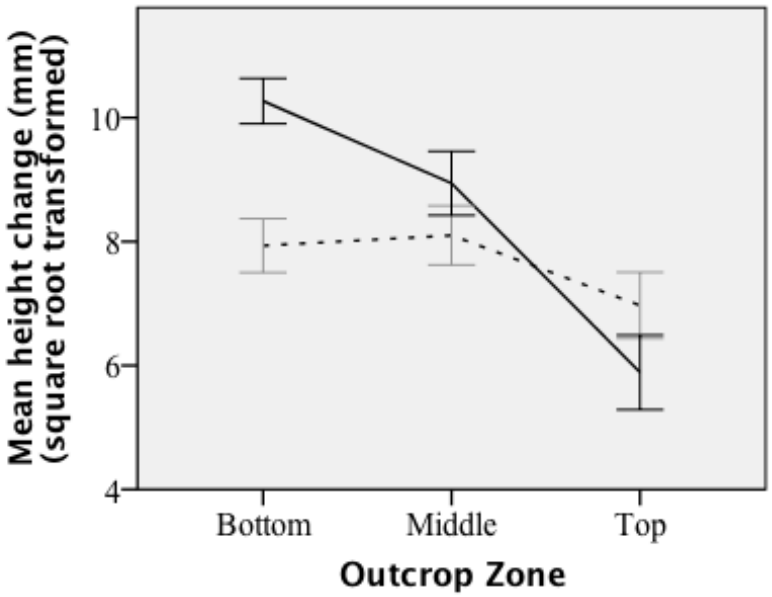

Figure 12. Variation of *L. californica* (solid line) and *L. gracilis* (dashed line) for the mean change in height in each outcrop zone. Error bars are +/- 1 standard error.

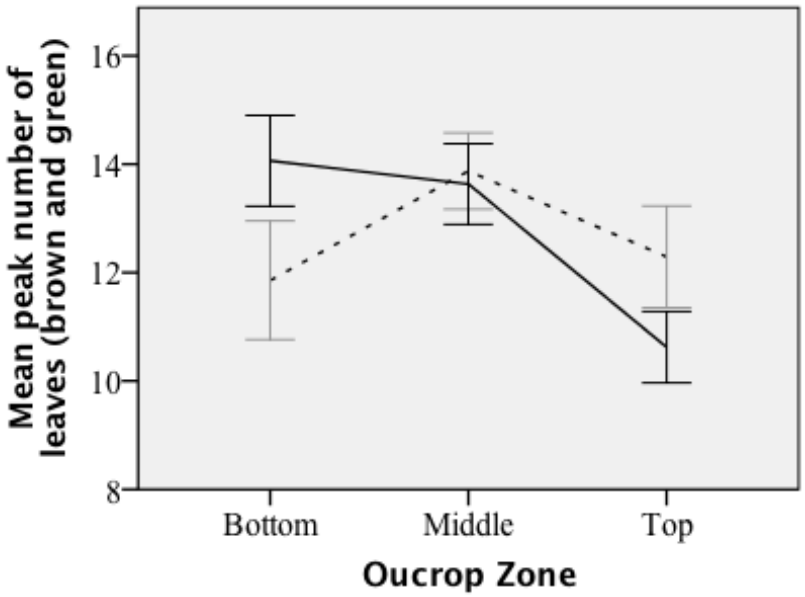

Figure 13. Variation of *L. californica* (solid line) and *L. gracilis* (dashed line) for the mean peak number leaves in each outcrop zone. Error bars are +/- 1 standard error.

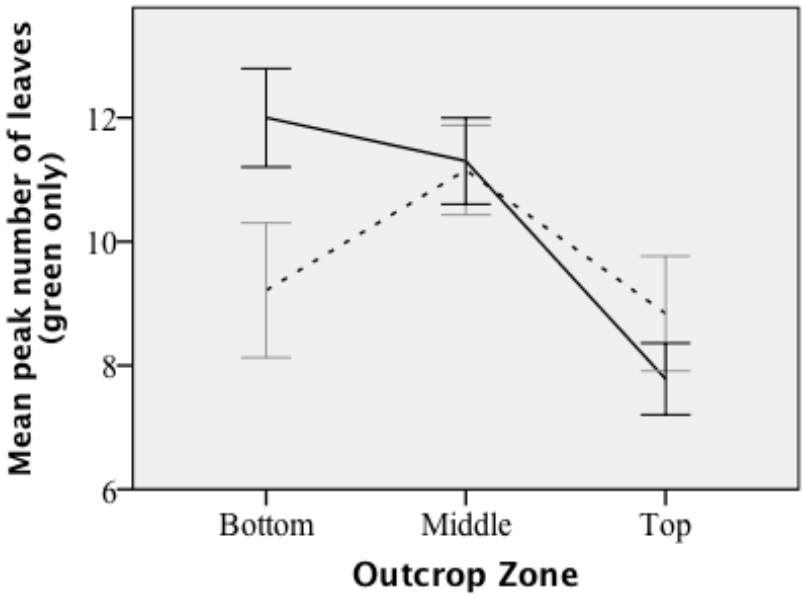

Figure 14. Variation of *L. californica* (solid line) and *L. gracilis* (dashed line) for the mean peak number green leaves in each outcrop zone. Error bars are +/- 1 standard error.

Survival---Kaplan-Meier tests revealed survival for *L. gracilis* was greater at the middle than the top ($P < 0.001$) and bottom ($P < 0.01$) of the slope (Figure 15). *Lasthenia californica* had greater survival at the bottom, and the lowest survival at the top of the hill, $P < 0.001$ for all three comparisons (Figure 16).

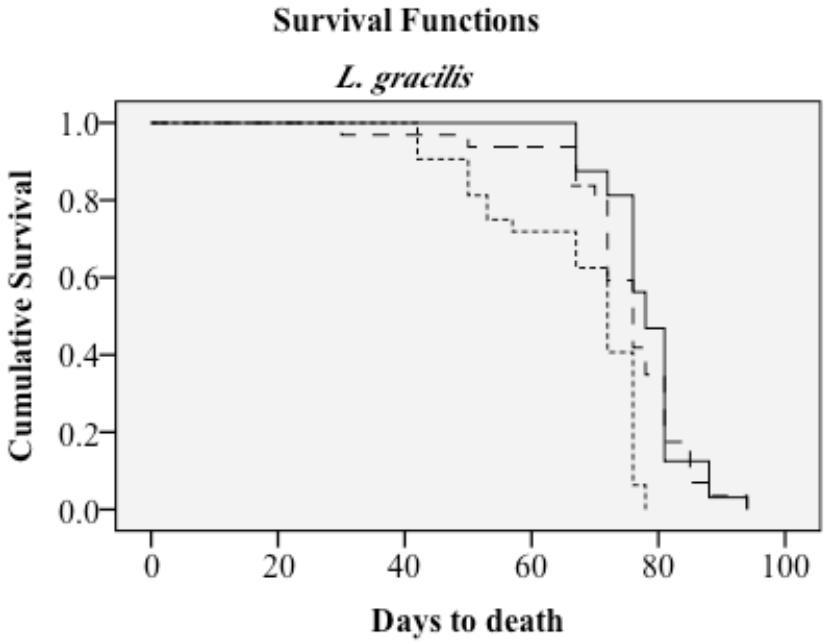

Figure 15. Survival over the season since transplant date for *L. gracilis* in each of the outcrop zones. Zones labeled as follows: solid line = middle, dotted line = top, and dashed line = bottom.

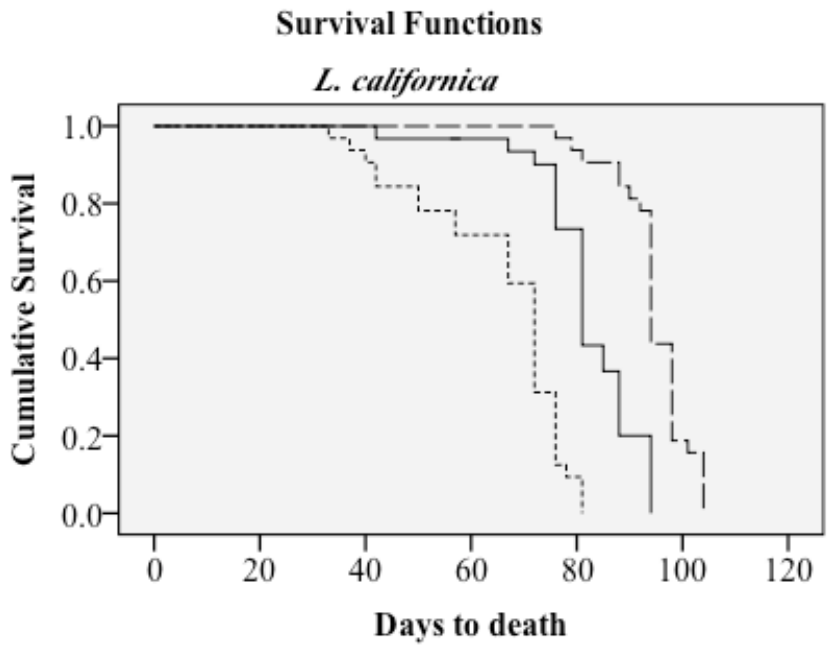

Figure 16. Survival over the season since transplant date for *L. californica* in each of the outcrop zones. Zones labeled as follows: solid line = middle, dotted line = top, and dashed line = bottom.

DISCUSSION

The study of local adaptation to variable edaphic conditions provides vital information to managers and planners of restoration projects. Reciprocal transplant studies used to examine local adaptation not only offer insight into plant evolution, they also are useful in testing the success of plant translocations and reintroductions for habitat restoration. Knowledge of seed source and adaptability to different locations of similar habitat are vital to the success of transplants in restoration projects (McKay et al., 2005). The presence of *L. californica* and *L. gracilis* growing in parapatry at Jasper Ridge Biological Preserve demonstrates how two cryptic reproductively isolated species can be

adapted to distinct areas along an edaphic gradient. My findings that all plants sampled at the bottom of the outcrop were *L. californica*, that all plants sampled form the top were *L. gracilis*, and that species was variable in the transition zone (also see Yost et al., 2012) show the boundary observed by Rajakaruna and Bohm (1999) is still present. Soil chemistry, plant survival, and fitness did vary greatly along the serpentine outcrop.

As expected, Ca:Mg consistently accounted for much of the soil chemistry variation by zone. Potassium also played a big role, but Na:K was not as consistent. I predicted nitrogen would play a larger role however; OM a predictor of ENR and ENR itself were among the top four variables of PC1. There were some significant variations of soil chemistry throughout the season, demonstrated by the changes in PC1 and PC2 by date and the repeated measures ANOVAs on the four soil variables accounting for most of the variance in PC1 (Ca:Mg, K, OM, and ENR). The same four edaphic variables accounted for most of the variation when PCA was performed with samples from all dates in one test (Yost et al., 2012). Linear regression analysis revealed significant variations through time for many of the soil variables (Yost et al., 2012), so I examined more closely the four variables accounting for most of the variation in PC1 (listed above) using repeated measures ANOVAs with deviation contrasts. These tests reveled Ca:Mg and K varied significantly ($P < 0.05$) throughout the season. Although OM and ENR did not vary significantly by date, they were significantly ($P < 0.001$) higher at the bottom and lower at the top. Moisture (VWC) is also important for plant fitness, and was significantly higher at the bottom than the top and middle of the outcrop ($P < 0.05$) as demonstrated in figure 17.

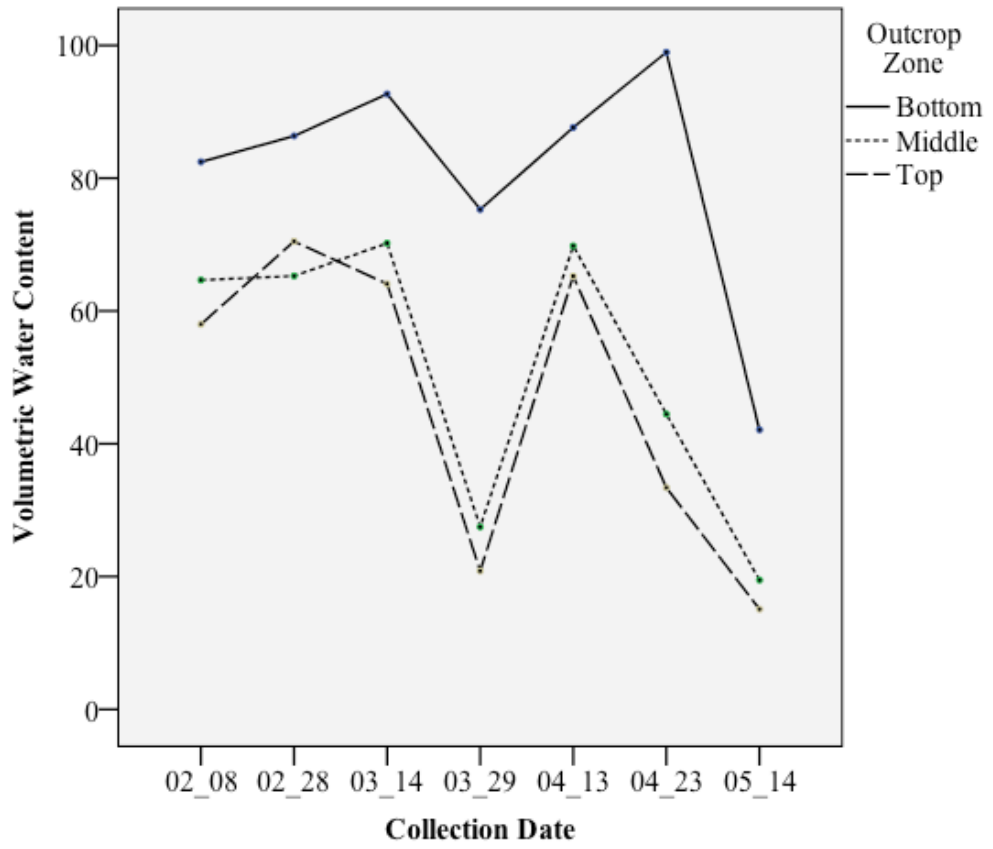

Figure 17. Variation in volumetric water content by outcrop zone and soil collection date.

As predicted, *L. californica* demonstrated greater fitness in its home range (bottom of the hillside) than *L. gracilis*. *Lasthenia gracilis* performed better in its home range (top of the hillside) than *L. californica*, but *L. gracilis* unexpectedly peaked in the transition zone between the two species. *Lasthenia californica* produced the most flower heads, total seeds, and viable seeds at the bottom and the least at the top of the outcrop as expected however; *L. gracilis* produced the most flowers and seeds in the transition zone and the least in their home range (top). Yost et al. (2012) further supported these results

(viable seeds only) with aster analysis where each species was found to have a home site advantage, but *L. gracilis'* fitness peaked in the transition zone. Survival was greatest for *L. californica* in its home range, but again *L. gracilis* performed best in the transition zone. Comparisons of the two species in each zone revealed significant differences between the *L. californica* and *L. gracilis* at the middle and bottom, but not at the top of the outcrop. There are several plausible explanations as to why *L. gracilis* peaked in the transition zone. First, *L. gracilis* may be able to acclimate to the drier, higher Ca:Mg conditions at the top of the hill, but thrive in more ideal moisture conditions seen in the transition zone. Another reason is there may be more competition from *Plantago erecta* that appears more abundant in *L. gracilis'* home range than in *L. californica's* (or in the transition zone) and less competition from *P. erecta* may give an edge for better success of *L. gracilis* in the transition zone. Thus, my hypothesis that both species would display greater fitness and survival home than away was partially supported.

Hydroponic studies are ideal for examining a plant's response to specific soil variables. For example, different races of *L. californica* have been documented to vary in survivorship when exposed to solutions of NaCl and $MgSO_4$ (Rajakaruna et al., 2003a). General edaphic and species interactions were also revealed using the aster model PC1 x species. *Lasthenia gracilis* had the highest fitness when PC1 values were low (higher K^+, lower Mg^{2+}), and *L. californica* displayed the greatest fitness when PC1 values were high (Yost et al., 2012).

Many variables I did not measure quantitatively such as herbivory, animal disturbance (e.g. gophers and ants), and pollination may also have affected the outcome

of my experiment. In order to account for disturbance the four trampled plants were removed from analyses. Herbivory was not determined to be significant because results remained the same in follow up MANOVAs where the six plants estimated to be victims of herbivory (> 2mm reduction in height, and above ground biomass = 0.0000g) were treated as missing data. Pollinators appeared to be present and consistent in all outcrop regions throughout the flowering season; however, species may have experienced an away-site disadvantage due to the relative lack of conspecific pollen donors. The variable of reproductive isolation (self-incompatibility) simply could not be controlled. As stated in the introduction *L. californica* and *L. gracilis* are also reproductively isolated by 7 to 10 day difference in flowering time (Rajakaruna and Bohm, 1999). This difference was noted in my experimental plants as well. Reproductive isolation in the two species was also supported by reduced pollen tube growth in preliminary crossing experiments (Rajakaruna and Whitton, 2004).

In a field study not all variables can be controlled for; however, my results support *L. californica* and *L. gracilis* are locally adapted to specific regions within the serpentine outcrop. These findings not only support site-specific tolerance, which can lead to speciation (Kruckeberg, 1986), they offer important resources for restoration planning. Successes and more often failures of plant reintroductions for restoration are not well documented (Drayton and Primack, 2012). Restoration efforts should be better documented; however, results from my study and other reciprocal transplant studies can help managers of restoration projects select suitable seed sources (McKay et al., 2005), transplant methods, and assessment of transplant success.

FUTURE DIRECTIONS

Two important variables including the replication of my study at other locations in the species' geographic range need to be further examined to demonstrate local adaptation in the two *Lasthenia* species. First, the germination stage is vital to the establishment and maintenance of a population. The germination rate of *L. californica* is generally less than 50% and just over 50% for *L. gracilis* in a laboratory environment (Barry, unpublished). The germination rates of seeds I started in the growth chamber were as follows: 232/542 *L. californica* and 283/502 *L. gracilis* seeds. Higher germination rates for *L. gracilis* were also observed by Rajakaruna (personal communication; Rajakaruna and Bohm, 1999). Failure of seed germination and the differences between germination rates should be better quantified in future experiments. Second, the viability of future generations should be tested to determine if an annual plant population is able to establish past the first season. I ran some preliminary germination trials on 200 seeds from each of my species x outcrop zone combinations (except for *L. californica* planted in *L. gracilis'* home range but, not enough dark viable seeds were produced to test) from this study. The trend was that greater numbers of seeds germinated from plants transplanted in their home range. Additionally, all of the viable seeds produced from transplants should be tested at the same time in the same growing facility in order to minimize any variability resulting from conducting germination trials across time and space. Finally, it is important to replicate this experiment at different locations where the two species grow in parapatry. Reciprocal transplant studies on these species are currently ongoing at Palmer Ranch (Monterey

county), Coyote Ridge (Santa Clara county), and in the greenhouse (with seeds and field collected soil from five additional locations) using seeds (Yost, personal communication). These experiments will provide replication, and will examine the germination stage in field and laboratory settings. Another advantage to using seeds in the field, although harder to monitor, is the full life cycle from germination to seed set is considered under the same conditions. I found that *L. gracilis* seeds germinated in slightly greater numbers than *L. californica* seeds when field collected, but when seeds from my reciprocal transplant experiment were tested more *L. californica* seeds from plants transplanted in their home range germinated (90/200) than *L. gracilis* seeds from transplants in their home range (58/200). Seedlings grown in greenhouse or growth chamber conditions may be at a disadvantage (e.g. transplant shock) to seedlings that germinated in the field. These great preliminary results provide additional insight on how the two species are adapted to different regions of the serpentine outcrop and the ongoing field-based reciprocal transplant studies will demonstrate what role the germination phase plays in local adaptation.

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