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EDAPHIC ADAPTATION MAINTAINS THE COEXISTENCE OF TWO CRYPTIC SPECIES ON SERPENTINE SOILS[1]

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- *Premise of the study:* Divergent edaphic adaptation can contribute to reproductive isolation and coexistence between closely related species, yet we know little about how small-scale continuous edaphic gradients contribute to this phenomenon. We investigated edaphic adaptation between two cryptic species of California wildflower, *Lasthenia californica* and *L. gracilis* (Asteraceae), which grow in close parapatry on serpentine soil.
- *Methods:* We reciprocally transplanted both species into the center of each species' habitat and the transition zone between species. We quantified multiple components of fitness and used aster models to predict fitness based on environmental variables. We sampled soil across the ridge throughout the growing season to document edaphic changes through time. We sampled naturally germinating seedlings to determine whether there was dispersal into the adjacent habitat and to help pinpoint the timing of any selection against migrants.
- *Key results:* We documented within-serpentine adaptation contributing to habitat isolation between close relatives. Both species were adapted to the edaphic conditions in their native region and suffered fitness trade-offs when moved outside that region. However, observed fitness values did not perfectly match those predicted by edaphic variables alone, indicating that other factors, such as competition, also contributed to plant fitness. Soil water content and concentrations of calcium, magnesium, sodium, and potassium were likely drivers of differential fitness. Plants either had limited dispersal ability or migrants experienced early-season mortality outside their native region.
- *Conclusions:* Demonstrating that continuous habitats can support differently adapted, yet closely related, taxa is important to a broader understanding of how species are generated and maintained in nature.

Key words: aster models; Asteraceae; edaphic adaptation; habitat isolation; *Lasthenia*; local adaptation; plant distributions; plant soil relations; reproductive isolation; serpentine.

Ecological factors play an important role in the generation and maintenance of species (reviewed in Givnish, 2010; Sobel et al., 2010). Darwin and Wallace first provided a foundation for understanding the contributions of natural selection and adaptation to the speciation process, and yet the direct connection between adaptation and reproductive isolation is still unclear (reviewed in Coyne and Orr, 2004; Schemske, 2010; Sobel et al., 2010). As organisms adapt to the myriad ecological pressures of their environments, selection for adaptive traits can lead to uniquely adapted genotypes (e.g., Clausen et al., 1941; Kruckeberg, 1951; Sork et al., 1993; reviewed in Linhart and Grant, 1996; Kawecki and Ebert, 2004; Leimu and Fischer, 2008). Divergent adaptation can affect numerous components of reproductive isolation and therefore contribute to the generation and maintenance of closely related species.

Habitat isolation is the reduction in gene flow between populations caused by spatial separation of the habitats to which they are differently adapted (Mayr, 1947). Since habitat isolation precludes the opportunity for mating, it is one of the earliest acting reproductive barriers and has a disproportionately large effect on total reproductive isolation (Ramsey et al., 2003; Kay, 2006). Habitat isolation requires both divergent adaptation in habitat affinity, in which migrants between habitats are selected against, and a reduction in the likelihood of mating for individuals living in the different habitats. On a landscape scale, habitat isolation can maintain allopatric ranges when successful expansion into another species' range is prevented (i.e., ecogeographic isolation, Ramsey et al., 2003; Angert and Schemske, 2005). On a local scale, within the range of normal dispersal, habitat isolation can facilitate sympatric or parapatric coexistence of close relatives (McNeilly and Antonovics, 1968; Gardner and MacNair, 2000; Sambatti and Rice, 2006; Matute et al., 2009).

Although it is clear that edaphic environments vary over small spatial scales (van der Putten et al., 2004) and can act as agents of divergent selection (Ettema and Wardle, 2002; Rajakaruna, 2003, Baythavong and Stanton, 2010), previous investigations of small-scale habitat isolation typically have considered taxa that occur on and off discrete soil types (e.g., serpentine, mine tailings). In these cases, plants occur well within mating distance of each other but are maintained as genetically distinct populations due to low fitness on the adjacent harsh soil (e.g., McNeilly, 1968; McNeilly and Antonovics, 1968; Antonovics and

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Bradshaw, 1970; Searcy and Macnair, 1993; Gardner and MacNair, 2000; Sambatti and Rice, 2006; Wright et al., 2006; Baythavong and Stanton, 2010). For example, serpentine soils often provide highly divergent habitats that impose strong selection for tolerance even in the face of gene flow from nontolerant populations (reviewed in Kruckeberg, 1984; Brady et al., 2005; O'Dell and Rajakaruna, 2011). Studies on contrasting soil types suggest that adjacent discrete habitats can drive or maintain divergence (Harrison et al., 2000; Anacker et al., 2011), but the contribution of continuous differences in habitat to reproductive isolation, especially over small scales, is much less understood.

To address the degree over which small scale continuous edaphic gradients can contribute to habitat isolation between closely related species, we investigated the coexistence of two nearly indistinguishable species of *Lasthenia* (Asteraceae) on a serpentine hillside. The parapatric distribution of *Lasthenia gracilis* (DC.) Green and *L. californica* DC. ex Lindl. has been stable for at least 30 years (Rajakaruna and Whitton, 2004; Bohm and Rajakaruna, 2006), and hybrids are rarely found, although they can be created in the greenhouse (J. M. Yost, unpublished data). Habitat isolation might be playing an important role in limiting gene flow between these taxa, since other more obvious reproductive barriers are unlikely and the species occur within such close proximity (inches from one another). Although many soil variables important for plant growth vary continuously across the ridge, spatially intensive sampling has failed to find any abrupt differences in soil characters that might be used to define the species' edaphic niches (Rajakaruna and Bohm, 1999). Rajakaruna and Bohm (1999) found that the bottom of the ridge is wetter and more ionically extreme (higher concentrations of toxic ions), whereas the top of the ridge is drier and more ionically benign. Small-scale variation in ion concentrations (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) and water availability may exert enough divergent selection within this serpentine ridge to maintain differently adapted species (Rajakaruna and Bohm, 1999; O'Dell and Claassen, 2006).

We sought to determine whether the coexistence of *L. gracilis* and *L. californica* could be explained by genetically based differences in habitat affinity. We attempted to isolate the edaphic variables driving divergence between the habitats and asked if soil variables change over time in each region. Finally, we asked whether selection or limited dispersal or both better explains the abrupt boundary between species. We hypothesized that different selection pressures and ecological specialization to soil conditions maintain the stable parapatric distribution of taxa and therefore contribute to habitat isolation. Alternatively, the distributions may appear stable due to the colonization history of the site and limited dispersal of seeds. We investigated these possibilities with a reciprocal transplant experiment across a small serpentine ridge (50×60 m). We measured soil variables twice a month to quantify the edaphic environment, and we genotyped germinating seedlings across the abrupt species boundary to look for evidence of dispersal.

MATERIALS AND METHODS

Study system—*Lasthenia* Cass. is a predominantly Californian genus of 21 taxa (Chan et al., 2001, 2002). The two most widespread members of the genus, *L. californica* DC. ex Lindl. and *L. gracilis* (DC.) Green, are self-incompatible spring annuals. *Lasthenia gracilis* was recognized only recently as meriting separate species status from *L. californica* based on molecular phylogenetic work (Chan et al., 2002). The only morphological difference between the two species is a subtle difference in pappus shape: *L. gracilis* typically has a flared, white pappus, whereas *L. californica* typically has a linear, brownish pappus (Chan et al., 2002). Both species occur on varied substrates, including alkali flats, serpentine soils, open grasslands, oak woodlands, and coastal bluffs (Ornduff, 1966; Rajakaruna and Bohm, 1999). *Lasthenia gracilis* occurs throughout southern California, whereas *L. californica* is predominantly found in northern California, but there is a large area of overlap in the ranges of the two species in central California. We have identified at least five sites where *L. gracilis* and *L. californica* grow side by side in sympatry or very close parapatry. At all mixed sites, the species are found on sloping hillsides, with *L. californica* in the lower swale portion of the hill and *L. gracilis* in the drier upland (J. M. Yost, unpublished data).

Jasper Ridge Biological Preserve (JR), 37°25′N and 122°2.5′W, is one such site where the two species co-occur in close parapatry. JR is a low ridge in the western foothills of the Santa Cruz Mountains at the base of the San Francisco Peninsula, San Mateo County, California. The serpentine ridge occurs at ca. 180 m in elevation and covers ca. 20 hectares. A fire road runs the length of the ridge and numerous studies (reviewed in Bohm and Rajakaruna, 2006), including ours, have established transects referenced from this road. Our study site occurs entirely to the west of the fire road on the southwest-facing slope of the ridge.

Previous work at Jasper Ridge has documented typical serpentine conditions including low K^{+} concentrations, low Ca to Mg ratios, and the presence of heavy metals (Kruckeberg, 1984; Rajakaruna and Bohm, 1999). The presence of high concentrations of Mg^{2+} in the soil limits the selective uptake of essential Ca^{2+}, resulting in difficult conditions for plant growth (Brady et al., 2005). The top and bottom of the ridge vary continuously in water availability, organic matter, ion concentrations, and heavy metals; yet, no abrupt shift has been observed in soil conditions that can explain the abrupt transition (over only meters) from *L. gracilis* at the top of the ridge to *L. californica* at the bottom of the ridge (Rajakaruna and Bohm, 1999).

Soils—*We collected a soil sample from each plot from the top 10 cm of soil every 2 wk from February through May. All soil samples were air dried and sent to A & L Western Agricultural Laboratories for analysis (Modesto, CA; soil test suite SNB1). Samples were analyzed for cation exchange capacity (CEC), organic matter (OM), estimated nitrogen release (ENR), pH, hydrogen (H^{+}), phosphorus (using Bray and Olsen methods), soluble salts, nitrogen (NO_3^{-}), sulfur (SO_4^{2-}), and extractable K^{+}, Mg^{2+}, Ca^{2+}, and Na^{+} (ppm). Additionally, every week from November to May, we measured volumetric water content of each plot by averaging three readings taken just outside each plot using a Spectrum Technologies TDR200 soil moisture meter.

Transplants—*In April 2009, we collected seed from ca. 20 *L. californica* individuals from the bottom of the serpentine ridge at JR (between 50–60 m below the fire road) and 20 *L. gracilis* individuals from the top of the serpentine ridge (between 5–20 m from the fire road). Before germination, we dipped all seeds in 1% bleach solution, rinsed them in deionized water, and placed them in petri dishes on wet filter paper. We placed the dishes at 4°C to simulate winter conditions, and after 4 d, we moved the dishes to a growth chamber (Conviron E7 Plant Growth Chamber, Winnipeg, Manitoba, Canada) with 12/12 h of light/dark with 18°C day and 12°C night temperatures. Approximately 2–6 d later when the radicle appeared, we transferred seedlings to 1-inch germination trays filled with a germination mixture (Sunshine mix #3 by SunGro Horticulture Canada, Vancouver, British Columbia, Canada). We watered the seedlings as needed to keep the germination mix moist. In mid-February 2010, we transplanted 20–50 mm tall seedlings into the field. We chose to plant seedlings instead of seeds to ensure that the plants survived through the germination stage. We, therefore, missed any selection acting on the germination phase and future work will address this shortcoming.

Our transplant plots spanned JR Trail Nine. We established four replicate plots (1×1 m) 5 m from the fire road at the top of the ridge, where *L. gracilis* occurs, four plots at the bottom of the ridge (58–64 m from the fire road), where *L. californica* occurs, and four plots at ca. 48 m from the fire road, in the transition zone between the two species. We randomly planted eight unrelated individuals of each species in an alternating pattern in each plot and tried to capture natural competitive interactions by not weeding the plots. Plant density in each plot was equivalent to natural densities. We watered the transplanted seedlings once a day for the first 3 d. To exclude the effects of transplant shock, we replaced any plant that died within the first 5 d with a new seedling.

Once the seedlings were established, we recorded survival every 2 wk. At the end of the season (May–June), we measured reproductive output by collecting and counting all flower heads and counting the total number of viable seeds

produced. Dark, full seeds were considered viable, while white or lighter, deflated seeds were considered inviable (N. Rajakaruna, personal observation).

***Dispersal*—**In February, March, and April 2011, we identified naturally occurring germinants to species along the 60 m transect from the fire road to the oak woodland boundary (this corresponds to Transect 1 in Rajakaruna and Bohm, 1999). We sampled a single individual at 0.25-m intervals from 0 to 30 m and from 50 to 60 m. We sampled more densely (0.2-m intervals) from 30 to 50 m, where the abrupt transition between species has been observed in mature plants. For preflowering seedlings, we collected young leaf tissue for genotyping with species-specific PCR. Starting in March and April, as plants began to flower, we differentiated the species using pappus morphology (Chan et al., 2002). To genotype leaf samples, we took advantage of a fixed 11-bp deletion in the ITS region of rDNA in *L. gracilis* (Chan et al., 2002) and developed species-specific primers for *L. californica* and *L. gracilis*. (*L. californica* forward: 5′-AGAACGACCCGTGAACTTGT, reverse: 5′-GGTTGCCCAAAGGGAAGT; *L. gracilis* forward: 5′-ATAGCAGAACGACCCGTGAA, reverse: 5′-CTCATGGTTGCCCAMGAAC). Genotyping to species required two PCRs—one with *L. californica* specific primers and one with *L. gracilis* specific primers. We froze leaf tissue prior to DNA extractions. We placed 2–3 mm of leaf tissue in 300 μL of a 10% Chelex solution (Chelex 100, Bio-Rad Laboratories, Hercules, California). We vortexed samples for 10 s, spun them briefly to ensure that plant material was in the solution, and then incubated them at 95°C for 20 min. After the incubation step, we vortexed the samples again for 10 s and briefly centrifuged them to separate contaminants and Chelex beads from the DNA in the supernatant. We diluted the supernatant from the Chelex extraction 1 : 1 with water and used it directly in the PCR. PCR consisted of 6 μL GoTaq Green PCR premix, 1.2 μL of each primer at 10 μmol/L, and 1 μL of diluted Chelex DNA for a total volume of 12 μL. The PCR program ran at 95°C for 1 min, followed by 30 cycles of 95°C for 1 min, 55°C for 30 s, and 72°C for 2 min, and finished with 72°C for 7 min. A positive result for one of the primer pairs indicates species identity. Hybrid plants will amplify with both primer pairs (J. M. Yost, unpublished data). Tissue from known species was concurrently extracted and used as positive controls. We binned the genotyping results by meter, resulting in 4–5 individuals per meter. If a meter position contained individuals from both species, we considered it a "mixed" location. Although seedlings begin germinating in November, young *Lasthenia* seedlings are indistinguishable from other species on the ridge, especially *Plantago erecta* (Plantaginaceae), and we were therefore unable to sample seedlings before February.

***Data analyses*—**Soil data from each plot from throughout the season were subjected to a principal component analysis to identify the major axes of variation separating the regions. Soil changes through time were analyzed with a series of linear regressions. Differences in the timing of mortality were analyzed with a survival analysis using a log-rank test in the program JMP (ver. 9.0.0, SAS Institute, Cary, North Carolina, USA).

To compare the average fitness of individuals of both species in the three regions of the hillside, we used a hierarchical modeling approach called aster analysis (Shaw et al., 2008). In most studies, overall fitness is calculated as survival multiplied by various components of fitness. Individuals that do not survive introduce many zeros into the distribution of overall fitness values. This nonnormal fitness distribution complicates and violates many traditional statistical tests, such as ANOVA. Nonparametric modeling approaches, such as GLM with a log link function, have also been used to analyze lifetime fitness data, but they are unable to estimate the effects of individual fitness components or fit unique distributions to fitness parameters. Aster models, developed for implementation in R (R Core Development Team, 2008), allow for joint analysis of multiple fitness components that have different underlying distributions (Geyer et al., 2007; Shaw et al., 2008). Therefore, aster modeling provides a biologically and statistically appropriate method for evaluating both overall fitness differences and the individual components of fitness that contribute to total fitness. Our lifetime fitness model contained four fitness components: survival to flowering, number of inflorescences, total number of seeds, and number of viable seeds. We fit a Bernoulli distribution to survival, zero-truncated Poisson distribution to inflorescence production, Poisson distribution to seed set, and Bernoulli distribution to viable seed set. In addition to fitting appropriate distributions to the model parameters, aster analysis accounts for the dependence of one variable on all previous fitness components, a common shortcoming of other analysis methods.

We built a series of nested models to test the effect of species and region on fitness. In a separate modeling effort, we substituted the first two principal components of the soil variables for region to explicitly test for the effect of edaphic conditions on fitness. To obtain PC values for each plot, we averaged soil variables for each plot through time and conducted the PCA on the means. Using a regional model and a soil PC model, we estimated fitness based on two different, biologically meaningful fitness components, the number of viable seeds produced and inflorescence production. We estimated fitness based on inflorescence production because we experimentally moved self-incompatible individuals away from potential mates and we wanted to know how plants might be capable of reproducing if fully pollinated. This latter approach is a more conservative estimate of habitat isolation since it excludes the low probability of encountering compatible pollen for a rare migrant. In both models, aster estimates of the fitness component of interest (viable seed count and inflorescence production) reflect the contribution of all earlier fitness components even though they are not specifically estimated in our models (Shaw et al., 2008).

We used likelihood ratio tests to determine the best-fit models. We then obtained maximum likelihood estimates and standard errors for viable seed set and inflorescence production for each species.

RESULTS

***Edaphic environment*—**The principal component analysis of 17 soil variables showed a continuous transition in edaphic habitats from the top to the bottom of the ridge. The first and second principal components (PC1 and PC2) explained 32.9% and 12.9% of the variation, respectively, for a total of 45.8% (Table 1, Fig. 1). PC1 described the major differences between regions. The highest loading variable was the Ca to Mg ratio, which was much lower at the bottom indicating a habitat deficient in Ca^{2+} and enriched in Mg^{2+}. The bottom region had more positive values of PC1 indicating more OM, ENR, Mg^{2+}, pH, CEC, and VWC but lower concentrations of Ca^{2+} and K^{+}. The top of the ridge had negative values for PC1 indicating a habitat that is high in Ca^{2+}, K^{+}, but low in OM, ENR, Mg^{2+}, pH, and CEC. The middle region was intermediate. Linear regressions reveal directional changes in soil variable through time. Those regions of the ridge that experienced significant increases or decreases in a particular soil variable are presented in Table 2. Nonsignificant results are presented in Appendix S1 (see Supplemental Data with the online version of this article).

***Survival*—**Survival analysis showed that there was a significant difference in the timing of mortality between the two species in

TABLE 1. Principal component analysis of soil variation across a serpentine hillside at Jasper Ridge Biological Reserve. Bold values represent loading scores above 0.5.

Soil character	PC1 (32.9%)	PC2 (12.9%)
Volumetric water content	0.48853	0.34497
Organic matter (%)	**0.75794**	0.21498
Estimated nitrogen release (lbs/acre)	**0.75483**	0.21609
pH	**0.61004**	–0.03363
Cation exchange capacity (meq/100 × *g*)	**0.67677**	–0.29373
Hydrogen (meq/100 × *g*)	–0.49078	–0.06023
Soluble salts (mmhos/cm)	0.19061	**0.61881**
Nitrogen as NO_3^- (ppm)	0.15799	**0.53520**
Phosphorus (Bray-ppm)	–0.10495	**0.50794**
Phosphorus (Olsen-ppm)	–0.02619	0.37351
Sulfur as (SO_4^{2-} (ppm)	0.30576	0.20835
Calcium (ppm)	**-0.65884**	–0.33565
Magnesium (ppm)	**0.73606**	–0.26433
Potassium (ppm)	**-0.74634**	0.07372
Sodium (ppm)	0.36739	**-0.61921**
Ca : Mg	**-0.91599**	0.00625
K : Na	**-0.68323**	0.35666

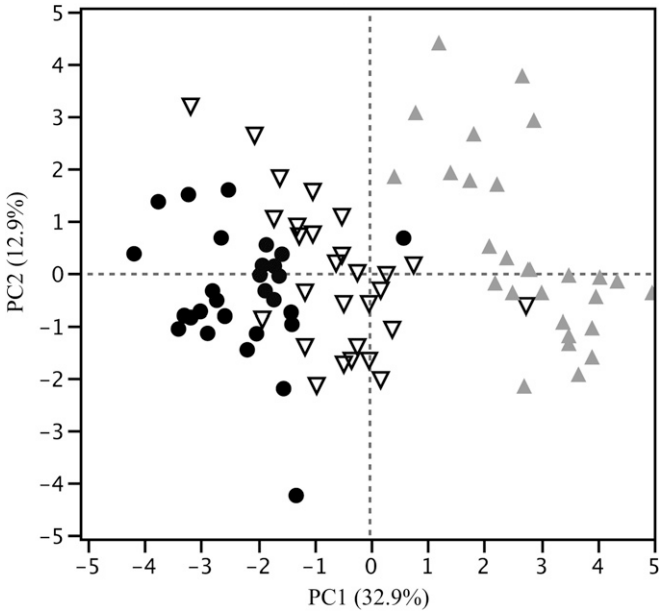

Fig. 1. Principal component analysis of 17 soil variables. The first two principal components (PCs) describe 45.8% of the variation in soil characteristics among the individual plots at Jasper Ridge. Points represent plot values for the top region (black circles), middle region (open triangles), and bottom region (closed triangles). See Table 1 for loading scores.

the bottom region and the middle region, but not in the top region (Fig. 2). *Lasthenia gracilis* died earlier than *L. californica* in the middle region (log-rank, $P = 0.0077$) and in the bottom region (log-rank, $P < 0.0001$).

***Overall fitness*—**We fit two different models to our fitness data (Table 3). In the regional model, we tested for an effect of species and region. The addition of the interaction term species × region to our model improved the fit significantly (Table 3). This is evidence that each species is uniquely adapted to its home site. We estimated fitness using two fitness components, viable seed set and inflorescence production, and we observed the same pattern in each. Therefore, we only show estimates for viable seed set (Fig. 3). *Lasthenia californica* in its native region produced more inflorescences and set more viable seed than in the other two regions. *Lasthenia gracilis* reached maximum fitness in the middle region, in both inflorescence production and viable seed set. Each species had a home site advantage in its native region (Fig. 3).

To explicitly test for the effect of edaphic conditions on plant fitness, we ran a separate aster model replacing region for the principal components of soil variables. By using the principal components, we took into account the variation among plots in each region and directly correlated edaphic variation with plant fitness. The model containing only the species × PC1 interaction term was the best-fit model and was used for subsequence analyses (Table 3). Adding the three-way interaction term of species × PC1 × PC2 was only marginally significant, and we chose not to include it. The significant interaction between PC1 and species indicates that the species are divergently adapted to edaphic factors. *Lasthenia gracilis* had the highest fitness in plots with the lowest PC1 values (higher and K^+, lower Mg^{2+}) (Fig. 4). For *L. californica*, high fitness was correlated with positive PC1 values (Fig. 4). The same pattern was observed using both viable seed set and inflorescences production, meaning that the rarity of conspecific mates had only a minor impact, if any, on fitness. We report the results from viable seed set only (Fig. 4).

TABLE 2. Edaphic changes through time. Linear regression analyses for soils collected every 2 wk at Jasper Ridge. Significant results are presented here. All analyses can be found in Appendix S1 (see Supplemental Data with the online version of this article).

Soil character	Slope	r^2	F	P
Volumetric water content				
Top	−6.02E-06	0.45754	21.92	<0.0001
Middle	−5.34E-06	0.368494	15.17	0.0006
Estimated nitrogen release (lbs/acre)				
Top	1.48E-06	0.13634	4.1044	0.0531
Phosphorus (Bray-ppm)				
Middle	−0.000002	0.224788	7.5392	0.0108
Phosphorus (Olsen-ppm)				
Middle	−9.64E-07	0.132141	3.9588	0.0572
Bottom	−1.26E-06	0.217246	7.2161	0.0124
Magnesium (ppm)				
Top	0.0001165	0.409614	18.039	0.0002
Middle	0.0001185	0.462786	22.3979	<0.0001
Calcium (ppm)				
Middle	6.54E-06	0.168258	5.2597	0.0302
Sodium (ppm)				
Bottom	1.80E-06	0.431419	19.7279	0.0001
Cation exchange capacity (meq/100 × g)				
Top	1.02E-06	0.38298	16.138	0.0004
Middle	9.67E-07	0.461116	22.2478	<0.0001
Nitrogen as NO_3^- (ppm)				
Top	−5.62E-07	0.180587	5.73	0.0242
Ca : Mg ratio				
Top	−6.49E-09	0.329397	12.7711	0.0014
Middle	−3.83E-09	0.21759	7.2307	0.0123
Bottom	−3.06E-09	0.224564	7.5295	0.0109
K : Na ratio				
Middle	−1.69E-07	0.402039	17.4811	0.0003
Bottom	−1.84E-07	0.291023	10.6725	0.0031

***Dispersal*—**Our results indicated that *L. gracilis* and *L. californica* seedlings occurred from 0–50.0 m and from 43.0–60.0 m along the ridge, respectively. There was a region of overlap between 43.0 m and 50.0 m along the ridge; however, there was no observed constriction of the mixed region between February and April as would be expected if late season mortality was responsible for maintaining species distributions. Of over 1000 plants genotyped, only two hybrids were found. These hybrids were collected during the March collection at 31.75 and 36.4 m.

DISCUSSION

Lasthenia as a genus lacks many of the exciting morphological differences that have motivated other studies of reproductive isolation. Among the 21 taxa in the genus, there are no obvious differences in flower morphology, scent, or color, and flowers are pollinated by a suite of generalist pollinators (Ornduff, 1966; Emery et al., 2012). The discovery of cryptic diversity within the genus and the wide range of edaphic tolerances present in the genus allow us to link more obscure but important mechanisms of ecological divergence, such as physiological tolerance, to reproductive isolation. Here we have investigated the role habitat isolation plays in the parapatric coexistence of

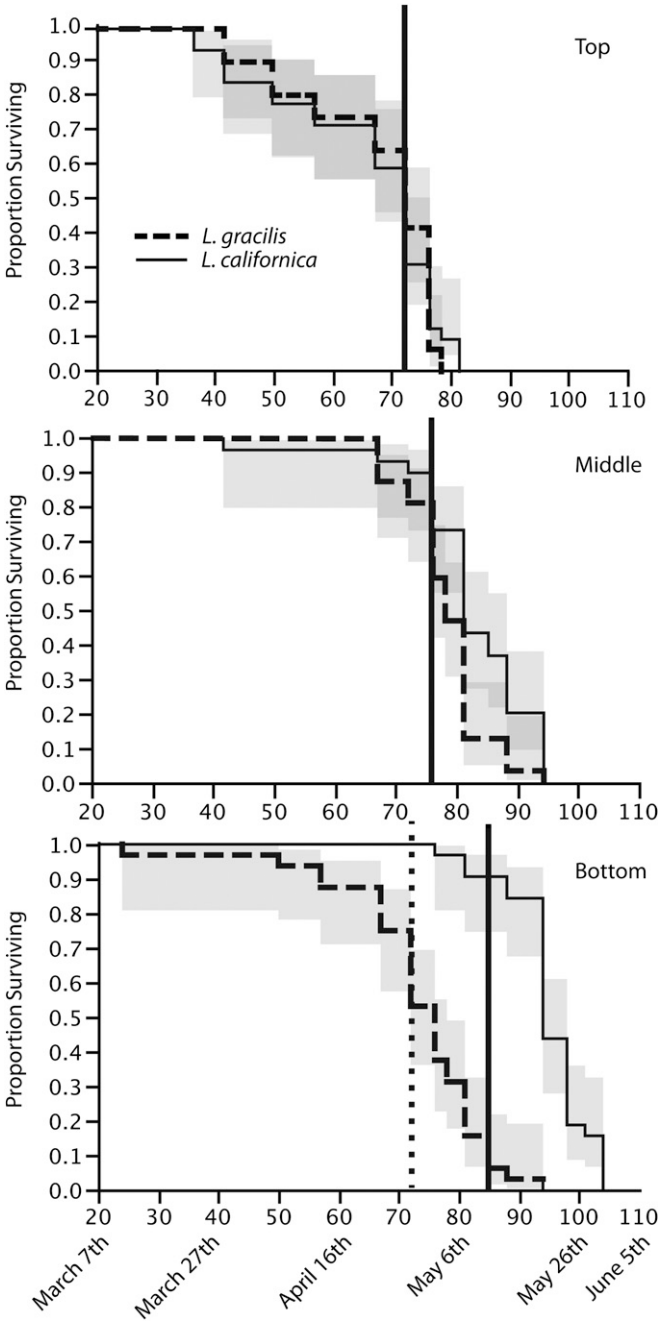

Fig. 2. Survival over time since planting date of the two species in transplanted regions. There was no difference in the timing of mortality between species at the top of the ridge (log-rank, $P = 0.9721$). There is a significant difference in timing of mortality between the species at the middle and bottom of the ridge (log-rank, $P = 0.0077$ and $P < 0.0001$) with *L. californica* surviving longer in these regions. Shaded regions are pointwise confidence intervals. The vertical solid line indicates peak flowering of *L. californica*. The dotted vertical lines indicates peak flowering of *L. gracilis*. When only one line is present, peak flowering for both species occurred on the same census date.

L. californica and *L. gracilis*. The first step in documenting habitat isolation is to show that species are differently adapted. Here we showed that *L. californica* and *L. gracilis*, two cryptic close relatives, can co-occur on a 60 m serpentine ridge because they are differently adapted to unique edaphic conditions within serpentine soils.

To understand how *L. californica* and *L. gracilis* could co-occur in such close proximity, we had to first document potential agents of divergent selection. The close associations plants have with soil implicate edaphic variables as important predictors of plant fitness (Ettema and Wardle, 2002). Our work confirmed that the top, middle, and bottom of the ridge vary continuously in edaphic conditions (Rajakaruna and Bohm, 1999). The most important variables contributing to the differences between the top and bottom of the ridge, as expressed in PC1, are the Ca to Mg ratio, K^+, OM, and ENR. The top of the ridge is drier but more hospitable for plant growth (higher Ca^{2+} and K^+) than the bottom of the ridge, which is wetter, but ionically harsher (high Mg^{2+}). We also document dynamic shifts in soil characteristics through time. The bottom region becomes more ionically hostile to plant growth as seen in decreasing Ca to Mg and K to Na ratios through time. The top and middle regions both undergo significant drying as compared to the bottom region. These changes could have implications for the timing of selection against migrants or maladapted genotypes. While the heterogeneous nature of serpentine soils is gaining appreciation (Kruckeberg, 1984; Baythavong and Stanton, 2010), the implications of this heterogeneity on plant fitness and reproductive isolation are not well understood.

Reciprocal transplant experiments reveal how isolated two taxa might be due to different ecological adaptations. Using aster models we predicted unconditional fitness means using a regional model and a soil PC model. On the basis of fitness estimates in both the regional and soil PC models, if *L. californica* were to disperse uphill (even a distance of 5 m) into the *L. gracilis* region, its fitness would decrease substantially and reach nearly zero at the top of the ridge. When we estimate fitness using inflorescence production, we find the same pattern as observed in the fitness estimates using viable seed set. This indicates that the low fitness of *L. californica* in the middle and top regions of the ridge is not due simply to a lack of successful pollinations. When we isolate the effects of edaphic variation in the soil PC model, *L. californica* shows a significant positive response in fitness to increasing PC1 values, showing that it is uniquely adapted to the conditions in the bottom region. Predicting plant fitness using PCs takes into account the heterogeneity present within each of our predefined regions, but excludes other biologically important predictors of fitness, such as plant community composition.

Modeling fitness based on region versus PCs resulted in two different patterns for *L. gracilis*. In the regional model, *L. gracilis* has maximum fitness in the middle region, whereas the soil PC model shows a negative linear relationship between PC1 and fitness. While PC1 values roughly correspond to our categories of region (top, middle, and bottom; Fig. 1), they do not encompass all of the variation among these categories. Other abiotic or biotic factors must be reducing the fitness of *L. gracilis* in the top region, such that it has higher fitness in the middle region. Competition with different plant assemblages is a possible explanation (Bischoff et al., 2006). We can, however, conclude that each species is differently adapted to the edaphic extremes found on the ridge, as expressed by PC1. If *L. gracilis* were to disperse downhill, a likely scenario for gravity-dispersed seeds, it would actually have higher fitness toward the middle of the ridge, despite the increasing PC1 values, but only to a point. In the bottom region, *L. gracilis* has low fitness compared to *L. californica*, according to both the regional model and soil

TABLE 3. Model comparisons to test for the effects of region, species, and principal components of the soil variables. The test deviance is twice the log likelihood ratio. Statistical comparisons were made between nested models. A significant analysis of deviance indicates improvement of the model following the addition of a new factor or interaction. Fitness estimates presented in Figs. 3 and 4 are from the best-fit models (in bold).

Model	Model df	Model deviance	Test df	Test deviance	*P*
Regional model					
Species + region	7	–92018			
Species + region + species × region	**9**	**–92122**	**2**	**104.6**	**<0.0001**
Soil PC model					
Species + PC1	6	–91967			
Species + PC1 + PC2	7	–91971	1	4.221	0.03993
Species + PC1 + PC2 + species × PC1	**8**	**–92030**	**1**	**58.278**	**<0.0001**
Species + PC1 + PC2 + species × PC1 + species × PC2	9	–92031	1	1.7396	0.1872
Species + PC1 + PC2 + species × PC1 + species × PC1 × PC2	11	–92037	3	7.6574	0.05365

PC model. The low fitness of *L. gracilis* in the bottom region is likely explained by the inability of *L. gracilis* to tolerate higher concentrations of Mg^{2+} and Na^{+}. This has been experimentally tested in hydroponic studies in which the two species were found to have genetically based differences in their ion physiologies (Rajakaruna et al., 2003b). When grown in ionically extreme conditions, *L. californica* accumulated more Mg^{2+}, Na^{+}, and Ca^{2+} in its tissues than did *L. gracilis*. The inability of *L. gracilis* to accumulate ions provides a potential mechanism for its low fitness in the bottom region. Ion accumulation is one mechanism plants use to take up water in ionically extreme soils

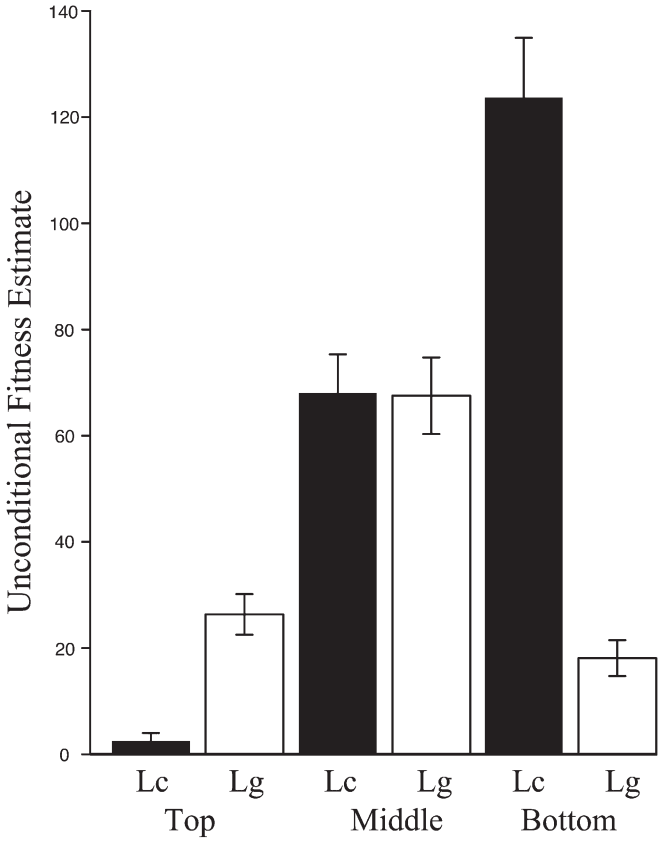

Fig. 3. Unconditional fitness estimates from the aster regional model presented in Table 3. *Lasthenia californica* (Lc) occurs at the bottom of the ridge and *L. gracilis* (Lg) occurs at the top of the ridge. Error bars are ±1 SE. The same pattern was observed in fitness estimates based on inflorescence production.

(Khan et al., 2000; Rajakaruna et al., 2003a; Ebrahimi and Bhatla, 2011; Nardini et al., 2011). In the transition zone, both models show that *L. gracilis* and *L. californica* have equal fitness, likely preventing one species from dominating. We find that regardless of whether we estimate fitness from inflorescence production or viable seed set, both species are at an advantage in their native region.

Initially, we had hoped to correlate the change in soil variables through time with the establishment of the abrupt boundary between the species. Collecting germinating seedlings across the ridge showed that the region of overlap (43–50 m) between the two species is established as early as February. Based on our transplant results, if there was a great amount of seed dispersal, we would expect at least some seeds to survive long enough to flower. However, the dispersal structures on *Lasthenia* seeds are reduced and are not expected to facilitate long-distance dispersal. We had originally hypothesized that possible migrants would experience late season mortality associated with the onset of the summer drought at the top of the ridge and more concentrated ionic conditions at the bottom. While the top and bottom regions do change in the expected way, our results suggest that the boundary is established much earlier in the season. It is possible that there is seed dispersal followed by early season (pre-February) mortality, which we did not estimate. Additionally, we chose to plant seedlings and therefore missed any selection against the seed/germination phase of the life cycle. Previous greenhouse experiments show that under increasing Na^{+} and Mg^{2+} concentrations *L. gracilis* seeds do not germinate as well as *L. californica* seeds (Rajakaruna et al., 2003b), leading us to conclude that our fitness estimates are conservative.

The narrow habitat use observed at JR is not generally characteristic of *L. gracilis* and *L. californica*, since both are known to occur in a wide variety of habitats, including alkali flats, serpentine soils, open grasslands, oak woodlands, and coastal bluffs (Ornduff, 1966; Rajakaruna and Bohm, 1999). It is possible that direct competition between the two species causes them to shift habitat use at sympatric sites, or alternatively, that only those populations with sufficient ecological differences can persist in the same area. We have observed similar habitat partitioning at other mixed sites throughout California. When found together, *L. californica* is consistently found in lower wetter depressions, and *L. gracilis* is found on higher hilltops, likely mirroring the difference we found at JR (J. M. Yost, unpublished data). However, the range of habitats in which both species occur at allopatric sites does not appear to follow the pattern observed at sympatric sites (Choe, 2007; J. M. Yost, unpublished data).

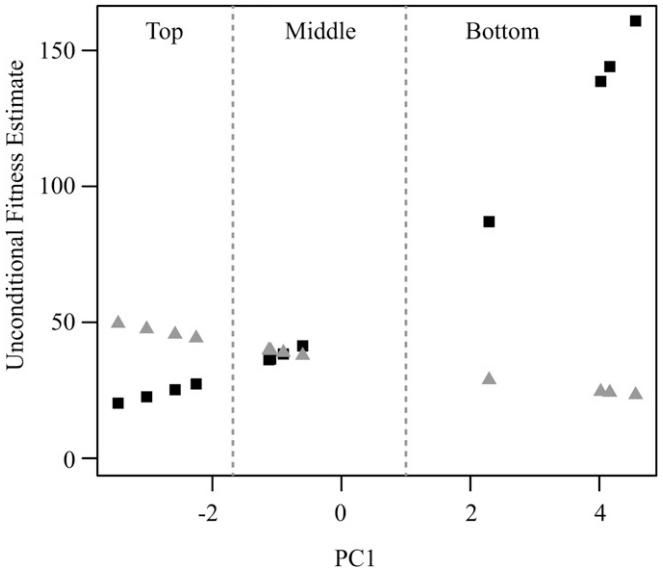

Fig. 4. Unconditional fitness estimates from the aster soil principal components model presented in Table 3. *Lasthenia californica* (solid squares) occurs at the bottom of the ridge and *L. gracilis* (gray triangles) occurs at the top of the ridge. The same pattern was observed in fitness estimates based on inflorescence production.

Habitat isolation is only one component of reproductive isolation. This study establishes the role of selection acting against potential migrants at JR, but there is also a documented difference in flowering time of 7–10 d between the top and bottom of the ridge (Rajakaruna and Bohm, 1999). In this case, the earlier drying soils at the top might indirectly cause a shift in flowering time, which further reduces gene flow between the species. Due to the likelihood of shared pollinators at JR, and the overlap of flowering time at the transition zone (~46 m), there may be postpollination barriers present between the taxa. Preliminary data shows that reduced pollen adhesion and pollen tube growth limit the number of hybrid seeds created (Rajakaruna and Whitton, 2004). Finally, our study did not measure the fitness of hybrid offspring. Even if the hybrids are viable in the greenhouse, the lack of suitable habitat for hybrids, or direct competition with parent taxa, might also function as postzygotic barriers in this system. Our future work will address these possibilities.

Habitat isolation creates a direct link between an organism's ecology and reproductive isolation (Ramsey et al., 2003; Angert and Schemske, 2005; Schemske, 2010; Kay et al., 2011). Recent reviews of plant speciation studies have highlighted the pervasiveness and importance of ecologically based reproductive barriers in general, and habitat isolation specifically (Lowry et al., 2008; Schemske, 2010). Habitat isolation is consistently found to be important between closely related populations or species on and off serpentine soil (Kay et al., 2011), but our work shows that even small-scale heterogeneity within a serpentine outcrop can generate large fitness trade-offs.

Demonstrating that continuous habitats can support differently adapted, yet closely related, taxa is important for a broader understanding of how species are maintained in nature. Detecting divergent adaptation has direct implications for the geographic scale of speciation, as habitat partitioning can contribute to reproductive isolation (Coyne and Orr, 2004; Schemske, 2010; Sobel et al., 2010). While the pervasive role of selection in creating and maintaining species is gaining appreciation (Givnish, 2010; Schemske, 2010; Sobel et al., 2010), this work demonstrates that selection can contribute to reproductive isolation over small spatial scales and continuous environmental gradients.

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