HOW ARE RARE SPECIES MAINTAINED?: REPRODUCTIVE BARRIERS

BETWEEN *LAYIA JONESII*, A RARE SERPENTINE ENDEMIC,

AND *L. PLATYGLOSSA*

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                                    Barriers Between *Layia jonesii*, A Rare Serpentine
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ABSTRACT

How Are Rare Species Maintained?: Reproductive Barriers Between *Layia jonesii*, A
Rare Serpentine Endemic, And *L. platyglossa*
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Reproductive barriers are vital to generating new species as well as maintaining distinct species. Investigating reproductive barriers between closely related plant taxa helps us to understand how these barriers are maintained, particularly between rare and widespread relatives. *Layia jonesii*, a rare San Luis Obispo County serpentine endemic, and *L. platyglossa,* a common coastal species, co-occur on serpentine derived hillsides and are interfertile. At these locations, *L. jonesii* is isolated to dry soils near serpentine rock outcrops and *L. platyglossa* is located on slightly deeper grassland soils surrounding the rock outcrops. On hillsides where they co-occur, I observe two morphologically distinct species, therefore the two species must be maintaining reproductive barriers, yet mechanisms that maintain this isolation are unknown. I studied this system to investigate possible mechanisms contributing to the maintenance of reproductive barriers. I hypothesize prezygotic reproductive isolation in this system is due to (1) habitat isolation due to local adaptation to differential edaphic environments on the hillside, (2) flowering time differences, and (3) reduced seed set resulting from hybrid crosses. To investigate the local adaptation of *L. jonesii* and *L. platyglossa,* I reciprocally transplanted both species into the center of each species' distribution. I also conducted a competition experiment to determine if *L. jonesii* is sensitive to resource competition beyond its natural distribution. To investigate flowering time differences, I tracked flowering time of both wild and reciprocally transplanted populations. I also performed controlled crosses to determine if heterospecific, or hybrid crosses, result in lowered seed set than conspecific crosses. The reciprocal transplants showed *L. platyglossa* is locally adapted to the grassland habitat. Local adaptation likely prevents *L. playtyglossa* from dispersing into the rock outcrop habitat. Results of the competition experiment revealed *L. jonesii* is sensitive to competition and this may contribute to its constrained distribution to shallow soils. Local adaptation and competition likely contribute to habitat isolation between the two species. I also documented stark differences in flowering time between the species which contributes to reproductive isolation by reducing pollen flow. Hybrid crosses also resulted in lowered seed set than conspecific crosses. These results suggest prezygotic barriers to reproduction likely maintain the majority of isolation between the two species. These results provide insight into mechanisms that maintain reproductive barriers between closely related taxa existing in similar habitats. The results also contribute to our understanding of how rare plants preserve genetic integrity near common and interfertile relatives.

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CHAPTER 1

INTRODUCTION

Charles Darwin was one of the first scientists to recognize the importance of barriers to gene exchange as vital to the origin and maintenance of species (Darwin, 1859). Since Darwin, many evolutionary biologists have studied and characterized reproductive barriers, including Mayr (1947), whose biological species concept is founded on the importance of reproductive barriers. Today, studies of reproductive barriers remain essential for understanding the generation and maintenance of species and for understanding biodiversity.

Reproductive barriers maintain reproductive isolation between species and are commonly divided into two broad categories: prezygotic and postzygotic barriers. Prezygotic barriers prevent the formation of a zygote. Types of prezygotic barriers in plants include ecogeographic or habitat isolation (Ramsey et al., 2003; Angert and Schemske, 2005), temporal isolation through flowering time differences (Lowry et al., 2008), pollinator isolation (Hodges et al., 2002; Miller et al., 2014), and postpollination isolation that can occur due to genetic incompatibilities of stigma and style (Kay, 2006). Post-zygotic barriers prevent gene flow after a zygote is formed and include hybrid inviability (Christie and Macnair, 1987) and reduced hybrid fitness (Kay, 2006). Many different types of reproductive barriers contribute to total reproductive isolation between taxa (Sobel and Chen, 2014).

The majority of reproductive isolation between plant taxa is due to prezygotic barriers to reproduction (Ramsey et al., 2003; Sobel et al., 2010). Previous studies show prezygotic barriers can contribute to greater than 99% of total reproductive isolation

between some plant species (Ramsey et al., 2003; Kay, 2006). A large portion of isolation between these species is due to ecological interactions between plants and their environment, rather than genetic incompatibilities between species. This research suggests differential ecological adaptation is not only crucial for maintaining species but is also a factor driving speciation (Schemske, 2010).

Habitat isolation, caused by differential adaptation between two plant species, reduces reproductive contact between the two species and therefore acts as a strong prezygotic isolating mechanism (Nosil et al., 2005; Sobel et al., 2010). Habitat isolation contributes to reproductive isolation both between closely related species and among differentially adapted populations, or ecotypes, of the same species (Clausen et al., 1947; Kruckeberg, 1951; Ramsey et al., 2003; Kay, 2006; Sobel et al., 2010). Habitat isolation has commonly been studied using systems in which populations are separated by large geographic or elevation differences (Nagy and Rice, 1997; Ramsey et al., 2003), or exist in drastically different edaphic conditions (relating to the soil i.e. serpentine and non-serpentine soils; Kruckeberg, 1951; Brady et al., 2005; Wright et al., 2006). Few studies have focused on the contribution of habitat isolation to reproductive isolation between species that occur within mating distance at a small, local scale along edaphic gradients (but see Yost et al., 2012).

Understanding how reproductive barriers maintain rare species that occur within mating distance of interfertile and widespread relatives is especially important. Repeated hybridization of a rare species with a more abundant relative can be detrimental to population growth of the rare species and could potentially lead to extinction (Levin, 1996). Few studies have investigated which prezygotic barriers to reproduction contribute

to reproductive isolation between rare and common taxa occurring within mating distance along an edaphic gradient. Investigating how rare species in these types of systems maintain prezygotic reproductive isolation near common and widespread relatives, and therefore preventing hybridization, can help inform conservation efforts.

Layia jonesii A. Gray, a rare serpentine endemic in San Luis Obispo, California and *Layia platyglossa* (Fisch. & C.A. Mey) A. Gray, a widespread species, co-occur on serpentine derived hillsides along an edaphic gradient. These two species are interfertile **(**Clausen, 1951**)** and occur within a few meters of each other (parapatrically) at multiple sites in San Luis Obispo (California Consortium of Herbaria at *ucjeps.berkeley.edu/consortium/*). At these sites, *L. jonesii* is primarily isolated to rocky, dry soils near serpentine rock outcrops while *L. platyglossa* occurs in grassland habitats surrounding rock outcrops (Figure 1). Because the two species are capable of hybridizing, I would expect that over time the rare species would become extinct through introgression with a much more common and widespread relative. However, at sites where these species co-occur, I observe two intact and morphologically distinct species. Here, I characterize prezygotic reproductive barriers that contribute to reproductive isolation between *L. jonesii* and *L. platyglossa*. I hypothesize prezygotic reproductive isolation in this system is due to (1) habitat isolation due to local adaptation to differential edaphic environments on the hillside, (2) temporal isolation due to flowering time differences, and (3) postpollination isolation through reduced seed set in hybrid crosses.

MATERIALS AND METHODS
Study System

The genus *Layia* Hook. & Arn. ex DC. consists of 14 spring-flowering annuals

endemic to California. Most species are self-incompatible, including *L. jonesii* A. Gray and *L. platyglossa* (Fisch. & C.A. Mey) A. Gray (Clausen, 1951). *Layia jonesii* and *L. platyglossa* are distinguished morphologically by pappus shape. *Layia jonesii* has a pappus of short scales, whereas *L. platyglossa* has pappus of long bristles. *Layia jonesii* is endemic to San Luis Obispo County, California on serpentine derived soils (Safford et al., 2005). The distribution of *L. jonesii* consists of only about 15 small, scattered populations from the city of San Luis Obispo north to Cayucos (California Consortium of Herbaria at *ucjeps.berkeley.edu/consortium/*). In contrast, *L. platyglossa* is a more widespread species ranging from Baja California to the northern coast ranges of California, occurring on a range of soil substrates including serpentine (Baldwin et al., 2012). Herbarium records document the two species growing sympatrically (intermixed populations) and parapatrically (adjacent populations) at multiple populations within San Luis Obispo County (California Consortium of Herbaria at *ucjeps.berkeley.edu/consortium/*).

The two species occur within meters of each other (parapatrically), which is well within mating distance, on serpentine derived hillsides in Reservoir Canyon Open Space (RC), San Luis Obispo County, California (35.284897, -120.618303). RC is part of a series of serpentine ridges that run parallel to the Santa Lucia Mountains in San Luis Obispo County. The ridge through RC is part of the Franciscan Complex, a coastal geologic formation consisting of mainly metamorphic substrates including serpentine. Multiple populations of both *L. jonesii* and *L. platyglossa* occur on serpentine derived soil within RC. At these populations, *L. jonesii* is restricted to rock outcrops while *L. platyglossa* occurs in grassland soils surrounding these rock outcrops. Although these two

species occur on different soils, there is little evidence of a stark line that divides the rock outcrop and grassland habitats. The two species exist along an edaphic gradient with some overlap in distribution toward the center of this gradient.

Soil Sampling

Soil was sampled from the four reciprocal transplant plots to characterize the soil chemistry at RC. I made three, 1L collections of soil from the top 15cm in each plot. The soil was tested at A&M Laboratories for organic matter percent, estimated nitrogen release (lbs/acre), phosphorus (Weak Bray and Olson Method, ppm), potassium (ppm), magnesium (ppm), calcium (ppm), sodium (ppm), soil pH, hydrogen (meq/100g), cation exchange capacity (meq/100g), and sulfur (ppm). I performed a principle component analysis with these soil characteristics with JMP.

To characterize physical characteristics of the soils at RC, I conducted one soil profile description in each of the grassland and rock outcrop habitats. Within each habitat, I described a soil directly adjacent to a reciprocal transplant plot. I described the depth, rock fragment content, texture, and structure for each horizon in both soil profiles. I also calculated the available water holding capacity for each soil profile using standard calculations based on texture and rock fragment content (Schoeneberger et al., 2012; Soil Survey Division Staff, 1993).

Reciprocal Transplant and Competition Experiment

I performed a reciprocal transplant between *L. jonesii* and *L. platyglossa* at RC. I established four reciprocal transplant plots (0.5m x 0.5m) on a serpentine hillside where

L. jonesii and *L. platyglossa* co-occur. Two plots were placed near the serpentine rock outcrop in previously documented *L. jonesii* habitat and two plots were placed in the grassland below the rock outcrop in *L. platyglossa* habitat. In each of the four plots, I planted two seeds from 16 maternal families of each species. In total, I planted 32 seeds of *L. jonesii* and 32 seeds of *L. platyglossa* for a total of 64 seeds per plot. I glued each seed to a color-coded toothpick to assist with tracking each plant through its life cycle. Each seed was randomly assigned a planting position within the plot. I planted the seeds in early November, 2013.

In addition to the reciprocal transplant experiment, I conducted a competition experiment. In both grassland transplant plots, I established two smaller sub-plots (weeded plots) adjacent to the reciprocal transplant plots in which I planted two additional seeds from the same 16 maternal families of *L. jonesii* for a total of 32 seeds per sub-plot. On a weekly basis, I weeded out all plants except for the planted *L. jonesii* seedlings.

Once the seeds germinated in both the reciprocal transplant and weeded plots, survival was recorded on a weekly basis until the end of the growing season. I quantified fitness by counting the number of viable seeds produced by each surviving individual for both the reciprocal transplant and competition experiments. Dark-colored, fully inflated seeds were considered viable. Pale-colored, deflated seeds were considered unviable. Germination and survival rates were analyzed using a generalized linear mixed model (PROC GLIMMIX) with SAS. I analyzed survival and viable seed set data from the reciprocal transplant and competition experiments using a 2-factor ANOVA with a randomized block design with JMP (ver 11.0.0, SAS Institute, Cary, North Carolina,

USA). I transformed the viable seed set with a square root before our analysis to account for non-constant variance in our dataset.

Flowering Time

I tracked flowering time in wild and reciprocally transplanted populations. To track flowering time in wild populations at RC, I established a plot (0.5m x 0.5m) in the center of each species distribution. I counted the number of opened and unopened heads within each plot weekly over a four-week period from mid-April to early May. An open head was defined as having at least one open disk floret. Using this data, I calculated the proportion of open heads per week in wild populations of *L. jonesii* and *L. platyglossa*. To track flowering time in reciprocally transplanted populations, I documented the number of open heads on individuals within the transplant plots on a weekly basis. I analyzed flowering time data from the reciprocal transplant using a 2-factor ANOVA with a randomized block design with JMP.

Controlled Crosses

Plants used for the controlled crosses were germinated from seeds collected at RC. I used 12 individuals of *L. jonesii* and 8 individuals of *L. platyglosssa.* As the individuals began to bloom, each head was randomly designated as one of three types of crosses. The first type of cross was a conspecific cross, meaning pollen from a different individual of the same species was used to pollinate the inflorescence. The second type of cross was a heterospecific cross, or hybrid cross, meaning pollen from a different species was used to pollinate the head. The third type of cross was a control meaning the head

was not manipulated and received no pollen. I performed the controlled crosses by rubbing open heads together every two days during the entire period the head was open. The average number of crosses per head was 4.17 with a range of 1-8. I counted the viable number of seeds produced by each cross. I analyzed the seed set for each type of cross using a 2-factor ANOVA with a randomized block design with JMP.

RESULTS

Soil Characteristics

To characterize the chemical edaphic environment on the serpentine hillside, we conducted a principle component analysis of 13 chemical soil characteristics in both the grassland and rock outcrop habitats. The first two principle components describe 67.3% of the variation in soil characteristics between the two habitats in RC (Table 1, Figure 2). The analysis shows there is little variation between the rock outcrop and grassland soils, but there is similar variation among soil characters within both soils. Low levels of essential nutrients like phosphorus, potassium, and calcium along with high levels of magnesium have loading scores above 0.5 and therefore drive variation within the soils. This pattern of nutrient content is characteristic of serpentine soils (Alexander et al., 2007).

To characterize the physical edaphic environment on the serpentine hillside, I conducted a soil pedon description in the rock outcrop and grassland habitats. Both soil profiles show evidence of soil development by the presence of an illuvial clay horizon (B_t). The rock outcrop and grassland soils were a similar depth, however, the outcrop soil contains a higher percentage of rock fragments in all solum horizons (horizons above the bedrock, C_r) compared to grassland soil. The presence of high levels of rock fragments in

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the solum reduces the available water holding capacity of rock outcrop soils. The available water holding capacity of the rock outcrop soil is about half of the water holding capacity of the grassland soil (rock outcrop soil 1.9 cm; grassland soil 3.75 cm; Table 2).

Reciprocal Transplant and Competition Experiment

To investigate if habitat isolation contributes to reproductive isolation between *L. jonesii* and *L. platyglossa*, we sought to determine if either species is locally adapted to specific habitats on the hillside in RC using a reciprocal transplant. To determine if the grassland and rock outcrop habitats effect plant fitness during the early portion of the life cycle, we tracked the germination and survival to flowering. Germination proportions of *L. jonesii* and *L. platyglossa* were similar across all habitats (Table 3 and 4; Figure 3). Proportion of *L. jonesii* individuals and *L. platyglossa* individuals surviving to flowering was similar across all habitats (Table 5 and 6; Figure 4). Both species survived longer, regardless if they flowered or not, in the grassland habitat. Individuals survived 24 days longer in the grassland habitat than the rock outcrop habitat. (F=6.2956, P=0.0142; Table 7).

We used viable seed set to determine the reproductive fitness of *L. jonesii* and *L. platyglossa* in the rock outcrop and grassland habitats in RC. Results from the reciprocal transplanted showed the reproductive fitness of both *L. jonesii* and *L. platyglossa* was higher in the grassland habitat than the rock outcrop habitat (F=25.16, P<0.0001; Table 8; Figure 6). *Layia jonesii* showed a somewhat higher fitness in both the rock outcrop and grassland habitats compared to *L. platyglossa*. Results from the competition experiment

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showed *L. jonesii* had a higher reproductive fitness when competition was removed in the weeded grassland plots than when competition was not removed in the un-weeded grassland plots (weeded grassland 298.3 viable seeds per individual, 95% CI 178.71-448.46; un-weeded grassland 46.47 viable seeds per individual, 95% CI 4-104.85; F= 18.0, P=0.0004; Figure 6).

Flowering Time

To determine if flowering time differences, or temporal isolation, contribute to reproductive isolation between *L. jonesii* and *L. platyglossa*, we tracked flowering time of reciprocal transplant and wild populations. Reciprocal transplant populations in both the rock outcrop and grassland habitats showed an earlier flowering time for *L. jonesii* than *L. platyglossa* (F=18.7, P=0.0003). In the rock outcrop plots, *L. jonesii* flowered 7.99±2.61 days earlier than *L. platyglossa*, and in the grassland plots *L. jonesii* flowered 5.98±2.17 days earlier than *L. platyglossa*. Both species showed a slight delay in flowering time in the grassland habitat compared to the rock outcrop habitat (Figure 7). Wild populations of *L. jonesii* also flowered earlier than wild populations of *L. platyglossa*. Peak flowering time for wild *L. jonesii* was about 15 days earlier than wild *L. platyglossa* (Figure 8).

Controlled Crosses

To determine if any postpollination reproductive barriers exist between *L. jonesii* and *L. platyglossa*, we conducted controlled crosses in the greenhouse. For both species, conspecific crosses resulted in a higher viable seed set than heterospecific crosses

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(F=80.70, P<0.0001). Because both species are self-incompatible, I am confident the viable seeds that resulted from heterospecific crosses are hybrid seeds rather than the result of a self-fertilization event. Control heads were not manipulated during the crosses and produced no viable seeds (Figure 9).

DISCUSSION

Local Adaptation

Prezygotic reproductive barriers contribute most strongly to reproductive isolation between plant taxa (Sobel et al., 2010; Schemske, 2010). Specifically, habitat isolation via differential adaptation acts as a strong reproductive barrier in many systems (e.g., between coastal and inland taxa (Lowry et al., 2008), high and low elevation taxa (Angert and Schemske, 2005), and edaphic specialists (Wright et al., 2006)), but it may also contribute to reproductive isolation on a more local scale where species occur parapatrically. At RC, the distinct distribution of two species in the genus *Layia* on a hillside – with *L. jonesii* occurring mainly near a rock outcrop and *L. platyglossa* occurring in the grassland, lead me to hypothesize that local adaptation to different edaphic conditions may control the observed distribution. The rock outcrop and grassland habitats present on the serpentine derived hillside in RC exist within meters of each other, but although the soils are chemically similar, they are physically distinct. The rock outcrop habitat soils are rocky, and therefore, have about half the available water holding capacity of the grassland soils. This means the rock outcrop habitat is a drier environment than the grassland habitat.

The reciprocal transplant results indicate that both *L. jonesii* and *L. platyglossa* germinate and survive to flowering time at equivalent rates in both the rock outcrop and the grassland habitat, suggesting there is no selection against migrants of either species between the two habitats at the germination or early seedling stage. At the reproductive stage, reproductive fitness data (viable seed set) from the reciprocal transplant shows *L. platyglossa* is less fit in the rock outcrop habitat than the grassland habitat. The lowered reproductive fitness of *L. platyglossa* in the rock outcrop habitat provides support for the hypothesis that *L. platyglossa* is locally adapted to grassland soils, and this likely prevents the species from expanding into the rock outcrop habitat. *Layia platyglossa* likely not as fit in the rock outcrop habitat because of the dry conditions.

To determine if the distribution of *L. jonesii* is constrained to the rock outcrop habitat due to higher levels of competition in grassland soils, I created weeded grassland plots and examined the effect of removing competition on the fitness of *L. jonesii*. *Layia jonesii* produced about six times more viable seeds in the weeded portion of the grassland plots than the non-weeded portion of the plot. These results show that *L. jonesii* is sensitive to competition from surrounding species. Reduced competitive ability of some serpentine endemics may be due to an evolutionary trade-off between serpentine tolerance and competitive ability (Kruckeberg, 1954; Kazakou et al., 2008; Kay et al., 2011). It hypothesized that serpentine soils serve as a kind of refuge from competition for many serpentine species which have a lowered ability to compete with species on non-serpentine soils (Alexander et al., 2007; Brady et al., 2005; Kruckeberg, 1954), but my results suggest competitive interactions could influence the distribution of serpentine endemics at an even finer scale. The dry rock outcrop habitats patchily distributed on

serpentine soils may serve as a refuge from competition for endemic species within the serpentine environments.

The evolution of serpentine tolerance to avoid competition may be an evolutionary "dead-end." Using molecular phylogenetic analyses of 23 genera (including *Layia*), Anacker et al. (2010) show that diversification rates are lower for serpentine endemic lineages than non-endemic lineages. The abrupt ecological boundaries and the island-like nature of serpentine environments drives speciation on to these soils, but once lineages evolve on to serpentine, the lack of specialized niches and the homogenous nature of these habitats may limit the further diversification (Rajakaruna, 2004). In addition, many serpentine endemics have small, isolated populations, and therefore, limited genetic diversity could potentially further constrain speciation of these linages (Stockwell et al., 2003; Leimu et al., 2006).

Unexpectedly, the reciprocal transplant results did not support my hypothesis of local adaptation of *L. jonesii* to the rock outcrop habitat. Although I saw the distribution of *L. jonesii* constrained to the rock outcrop habitat during the previous year, my results indicate *L. jonesii* has a higher fitness in the grassland than the rock outcrop habitat. These results could be related to the extreme drought that coincided with my reciprocal transplant in spring 2014. During spring 2014, I observed that the distribution of *L. jonesii* expanded out from the rock outcrop habitat into the grassland habitat. Extreme drought likely reduced the survival of many annual species in the grassland habitat, and therefore, competition may have been lower in that habitat during the drought than in previous years. Reduced competition may have allowed the *L. jonesii* individuals in my reciprocal transplant plots to take advantage of the more conducive growing environment,

like higher water availability, found in grassland soils than rock outcrop soils. The hypothesis that increased rainfall can lead to increased productivity and therefore increased competition on serpentine soils is supported by other studies. Harrison et al. (2006) found that increased rainfall and the corresponding increased productivity was negatively correlated with serpentine endemic richness on serpentine rock outcrops in California. This result provides validity to my hypothesis that increased fitness of *L. jonesii* in the grassland habitat could be due to reduced rainfall and therefore reduced competition in that habitat.

The expansion of *L. jonesii* during a drought year suggests the distribution of the two species is dynamic from year to year based on annual environmental conditions. Because *L. jonesii* occurs on rocky, dry soils near serpentine rock outcrops, it may be more drought tolerant than *L. platyglossa*. This pattern suggests temporal selection maintains the distribution of the two populations on serpentine derived hillsides, where expansion of the rare serpentine endemic *L. jonesii* is favored during dry years but expansion of the common *L. platyglossa* is favored during wet years. It is hypothesized that the dry conditions of serpentine soils are a major evolutionary pressure driving serpentine tolerance (Alexander et al., 2007; Gardner and Macnair, 2000; Brady et al., 2005), and previous studies provide support for the hypothesis that serpentine endemics are more drought tolerant than non-serpentine or *bodenvag* species (Hughes et al., 2001; Wu et al., 2010). The local expansion of a serpentine endemic during drought conditions suggests serpentine endemics are drought tolerant and may be well adapted to survive a prolonged drought scenario due to climate change. Serpentine endemic species also share morphological features that are characteristic of drought tolerance such as succulent

leaves and deep root systems that could help them persist through future droughts (Brady et al., 2005a; Kazakou et al., 2008; Kay et al., 2011). A thorough morphometric and physiological comparison between *L. platyglossa* and *L. jonesii* could elucidate characteristics associated with drought that may allow the rare *L. jonesii* to better survive drought conditions in the rock outcrop habitat than the more common *L. platyglossa*.

Edaphic specialization and highly local endemism is common in the genus *Layia*, with many rare species confined to specific soil substrates including *L. discodea* and *L. jonesii* on serpentine soils, *L. carnosa* on dune soils, *L. munzii* on alkali soils, and *L. leucopappa* on alluvial clay soils. *Layia jonesii, L. munzii,* and *L. leucopappa* are morphologically similar sister taxa and are readily interfertile (Baldwin, 2006; Clausen, 1951). Differential edaphic adaptation maintains these species as geographically distant, allopatric populations, and therefore, differential adaptation rather than genetic incompatibilities likely acts as the strongest barrier to reproduction among these species.

Flowering Time

I sought to determine if there were flowering time differences between the two species that could potentially contribute to reproductive isolation. Results show there was a significant flowering time difference between *L. jonesii* and *L. platyglossa* when the two species are grown in common habitats in the reciprocal transplant plots. In both rock outcrop and grassland plots, *L. jonesii* flowered about a week earlier than *L. platyglossa*. We documented a similar pattern in wild populations of *L. jonesii* in the rock outcrop habitat and *L. platyglossa* in the grassland habitat. Peak flowering time of *L. jonesii* was about two weeks earlier than *L. platyglossa* in the wild populations. The greater

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difference in flowering time when the two species grew in different habitats (wild populations), than when grown in the same habitat (reciprocal transplant plots) suggests that both environmental and genetic factors contribute to flowering time as a reproductive barrier. *Layia jonesii* flowers earlier in the rock outcrop habitat, where rocky soils become dry earlier in the growing season, than *L. platyglossa* in grassland soils which hold more moisture. This explanation is also supported by reciprocal transplant data. Flowering time for both *L. jonesii* and *L. platyglossa* was delayed in the grassland habitat compared to the rock outcrop habitat. For both species, earlier flowering time in the dry outcrop habitat is likely evidence of a plastic adaptation to ensure seed development by avoiding drought.

Shifts to earlier flowering time are often associated with adaptation to serpentine. Shifts in phenology likely evolved as mechanism to reproduce early in the season in order to avoid the drought conditions often found on serpentine soils (Brady et al., 2005; Kay et al., 2011). This pattern has been observed across multiple families. Serpentine endemic species in the genus *Mimulus, Helianthus,* and *Collinsia* flower earlier than close congeners, and this difference in flowering time contributes to reproductive isolation between species in these systems (Gardner and Macnair, 2000; Sambatti and Rice, 2006; Wright et al., 2006). *Layia jonesii* potentially evolved earlier flowering time as a mechanism to avoid drought, and this shift in flowering time likely has allowed this rare species to persist and maintain reproductive isolation while growing within mating distance of an interfertile congener. Flowering time differences can reduce the gene flow by reducing the amount of pollen transferred between two taxa (Sobel et al., 2010). Future studies could examine pollen movement dynamics between *L. jonesii* and *L.*

platyglossa in the field by utilizing fluorescent pollen dye and tracking movement of pollen between and within species by pollinators (Campbell and Waser, 1989).

Although flowering time differences between *L. jonesii* and *L. platyglossa* likely acts as a strong barrier to gene flow, there is some overlap in flowering time, and therefore, the barrier does not completely prevent gene flow. Both *L. jonesii* and *L. platyglossa* are morphologically similar in flower size, color, and color pattern and therefore likely share pollinators. Because the two species occur within meters of each other on serpentine derived hillsides at RC and other populations, some pollen transfer may occur during periods of flowering time overlap. At RC, I observed a diverse suite of pollinators, including beetles, sweat bees, and bumble bees, moving freely between individuals of the same species and between individuals of different species when blooming near each other at the same time.

Controlled Crosses

Controlled crosses show hybrid crosses between *L. jonesii* and *L. platyglossa* (heterospecific crosses) result in significantly lower seed set than crosses within the same species (conspecific crosses). Heterospecific crosses produced about 90% less seeds than conspecific crosses. These results show postpollination isolation contributes to reproductive isolation in between *L. jonesii* and *L. platyglossa,* although the mechanism causing postpollination isolation between the two species is unknown. Postpollination isolation can be caused by multiple mechanisms including pollen and stigma incompatibilities where heterospecific pollen grains do not germinate or grow well in stigmatic tissue (Kay, 2006), and pollen competition where heterospecific pollen tubes

grow more slowly than conspecific pollen tubes (Carney et al., 1996). Examining pollen tube growth in stigmas of heterospecific crosses could help determine the postpollination mechanism that contributes to reproductive isolation between *L. jonesii* and *L. platyglossa*.

My initial investigations into the viability of hybrid seeds that resulted from the few successful heterospecific crosses indicate that the seeds of both maternal parents germinate readily and develop to the reproductive stage. Planting these hybrid seeds in the field could elucidate patterns of hybrid survival fitness across the hillside and help me understand which mechanisms contribute to post-zygotic reproductive barriers between the species. It is possible hybrid individuals have a low survival and fitness in the field, which would act as a barrier to continued gene flow between the species.

CONCLUSIONS

My results show that prezygotic reproductive barriers exist between *L. jonesii* and *L. platyglossa* in RC. The strongest barriers to reproduction at this site are likely temporal isolation and postpollination isolation. Temporal isolation contributes to reproductive isolation through flowering time differences because *L. jonesii* flowers earlier than *L. platyglossa*. Post-pollination isolation due to genetic incompatibilities contribute to reproductive isolation through lowered seed set in hybrid crosses. Future studies could use genetic markers or other molecular techniques (RAPD, allozymes, etc.) to investigate true gene flow between the two *L. jonesii* and *L. platyglossa*.

My results also show that local adaptation may maintain the distribution of *L. jonesii* and *L. platyglossa* on the hillside. Local adaptation of *L. platyglossa* to the

grassland habitat prevents this species from expanding into the rock outcrop habitat. *Layia jonesii* is sensitive to resource competition from species in grassland habitat, and this may contribute to its constraint to drier rock outcrop habitat. Although local adaptation maintains these species parapatrically, habitat isolation is likely not a strong reproductive barrier in this system. My results also suggest that rocky, dry serpentine rock outcrops with sparse vegetation may act as refuges for *L. jonesii* and potentially other competition-sensitive rare serpentine endemics.

My research provides insight into the mechanisms that prevent gene flow between two closely related species. These same mechanisms, like temporal isolation and postpollination isolation, have also been found to maintain rare serpentine species in other systems as well (Gardner and Macnair, 2000; Sambatti and Rice, 2006), but my research shows these same mechanisms also act similarly on a fine spatial scale across an edaphic gradient. Understanding how these mechanisms maintain reproductive isolation between a rare species with limited distribution and a more widespread species is especially important. Rare species are vulnerable to the homogenizing effects of gene flow and have the potential to become extinct when occurring near interfertile relatives. Prezygotic barriers provide substantial isolation from more widespread species and help preserve genetic integrity of rare species.

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APPENDICES
A. Tables

Table 1. Principal component analysis of chemical soil characteristics between rock outcrop and grassland habitats on a serpentine hillside in Reservoir Canyon. Bold values represent loading scores above 0.5.

Soil Character	PC1 (35.8%)	PC2 (31.5%)
Organic Matter (%)	-0.131496892	**0.932253751**
ENR (lbs/A)	-0.129973069	**0.916607008**
Phosphorus - Weak Bray (ppm)	**0.831659909**	-0.253545207
Phosphorus - Olsen Method (ppm)	**0.763682855**	-0.20125828
K (ppm)	**0.792500946**	-0.146389921
Mg (ppm)	0.082588052	**0.935110863**
Ca (ppm)	**0.852161306**	0.240069183
Na (ppm)	-0.003191771	**0.603536605**
Soil pH	**-0.869018537**	-0.338342157
H (meq/100g)	-8.29E-17	2.17E-16
CEC (meq/100g)	0.31159572	**0.910575831**
Sulfur (ppm)	**0.665912481**	0.055550232
Ca:Mg (%)	**0.824247913**	-0.138850573

Table 2. Soil pedon descriptions for soil pits in the rock outcrop and grassland habitats. Available water holding capacity (AWHC) in cm of water for the entire soil profile is shown above the table.

ROCK OUTCROP (AWHC=1.9 cm)

Horizon	Depth (cm)	Rock Fragments	Texture	Structure
A	0-1.5	<15%	Clay	granular
Bt	1.5-6	50%	Clay	blocky
Bt/C	6-20	65%	Clay	blocky
Cr	20+	-	-	-

GRASSLAND (AWHC=3.75 cm)

Horizon	Depth (cm)	Rock Fragments	Texture	Structure
A	0-5	<15%	Clay	granular
Bt	5-25	<15%	Clay	blocky
Cr	25+	-	-	-

Table 3. Fixed effect tests of germination of *L. jonesii* and *L. platyglossa* in reciprocal transplant plots in the rock outcrop and grassland habitats.

Effect	Num DF	Den DF	F	Pr > F
Habitat	1	217	0.08	0.7765
Species	1	30	1.64	0.2098
Habitat*Species	1	217	1.39	0.2396

Table 4. Fixed effect tests of germination of *L. jonesii* in the grassland and weeded plots (treatments).

Effect	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Treatment	1	1	0.011735	0.9137

Table 5. Fixed effect tests of proportion surviving to flowering of *L. jonesii* and *L. platyglossa* in reciprocal transplant plots in the rock outcrop and grassland habitats.

Effect	Num DF	DenDF	F	Pr>F
Habitat	1	51	1.39	0.2432
Species	1	26	1.46	0.2371
Habitat*Species	1	51	1.25	0.2696

Table 6. Fixed effect tests of survival to flowering of *L. jonesii* in the grassland and weeded grassland plots (treatments).

Effect	Nparm	DF	L-R ChiSquare	Prob>ChiSq
Treatment	1	1	0.01	0.92

Table 7. Fixed effect tests of number of days surviving of *L. jonesii* and *L. platyglossa* in the reciprocal transplant plots in the rock outcrop and grassland habitats. Bolded values represent significant p-values.

Effect	Nparm	DF	DFDen	F Ratio	Prob > F
Habitat	1	1	75.89	6.2956	**0.0142**
Species	1	1	0.441	0.0371	0.9018
Habitat*Species	1	1	75.89	0.8164	0.3691

Table 8. Fixed effect tests of the number of viable seeds produced by *L. jonesii* and *L. platyglossa* individuals in the reciprocal transplant plots in the rock outcrop and grassland habitats. Bold values represent significant p-values.

Effect	Nparm	DF	DFDen	F Ratio	Prob > F
Habitat	1	1	42.48	25.1585	**<0.0001**
Species	1	1	18.23	1.6481	0.2153
Habitat*Species	1	1	42.48	0.0828	0.775

Table 9. Fixed effect tests of the number of viable seeds produced by *L. jonesii* in the grassland and weeded grassland plots (treatments). Bolded value represents a significant p-value.

Effect	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	854.103	18.00	**0.0004**

Table 10. Least square means, standard errors and confidence intervals for transformed fitness values (number of seeds produced per individual) of *L. jonesii* and *L. platyglossa* in the reciprocal transplant plots in the rock outcrop and grassland habitats.

Species	Habitat	Least Square Mean of Viable Seeds	SqRt(Least Square Mean of Viable Seeds)	Standard Error of SqRt (Least Sq Mean)	Lower 95% CI	Upper 95% CI
L. jonesii	grassland	46.47	6.82	0.60	31.07	64.95
L. jonesii	rock outcrop	8.43	2.90	0.67	2.40	18.12
L. platyglossa	grassland	31.79	5.64	0.79	16.40	53.23
L. platyglossa	rock outcrop	4.62	2.15	0.89	0.12	15.6

Table 11. Least square means, standard errors, and confidence intervals for fitness values (number of seeds produced per individual) of *L. jonesii* in the grassland and weeded grassland plots.

Treatment	Least Sq Mean	Std Error	Lower 95% CI	Upper 95% CI	Mean
Grassland	37.45	1.97	4	104.85	46.42
Weeded	298.34	1.87	178.71	448.46	268.36

Table 12. Fixed effect tests for flowering time of *L. jonesii* and *L. platyglossa* in the reciprocal transplant plots in the rock outcrop and grassland habitats. Bold values represent significant p-values.

Effect	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Habitat	1	1	206.49	9.4	**0.0057**
Species	1	1	410.98	18.7	**0.0003**
Habitat*Species	1	1	61.95	2.81	0.1073

Table 13. Fixed effect tests for the controlled greenhouse crosses. Cross types include conspecific, heterospecific, and control crosses. Bolded values represent significant p-values.

Effect	DF	F Ratio	Prob > F
Species	1	13.7336	**0.0003**
Cross Type	2	86.0074	**<.0001**
Species*Cross Type	2	13.5956	**<.0001**

B. Figures
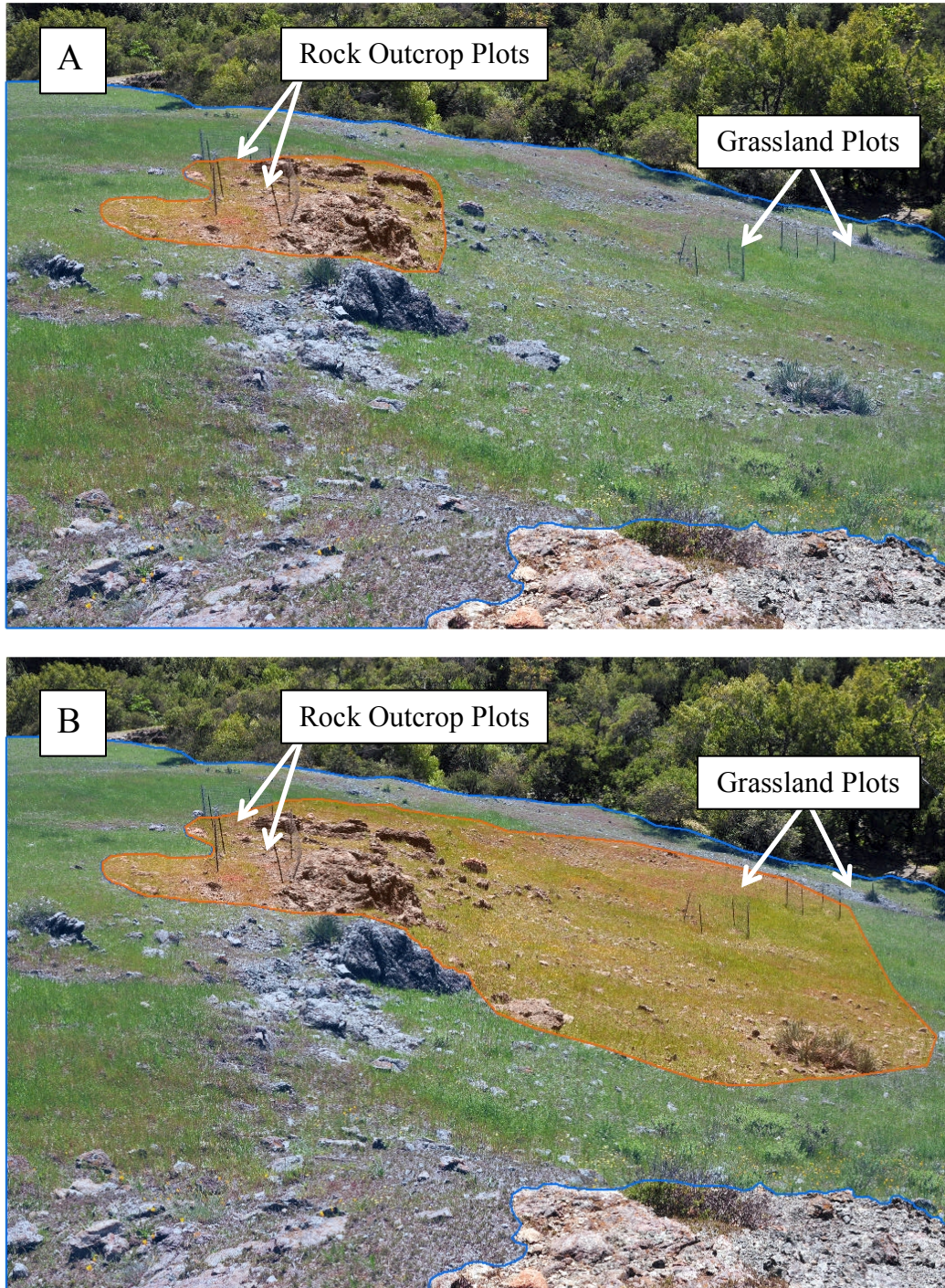
Figure 1. The distribution of the rare *L. jonesii* during spring 2013 (A) and spring 2014 (B) at RC. *Layia jonesii* distribution is shown in orange and *L. platyglossa* distribution is show in blue. During spring 2013, *L. jonesii* was confined to the rock outcrop but during spring 2014 it expanded into the grassland habitat. The placement of the reciprocal transplant plots is also shown.
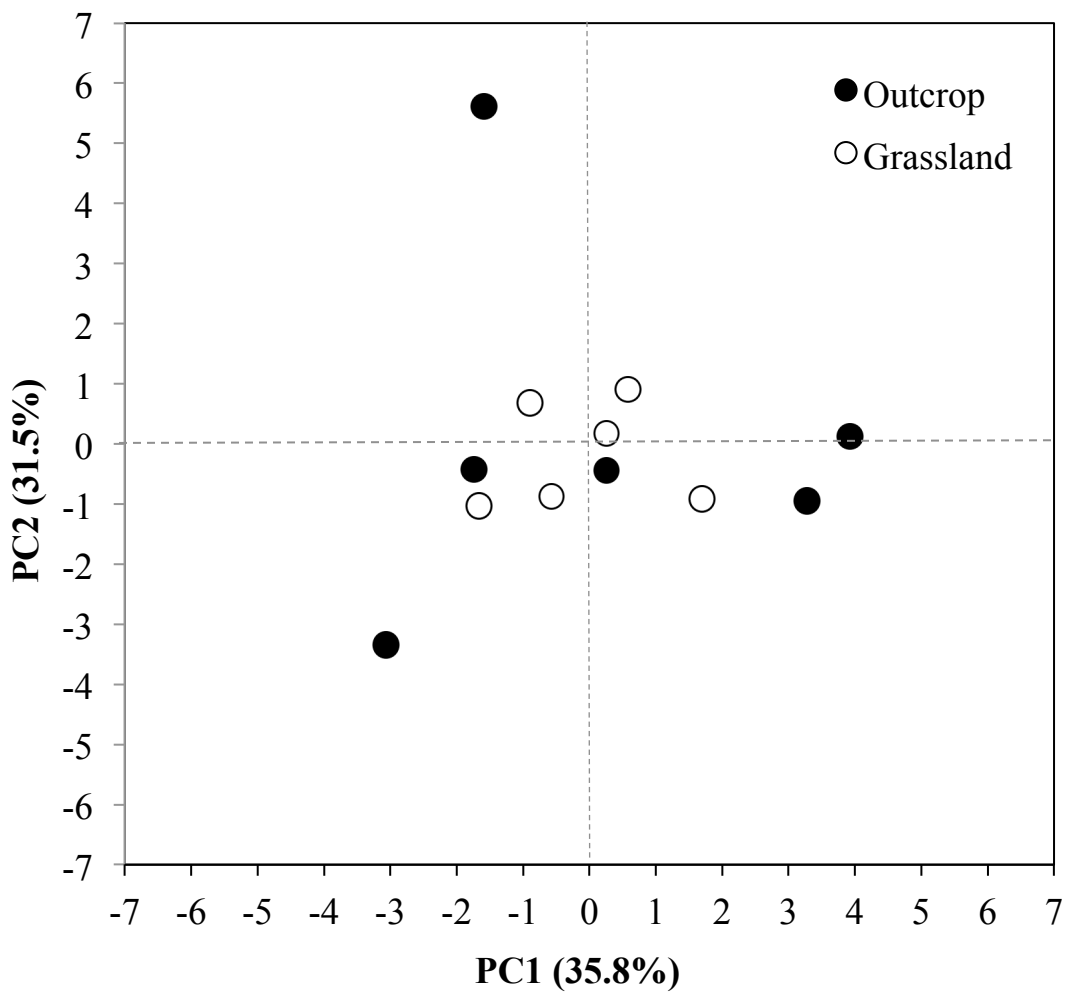
Figure 2. Principle component analysis of the 13 soil characteristics. The two principal components (PCs) describe 67% of the variation in soil characteristics between the two habitats at RC. The points in the plot above represent individual soil samples from the outcrop habitat (black circles) and the grassland habitat (white circles). See Table 1 for loading scores.
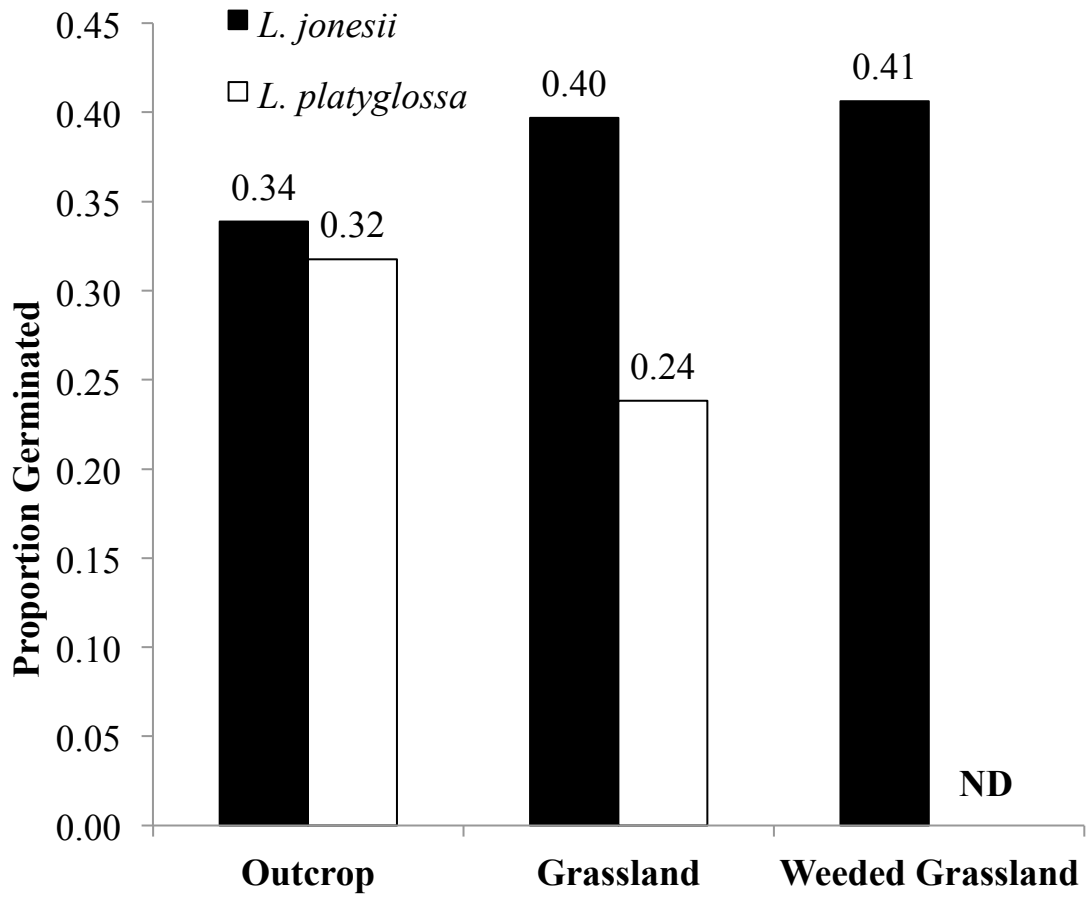
Figure 3. The proportion of germinated seeds of total seeds planted in the rock outcrop, grassland, and weeded grassland reciprocal transplant plots for both *L. jonesii* (outcrop n=62, grassland n=63, weeded grassland n=64) and *L. platyglossa* (outcrop n=63, grassland n=63). Value above the bar represents the proportion of germinated seeds of total seeds planted in each plot. *Layia platyglossa* was not grown in the weeded grassland plots.
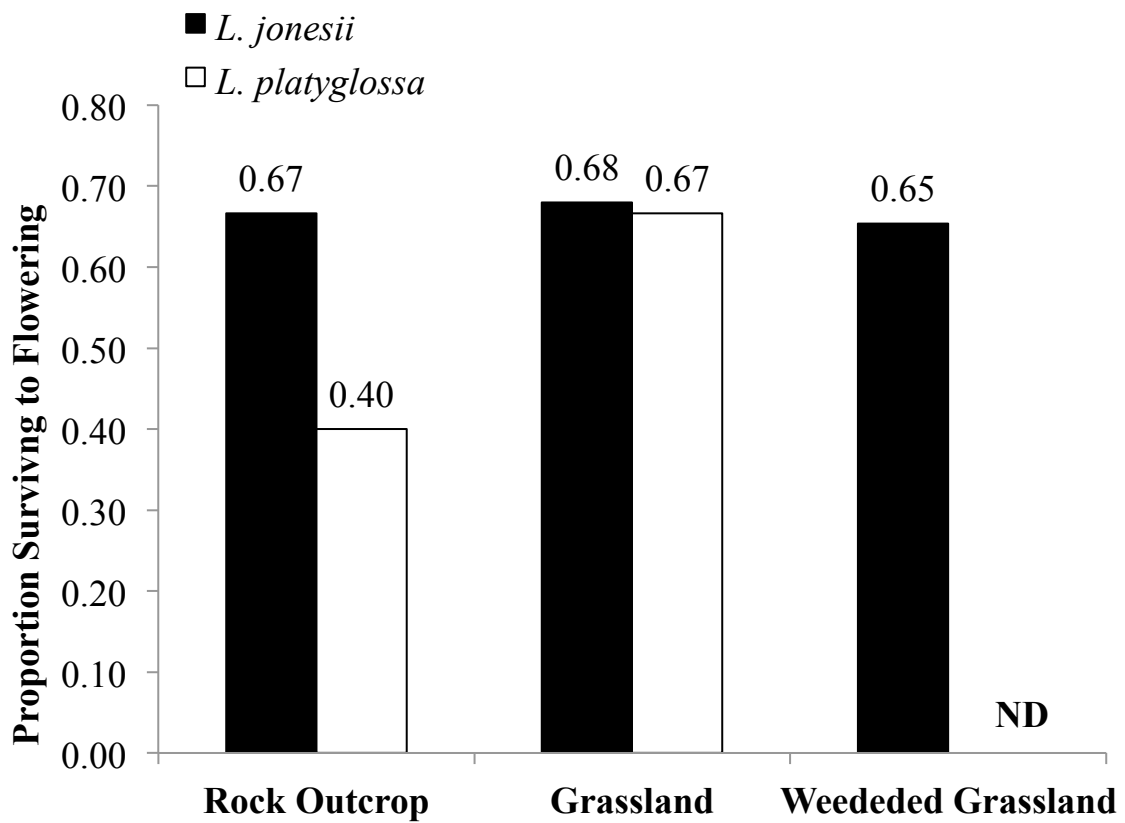
Figure 4. The proportion of germinated seeds surviving to flowering in the rock outcrop, grassland, and weeded grassland reciprocal transplant plots for both *L. jonesii* (outcrop n=21, grassland n=25, weeded grassland n=26) and *L. platyglossa* (outcrop n=20, grassland n=15) in RC. Values above the bar represent the proportion of germinated seeds surviving to flowering in each plot. *Layia platyglossa* was not grown in the weeded grassland plots.
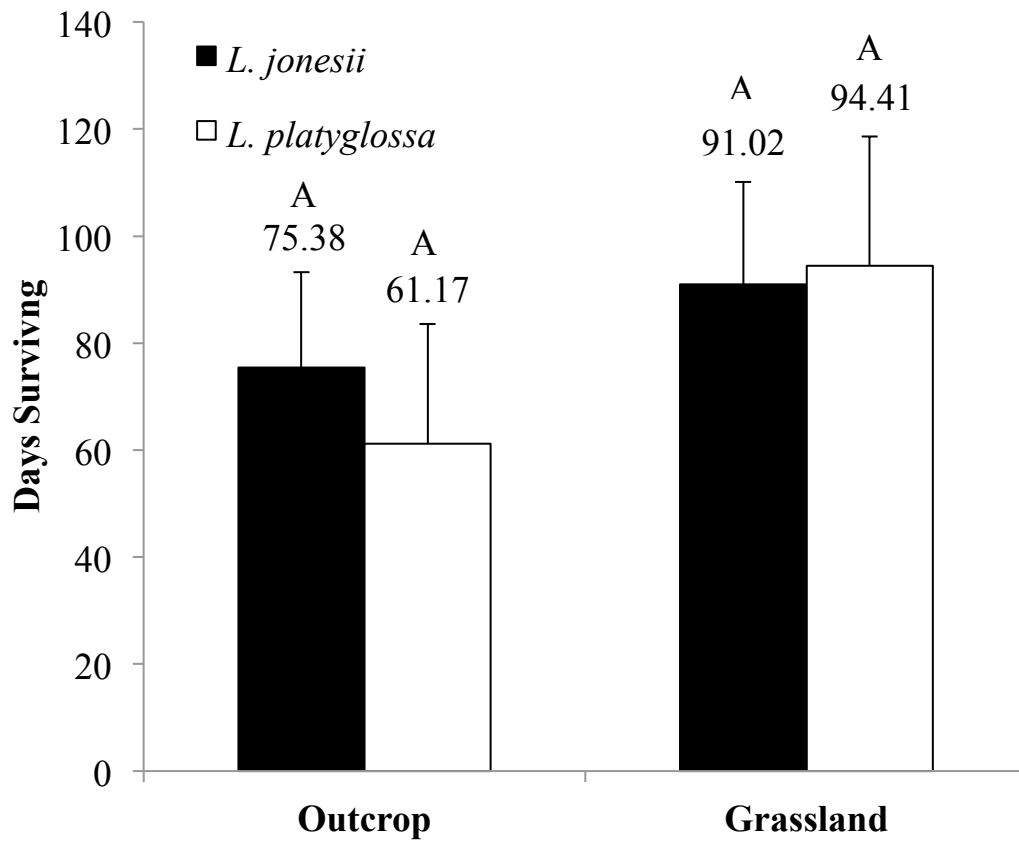
Figure 5. The number of days surviving for germinated seedlings of both *L. jonesii* (outcrop n=21, grassland n=25) and *L. platyglossa* (outcrop n=20, grassland n=15) individuals in the rock outcrop and grassland habitats. Values above the bar represent the least square mean. Means with identical letters are not significantly different in Tukey HSD comparisons (P < 0.05). Error bars represent 1 unit of standard error.
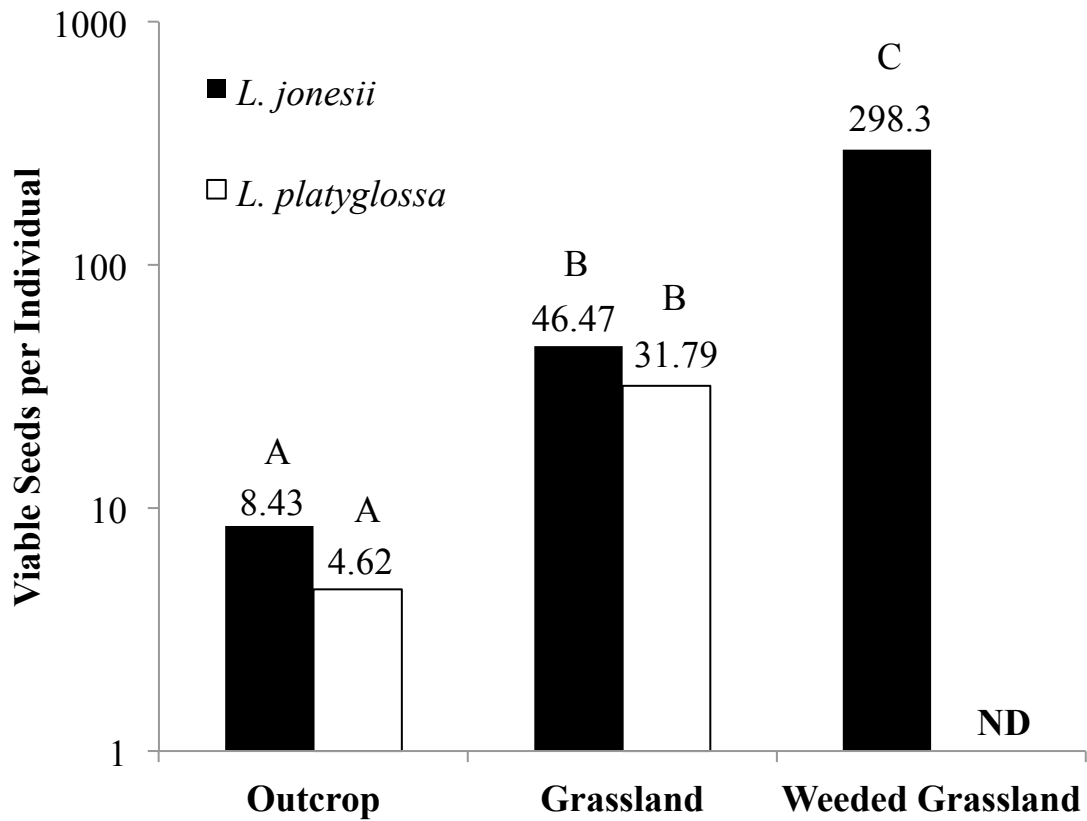
Figure 6. Number of viable seeds produced per individual plant in the rock outcrop, grassland, and weeded grassland reciprocal transplant plots of both *L. jonesii* (outcrop n=14, grassland n=17, weeded grassland n=17) and *L. platyglossa* (outcrop n=10, grassland n=8) in RC. Only individuals that survived to flowering were included in this analysis. Values above the bars represent the least squares mean. Means with identical letters are not significantly different in Tukey HSD comparisons (P < 0.05). Both species had significantly higher viable seed set in the grassland habitat than the rock outcrop habitat. See Table 10 for standard errors of each mean. *Layia platyglossa* in the weeded grassland plots.
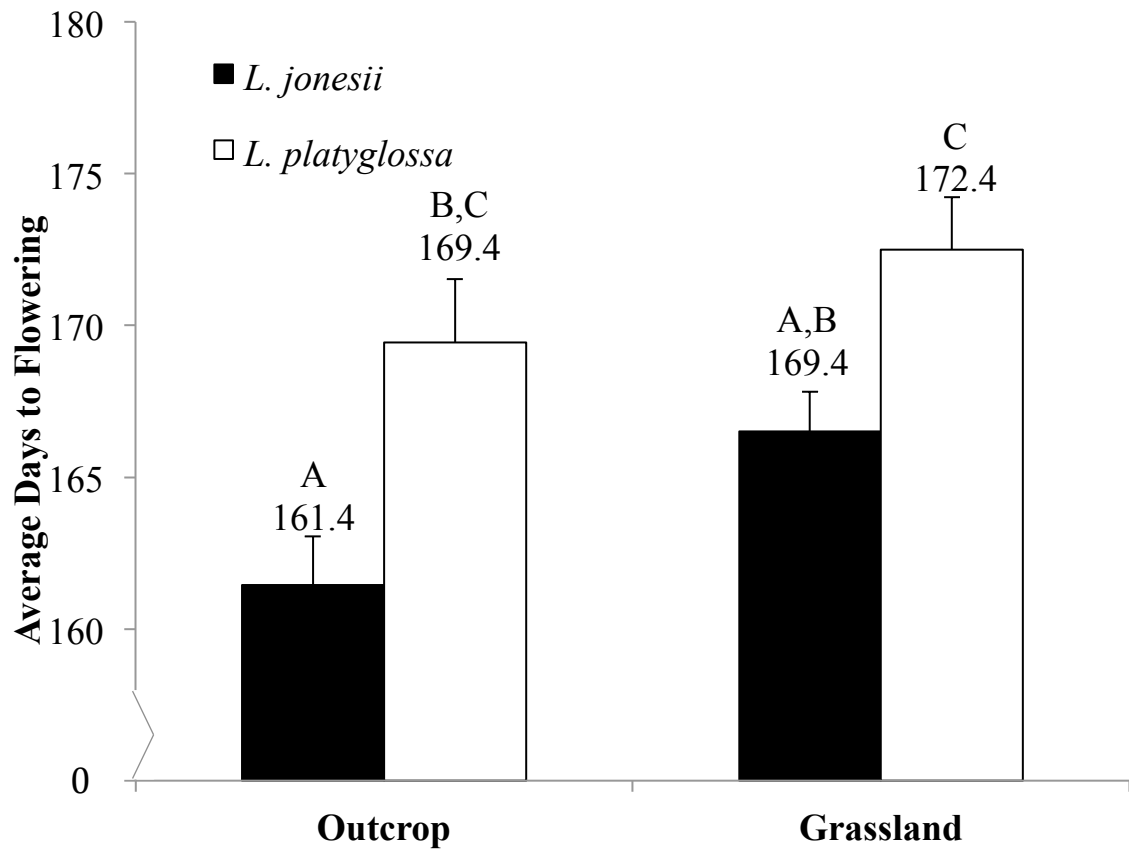
Figure 7. Average days to flowering for individual plants in the rock outcrop and grassland reciprocal transplant plots for both *L. jonesii* (outcrop n=14, grassland n=17) and *L. platyglossa* (outcrop n=10, grassland n=8). Values above the bars represent the least squares mean. Means with identical letters are not significantly different in Tukey HSD comparisons (P < 0.05). Error bars represent 1 unit of standard error.
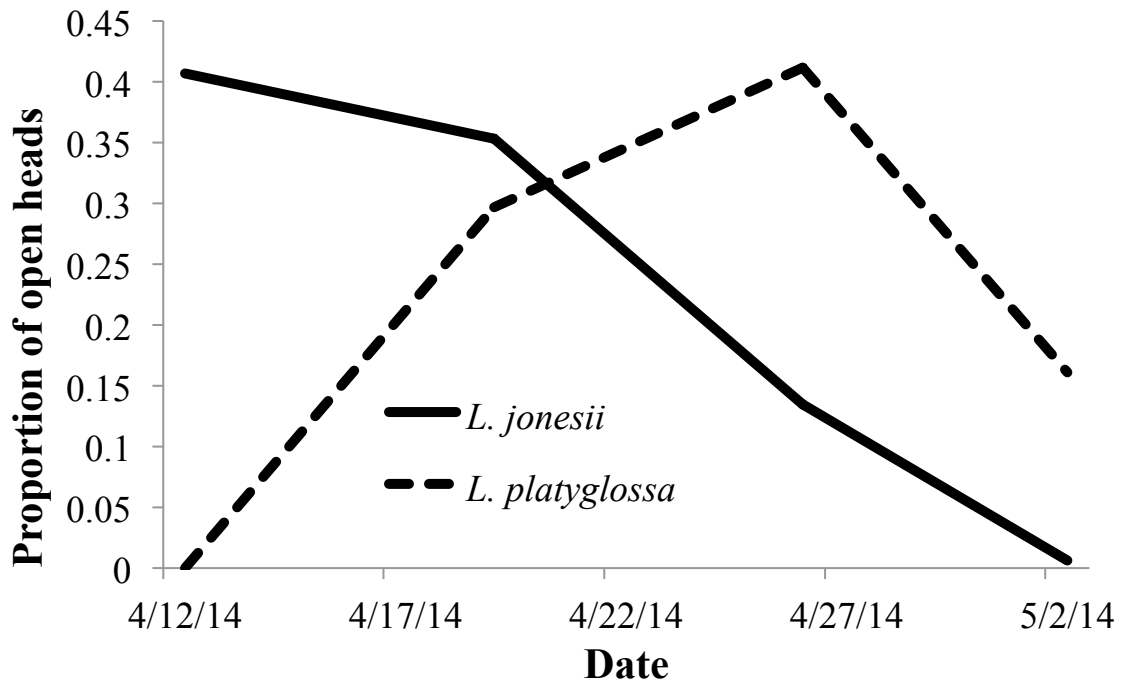
Figure 8. Flowering time differences in wild populations of *L. jonesii* (n=153) and *L. platyglossa* (n=74) at RC during spring 2014.
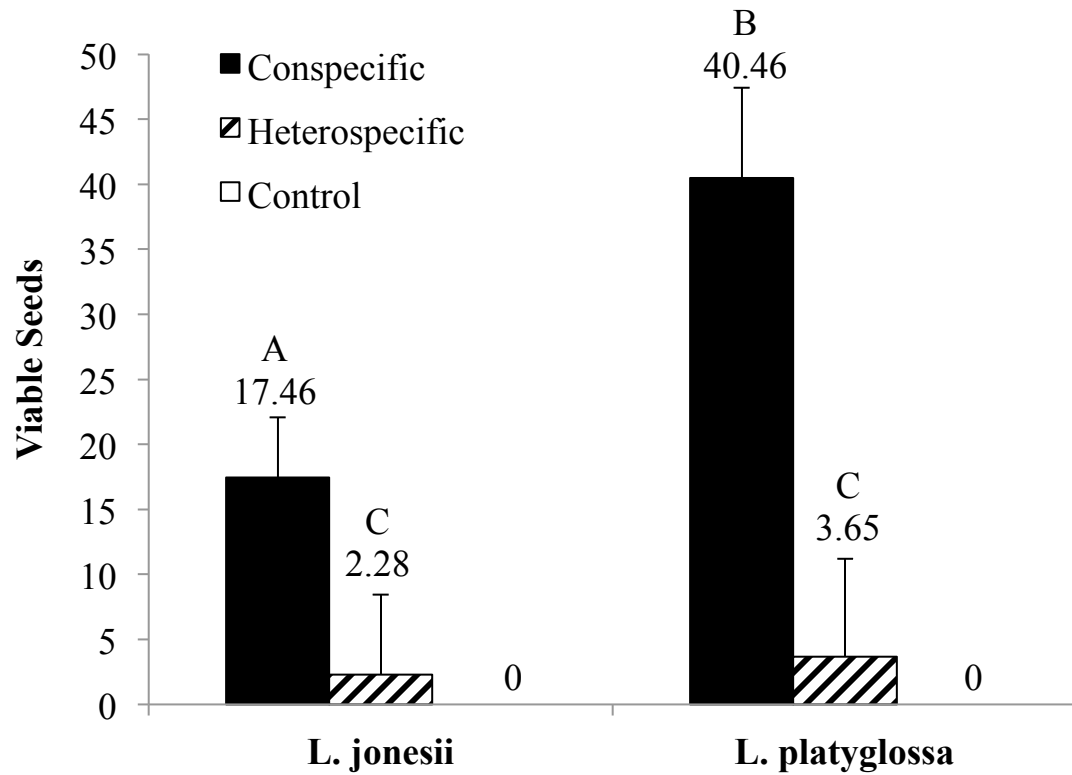
Figure 9. Number of viable seeds produced by each species in the conspecific, heterospecific (hybrid), and control crosses performed in the greenhouse with *L. jonesii* (conspecific n=33, heterospecific n=24, control n=20) and *L. platyglossa* (conspecific n=23, heterospecific n=24, control n=23). Values above the bars represent the least squares mean. Means with identical letters are not significantly different in Tukey HSD comparisons (P < 0.05). Error bars represent 1 unit of standard error.