The Phenotypic, Genotypic, & Environmental Drivers of Local Adaptation to a Harsh Granite Outcrop Environment in *Mimulus*

by

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SUMMARY

- A primary goal in evolutionary biology is to understand what traits, genes, and environmental variables drive local adaptation. This is difficult since many traits and genes diverge simultaneously between populations.
- Here we investigate the traits, genetic regions, and ecological variables that underlie *Mimulus laciniatus'* adaptation to granite outcrops. We measure selection on flowering time, flower size, and leaf shape in a reciprocal transplant using *M. laciniatus x M. guttatus* F_4 hybrids, test whether a previously mapped pleiotropic life-history quantitative trait locus (QTL) controls fitness in the field, and use fine scale measurements of soil moisture and herbivory to determine the environmental drivers of adaptation.
- We find that *M. laciniatus* and *M. guttatus* exhibit a fecundity trade-off, strong selection for earlier flowering in granite outcrops, and advantageous flowering time plasticity. The direction of selection differs before vs. after flowering on our life-history QTL, and differences in drought & herbivory drive survival differences between habitats.
- We conclude that *M. laciniatus* and *M. guttatus* are locally adapted to dramatically different microhabitats. While early flowering time underlies plant fitness in *M. laciniatus'* seasonally dry environment, herbivore resistance and plant size are advantageous in a competitive mesic environment like *M. guttatus'*.

Key Words: local adaptation, flowering time, *Mimulus*, plasticity, drought, mating system, herbivory, phenotypic selection

INTRODUCTION

Closely related taxa often occupy ecologically disparate habitats. How do different taxa adapt to these new and initially stressful environments? What genes evolve in response to novel selection pressures and what traits do they influence? And which environmental variables act as selective forces? These are exciting questions since adaptation to new habitats can maintain genetic variation within species (Cain & Sheppard, 1953; Levene, 1953; Rausher, 1983; Gillespie & Turelli, 1988; Hedrick, 2006) and eventually lead to ecological speciation (Mayr, 1947; Mayr, 1949; Schluter, 2001; Coyne & Orr, 2004; Rundle & Nosil, 2005). Many reciprocal transplant studies have demonstrated local adaptation, but few have elucidated the specific traits, genes, and environmental variables that drive this adaptation (reviewed in Kawecki & Ebert, 2004; Hereford, 2009; Salvolainen et al., 2013). Conversely, the genetic basis of many potentially adaptive traits has been uncovered in the lab, but the fitness effects of these loci are rarely tested in nature (Johanson et al., 2000; Nachman et al., 2003; Colosimo et al., 2005; Rosenblum et al., 2010; Chan et al., 2010; Jones et al., 2012).

Differential adaptation can arise through fitness trade-offs at individual loci (antagonistic pleiotropy) or conditional neutrality where an allele has a fitness advantage in its native environment but is neutral in the other (Anderson et al., 2011b). Antagonistic pleiotropy can maintain genetic variation within a species, but conditional neutrality will not. To determine which process is responsible for differential habitat adaptation it is necessary to test the fitness effects of alleles in their natural environments (Anderson et al., 2011b). Combining controlled crosses and field experiments can disentangle loci from their genetic backgrounds, elucidate the individual traits and loci that contribute to adaptation, and test whether differential habitat adaptation is produced by antagonistic pleiotropy.

Plant species are excellent systems for studying the genetics of differential habitat adaptation. Because of their sessile lifestyle, plants often experience strong divergent selection across heterogeneous environments on a small geographic scale (Kalisz, 1986; Schmitt & Antonovics, 1986; Stewart & Schoen, 1987; Robichaux, 1990). This sessile nature also makes plants the best organisms for conducting reciprocal transplant experiments where the fitness of different genotypes can be directly measured in their

native habitats. The *Mimulus guttatus* species complex is an excellent system for studying local adaptation because it is a closely related group of wildflowers that are largely inter-fertile and occupy a variety of edaphic environments across Western North America. Most members of the species complex occur in moist seeps and streambeds, but some have colonized rapidly drying habitats such as serpentine soils, copper mine tailings, and granite outcrops (MacNair & Gardner, 1998; Wu et al., 2007; Ferris et al., 2015; Wright et al., bioRxiv). Granite outcrops are harsh habitats characterized by shallow rocky soils, high light intensity, extreme temperatures, and low soil water retention (Ferris et al., 2014). The Sierra Nevada natives *M. laciniatus* (Figure 1b) and the recently described *M. filicifolius* (Sexton et al., 2013, Ferris et al., 2014) specialize on granitic habitat (Figure 1a) growing in thin strips of moss and gravel where few other plant species can survive. *Mimulus laciniatus* and *M. filicifolius* share several traits that are likely adaptive in rocky outcrops that dry rapidly once seasonal snowmelt is gone: rapid flowering (Friedman & Willis, 2013; Ferris et al., 2016), a self-fertilizing mating system (Fenster & Ritland, 1994; Ferris et al., 2014), small plant size, and lobed leaf shape (Figure S1a, Ferris et al., 2015). In contrast the close relative, and purported progenitor of the species complex, *M. guttatus* is round-leaved (Figure S1b), larger, later-flowering, and largely outcrossing (Figure 1c). *Mimulus guttatus* occurs in deeper, densely populated soils in moist seeps and meadows (Figure 1d) often within meters of *M. laciniatus'* granite outcrops (Ferris et al., 2016).

Early flowering allows plants to reproduce before the onset of summer drought and has been shown to be adaptive across plant taxa (Kiang & Hamrick, 1987; Fox, 1990, Macnair & Gardner, 1998; Hall & Willis, 2006; Willis et al., 2008; Anderson et al., 2012). Plant taxa occupying harsh, marginal habitats also often have self-fertilizing mating systems (Stebbins, 1970; Kiang & Hamrick, 1987; MacNair & Gardner, 1998; Mazer et al., 2010; Wu et al., 2010). Small flower size and rapid floral development are correlated with self-fertilization and may reduce floral tissue transpiration in dry environments (Galen, 1999). Additionally, self-fertilization provides reproductive assurance in marginal environments often sparsely populated by pollinators (Stebbins, 1970; Piper et al., 1986; Cunningham, 2000; Fausto et al., 2001). In a seasonally dry environment like *M. laciniatus'* small plants Since lobed leaf shape is unique to rocky

outcrop *Mimulus* taxa (Ferris et al., 2015), and is seemingly an example of parallel evolution (Ferris et al., 2014), it is likely adaptive in this habitat. Lobed leaves have thinner boundary layers and are cooled more efficiently by convection than round leaves which should decrease the amount of transpiration required for cooling when leaves are heated above ambient temperature by direct sunlight (Vogel, 1968; Givnish, 1978; Schuepp, 1993; Nobel, 2005). Lobed leaves also have lower hydraulic resistance (R_{leaf}) and therefore less drought stress prone tissue than round leaves (Nicotra et al., 2011). A thinner boundary layer & lower R_{leaf} should be advantageous in *M. laciniatus'* dry, exposed granite outcrops.

To understand adaption to granite outcrops in *Mimulus* we asked five questions: (1) Are *M. laciniatus* & *M. guttatus* differentially adapted and if so are there fitness trade-offs? (2) What traits are involved in this adaptation? (3) Are these traits phenotypically plastic? (4) Is differential adaptation due to antagonistic pleiotropy at the single locus level? And (5) what environmental variables drive adaptation local adaptation in these species? To address these questions, we performed a large-scale reciprocal transplant experiment with *M. laciniatus*, *M. guttatus*, and outbred F_4 hybrids planted in native granite and meadow habitats. Trait associations present in the parental species are broken up by recombination and independent assortment in the F_4's allowing the measurement of selection on individual phenotypes. We measured fitness differences between *M. laciniatus* & *M. guttatus*, and the strength of selection on flowering time, flower size, leaf size, and leaf shape in the F_4's. To address whether differential habitat adaptation is due to antagonistic pleiotropy, we examined the fitness consequences of a large effect pleiotropic QTL (LG8b) that controls differences in these traits between sympatric populations of *M. laciniatus* and *M. guttatus* in the greenhouse (Ferris et al., 2016). Fine scale measurements of soil moisture and herbivory were taken over the course of the experiment to examine the ecological drivers of adaptation.

MATERIALS AND METHODS

Construction of the hybrid population

In summer of 2008, 20 individuals were collected from each parental population, White Wolf (WLF, *M. laciniatus*) and Yosemite Overlook (YVO, *M. guttatus*) along Tiago Road in Yosemite National Park, CA. The parental inbred lines, WLF47 and YVO6,

were developed through hand pollinated self-fertilization and single seed descent for 7 and 5 generations respectively. Outbred F_4 hybrid seeds were created by first self-fertilizing F_1 hybrids to generate a large F_2 population. Two hundred F_2's were randomly paired and reciprocally crossed to generate 100 families. Then 200 F_3's were randomly paired and reciprocally crossed repeated to create 100 F_3 families. We pooled ~30 seeds from each maternal F_3 to create one large outbred F_4 family. F_4 seeds were then distributed randomly throughout experimental blocks.

Reciprocal transplant experiment

In April of 2013, 400 *M. laciniatus* (WLF47), 400 *M. guttatus* (YVO6), and 3000 F_4's were planted in open soil flats in the UC Davis greenhouses. Seeds were cold stratified at 4°C for 10 days, then left in the greenhouse to germinate for one week. At the cotyledon stage seedlings were transplanted one inch apart into 50 randomized blocks of 19 plants each (2 WLF47, 2 YVO6, 15 F_4) at each of four field sites along Tioga Road in Yosemite NP, CA. The two *M. laciniatus* habitat sites, Olmstead Point (Granite 1, Figure 1a) and Yosemite Creek (Granite 2), are undisturbed granite outcrops with native *M. laciniatus* growing on moss at elevations of 8,500 and 7,500 feet respectively. The *M. guttatus* sites, Little Meadow (Meadow 1) and Crane Flat (Meadow 2, Figure 1c), are undisturbed meadows with native *M. guttatus* growing near a standing seep. They occur at 6,200 and 6,000 feet respectively. Sites were chosen to maximize the similarity between the developmental stages of transplanted and native plants.

Seedlings that died within a week of transplant were replaced so that transplant shock would not affect survivorship. Experimental plants were censused every other day May - August 2013 for survival and flowering time. On the day of first flower, flowering time, corolla width, and plant height were recorded. So as not to damage plants before flowering, the first flower from each surviving plant was placed in silica gel for DNA extraction as it senesced. Morphological measurements were taken with a small metal ruler in millimeters. Leaf shape was not measured until plants began to senesce to avoid damaging plants before they set seed. Leaf area and degree of lobing were determined by digitally scanning leaves and performing convex hull analysis in ImageJ as described previously (Ferris et al., 2015). Briefly, the convex hull analysis consists of comparing the area of each leaf's convex hull (the shape created by connecting the outermost points

of a leaf) to the leaf's true area and dividing this difference in area by the convex hull area to control for size. Fruit number was counted after senescence as a measure of lifetime fitness.

Local adaptation and phenotypic selection

To test for local adaptation between *M. laciniatus* and *M. guttatus* we conducted a logistic regression with survival to flowering as the dependent variable and species, habitat, and their interaction as independent variables. We performed two-tailed t-tests to determine whether mean survival to flowering and fruit number significantly differed between *M. laciniatus* and *M. guttatus* by habitat. To detect phenotypic plasticity in flowering time, flower size, plant height, leaf area, and leaf shape we conducted factorial ANOVAs with habitat (Granite vs. Meadow) & genotypic class (*M. laciniatus*, *M. guttatus*, & F_4) as independent variables. To detect genotype by environment (GxE) interactions we tested for interactions between habitat and genotypic class. To correct for multiple testing we used a Bonferroni correction ($\alpha = 0.01$). Phenotypic correlations were measured among traits for each habitat type using a restricted maximum likelihood model (REML) in JMP (JMP®, Version *10*. SAS Institute Inc., Cary, NC, 1989-2007).

To measure the strength of selection on individual phenotypes we conducted linear and quadratic selection analysis (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987). We regressed fitness simultaneously on flowering time, corolla width, leaf area, and leaf shape in our F_4 population. To control for excess zeros in our count data we used a zero-inflated Poisson regression model on individual phenotypes (as in Anderson et al., 2012). Zero-inflated Poisson models control for excess zeros in count data by simultaneously breaking up the data into a logistic regression on whether a plant set seed or not, and performing a Poisson regression on the count data for plants that did set seed (Ridout et al. 1998). Height at first flower was excluded because of its positive correlation with flower and leaf size (Table S2). All phenotypes were standardized to a mean of 0 and standard deviation of 1 to enable comparison of traits measured in different units.

Detecting fitness QTL's in the field

To determine whether local adaptation between *M. laciniatus* and *M. guttatus* is due to antagonistic pleiotropy we tested the effect of the major pleiotropic life-history QTL,

LG8b, on fitness in the field. LG8b controlled species differences in flowering time, flower size, and leaf shape in a cross between different *M. laciniatus* and *M. guttatus* populations (SHL x SHG) in the greenhouse (Ferris et al., 2016). We hypothesized that LG8b would contribute to fitness in our reciprocal transplant experiment because it affected all three of our focal traits and was the largest effect QTL for each trait in our greenhouse QTL mapping experiment (Ferris et al., 2016). DNA was extracted from dried floral tissue from 563 F_4's (Granite1:273, Granite2:122, Meadow1:45, and Meadow2:123). Consequently, only F_4's that survived to flower were genotyped at a single exon-primed intron crossing (EPIC) marker derived from expressed sequence tags (ESTs), *MgSTS538* (Fishman et al., 2008), within the LG8b QTL region (Ferris et al., 2016). We screened parental inbred lines for PCR fragment length polymorphism at *MgSTS538*. Primers for this marker are found at http://www.mimulusevolution.org. PCR products underwent capillary electrophoresis and fragment analysis on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment size was scored in GeneMarker (Soft Genetics, State College, PA, USA).

To test for an association between LG8b genotype and phenotypic variation in flowering time, flower size, and leaf shape in the field we performed one-way ANOVAs with *MgSTS538* genotype as the independent variable in R (R Development Core Team 2008). We analyzed genotype data for each site separately. To test for selection on LG8b before flowering we used a Chi-squared test to detect skew in the genotypic ratios among plants that survived to flower at each site. In the absence of selection at LG8b we expect the genotypic ratios to be 1:2:1 (*LL*:*GL*:*GG*). If there was selection at LG8b before flowering, then our observed genotypic ratio should significantly differ from the expected ratio. To test for selection on LG8b after flowering we performed a zero inflated Poisson regression of fruit number on LG8b genotype. To correct for multiple tests across population sites in our ANOVA, Chi-squared, and regression analyses we used the Bonferroni correction ($\alpha = 0.0125$).

Environmental census

In addition to phenotype, fitness, and genotype data we also collected fine scale soil moisture and herbivore damage data in each block across each transplant site. This allows us to test whether these environmental variables are associated with plant fitness

differences on a fine spatial and temporal scale within and between habitat types. Percent soil moisture was measured weekly in each experimental block May through August using a Decagon soil moisture probe. We measured herbivory in each block at the time of plant senescence by recording the presence or absence of damage on the first true leaf. To determine whether soil moisture and leaf damage affected survival across habitats we ran a mixed effects repeated measures model using the nlme package in R (R Core Development Team 2008) with habitat type, soil moisture, leaf damage, and time as fixed independent variables, and block as a random effect nested within site, nested within habitat. To determine whether different environmental variables drove survival in granite vs. meadow sites we ran separate mixed effects repeated measures models on the survival data from each habitat. We determined the best-fit model using AIC model selection criteria (Zuur et al., 2009).

RESULTS

M. laciniatus & *M. guttatus are phenotypically plastic and locally adapted*

Our logistic regression of survival to flowering detected a significant interaction between habitat and species indicating that parental lines are locally adapted (Table S3). In addition, *M. laciniatus* had significantly higher survival than *M. guttatus* in its native granite (0.23 > 0.12, p-value < 0.0001), but the species had similar survival in *M. guttatus'* meadows (*M. guttatus* = 0.09, *M. laciniatus* = 0.10, p-value = 0.72, Figure 2a). However, a fitness trade-off in fecundity was indicated by crossing reaction norms (Figure 2b). *M. laciniatus* has significantly higher mean fruit number in granite habitat (mean = 0.48, SE = 0.05) than *M. guttatus* (mean = 0.10, SE = 0.022, p-value <0.0001), while *M. guttatus* has marginally significantly higher mean fruit number than *M. laciniatus* in its meadows (*M. guttatus* = 0.05, SE = 0.022, *M. laciniatus* = 0.007, SE = 0.007, p-value = 0.055).

To understand how ecologically important traits varied between habitats and species we calculated the mean trait value and standard error for F_4's, *M. laciniatus*, and *M. guttatus* in each habitat and transplant site. In granite habitat phenotypic differences between genotypes are in the expected direction with *M. laciniatus* flowering earlier (by 3.29 days), being smaller flowered (by 2.88 mm) and leaved (by 51.84 mp), and more lobed (by 0.07) than *M. guttatus* (Table 1). In the meadow habitat the direction of trait

differences was the same, except that *M. laciniatus* flowered 3.5 days later than *M. guttatus* (Table 1,).

There was significant phenotypic plasticity in all traits except leaf area, significant GxE in flowering time (p-value = 0.007), and marginally significant GxE in plant height (p-value = 0.022) and flower size (p-value = 0.071). Within each genotypic class (F_4, *M. laciniatus*, *M. guttatus*) plants flowered earlier (8.57, 8.8, 2.05 days, p-value < 0.001) and were shorter (4.63, 17.94, 43.23 mm, p-value < 0.001) in *M. laciniatus'* granite habitats than in *M. guttatus'* meadows (Table 1, Figure 3). Interestingly *M. laciniatus* was significantly more plastic in flowering time, but less plastic in height than *M. guttatus* (p-value = 0.007, 0.022, Figure 3a&c). F_4's and *M. laciniatus* had larger flowers (2.2 mm) in granite, while *M. guttatus* had larger flowers (1 mm) in meadows (p-value < 0.001). *M. laciniatus* & F_4's showed a slight, but significant (p-value = 0.002) increase in leaf lobing in the granite habitat (Tables 1, Figure S1 c). Leaf shape was highly plastic between the greenhouse and the field (Figure S1 d&e). Hybrids in the greenhouse displayed variable leaf lobing among individuals (S.D.= 0.13, Ferris et al. 2016), but there were few lobed F_4's in the field (S.D.=0.003, Figure S1 d&e).

We examined phenotypic correlations among traits in the F_4's (Table S2). In both habitats leaf area, flower size, and height at first flower were highly positively correlated (r > 0.5), while leaf area and shape are slightly positively correlated (granite r = 0.079, meadow r = 0.136), suggesting a genetic correlation among these traits in both habitats. Flowering time was uncorrelated with other traits in granite (Table S2), but positively correlated with height (r = 0.110) and leaf area (r = 0.128) in meadows. This difference suggests that the genetic architecture of flowering time may differ between habitats.

Early flowering time is adaptive in granite outcrops

To assess selection on individual phenotypic traits in each habitat we performed linear and quadratic selection analysis in our outbred F_4 population. In the Poisson regression, which tested for associations between phenotypic value and fruit number among plants that set seed, we found significant directional and quadratic selection coefficients. There was significant directional selection for earlier flowering in the granite habitat and increased flower width in both habitats (Table 2). There was no significant directional selection on leaf area or leaf shape at the habitat level, but there was positive selection on

leaf shape in the Meadow 2 site (Table 2). We detected significant negative quadratic selection on leaf area in the granite habitat and on leaf shape in the meadow habitat (Table 2). Negative quadratic selection indicates that there may be disruptive selection acting on leaf area in granite and leaf shape in meadows. The lack of significant directional selection on leaf shape was due to a lack of expressed variation in leaf shape (Figure S1). To test whether selection operated differently on traits between habitats and sites we tested for interactions between trait & habitat, and trait & site. We found significant interactions between flowering time and both habitat type & site (Table 2).

In the logistic regression we tested whether phenotypic values were correlated with the probability that an individual F_4 set seed or not. We found positive selection on leaf area in meadow habitat and significant negative quadratic selection on leaf area in Granite 1 and Meadow 1 (Table 3). Negative quadratic selection indicates the presence of stabilizing selection. We detected significant interactions between population and leaf area (Table 3). We recognize that selection gradient values could be due to selection on unmeasured correlated characters.

Pleiotropic greenhouse QTL only associated with flower size in the field

Before assessing whether there is directional selection on our major pleiotropic greenhouse QTL on LG8 we wanted to confirm that LG8b significantly contributed to our phenotypes in the field. This is important because (1) we used a different population cross in our previous greenhouse experiment (SHLxSHG) than in the field (WLFxYVO), and (2) QTL's are often environmentally unstable (Wan et al. 2005, Messmer et al. 2009, Anderson et al. 2011, Desmarais et al. 2013). To determine whether our major pleiotropic greenhouse QTL controls phenotypic variation in our field F_4's we looked for a significant association between genotype at LG8b and phenotype using three one-way ANOVAs. In our greenhouse experiment LG8b was the largest effect QTL for flowering time, flower size, and leaf shape. However in our reciprocal transplant LG8b genotype was significantly associated with flower size in all sites, but not with flowering time or leaf shape (Table S4).

Selection on major QTL differs before vs. after flowering

To test whether there was selection before flowering at the LG8b locus we looked for a skew from neutral (1:2:1) genotypic ratios in plants that survived to flowering. We found

significant genotypic skew at two of four transplant sites: Granite 1 (p-value < 0.001) and Meadow 2 (p-value <0.001). There was an excess of *M. guttatus* homozygotes (GG) in both sites (Figure 4a&d). To test whether LG8b genotype affected fecundity in the field we performed one-way ANOVAs. *M. laciniatus* homozygotes (LL) had higher fruit number than *M. guttatus* homozygotes in Granite 2 (Figure 4f), however this trend was not significant due to low survival (Figure 4f). At Meadow 2 both heterozygotes (GL) and *M. laciniatus* homozygotes (LL) produced significantly more fruits than *M. guttatus* homozygotes (GG) (Table S5, Figure 4h). Collectively our results suggest that while the LG8b *M. guttatus* allele is advantageous in both habitats before flowering, the *M. laciniatus* allele may have a fecundity advantage in both habitats after flowering.

Drought drives survivorship everywhere while herbivory affects meadows

In our best fit mixed effects model soil moisture and leaf damage significantly affected plant survival when all sites were analyzed together (Table 4). A significant interaction between soil moisture & habitat indicates that soil moisture affects survival differently in each habitat over time (Table 4). Specifically, while soil moisture is in fact higher in granite outcrops at the beginning of the growing season, it then decays rapidly over a course of one week mid-summer (Figure 5a). In contrast, soil moisture in the meadow habitat decays in a gradual linear fashion over the course of the season (Figure 5b). Survival tracks soil moisture with a slight delay in both granite and meadow habitats (Figure 5c&d). When each habitat type was analyzed separately soil moisture was still significantly associated with survival in both, but leaf damage only significantly affected survival in meadows (Table 4). There was also significantly more herbivore damage, and therefore more herbivore pressure, in the meadow habitats than in granite (p-value < 0.001, Figure S2).

DISCUSSION

Since the pioneering reciprocal transplant experiments of Clausen, Keck, and Hiesey (1941) local adaptation has been repeatedly demonstrated in plants (Hereford, 2009). However the traits that are responsible for differential adaptation, the genes that underlie them, and the selective forces driving it are still poorly understood (Salvolainen et al., 2013). To test the adaptive significance of early flowering time, small flower size, and lobed leaf shape in *M. laciniatus* we performed a reciprocal transplant in the Sierra

Nevada Mountains of California with an outbred *M. laciniatus x M. guttatus* F_4 population, measured selection on phenotypes and genotypes, and collected fine scale soil moisture and herbivory data.

Flowering time underlies local adaptation

We found that *M. laciniatus* and *M. guttatus* are locally adapted to their respective habitats (Figure 1). *M. laciniatus* parents were significantly more likely to survive to flowering and produce more fruits in granite outcrops than *M. guttatus* parents (Table 4, Figure 2a). Interestingly in meadows we saw no difference in survival to flowering between species, but *M. guttatus* produced more fruits than *M. laciniatus* in its native habitat (Figure 2b). Our results agree with an earlier study which found that *M. laciniatus* had higher survival to flowering in granite than *M. guttatus*, but that the two performed equally well in meadows (Peterson et al., 2013). In fact many reciprocal transplant studies have found that one species or population does best in both habitats (Hereford et al., 2009) and this observation elicits the question, why doesn't the population with highest overall fitness occur in both habitats? There are several reasons why reciprocal transplant results may not reflect the distribution of naturally occurring populations: (1) is that one species or population is actually superior and in the process of taking over the others' habitat, (2) dispersal is limiting and therefore the taxon with higher fitness cannot colonize the others' habitat, (3) limits of the transplant study design, for example natural competitive environments are often not well replicated in experimental transplants for logistical reasons, and (4) lifetime fitness was not measured. Our results illustrate the fourth scenario well since our fecundity results show a trade-off in lifetime fitness between species that was not observable with survival data alone.

Our selection analysis found that strong directional selection on earlier flowering time in granite outcrops underlies *M. laciniatus'* adaptation to its harsh habitat (Table 2). Earlier flowering time is critical for drought escape in many annual plants that occupy seasonally dry environments (Kiang & Hamrick, 1978; Fox, 1990; Eckhart et al., 2004; Hall & Willis, 2006; Franks et al., 2007; Anderson et al., 2012). Early flowering allows *M. laciniatus* to reproduce before the rapid onset of seasonal drought in granite outcrops (Figure 5a&b). In contrast, in continually moist environments we expect selection for later flowering or stabilizing selection on midseason flowering since later flowering

plants are larger and more fecund (Mitchell-Olds, 1996; Hall & Willis, 2006; Anderson et al., 2012). Although we found evidence that selection acts differently on flowering time in granite outcrops vs. meadows (Table 2), we did not detect selection for later flowering in the *M. guttatus* habitat. This may be because meadows dried out earlier than normal due to the 2013 California drought (Swain et al., 2014). Selection for earlier flowering has been previously detected in annual populations of *M. guttatus* (Hall & Willis, 2006).

We detected positive directional selection on flower size in both habitats (Table 2), indicating this trait does not contribute to differential habitat adaptation between *M. guttatus* and *M. laciniatus.* In a study of *M. guttatus* inland annuals vs. coastal perennials, Hall and Willis (2006) also detected a pattern of selection for larger flowers across habitats. Increased flower size likely facilitates increased outcrossing via pollinator visitation, which would alleviate effects of inbreeding depression in both habitats. Instead of being adaptive, the difference in floral size between the parental species may be due to selection on a correlated trait such as whole plant size or flowering time.

Greater flowering time plasticity in *M. laciniatus*

There is much debate in the literature about the role of phenotypic plasticity in adaptive evolution (Via et al., 1995; Schmitt et al., 1995; Dudley et al., 1996; Ghalambor et al., 2007; Ghalambor et al., 2015). We discovered both phenotypic plasticity and GxE in flowering time, plant height, and flower size (Figure 3). The presence of genetic variation in height, flower size, and flowering time plasticity (GxE) indicates that phenotypic plasticity in these traits has the potential to respond to selection. Plants in all genotypic classes (F_4, *M. laciniatus*, *M. guttatus*) flowered earlier and were shorter in granite (Table 1). However *M. laciniatus* had significantly greater flowering time plasticity than *M. guttatus* (Figure 3a), and this ability to better respond to environmental cues is likely advantageous given the rapid decreases in soil moisture and survival that characterize its native granite habitat (Figure 5a&b). On the other hand, the slow decline in soil moisture throughout the season in *M. guttatus'* meadows seems unlikely to select for increased flowering time plasticity (Figure 5 c&d).

We also found evidence of disadvantageous phenotypic plasticity in flower size. While *M. guttatus* had larger flowers in meadows than in the granite outcrops, *M. laciniatus* & F_4's displayed the opposite pattern (Figure 3b). Larger flowers were selected

for in both habitats and therefore the decrease in *M. laciniatus'* flower size in meadows relative to granite suggests that *M. laciniatus* was at a competitive disadvantage. The significantly smaller size of *M. laciniatus* plants and leaves (Figure 3c&d) in meadows compared to the native *M. guttatus* reinforces the idea that *M. laciniatus* struggled to compete there. In the granite outcrop habitat *M. laciniatus* was only slightly smaller in stature and leaf size than *M. guttatus*. There was also selection for larger leaves in the meadow habitat (Table 3) indicating that larger plants were more likely to set seed. *Mimulus guttatus'* meadows have a much higher plant density than *M. laciniatus'* relatively depauperate granite outcrops (K. Ferris, personal observation), and small plants would be shaded out by other more vigorous species. Therefore even though the two species were equally likely to survive to flowering in meadows, competition for light and other resources could explain *M. laciniatus'* poor fecundity in *M. guttatus'* habitat and the species trade-off in lifetime fitness.

Contrary to our expectations, we found no directional selection on leaf shape in three out of four transplant sites, likely due to a lack of phenotypic variation in the field (Figure S1e). In the greenhouse our *M. laciniatus x M. guttatus* hybrid population had a leaf shape standard deviation 43X greater than the standard deviation of our field hybrids. This lack of variation may be a plastic response to the severe California drought of 2013 (Swain et al., 2014) since both experimental plants and native *M. laciniatus* were uncharacteristically small and unlobed (Table S1; K. Ferris, personal observation). This extreme weather may also explain why overall plant fitness was lower in the meadow sites than in the granite outcrops (Figure 2); even the native *M. guttatus* populations were visibly stressed. Due to a lack of variation, our results can neither reject nor confirm that lobed leaves are adaptive.

Life-history QTL does not contribute to local adaptation via antagonistic pleiotropy

If antagonistic pleiotropy at LG8b was responsible for the fecundity trade-off between the species (Figure 2b), we would expect *M. laciniatus* homozygotes to have higher fitness than *M. guttatus* homozygotes in granite outcrops, but lower fitness in meadows. Instead we see that before flowering *M. guttatus* homozygotes at LG8b experienced a selective advantage in both habitats (Figure 4a-d), while after flowering *M. laciniatus* homozygotes enjoyed a slight fecundity advantage (Table S5, Figure 4e-h). One caveat is

that we did not empirically measure the genotype frequency at LG8b in F_{4s} before selection, and therefore cannot be positive that it was originally at equilibrium. However, while segregation distortion could account for the *M. guttatus* genotypic skew before flowering, it could not explain the subsequent advantage of *M. laciniatus* alleles in our fecundity analysis.

We found that LG8b controlled variation in flower size, but not flowering time or leaf shape in the field. Several things could account for this QTL controlling different traits in the greenhouse and the field. LG8b was mapped in an F_2 population created from a different population cross than we used to construct our field F_4 population. It is possible that different QTL's underlie flowering time among populations of *M. laciniatus* and that consequently LG8b does not contribute to genetic variation in flowering time in our F_4 cross. Alternatively, LG8b may be environmentally unstable. There are many examples of different QTLs explaining the same trait across different environments (Paterson et al., 1991; Wan et al., 2005; Messmer et al., 2009; Anderson et al., 2011). This phenomenon is attributed to genotype-by-environment interactions (Des Marais et al., 2013). A third possibility is that LG8b is composed of several linked genes that independently control flowering time, flower size, & leaf shape and that these loci have been broken up by additional recombination in our F_4 population.

We conclude that while LG8b contributes to divergence in many traits between sympatric *M. laciniatus* and *M. guttatus* in the greenhouse, it does not contribute to differential habitat adaptation in our experiment via antagonistic pleiotropy. Instead, LG8b controls flower size in the field and is under conflicting selection before vs. after flowering. Large flowered *M. guttatus* genotypes seem to have an advantage before flowering, while small flowered *M. laciniatus* LG8b genotypes have a slight advantage after selection. Flower size is correlated with plant height and leaf size (Table S2). Perhaps larger plants were better able to produce flowers in response to early decreases in soil moisture, but as the drought increased being large became costly. Conflicting viability and fecundity selection on flower size has previously been found within a population of *M. guttatus* (Mojica et al., 2012), although in that study small flowered genotypes were more likely to survive to flower while plants with large flowers set more seed. Conflicting life-history selection on the LG8b QTL may make it difficult for flower

size to respond to directional selection and may ultimately act as an evolutionary constraint.

Habitat differences in soil moisture & herbivory drive local adaptation

Decreasing soil moisture drove survival on a fine spatial and temporal scale across all sites (Figure 5). However, a significant interaction between soil moisture and habitat type demonstrated that soil moisture decayed and interacted with survival differently in granite vs. meadow habitats (Table 4, Figure 5). In *M. guttatus'* meadows soil moisture decayed in a slow linear fashion (Figure 5c&d), while in *M. laciniatus'* granite outcrops soil moisture remained high and constant early in the season and then dropped precipitously mid-summer (Figure 5a&b). Different soil moisture regimes should select for different plant life histories. A fast cycling, drought escape strategy should be advantageous in the granite outcrops while a longer lived, more water-use-efficient strategy should be advantageous in the meadow habitat. While we have no water use efficiency data for these species, the granite habitat's water regime has clearly selected for *M. laciniatus'* rapid, early flowering life history. However, drought patterns do little to explain *M. guttatus'* higher fitness in meadows.

We also found a difference in herbivory pressure between habitats with herbivory significantly impacting survival only in *M. guttatus'* meadows (Table 4). There was significantly less herbivore damage overall in granite. Therefore, *M. laciniatus* may not be adapted to herbivory which could contribute to the fecundity trade-off between *M. laciniatus* & *M. guttatus* in the meadow habitat. Increased herbivory (Figure S2) and *M. laciniatus'* inability to reach a competitive size in meadows (Table 1, Figure 3b,c,&d) seem to be causing a lifetime fitness trade-off between species in *M. guttatus'* habitat despite similar survival rates. In summary our data show that differences in soil moisture regime and herbivory between *M. laciniatus* & *M. guttatus*' habitats drive local adaptation.

Conclusions

Understanding the traits, genes, and ecological variables driving differential habitat adaptation is a key component of ecology and evolutionary biology, and is particularly relevant in light of the future increases in temperature and precipitation stress predicted to accompany global climate change. We found that *M. laciniatus* and its close relative *M.*

guttatus are locally adapted to microhabitats that differ dramatically in drought regime and herbivory. Our results indicate that while early flowering time is critical for plant fitness in *M. laciniatus'* harsh seasonally dry environment, other traits such as herbivore resistance and plant size are more important in a competitive mesic environment like *M. guttatus'*. Additionally we find genetic variation for phenotypic plasticity in all traits and advantageous flowering time plasticity in *M. laciniatus* indicating that plasticity can respond to selection in this system. A major pleiotropic life-history QTL does not control adaptive species differences via antagonistic pleiotropy in the field suggesting that the genetic architecture of adaptation may be more complex in this system. Few other studies have combined large-scale reciprocal transplants, phenotypic selection analysis, selection on trait QTLs, and fine scale environmental data to holistically understand local adaptation.

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AUTHOR CONTRIBUTIONS

KF designed & performed controlled crossing, field and genotyping experiments, collected and analyzed all data, and wrote the first draft of the manuscript. JW helped with experimental design and provided feedback on the manuscript.

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Table 1. Phenotypic trait means and standard errors calculated for day of first flower (days), plant height (mm), flower width (mm), leaf are (megapixels), and leaf shape *M. laciniatus's* granite outcrop versus *M. guttatus's* meadow habitat type in F_4's, *M. laciniatus*, and *M. guttatus*. Significant phenotypic plasticity in a trait is indicated by "*" and significance codes are as follows: p-value < 0.001 = ***, 0.01= **,0.05 =*.

Plant	Trait	Mean Granite	SE Granite	Mean Meadow	SE Meadow
F4	Days to 1st Flower	77.85***	0.263	86.42***	0.774
	Height	40.55***	0.789	45.18***	1.764
	Flower Width	8.45***	0.131	6.292***	0.246
	Leaf Area	2009	157.6	3203	281.2
	Leaf Shape	0.13**	0.002	0.12**	0.004
M. laciniatus	Days to 1st Flower	76.96***	0.505	85.78***	1.327
	Height	28.59***	1.089	46.53***	4.766
	Flower Width	6.075***	0.253	3.875***	0.467
	Leaf Area	1068	144.0	1307	248.2
	Leaf Shape	0.18**	0.011	0.17**	0.018
M. guttatus	Days to 1st Flower	80.25***	0.743	82.3***	1.739
	Height	31.23***	1.667	74.46***	7.032
	Flower Width	8.8***	0.42	9.835***	0.473
	Leaf Area	1120	103.1	3524	647.1
	Leaf Shape	0.11	0.006	0.11	0.01

Table 2. Results of phenotypic selection analysis on flowering time, flower width, leaf area, and leaf shape using a zero-inflated poisson regression. This table contains the regression coefficients from the poisson regression portion of the model on fruit count. β represents the selection gradient for each phenotypic trait in each habitat type and population. Significance codes are as follows: p-value < 0.001 = ***, 0.01= **,0.05 =* .

Trait	β Granite	β Meadow	β G1	β G2	β M1	β M2
Days to 1st Flower	-0.857***	-0.246	-0.847***	-1.212***	-0.454	-0.455**
Flower Width	0.15*	0.38*	0.123	0.182	0.109	0.508**
Leaf Area	0.052	0.408	0.247	0.085	-	0.406
Leaf Shape	0.054	0.255	0.061	-0.042	-1.01	0.389*
Block	-0.004	-0.030*	0.004	-0.006	0.019	-0.038**
Leaf Area non-linear (γ')	-0.016*	-	-0.397*	-	-	-
Leaf Shape non-linear (γ')	-	-0.379**	-	-	-	-0.336*
Flowering Time (γ')	-	-	-	-	-	-0.342*
Days to 1st Flower*Habitat	-1.44**	-	-	-	-	-
Days to 1st Flower*Population	-	-	0.669***	-0.415	-0.017	-

Table 3. Results of phenotypic selection analysis on flowering time, flower width, leaf area, and leaf shape using a zero-inflated poisson regression. This table contains the regression coefficients from the logistic regression portion of the model on whether plants set seed or not. β represents the selection gradient for each phenotypic trait in each habitat type and population. Significant codes are as follows: p-value < 0.001 = ***, 0.01= **,0.05 =* .

Trait	β Granite	β Meadow	β G1	β G2	β M1	β M2
Days to 1st Flower	-381.9	-0.262	1.569	-196.2	-9.532	-3.959
Flower Width	243.9	-0.615	-0.242	225.7	0.070	0.939
Leaf Area	-534.3	4.055*	1.968	-280.9	-	4.76*
Leaf Shape	-54.19	0.077	0.116	-41.99	-34.67	1.665
Block	1.492	-0.205	0.351**	-1.567	0.622	-0.383*
Leaf Area non-linear (γ')	-	-	-3.1**	-	-	-
Leaf Area*Population	-	-	1.903*	-3.0741	10.20*	-

Table 4. Results of the linear mixed effects model testing how environmental variables affected survival across sites and time given as the equations with the highest AIC criteria. In the model description SM = Soil Moisture, LD = Leaf Damage, T=Time, and H=Habitat. "k" is the number of parameters in the model, "Log(L)" is the log likelihood, "AIC" score, "Δi" is the difference between the AIC score of the current & top models, "wi" is the model weight, Marginal R^2 is the contribution of the fixed effects, Conditional R^2 is the total contribution of both random and fixed effects.

Model	k	Log(L)	AIC	Δi	wi	Marginal R^2	Conditional R^2
Survival Total ~ SM + LD + T + H + SM*T + SM*H + LD*SM + SM*T*H	10	977.1	-1912	0	1	0.802	0.833
Survival Granite ~ SM + T + SM*T	5	399.6	-774.8	0	1	0.853	0.856
Survival Meadow ~ SM + T + LD + SM*T	6	962.4	-1896	0	0.607	0.627	0.692

Figure 1. Images of (a) granite outcrop habitat (Granite 1), (b) *M. laciniatus* growing in its native granite habitat, (c) meadow habitat (Meadow 2), and (d) *M. guttatus* growing in its native meadow habitat.

Figure 2. Reaction norm plots of (a) mean survival & (b) fecundity of *M. laciniatus* (red) and *M. guttatus* (blue) parental inbred lines in granite (*M. laciniatus*) and meadow (*M. guttatus*) habitats.

Figure 3. Reaction norm plots demonstrating phenotypic plasticity between granite and meadow habitats for (c) mean flowering time (d) mean corolla width (e) mean plant height (f) mean leaf area for each genotypic class: *M. laciniatus* (red), *M. guttatus* (blue), and F_4's (purple).

Figure 4. Genotype frequencies of the LG8b QTL before flowering in (a) Granite 1 (b) Granite 2 (c) Meadow 1 (d) Meadow 2 sites. Fitness of LG8b genotypes after flowering in (e) Granite 1 (f) Granite 2 (g) Meadow 1 and (h) Meadow 2 sites. LL = *M. laciniatus* homozygote, LG = heterozygote, and GG = *M. guttatus* homozygote.

Figure 5. Percent soil moisture (gray) and proportion plants surviving (black) measured weekly over the two month duration of the experiment on a block by block basis across the (a) *M. laciniatus* habitat (red) Granite 1 and (b) Granite 2 sites (c) *M. guttatus* habitat (blue) Meadow 1 and (d) Meadow 2 sites.

Table S1. Phenotypic trait means and standard errors (SE) calculated for day to first flower (days), plant height (mm), flower width (mm), leaf area (megapixels), and leaf shape in each reciprocal transplant site (G1, G2, M1, M2) separately in F_4's, *M. laciniatus*, and *M. guttatus.*

Plant	Trait	Mean G1	SE G1	Mean G2	SE G2	Mean M1	SE M1	Mean M2	SE M2
F4	Days to 1st Flower	80.13	0.305	73.81	0.313	86.42	0.774	89.85	0.654
	Height	38.50	0.959	44.21	1.332	45.18	1.764	88.45	3.47
	Flower Width	8.785	0.169	7.785	0.192	6.293	0.246	6.91	0.253
	Leaf Area	1326.4	54.37	2686.6	311.8	1309	77.04	3899.4	264.8
	Leaf Shape	0.12	0.002	0.12	0.049	0.12	0.003	0.12	0.004
M. laciniatus	Days to 1st Flower	77.91	0.656	74.83	0.551	84.26	1.754	87.82	1.98
	Height	27.38	1.123	31.53	2.494	33.31	3.1	64.72	8.744
	Flower Width	6.382	0.283	5.07	0.502	4.172	0.669	3.578	0.668
	Leaf Area	915.3	104.9	2109.6	992.6	1022.1	209.607	1741.8	284.82
	Leaf Shape	0.166	0.010	0.22	0.018	0.13	0.013	0.18	0.019
M. guttatus	Days to 1st Flower	82.03	0.671	75.31	1.283	82.76	2.078	93	2.233
	Height	28.81	1.656	37.71	3.740	50.5	4.955	94.19	9.938
	Flower Width	8.823	0.478	8.725	0.926	8.608	0.632	10.85	0.597
	Leaf Area	888.1	79.43	1497.8	185.2	1490.4	214.2	3834.5	413.2
	Leaf Shape	0.1	0.005	0.1	0.008	0.11	0.014	0.1	0.008

Table S2. Phenotypic correlation matrix from REML analysis among all traits at granite vs. meadow sites separately. The granite habitat correlations are in the bottom matrix in black and the meadow habitat correlations are on the top in gray.

	Days to Flowering	Stem Length	Flower Width	Leaf Area	Shape
Days to Flowering	**1**	0.11	0.023	0.128	-0.058
Stem Length	-0.073	**1**	0.557	0.534	0.010
Flower Width	0.047	0.677	**1**	0.305	0.0002
Leaf Area	-0.088	0.525	0.34	**1**	0.135
Shape	-0.052	0.046	0.08	0.08	**1**

Table S3. Results of a logistic regression of survival to flowering on species and habitat to test for local adaptation. The model was Survival to Flowering ~ Habitat (Granite or Meadow) + Species (*M. guttatus* or *M. laciniatus*) + Habitat*Species.

Independent Variables	β'	z-value	p-value
Plant(L)	0.092	0.356	0.722
Habitat(L)	0.265	1.071	0.284
Plant(L)*Habitat(L)	0.964	2.876	0.004**

Table S4. Results of One-Way ANOVA's testing whether genotype at LG8b affected F_4 flowering time, flower width, leaf shape, and leaf area in a reciprocal transplant experiment in the field. We report the degrees of freedom (Df), sum of squares (Sum Sq), mean square (Mean Sq), F-statistic, and p-value for each phenotype measured. Significance codes are as follows: p-value <0.001 = ***, 0.01 = **, 0.05 = *.

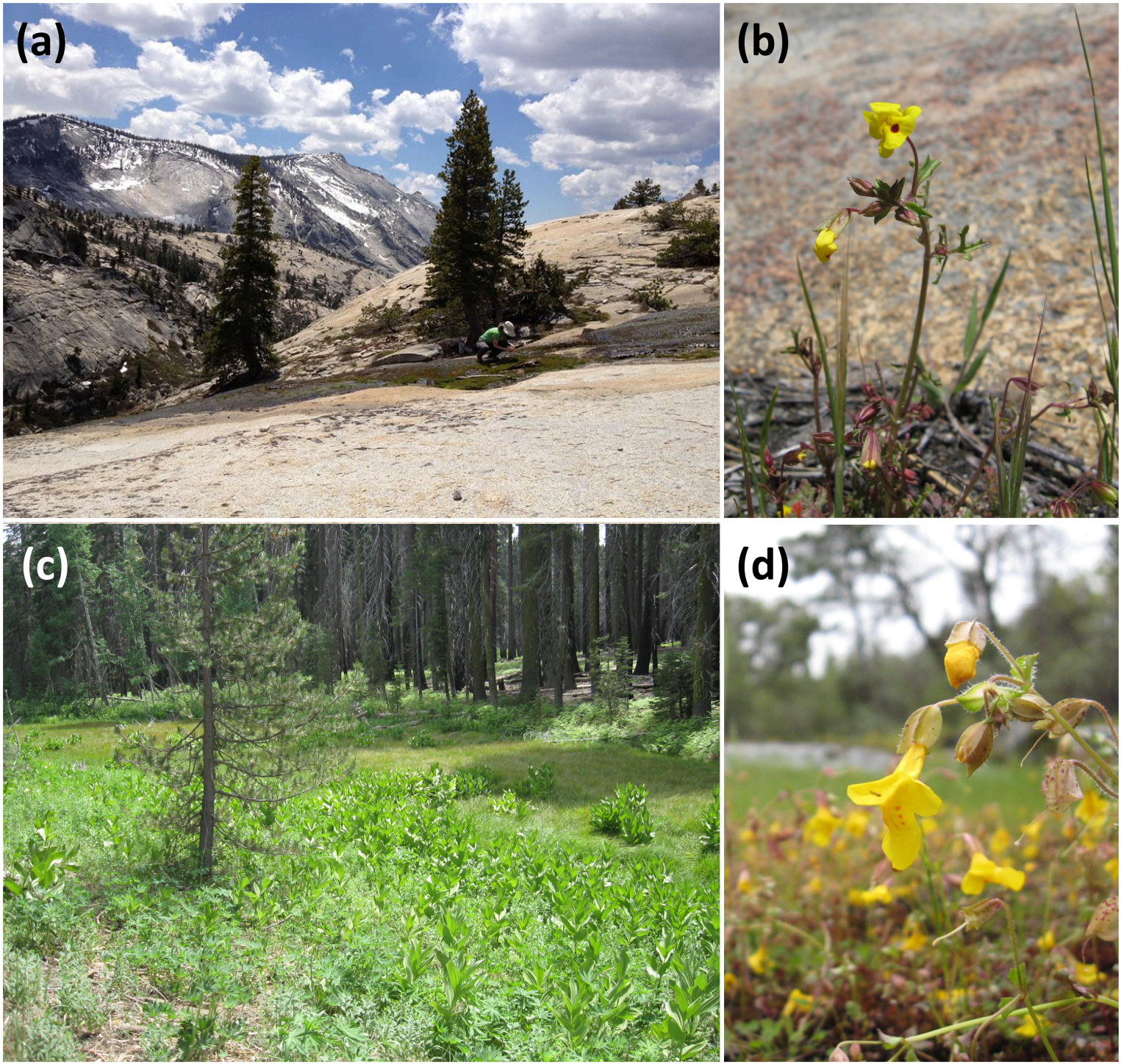
Phenotype	Model	Df	Sum Sq	Mean Sq	F-value	p-value
Flowering Time	LG8b genotype	2	137	68.71	1.098	0.335
	Residuals	384	24036	62.6		
Flower Width	LG8b genotype	2	1152	576.1	4.214	0.0155*
	Residuals	362	49488	136.7		
Leaf Shape	LG8b genotype	2	0.0008	0.0004	0.175	0.839
	Residuals	332	0.744	0.002		
Leaf Area	LG8b genotype	2	2.039e^07	10196269	2.025	0.134
	Residuals	332	2.275e^09	6853908		

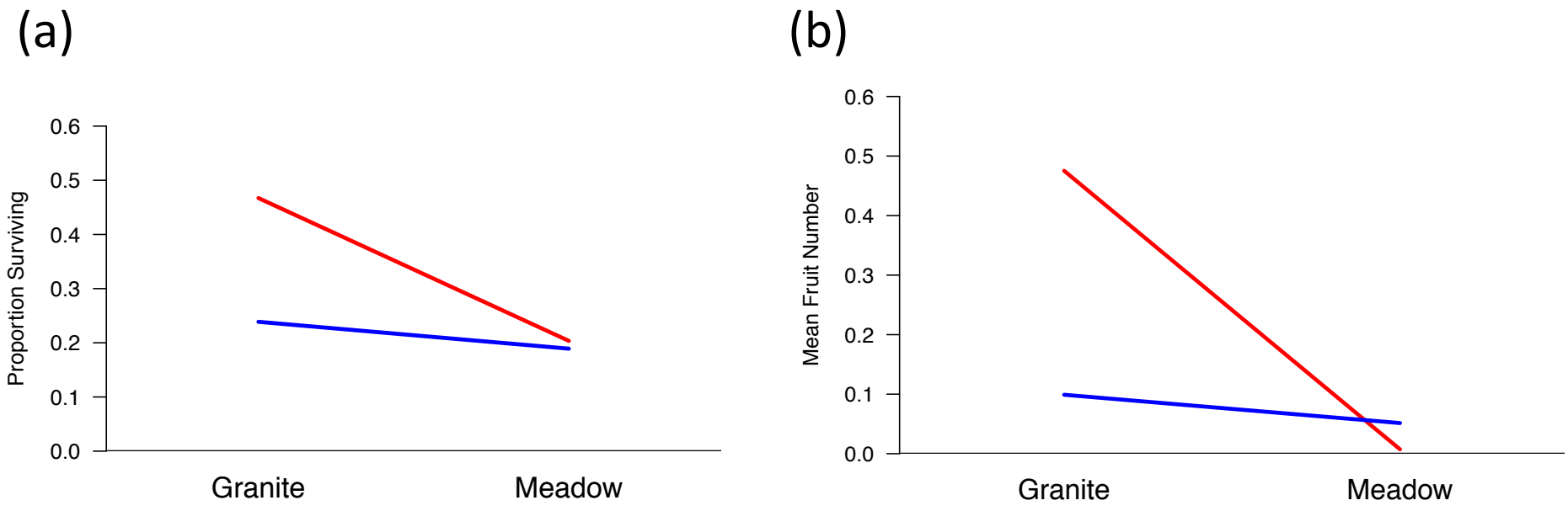
Table S5. Zero inflation poisson regression of fruit number on genotype at QTL LG8b. Genotype has three levels: GG (*M. guttatus* homozygote), GL (heterozygote), and LL (*M. laciniatus* homozygote)

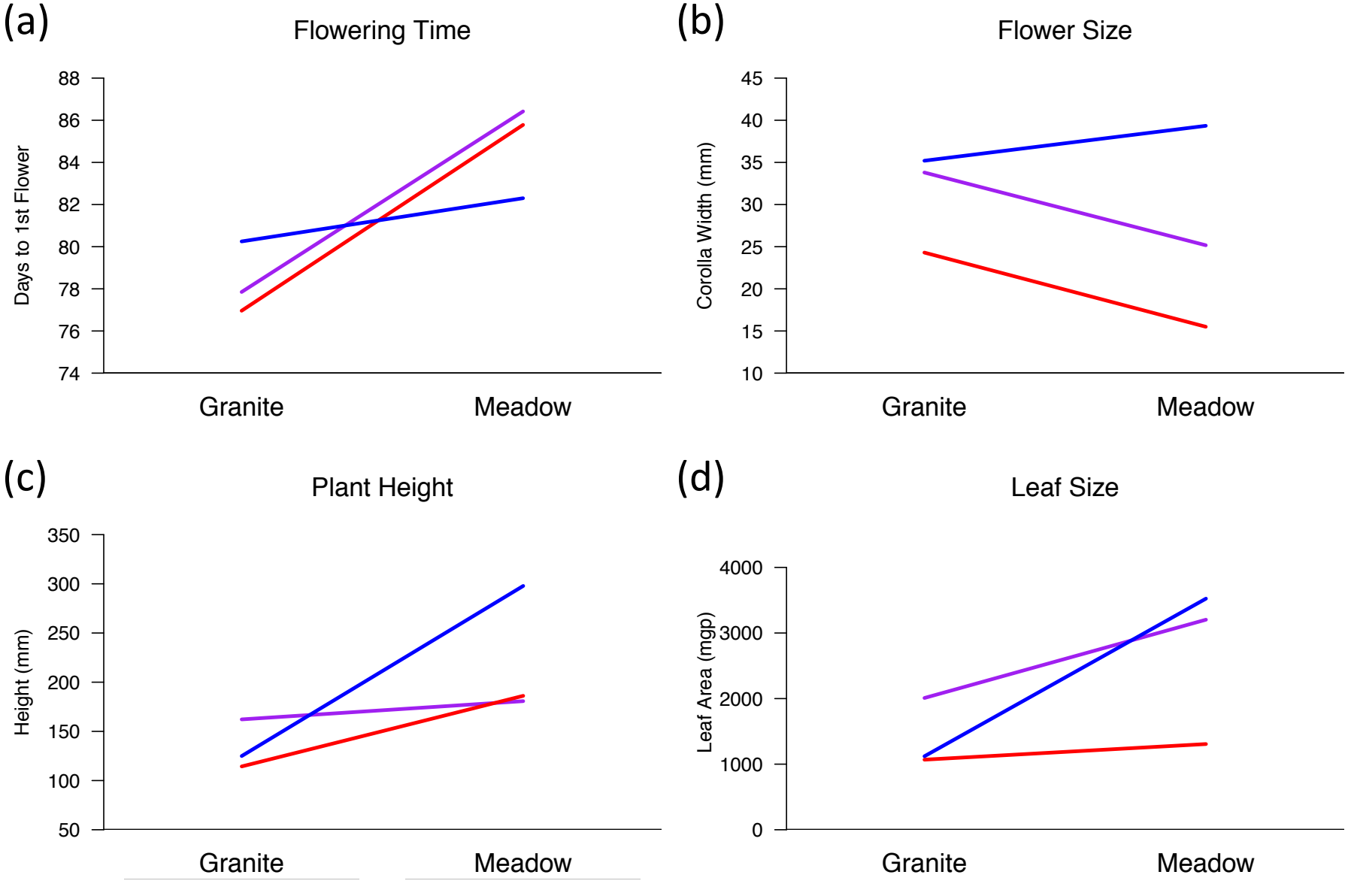
Dataset	Genotype	β	z-value	p-value
All populations	GL	**0.387**	**2.303**	**0.021***
	LL	0.462	1.36	0.174
Granite	GL	0.233	1.172	0.241
	LL	0.319	0.946	0.344
Meadow	GL	**0.785**	**2.325**	**0.02***
	LL	0.884	1.464	0.143
Granite 1	GL	0.189	0.832	0.405
	LL	0.099	0.386	0.699
Granite 2	GL	2.049	1.7	0.089
	LL	1.427	0.947	0.344
Meadow 1	GL	NA	NA	NA
	LL	NA	NA	NA
Meadow 2	GL	**0.878**	**2.049**	**0.040***
	LL	**1.017**	**1.934**	**0.053***

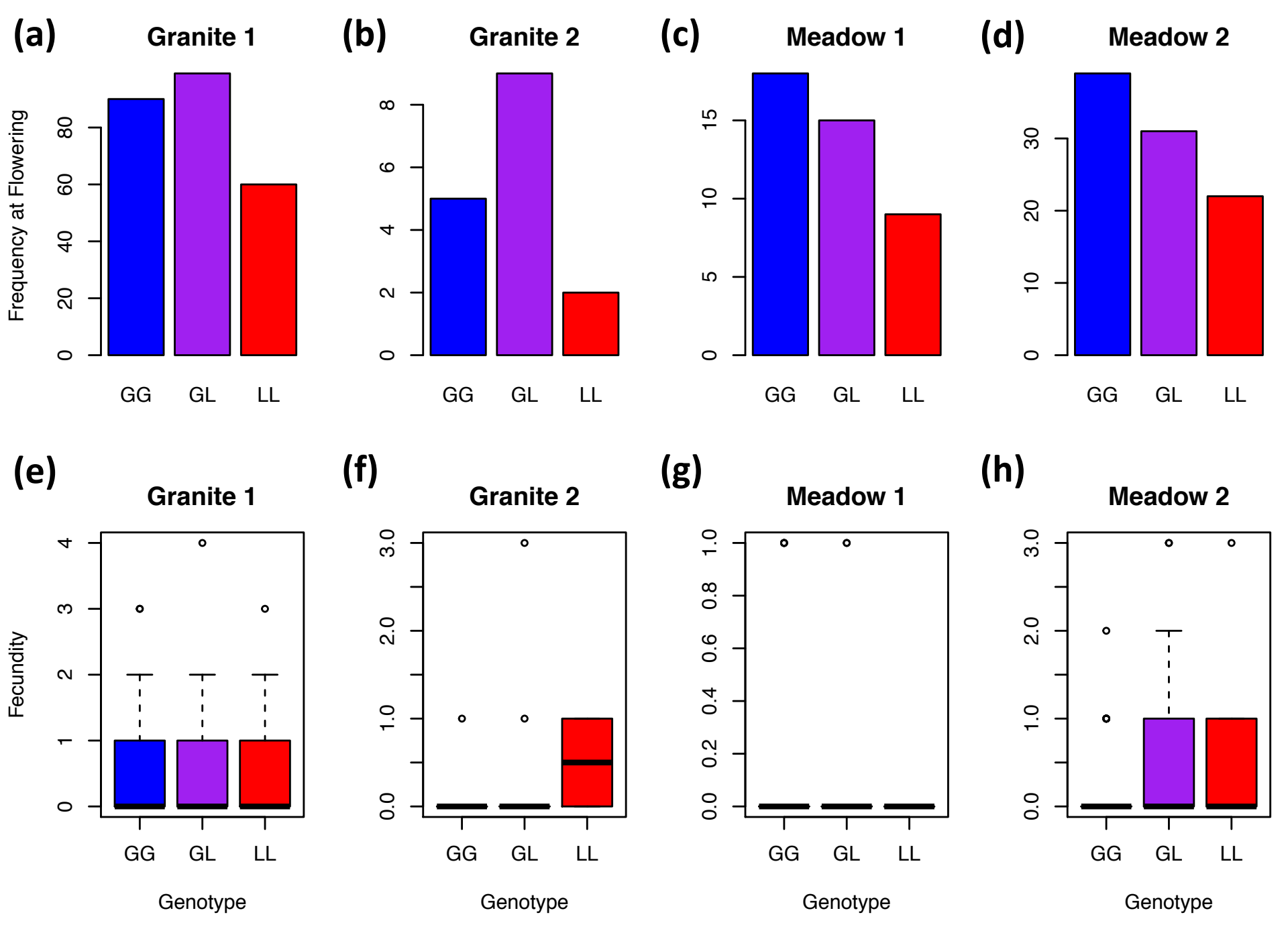
Figure S1. Images of (a) an *M. laciniatus* leaf and (b) an *M. guttatus* leaf. (c) Mean leaf shape of each genotypic class in each habitat. Images of *M. laciniatus* x. *M. guttatus* hybrid leaves on the same scale in (d) the greenhouse and (e) the field.

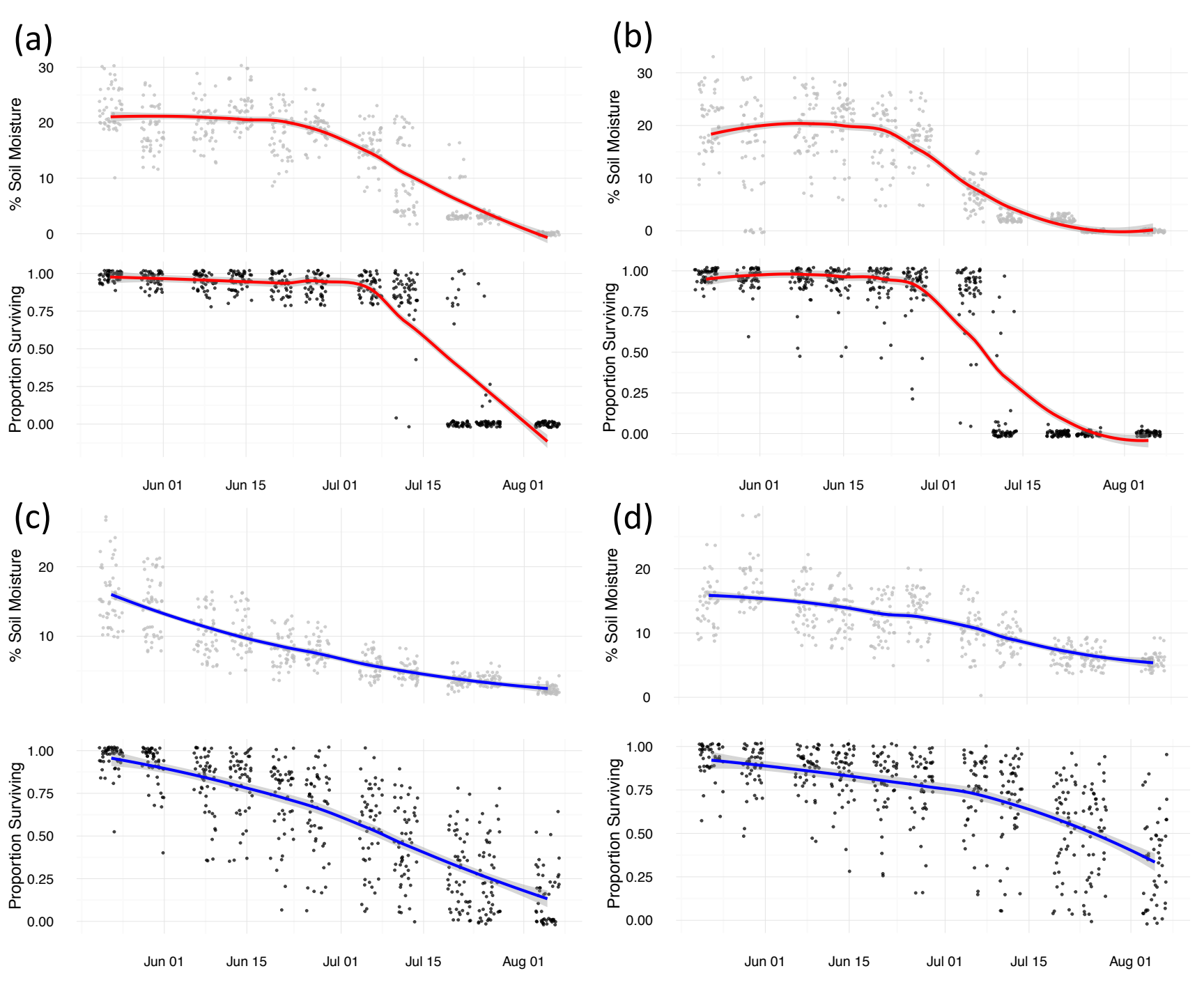
Figure S2. A boxplot of the number of herbivore damaged leaves per experimental block at the time of plant senescence in granite vs. meadow habitat.


(a)
(b)
(c)
(d)


(a)
Proportion Surviving
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Granite
Meadow
(b)
Mean Fruit Number
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Granite
Meadow


(a)
Flowering Time
Days to 1st Flower
88
86
84
82
80
78
76
74
Granite
Meadow
(b)
Flower Size
Corolla Width (mm)
45
40
35
30
25
20
15
10
Granite
Meadow
(c)
Plant Height
Height (mm)
350
300
250
200
150
100
50
Granite
Meadow
(d)
Leaf Size
Leaf Area (mgp)
4000
3000
2000
1000
0
Granite
Meadow


(a)
Granite 1
Frequency at Flowering
0
20
40
60
80
GG
GL
LL
(b)
Granite 2
0
2
4
6
8
GG
GL
LL
(c)
Meadow 1
0
5
10
15
GG
GL
LL
(d)
Meadow 2
0
10
20
30
GG
GL
LL
(e)
Granite 1
Fecundity
0
1
2
3
4
GG
GL
LL
Genotype
(f)
Granite 2
0.0
1.0
2.0
3.0
GG
GL
LL
Genotype
(g)
Meadow 1
0.0
0.2
0.4
0.6
0.8
1.0
GG
GL
LL
Genotype
(h)
Meadow 2
0.0
1.0
2.0
3.0
GG
GL
LL
Genotype


(a)
(b)
(c)
(d)
% Soil Moisture
Proportion Surviving
30
20
10
0
1.00
0.75
0.50
0.25
0.00
Jun 01
Jun 15
Jul 01
Jul 15
Aug 01