RUNNING TITLE: Fluctuating selection on Monkeyflowers

TITLE: Short-term fluctuating and long-term divergent selection on sympatric Monkeyflowers: insights from decade-spanning reciprocal transplants

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KEYWORDS (5-8): adaptation, phenology, speciation, divergence, environmental variation, evolution, natural selection, temporal variation

SUMMARY:

- Sympatric species often adapt to distinct microhabitats, leading to reproductive isolation and phenotypic diversity. However, temporal variation in selection may cause local maladaptation and species boundary breakdown, especially in years with large climatic events leading to episodic selection. Repeated reciprocal transplants can reveal short and long-term patterns of natural selection.
- To examine evolutionary trajectories of sympatric Monkeyflowers adapted to different niches, *Mimulus guttatus* and *M. laciniatus,* we performed three replicated transplants and combined them with previous experiments to leverage a dataset of five transplants spanning 10 years. We performed phenotypic selection analyses on parents and hybrids in parental habitats in Yosemite NP, CA during years of differing snowpack. If there is ecological isolation, then we predicted local adaptation and divergent phenotypic selection between habitats in line with species' differences.
- We found interannual fluctuations in selection, often not in predicted directions. Episodic selection due to extreme high snowpack caused a reversal of local adaptation and contributed to overall maladaptation of *M. guttatus*. However, a combined-year analysis detected longer-term divergent selection on flowering time, a key temporally isolating and adaptative trait, in agreement with species' differences.
- In conclusion, even with annual fluctuations, longer-term divergent selection may still promote species boundaries.

INTRODUCTION

Closely related sympatric species often occupy distinct habitats within areas of geographic overlap. Adaptation to differing environments may generate pre- and/or post-zygotic isolation via selection against immigrants (e.g. habitat isolation) or intermediate hybrids (e.g. extrinsic post-zygotic isolation; Rundle & Nosil, 2005). Divergent adaptation also increases the chance of species coexistence and persistence in sympatry (Coyne & Orr, 2004; Rundle & Nosil, 2005; Schluter, 2009). Given the importance of natural selection in the generation and maintenance of species, it follows that much of phenotypic diversity is likely due to environmental adaptation (Schluter, 2000, 2009). As speciation progresses, multiple forms of reproductive isolation

accumulate between divergent lineages hindering measurement of individual barriers (Coyne & Orr, 2004). Once species are partially isolated, neutral processes also cause the accumulation of phenotypic divergence. Experimental manipulation is necessary to determine whether habitat isolation is a key barrier and which traits are involved in adaptive divergence (Ramsey *et al.*, 2003; Hall & Willis, 2006; Lowry *et al.*, 2008). Measuring phenotypic selection on closely related sympatric species in a reciprocal transplant between native environments could identify whether divergent adaptation is important for reproductive isolation and speciation (Lowry *et al.*, 2008; Anderson *et al.*, 2015).

Natural populations are subject to spatial and temporal variation in selection (Siepielski *et al.*, 2009) which may influence the strength of reproductive isolation and species boundaries (Tataru *et al.*, 2023). Additionally, current environmental conditions may be different than the conditions during original divergence (Coyne & Orr, 2004; Rundle & Nosil, 2005). If environmental adaptation is important for reproductive isolation and maintenance of species boundaries, then we expect each species to have higher fitness in its habitat and to detect divergent phenotypic selection in the direction of species' differences (Anderson *et al.*, 2015), indicating habitat isolation and local adaptation to current conditions. However, if species are no longer adapted to their niches due to climate change, then we expect to find local maladaptation (Crespi, 2000) and selection for the non-local species' phenotype (Wilczek *et al.*, 2014; Kooyers *et al.*, 2019; Anderson & Wadgymar, 2020). In this scenario, spatially varying selection is acting contrary to reproductive isolation and could erode species boundaries by driving each towards the other's phenotypic optimum. Despite divergent spatial selection within one generation, temporal fluctuations could cause decreased reproductive isolation and eventual breakdown of species boundaries.

A great deal of temporal fluctuation in the direction and strength of natural selection across taxa has been found in nature, largely due to climatic factors (Siepielski *et al.*, 2009). Variation in selection in longitudinal studies is usually measured on a seasonal or annual timescale, a short-term measurement of population evolution. This leads to the question of how inter-annual fluctuations affect long-term evolutionary change in species and populations (Wadgymar *et al.*, 2017). A synthesis of phenotypic selection studies by Kingsolver & Diamond (2011) found that

while temporal variation in selection is common, it does not significantly inhibit long-term directional selection. Therefore, even with fluctuations in the direction of selection, there may be an overall positive or negative trend over a long enough time period. Wadgymar *et al.* (2017) found that interannual fluctuations in the direction of selection on the perennial plant *Boechera stricta* gave rise to long-term patterns consistent with stabilizing selection and local adaptation. How short versus long-term patterns of variable natural selection compare and impact species' divergence and reproductive isolation is not currently well understood.

Another understudied agent of evolutionary change is episodic selection resulting from sudden and extreme environmental events (e.g. drought, heatwave, precipitation events, fire, pest outbreak). Long-term studies which capture the evolutionary responses to these dramatic ecological changes are critical for understanding the maintenance of species boundaries. Through their long-term study of the Galapagos finches, Grant & Grant (1993) were able to illustrate how an extreme El Niño year altered patterns of selection on beak size and rates of interspecific gene flow. Strong episodes of selection such as this may drive evolutionary change by affecting the long-term trajectory of species evolution and biodiversity (Grant & Grant, 1993). Extreme climatic events are predicted to become more frequent from anthropogenic climate change (Bailey & van de Pol, 2016).

The *Mimulus guttatus* species complex, a group of closely related wildflowers, is well-suited for studies of natural selection on species boundaries and reproductive isolation (Wu *et al.*, 2008). *Mimulus guttatus* and *M. laciniatus* (syn. *Erythranthe guttata* and *E. laciniata*) are locally adapted to contrasting microhabitats throughout the central and southern Sierra Nevada, CA and are incompletely reproductively isolated (Vickery, 1964; Tataru *et al.*, 2024). *Mimulus guttatus* occurs in moist meadows and seeps while *M. laciniatus* occurs on rocky granite outcrops (Ferris *et al.*, 2014; Ferris & Willis, 2018). Granite habitats are harsh with shallow soils, high light intensity, extreme temperatures, and a short growing season (Ferris *et al.*, 2014). *Mimulus laciniatus* has adapted with a smaller plant size, lobed leaves, self-fertilizing mating system, reduced floral size, and earlier flowering phenology (DeMarche *et al.*, 2013; Ferris & Willis, 2018). Typical *M. guttatus* populations are largely outcrossing with entire leaves, larger flowers and plant sizes (Awadalla & Ritland, 1997; Ferris *et al.*, 2014). Flowering time is a key

premating barrier and contributor to local adaptation in the *Mimulus guttatus* species complex (Hall & Willis, 2006; Lowry *et al.*, 2008; Ferris *et al.*, 2017; Mantel & Sweigart, 2019).

Here, we build on previous work investigating spatial and temporal variation in natural selection on interspecific divergence between *Mimulus guttatus* and *M. laciniatus* to provide five years of repeated reciprocal transplant experiments under dramatically varying abiotic conditions (Figure 1). Previously, hybrids and parents were transplanted into each species' habitat in Yosemite National Park, CA during years of historically low (Ferris & Willis, 2018) and high snowpack (Tataru et al. 2023). Weaker divergent selection on a key isolating barrier, flowering time, was found during the high snowpack year which could erode species differentiation. We repeated these transplants during similarly low snowpack years (2021, 2022) and a year of exceptionally high snowpack (2023; Figure 1a,b). In this study, we address the following questions: (1) Are *M. guttatus* and *M. laciniatus* locally adapted? (2) Does the strength and direction of selection on adaptive and reproductively isolating phenotypes fluctuate? How do short versus long-term patterns of divergence compare? (3) How does episodic selection affect the trajectory of species divergence? By examining both interannual variation and overarching signatures of selection over a 10 year period, we can better elucidate the effects of a changing climate on the maintenance of biodiversity.

MATERIALS AND METHODS

Repeated reciprocal transplant design

The reciprocal transplants used hybrids created from inbred *M. guttatus* (YVO 18; 37.723366, -119.746433; 5,395 ft elevation) and *M. laciniatus* lines (WLF 47; 37.841533, -119.59385; 7993 ft elevation; Figure 1c). Due to seed availability, we used a different line from the same *M. guttatus* population than was used previously (YVO 6; Ferris & Willis, 2018; Tataru *et al.*, 2023). Parents were crossed to generate F_1 hybrids (WLF 47 maternal x YVO 18 paternal), which were self-fertilized to generate the F_2 seeds used in 2021. 500 F_2 hybrids were self-fertilized and propagated with single seed descent to produce the F_3 seeds used in 2022 and to develop the 300 F_6 lines used in 2023. In 2021 and 2022, we pooled seeds from 200 hybrid maternal families for use in the transplant experiment. To prevent inbreeding depression in parental genotypes in 2023, we intercrossed inbred lines within each parental population (WLF and YVO).

Our repeated reciprocal transplants took place in 2021, 2022, and 2023 in Yosemite National Park, CA, USA. We replicated the experiments performed in 2013 and 2019 using the same four study sites (Figure 1c): two granite outcrops (Granite 1 [37.810700, -119.485200; 8,436 ft elevation], Granite 2 [37.843702, -119.573120; 8,117 ft elevation]) and two meadows (Meadow 1 [37.767781, -119.772720; 6,605 ft elevation], Meadow 2 [37.755968, -119.803031; 6,163 ft elevation]. In 2021 and 2022, each site comprised of 50 randomized blocks of 18 and 24 individuals, respectively, in a 4×6 formation with one inch spacing between individuals. In 2021, blocks contained 6 F_2 hybrids and 6 of each parent and Granite 2 comprised 75 instead of 50 blocks. The 2021 experiment totaled 600 individuals of each genotype (*M. guttatus*, *M. laciniatus*, F_2 hybrids) in meadow habitat and 750 of each genotype in granite habitat with 4,050 total plants. In 2022, each randomized block contained 16 F_3 hybrids with 4 of each parent, totaling 1,600 F_3 hybrids and 400 parents per habitat type and 4,800 overall experimental plants. In 2023, local parental genotypes originating from each site were planted only into their origin site, to compare the fitness of local *M. guttatus* and *M. laciniatus* parents with the parents of experimental hybrids originating from nearby sites (WLF and YVO). We used 2–3 intrapopulation parental crosses and original inbred lines, distributed evenly across blocks and sites, which did not differ in mean seed count and were combined for downstream analyses (Table S1). Each site had 60 blocks of 35 individuals in a 5×7 formation, composed of 3–4 of parental genotypes (YVO, WLF, and local), and 16–23 F_6 hybrids for 200 of each parent and 1,500 F_6 hybrids (5 replicates of 300 F_6 lines) at each site. There were a total of 1,200 of each parent and 3,000 F_6 hybrids in each habitat type with 8,400 overall experimental plants.

We cold-stratified parental *M. laciniatus* and hybrid seeds for 10 days and parental *M. guttatus* seeds for 5 days at 4°C to break dormancy and synchronize germination (Sunshine Mix #4 Professional Growing Mix, Sun Gro) for one week in greenhouses at the University of California (UC) Davis (2021) and UC Merced (2022–2023). Following germination, cotyledons were planted into block designs described above. Mortality within the first week was attributed to transplant shock and dead germinates were replaced.

Data collection

To gain an understanding of microhabitat variation between and within sites, environmental measurements were taken weekly from experimental blocks. We measured soil moisture (%) using the SM150T Soil Moisture Sensor (Dynamax), soil surface temperature (°F) using a laser thermometer, and light intensity (µmol m-2 s-1) using a MQ-200X Sunlight Quantum Meter (Apogee Instruments). We recorded survival, herbivory (presence or absence), and phenotypes every other day until the end of the growing season. At first flower emergence, we recorded the date and measured plant height (to apical meristem; mm), stigma and anther lengths (mm), and corolla width (mm). Flowering time was determined as the number of days from transplant to the first open flower. Herkogamy (i.e. stigma-anther separation) was calculated by subtracting stigma length from longest anther length, as a metric of outcrossing ability. To assess leaf shape, the first true leaf was collected one week after flowering. Leaves were digitally scanned and analyzed to calculate leaf lobing index, a measure of degree of lobing, using ImageJ (Schneider *et al.*, 2012; Ferris *et al.*, 2015).

Assessing fitness metrics

We collected fruits and counted fruit and seed number, and calculated their correlation using Kendall's correlation analysis. Plants that produced flowers but did not produce fruit or seed were indicated with a value of zero. To assess local adaptation, we used three metrics of fitness for parental genotypes: survival to flowering ('survival'), mean seed number per reproductive individual ('fecundity'), and mean seed number per planted seed ('total fitness'). To assess long term patterns of fitness and local adaptation, we averaged survival, fecundity, and total fitness across years (2013, 2019, 2021, 2022, 2023) for each habitats and species. Data from 2013 was omitted from averaged fecundity and total fitness calculations because only fruit number was counted.

Environmental data analysis

To find associations between environmental variables and plant survival in each habitat (granite or meadow), we used linear mixed effects models with survival as the dependent variable and soil moisture, time, soil temperature and light levels as fixed independent variables, and block as a random effect. Survival in this analysis was calculated as the proportion surviving in each block during each environmental survey. We tested for interactions between independent

variables and performed model selection for the best-fit model using AIC selection criteria using R packages *nlme* v3.1 (Pinheiro & Bates, 2000; Pinheiro *et al.*, 2023) and *MuMIn* v1.47.5 (Bartoń, 2018). To test whether foliar herbivory and habitat are independent, we used chi-squared tests to test for correlation and created contingency tables. To understand the effect of herbivory on plant fitness, we used ANOVA models using seed count as the dependent variable, and herbivory presence/absence, habitat, site, and block as independent variables and interactions between them, and performed model selection using the R packages *stats* v3.6.2 (R Core Team, 2023) and *MuMIn* v1.47.5 (Bartoń, 2018).

Phenotypic selection analyses

To investigate plasticity between habitats in parental genotypes, we compared trait means using *t*-tests and calculated the coefficient of variation (CV; standard deviation divided by mean; (Pélabon *et al.*, 2020) using the R package *stats* v3.6.2 (R Core Team, 2023). Although hybrid composition may differ genetically between habitats, we compared trait means of hybrids (aggregated) for a putative indication of plasticity. To investigate linkage between traits, we created a phenotypic trait correlation matrix using hybrid individuals, grouped separately by habitat and year, using R packages *Hmisc* v5.1-1 (Harrell Jr, 2023) and *corrplot* v0.92 (Simko, 2021). We found generally weak correlations ($r < 0.50$) between traits, except between leaf area and plant height or flower width in 2022 and 2023 (Figure S2, S3, S4). Therefore, leaf area was removed from following phenotypic selection analyses.

To understand the strength and direction of selection on traits, we used multivariate linear and quadratic phenotypic selection analyses on hybrid datasets. Quantitative traits (leaf lobing, plant height, outcrossing, flower width, flowering time) were standardized to a mean of 0 and s.d. of 1. We used seed count as the dependent variable with plant height, days to flowering, flower width, herkogamy, leaf lobing, and their interactions as fixed independent variables, and block nested in site as a random effect. We used two types of models to account for overdispersion of zeros in seed count using the R package *glmmTMB* v1.1.8 (Brooks *et al.*, 2017; Magnusson *et al.*, 2017). Multivariate negative binomial models were used to assess whether a trait is associated with the production of any seeds. Additionally, we used zero-truncated Poisson analysis to assess whether traits are associated with the number of seeds produced (i.e. omitting zero values). We performed

model selection for the best-fit model using the R package *MuMIn* v1.47.5 (Bartoń, 2018). Phenotypes and interactions included in the top model were considered to be under selection. If a trait did not appear in the model, we consider there to have been no selection on that phenotype. To extend the comparison to the 2013 experiment which used only fruit number, we repeated all analyses using fruit number as the dependent variable. Lastly, we repeated phenotypic selection analyses on a combined dataset of hybrid genotypes from all years (2013–2023) using fruit number as the dependent variable with plant height, days to flowering, flower width, leaf lobing, and their interactions as fixed independent variables, and site and year as random effects. To visualize the fitness-phenotype relationships we plotted selection gradients using the R package *visreg* (Breheny & Burchett, 2017).

RESULTS

Soil moisture always influences survival

Fine-scale environmental variation was significantly tied to survival trends in both habitats across years (Figure 2, Table S2). Soil moisture and time (day) were significantly associated with survival across all years and habitats ($p < 0.001$). Best-fit models for *M. guttatus*'s meadows in 2021 and 2022 also included light intensity ($p < 0.001$), and 2022 included soil temperature. In our extreme snowpack year (2023), meadow habitat differed from other years where temperature, but not light, significantly affected survival ($p < 0.001$; Table S2). In *M. laciniatus's* granite habitat in 2021 and 2022, time, soil moisture, light and temperature all significantly impacted survival ($p < 0.0001$; Table S2). In 2023, light and temperature were in the best fit model but light was not significant ($p = 0.5672$). We found that temperature was significantly associated with survival in meadow habitat in high, but not low snowpack years. In granite, light significantly affected survival in low, but not high snowpack years. Across habitats in 2023, light was not associated with survival. Further, 2021–2023 differed from 2013 and 2019 where soil moisture was the only significant environmental factor; however, light and temperature were not measured in 2013.

The extreme high snowpack year (2023) differed from other years in several aspects. Soil moisture declined gradually in both habitats while light and surface temperatures were lower (Figure 2). In granite in 2023, there was no characteristic temperature spike usually coinciding

with mortality (Figure 2b). The growing season was delayed by six weeks or more in both habitats due to the snowpack, with surveys not beginning in granite until previous years' surveys had ended. Unlike in previous years plants were still alive in meadows at the end of the growing season: 41.7% (Meadow 1) and 50.7% (Meadow 2) alive at the last survey before snowfall (Figure 2;). Of the individuals still alive in Meadow 1, many had not flowered (0% of local genotype, 31.0% of hybrids, 44.1% of *M. guttatus*, 70.7% of *M. laciniatus*). Meadow 2 was similar (0% of local genotype, 47.3% of hybrids, 40.4% of *M. guttatus*) except most individuals of *M. laciniatus* flowered (92.0 %). The 2023 growing season was longer in granite (Granite 2013-2022: 33-87 days; 2023: 112-113 days) but comparable to previous years in meadow (Meadow 2013-2022: 74-160 days; 2023: 119-146 days).

Herbivory is always higher in meadows

As in previous studies (Ferris & Willis, 2018; Tataru *et al.*, 2023) herbivory pressure differed significantly between habitats in all years with greater herbivory in *M. guttatus'*s meadows : 2021 (meadow 22%, granite 1.97%; χ^2=552.3, d.f. = 1, $p < 0.0001$), 2022 (meadow 37.75%, granite 2.58%; χ^2=921.78, d.f. = 1, $p < 0.0001$), and 2023 (meadow 23.17%, granite 8.74%; χ^2=325.91, d.f. = 1, $p < 0.0001$). Only in 2021 did the best-fit ANOVA model of the effect of herbivory on total fitness include herbivory, site, and their (Table S3). Overall, herbivory pressure was spatially stable but its effect on fitness varied temporally.

M. guttatus appears maladapted to its meadows

Mimulus laciniatus had higher survival to flowering than *M. guttatus* in both habitats in all three years, similar to 2013 (Figure 3a). In 2019 when there was extremely low overall survival of parental genotypes. With all five years combined, *M. laciniatus* had higher average survival in both habitats (Figure 3a). Patterns of fecundity differed between years with parental species having higher fecundity in their native habitats in 2021 indicating local adaptation and selection against immigrants (Figure 3b). However, in 2022, *M. laciniatus* had higher fecundity in both habitats, and in 2023, we found reciprocal local maladaptation where *M. guttatus* had higher fecundity than *M. laciniatus* in granite and vice versa in meadows. With years combined, we found overall evidence of local maladaptation in mean fecundity (Figure 3b); however, if the extreme snowpack of 2023 is excluded then local adaptation in fecundity is seen (Figure S1). For

total fitness, *M. laciniatus* and *M. gutta*tus were equal in granite in high snowpack years (2019 and 2023), but *M. laciniatus* had the home-site advantage in low snowpack years (2013, 2021, 2022). Total fitness in meadow was not as predictable with *M. laciniatus* having higher total fitness for all three recent years but lower or similar fitness in 2013 and 2019. Overall, *M. laciniatus* had higher total fitness in both habitats (Figure 3c, S1).

Phenotypes are adaptively plastic between habitats

Parental genotypes exhibited plasticity in phenotypic traits between habitats, with the non-native species exhibited phenotypic plasticity in the direction of the local species' phenotype. In 2021 in its nonnative granite habitat, *M. guttatus* had significantly shorter mean flowering time and smaller plant height, more similar to the native *M. laciniatus* trait values (Table 2). In 2022 in granite habitat, *M. guttatus* had significantly smaller leaf area and increased leaf lobing which are both *M. laciniatus*-like traits (Table 3). In 2023, phenotypic plasticity moved *M. guttatus* to more *M. laciniatus*-like values in all traits with shorter flowering time, smaller plant height, smaller flowers, smaller leaves with increased lobing in granite habitat (Table 4). In all three years, *M. laciniatus* grew more *M. guttatus*-like when growing in its non-native meadow habitat with significantly longer flowering time, taller plant height, larger leaf area (Tables 3, 4, 5). Additionally in 2022 it had reduced leaf lobing like *M. guttatus*, but not in 2021 and 2023 (Table 3). These results are similar to 2013 with plasticity in every trait except *M. laciniatus*'s flower size, in the direction of the local species' phenotype (Ferris & Willis 2018).

Similarly, mean hybrid phenotypes were different between habitats with shorter flowering time, smaller plant height, smaller leaf area in hybrids grown in the granite habitat. In 2021, hybrids in granite exhibited reduced herkogamy, in the direction of the *M. laciniatus*, but reduced leaf lobing unlike *M. laciniatus* (Table 2). For hybrids in 2022, we found reduced herkogamy, smaller flower width and increased leaf lobing in granite, all in the *M. laciniatus* direction (Table 3). In 2023, we found that hybrids had smaller flowers but increased herkogamy in granite, (Table 4). In 2019, hybrids in granite differed by having larger flowers and larger leaf area relative to meadow (Tataru et al. 2023). In contrast, there were no significant differences between hybrids in granite and meadow habitats in 2013 (Ferris & Willis 2018). Overall, an

earlier mean flowering time in granite is common throughout years (2013–2023) across hybrids and parents.

Interannual fluctuation but long-term directional selection

We found temporal fluctuation in the strength and direction of selection on each of our quantitative traits. Based on observed trait differences between species, we predicted selection in meadow habitat for later flowering time, increased flower width, decreased leaf lobing, increased plant height, and increased herkogamy, with the inverse direction of selection in granite habitat (Figure 4a). In our comparison of low snowpack years, we found less divergent selection between habitats in the direction of species differences in 2021 relative to 2013. We were not able to assess selection between habitats in 2022 due to insufficient seed production in granite. We generally found weaker selection in *M. guttatus*'s meadow habitat in 2022 relative to 2021, contrary to expectations as 2022 was slightly drier. For certain traits, we found a very strong signature of selection in 2023 relative to other years (e.g. flower width, leaf lobing, plant height).

In the negative binomial selection analysis, measuring whether a trait influenced the production of any versus zero seeds, we found consistent selection for earlier flowering in both habitats across years, except in 2022 where flowering time was absent in the best-fit model (Figure 4b; Table 5, S4). In all years except 2021, there was stronger selection for earlier flowering in granite, matching predictions. We found similar selection for smaller flowers in 2021 in both granite and meadow habitats, but strong selection for larger flowers in 2022 and 2023 which echoed results in 2013 (granite) and 2019 (meadow). There was selection for reduced leaf lobing in meadows across years, matching predictions. In granite habitat, there was selection for increased leaf lobing in 2013, matching predictions, however leaf shape did not impact fitness in other years. As predicted we found selection for taller plants in every year in meadows. There was selection for taller plants in granite in 2021 and 2023 which was unexpected, but selection for smaller plants in 2013. There was selection for increased herkogamy as predicted from the outcrossing mating system of *M. guttatus* in 2023 in meadow habitat.

In the zero-truncated Poisson selection analysis, which measures the influence of a trait on seed number excluding plants with zero seeds, we found divergent selection in the opposite direction

of predictions in many years. In contrast to our predictions and 2013 results, we found stronger selection for earlier flowering in meadow habitats than granite in 2021, 2019, and 2023 (Figure 4c; Table 6, S4). In the high snowpack years (2019 and 2023), selection was actually reversed from our predictions with later flowering increasing fitness in granite and earlier flowering advantageous in meadows. Across all years and habitats, we found that having larger flowers and taller plant height resulted in higher fitness, except for weak selection for smaller flowers in 2023 in meadows and strong selection for shorter plants in granite in 2023. Smaller plants were never selected for in meadows. There was selection against leaf lobing in both habitats in 2021 and strong selection against lobing in granite in 2023, and this is different from our predictions (Figure 4a) and previous results. We found divergent selection on herkogamy in 2021 and 2023 with stronger selection in granite which matches the native *M. lacinaitus's* self-fertilizing mating system. Also in line with our predictions, there was selection for reduced herkogamy in 2022 and 2023 in meadow. We found that β values derived from fruit number were similar to those from seed number in both strength and direction (Table S5 and S6). This is likely attributed to a strong positive correlation between seed and fruit number (Table S7).

Although we see interannual fluctuations in the strength and direction of selection on all traits, when we combined data across years we found long term patterns of directional selection (Table 7, Figure 5, S5). In both zero-truncated Poisson and negative binomial analyses, we found long term selection for earlier flowering time and larger plant sizes in both habitats, with stronger selection in granite. Further, we found selection for smaller flowers in both habitats which does not match our predictions. However, we found selection for more lobed leaves in granite and negligible selection on leaf shape in meadow habitat. Herkogamy was not included in the combined dataset due to its absence in 2013. Therefore, while annual fluctuations make selection on our traits seem inconsistent, we do find longer term divergent selection in the direction of species' differences in both flowering time and leaf shape.

DISCUSSION

In this study, we investigated spatial and temporal variation in selection on two sympatric Monkeyflower species, *M. guttatus* and *M. laciniatus*. We compared multiple large-scale replicated reciprocal transplants conducted in low (2013, 2021, 2022) and high (2019, 2023)

snowpack years including one extreme year resulting in episodic selection (Figure 1a,b). Using experimental hybrids, we found fluctuating selection on phenotypic traits involved in reproductive isolation and environmental adaptation. Our results suggest that *M. guttatus* is maladaptated to its meadow habitat across years and that habitat isolation may therefore be asymmetric. Within each year, phenotypic selection was often contrary to our predicted directions but we saw a longer-term trend of divergent selection in the direction of species' differences in both flowering time, a key reproductive isolating trait, and leaf shape. Finally, we find that episodic selection, caused by an extreme snowfall event, may disrupt local adaptation and impact species boundaries.

M. guttatus is locally maladapted

Organisms are expected to have a fitness advantage in their home environment because spatial heterogeneity should favor the evolution of local adaptation (Hastings, 1983). However, temporal changes in environmental conditions are theorized to constrain adaptation if there are opposing selection pressures over time (Stearns, 1992) which may negatively impact reproductive isolation and species boundaries. We predicted that if habitat isolation is an important reproductive isolating barrier between our sympatric *Mimulus*, then there would be a fitness advantage of the local species in each habitat. Instead, we found evidence of local maladaptation of *M. guttatus* with *M. laciniatus* having higher total fitness in both habitats across time (Figure 3c). Annual fluctuations in snowpack did influence the relative fitness of the parent species in granite with equal fitness of *M. laciniatus* and *M. gutta*tus in high snowpack years, but in low years *M. laciniatus* had the home-site advantage (Figure 3c).

Although local adaptation is thought to be ubiquitous in natural populations (Hereford, 2009), fitness trade-offs are not always found in reciprocal transplants (Bennett & Lenski, 2007; Lowry *et al.*, 2009). It is possible that our reciprocal transplant did not detect existing trade-offs or that adaptation has evolved in *M. laciniatus* without fitness trade-offs across environments (Fry, 1996; Leimu & Fischer, 2008). A failure to detect local adaptation of *M. guttatus* to its native habitat could be attributed to germinating seeds in greenhouse conditions and transplanting as cotyledons, and/or the reduction of competition in experimental blocks. Transplanting as cotyledons may not account for species differences in seed survival, dormancy, and germination.

Parental genotypes may have been negatively impacted by inbreeding, affecting *M. guttatus* more than the highly self-fertilizing *M. laciniatus* (93–97%; Ferris *et al.*, 2014). However, this seems unlikely because outbred and inbred parental genotypes performed similarly in 2023 (Table S1).

Asymmetry in local adaptation or a lack of trade-offs, has been found in other transplant experiments (Hereford, 2009; Gosden *et al.*, 2015; Latreille & Pichot, 2017; Toll & Willis, 2018). The universal fitness advantage of *M. laciniatus* could be due to adaptive phenotypic plasticity (Ghalambor *et al.*, 2015). We found that both *M. guttatus* and *M. laciniatus* exhibit plasticity in the direction of the local species' phenotype in each habitat (Tables 2, 3, & 4). *Mimulus laciniatus's* occupation of a harsh, highly variable environment may have driven the evolution of adaptive plasticity (Via & Lande, 1985) allowing it to thrive in both habitats. However, *M. guttatus* was more plastic than *M. laciniatus* in all phenotypes except fitness indicating that plasticity, in the traits we measured, is unlikely to account for the lack of fitness trade-offs. Alternatively, *M. guttatus* may no longer be at its adaptive optima. Local adaptation is predicted to evolve in an annual outcrossing plant with large effective population sizes like *M. guttatus*, but may be constrained by natural selection, gene flow, genetic drift, low genetic diversity, or the genetic architecture of underlying traits (Kawecki & Ebert, 2004; Anderson *et al.*, 2011). Our results could indicate an adaptive lag where, due to recent rapid climate change, the adaptive landscape of *M. guttatus's* habitat has changed to be more *M. laciniatus*-like and *M. guttatus*'s evolutionary response has not kept pace (Lane *et al.*, 2012; Mills *et al.*, 2013; Kooyers *et al.*, 2019). This pattern of adaptive lag and local maladaptation has been found in other populations of *M. guttatus* (Kooyers *et al.*, 2019). Over time this trend of adaptive lag could erode species boundaries by allowing colonization of *M. guttatus's* meadow by *M. laciniatus* potentially increasing hybridization or worse, lead to population extinction.

Annual fluctuation in natural selection does not erase signatures of long-term divergence

Temporal and spatial variation in the strength and direction of selection has been well characterized as a mechanism that maintains genetic variation within species (Hereford, 2009; Siepielski *et al.*, 2009, 2017; Bergland et al. 2014; Bitter et al. 2023). However, few studies have empirically tested how fluctuating selection impacts the strength of reproductive isolation

between sympatric species (but see Campbell & Powers, 2015). We uncovered annually fluctuating selection on traits between low and high snowpack years, with more consistent patterns of selection on plant height, leaf lobing, flowering time and plant height. There was selection for earlier flowering time in granite across most years (Figure 4b,c). However, in high snowpack years, later flowering was associated with higher seed number in *M. laciniatus's* granite habitat which is in the opposite direction of species' differences (Figure 4c). This reflects a relaxation of divergent selection in high snowpack years. *Mimulus guttatus* that flower later in the season tend to produce higher seed (Mojica & Kelly, 2010; Mojica *et al.*, 2012), particularly during a longer, high snowpack growing season with lowered desiccation risk. Selection on leaf lobing also fluctuated across years but typically with selection patterns opposite predictions (Figure 4c).

Annual selection fluctuated unpredictably for flower width with combined selection for smaller flowers (Figure 4, Table 7), counter to species differences where *M. guttatus* has larger flowers than *M. laciniatus*. Flower size affects reproductive success and can contribute to population differentiation and reproductive isolation (Bradshaw *et al.*, 1995; Brunet, 2009; Schiestl & Schluter, 2009; Venail *et al.*, 2010; Krizek & Anderson, 2013). Flower size is often correlated with plant size indicating that early flowering, small stature, and small flowers are linked (Troth *et al.*, 2018). An antagonistic pleiotropy has been demonstrated in *M. guttatus* where later flowering alleles also increase plant and flower size (Mojica *et al.*, 2012; Monnahan & Kelly, 2015). Although plants with larger flowers have increased fecundity, they also lower viability, resulting in overall selection for smaller flowers (Mojica & Kelly, 2010). Although we saw selection for early flowering and small flowers, we find that selection consistently favored larger plants in both habitats across years suggesting that in our species' these traits are genetically distinct (Table 7). Although we initially predicted selection for smaller plant sizes in granite based on *M. laciniatus's* short stature, our findings suggest that larger plants always have higher fecundity. Size at reproduction is a key determinant of fecundity across taxa (Aarseen & Taylor, 1992; Aarssen & Jordan, 2001; Kingsolver & Pfennig, 2004; Troth *et al.*, 2018). Despite selection for larger sizes, average plant height was still smaller in granite than meadow habitats (Tables 2, 3, & 4), likely constrained by limited water and nutrients in rocky outcrops. We found

weak selection on herkogamy perhaps indicating that selection on stigma-anther distance is no longer as strong as during the earlier stages of speciation (Harder & Johnson, 2009).

Even with interannual fluctuations, we found longer-term divergent selection on flowering time and leaf shape between habitats when we combined data across years (Table 7, Figures 5, S5). In the combined analysis, there was stronger selection for early flowering in granite than meadow habitat which aligns with our predictions based on species' differences. We also found a distinct signature of divergent selection on leaf lobing in predicted directions. Lobed leaf shape is thought to be involved in adaptation to dry, exposed habitats like *M. laciniatus's* rocky outcrop environment (Nicotra *et al.*, 2011; Ferris, 2019) and therefore may also be involved in habitat adaptation and isolation (Ferris & Willis, 2018; Tataru *et al.*, 2023). A signature of overall divergent selection on these key traits in across transplants suggests ongoing adaptive differentiation and the potential for long-term maintenance of species boundaries (Chapurlat *et al.*, 2020).

Episodic selection weakens species divergence

The high Sierra Nevada snowpack of 2023 resulted in the delayed onset of spring in mid to high elevations by approximately 4–6 weeks and dramatically impacted patterns of soil moisture, light intensity, and surface temperature (Figure 2). In shifting the growing season the extreme snowpack contributed to local maladaptation between parental species (Figure 3b), as evidenced by a signature of local adaptation in fecundity reappearing when 2023 was excluded (Figure S1). Our results indicate that in *M. guttatus's* meadow habitat, this delay was detrimental to hybrids and *M. guttatus* by delaying flowering phenology which negatively impacted fitness. Additionally, none of the experimental local meadow genotypes flowered before snowfall, indicating a negative effect on native populations. However, many meadow plants remained alive at the first snowfall indicating potential facultative perenniality. Shorter day lengths were experienced in the late shifted season and critical photoperiod thresholds may not have been met to initiate the transition flowering (Friedman & Willis, 2013; Fishman *et al.*, 2014; Kenney & Sweigart, 2016). End-of-season drought stress may also typically act as a flowering cue in these populations (Kooyers *et al.*, 2015; Mantel & Sweigart, 2019), however in 2023 soil moisture in the meadows remained higher than in previous years (Figure 2). The seasonal delay did not

appear to negatively affect the fitness of *M. laciniatus*, likely because *M. laciniatus* flowers rapidly under shorter daylengths (Friedman & Willis, 2013; Ferris & Willis, 2018).

In granite, the delayed season in 2023 fostered a more favorable environment for the non-native *M. guttatus* with high water availability, lower light intensity and surface temperatures, and a longer growing season than is typical in the harsh habitat (Figure 2). This led to higher *M. guttatus* survival in granite than usual (Table 1). Although *M. guttatus* still had lower survival than *M. laciniatus*, it had remarkably high fecundity (Figure 3b) resulting in similar total fitness between the two species in granite which was unprecedented in previous years (Figure 3c). In contrast to the meadow habitat, all plants had senesced and many had set seed by the first snowfall in granite habitat in 2023. This suggests that drought stress is more important than critical photoperiod as a flowering cue in *M. lacinaitus's* habitat. It has also been shown that high-elevation populations of *M. laciniatus* have increased plasticity in critical photoperiod (Love & Ferris, 2024).

Our results demonstrate how an extreme climatic event may disrupt species boundaries. This atypical year produced unusually strong selection opposite of predicted directions on key life-history and morphology traits involved in adaptation and reproductive isolation: flowering time, flower size and leaf lobing (Figure 4). In the long term, episodic selection could expand or contract *M. guttatus* or *M. laciniatus'* ranges, encourage the emergence of hybrid swarms or a new hybrid species (Grant & Grant, 1993), or shift their adaptive potential (Campbell-Staton *et al.*, 2017). The rarity and unpredictability of extreme climatic events complicates predictions about the evolutionary response to climate change. Surveys of plant populations after extreme climatic events can monitor changes in the selective landscape and responses (Anderson, 2016; Bailey & van de Pol, 2016). In species with limited ranges, like the Sierra Nevada endemic *M. laciniatus*, will these events influence extinction risk? More longitudinal field studies are needed to understand long-term evolutionary consequences of extreme climatic events and changing climatic patterns on native plant species. For instance, do these events drive evolutionary change and does this outweigh selection acting during 'normal' periods? For species with a limited range, such as the Sierra Nevada endemic *M. laciniatus*, will these events influence extinction risk of populations?

Conclusions

Our decade-spanning repeated reciprocal transplants provide greater insight into the impacts of fluctuating selection on species' boundaries. The foreign advantage of *M. laciniatus* in the non-native meadow habitat across years suggests either a low cost of adaptation to the granite habitat or recent change in the shape of the adaptive landscape of the meadow habitat leading to current maladaptation of *M. guttatus*. Furthermore, longer-term divergent selection on flowering time found in our combined phenotypic selection analysis should reinforce temporal and habitat isolation between these species. However, the lack of consistent divergent selection on other traits involved in reproductive isolation and local adaptation could lead to eventual species fusion. Finally, a bout of episodic natural selection due to extreme snowfall shifted population dynamics in a way that may have long term impacts on species' range, abundance, and gene flow. Our study illustrates the strength of integrating manipulative field experiments across multiple spatial and temporal scales to gain a deeper understanding of the maintenance of biodiversity in the face of environmental change (Wadgymar *et al.*, 2017; Oakley *et al.*, 2023).

ACKNOWLEDGEMENTS

This work was financially supported by the National Institute of General Medicinal Sciences of the National Institute of Health (NIH) under award number R35GM138224 to KGF. We thank the Yosemite National Park Service for permit support (YOSE-2021-SCI-0033, YOSE-2022-SCI-0051, YOSE-2023-SCI-0011), and Breeanne Jackson at Yosemite Field Station (doi:10.21973/n3v36c) for logistical support and providing accommodation. We thank Johanna Schmitt at the University of California (UC) Davis, and Danielle Edwards and Jason Sexton at UC Merced for providing cold-stratification and greenhouse resources. We also thank members of the Ferris Lab who assisted the project, Natalie Gonzalez and Jessica Scales. Lastly, we acknowledge the original inhabitants of the unceded land on which our research was conducted, the Southern Sierra Miwuk Nation, Bishop Paiute Tribe, Bridgeport Indian Colony, Mono Lake Kutzadikaa, North Fork Rancheria of Mono Indians of California, Picayune Rancheria of the Chukchansi Indians and the Tuolumne Band of Me-Wuk Indians.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

KGF conceived the study and CMD, DT, BAR, and JKS contributed to experimental design. CMD, BAR, JKS, MD, RD, SD, FMP, and LM performed the reciprocal transplant experiments. CMD completed data analysis and drafted the manuscript with input from KGF. All authors contributed to and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in [repository name] at http://doi.org/[doi], reference number [reference number], upon acceptance of the manuscript.

REFERENCES

Aarseen LW, Taylor DR. **1992**. Fecundity allocation in herbaceous plants. *Oikos* **65**: 225–232.

Aarssen LW, Jordan CY. **2001**. Between-species patterns of covariation in plant size, seed size, and fecundity in monocarpic herbs. *Ecoscience* **8**: 471–477.

Anderson JT. **2016**. Plant fitness in a rapidly changing world. *New Phytologist* **210**: 81–87.

Anderson JT, Eckhart VM, Geber MA. **2015**. Experimental studies of adaptation in *Clarkia xantiana*: III: Phenotypic selection across a subspecies border. *Evolution* **69**: 2249–2261.

Anderson JT, Wadgymar SM. **2020**. Climate change disrupts local adaptation and favours upslope migration. *Ecology Letters* **23**: 181–192.

Anderson JT, Willis JH, Mitchell-Olds T. **2011**. Evolutionary genetics of plant adaptation. *Trends in Genetics* **27**: 258–266.

Awadalla P, Ritland K. **1997**. Microsatellite variation and evolution in the *Mimulus guttatus* species complex with contrasting mating systems. *Molecular Biology and Evolution* **14**: 1023–1034.

Bailey LD, van de Pol M. **2016**. Tackling extremes: Challenges for ecological and evolutionary research on extreme climatic events. *Journal of Animal Ecology* **85**: 85–96.

Bartoń K. **2018**. MuMIn: Multi-Model Inference. R package version 1.42.1. https://CRAN.R-project.org/package=MuMIn.

Bennett AF, Lenski RE. **2007**. An experimental test of evolutionary trade-offs during

temperature adaptation. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 8649–8654.

Bradshaw HD, Wilbert SM, Otto KG, Schemske DW. **1995**. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* **376**: 762–765.

Breheny P, Burchett W. **2017**. Visualization of Regression Models Using visreg. *The R Journal* **9**: 56–71.

Brooks ME, Kristensen K, Benthem KJ van, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM. **2017**. Modeling Zero-Inflated Count Data With glmmTMB. bioRxiv preprint. *bioRxiv*.

Brunet J. **2009**. Pollinators of the Rocky Mountain columbine: Temporal variation, functional groups and associations with floral traits. *Annals of Botany* **103**: 1567–1578.

Campbell-Staton SC, Cheviron ZA, Rochette N, Catchen J, Losos JB, Edwards S V. 2017. Winter storms drive rapid phenotypic, regulatory, and genomic shifts in the green anole lizard. *Science* **357**: 495–498.

Campbell DR, Powers JM. **2015**. Natural selection on floral morphology can be influenced by climate. *Proceedings of the Royal Society B: Biological Sciences* **282**.

Chapurlat E, Le Roncé I, Ågren J, Sletvold N. **2020**. Divergent selection on flowering phenology but not on floral morphology between two closely related orchids. *Ecology and Evolution* **10**: 5737–5747.

Coyne JA, Orr HA. **2004**. *Speciation*. Sunderland, Massachusetts: Sinauer Associates.

Crespi BJ. **2000**. The evolution of maladaptation. *Heredity* **84**: 623–629.

DeMarche ML, Rice KJ, Sexton JP. **2013**. Niche partitioning between close relatives suggests trade-offs between adaptation to local environments and competition. *Ecology and Evolution* **3**: 512–522.

Ferris KG. **2019**. Endless forms most functional: uncovering the role of natural selection in the evolution of leaf shape. *American Journal of Botany* **106**: 1532–1535.

Ferris KG, Barnett LL, Blackman BK, Willis JH. **2017**. The genetic architecture of local adaptation and reproductive isolation in sympatry within the *Mimulus guttatus* species complex. *Molecular Ecology* **26**: 208–224.

Ferris KG, Rushton T, Greenlee AB, Toll K, Blackman BK, Willis JH. **2015**. Leaf shape evolution has a similar genetic architecture in three edaphic specialists within the *Mimulus*

guttatus species complex. *Annals of Botany* **116**: 213–223.

Ferris KG, Sexton JP, Willis JH. **2014**. Speciation on a local geographic scale: The evolution of a rare rock outcrop specialist in *Mimulus*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369**: 27–29.

Ferris KG, Willis JH. **2018**. Differential adaptation to a harsh granite outcrop habitat between sympatric *Mimulus* species. *Evolution* **72**: 1225–1241.

Fishman L, Sweigart AL, Kenney AM, Campbell S. **2014**. Major quantitative trait loci control divergence in critical photoperiod for flowering between selfing and outcrossing species of monkeyflower (*Mimulus*). *New Phytologist* **201**: 1498–1507.

Friedman J, Willis JH. **2013**. Major QTLs for critical photoperiod and vernalization underlie extensive variation in flowering in the *Mimulus guttatus* species complex. *New Phytologist* **199**: 571–583.

Fry JD. **1996**. The Evolution of Host Specialization: Are Trade-Offs Overrated? *The American Naturalist* **148**: S84–S107.

Ghalambor CK, Hoke KL, Ruell EW, Fischer EK, Reznick DN, Hughes KA. **2015**. Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**: 372–375.

Gosden TP, Waller JT, Svensson EI. **2015**. Asymmetric isolating barriers between different microclimatic environments caused by low immigrant survival. *Proceedings of the Royal Society B: Biological Sciences* **282**.

Grant BR, Grant PR. **1993**. Evolution of Darwin's Finches Caused by a Rare Climatic Event. *Proceedings of the Royal Society B: Biological Sciences* **251**: 111–117.

Hall MC, Willis JH. **2006**. Divergent Selection on Flowering Time Contributes To Local Adaptation in *Mimulus Guttatus* Populations. *Evolution* **60**: 2466.

Harder LD, Johnson SD. **2009**. Darwin's beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytologist* **183**: 530–545.

Harrell Jr F. **2023**. Hmisc: Harrell Miscellaneous. R package version 5.1-2.

Hastings A. **1983**. Can spatial variation alone lead to selection for dispersal? *Theoretical Population Biology* **24**: 244–251.

Hereford J. **2009**. A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist* **173**: 579–588.

Kawecki TJ, Ebert D. **2004**. Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Kenney AM, Sweigart AL. **2016**. Reproductive isolation and introgression between sympatric *Mimulus* species. *Molecular ecology* **25**: 2499–2517.

Kingsolver JG, Diamond SE. **2011**. Phenotypic selection in natural populations: What limits directional selection? *American Naturalist* **177**: 346–357.

Kingsolver JG, Pfennig DW. **2004**. Individual-level selection as a cause of cope's rule of phyletic size increase. *Evolution* **58**: 1608–1612.

Kooyers NJ, Colicchio JM, Greenlee AB, Patterson E, Handloser NT, Blackman BK. **2019**. Lagging adaptation to climate supersedes local adaptation to herbivory in an annual monkeyflower. *American Naturalist* **194**: 541–557.

Kooyers NJ, Greenlee AB, Colicchio JM, Oh M, Blackman BK. **2015**. Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytologist* **206**: 152–165.

Krizek BA, Anderson JT. **2013**. Control of flower size. *Journal of Experimental Botany* **64**: 1427–1437.

Lane JE, Kruuk LEB, Charmantier A, Murie JO, Dobson FS. **2012**. Delayed phenology and reduced fitness associated with climate change in a wild hibernator. *Nature* **489**: 554–557.

Latreille AC, Pichot C. **2017**. Local-scale diversity and adaptation along elevational gradients assessed by reciprocal transplant experiments: lack of local adaptation in silver fir populations. *Annals of Forest Science* **74**.

Leimu R, Fischer M. **2008**. A meta-analysis of local adaptation in plants. *PLoS ONE* **3**: 1–8.

Love JM, Ferris KG. **2024**. Local adaptation to an altitudinal gradient: The interplay between mean phenotypic trait variation and phenotypic plasticity in *Mimulus laciniatus*. *Perspectives in Plant Ecology, Evolution and Systematics* **63**: 125795.

Lowry DB, Hall MC, Salt DE, Willis JH. **2009**. Genetic and physiological basis of adaptive salt tolerance divergence between coastal and inland *Mimulus guttatus*. *New Phytologist* **183**: 776–788.

Lowry DB, Rockwood RC, Willis JH. **2008**. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution* **62**: 2196–2214.

Magnusson A, Skaug HJ, Nielsen A, Berg CW, Kristensen K, Maechler M, Bentham KJ

van, Bolker BM, Brooks ME. **2017**. glmmTMB: Generalized Linear Mixed Models using Template Model Builder. R package version 0.1.3.

Mantel SJ, Sweigart AL. **2019**. Divergence in drought-response traits between sympatric species of *Mimulus*. *Ecology and Evolution* **9**: 10291–10304.

Mills LS, Zimova M, Oyler J, Running S, Abatzoglou JT, Lukacs PM. **2013**. Camouflage mismatch in seasonal coat color due to decreased snow duration. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 7360–7365.

Mojica JP, Kelly JK. **2010**. Viability selection prior to trait expression is an essential component of natural selection. *Proceedings of the Royal Society B: Biological Sciences* **277**: 2945–2950.

Mojica JP, Lee YW, Willis JH, Kelly JK. **2012**. Spatially and temporally varying selection on intrapopulation quantitative trait loci for a life history trade-off in *Mimulus guttatus*. *Molecular Ecology* **21**: 3718–3728.

Monnahan PJ, Kelly JK. **2015**. Naturally segregating loci exhibit epistasis for fitness. *Biology Letters* **11**.

Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL, Tsukaya H. **2011**. The evolution and functional significance of leaf shape in the angiosperms. *Functional Plant Biology* **38**: 535–552.

Oakley CG, Schemske DW, McKay JK, Ågren J. **2023**. Ecological genetics of local adaptation in *Arabidopsis*: An 8-year field experiment. *Molecular Ecology* **32**: 4570–4583.

Pélabon C, Hilde CH, Einum S, Gamelon M. **2020**. On the use of the coefficient of variation to quantify and compare trait variation. *Evolution Letters* **4**: 180–188.

Pinheiro J, Bates D. **2000**. *Mixed-Effects Models in S and S-PLUS*. ew York: Springer.

Pinheiro J, Bates D, R Core Team. **2023**. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-163.

Ramsey J, Bradshaw HD, Schemske DW. **2003**. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**: 1520–1534.

Rundle HD, Nosil P. **2005**. Ecological speciation. *Ecology Letters* **8**: 336–352.

Schiestl FP, Schluter PM. **2009**. Floral isolation, specialized pollination, and pollinator behavior in orchids. *Annual Review of Entomology* **54**: 425–446.

Schluter D. **2000**. *The Ecology of Adaptive Radiation*. Oxford Series in Ecology and Evolution.

Schluter D. **2009**. Evidence for ecological speciation and its alternative. *Science* **323**: 737–741.

Schneider CA, Rasband WS, Eliceiri KW. **2012**. NIH Image to ImageJ: 25 years of Image Analysis. *Nature Methods* **9**: 671–675.

Siepielski AM, Dibattista JD, Carlson SM. **2009**. It's about time: The temporal dynamics of phenotypic selection in the wild. *Ecology Letters* **12**: 1261–1276.

Siepielski AM, Morrissey MB, Buoro M, Carlson SM, Caruso CM, Clegg SM, Coulson T, Dibattista J, Gotanda KM, Francis CD, *et al.* 2017. Precipitation drives global variation in natural selection. *Science* **355**: 959–962.

Simko WT. **2021**. R package 'corrplot': Visualization of a Correlation Matrix. (Version 0.92).

Stearns S. **1992**. *The evolution of life histories*. Oxford, UK: Oxford University Press.

Tataru D, De Leon M, Dutton S, Machado-Perez F, Rendahl A, Ferris KG. **2024**. Fluctuating selection in a Monkeyflower hybrid zone. *bioRxiv*: 1–33.

Tataru D, Wheeler EC, Ferris KG. **2023**. Spatially and temporally varying selection influence species boundaries in two sympatric *Mimulus*. *Proceedings of the Royal Society B: Biological Sciences* **290**.

Toll K, Willis JH. **2018**. Hybrid inviability and differential submergence tolerance drive habitat segregation between two congeneric monkeyflowers. *Ecology* **99**: 2776–2786.

Troth A, Puzey JR, Kim RS, Willis JH, Kelly JK. **2018**. Selective trade-offs maintain alleles underpinning complex trait variation in plants. *Science* **361**: 475–478.

Venail J, Dell'Olivo A, Kuhlemeier C. **2010**. Speciation genes in the genus *Petunia*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 461–468.

Via S, Lande R. **1985**. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 505–522.

Vickery RK. **1964**. Barriers to gene exchange between members of the *Mimulus guttatus* complex (Scrophulariaceae). *Evolution* **18**: 52.

Wadgymar SM, Daws SC, Anderson JT. **2017**. Integrating viability and fecundity selection to illuminate the adaptive nature of genetic clines. *Evolution Letters* **1**: 26–39.

Wilczek AM, Cooper MD, Korves TM, Schmitt J. **2014**. Lagging adaptation to warming climate in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 7906–7913.

Wu CA, Lowry DB, Cooley AM, Wright KM, Lee YW, Willis JH. **2008**. *Mimulus* is an emerging model system for the integration of ecological and genomic studies. *Heredity* **100**: 220–230.

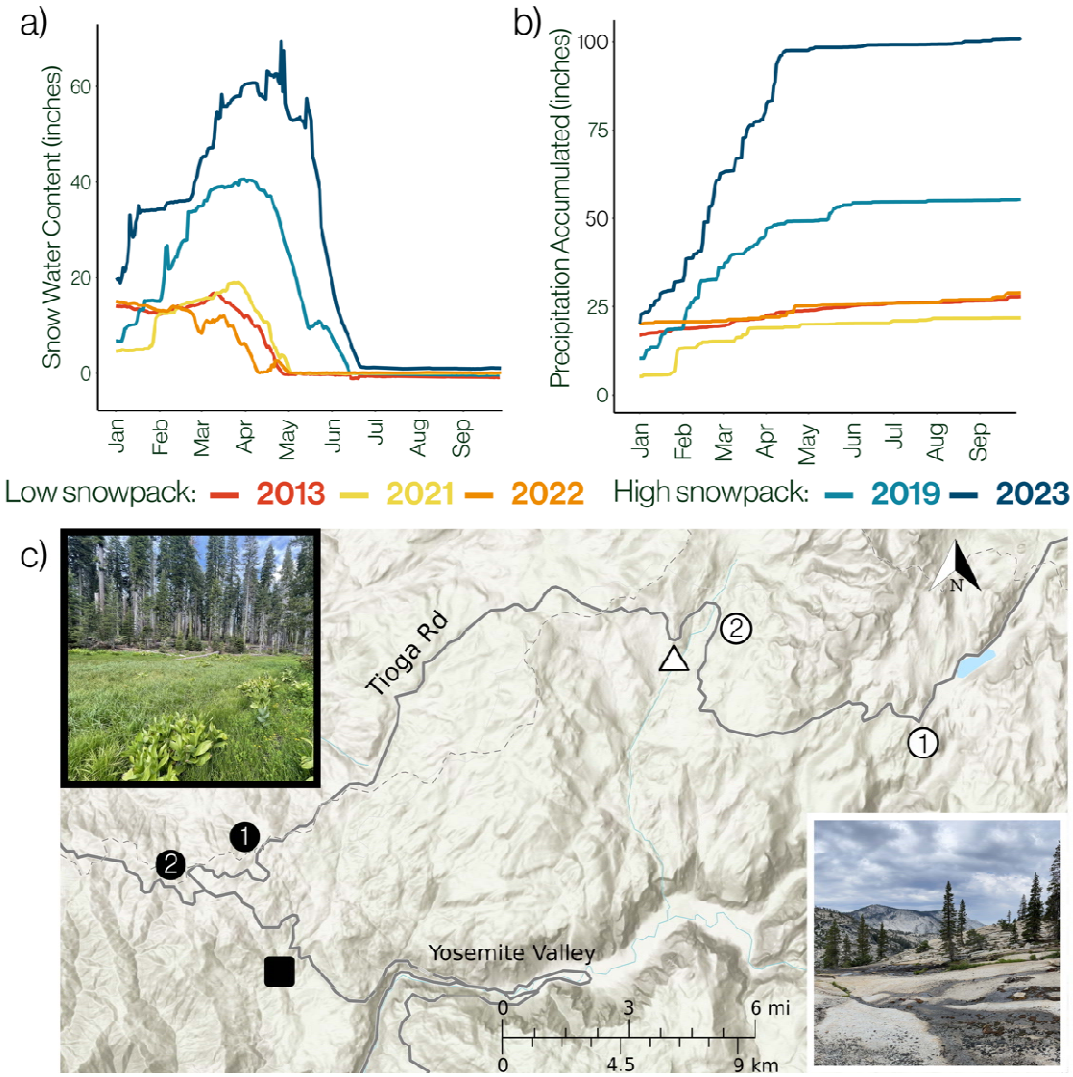

Figure 1. Temporal and spatial scale of the experimental design. Annual changes in a) snow water content and b) accumulated precipitation during experimental years of low snowpack (2013, 2021, 2022) and comparatively higher snowpack (2019 and 2023) with the c) spatial layout of experimental sites: meadow 1 and 2 (filled circles) and granite 1 and 2 (open circles). Nearby parental populations are shown: *M. guttatus* YVO (filled square) and *M. laciniatus* WLF (open triangle). Representative meadow and granite habitats are shown in the insets. Water data are averaged from the California Department of Water Resources data stations GIN, WHW, and TUM, located nearby experimental sites along Tioga Road in Yosemite National Park (cdec.water.ca.gov).

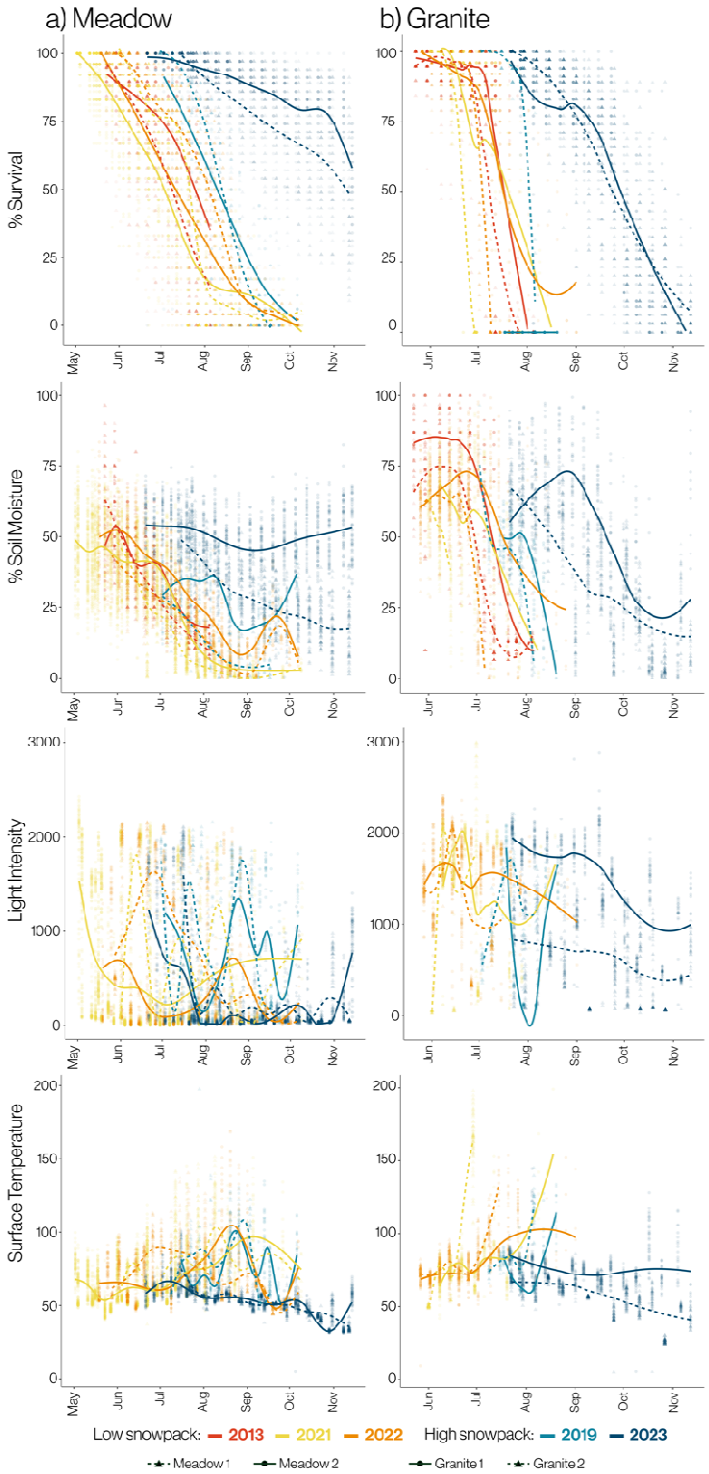

Figure 2. Seasonal changes in survival (%), soil moisture (%), light intensity (µmol m-2 s-1), and soil surface temperature (°F) in each block per site a) meadow and b) granite habitats in low snowpack years (2013, 2021, 2022) and high snowpack years (2019, 2023). Data from 2013 and 2019 are published in Ferris and Willis 2018 and Tataru *et al*. 2023 and re-analyzed here.

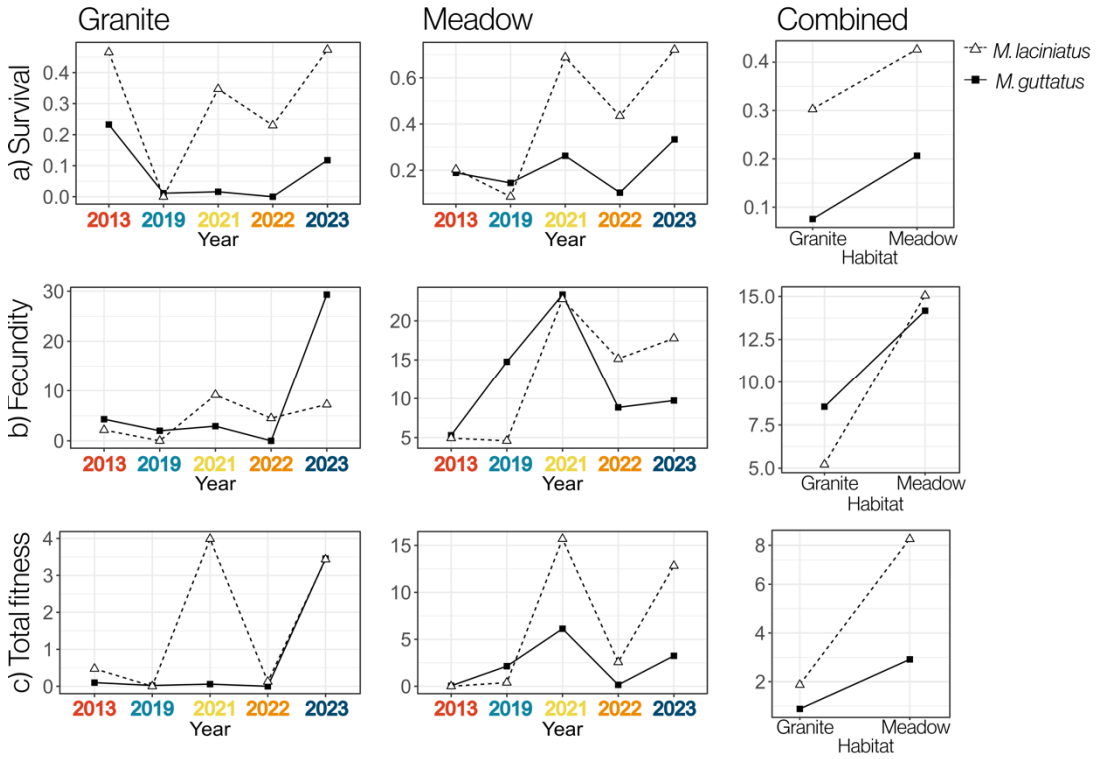

Figure 3. a) Survival to flowering, b) fecundity (mean seed number per reproductive individual), and c) total fitness (mean seed number per planted seed) of *Mimulus guttatus* (filled square, solid line) and *M. laciniatus* (open triangle, dashed line) in granite habitat, meadow habitat, and averaged across years. Calculations of fecundity and total fitness for 2013 are based on fruit number because seed number was not recorded; 2013 was omitted from averaged fecundity and total fitness. Values from 2013 and 2019 are adapted from Ferris and Willis 2018 and Tataru *et al*. 2023.

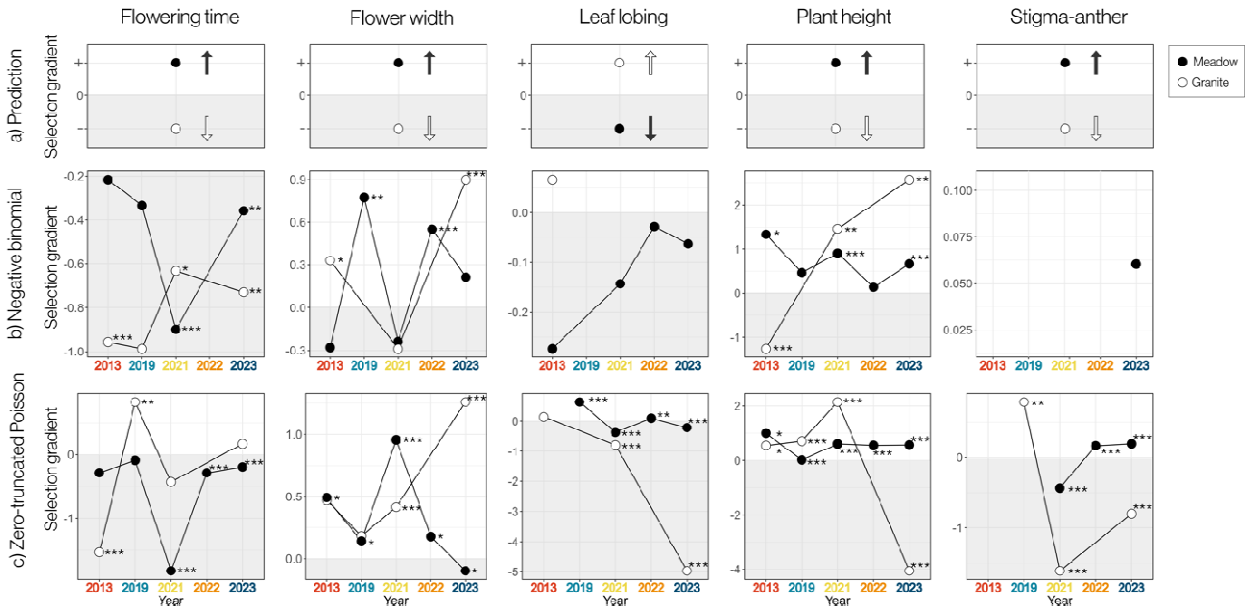

Figure 4. Visual representation of selection gradients (β) measured for hybrids during experimental years in granite (open circles) and meadow (filled circles) habitats. Shown are a) predicted values in each habitat based on native species trait differences and estimated values from b) negative binomial models and c) zero-truncated Poisson models. Significance level is indicated with asterisks and negative graph areas are shaded grey for clarity. The absence of a value during an experimental year indicates that the trait was not present in the best-fit model. All β values are based on seed number, except for values from 2013 which are based on fruit number, as seed number was not recorded. Values from 2013 and 2019 are adapted from Ferris and Willis 2018 and Tataru *et al.* 2023.

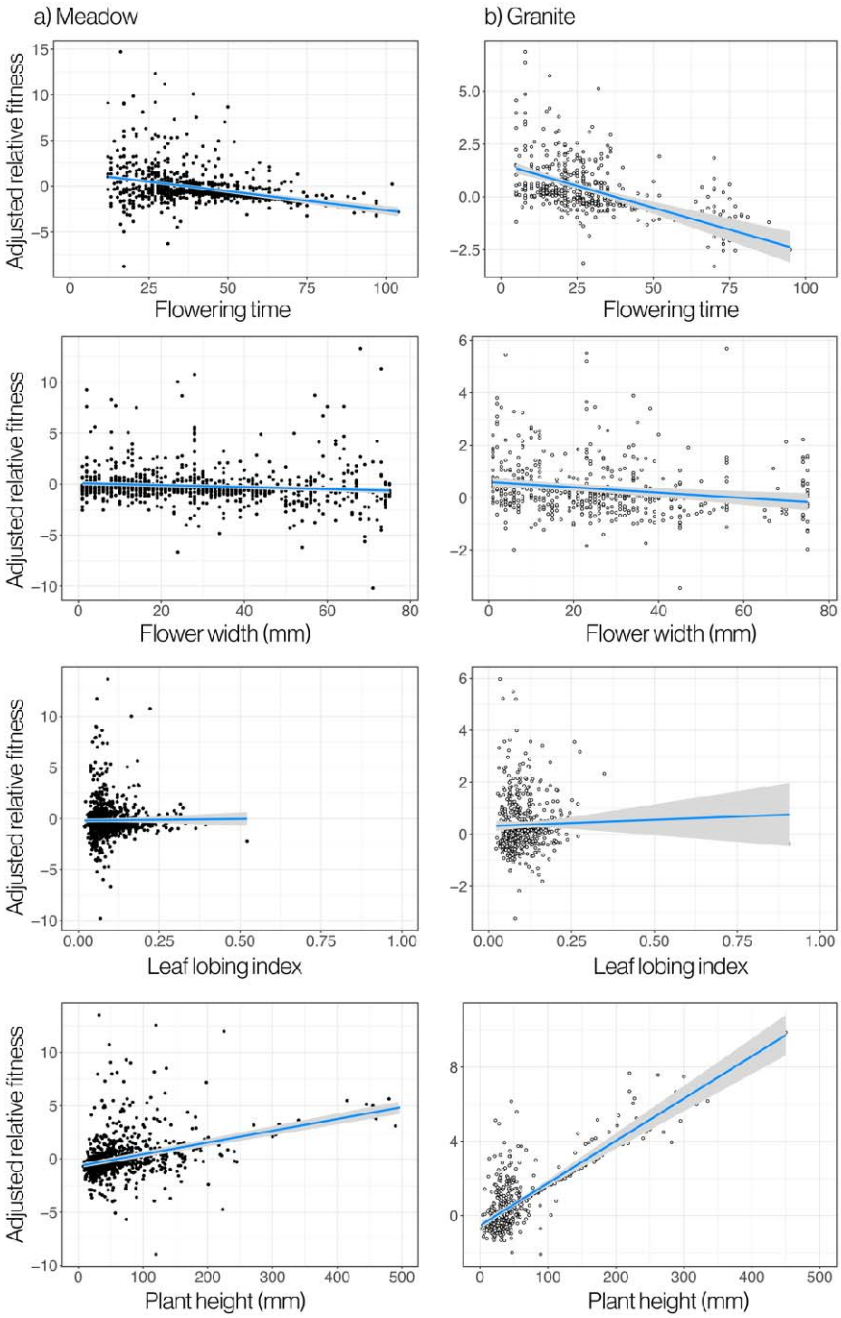

Figure 5. Patterns of cumulative selection with all years combined in a) meadow (filled circles) and b) granite (open circles) habitat in a zero-truncated Poisson analysis on flowering time (days from planting), width of first flower (mm), leaf lobing index, and plant height at flowering (mm to apical meristem). Y-axes show adjusted relative fitness (fruit number), statistically corrected for other variables included in the models, and the x-axes show unstandardized trait values for biological relevance. Data from 2013 and 2019 are published in Ferris and Willis 2018 and Tataru *et al.* 2023 and re-analyzed here.

	2021			2022			2023			
	F_2	*M. guttatus* (YVO18)	*M. laciniatus* (WLF47)	F_3	*M. guttatus* (YVO18)	*M. laciniatus* (WLF47)	F_6	*M. guttatus* (YVO)	*M. laciniatus* (WLF)	"Local"
Meadow	63%	26%	69%	21%	10%	44%	32%	33%	72%	0%
Granite	46%	2%	35%	1%	0%	23%	8%	12%	47%	0%

Table 1. Percentage surviving to first flower of hybrids, *M. guttatus* (YVO), *M. laciniatus* (WLF), and local site native genotypes (2023 only).

		Meadow			Granite		
Genotype	**Trait**	**Mean**	**SE**	**CV**	**Mean**	**SE**	**CV**
F_2	Fl. time	31.58***	0.61	0.45	14.57***	0.31	0.43
	Height	57.52***	1.40	0.57	28.43***	0.63	0.45
	Fl. Width	5.89	0.11	0.37	6.09	0.12	0.36
	Leaf area	48864.77**	3838.85	1.57	33811.16**	2769.86	1.52
	Lobing	0.08***	0.00	0.45	0.07***	0.00	0.37
	Herkogamy	-1.34***	0.05	-0.73	-0.86***	0.07	-1.36
***M. guttatus* (YVO 18)**	Fl. time	44.59*	0.99	0.34	35.08*	4.23	0.42
	Height	74.65***	2.56	0.46	20.56***	3.50	0.51
	Fl. Width	7.49	0.22	0.33	5.88	0.81	0.36
	Leaf area	50215.94***	6314.38	1.47	11519.75***	1912.33	0.94
	Lobing	0.08*	0.00	0.68	0.06*	0.00	0.33
	Herkogamy	-1.63	0.10	-0.76	-0.94	0.31	-0.94
***M. laciniatus* (WLF 47)**	Fl. time	26.7***	0.49	0.45	19.21***	0.54	0.48
	Height	34.04***	0.75	0.49	20.41***	0.72	0.57
	Fl. Width	1.96***	0.05	0.49	2.55***	0.15	0.73
	Leaf area	16642.84**	1268.08	1.46	12059.12**	1095.45	1.07
	Lobing	0.08***	0.00	0.47	0.07***	0.03	0.40
	Herkogamy	-0.92	0.10	-1.17	-0.74	0.08	-1.01

Table 2. Trait means, standard error (SE), standard deviation (SD), and coefficient of variation (CV) from the 2021 experiment. Significant variation in trait means between habitat is indicated by asterisks (p-value < 0.001***; 0.01**; 0.05*).

		Meadow			Granite		
Genotype	**Trait**	**Mean**	**SE**	**CV**	**Mean**	**SE**	**CV**
F_3	Fl. time	59.92***	0.70	0.21	44.19***	3.10	0.32
	Height	54.89***	2.07	0.69	13.14***	1.52	0.53
	Fl. Width	5.52***	0.13	0.41	3.34***	0.30	0.41
	Leaf area	1013.85***	77.72	1.31	208.62***	27.30	1.09
	Lobing	0.07***	0.00	0.33	0.09***	0.00	0.24
	Herkogamy	-1.35*	0.05	-0.67	-0.92*	0.17	-0.77
***M. guttatus* (YVO 18)**	Fl. time	72.66	1.89	0.17	-	-	-
	Height	69.15	5.22	0.48	-	-	-
	Fl. Width	7.73	0.36	0.29	-	-	-
	Leaf area	1788.03***	220.71	0.71	169.69***	22.08	0.47
	Lobing	0.05**	0.00	0.27	0.08**	0.00	0.22
	Herkogamy	-2.40	0.13	-0.33	-	-	-
***M. laciniatus* (WLF 47)**	Fl. time	44.53***	0.83	0.25	34.65***	1.08	0.30
	Height	15.61***	0.99	0.84	7.16***	0.42	0.57
	Fl. Width	1.25	0.08	0.74	1.22	0.14	0.97
	Leaf area	294.09***	22.64	0.97	97.56***	4.31	0.41
	Lobing	0.08*	0.00	0.30	0.09*	0.00	0.29
	Herkogamy	-0.85	0.05	-0.65	-1.01	0.11	-0.71

Table 3. Trait means, standard error (SE), standard deviation (SD), and coefficient of variation (CV) from the 2022 experiment. Significant variation in trait means between habitat is indicated by asterisks (p-value < 0.001***; 0.01**; 0.05*)

		Meadow				Granite			
Genotype	**Trait**	**Mean**	**SE**	**SD**	**CV**	**Mean**	**SE**	**SD**	**CV**
F_6	Fl. time	43.04***	0.28	15.3	0.36	35.77***	0.36	19.67	0.55
	Height	91.71***	1.01	55.14	0.60	25.22***	0.27	14.81	0.59
	Fl. Width	5.62***	0.04	2.23	0.40	4.68***	0.04	2.35	0.50
	Leaf area	14342.24***	287.00	15722.42	1.10	6183.81***	173.97	9528.94	1.54
	Lobing	0.08	0.00	0.05	0.63	0.08	0.00	0.06	0.75
	Herkogamy	-0.34*	0.03	1.4	-4.12	-0.4	0.02	1.09	-2.73
***M. guttatus* (YVO)**	Fl. time	52.94**	0.61	12.14	0.23	44.6**	0.78	15.49	0.35
	Height	76.17***	1.75	34.94	0.46	34.56***	0.84	16.78	0.49
	Fl. Width	9.92*	0.13	2.68	0.27	8.69*	0.16	3.1	0.36
	Leaf area	18876.1***	942.86	18833.71	1.00	11853.5***	655.40	13091.55	1.10
	Lobing	0.07**	0.00	0.03	0.43	0.08**	0.00	0.02	0.25
	Herkogamy	-0.66	0.08	1.5	-2.27	-1.11	0.07	1.41	-1.27
***M. laciniatus* (WLF)**	Fl. time	24.28***	0.71	14.19	0.58	20.68***	0.92	18.36	0.89
	Height	58.72***	1.72	34.45	0.59	21.16***	0.56	11.16	0.53
	Fl. Width	3.01	0.08	1.59	0.53	3.37	0.07	1.44	0.43
	Leaf area	17699.42***	850.63	16991.34	0.96	8788.1***	327.31	6537.97	0.74
	Lobing	0.15**	0.00	0.08	0.53	0.11**	0.00	0.07	0.64
	Herkogamy	-0.36	0.05	0.98	-2.72	0.12	0.05	0.92	7.67

Table 4. Trait means, standard error (SE), standard deviation (SD), and coefficient of variation (CV) from the 2023 experiment. Significant variation in trait means between habitat is indicated by asterisks (p-value < 0.001***; 0.01**; 0.05*)

	Meadow			Granite	
	2021	**2022**	**2023**	**2021**	**2023**
Trait	**β F2**	**β F3**	**β F6**	**β F2**	**β F6**
Flowering time (FT)	-0.900***	-	-0.358**	-0.632*	-0.730**
Flower width (FW)	-0.239	0.551***	0.213	-0.288	0.893***
Leaf lobing (LL)	-0.143	-0.028	-0.062	-	-
Herkogamy (H)	-	-	0.060	-	-
Plant height (PH)	0.903***	0.137	0.662***	1.461**	2.571**
FW x PH	-0.291*	-	-0.251*	-1.057	-
FT x H	-	-	0.237*	-	-
LL x PH	-0.232	0.096*	-	-	-
FT x PH	-	-	-	-	-
FW x LL	-	-	-0.483**	-	-
H x PH	-	-	-	-	-
FT x FW	-	-	-	-	-
n =	200	258	775	166	214

Table 5. Negative binomial analysis on whether or not a plant produced seeds. Values indicate the selection gradient (β), or strength of selection. Asterisks indicate significance of selection in a trait: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Missing values indicate traits which were not included in the best-fit model.

	Meadow			Granite	
	2021	**2022**	**2023**	**2021**	**2023**
Trait	**β F2**	**β F3**	**β F6**	**β F2**	**β F6**
Flowering time (FT)	-1.827***	-0.289***	-0.195***	-0.432	0.173
Flower width (FW)	0.957***	0.178*	-0.097*	0.415***	1.254***
Leaf lobing (LL)	-0.374***	0.094**	-0.216***	-0.794***	-4.978***
Herkogamy (H)	-0.436***	0.167***	0.195***	-1.610***	-0.800***
Plant height (PH)	0.615***	0.563***	0.577***	2.15***	-4.056***
FT x FW	1.964***	0.542***	0.317***	-	-0.854***
FT x H	-0.956***	-0.314***	0.311***	-2.022***	-
FT x PH	-0.651***	-0.420***	-0.097*	1.110*	-
FW x LL	0.715***	-	-0.423***	-	1.667***
FW x PH	-0.601***	-0.154***	-0.126***	-	-1.700***
LL x PH	-0.186***	-	0.241***	-1.517***	-8.017***
H x PH	0.401***	0.072**	-0.072***	0.769***	-1.978***
n =	128	134	202	87	51

Table 6. Zero-truncated Poisson analysis on number of seeds produced if any were produced. Values indicate the selection gradient (ß), or strength of selection. Asterisks indicate significance of selection in a trait: ***p < 0.001; **p < 0.01; *p < 0.05. Missing values indicate traits were not included in the best-fit model.

Trait	Negative binomial		Zero-truncated Poisson	
	Meadow	**Granite**	**Meadow**	**Granite**
Flowering time (FT)	-0.428***	-0.807***	-1.082***	-1.122***
Flower width (FW)	-0.069*	-0.091**	-0.239***	-0.192*
Leaf lobing (LL)	-	-	0.056	0.198
Plant height (PH)	0.602***	0.853***	0.962***	1.056***
FT x FW	-0.093*	-	-0.218***	-0.163
FT x PH	-0.150***	-0.570***	-0.114	-0.348**
FT x LL	-	-	-	-0.305**
FW x LL	-	-	-0.059	0.096
FW x PH	-	0.118**	0.076	0.195*
LL x PH	-	-	0.040	0.472
FT x FW x LL	-	-	-	0.277**
FT x LL x PH	-	-	-	-0.223*
FT x FW x PH	-	-	0.109*	-
FW x LL x PH	-	-	-0.162*	-0.304***
n =	2,014	992	998	547

Table 7. Phenotypic selection analyses on a combined dataset of 2013, 2019, 2021, 2022, and 2023 using fruit number as a metric of fitness. Values indicate the selection gradient (ß), or strength of selection. Asterisks indicate significance of selection in a trait: ***p < 0.001; **p < 0.01; *p < 0.05. Missing values indicate traits were not included in the best-fit model. Herkogamy was excluded due to its absence from the 2013 dataset. Data from 2013 and 2019 are published in Ferris and Willis 2018 and Tataru *et al*. 2023 and re-analyzed here.

Supplementary Information

Fig S1 Combined survival to flowering, fecundity, and total fitness excluding 2023.

Fig S2 Trait correlation matrices for F2 hybrids in 2021 in meadow and granite habitats.

Fig S3 Trait correlation matrices for F3 hybrids in 2022 in meadow and granite habitats.

Fig S4 Trait correlation matrices for F6 hybrids in 2023 in meadow and granite habitats.

Fig S5 Patterns of cumulative selection with all years combined in meadow and granite habitats in a negative binomial analysis.

Table S1 Intrapopulation parental crosses used in the 2023 experiment.

Table S2 Model selection for best fit models of plant survival and environmental variables.

Table S3 Model selection for best fit models of hybrid traits.

Table S4 Model selection for best fit models of herbivory.

Table S5 Negative binomial analysis on whether or not a plant produced fruits.

Table S6 Zero-truncated Poisson analysis on number of fruit produced if any were produced.

Table S7 Strength of correlations between fruit number and seed number.

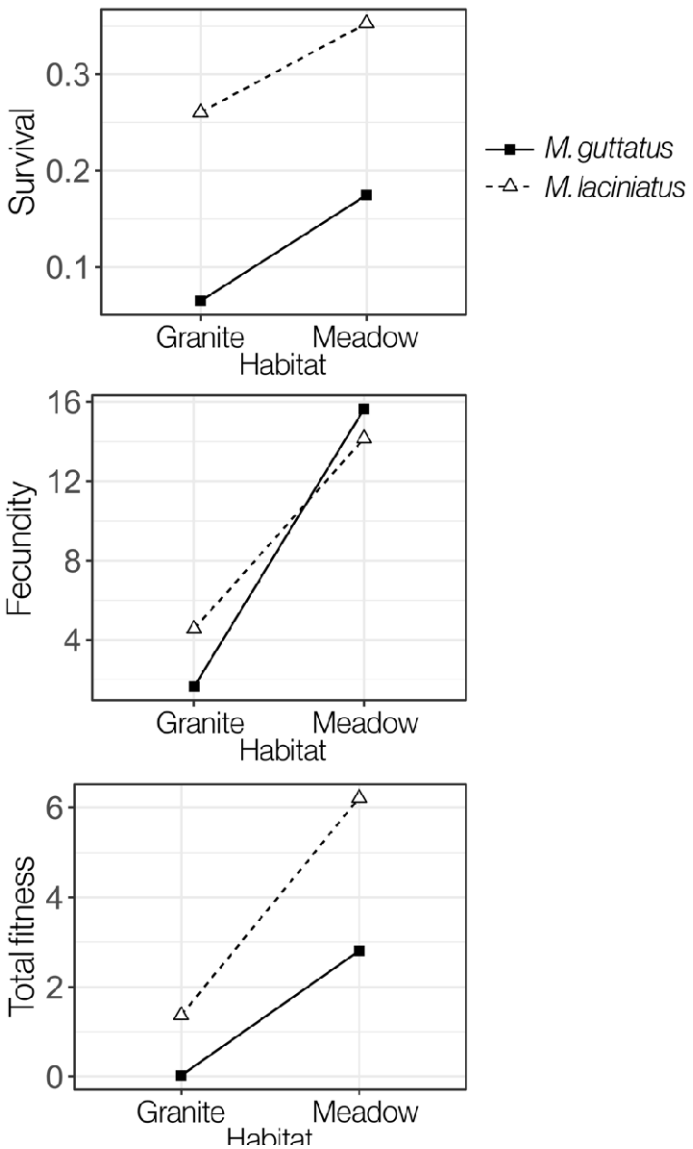

Figure S1. Combined survival to flowering, fecundity (mean seed number per reproductive individual), and total fitness (mean seed number per planted seed) of *Mimulus guttatus* (filled square, solid line) and *M. laciniatus* (open triangle, dashed line) in granite and meadow habitat from 2013-2022 (excluding 2023). 2013 was omitted from fecundity and total fitness calculations because seed number was not recorded, only fruit number. Values from 2013 and 2019 are adapted from Ferris and Willis 2018 and Tataru *et al*. 2023.

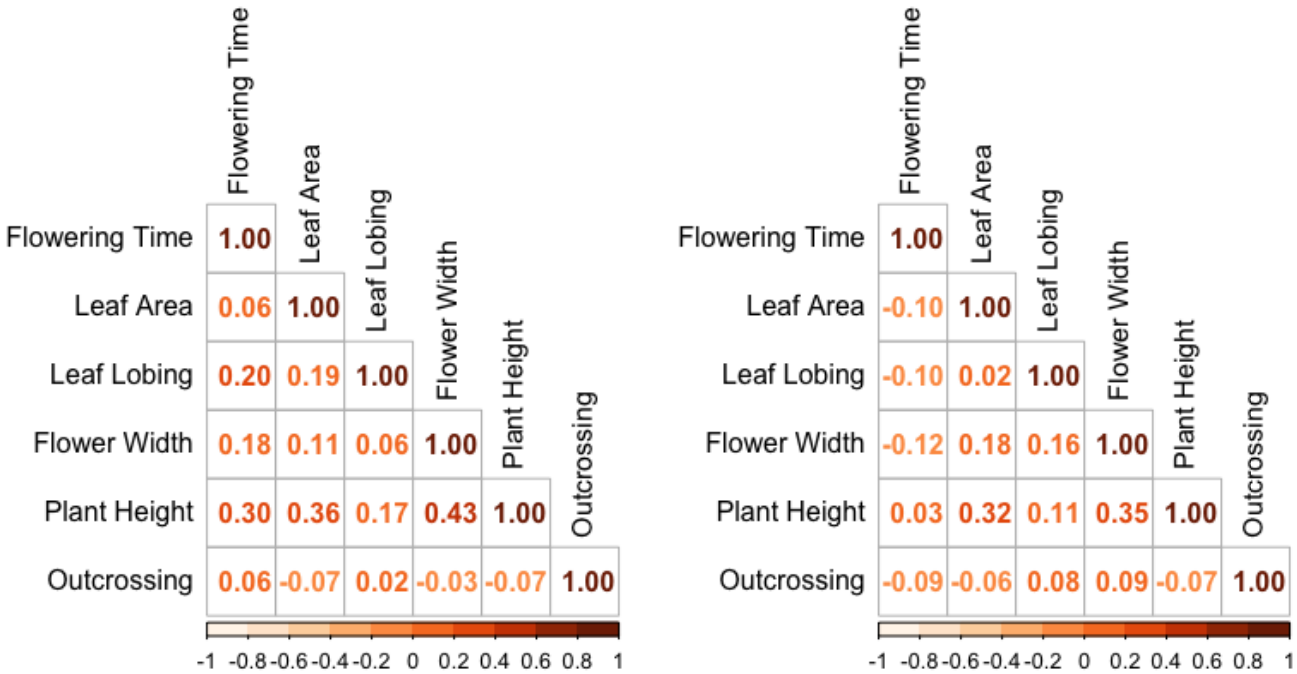

Figure S2. Trait correlation matrices for F2 hybrids in 2021 in meadow (left) and granite (right) habitats.

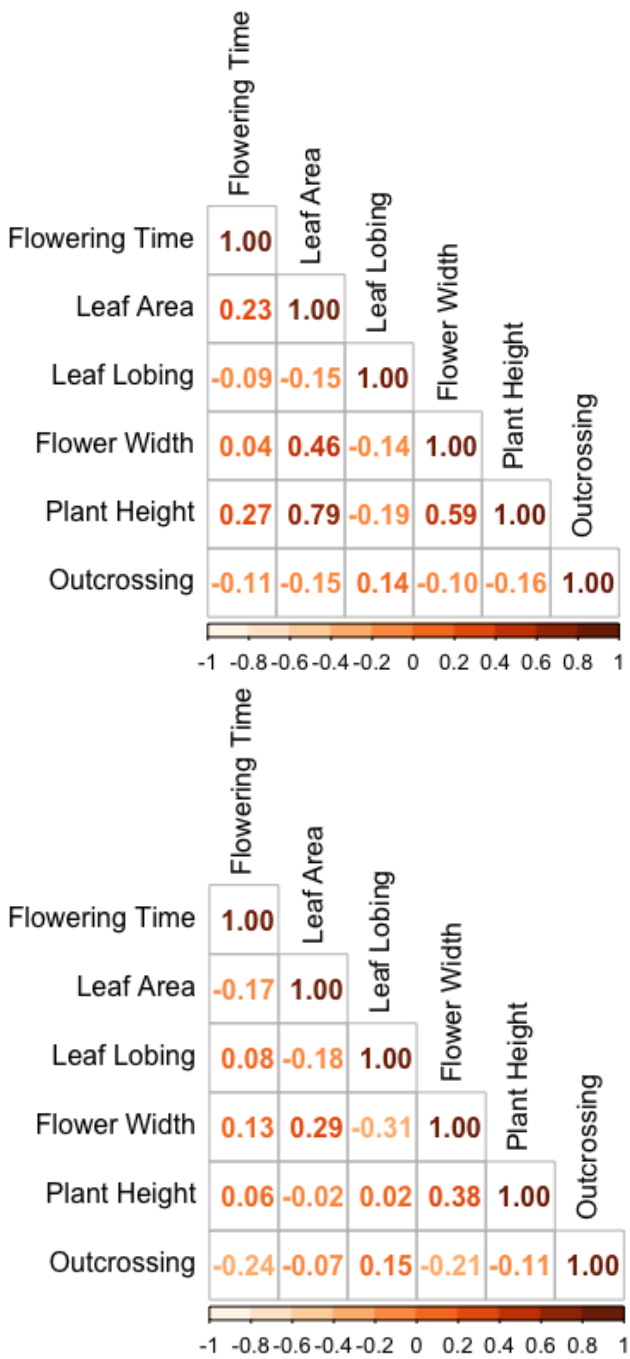

Figure S3. Trait correlation matrices for F3 hybrids in 2022 in meadow (left) and granite (right) habitats.

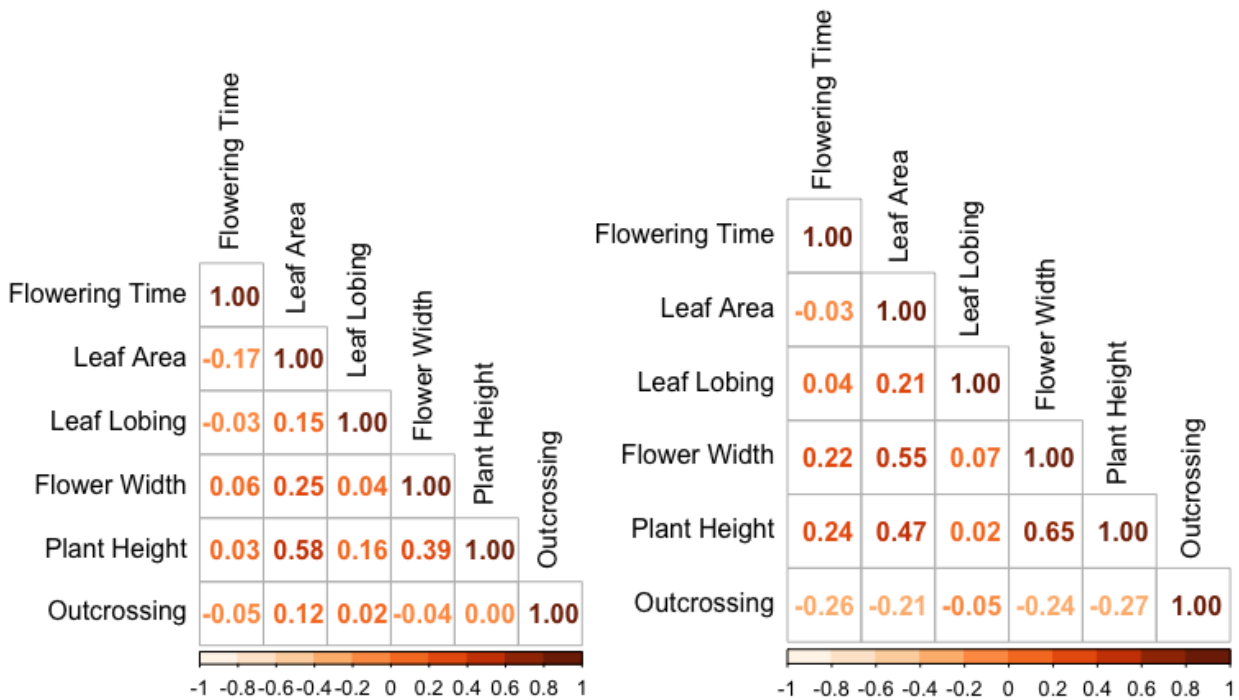

Figure S4. Trait correlation matrices for F6 hybrids in 2023 in meadow (left) and granite (right) habitats.

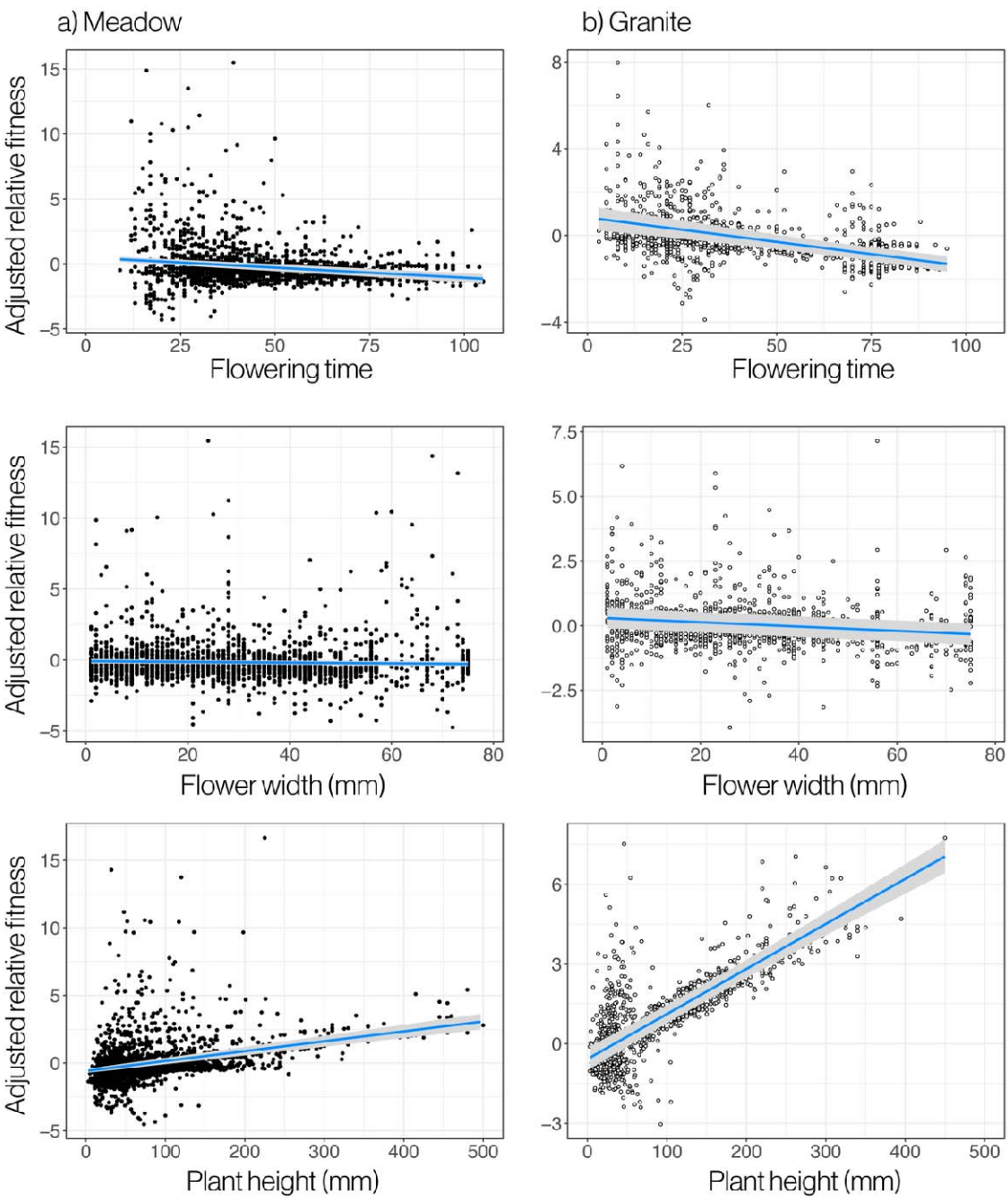

Figure S5. Patterns of cumulative selection with all years combined in a) meadow (filled circles) and b) granite habitats (open circles) in a negative binomial analysis on flowering time (days from planting), width of first flower (mm), leaf lobing index, and plant height at flowering (mm to apical meristem). Y-axes show adjusted relative fitness (fruit number), statistically corrected for other variables included in the models, and the x-axes show unstandardized trait values for biological relevance. Data from 2013 and 2019 are published in Ferris and Willis 2018 and Tataru *et al.* 2023 and re-analyzed here.

Habitat	Genotype	Survival to flowering	%Survival	Seed count	Mean Seed Count	Seed SD	N
Granite	WLF60xWLF47	81	44	585	7.31	21.27	184
	WLF61xWLF47	108	66	784	7.26	12.78	164
	WLF47 inbred	0	0	0	NA	NA	51
						Total:	**399**
	YVO18xYVO26	0	0	0	NA	NA	20
	YVO25xYVO18	12	8	417	32.08	67.69	159
	YVO26xYVO18	35	18	961	27.46	51.83	200
	YVO18 inbred	0	0	0	NA	NA	20
						Total:	**399**
Meadow	WLF60xWLF47	97	58	1760	17.25	32.41	166
	WLF61xWLF47	156	83	3201	20.13	33.95	187
	WLF47inbred	35	76	156	4.46	8.43	46
						Total:	**399**
	YVO18xYVO26	26	47	208	8	16.78	55
	YVO25xYVO18	26	23	378	14.54	47.43	114
	YVO26xYVO18	55	32	687	12.27	27.66	171
	YVO18 inbred	26	44	21	0.81	2.77	59
						Total:	**399**

Table S1. Intrapopulation parental crosses used in the 2023 experiment. Due to insufficient seed stock of single crosses of YVO and WLF, we used 2-3 crosses and original inbred lines, distributed evenly across blocks and sites. With habitats combined, WLF genotypes did not differ significantly in total seed count ($F = 2.324$, $df = 2$, $p = 0.099$). Similarly, YVO genotypes also did not differ significantly ($F = 1.944$, $df = 3$, $p = 0.124$).

Year	Habitat	Model	df	Log(L)	AIC	delta(i)	wi
2021	meadow	survival ~ time + light + soil + time:soil	7	1338.307	-2662.6	4.56	0.869
2021	granite	survival ~ time + light + soil + temp + time:light + light:temp	9	127.115	-235.9	0.38	0.546
2021	both	survival ~ time + light + soil + temp + time:light + time:soil + light:temp + soil:temp	11	1232.41	-2442.8	13.76	0.997
2022	meadow	survival ~ time + light + soil + temp + time:temp	8	608.991	-1201.9	1.12	0.608
2022	granite	survival ~ time + light + soil + temp + time:temp + light:soil + soil:temp	10	625.324	-1230.4	1.4	0.668
2022	both	survival ~ time + light + soil + temp + time:temp + light:soil + soil:temp	10	882.345	-1744.6	22.24	1
2023	meadow	survival ~ time + soil + temp + soil:temp	7	1580.3	-3146.6	2.4	0.666
2023	granite	survival ~ time + light + soil + temp + time:light + time:soil + time:temp + soil:temp	11	656.63	-1291.1	1.5	0.502
2023	both	survival ~ time + soil + temp + time:soil + time:temp + soil:temp + time:soil:temp	10	1190.095	-2360.1	4.69	0.886

Table S2. Best-fit models of plant survival and environmental variables. For each analysis, we report k, the number of parameters; Log(L), the likelihood score; AIC score, delta(i), the difference between that model's AIC and the top model's AIC; and wi, the model weight.

Year	Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
2021	Herbivory	1	50597	50597	25.25	5.43E-07	***
	Site	3	604179	201393	100.52	< 2e-16	***
	Herbivory*Site	3	75224	25075	12.52	4.10E-08	***
2022	Block	1	153	152.6	0.803	0.37046	
	Site	1	5	4.5	0.024	8.78E-01	**
	Block*Site	1	1961	1961.3	10.323	0.00139	*
2023	Block	1	1048	1048	2.193	0.13887	
	Site	1	4412	4412	9.23E+00	0.00242	**
	Block*Site	1	1832	1832	3.832	0.05049	

Table S3. Best-fit models of plant fitness and herbivory.

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Genotype	Habitat	Metric	Best-fit model	df	Log(L)	AIC	delta(i)	wi
Zero-truncated Poisson (seed)								
F2	granite	seed	seed1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:H + FT:PH + LL:PH + H:PH	12	-343.824	715.9	1.98	0.338
F2	meadow	seed	seed1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:FW + FT:H + FT:PH + FW:LL + FW:PH + LL:PH + H:PH	15	-1944.783	3923.9	28.56	1
F3	meadow	seed	seed1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:FW + FT:H + FT:PH + FW:PH + H:PH	13	-633.098	1295.2	0.85	0.329
F6	meadow	seed	seed1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:FW + FT:H + FT:PH + FW:LL + FW:PH + LL:PH + H:PH	15	-1332.565	2697.7	2.47	0.771
F6	granite	seed	seed1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:FW + FW:LL + FW:PH + LL:PH + H:PH	13	-349.91	735.7	0.2	
Negative binomial (seed)								
F2	granite	seed	seed ~ FT + FW + PH + (1 | Site/Block) + FW:PH	9	-412.503	844.2	0.04	
F2	meadow	seed	seed ~ FT + FW + LL + PH + (1 | Site/Block) + FW:PH + LL:PH	11	-744.987	1513.4	0.05	0.044
F3	meadow	seed	seed ~ FW + LL + PH + (1 | Site/Block) + LL:PH	9	-663.336	1345.4	0.86	

F6	meadow	seed	seed ~ FT + FW + LL + H + PH + (1 | Site/Block) + FT:H + FW:LL + FW:PH	13	-1188.134	2402.7	0.5	
F6	granite	seed	seed ~ FT + FW + PH + (1 | Site/Block)	8	-299.661	616	0.8	
Zero-truncated Poisson (fruit)								
F2	granite	fruit	fruit1 ~ FT + PH + (1 | Site/Block)	5	-126.834	264.3	0.17	0.05
F2	meadow	fruit	fruit1 ~ FT + FW + LL + H + PH + (1 | Site/Block) + LL:PH + H:PH	10	-301.67	624.9	1.13	0.104
F3	meadow	fruit	fruit1 ~ PH + (1 | Site/Block)	4	-101.927	212.1	0.64	0.055
F6	meadow	fruit	fruit1 ~ FT + FW + H + PH + (1 | Site/Block) + H:PH	8	-302.086	620.7	0.44	0.085
F6	granite	fruit	fruit1 ~ FT + FW + PH + (1 | Site/Block) + FT:PH	7	-66.555	148.7	0	
Combined	meadow	fruit	fruit1 ~ FT + FW + LL + PH + (1 | Site) + (1 | year) + FT:FW + FT:PH + FW:LL + FW:PH + LL:PH + FT:FW:PH + FW:LL:PH	14	-1265.383	2559.2	1.13	0.093
Combined	granite	fruit	fruit1 ~ FT + FW + LL + PH + (1 | Site) + (1 | year) + FT:FW + FT:LL + FT:PH + FW:LL + FW:PH + LL:PH + FT:FW:LL + FT:LL:PH + FW:LL:PH	16	-593.575	1220.2	0.35	0.115
Negative binomial (fruit)								
F2	granite	fruit	fruit ~ PH + (1 | Site/Block)	6	-242.524	497.6	0.31	
F2	meadow	fruit	fruit ~ FT + FW + H + PH + (1 | Site/Block) + FT:FW + FW:PH + H:PH	12	-399.2	824.1	0.07	0.065

F3	meadow	fruit	fruit ~ FW + LL + (1 | Site/Block)	7	-289.138	592.7	0	
F6	meadow	fruit	fruit ~ FT + FW + LL + PH + (1 | Site/Block) + FT:PH	10	-811.743	1643.8	0.71	0.09
F6	granite	fruit	fruit ~ FT + FW + PH + (1 | Site/Block) + FT:PH	9	-194.445	407.8	0.77	
Combined	meadow	fruit	fruit ~ FT + FW + PH + (1 | Site) + (1 | year) + FT:FW + FT:PH	10	-2567.225	5154.6	0.2	0.063
Combined	granite	fruit	fruit ~ FT + FW + PH + (1 | Site) + (1 | year) + FT:PH + FW:PH	10	-1220.19	2460.6	0.41	

Table S4. Model selection for best-fit models of hybrid traits in 2021 (F2), 2022 (F3), 2023 (F6), and with years combined (2013, 2019, 2021, 2022, 2023). For each analysis, we report df, degrees of freedom; Log(L), the likelihood score; AIC score, delta(i), the difference between that model's AIC and the top model's AIC; and wi, the model weight. FT, flowering time; FW, flower width; LL, leaf lobing; PH, height; H, for herkogamy (stigma-anther separation).

	Meadow			Granite	
	2021	**2022**	**2023**	**2021**	**2023**
Trait	**β F2**	**β F3**	**β F6**	**β F2**	**β F6**
Flowering time (FT)	-0.90524***	-	-0.28166**	-	-0.7680*
Flower width (FW)	-0.07251	0.31918***	-0.22248*	-	0.5890***
Leaf lobing (LL)	-	0.12841	0.18433*	-	1.5173***
Herkogamy (H)	-0.04269	-	-	-	-
Plant height (PH)	0.7464***	-	0.39770***	0.5202*	-
FW x PH	-0.20885*	-	-	-	-
LL x PH	-	-	-	-	-
FT x PH	-	-	-0.25262**	-	-0.5164
FW x LL	-	-	-	-	-
H x PH	0.23229*	-	-	-	-
FT x FW	0.25901	-	-	-	-
n =	201	258	776	165	214

Table S5. Negative binomial analysis on whether or not a plant produced fruits. Values indicate the selection gradient (β), or strength of selection. Asterisks indicate significance of selection in a trait: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Missing values indicate traits which were not included in the best-fit model.

	Meadow			Granite	
	2021	**2022**	**2023**	**2021**	**2023**
Trait	**β F2**	**β F3**	**β F6**	**β F2**	**β F6**
Flowering time (FT)	-0.92254***	-	-1.3223**	-0.8667**	-0.7924***
Flower width (FW)	-0.31538***	-	0.7348***	-	-0.40193***
Leaf lobing (LL)	0.11745	-	-	-	-
Herkogamy (H)	-0.04592	-	-	-	0.22461*
Plant height (PH)	0.62646***	0.4410***	1.6214**	1.4328***	0.49728***
FT x FW	-	-	-	-	-
FT x H	-	-	-	-	-
FT x PH	-	-	-1.0061	-	-
FW x LL	-	-	-	-	-
FW x PH	-	-	-	-	-
LL x PH	-0.15017*	-	-	-	-
H x PH	0.24256**	-	-	-	-0.15321**
n =	150	171	79	107	274

Table S6. Zero-truncated Poisson analysis on number of fruit produced if any were produced. Values indicate the selection gradient (ß), or strength of selection. Asterisks indicate significance of selection in a trait: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Missing values indicate traits were not included in the best-fit model.

Year	Habitat	τ	p	n
2021	Meadow	0.56	0.001	1296
	Granite	0.59	0.001	809
2022	Meadow	0.64	0.001	545
2023	Meadow	0.75	0.0001	1393
	Granite	0.69	0.0001	480

Table S7. Strength of correlations between fruit number and seed number calculated using Kendall's correlation analysis.